PHYTOCHEMICAL AND ANTI BACTERIAL STUDIES ON THE STEM BARK OF *LANNEA BARTERI.* (OLIV.) ENGL. (ANACARDIACEAE)

BY

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(MSc/PHARM-SCI/25136/2012-2013)

DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT, FACULTY OF PHARMACEAUTICAL SCIENCES, AHMADU BELLO UNIVERSITY, ZARIA

NOVEMBER, 2015

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A THESIS SUBMITTED TO THE SCHOOL OF POSGRADUATE STUDIES, AHMADU BELLO UNIVERSITY ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER IN SCIENCE DEGREE OF PHARMACOGNOSY

DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT;
FACULTY OF PHARMACEAUTICAL SCIENCES,
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NOVEMBER, 2015

DECLARATION

I declare that the work in this dissertation entitled, Phytochemical and Antibacterial Studies of Stem bark of *Lannea barteri* (Oliv.) Engl. (Anacardiaceae) has been carried out by me in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, under the supervision of Dr. Umar Adam Katsayal and Dr. Umar Habibu Danmalam. The information derived in the literature has been duly acknowledged in the text and list of references provided. No part of this dissertation has been previously presented for another higher degree or diploma at this or other institutions.

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Ibrahim SABO Date

CERTIFICATION

This dissertation entitled **Phytochemical and Antibacterial Studies of Stem bark of *Lannea barteri* (Oliv.) Engl. (Anacardiacea)** by Ibrahim SABO meets the regulations governing the award of the degree of Masters of Science in Pharmacognosy of Ahmadu Bello University, and is approved for its contribution.

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DEDICATION

To Malam Lawan and Alhaji Ibrahim Sabo Katifa for their positive contributions to my life which led to the achievement of this.

ACKNOWLEDGEMENT

I acknowledge the sufficient grace of God that has sustained me through the easy and difficult moments encountered during the course of this research.

I am sincerely grateful to my supervisors Dr. U.A. Katsayal and Dr. U.H. Danmalam for their contributions towards the realization of this work.

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Finally to all the lecturers and staff in the Department of Pharmacognosy and Drug Development, Department of Microbiology, Herbarium unit of Department of Biological Sciences, Faculty of Pharmaceutical Sciences and to all who helped me in diverse ways I say "May God bless you abundantly".

ABSTRACT

Lannea bartei (Oliv.) Engl. is a plant with medicinal and commercial uses found usually in the tropical regions like Africa especially Ivory Coast. The plant is used traditionally to treat various diseases including wound healing and as anti diarrhoea. This research is aimed at investigating the wound healing claim which is due to anti bacterial property of the stem bark of the plant. Powdered stem bark of the plant (2 kg) was extracted with methanol using maceration technique and part of the crude extract obtained (300 g) was used for phytochemical screening and anti bacterial assay against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Salmonella typhi*. Physical constant determination and the chemo microscopical features of the powdered stem bark were also carried out. 50 g of the crude methanolic extracts was dissolved in warm water, filtered and the filtrate was partitioned with hexane, ethyl acetate and n-butanol to obtained the respective fractions of the organic solvents hexane, ethyl acetate, n-butanol and aqueous fractions which were equally used for antibacterial assay. The aqueous fraction and the resultant three fractions obtained after partitioned were tested against *S. aureus, B. subtilis, E. coli and S. typhi.* Most of them were active but the ethyl acetate fraction was more active and was subjected to column and thin layer chromatography leading to the isolation of an oily substance which is yellowish in colour named compound A. The compound was also subjected to antibacterial assay and was found to be active against *S. aureus, B. subtilis, E. coli and S. typhi.* Specific test and FTIR result on the compound A shows the evidence of presence of phenolic group in the compound. The stem bark of *L. barteri* posseses anti bacterial activity and this is attributed to the presence of various classes of compounds which were proven by this research to be present. These compounds include phenols, alkaloid, coumarins etc.

TABLE OF CONTENT

Cover page----------i

Title page----------iii

Declaration----------iv

Certification----------v

Dedication----------vi

Acknowledgment----------vii

Abstract----------viii

Table of content----------x

List of figures----------xvi

List of tables----------xvii

List of plates----------xviii

List of abbreviation----------xix

1.0 INTRODUCTION----------1

1.1 Medicinal plants……1

1.2 Medicinal Plants with Antibacterial Activities.....2

1.3 Statement of Research problem----------3

1.4 Justification of the Research----------4

1.5 Statement of the Research Hypothesis----------4

1.6 Overall Aim of the work----------5

1.7 Specific Aims----------5

2.0 LITERATURE REVIEW----------6

2.1 Prevalence of bacterial infections----------6

2.2 Burden of Bacterial Infections----------7

2.3 Characteristics features of Bacteria----------9

2.4 Classification of Bacterial infections----------10

2.5 Current treatment to Bacterial infections----------12

2.6 Limitations of current Bacterial infections----------13

2.7 Antibacterial Drug resistance----------14

2.8 Mechanisms of Antibacterial Drug resistance----------16

2.9 Plant Derived Agents with Antimicrobial Properties----------18

2.10 Description of *L. barteri*----------22

2.11 Ethno medical uses of *L. barteri*----------25

2.12 Reported Constituents from *L. barteri*----------25

2.13 Some Isolated chemical constituents from the family Anacardiacea ----------26

3.0 MATERIALS AND METHOD----------27

3.1 List of material----------27

3.2 List of Equipment----------27

3.3 List of Media----------27

3.4 List of chemicals ----------27

3.5 Plant Collection, Identification and Preparation----------28

3.5.1 Collection of plant material----------28

3.5.2 Identification of plant material----------28

3.5.3 Preparation of the stem bark----------28

3.6 Pharmacognsostic studies of the stem bark----------28

3.6.1 Determination of moisture constant----------28

3.6.2 Determination of Ash value----------29

3.6.3 Determination of acid-insoluble Ash Value----------29

3.6.4 Determination of water soluble Ash----------30

3.6.5 Water soluble extractives (cold maceration method)----------30

3.6.6 Ethanol soluble extractives (cold maceration method----------31

3.7 Chemo-microscopic studies of the stem bark----------31

3.7.1 Test for lignin----------31

3.7.2 Test for cellulose----------31

3.7.3 Test for starch----------32

3.7.4 Test for Tannins----------32

3.7.5 Test for Gums and Mucilage----------32

3.7.6 Test for fats and oils----------32

3.7.7 Test for calcium oxalates and calcium carbonates----------32

3.8 Extraction of *Lannea barteri* stem bark----------33

3.9 Fractionation of methanolic extract----------33

3.10 Preliminary Phytochemical Screening----------33

3.10.1 Test for Carbohydrates----------33

3.10.2 Test for Anthracene Derivatives----------34

3.10.3 Test for Unsaturated steroid and triterpenes----------34

3.10.4 Test for glycosides----------34

3.10.5 Test for tannins----------35

3.10.6 Test for flavonoids----------36

3.10.7 Test for alkaloids----------36

3.11 Antibacterial Activities of the Organic Solvents Fractions ----------37

3.11.1 Test Organisms----------37

3.11.1 Culture media----------37

3.11.3 Preparation of the Standard Inocula of the Organisms ----------37

3.11.4 Determination of inhibitory activity (Sensitivity test) of the extract using agar well diffusion method.----------38

3.11.5 Determination of minimum inhibitory concentration (MIC)----------38

3.11.6 Determination of minimum bactericidal concentration (MBC)----------39

3.12 Column Chromatography----------39

3.13 Determination of Zone of Inhibition, MIC and MBC for Compound A----------40

3.14 Isolation of compound A----------40

4.0 RESULTS----------43

4.1 Pharmacognostic properties of *L. Barteri*----------43

4.2 Chemo microscopy results----------43

4.3 Extractiion of *Lannea barteri* stem bark----------45

4. 4 partitioning of *Lannea barteri* stem bark----------45

4.5 Preliminary Phytochemical screening ----------45

4.6 Thin layer chromatography of extract and fractions----------47

4.7 Diameter Zone of Inhibition----------49

4.8 Minimum Inhibitory Concentrations (MIC) (mg/ml)----------51

4.9 Minimum Bactericidal Concentrations (MBC) (mg/ml)----------53

4. 10 Column chromatography of ethyl acetate fraction----------55

4.11 Isolation of compound----------57

4.12 Diameter Zone of Inhibition, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration for the Compound A----------59

4.13 Fourier Transformed-Infra Red (FT-IR) Spectroscopy----------61

5.0 DISCUSSION----------63

6.0 SUMMARY, CONCLUSION AND RECOMENDATION----------69

6.1 Summary----------69

6.0 Conclusion----------70

6.3 Recommendations----------71

REFERENCES----------72

APPENDICES----------82

LIST OF FIGURES

Fig 3.1: Fractionating chart for the stem bark of *L. barteri* ----------41

Fig 3.2: column chromatography chart showing the steps in isolation of compound A----------42

Fig 4.1: Fourier Transformed Infrared Spectra of compound A----------62

LIST OF TABLES

Table 4.1: Chemo –microscopy results----------44

Table 4.2 Preliminary phytochemicla screening----------46

Table 4.3: Zone of inhibition(mm) at varying concentration (mg/ml) of the extract----------50

Table 4.4: Minimum inhibitory concentrations (mg/ml) ----------52

Table 4.5: minimum bactericidal concentrations mg/ml----------54

Table 4.6 Determination of zone of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration for the isolated compound----------56

LIST OF PLATES

PLATE I: *Lannea barteri* in it habitat----------24

PLATE II: TLC profile of ethyl acetate fraction ----------48

PLATE III : TLC profile of Hexane fraction.----------48

PLATE IV: TLC profile of column fractions from 31-35----------56

PLATE V: TLC profile of the isolated compound----------58

PLATE VI: TLC profile of the isolated compound sprayed with specific detecting

Reagent (Ferric chloride). ----------58

LIST OF ABBREVIATION

Fig.	Figure
Mg	Milligram
Mm	Millimetre
TLC	Thin layer chromatography
R_f	Retention factor
IR	Infrared ray
FTIR	Fourier Transformed Infrared Ray
MIC	Minimum inhibitory concentrations
MBC	Minimum Bactericidal concentrations
MDR	Multi Drug Resistance

CHAPTER ONE

1.0 INTRODUCTION

1.1 Medicinal Plants

Medicinal plants have also been of importance in the health care system of local communities as the main source of medicine for the majority of the rural population. Plants have not only nutritional value but also, in the eyes of the local people, they have medicinal and ritual or magical values (Adewunmi *et al*., 2001). Plants have been a major source of medicine for human kind. According to available information, a total of at least 35000 plants species are widely used for medicinal purposes. The demand for traditional herbs is increasing very rapidly, mainly because of the harmful effects of synthetic chemical drugs. The global clamor for more herbal ingredients creates possibilities for the local cultivation of medicinal and aromatic crops as well as for the regulated and sustainable harvest of wild plants. Such endeavors could help raise rural employment in the developing countries, boost commerce around the world and perhaps contribute to the health of millions (Anita, 2004). Nigeria is endowed with an enormous diversity of animals and plants, both domesticated and wild, and an impressive variety of habitats and ecosystems. This heritage sustains the food, medicinal, clothing, shelter, spiritual, recreational, and other needs of her population (Odugbemi and Akinsulire, 2006). This biodiversity also ensures the essential ecological functions on which life depends, including a steady supply of clean water, nutrient cycling, and soil maintainance. It is the treasure house from which future food needs, cures diseases, and elements for knowledge and technology will be found. Plants have provided the basis for traditional treatment for different types of diseases and still offer an enormous potential source of new chemotherapeutic agent (Adewunmi *et al*.,

2001). This however requires extraction of the bioactive molecules of pharmacological importance present following purification and identification procedures as well as toxicological studies.

Plants have long been used by man to maintain health and well-being (Kafaru, 1994). The healing power of plants date back to many years (Kafaru, 1994). In Nigeria, application of medicinal plants especially in traditional medicine is currently well- acknowledged and established as a viable profession (Kafaru, 1994). Pharmacognosy as a field of study is concerned with description and identification of drugs both in the whole state and in powder. Such branch of pharmacognosy found importance, particularly for pharmacopoeial and quality control purposes (Evans, 1996). Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs (Preto- rious and Watt, 2001). The local use of natural plants as primary health remedies, due to their pharmacological properties, is quite common in Asia, Latin America and Africa (Bibitha *et al*., 2002).

1.2 Medicinal Plants with Antibacterial Activities

There are reports of the use of medicinal plants against bacteria by different ethnic communities throughout the world. The use of herbs has always been the popular remedy against bacteria especially in the tropical and subtropical regions of the world. These medicinal plants having antagonistic efficacy against bacteria have been evaluated pharmacologically and several active components have been isolated. The medicinal plants that has been pharmacologically investigated using various models and parts of the plants include the following; *Allium cepa, Allium sativum, Pimpinellaanisum, Sassafras albidum, Morindacitrifolia, Gaultheria procumbens, Zingiberofficinale* Roscoe; occasionally *Z. Capitatum,*Siegesbeckia

Orientalis Linn., *BerberistinctoriaLesch. Justiciabetonica Linn*.(Sasikumar *et al*.,2006), *Saturejabakhtiarica* (Ahanjan *et al.,* 2014).*Anaphalis margaritacea* (L.) Benth & Hook.f., *Grindelia squarrosa* (Pursh) Dunal, *Apocynum androsaemifolium* L., *Arctostaphylosuva-ursi* (L.) Spreng, *Cornuscanadensis* L., *Rauvolfia serpentine, Tageteserecta, Xanthium strumarium* L. (Hassan *et al*., 2014).

1.3 Statement of Research Problem

Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs (Silver, 1993). It is therefore very necessary that the search for newer antibiotic sources be a continuous process. The prevalence of drug resistant bacteria is becoming a worldwide problem with implications for treatment of patients. Moreover, more effort should be made to seek anti microbial agent effective against pathogenic micro organism resistant to current treatment (Turkoglu *et al*., 2006). According to various medical literatures, several adverse drug resistant bacteria and fungal pathogens have complicated further the treatment of infectious diseases in immune compromised, AIDS and cancer patients (Diamond, 1991). Plants extracts like that of *L. barteri* showing target sites other than used by antibiotics will be active against drug resistant bacteria. However very little information is available on such activity of medicinal plants.

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients in developing countries (Al-Bari *et al*., 2006). The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced

susceptibility to antibiotics raised the spectre of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies (Zy *et al*., 2005; Rojas *et al*., 2006).

1.4 Justification of the Research

For a long time, plants have been an important source of natural products for human health. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties (Adriana *et al*., 2007). The practice of complementary and alternative medicine is now on the increase in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections. (Vijaya and Ananthan, 1997; Dilhuydy and Patients, 2003). Plants are the cheapest and safer alternative sources of antimicrobials (Pretorius and Watt, 2001; Sharif and Banik, 2006; Doughari *et al*., 2007).

Since the medicinal properties and chemical information on *L. barteri* is not well documented and the plant is used without scientific authentication, this research would provide comprehensive chemical information about the stem-bark of the plant and also provide information about the potent constituents responsible for it antibacterial activity.

1.5 Statement of the Research Hypothesis

Stem bark of *L. barteri* contains bioactive constituents which have antibacterial activity.

1.6 Overall Aim of the Project

To investigate the anti bacterial properties of the stem bark of *L. Barteri.*

1.7.1 Specific Aim 1

- To evaluate the pharmagonostic features of *L. barteri* stem bark.

1.7.2 Specific Aim 2

- To screen for the phytochemical constituents present in the stem bark of *L. barteri.*

1.7.3 Specific Aim 3

- To screen the anti bacterial properties of the crude plant extract and its fractions.

1.7.4 Specific Aim 4

- To isolate chemical compound(s) from the active fraction(s) obtained from the crude extract of *L. baretri* stem bark

1.7.6 Specific Aim 5

- To carry out Fourier transformed infrared red (FTIR) analysis on the isolated compound.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Prevalence of Bacterial Infections

Bacterial infections especially urinary tract infection (UTI) has been estimated that symptomatic UTI occurs in as many as 7 million people visiting emergency units and 100,000 hospitalizations annually in USA (Schappert, 1997). UTI has become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections, and it is the second most common cause of bacteraemia in hospitalized patients (Stamm, 2002; Weinstein, 1997). In Nigeria, the prevalence varies from location to locations, in Nasarawa, the prevalence has been reported to be up to 30% of the populations (Kolawale *et al.*,2009). 35.5% rate was recorded by Ebie *et al*., (2001) in Rukuba Military Cantonment, Jos, Plateau State. Similarly Mbata (2007) recorded 77.9% among Prison inmates in Nigeria.

The commonest bacteria found in infected wound are *Pseudomonas spp, Escherichia coli, Staphylococcus aureus* and their prevalence in Nigerian tertiary hospitals (Unversity Teaching Hospitals) between 1995-2001 were recorded as *Pseudomonas spp*.-29.9%, *S. aureus* (27.5 %). *E. coli* (7 %) (Lateef *et al.*,2003).

In Ibadan, an increase prevalence of eczema, idiopathic pruritus, urticaria, connective tissue diseases, and fixed drug eruptions was reported. Infections, such as scabies, candidiasis, and tinea versicolor, had also increased. Pyoderma, leprosy, onchocerciasis, and dermatophytoses showed a decline. Psoriasis was uncommon, although there was a slight increase in prevalence. Vitiligo and alopecia were stable. Cutaneous tuberculosis, such as lupus vulgaris, was rare (Ogunbiyi *et al.,* 2004).

2.2 Burden of Bacterial Infections

Human beings are constantly in contact with a myriad of micro-organisms in the environment. However, they are in even more intimate contact with an enormous number of micro-organisms that inhabit their bodies. There are thousands of species of bacteria which cause variety of diseases in human beings. In recent years infections caused by bacteria resistant to multiple antibiotics have been a significant problem. Some commonly encountered pathogens have been associated with some of the human diseases. Methicillin resistant *Staphylococcus aureus* (MRSA) has been troubling hospital services all over the world (Archibald *et al*., 1997; Smith *et al.*, 1999).

Staphylococcus aureus (literally "Golden Cluster Seed") is the most common cause of Staph infection. It is a spherical bacterium, frequently living on the skin or in the nose of a person that can cause a range of illness from minor skin infections, such as pimples, impetigo, boils, cellulites and abscesses, to life- threatening diseases, such as pneumonia, meningitis, endocarditis, and septicemia. It is a facultatively anaerobic, gram positive bacteria, which causes food poisoning and usually grow on the nasal membrane and skin. It is also found in the gastrointestinal and urinary tracts of warm blooded animals. Its incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections. Every year, about 500,000 patients in American hospitals contact a Staphylococcal infection. Staphylococcal resistance to penicillin is mediated by penicillinase (a form of β-lactamase) production: an enzyme which breaks down the β-lactam ring of the penicillin molecule. Penicillinase-resistant penicillins such as Methicillin, oxacillin, cloxacillin, dicloxacillin and flucloxacllin are able to resist degradation by staphylococcal penicillinase. Vancomycin-resistant

S. aureus (VSRA) is a strain that became resistant to the glycopeptides. The first case of Vancomycin-intermediate *S. aureus* was reported in Japan (Black, 2000 Cheesbrough, 2000).

Also the worldwide emergence of *E. coli* and many other β- lactamase producers became a major therapeutic problem (Ferreira *et al.*, 2004). *E.coli* is a gram negative bacteria usually motile. It is an extremely versatile opportunistic pathogen (Cheesbrough, 2000) that causes septic mias and can infect the gall bladder, meninger, surgical wound, skin lesions, and the lungs especially in debilitate and immuno-deficient patients (Black, 2000).

Another highly infectious microbe *Salmonella typhimurium* also causes serious diseases. Salmonella genus of rod-shaped, Gram-negative, non-spore-forming, predominantly motile enterobacteria. The genus Salmonella was ultimately named after Daniel Elmer Salmon, an American veterinary pathologist. Salmonella infections are zoonotic and can be transferred between humans and non-human animals. Many infections are due to ingestion of contaminated food. Salmonella infection may spread from the intestines to the blood stream, and then to other body sites and can cause death unless the person is treated promptly with antibiotics. The elderly, infants, and those with impaired immune systems are more likely to develop severe illness. It is estimated that every fifth person in Germany is a carrier of Salmonella. In the USA, there are approximately 40,000 cases of Salmonella infection reported each year. According to the World Health Organization, over 16 million people worldwide are infected with typhoid fever each year, with 500,000 to 600,000 fatal cases (Cheesbrough, 2000), In Africa, *S. typhi* is the major cause of typhoid, and it has an estimated crude incidence of 362 cases per 100,000 persons per year (Buckle et *al*., 2012).

2.3 Characteristics Features of Bacteria

Bacteria are microscopic, single-celled organisms found in air, water, soil, and food. They live on plants, insects, animals, pets, and even in the human digestive system and upper respiratory tract. There are thousands of kinds of bacteria, but only a few actually cause disease in humans.

Bacteria are frequently identified by their shape, the makeup of their cell walls, and their ability to grow in air. They can be round (such as staphylococci or streptococci), rod-shaped (such as bacillus or E. coli), or corkscrew-shaped (Borrelia species). In most cases, bacteria have cell walls that provide a target for many antibiotics (antibiotics easily identify bacteria) (Gold and Eisenstein, 2000).

They are also classified by their color after a Gram stain is applied. Gram-positive bacteria stain blue, while Gram-negative bacteria stain pink (Gold and Eisenstein, 2000).

Gram-negative bacteria cell walls contain a substance known as lipopolysaccharide (LPS), a highly inflammatory chemical that provokes an immune response in the human body. LPS is responsible for triggering the overreaction of the host immune system, which results in the release of oxygen and nitrogen species, cytokines, and other pro-inflammatory mediators (Gold and Eisenstein, 2000).

2.4 Classification of Bacterial Infections

Bacterial infections are classified by the causative agent, as well as the symptoms and medical signs produced. Symptomatic infections are apparent, whereas an infection that is active but does not produce noticeable symptoms may be called inapparent, silent, or subclinical. An infection that is inactive or dormant is called a latent infection. A short-term infection is an *acute* infection. A long-term infection is a chronic infection (Kayser *et al.*,2005).

2.4.1 Occult infection

Occult infection is a hidden infection first recognized by secondary manifestations. Dr. Fran Giampietro discovered this type, and coined the term "occult infection" in the late 1930s. An infection first recognised by secondary manifestations—e.g., increased neutrophils in the circulation or fever of unknown origin—often caused by a bacterial infection in an obscure site. e.g., a subphrenic or other intra-abdominal region (Ryan and Ray, 2004).

2.4.2 Infectious or not

One way of proving that a given disease is "infectious", is to satisfy Koch's postulates (first proposed by Robert Koch), which demands that the infectious agent be identified only in patients and not in healthy controls, and that patients who contract the agent also develop the disease. These postulates were first used in the discovery that Mycobacteria species cause tuberculosis. Koch's postulates cannot be applied ethically for many human diseases because they require experimental infection of a healthy individual with a pathogen produced as a pure culture. Often, even clearly infectious diseases do not meet the infectious criteria. For example, *Treponema pallidum*, the causative spirochete of syphilis, cannot be cultured *in vitro*. However the organism can be cultured in rabbit testes. It is less clear that a pure culture comes from an animal source

serving as host than it is when derived from microbes derived from plate culture (Kayser *et al.*,2005).

Epidemiology is another important tool used to study disease in a population. For infectious diseases it helps to determine if a disease outbreak is sporadic (occasional occurrence), endemic (regular cases often occurring in a region), epidemic (an unusually high number of cases in a region), or pandemic (a global epidemic) (Kayser *et al.*,2005).

2.4.3 Contagious Bacterial Infections

Infectious diseases are sometimes called contagious disease when they are easily transmitted by contact with an ill person or their secretions (e.g. influenza). Thus, a contagious disease is a subset of infectious disease that is especially infective or easily transmitted. Other types of infectious/transmissible/communicable diseases with more specialized routes of infection, such as vector transmission or sexual transmission, are usually not regarded as "contagious," and often do not require medical isolation (sometimes loosely called quarantine) of victims. However, this specialized connotation of the word "contagious" and "contagious disease" (easy transmissibility) is not always respected in popular use (Kayser *et al.*,2005).

2.4.4 Anatomic Location

Infections can be classified by the anatomic location or organ system infected, including:

- Urinary tract infection
- Skin infection
- Respiratory tract infection
- Odontogenic infection (an infection that originates within a tooth or in the closely surrounding tissues)

- Vaginal infections
- Intra-amniotic infection

In addition, locations of inflammation where infection is the most common cause include pneumonia, meningitis and salpingitis (Kayser *et al.*,2005).

2.5 Current Treatment for Bacterial Infections

Antibiotics are the mainstay of bacterial treatment (Archer and Polk, 20014). The goal of these drugs is to kill invading bacteria without harming the host. Antibiotic effectiveness depends on mechanism of action, drug distribution, site of infection, immune status of the host, and resistance factors of bacteria (Archer and Polk, 20014; Roden, 2004).

Antibiotics work through several mechanisms. Some (such as vancomycin and penicillin) inhibit formation of bacterial cell walls. Erythromycin, tetracycline, and chloramphenicol interrupt protein synthesis. Others inhibit bacterial metabolism (sulfa drugs) or interfere with DNA synthesis (ciprofloxacin, rifampin) and/or cell membrane permeability (polymyxin b) (Conte, 2002).

When antibiotics were discovered in the 1940s, they were incredibly effective in bacterial infection treatment. Over time, however, many antibiotics have lost effectiveness, against common bacterial infections because of increasing drug resistance (Barie, 1998; Domin, 1998). Bacteria may be naturally resistant to different classes of antibiotics or may acquire resistance from other bacteria through exchange of resistant genes. Indiscriminate, inappropriate, and prolonged use of antibiotics have selected out the most antibiotic-resistant bacteria (Petrosillo, 2002; van der Waaij, 2000). Antibiotic-resistant strains have emerged in hospitals, long-term care facilities, and communities worldwide (Flaherty, 1996; Jacobs, 1999; Levin, 2003).

For example, *S. aureus* is a common bacterial pathogen that causes pneumonia, skin and urinary tract infections, as well as blood and surgical site infections. Some strains that are resistant to all current antibiotics, including vancomycin, have emerged in the United States and Japan. Similarly, Audu and Kudi, (2004) identified *S. aureus* as the leading entiologic agent in urinary tract infection in Nigeria. It constituted as high as 65.8% of cases in women suspected of UTI compared to men (34.2%). Antibiotic-resistant organisms lead to increased hospitalizations, health costs, and mortality (Amsden, 2004; Apfalter, 2003; Austin, 1999; Baggett, 2004; Barie, 1998; Bonten, 2001; Borer, 2002; Tasota, 1998).

Besides increased drug resistance, high-dose and prolonged antimicrobial therapy can eliminate helpful bacterial flora and predispose people to infection (Carson, 2003; Guarner, 2003). A common adverse effect of antibiotics is diarrhea, which can lead to loss of essential vitamins and minerals, especially vitamin K, magnesium, and zinc (Briend, 1988; Brunser, 1977; Fontaine, 1996; Guerrant, 2000). Other adverse effects of antibiotic therapy include vitamin deficiencies, seizures, allergic shock (in people who are allergic to antibiotics), autoimmune disease, decreased platelets, kidney injury, drug/drug interaction, and death (Roden, 2004).

2.6 Limitations to Current Bacterial Treatment

For the past 70 years, antimicrobial drugs, such as antibiotics, have been successfully used to treat patients with bacterial and infectious diseases. Over time, however, many infectious organisms have adapted to the mechanism of resistance, making the drugs less effective (Archer and Polk, 20014).

In the presence of an antimicrobial, microbes are either killed or, if they carry resistance genes, survive. These survivors will replicate, and their progeny will quickly become the dominant type throughout the microbial population (Briend, 1988; Carson, 2003; Guarner, 2003).

Gram-negative bacterial infections can be difficult when treating, because of several unique features of these bacteria. For example, the unique nature of their cell wall makes them resistant to several classes of antibiotics. Infections have typically been treated with broad-spectrum antibiotics, such as beta-lactams followed by carbapenems. However, even these drugs have become ineffective against some bacteria, leaving healthcare providers no choice but to use older drugs, such as colistin, which can have toxic side effects (Conte, 2002).

2.7 Anti Bacterial Drug Resistance

Antibacterial agents inhibit the growth of bacteria and may rapidly kill them by disrupting one or more of their essential cellular functions. For example, depending on the type of antibacterial agent, the mechanism of activity may result in: inhibition of the production of proteins or cell wall materials; inhibition of DNA replication; disruption of cell membrane activities that maintain chemical balance. Bacteria are usually grouped according to various attributes such as the structure of their outer coverings and their metabolic functions. The primary classification of bacteria is based on their staining properties, which, for almost all types of bacteria, divides them into Gram-positive or Gram-negative groups. Those called Gram-positive have a cell membrane plus a thick layer of cell wall material (peptidoglycan) lying outside the membrane. In contrast, Gram-negative bacteria have a cell membrane, a relatively thin layer of peptidoglycan and then an outer membrane. These major structural differences result in different patterns of susceptibility to antibacterial agents because the outer coverings of the bacteria affect access to the sites where they exert their activity (Archer and Polk, 2014).

Therefore, each group of bacteria is usually susceptible to the actions of only a limited range of antibacterial agents and show inherent (i.e. normal) resistance to the actions of others. Moreover, bacteria have the ability to acquire resistance to one or more antibacterial agents to

which they would normally be susceptible. Acquired resistance can arise by mutations that can occur during replication or by gaining genes encoding a mechanism of resistance from other bacteria. The ease with which resistance can be acquired varies between bacterial types. Unfortunately, some of the types of bacteria that are normally not susceptible to many antibacterial agents are also easily able to acquire resistance to others. The result is multidrug resistance. In extreme cases, bacteria can show resistance to most or all of the agents that would commonly be used to treat them. In addition, each acquired mechanism of resistance may render the bacterium resistant to many or all antibacterial agents of the same type (class) and sometimes confers resistance to agents from many classes. This is called cross-resistance (Archer and Polk, 2014).

The genes encoding some mechanisms of resistance are sometimes linked in such a way that they are transferred all together between organisms. This is often referred to as co-resistance. Each time an antibacterial agent is used to treat an infection, there is a risk that the agent will select, in the population of infecting bacteria, for bacteria that are resistant to it, thus causing unresolved infection in the patient undergoing treatment. The agent will also select for resistant bacteria in the patient's commensal flora, thus resulting in colonisation by resistant bacteria, which may subsequently be responsible for another infection at the same or another body site. In both cases, these resistant bacteria will have the possibility to spread to other patients, especially within hospitals. Thus, increasing rates of resistance to an antibacterial agent and to all other agents that are rendered inactive by common mechanisms of resistance is an inevitable consequence of its use. In the last 10–20 years, multidrug resistance has emerged in many frequently encountered pathogenic bacteria. In extreme cases, these bacteria are not susceptible

to any licensed antibacterial agent or are susceptible only to those that are more toxic to the patient than the more commonly used drugs (Flaherty, 1996).

2.8 Mechanism of Antibacterial Resistance to Drugs.

Antibiotic resistance can be attained through intrinsic or acquired mechanisms. Intrinsic mechanisms are those specified by naturally occurring genes found on the host's chromosome, such as, AmpC β-lactamase of gram-negative bacteria and many multi drug resistance (MDR) efflux systems. Acquired mechanisms involve mutations in genes targeted by the antibiotic and the transfer of resistance determinants borne on plasmids, bacteriophages, transposons, and other mobile genetic material. In general, this exchange is accomplished through the processes of transduction (via bacteriophages), conjugation (via plasmids and conjugative transposons), and transformation (via incorporation into the chromosome of chromosomal DNA, plasmids, and other DNAs from dying organisms) (Levy and Marshall, 2004).

Although gene transfer among organisms within the same genus is common, this process has also been observed between very different genera, including transfer between such evolutionarily distant organisms as gram-positive and gram-negative bacteria (Courvalin, 1994). Plasmids contain genes for resistance and many other traits; they replicate independently of the host chromosome and can be distinguished by their origins of replication. Multiple plasmids can exist within a single bacterium, where their genes add to the total genetics of the organism. Transposons are mobile genetic elements that can exist on plasmids or integrate into other transposons or the host's chromosome. In general, these pieces of DNA contain terminal regions that participate in recombination and specify proteins (e.g. transposase or recombinase) that facilitate incorporation into and from specific genomic regions. Conjugative transposons are unique in having qualities of plasmids and can facilitate the transfer of endogenous plasmids

from one organism to another. Integrons contain collections of genes (gene cassettes) that are generally classified according to the sequence of the protein (integrase) that imparts the recombination function (Mazel, 2006). They have the ability to integrate stably into regions of other DNAs where they deliver, in a single exchange, multiple new genes, particularly for drug resistance. The superintegron, one which contains hundreds of gene cassettes (representing about 3% of the host's genome), is distinct from other integrons; it was first identified in Vibrio cholerae (Mazel *et al.*, 1998).

Although Alexander Fleming selected and described mutants resistant to penicillin soon after he had discovered the antibiotic, no one could have predicted the speed with which bacteria would acquire the capacity for dealing with multiple antibacterial agents. Plasmids, specifying a collection of individual antibiotic resistance determinants (originally termed resistance factors, R factors), were initially described as the vehicle used to rapidly spread resistance traits among bacteria. Strains of Shigella bearing the self-replicating and self-transfer- able plasmids were easily selected and propagated during a period of considerable sulfonamide use in Japan after World War II (Watanabe, 1963). Antibiotics such as streptomycin, chloramphenicol, and tetracycline were subsequently introduced for the treatment of the sulphonamide-resistant organisms. Shigella and *E. coli* strains bearing resistance to all four agents, however, were recognized in 1955 (Watanabe, 1963). Now, more than 60 years after antibiotics were first introduced into clinical practice, the prescience of Fleming has come to fruition as the infectious disease community has yet to identify an antibiotic that has managed to circumvent the development of resistance.

2.9 Plant Derived Agents with Antimicrobial Properties

Plant-derived compounds of therapeutic value are mostly secondary plant metabolites traditionally used for medicinal purposes. They have a wide activity range, according to the species, the topography and climate of the country of origin, and may contain different categories of active principles. Variations in the chemical composition modifies their antimicrobial activity. Some main categories of phytochemicals extracted from medicinal plants have been reported with their pharmacological activities (Assob *et al.,* 2011; Ahmad *et al* 2011).

2.9.1 Flavonoids: Previously called bioflavonoids and include aromatic compounds, are phenolic structures ubiquitous in photosynthesizing cells and are commonly found in fruit, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey. For centuries, preparations containing these compounds as the principal physiologically active constituents have been used to treat human diseases. The basic structural feature of flavonoid compounds is the 2-phenyl-benzopyrane or flavane nucleus, consisting of two benzene rings linked through a heterocyclic pyrane ring. In total, there are 14 classes of flavonoids, differentiated on the basis of the chemical nature and position of substituents on the different rings. The antibacterial properties of flavonoids are thought to come from the ability to form complexes with both extracellular and soluble proteins, as well as with bacterial membranes (Cowan, 1999; Fowler *et al*., 2011).

A synergy has been demonstrated between active flavonoids as well as between flavonoids and existing chemotherapeutics, even if the reports of activity in the field of antibacterial flavonoid research are widely conflicting, probably owing to inter- and intra-assay variation in susceptibility testing. Future optimization of these compounds through structural alteration may allow the development of a pharmacologically acceptable antimicrobial agent or group of agents.

Existing structure–activity data suggest that it might be possible, for example, to prepare a potent antibacterial flavanone by synthesizing a compound with halogenation of the B ring as well as lavandulyl or geranyl substitution of the A ring. Also, it is worth noting that by elucidating flavonoid biosynthetic pathways it would be possible to produce structural analogs of active flavonoids through genetic manipulation. Numerous research groups have sought to elucidate the antibacterial mechanisms of action of selected flavonoids; the activity of quercetin has been at least partially attributed to the inhibition of DNA gyrase, whereas sophoraflavone G and (-)-epigallocatechin gallate inhibit cytoplasmic membrane function, and licochalcones A and C inhibit energy metabolism (Fabricant and Fransworth 2001).

Phenolics and Polyphenols compounds are widely distributed in plants, where they protect the plants from microbial infections. They have potential anti-oxidative properties but are also potent anti-infectives. They are a large group of aromatic compounds, consisting of flavones, flavanoids and flavanols containing one carbonyl group, quinones with two carbonyl groups, tannins, polymeric phenolic substances, and coumarins, phenolic compounds with fused benzene and pyrone groups (Cowan, 1999; Fowler *et al*., 2011).

Flavones and their derivatives represent an antibacterial therapeutic possibility to disrupt bacterial envelopes. The catechins are included among the flavan-3-ols or flavanols, present in different plants, particularly in tea-plant *Camelia sinensis*, where they form complexes with the bacterial cell wall and are active on intestinal microorganisms. Biological assays indicated the inactivation of specific bacterial enzymes by several of these compounds. Moreover significant synergy was also observed between theaflavin and epicatechin against important nosocomial Gram-negative pathogens (Betts *et al*., 2011).

Quinones (aromatic rings with two ketone substitutions), ubiquitous in nature, are another significant group of secondary metabolites with potential antimicrobial properties. They provide a source of stable free radicals and irreversibly complex with nucleophilic amino acids in microbial proteins determining loss of their function. Anthraquinones in particular had a large spectrum of antibacterial (also antimycobacterial) activity, based on inactivation and loss of function of bacterial proteins, such as adhesins, cell wall polypeptides and membrane-bound enzymes, consequently leading to the death of the pathogens. (Fabricant and Fransworth 2001).

Tannins are a group of polymeric phenolic substances found in almost every plant part characterized by antibacterial activity owing to inactivation of bacterial adhesins, enzymes, cell envelope and transport proteins. Recently, gallotannin-rich plant extracts demonstrated inhibitory activities on different bacteria attributable to their strong affinity for iron and to the inactivation of membrane-bound proteins (Engels *et al*., 2011).

Hydrolysable and condensed tannins, derived from flavanols, and called proanthocyanidins, exert antimicrobial activity by antiperoxidation properties inhibiting in particular the growth of uropathogenic *E. coli*. Anthocyanidin synthesis occurs in plants on the cytoplasmic leaflet of the endoplasmic reticulum and then accumulates in the large central vacuole; in many plants, anthocyanidins might occur in oligomeric form and in this case they are called proanthocyanidins. Depending on the type of bond between the oligomer-forming anthocyanidin molecules, two general types (A and B) of proanthocyanidins are distinguished. In less common A-type proanthocyanidins, two bonds are formed between 2β-7 and 4β-8 carbon of oligomer-forming molecules; in B-type, only one 4β-8 bond is formed. The beneficial effects of

anthocyanins on human health have been known at least from the 16th century, when blackberry juice was used in the treatment of mouth and eye infections. However, only few studies have focused on the antimicrobial activity of these compounds. Recently, Cisowska *et al.*, (2011) described the anthocyanin profile of action of different fruits, mainly berries, but also red grapes and, by consequence, red wine, also containing stilbenoid resveratrol, indicating a superior activity against Gram-positive bacteria (Cisowska *et al* ., 2011)

2.9.2 Alkaloids Alkaloids are heterocyclic nitrogen compounds characterized by different antimicrobial activities. The analysis of the leaf extracts of *Gymnema montanum* and of ethanol extract of *Tabernaemontana catharinensis* root bark revealed an antimicrobial activity in the first case due to an activity depending upon the chemical composition of the extracts and membrane permeability of the microbes, and in the second case linked to indole alkaloids responsible for the observed antibacterial and antidermatophytic activity. Diterpene alkaloids, commonly isolated from the plants of the Ranuncolaceae group, had antimicrobial properties. Berberine, an isoquinoline alkaloid, present in roots and stem-bark of Berberis species, is a hydrophobic cation widely used in traditional medicine owing to its activity against bacteria, fungi, protozoa and viruses.It accumulates in cells driven by the membrane potential and is an excellent DNA intercalator active on several microorganisms with a target on RNA polymerase, gyrase and topoisomerase IV and on nucleic acid (Yi *et al*.,2007).

2.9.3 Terpenes Terpenes compounds are also referred to as isoprenoids and their derivatives containing additional elements, usually oxygen, are called terpenoids. The antibacterial activity of some monoterpenes (C10), diterpenoids (C20), sesquiterpenes (C15), triterpenoids (C30)and their derivatives was recently reviewed. The results obtained illustrate the strong structure–

function influence of the antibacterial potential of terpenes. Diterpenoids, such as sesquiterpenes, isolated from different plants exhibited bactericidal activity against Gram-positive bacteria and inhibited the growth of *M. tuberculosis*. The mechanism of action of terpenoids is not fully understood, but is speculated to involve membrane disruption by the lipophilic compounds (Termentzi *et al*., 2011).

2.9.4 Coumarins One known coumarin, scopoletin, and two chalcones were isolated as antitubercular constituents of the whole plant *Fatoua pilosa* (Garcia *et al*., 2012).

Also, spices and aromatic plants have an antimicrobial effectiveness that depends on the kind of plant, its composition and concentration of essential oils, often rich in monoterpens and sesquiterpenes. Studies analyzing the antimicrobial activity of essential oil of *Allium sphaerocephalon* inflorescenses revealed the accordance with the popular use of plants belonging to the *Allium* genus in traditional medicine, indicating the importance of aroma precursors (cysteine sulfoxides) for a potent biologic activity (shahid *et al*., 2011).

2.10 Description of *Lannea barteri*

Lannea barteri belongs to the family Anacardiacea. It is a deciduous tree with a spreading crown; it can grow from 5 - 18 metres tall. The bole is usually straight and clear of branches for several meters, it can be up to 40cm in diameter with a thick bark. Flowers are unisexual and usually regular with 8 stamens, 4 cell ovary and 4 stigmas. Terminal leaflet with long stalk, the fruit are cylindrical (Jansen, 2005).

The plant is often harvested from the wild for local use. It is an important source of a red-brown

dye that is used in traditional dyeing in Africa and also supplies food, medicines and materials (Garba *et al.,* 2015).



Plate I; *Lannea barteri* in its natural habitat

2.11 Ethno medical uses of *Lannea barteri*

The bark is stomachic. A decoction is drunk as a treatment for gastric pains, diarrhoea, oedema, paralysis, epilepsy and madness (Kone *et al*., 2011).

When combined with the some spices (Yoruba, species) it is used as a vermifuge. The bark is used externally to treat ulcers, sores and leprosy. A root decoction is taken to cure hernia.The root is ground, wrapped in the leaves of an unknown species, and applied as a poultice on wounds. A leaf decoction is taken to cure haemorrhoids. (Jansen, 2005).

(Garba *et al.*, 2015) report that the stem bark of *L. barteri* is used in the treatment of epilepsy, gastritis, childhood convulsions among other uses in northern Nigeria for many years. The popularity of its efficacy is well established among the Traditional Medical Practitioners.

2.12 Reported Constituents from *Lannea barteri*

The phytochemical analysis of the roots and stem bark extracts by (Kone *et al*., 2011) reported the presence of steroids, triterpenoids, saponins, polyphenols, flavonoids, tannins, alkaloids and quinoine.

Quantitative estimation proved that both extracts of roots and stem bark have considerably high Constitutions of phenolic compounds. Similar total phenolic contents were obtained for the stem bark and roots with respectively 254.46 and 254.96 μg/g GAE (Kone *et al*., 2011).

2.13 Some Isolated chemical constituents from the family Anacardiacea

Four flavonoids named as 6,7-(2",2"-dimethyl chromeno)-8-g,g-dimethyl allylflavanone, 3',4'dihydroxy-7,8(2",2"-dimethylchromeno)-6-g,g dimethyl allylflavonol, 7-methyltectorigenin, Irisolidone, have been isolated from leaves of *Lannea acida* (Garba *et al.*, 2015).

Two dihydroflavonols, (2R,3S)-(+)-3',5-dihydroxy-4',7-dimethoxydihydro¯avonol and (2R,3R)-(+)-4',5,7- trimethoxydihydro¯avonol were isolated from the stem bark of *Lannea coromandelica*, along with the known (2R,3R)-(+)-4',7- di-O-methyldihydroquercetin, (2R,3R)-(+)-4',7-di-O-methyldihydrokaempferol and (2R,3R)-(+)-4'-O-methyldihydroquercetin.

All the compounds were isolated for the first time from the genus Lannea (Islam and Tahara, 2000). Two alkylphenols [cardonol 7 (1) and cardonol 13 (2)], and three new dihydroalkylhexenones were also isolated from *lanne aedulis* (ueroz *et al*, 2003).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 List of Materials

measuring cylinder, , microscope slides, test tubes, incubator, calibrated ruler, plates, sterilized wire loop, column tube, beakers, Silica gel (for column chromatography) made by Qualikem India.

3.2 List of Equipment

Equipment include mechanical shaker, column for column chromatography, water bath, furnace, microscope, Bunsen burner.

3.3 List of media

The media used in research were Nutrient agar and Mueller Hinton broth.

3.4 List of Chemicals

All the chemicals used were of high quality obtained from agent of Sigma Aldrich and JHD. Some of these chemicals include methanol, ethyl acetate, chloroform, hexane, n-butanol, coloring reagent, detecting/spraying reagents for TLC.

3.5 Plant Collection, Identification and Preparation

3.5.1 Collection of the Plant Material

Sample of *L. barteri* was collected in the month of August 2014 from Fan-taki area, Bomo Village of Sabon Gari Local Government, Kaduna state, Nigeria.

3.5.2 Identification of of *Lannea barteri*

After collection of plant sample, it was taken to Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria. The plant was identified as *L. barteri* by Mallam Musa.

3.5.3 Preparation of the Stembark of *L. barteri.*

The stem bark was obtained by making longitudinal and transverse incisions through the outer layer of the stem bark of the plant followed by peeling. The stem bark collected was further dried at room temperature for about 3 weeks and then pounded with the aid of mortar and pestle to obtain the semi powdered form of the plant material which was stored in air – tight container prior to use.

3.6 Determination of Pharmacognsostic Properties of *L. barteri* Stembark

3.6.1 Determination of Moisture Content

A nickel crucible was heated to a constant weight and the exact weight was taken and allowed to cool in desiccators. 3 g of powdered drug was weighed into the crucible and was heated in an oven at 105^{o}C for about one hour; it was later heated and weighed at 30 minutes intervals until a

constant weight was achieved. The percentage of the moisture – content was calculated with reference to the initial weight of the powdered drug (WHO, 2011).

3.6.2 Determination of Ash Value

A nickel crucible was heated at 105^{o}C to a constant weight and the accurate weight was recorded, the crucible was cooled in desiccators.

2 g of powdered drug was weighed in to crucible and was heated until it is moisture free and charred, the heat was gradually increased until the carbon vaporised and the residue turns to white. It was cooled in desiccators and weighed; it was heated and weighed continuously until the weight of residue was constant. The percentage of Ash value was calculated with reference to the initial weight of the drug (WHO, 2011).

A detail of the calculation is in the appendix.

3.6.3 Determination of Acid-insoluble Ash Value

The crucible together with the ash from above experiment was transferred to a beaker containing 25 ml of dilute hydrochloric acid. It was then boiled for 5 minutes and filtered through an ash less filter paper. The funnel along with the filter paper were allowed to dry in an oven at 105^{o}C, the filter paper together was transferred in to clean and weighed crucible and were heated till the ash- less filter paper completely charred. The crucible and its content were cooled in desecrator, weighed and the reading was recorded. The Acid-insoluble ash was calculated with reference to the initial weight of the powdered drug (WHO, 2011).

3.6.4 Determination of Water Soluble Ash

The ash obtained from above experiment (3.6.3) was transferred to a beaker containing 25 ml of water. It was then boiled for 5 minutes and filtered through an ash less filter paper. The funnel along with the filter paper was allowed to dry in an oven at 105^{o}C. The filter paper together was transferred in to clean and weighed crucible and were heated till the ash - less filter paper was completely charred. The crucible and its content were cooled in desiccators and weighed and the reading was recorded. The water soluble ash was calculated with reference to the initial weight of the powdered drug (WHO, 2011).

A detail of the calculation is in the appendix.

3.6.5 Determination of Water Soluble Extractive Values

Sample (5 grams) of powdered plant was macerated in 100 ml of water in flask and then shaken frequently for six hours using shaker. It was allowed to stand for another 18 hours and filtered immediately. 25 ml of the filtrate was evaporated to dryness in evaporating dish and dried at 105^{o}C to constant weight (WHO, 2011). The water soluble extractive value was determined as:

Water soluble extractive value = $\frac{Weight\ of\ extract\ x\ 4}{weight\ of\ sample} x\ 100$

A detail of the calculation is in the appendix.

3.6.6 Determination of Ethanol soluble extractives (cold maceration method)

Sample (5grams) of powdered plant was macerated in 100 ml of ethanol in a flask and then shaken frequently for six hours using shaker. It was allowed to stand for 18 hours and filtered immediately. 25 ml of the filtrate was evaporated to dryness in evaporating dish and dried at 105^{o}C to constant weight (WHO, 2011). Alcohol soluble extractive value was calculated in (percentage) with reference to the initial weight of the extract as;

Alcohol extractive value = $\frac{Weight\ of\ extract\ x\ 4}{weight\ of\ sample} x\ 100$

A detail of the calculation is in the appendix.

3.7 Determination of Chemo-microscopic Properties of the Stem-bark of *L. barteri*

3.7.1 Test for Lignin

Cleared powdered sample was separately placed on the slide and a drop of phloroglucinol was added followed by a drop of concentrated hydrochloric acid and was viewed for the presence or appearance of red stain to confirm lignin is present (WHO, 1998).

3.7.2 Test for cellulose

Small amount of powdered drug was separately placed on a slide and a drop of N/50 iodine was added, it was left for a minute, and a drop of 66% sulphuric acid was added in drop. It was viewed for the appearance of bluish colour to confirm the presence of cellulose (WHO, 1998).

3.7.3 Test for Starch

N/50 iodine was added to a small portion of the cleared powdered drug and was viewed for the appearance of blue-black or reddish – blue colouration to confirm the presence of starch (WHO, 1998).

3.7.4 Test for Tannins

Ferric chloride solution (5%) was added to a small portion of the cleared material and was viewed for the presence of greenish black colour to confirm the presence of tannins (WHO, 1998).

3.7.5 Test for Gums and Mucilage

A drop of ruthenium red was added to the cleared powdered drug and was viewed for the presence of pink colouration to confirm the presence of gums and mucilage (WHO,1998).

3.7.6 Test for Fats and Oils

A drop of Sudan (IV) reagent was added to a cleared powdered drug and allowed to stand for a minute. It was viewed for the presence orange red colour to confirm the presence of fats and oils (WHO, 1998).

3.7.7 Test for Calcium Oxalates and Calcium Carbonates

HCl was added to a cleared portion of powdered drug and effervescence observed for the presence of calcium carbonate, while the dissolution of the crystals upon addition of concentrated hydrochloric acid indicate the presence of calcium oxalate crystals (WHO, 1998).

3.8 Extraction of the stem bark of *Lannnea barteri*

Powdered stem bark (2kg) of the plant was subjected to maceration technique using methanol for 2 weeks at room temperature. The mixture was filtered and the filtrate was dried on water bath at 40^{o}C to obtain a dark brown gummy residue which was transferred into a glass tube container and labelled methanol extract, wrapped with aluminium foil and kept inside desiccators.

3.9 Fractionation of Methanolic Extract of *Lannea barteri* Stem-bark

Solvent – solvent fractionation was carried out using protocol design by Kupchan and Tsou (1973) and modified version of (Wagenen *et al*., 1993).

The crude extract (50g) was suspended in hot water and filtered. The filtrate solution was then partitioned successively using the following solvents of increasing polarity. n-hexane (500ml x2), ethyl acetate (500ml x2) and n- butanol (500ml x2). All the fractions were evaporated to dryness at low temperature of 39^{o}C on water bath and kept in air tight container for further analysis.

3.10 Preliminary Phytochemical Screening of the Fraction of the Stembark of *Lannea barteri*

Small portion of the extract and fraction was dissolved in water and the preliminary phytochemical studies of the four fractions 1, 2, 3 and 4 were carried out using the procedures outlined in (Evans, 1996).

3.10.1 Test for Carbohydrates

Molish test was employed to detect the presence of carbohydrate, Few drops of Molish reagent was added to a small portion of the dissolved fractions in a test tube and concentrated sulphuric

acid was added down the side of the test tube to form a lower layer. A reddish-colored ring at the interphase indicates the presence of carbohydrates.

3.10.1.1 Fehling's Test

0.5 mg of the sample in 5 ml of water was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a color reaction.

3.10.2 Test for Anthracene Derivatives

Bontrager's Test was used to determine the presence of anthracene derivatives in the fractions. 5 ml of chloroform was added to a portion of the dissolved fraction in a test tube and was shaken for at least 5 minutes. This was filtered and the filtrate shaken with equal volume of 10% ammonia solution. Bright pink colour in the chloroform (lower) layer indicates the presence of free anthraquinones (Evans, 1996).

3.10.3 Test for Unsaturated Steroid and Triterpenes

3.10.3.1 *Liebermann-Buchard test*

To a portion of the fraction, equal volume of acetic acid anhydride was added and mixed gently. 1 ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. Colour change was observed immediately and over a period of one hour. Blue-green colour in the upper layer and reddish pink or purple colour indicate the presence of triterpene (Evans, 1996).

3.10.3.2 *Salkwoski test for Unsaturated Sterols*

To a small portion of the dissolved fraction, 2 drops of concentrated sulphuric acid was added at the side of the tube. Immediate colour changes were observed at the interphase of the extract and sulphuric acid. The immediate colour changes lasted for over one hour period (cherry red colour) usually indicates the presence of unsaturated sterols (Evans, 1996).

3.10.4 Test for Cardiac Glycosides

Keller-Killiani test was employed to detect the presence or absence of cardiac glycosides in the fractions. One millilitre of glacial acetic acid containing traces of ferric chloride solution was added to a portion of the dissolved fraction. This was then transferred in to 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. It was carefully observed for a purple-brown ring. This indicates the presence of deoxy sugars and pale green colour in the upper acetic acid layer indicates the presence of cardiac glycosides (Evans, 1996).

3.10.5 Test for Tannins

Ferric chloride test and Bromine water test were employed to detect the presence or absence of Tannins in the fraction.

3.10.5.1 *Ferric ChlorideTtest*

To a portion of the extract, 3 drops of ferric chloride solution were added. A greenish-black precipitate indicates the presence of condensed tannins while hydrolysable tannins give a brownish or brownish- blue precipitate (Evans, 1996).

3.10.5.2 *Bromine Water test*

Few drops of bromine water were added to the fraction in a test tube. A buff coloured precipitate indicates the presence of condensed tannins while hydrolysable tannins give none at all (Evans, 1996).

3.10.6 Test for Flavonoids

Sodium hydroxide test and Shinoda test were employed to detect the presence or absence of flavonoid in the fraction

3.10.6.1 *Sodium Hydroxide test*

Few drops of 10% sodium hydroxide were added to the extract. Yellow coloration indicates presence of flavonoid (Evans, 1996).

3.10.6.2 *Shinoda Test*

A portion of the fraction was dissolved in 2ml of 50% methanol in the heat. Metallic magnesium chips and few drops of concentrated hydrochloric acid were added. Appearance of red colour indicates the presence of flavonoids (Evans, 1996).

3.10.7 Test for alkaloids

Dragendorff's test and Mayer's test were employed to detect the presence or absence of alkaloids.

3.10.7.1 *Dragendoff's Test*

To a portion of the fraction, few drops of Dragendoff's reagent were added. A reddish brown precipitate indicates the presence of alkaloids (Evans, 1996).

3.10.7.2 *Mayer's Test*

To a portion of the fraction, few drops of Mayer's reagent were added. A cream precipitate indicates the presence of alkaloids (Evans, 1996).

3.11 Antibacterial Activities of the Organic Solvents Fractions

3.11.1 Test Organisms

The test organisms used for this analysis were clinical isolates of bacteria obtained from Department of Microbiology, Ahmadu Bello University, Zaria. The isolates were; *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Salmonella typhi*.

3.11.2 Preparation of the Standard Inocula of the Organisms

The test organisms were prepared by streaking on a freshly prepared nutrient agar plates to obtain discrete colonies. A colony was picked with a sterile wire loop and transferred aseptically in to a small bijou bottle containing sterile normal saline, it was then shaken to dissolve completely and the turbidity was compared with that of a McFarland turbidity standard scale 0.5 which is equivalent to a bacterial cell density of 1.5×10^8 CFU/ML.

NB

McFarland turbidity standard is a combination of 1 % $Bacl_2$ and 1 % of H_2SO_4.

3.11.3 Culture Media

The culture media used for the analysis include Mueller Hinton agar (MHA), Mueller Hinton broth (MHB) and nutrients agar. The mentioned media were used for sensitivity test, determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC). All the media were prepared according to manufacturer's instructions and sterilized by autoclaving at 121^oC for 15 minutes.

3.11.4 Determination of Antibacterial activity of the Fractions Using Agar Well Diffusion Method.

The antimicrobial activity of fractions was evaluated using Agar Well Diffusion Method with minor modifications. One gram each of the butanol and aqueous fraction were diluted in 10 ml of water giving the concentrations of 100, 50, 25 and 12.5 mg/ml, while 0.02 gram of hexane and ethyl acetate fraction were dissolved in 10 ml of D.M.S.O and water, giving varying concentrations of 20, 10, 5 and 2.5 ml/mg. the bacterial strains was swabbed on the Nutrient agar plates. Wells of 5 mm diameter were punched into the agar plates with the help of sterilized cork borer (5 mm). Using a micropipette, the fractions were added to the wells made in the plate. The plates were incubated aerobically in an upright position at 37±2 °C for 24-48 h. Antimicrobial activities of the fractions were evaluated by measuring the zone of inhibition (mm) against the tested bacteria. The test was carried out using Ciprofloxacin (10 µg) as a control (Rubina, 2011).

3.11.5 Determination of Minimum Inhibitory Concentration (MIC) of the Fractions of Crude methnol Extract of *Lannea barteri* Stembark

MIC was determined using broth dilution method. The lowest concentration of the fractions showing inhibition for each organism were serially diluted in the test tube containing Mueller Hinton broth .The bacterial strains were inoculated in tubes with equal volume of nutrient broth and fractions. The tubes were incubated at 37 °C for 24-48 h. Three control tubes were maintained for each strain (media control, organism control and extract control). The lowest concentration (highest dilution) of the fractions that produced no visible growth (no turbidity) when compared with the control tubes were considered as initial MIC. The dilutions that showed

no turbidity were incubated further for 24 h at 37 °C. The lowest concentration that produced no visible turbidity after a total incubation period of 48 h was regarded as final MIC (Rubina, 2011).

3.11.6 Determination of Minimum Bactericidal Concentration (MBC) of the Fractions Methanol Extract of *Lannea barteri* Stembark

MBC value was determined by sub culturing the test dilution [which showed no visible turbidity] on to freshly prepared nutrient agar media. The plates were incubated further for 18-42 h at 37 °C. The highest dilution that yielded no single bacterial colony on the nutrient agar plates was taken as MBC (Rubina, 2011).

3.12 Column Chromatography of the Ethyl acetate Fraction of *Lannea barteri* Stembark

Silica gel G of (60- 120 µm mesh size) (100 g) was carefully packed using wet method in a heavy walled glass tube leaving sufficient head space. The column was allowed to settle for 3 hours to allow the silica gel (stationary phase) settle sufficiently. 2 g of the ethyl acetate fraction pre-adsorbed on silica gel was loaded onto the packed adsorbent and allowed to stabilize for 3 hours before elution begins.

Gradient elution of the solvents (mobile phase) was used. Starting with chloroform 100% as the first or initial eluent. Subsequently, the polarity was increased as chloroform: methanol 9:1, 4:1, 7:3, 3: 2, and 1:1.

Fractions of 20 ml each were collected and allowed to evaporate at room temperature. Each fraction was numbered in accordance with how they were collected.

The column fractions were monitored on TLC, and visualizing with U.V light, *P*-anisaldehyde and 10 % sulphuric acid were the general detecting reagents used. Fractions that showed same

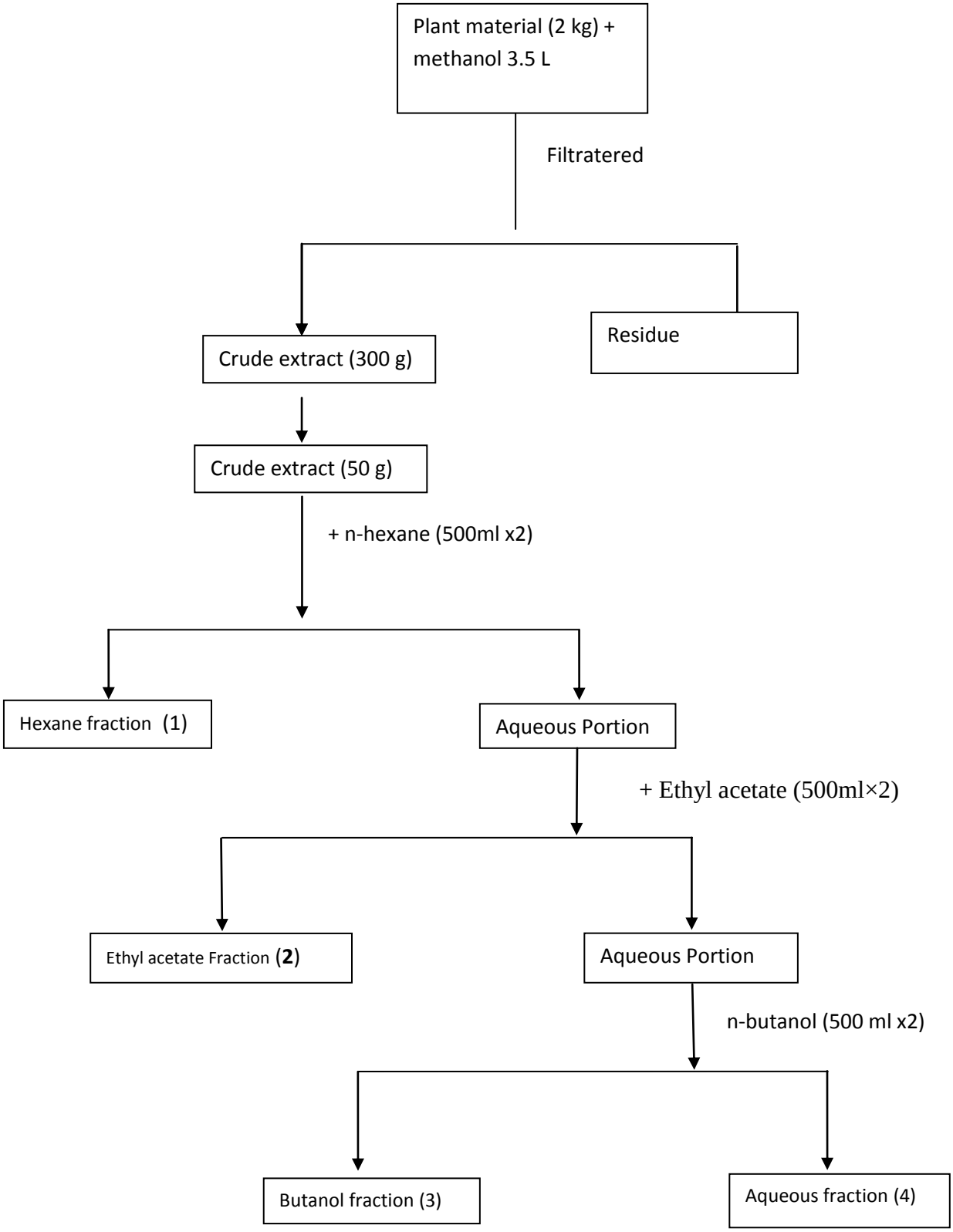
spot, colour and at the same R_f value were combined together in one beaker. Further purification was carried out using preparative thin layer chromatography using chloroform ; methanol 8-2) as a solvent system.

3.13 Isolation of Compound A

Fractions 31-35 was yellow colour mass. It was dissolved in methanol and spotted on glass preparative TLC. It was later developed again in chloroform - methanol (4:1) for about 30 minutes. It was scraped using the clean razor blade. This was later dissolved in absolute methanol filtered using filter paper. The liquid filtrate was then placed inside clean beaker and allowed to evaporate under room temperature.

3.14 Determination of Zone of Inhibition, MIC and MBC for Compound A

The above procedure used in determining the zone of inhibition, MIC and MBC of the fractions was used to determine the Zone of inhibition, MIC and MBC of compound A. 0.02 g of the compound was diluted in10 ml of water giving the varying Concentrations of 20 mg/ml, 10 mg/ml, 5 mg/ml and 2.5 mg/ml instead of 100 mg/ml, 50 mg/ml, 25mg/ml and 12.5mg/ml. These implied 4,2,1,0.5mg of the Compound A.


Plant material (2 kg) + methanol 3.5 L
Filtratered
Residue
Crude extract (300 g)
Crude extract (50 g)
+ n-hexane (500ml x2)
Hexane fraction (1)
Aqueous Portion
+ Ethyl acetate (500ml×2)
Ethyl acetate Fraction (2)
Aqueous Portion
n-butanol (500 ml x2)
Butanol fraction (3)
Aqueous fraction (4)

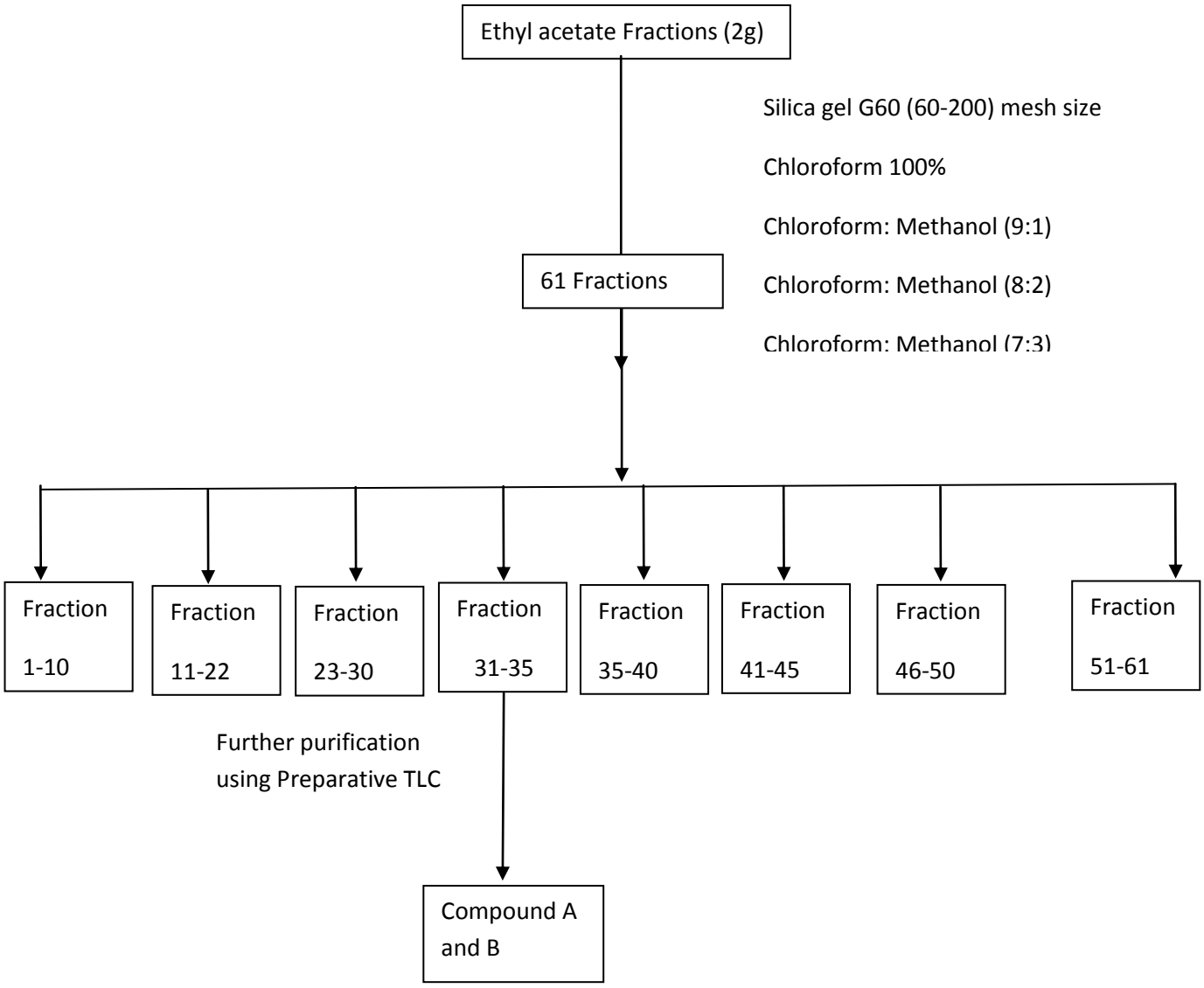


Fig 3.3; Column chromatography chart showing the procedure for the isolation of compound A

CHAPTER FOUR

4.0 RESULTS

4.1 Pharmacognostic Properties of *Lannea barteri* Stem bark

The average moisture content of the stem bark was calculated to be 8.67 ± 0.19 (appendix I).

The ash value was calculated to be 8.675 ± 0.17, acid insoluble ash 1.33 ± 0.17 and water soluble ash 1.00 ±0.00 (appendix II, III and IV) for the details of calculations. Also the ethanol extractive value was calculated to be 15.45 ± 0.27 and that of water soluble extractives 17.4 ± 0.40 (appendix V and VI).

4.2 Chemomicroscopy

The powdered stem bark showed the presence lignin, starch, tannins, Gums and mucilage's, fats and oils but calcium carbonate and calcium oxalate crystals were not found (Table 4.1).

Table 4.1: Chemomicroscopic results of *L. barteri* stem bark

Chemo microscopic tests	Results
Cellulose test	Present
Lignin test	Present
Starch test	Present
Test for tannins	Present
Gums and mucilage	Present
Fats and oils	Present
Calcium carbonates	Absent
Calcium oxalate crystals	Absent

4.3 Extraction of the stem bark of *Lannea barteri*

The extraction of 2 kg of stem bark of *Lannea barteri* yielded 300 g of methanolic extract. Indicating the yield of 15 %.

4. 4 Partitioning of the methanol extract of stem bark of *Lannea barteri*

The partitioning of 50g of methanol extract with hexane, ethyl acetate, and n-butanol yielded 0.10, 4.46, 4.83 and aqueous portion 25.8g. These implied 0.2, 8.92, 9.66 and 51.60% respectively.

4.5 Preliminary Phytochemical Screening of the Fractions

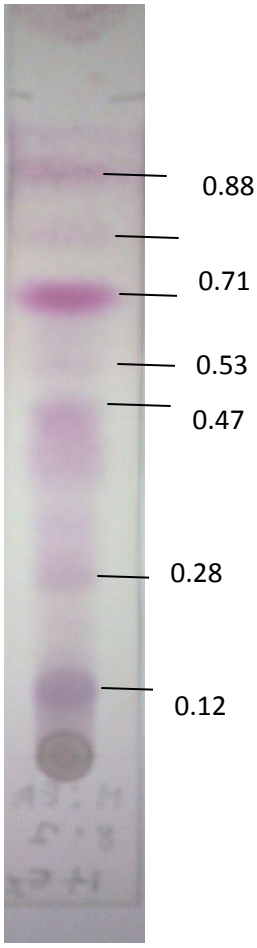
The preliminary phytochemical screening revealed the presence of carbohydrates, anthracene derivatives, unsaturated steroids and triterprnes, cardiac glycosides, saponin, tannins, flavonoids and alkaloids (Table 4.2).

Table 4.2: Preliminary Phytochemical Screening Result of *Lannea barteri* Stem-bark

Constituents	Aqueous	n-butaol	Ethyl acetate	Hexane
Carbohydrates				
Molish test	present	Present	Absent	Absent
Fehling solution test	Present	Present	Absent	Absent
Anthracene derivatives				
Bontragers test	Present	Absent	Present	Absent
Steroid & tritepenes				
Liebermann Buchard test	Present	Present	Present	Present
Salkwoski test	Present	Present	Present	Present
Cardiac glycosides				
Keller-Killiani test	Present	Present	Present	Absent
Saponins				
Frothing test	Present	Present	Present	Absent
Tannins				
Ferrric chloride test	Present	Present	Present	Absent
Bromine water test	Present	Present	Present	Absent
Flavonoids				
Shinoda test	Present	Present	Present	Absent
Sodium hydroxide test	Present	Present	Present	Absent
Alkaloids				
Mayer's test	Present	Absent	Absent	Present
Dragendorff's test	Present	Absent	present	Present

4.6 Thin layer Chromatography of Extract and Fractions of *L. barteri* Stem-bark

Thin layer chromatography analysis of hexane fraction in hexane – ethyl acetate (4:1) revealed the presence of 8 major spots. The ethyl acetate fractions developed in chloroform – methanol (4:1) revealed 4 major spots (Plate I and II).



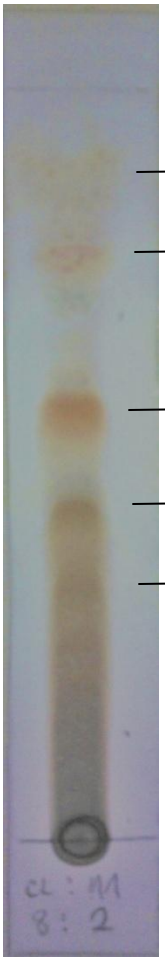


PLATE I **PLATE II**

PLATE II TLC profile of Hexane fraction developed in Hexane - ethyl acetate (8:2) and sprayed with 10% sulphuric acid in methanol.

PLATE III: TLC profile of ethyl acetate fraction developed in chloroform- methanol (4:1) and sprayed with 10% sulphuric acid in methanol.

4.7 Diameter Zone of Inhibition

The results in table 4.4 show that *Staphylococcus aureus* was susceptible to hexane, ethyl acetate, butanol and aqueous fractions with diameter zone of inhibition ranging from 10-20 mm at varying concentrations of 100, 50, 25 and 12.5 mg/ml. Similarly, *Bacillus subtilis* was susceptible to all the two fractions with diameter zone of inhibition range of 11 – 17 mm at the varying concentrations.

Escherichia coli show susceptibility to only hexane fraction at concentrations of 20 and 10 mg/ml with diameter of inhibition 19 and 14mm respectively.

Salmonella. typhi was susceptible to none of the fractions at all the varying concentrations (Table 4.3).

Table 4.3: Diameter Zone of Inhibition (mm) at varying concentrations (mg/ml) of the fractions

Test organisms	n-hexane				Ethyl acetate				n-butanol				Aqueous				C
	2.5	5	10	20	2.5	5	10	20	12.5	25	50	100	12.5	25	50	100	
S. aureus	0	0	12	15	0	12	15	18	10	13	16	18	13	15	17	20	40
B. subtilis	0	0	11	12	0	11	14	16	0	11	14	16	0	12	14	17	45
E coli	0	0	14	19	0	0	0	0	0	0	0	0	0	0	0	0	38
S. typhi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38

Key; C= control (ciprofloxacin), **0 = No activity**

4.8 Minimum Inhibitory Concentrations (MIC) (mg/ml)

The MIC of hexane fraction was observed at 10 mg/ml for *S. aureus* and *E. coli* and no activity was observed against *B. subtilis* and *S. typhi.*

The MIC of ethyl acetate fraction was observed at 5 mg/ml while the MIC of butanol and aqueous fractions was observed at 12.5 mg/ml for *S. aureus* and *B. subtilis* (Table 4.4).

Table 4.4: Minimum Inhibitory Concentrations (MIC) (mg/ml)

Organisms	Hexane	Ethyl acetate	Butanol	Aqueous
S. aureus	10	5	12.5	12.5
B. subtilis	10	5	25	25
E. coli	10	N/C	N/C	N/C
S. typhi	N/C	N/C	N/C	N/C

N/C = Not Carried out

4.9 Minimum Bactericidal Concentrations (MBC) (mg/ml)

The MBC of the hexane fraction was observed at 20 mg/ml for *S. aureus, E. coli* and *B. subtilis*. And no activity was observed against *B. subtilis* and *S. typhi.*

Similarly the MBC for ethyl acetate was observed at 10 mg/ml for *S. aureus* and *B. subtilis* while the MBC of butanol and aqueous fractions were observed to be 25 mg/ml for *S. aureus* and 50 mg/ml for *B. subtilis* which is the concentration required to inhibit the activity of the bacteria or completely killed the bacteria. And no activity was observed against *E. coli* and *S. typhi* (Table 4.5).

Table 4.5: Minimum Bactericidal Concentrations mg/ml

Organisms	Hexane	Ethyle aceate	Butanol	Aqueous
S. aureus	20	10	25	25
B. subtilis	20	10	50	50
E. coli	20	N/C	N/C	N/C
S. typhi	N/C	N/C	N/C	N/C

N/C = Not carried out

4.10 Column Chromatography of Ethyl acetate Fraction

The column chromatography of ethyl acetate fraction yielded 61 fractions which were pooled together based on their TLC profile to obtain 8 combined fractions. Plate IV below is the chromatogram of fraction 30 – 35 which was purified using preparative thin layer chromatography to obtain white crystals weighing 20mg. It was soluble in chloroform, methanol and ethanol.



PLATE IV: TLC profile of column fractions from 31-35 developed in chloroform - methanol (4:1) and sprayed with 10% sulphuric acid in methanol.

4.11 Isolation of Compound A

Compound A was isolated using preparatory thin layer chromatography developed in a solvent system (chloroform-methanol 4:1) at room temperature and the targeted compound was scraped using scraper. Pure yellowish oily substance named compound A was obtained (Plate IV).



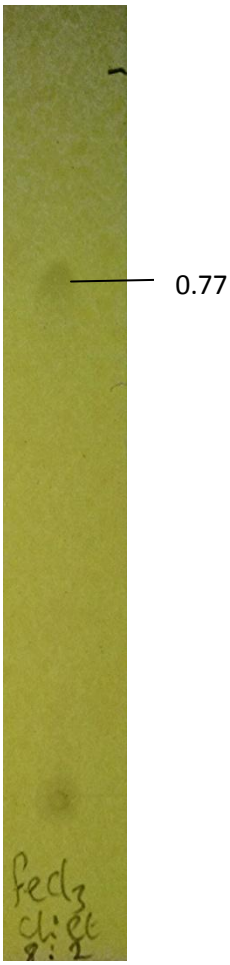


PLATE VII **PLATE VIII**

PLATE V: TLC profile of the isolated compound developed in Chloroform -ethanol (4:1) and sprayed with 10% sulphuric acid in methanol.

PLATE VI: TLC profile of the isolated compound developed in Chloroform - methanol (4:1) and sprayed with Ferri

4.12 Diameter Zone of Inhibition, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration for the Compound A

The isolated compound sowed anti bacterial activity against all the Gram positive and Gram negative bacteria at 20 and 10 mg/ml with diameter of inhibition range of 11 – 20 mm. The highest diameter zone of of inhibition was observed to be 20 mm at the concentrations of 20 mg/ml while the least diameter zone of inhibition was observed at 10 mg/ml which is 11mm. None of the Gram positive and Gram negative bacteria was susceptible to compound A at concentrations of 5 and 2.5 mg/ml for all the gram positive and gram negative bacteria tested (Table 4.6).

The MIC of the isolated compound against *S. aureus*, *B. subtilis*, *E. coli* and *S. typhi* was 10 mg/ml (Table 4.6).

The MBC of the isolated compound was observed to be 20 mg/ml against *S. Aureus*, *B. subtilis*, *E. coli* and *S. typhi* (Table 4.6).

Table 4.6 Determination of Zone of Inhibition, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration for the Isolated Compound

	Zone of inhibition					MIC	MBC
Test organisms	**Compound**				**Control**	**Compound**	**Compound**
	2.5	**5**	**10**	**20**			
S. aureus	0	0	12	16	40	10	20
B. subtilis	0	0	11	15	45	10	20
E. coli	0	0	14	20	38	10	20
S. typhi	0	0	11	15	38	10	20

Control= ciprofloxacin

4.12 Fourier Transformed-Infra Red (FT-IR) Spectroscopy

The FT-IR spectra of compound A revealed a total of 11 major bands (Fig. 4.1). Absorbance in the functional group region was observed at 2929.97 cm^{-1}indicating C-H vibration occurring in the molecule. Absorbance at 3351.43 cm^{-1} is an indicative of O-H stretching vibration.

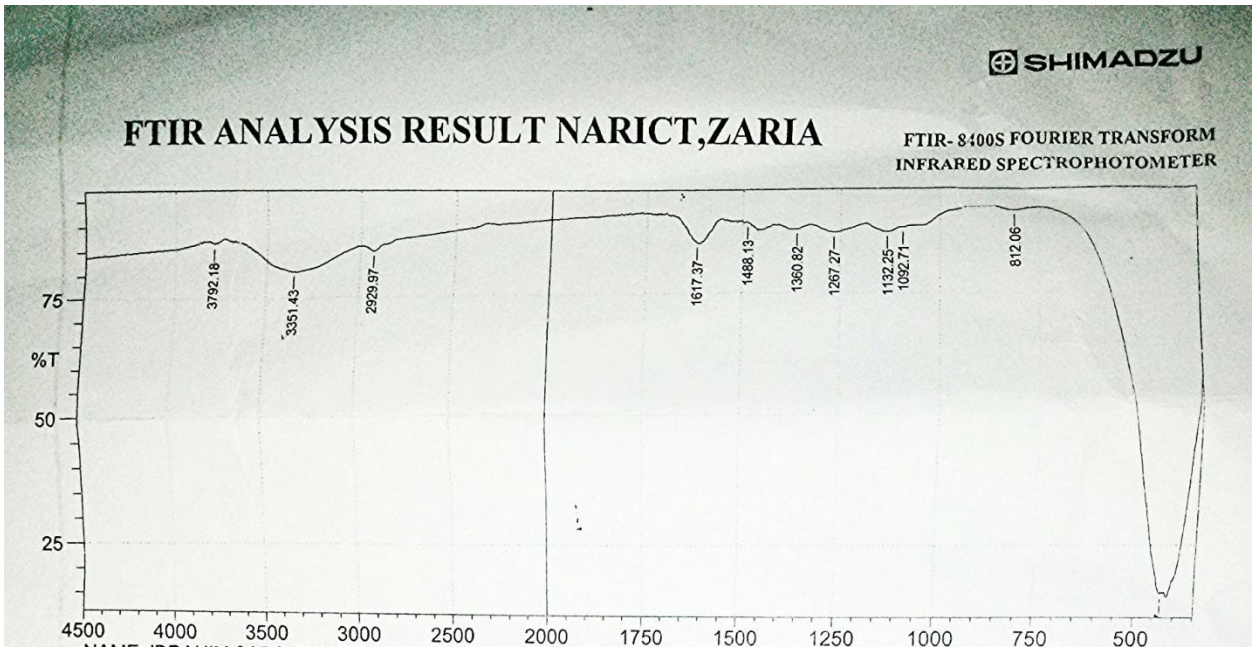


Fig 4.1: Fourier Transformed Infrared Spectra of compound A

CHAPTER 5

5.0 DISCUSSION

The results for phytochemical screening showed the presence of flavonoids, alkaloids, steroids and triterpenes, tannins and saponins. Similar component except alkaloids were confirmed by (Kone *et al*., 2011) to be present in the stem bark of *L. barteri.* This difference may be due to age of the plant, physiological variations, environmental conditions, geographic variations, genetic factors (Figueiredo *et al*., 2008) or evolutional differences of the plant or the presence of an antagonist in the extract. The information on the presence or absence and the type of phytochemical constituents especially the secondary metabolites are useful taxonomic keys in identifying a particular species and distinguishing it from a related species, thus helping in the delimitation of taxa (Jonathan and Tom, 2008).

Alkaloids, the most revered of all the phytochemicals and have bactericidal effects (Okwu and Okwu, 2004) are present in 3 of the fractions, alkaloids are said to be pharmacologically active and their actions are felt in the autonomic nervous system, blood vessels, promotion of diuresis, respiratory system, gastrointestinal tract, uterus, malignant diseases, infections and malaria (Trease and Evans, 1989). Also flavonoids which are reported to be important antimicrobial component (Chung *et al*., 1998; Karou *et al*., 2005) were present in the stem bark of *L. Barteri.* This explains the anti bacterial property of the plant. Saponin, which is responsible for numerous pharmacological properties (Estrada *et al*., 2000) was also tested positive in most of the fractions. Saponins are considered a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effect (Liu and Henkel, 2002). They lower the

cholesterol level; have anti-diabetic and anti-carcinogenic properties (Trease and Evans, 1989). In addition, Saponins are expectorants, cough suppressants and for haemolytic activities (Sofowora, 1993; Okwu, 2005).

The anti bacterial property of the plant is attributed to the presence of these constituents. Similar components were found in some members of the family. *Spondias mombin. L* (Shittu *et al*., 2014), *Lannea coromandelica* (Islam and Tahara, 2000), *Lannea welwitschii* (Groweiss, *et a*l., 1997), *Lannea edulis* (Queiroz, *et al*., 2003).

Moisture is an evitable component of crude drug, which must be eliminated as much as possible. The determination of the moisture content is very important especially during storage. High percentage may activate enzymes that can catalyse the breakdown of medicinally active chemical compounds. The moisture content of *L. barteri* was calculated in this research to be 8.6 ±0.19. The moisture content of *Lannea coromandelica* was calculated by (Avinash *et al.*, 2011) to be 9.4 %. The value 8.6 ±0.19 is within the acceptable limits of about 6 to 15 % for most vegetable drugs (Kunle, 2000). Low moisture content reduces errors in the estimation of the actual weight of drug material, reduces components hydrolysis by reducing the activities of hydrolytic enzymes which may destroy the active components, and also reduces the proliferation of microbial colonies and therefore minimize the chance of spoilage due to microbial attack (Shellard, 1958).

When a plant material is subjected to high temperature, the organic matter is destroyed and the inorganic residue known as the ash or total ash is that all left. The total ash represents the physiological and this consists of carbonates, chlorides and silicate (African pharmacopeia, 1985). The total ash value for *L. barter*i was calculated in this research to be 8.65 ± 0.17 which is

moderate and showed diagnostic purity index. It represents the physiological ash and non-physiological ash. Physiological ash is the ash inherent in the plant due to biochemical processes and the non-physiological is contaminants from the environment. These may be carbonates, phosphates, nitrates, sulphates, chlorides.

Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the washed insoluble matter left on ash-less filter paper. The acid insoluble ash for *L. barteri* was calculated in this research to be 1.33 ± 0.17 which is an indication that the stem bark contains some amount of dirt in lower quantity.

The water soluble ash was determined by calculating the differences between the total ash obtained and residue remaining after treatment of the total ash with water. The value obtained after calculating is 1.00 ± 0.00 % and that of *L. Coromandelica* was found to be 2.1 % (Avinash *et al.,* 2011).

Extractive values of crude drugs are important for their evaluation. The determination is to measure the amount of constituents which are extractable by the solvents under specified conditions. The ethanol and water extractive values are 15.45 ± 0.27 and 17.20 ± 0.04 respectively. The extractive values showed that the stem bark contained a larger portion of constituents that are soluble in methanol and water as the extractive values in both the solvents are relatively high. Thus, the use of water for this herb will be more beneficial with regard to its high extractive values.

Antimicrobial agent if in contact with any organism that is susceptible to it at a concentration cidal or static to the organism should make the population of the organism to reduce gradually until such a time that the medium may become sterile. The results in table 4.1 show that the butanol, ethyl acetate and aqueous fraction were active against gram positive only. It has been reported that plant extracts are more active against Gram positive bacteria than Gram negative bacteria (Jigna and Sumitra, 2006). However, the Gram negative were susceptible to the hexane fraction and the isolated compound, this is at disparity with an earlier report by (Jigna and Sumitra, 2006). This development may be attributed to the variation of the constituent of the fractions which were able to inhibit the growth of the bacteria. *L. barteri* has a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids which have antibacterial properties (Abeysinghe *et al*., 2004; Bandaranayake, 2002). Secondary metabolites present in the plants may play a role in plants' defence through cytotoxicity towards pathogenic microorganisms (Briskin, 2000).

The ability of the hexane fraction and the isolated compound to act against the Gram negative may be attributed to the better solubility of the active components (de Boer *et al.*, 2005) or resistance from the bacteria to the components of ethyl acetate, butanol and aqueous fractions. The ability of bacteria to develop resistant to antimicrobial agents has become a significant problem in the treatment and control of bacterial infectious diseases. Probably these two Gram negative bacteria may have evolved a number of different mechanisms to resist the anti bacterial constituents present in those fractions.

The isolated compound was active against both Gram positive and Gram negative bacteria tested but the ethyl acetate fraction from which the compound was isolated was not active on Gram

negative. This shows that the isolated compound was not able to express itself in synergic form but express it activity as an entity.

The low MIC value observed for *S. typhi* is a good indication of high efficacy against this bacterium. This outcome is remarkable considering that typhoid fever (caused by *S. typhi*) is on the rise and also becoming recalcitrant to first line antibiotics for its treatment in developing countries, including Nigeria. While that of *B. subtilis* was moderately high. High MIC may be an indication of low efficacy or that the organisms have the potential for developing resistance to the bioactive compounds (Doughari *et al.*, 2007). The MIC of compound A is 10 mg/ml for all the bacteria tested while the MBC is 20 mg/ml for all bacterial species tested. These results indicated that A had antimicrobial potency, may represent a new type of bacteriostatic agent (Table 4.6).

The ability of the solvent system (Hexane - Ethyl acetate 4:1) to move and separate the component of hexane fraction as shown in plate II is due the nature of the component in that fraction. Non polar components such as triterpenes, steroid, fats and oils are reported to be part of component of hexane fraction (Green *et al.*, 2011). The chromatogram shows 9 visible spots in which two spots are clearly separated from each other.

The preliminary phytochemical screening revealed the presence high polar phenolic compounds like tannins which may be the reasons why the solvent system (Hexane: Ethyl acetate 4:1) was not able to move any component from the baseline in the ethyl acetate fraction. It is known that phenolic compound especially flavonoids and tannins in plant are usually found in ethyl acetate fraction (Wu *et al.*, 2005). This development leads to formation of new solvent system

(chloroform: methanol 8: 2) which was able to move and separate the component of the fraction giving 9 visible spots.

The column chromatography of 2g of ethyl acetate fraction yielded 61 fractions which were pooled together base on the similarities of the content to form 8 fractions. Fraction 31-35 was further purified using preparatory thin layer chromatography developed in a solvent system (chloroform: methanol 4:1) to get a yellowish oily liquid which was developed using various solvent system and sprayed with general detecting reagent to confirm it as one single spot.

The IR interpretation used absorption and shape. The saturated hydrocarbon CH stretching absorptions all occur below 3000 cm^{-1}. The spectra in figure 4.1 showed that there is absorption at 2929.97cm^{-1} which indicate the presence of C-H. When the absorption is between 2900 -3000 cm^{-1} , the C-H is alkyl Sp^3 C-H bond (Coates 2000). The CH stretch vibrations is for methylene and is the most characteristic in terms of recognizing the compound as an organic compound containing at least one aliphatic fragment or centre (Coates, 2000).

The absorption at 3351.4cm^{-1} is broader in shape which indicates the presence OH group and the OH group is linked to normal "polymeric" OH. Absorptions between 3200-3670 cm^{-1} is an indication of OH group and absorptions that fall at or between 3400-3200cm^{-1} is a normal polymeric OH (Coates, 2000).

Absorptions at or between the region of (1600 – 1450 cm^{-1}) indicate Aromatic. Therefore, the absorption at the region of 1617.37cm^{-1} indicate the presence of C= C functional group (Coates, 2000).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMENDATION

6.1 Summary.

This study evaluated physicochemical parameters of the stem bark of *L. barteri*, the anti-bacterial activity of the stem bark with the aim of identifying and isolating one of its chemical constituents responsible for the activity.

The preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, carbohydrates, saponin, triterpenes and glycosides. Most of which were reported by previous literatures to posseses anti-bacterial properties.

The physicochemical parameters of the stem bark were found to be; moisture content (8.67 %), Ash value (8.65 %), Acid insoluble ash (1.3 %), Water soluble ash (1 %), Ethanol extractive (15.4 %) and Water extractive (17.4 %).

The stem bark (2 kg) was extracted with methanol and further partitioned with hexane, ethyl acetate and n-butanol respectively, yielding four fractions including aqueous fraction (as the last fraction). The four organic solvent fractions were tested against two Gram positive bacteria (*S. aureus* and *B. subtilis*) and two Gram negative (*E. coli* and *S. typhi*). The ethyl acetate fraction was taken to column chromatography, which afforded a yellowish oily liquid named compound A. The infra red analysis of the compound revealed the presence of hydroxyl (OH), aromatic ring and saturated carbon.

The compound A tested against the two Gram positive bacteria and two gram negative bacteria was found to be active against both types of bacteria at 20 and 10 mg/ml concentrations respectively.

6.2 Conclusion

The therapeutic potential of the stem bark of *Lannea barteri* is attributed to classes of active constituents present in the stem bark such as alkaloid, flavonoids, tannins and phenolic etc. which may be acting in synergy or individually.

The anti-bacterial activity carried out in this study lend credence to the traditional claim about the wound healing property of the stem bark of *L. barteri*

The column chromatographic analysis of the ethyl acetate fraction led to the isolation of compound which posseses anti-bacterial activity on both gram positive and gram negative bacteria tested.

The FT-IR analysis and the specific test using thin layer chromatography carried out on compound A has indicated it to be a phenolic compound.

6.3 Recommendations

Traditionally the stem bark of *L. barteri* is used in wound healing and anti- diarrhoea and this research validate the wound healing claim. Further studies should be carried out on the anti diarrhoeal claim and other uses of not only the stem bark of the plant but other parts of which may probably posses some therapeutic activities.

Also further studies should be done on the standardization and subsequent preparation of the fractions into appropriate and acceptable dosage forms that will aid the use of the stem bark as antibacterial agent.

To the best of my knowledge, no any chemical compound has been isolated from the stem bark of this plant until now. Therefore, there is a need for further investigation into the chemical constituents of the plant. This will unearth the medicinal potential of the plant and also provide a basis for its recognition as an antibacterial agent.

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APPENDIX I

Determination of the moisture content

	1	2	3
Weight of the Empty crucible	44.75	49.75	47.17
Weight of the Crucible + sample B4 heating	47.75	52.75	50.17
Weight of the Crucible +sample After heating	47.49	52.48	49.92
Weight of the sample	2.74	2.73	2.75
Weight of the moisture	0.26	0.27	0.25
% Moisture content	0.26/3x100	0.27/3x100	0.25/3x100
	8.67%	9.0%	8.33%
Average moisture Content %	8.67 ± 0.19		

APPENDIX II

Determination of Ash Value

	1	2	3
Constant weight Of the crucible	38.67	39.62	37.83
Weight of the crucible +2g of sample	40.67	41.62	39.83
Weight of crucible + Ash	38.85	39.79	38.00
Weight of the Ash	0.18	0.17	0.17
Ash Value %	0.18/2x100	0.17/2x100	0.17/2x100
	9%	8.5%	8.5%
Average Ash Value %	8.65 ± 0.17		

APPENDIX III

Determination of Acid insoluble Ash

	1	2	3
Weigh of empty crucible	39.62	37.83	38.67
Weight of crucible + Sample	41.62	39.83	40.67
Weight of crucible + Acid insoluble Ash	39.65	37.85	38.70
Acid insoluble Ash	0.03	0.02	0.03
%Acid insoluble Ash	0.03/2x100	0.02/2X100	0.03/2X100
	= 1.5%	=1.0%	=1.5%
Average %	1.33 ± 0.17		

APPENDIX IV

Determination of water soluble Ash

	1	2	3
Weight of the empty Crucible	37.83	37.83	37.83
Weight of the crucible + Sample	39.83	39.83	39.83
Weight of the crucible + Ash	38.00	38.00	38.00
Weight of the crucible + Water insoluble ash	37.98	37.98	37.98
Weight of water Insolube Ash	0.15	0.15	0.15
Weight of the Ash	0.17	0.17	0.17
Weight of water soluble Ash	0.02	0.02	0.02
% weight of water Soluble Ash	0.02/2x100	0.02/2x100	
= 1.00 ± 0.00			

APPENDIX V

Alcohol extractive Values

5g of the powdered plant material was used in 100ml of 100% ethanol

Description	**1**	**2**	**3**
Constant weight of the crucible (g)	65.51	65.51	66.80
Weight of the crucible and the content after evaporating(g)	65.70	65.70	67.00
Alcohol extractive content (g)	0.19	0.19	0.20
Alcohol extractive value %	15.2%	5.2%	16%
Average mean (%)		15.45 ± 0.27	

APPENDIX VI

Determination of water extractive values

5g of the powdered plant material was used in 100ml of 100% water

Description	**1**	**2**	**3**
Constant weight of the crucible(g)	67.46	68.0	65.51
Weight of the crucible and the content after evaporating(g)	67.67	68.22	65.72
Alcohol extractive content (g)	0.21	0.22	0.21
Alcohol extractive value (%)	16.8	17.6	16.8%
Average mean (%)	17.20 ± 0.40		