**ANTIMICROBIAL PROPERTY OF THE HEXANE EXTRACT FROM THE PODS OF *ACACIA NILOTICA* (L.) DEL. (MIMOSACEAE)**

**BY**

**YAHAYA IBRAHIM**

**DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT,**

**FACULTY OF PHARMACEUTICAL SCIENCES, AHMADU BELLO UNIVERSITY,**

**ZARIA.**

**JANUARY, 2015.**

**ANTIMICROBIAL PROPERTY OF THE HEXANE EXTRACT FROM THE PODS OF *ACACIA NILOTICA* (L.)**

**DEL. (MIMOSACEAE)**

**BY**

**YAHAYA IBRAHIM B.Sc ABU (2010) (MSc/PHARM-SCI/13316/2011-12)**

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

**DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT,**

**FACULTY OF PHARMACEUTICAL SCIENCES AHMADU BELLO UNIVERSITY, ZARIA NIGERIA.**

**JANUARY, 2015.**

# DECLARATION

I, YAHAYA IBRAHIM solemnly declare that this project titled Antimicrobial property of the hexane extract from the pods of *A. nilotica* (l.) Del. (Mimosaceae) is a record of my research under the supervision of Prof A. Agunu and Dr A. Ahmed in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria and to the best of knowledge it has never been published or presented for any degree. All references therein have been dully acknowledged.

**Yahaya Ibrahim Date**

# CERTIFICATION

This thesis entitled ANTIMICROBIAL PROPERTY OF THE HEXANE EXTRACT FROM

THE PODS OF *A. NILOTICA* (L.) DEL. (MIMOSACEAE)byYahaya Ibrahimmeets the regulations governing the award of Master of Science in Pharmacognosy, Ahmadu Bello University Zaria, and is approved for its scientific knowledge and literary presentation.

## Prof A. Agunu B. Pharm, M.Sc, Ph.D Date

Chairman, supervisory committee Department of Pharmacognosy and Drug Development,

Ahmadu Bello University, Zaria.

## Dr A. Ahmed B.Sc, M.Sc, Ph.D Date

Member, supervisory committee Department of Pharmacognosy and Drug Development,

Ahmadu Bello University, Zaria.

## Dr G. Ibrahim B.Sc, M.Sc, Ph.D Date

Head, Department of Pharmacognosy and Drug Development

Ahmadu Bello University, Zaria.

## Prof A. Z Hassan Date

Dean, School of Postgraduate Studies Ahmadu Bello University, Zaria**.**

# DEDICATION

This work is dedicated to ALLAHU(SWT), creator of Heaven and Earth for giving me the strength and courage to embark on this research.

# ACKNOWLEDGEMENT

First and foremost, my gratitude goes to Almighty Allah for his endless blessings guidance and protection, for keeping me in good health and giving me the strength to withstand the pressure during the cause of my work.

My sincere gratitude goes to my supervisors Prof A. Agunu and Dr A. Ahmed for all their support and encouragement during the cause of my research and for standing by me at times of difficulty. Thank you sirs.

My special appreciation goes to Dr. G. Ibrahim, the Departmental Postgraduate Coordinator, and the entire staff of Department of Pharmacognosy and Drug Development. I need not forget the relentless efforts of Mr Emmanuel Mshelia (UsmanDanfodio University, Sokoto) for his assistance during the NMR analysis, thank you sir.

My special gratitude goes to my parents, Sqnldr G.S Yahaya (rtd), HajiyaHajara, HajiyaSaratu, and Hajiya Fatima. My brothers Mahmud, Saidu, Haruna, Saeed (jr), Sageer and Ameer. My sisters Fatima, Balkisu, Rahmatu and Amina. Words alone cannot express how grateful i am, Thank you for all your support and encouragement.

Lastly, I will like to shout a big thank you to my friends and colleagues in persons of Abba Anas, UsmanJajere, Abubakar Suleiman Umar, Saifullah Umar, Nusa Sadisu, AliyuAshafa, Nusa k Asmau, Ibrahim Hadiza, AbdulrahmanAdama, AbubakarAsmau, Moveh Patience, among others. You really inspired me toward achieving my dreams, God bless you all.

# ABSTRACT

*Acacia nilotica*is a multipurpose plant belonging to the family Mimosaceae. Commonly known as Prickly acacia in English and *Bagaruwa* in Hausa, It has medicinal properties which include antimalaria, antimicrobial, anti-inflammatory, anticancer, etc. Traditionally, the pods are used for stomach disorder, foot and mouth disease, sore throat, dysentery, among others. This research was aimed at isolating from the pod of this plant and testing for its antimicrobial activity which will justify the claim for its usage. The pods were extracted using n-hexane and the extract was subjected to column chromatography which afforded a compound tagged CY2 which was analysed by spectroscopical means and suggested to be **Pentacosane-dioic acid**. Antimicrobial studies of the crude extract and the isolated compound against some pathogens were conducted and compared with standard drugs (Ciprofloxacin and Fluconazole). The result showed that the pod have antimicrobial activities, five bacterial strains (*Staphylococcus aureus, Strptococcuspyrogenes, Eschericia coli, Shegiliadysenteriae, Salmonella typhi,)* and one fungal strain (*Candida albicans*) were susceptible to both the crude extract and the isolated compound, The isolated compound tend to have a higher activity compared to the crude extract but not as potent as the control drugs used which showed better activity among all the microbes used. A bacteria strain (*Corynebactoriumulcerans*) and a fungal strain (*Candida krusei*) among the test organisms tend to be resistant to the crude extract as well as the isolated compound.

# TABLE OF CONTENTS

Title Page

Title page ii

[Declaration iii](#_TOC_250025)

[Certification iv](#_TOC_250024)

[Dedication v](#_TOC_250023)

[Acknowledgement vi](#_TOC_250022)

[Abstract vii](#_TOC_250021)

[Table of contents viii](#_TOC_250020)

[List of tables. xi](#_TOC_250019)

[List of figures xii](#_TOC_250018)

[List of plates xiii](#_TOC_250017)

[List of appendices. xiv](#_TOC_250016)

Abbreviations xv

[CHAPTER ONE](#_TOC_250015)

* 1. [Introduction 1](#_TOC_250014)
  2. [Plants and medicine 1](#_TOC_250013)
  3. Traditional medicine 5
  4. [African Traditional Medicine (ATM) 6](#_TOC_250012)
  5. [Traditional Chinese Medicine (TCM). 7](#_TOC_250011)
  6. [Challenges in Medicinal plants research 7](#_TOC_250010)
     1. [The role of plants in human history 8](#_TOC_250009)
     2. [The role of plant-derived compounds in drug development. 9](#_TOC_250008)
     3. [Plants as sources of antimicrobial agents 9](#_TOC_250007)
     4. [Statement of research problem 11](#_TOC_250006)
     5. [Justification 11](#_TOC_250005)
     6. [Hypothesis 11](#_TOC_250004)
     7. [General aim 11](#_TOC_250003)
     8. [Objectives 11](#_TOC_250002)

[CHAPTER TWO](#_TOC_250001)

* 1. [Literature review 12](#_TOC_250000)
  2. Botanical description of Mimosacea 12
  3. Economic and medicinal importance of Mimosaceae family 12
  4. Chemistry of acacia specie 13
  5. Description of Acacia nilotica 14
  6. Chemistry of Acacia nilotica 15
  7. Some economic importance of Acacia nilotica 18

CHAPTER THREE

* 1. Materials and method 21
  2. Equipments used. 21
  3. Collection and identification of plant material. 21
  4. Preparation of plant material. 21
  5. Extraction of plant material. 22
  6. Chromatographic studies. 22
     1. Solvents used. 22
     2. TLC solvent system. 22
     3. TLC spray reagent. 22
     4. TLC profiles of crude extract. 23
     5. Column chromatography of crude extract. 23
        1. Determination of melting point of compound CY2. 24
        2. Spectroscopic analysis 24
        3. Antimicrobial studies. 24
        4. Preparation of stock solution. 25
        5. Media for microbial growth. 25
  7. Zones of inhibition. 25
     1. Minimum inhibitory concentration. 25
     2. Minimum Bactericidal Concentration/ Minimum Fungal Concentration. 26

CHAPTER FOUR

* 1. Results. 28
  2. Extraction. 28
  3. TLC Profiles of crude extract. 28
  4. Melting point of compound CY2. 34
  5. Spectroscopical properties of compound CY2. 35
  6. Results of the Antimicrobial studies. 41

CHAPTER FIVE

1. Discussion. 44

CHAPTER SIX

1. SUMMARY, CONCLUSION AND RECOMMENDATION. 48
   1. Summary 48
   2. Conclusion. 49
   3. Recommendation. 49

REFERENCES 50

# LIST OF TABLES

## Table Title Page

* 1. 1HNMR spectral data of compound CY2 and literature reported in ppm

(In CDCl3) 38

* 1. 13CNMR spectral data of compound CY2 and literature reported in ppm

(In CDCl3) 39

* 1. Sensitivity Test and Zones of Inhibition of *A. nilotica,* bothhexane extracts

and CY2 against Some Microbes 41

* 1. Minimum inhibitory concentration (MIC) of the compound and crude

extract against the test microbes 42

* 1. Minimum bactericidal/ fungal concentration of the crude extract and

CY2 against the test organisms 43

# LIST OF FIGURES

|  |  |  |  |
| --- | --- | --- | --- |
| **Figure** | **Title** |  | **Page** |
| 1.1 | Somephytochemical constituents isolated from plants |  | 3 |
| 1.2 | Somephytochemical constituents isolated from plants |  | 4 |
| 2.3 | Somephytochemical constituents from *Acacia nilotica*17 |  |  |
| 2.4 | Somephytochemical constituentsfrom *Acacia nilotica* | 18 |  |
| 4.5 | 1HNMR Spectrum of compound CY2 in CDCl3 showing integration |  | 35 |
| 4.6 | 1HNMR spectrum of compound CY2 (EXPANDED) in CDCl3 |  | 36 |
| 4.7 | 13CNMR spectrum of compound CY2 showing chemical shifts |  | 37 |
| 4.8 | Proposed structure of compound CY2 |  | 40 |

# LIST OF PLATES

## Plates Title Page

1. *Acacia nilotica* tree in its Natural Habitat 21
2. Acacia*nilotica* fruits showing the pods 22

|  |  |  |
| --- | --- | --- |
| III TLC chromatograms of hexane crude extract of *A. nilotica*pod |  | 29 |
| IV Fractions 20-23 collected from column chromatography pooled together |  | 30 |
| V Chromatogram of fraction 20-23 pooled together coded as C |  | 31 |
| VI Chromatogram of fraction C purified on a smaller column showing single spot on fractions 2-4 coded as CY2 | 32 |  |
| VII Fractions 2-4 of CY2 combined and developed in Hex: EtOAc (8:2), showing a single spot sprayed with anisaldehyde/H2SO4 | 33 |  |

# LIST OF APPENDICES

## Appendix Title Page

* 1. Petri dishes showing zones of inhibition of the extract against the test microbes 56
  2. Petri dishes showing zones of inhibition of the extract against the test microbes 57
  3. Petri dishes showing zones of inhibition of CY2 against the test microbes 58
  4. Petri dishes showing zones of inhibition of CY2 against the test microbes 59

# ACRONYMS AND ABREVIATIONS

|  |  |
| --- | --- |
| **Acronyms** | **Meanings** |
| ATM | African Traditional Medicine |
| CFU | Colony formed per unit |
| 13CNMR | Carbon- 13 Nuclear Magnetic Resonance |
| CY2 | Compound isolated |
| DMSO | Dimethylsulfoxide |
| Eg | Example |
| Etc | Et cetera |
| *et al* | And co-workers |
| EtOAc | Ethyl acetate |
| H2SO4 | Sulphuric acid |
| HTS | High throughput synthesis |
| MBC | Minimum Bactericidal Concentration |
| MDR | Multidrug resistant |
| MFC | Minimum Fungal Concentration |
| MIC | Minimum Inhibitory Concentration |
| NS | Normal Saline |
| Ppm | Parts per million |
| 1HNMR | Proton Nuclear Magnetic Resonance |
| TLC | Thin Layer Chromatography |
| TM | Traditional Medicine |
| WHO | World Health Organisation |

## Chapter one

## Introduction

## Plants and Medicine

Plants used in the treatment of diseases are as old as civilization (Fabricant and Farnsworth, 2001) and traditional medicine is still a major part of habitual treatments of different maladies (Alviano and Alviano, 2009). This plant-based traditional medicine system continues to play

DQ HVVHQWLDO UROH LQ KHDOWK FDUH ZLWK DERX

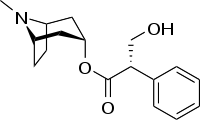
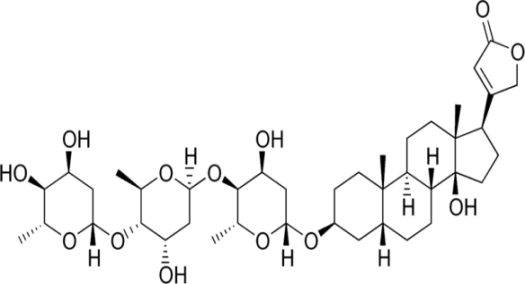
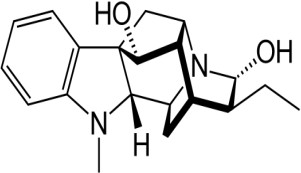
traditional medicines for their primary health care (Christiana *etal.,* 2012). Plants are considered as one of the main sources of biologically active materials. It has been estimated that about 50% of the prescription products in Europe and USA are originating from natural products including plants or their derivatives (Cordell, 2002; Newman *et al*., 2003).In spite of the recent domination of the synthetic chemistry as a method to discover and produce drugs, the potentials of bioactive plants or extracts to provide new and novel products for disease treatment and prevention is still enormous (Raskin*etal*., 2002). One of the most challenging pursuits in the realm of pharmaceutical and medical sciences is the search for newer and more potent drugs with little side effects, self-administrable, less expensive and completely reversible. Most of these properties are observed in drugs plants of natural origin. Medicinal plants, which form the bedrock of traditional medicine, have in the last few decades been the subject of very intense pharmacological studies (Unny*et al*., 2003).

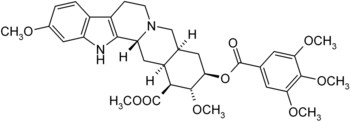
Whole plants or plant parts are used in traditional medicinal system where the synergistic effects of many constituents are evaluated on the whole human body inflicted with diseases (Rao, 2001). New drugs formulated on basis of organic chemistry and biochemistry-focuses

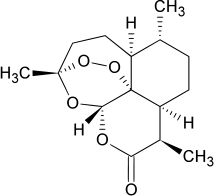
attention on the use of the purified compounds and there molecular structures modified to synthesize modern drugs, helpful to cure specific illnesses or diseases (Krikorian, 1998).

Many of the medicinal plants recorded in%XUNLOO¶V GLFWLRQDU

recently using modern equipments and techniques to analyze their chemistry, to isolate new compounds and to formulate new drugs. An analysis of drugs approved by the Food and Drug Administration in the United States over 12-year period (1983-1994) showed that 157 of 520 drugs (30%) were natural products or their derivatives (Cragg*et al*., 1997).Herbal remedies have been applied for treatment of many ailments since ancient times all over the world and about 25% of current drugs are derived from plants. (Farnsworth *et al.,* 1985) indicated that globally there were 119 compounds from 90 plants, which were used as single-entity medicinal agents. However, only about 20% of the plants with claimed bioactivities have been subjected to bioassay screening (Houghton, 2001). Some of the modern drugs derived from natural products such as plants include (1) Hyoscyamine (*Hyoscyamusniger*), (2) Ajmaline(*Rauwolfia spp.*), (3) Digitoxin (*Digitalis purpurea*), (4) Reserpine(*Rauwolfiaserpentina*), (5) Artemisinin (*Artemisia annua*), (6) Caffeine (*Coffeaarabica*), (7) Ephedrine(*Ephedra sinica*), (8) Morphine(*Papavasomniferum*), (9) Papaverine (*Papavasomniferum*), (10) Physostigmine (*Physostigmavenenosum*). Surprisingly, as leading developed countries race to colonize space and other planets, there are no authoritative estimates for the number of species of plants, insect, fungi, and marine organisms on this planet (Wilson, 1988). There is a significant need to enhance technology of drug discovery with respect to natural products.

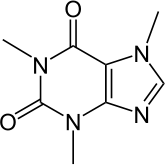
**1 2**

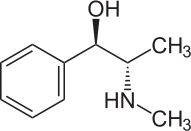
**3**

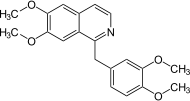
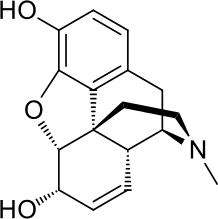
**4**

**5**

**Fig 1(1-5):** Some phytochemical constituents isolated from Plants (Evans, 2009).



**6 7**

**8**

**9**

**10**

**Fig 2(6-10):**Somephytochemical constituents isolated from Plants(Evans, 2009).

## Traditional medicine (TM)

The pharmacological treatment of disease began a long time ago with the use of herbs (Schulz *et al*., 2001). Methods of folk healing throughout the world commonly use herbs as part of their tradition. Traditional Medicine (TM) is an aspect of ethno-botany and ethno-medical studies. It is a practice by which, ailments are cured or treated by the use of raw materials from plants. Ethno-botany is a very broad discipline and it includes all sorts of human-plant interactions (Manzoor*et al*., 2006). It is a study of how people of a particular culture and region make use of indigenous plants. Ethno-medical study then conceptualized ethno-botany based on the medicinal use of plants and epidemiology. Epidemiology however, is the study of how often diseases occur in different group of people and why. All these concepts are then summarize as the use of plants in primitive societies (Manzoor*et al*., 2006)*.*The World Health Organization (WHO, 2011) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today (Fahn, 1983). Traditional medicine is the synthesis of therapeutic experience of generations of practising physicians of indigenous systems of medicine. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. The earliest recorded evidence of their use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The classical Indian texts include *Rigveda*, *Aryuveda*, *Charaksamhita*and *Sushrutasamhita*. The herbal medicines/traditional medicaments have, therefore, been derived from rich traditions of ancient civilizations and scientific heritage.

## African Traditional Medicine (ATM)

Medicinal plant knowledge is very similar to all other local knowledge; it is an important aspect of a particular cultural system (Antweiler, 1998; Vandebrock*et al.,*2004). Local knowledge is not always evenly distributed and it is not every member of a particular group or community that gets the knowledge. This is certainly true for medicinal plant knowledge, were a clear distinction can be drawn between specialist (Traditional healers) who posses more in depth knowledge of the practices. In Nigeria, medicinal plant knowledge is always kept within the close family circle and is passed on from one generation to another (Atawodi*et al*., 2002). Similarly, in other African countries like Cameroon, the knowledge of medicinal plants is considered secretive and healers claim to have learnt the techniques from a host of relatives (Ryan, 1998). However, in Zambia majority of the people are thought by family members, while others either get it through ancestral spirits or from other healers. In general, the knowledge of healing is tends to be a closely guarded family secrete which is handed down through kinship (Ndubani and Hojab, 1999). In other Countries like Malawi, many healers have assistant who are apprentice in training (Courtright, 1995). Many healers in Tanzania reported that they receive their information on new potentially useful medicinal plants during their sleep. They also communicate sometimes with other healers about the plants they use and the mode of administration (Gessler*et al*., 1995a). The family seemed to be the most important source to acquire knowledge, followed by ancestral spirit and experienced traditional healers outside the family (Gessler*et al*., 1995b). In Nigeria, traditional medicine continues to be widely used by majority of the rural communities. The administration of native drugs has been in the hands of native herbalist who are quite often old people living in rural communities.

## Traditional Chinese medicine (TCM)

Traditional Chinese medicine has been used by Chinese people from ancient times. Although animal and mineral materials have been used, the primary source of raw materials is natural. Of the more than 12 000 items used by traditional healers, about 500 are still in common use today (Li, 2000). Natural products are used only after some kind of processing,which may include, for example, stir-frying or soaking in vinegar or wine. In clinical practice, traditional diagnosis may be followed by the prescription of a complex and often individualized remedy. Traditional Chinese medicine is still in common use in China. More than half the population use traditional medicines regularly, with the highest prevalence of use in rural areas. About 5000 traditional remedies are available in China; they account for approximately one fifth of the entire Chinese pharmaceutical market (Li, 2000).

## Challenges in Medicinal Plants Research

Despite the great contribution of the plant Kingdom, it has only been haphazardly investigated. A few plants have been exhaustively studied while many have not been studied at all. It has been estimated that only 5-15% of the approximately 350,000 species of higher plants have been systematically investigated for the presence of bioactive compounds (Cragg, *et al*., 1997). It is well known that some of these important sources of drugs are fast getting extinct due to many factors which include agricultural development, indiscriminate destruction of flora and so on (Rukangira, 2009). There is a growing trade demands for cheaper healthcare products and new plant-based therapeutic markets in preference to more expensive target-specific drugs and biopharmaceuticals. This habitat loss is the greatest immediate threat to biodiversity and as such it has stimulated positive legal as well as research interest in order to document the potentials of these plants before they disappear completely or loss of genetic diversity.

Folk healers and their orally transmitted traditions are more vulnerable than medicinal plants themselves because many healers/indigenous knowledge holders aged and are dying or being killed in ethnic, religious or political crises with their knowledge left unrecorded. Traditional medicine(TM) is well known to be associated with secrecy (Sofowora, 2008), families with this knowledge would like to keep their information and knowledge to themselves for fear of being marginalized in the race to exploit the commercial values of their medicine. Nowadays, younger generation show less interest in acquiring knowledge of plants from parents due to expansion of modern education and to some extent modern medicine (Welderima, 2009). Despite the long history of success in the natural products as drugs, during the past couple of decades, research into natural products has experienced a steady global decline. This decline could partly be attributed to the introduction of high-throughput synthesis (HTS) and combinatorial chemistry (Eldridge *et al.*, 2002). HTS is aimed at inexhaustible supply of compound libraries, a promise which is yet to be fulfilled.

## The role of plants in human history

Over the centuries humans have relied on plants for basic needs such as food, clothing, and shelter, all produced or manufactured from plant matrices (leaves, woods, fibers) and storage parts (fruits, tubers). Plants have also been utilize for additional purposes, namely as arrow and dart poisons for hunting, poisons for murder, hallucinogens used for ritualistic purposes, stimulants for endurance, and hunger suppression, as well as inebriants and medicines. Most compounds responsible for the potency of arrow and dart poisons belong to three plant chemical groups, namely the alkaloids (e.g., strychnine from *Strychnos*species) cardiac glycosides (e.g., qouabain from *Strophanthus*species), andsaponins (e.g., a monodesmoside glycoside from *Clematis* species). The plant chemicals used for these latter purposes are largely the secondary metabolites, which are derived biosynthetically from plant primary

metabolites (e.g., carbohydrates, amino acids, and lipids) and are not directly involved in the growth, development, or reproduction of plants. These secondary metabolites can be classified into several groups according to their chemical classes, such alkaloids, terpenoids, and phenolics (Harborne, 1973).

## The role of plant-derived compounds in drug development

Despite the recent interest in drug discovery by molecular modelling, combinatorial chemistry, and other synthetic chemistry methods, natural-product-derived compounds are still proving to be an invaluable source of medicines for humans. The importance of plants in modern medicine has been discussed in recent reviews and reports (Fowler, 2006; James*et al.*, 2006). Other than the direct usage of plant secondary metabolites in their original forms as drugs, these compounds can also be used as drug precursors, templates for synthetic modification, and pharmacological probes.

## Plants as sources of antimicrobial agents

Infectious disease is one of the major causes of death accounting for approximately one-half of all deaths in tropical countries. Death from infectious diseases was ranked 5th in 1981, became the 3rd leading cause of death in 1992, with an increase to about 58% (Venkataswamy*et al*., 2010). The clinical efficacy of many of the antibiotics is being threatened by the emergence of multidrug resistant pathogens (Doss *et al*., 2009). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases (Anand*et al*., 2011).

The potentials of higher plants as sources of new therapeutic drugs are still largely unexplored. Among the estimated ¶- 50 0,0 00 plant species worldwide, only a small

percentage have been adequately investigated for their phytochemicals and biological or pharmacologicalscreening. Compounds of natural or synthetic origin have been the source of innumerable therapeutic agents (Mahesh and Satish, 2008). Medicinal plants are a rich source of antimicrobial agents. Plants are used medicinally in different countries and are the source of potential and powerful drugs.A wide range of medicinal plants are used to get different phytochemicals which possess different medicinal properties against different microbes. Although hundred of plants species have been tested for antimicrobial properties, the majority of these have not been adequately evaluated.

The problems associated with infectious microorganisms which were thought to have been controlled by antibiotics have led to re-emergence of more virulent microorganisms in a new form of resistant strains (Levy and Marshal, 2004; Udobi*et al.,* 2008). The problem thus makes it mandatory for mankind to seek new antimicrobial agents and/or new effective ways of treating infectious diseases caused by microorganisms such as the drug resistant bacteria (Salvat*et al*., 2008). One of the possible basic approaches to cure and control infections caused by multiple drug resistant (MDR) strains of bacteria is to explore the medicinal properties of herbs and higher plants.The use of medicinal plants as treatments against microbial invasion can be traced back to early civilizations in China, India and the Near East,

thus making phytomedicineunGRXEWOHVVO\ sRolQdeHst proRfeIssi onPs (DMaQheNshLaQndG¶

Satish, 2008). Medicinal plants have been used for centuries as remedies for human related diseases and have provided new sources of phytochemical compounds with biological activity as antimicrobial agents (Das *et al*., 2010). Antibiotics have undesirable side effects while the emergence of previously uncommon infections is also a serious medical problem (Marchese and Shito, 2001). Over 75 % of the antibacterial agents in clinical use are of natural origin and most of them are obtained from fungal sources (Newman *et al.,* 2003).

## Statement of research problem

The pods of *A. nilotica* are used in traditional medicine without any validation.

## Justification

$ERXW RI WKH ZRUOG¶V LQKDELWDQWV DUH U

primary health care (WHO, 2002).An estimate shows that over 50% of the best-selling pharmaceuticals in use today are derived from natural products(Newman *et al.,* 2003).It has also been estimated that only 5-15% of the approximately 350,000 species of higher plants have been investigated for the presence of bioactive compounds (Cragg*et al.,* 1997).

## Hypothesis

 *Acacia nilotica*pod does not have constituents with antimicrobial activity.

## General aim

 To establish the scientific basis for the use*of A. nilotica*pods in the treatment of infections such as Dysentery, sore throat, foot and mouth disease and stomach pain.

## Objectives

 Identification of the plant hexane extract of *A. nilotica*pod*.*

 To establish the antimicrobial property of the hexane extract and compound from*A. nilotica*pod.

# CHAPTER TWO

## Literature review

* 1. **Botanical Description of Mimosaceae**

The plant family comprises of mainly herbs, shrubs, trees, twiners and climbers. They vary in sizes from the smallest plants of the desert and arctic or alpine region to the tallest trees of the rain forest. They are characterised by large seeds and pods types of fruit. The pods possess parietal placentation along the adaxial suture with ovules two or many in the various species. Leaves are alternate, simple to compound, pinnate, rarely palmate or bipinnate, unifoliate, trifoliate and sometimes phyllodic or reduced to tendrils. Flowers are bisexual with a superior carpels, arranged singly or in racemes, regular, zygomorphic or irregular and hypogynous or slightly perigynous (Muhammad *et al.,* 2001; Dutta, 2005; Martin *et al*., 2006).

## Economic and Medicinal Importance Mimosaceae

They are used as pulses, vegetables, as natural fertilizers, source of timber, gums, and tannins. Legumes are important to agronomy due to their symbiotic capacity for biological nitrogen fixation. They possess tubercles containing bacteria root which is responsible for their nitrogen fixing bacteria properties (Dutta, 2005; James *et al*., 2006).

Medicinally, the plant family has been reported in scientific literature for their antihyperglycemic and antinociceptive activities. Several species of the family including *Alysicarpus* species, *Crotalaria* species, *Lephrosa* species, *Buteaparviflora, Cajanuscajan, Mucunapruriens* and *Purariatuberosa* are been utilized traditionally for treatment of skin eruptions, rheumatic pains, and in treatment of syphilis, gastritis, cough, fever, ringworm, epilepsy, dysentery, mouth ulcers, as vermicide and contraceptive. Species such as *Entadaphaseoloides, Buteamanosperma*, and *Bauhinia forticata* have been reported to exhibit

antidiabetic, antihyperglycemic, and hypoglycemic effects respectively (Fennel *et al.,* 2004; Alesiani*et al.,* 2007; Richa, 2010; Rahmatullah*et al.,* 2010 andMeghendra and Ashwani, 2013). *Parkia* species have been reported to possess antioxidant, antimicrobial, gastroprotective and hypertensive effects. Generally, species of Mimosaceae family are known to yield resins, balsams and dyes with few possessing astringent, narcotic, emetic, purging, tonic and restorative properties (Meghendra and Ashwani, 2013; Vivianne*et al*., 2013).

* 1. **Chemistry of *Acacia* species**

Several secondary metabolites such as hydrolysable and condensed tannins, flavonoids, terpenes, alkaloids, cyanogenic glycosides and gums have been reported in *Acacia* species. Flavonoids such as 3,4-GLK\GUREHQ]RL-Ftet rahDydFroLxyG-4-Vm eth ox yfl av an -3- ol,¶ ¶

3,4-GLK\GUR[\EHQ]R-LFtr ihydrDoxFy-L G -Vd im ¶e thox yfl av on e, a¶nd ¶ ¶

trihydroxyflavone have been isolated and characterised from *Acacia* VSHFLHV 6- WLJPDV

VLWRVW-HsitUostReroOl- 3-O -ȕ-Dȕ-glucoside, stigmasterol-3-O-ȕ-D-glucoside, lupenone, taraxerone, apigenin, luteolin, quercetin, gallic acid, methyl gallate and salicylic acids have been reported from the aerial parts of *A. cochliacantha*. Compounds such as 3,4- dihydroxybenzoic acid, 2,3-trans- -t etr¶ah yd rox¶ydihydroflavonol, 3,3-hydroxyl-4-

PHWKR[\EHQ]RLF DFLG- SPHQHWDKK\O\G UHRV[W\HIU-tO etrD ahYy dRr oQx y-H ¶

3-methoxyflavone, 3,4-GLK\GUR[\EHQ]RLF DFL-pGen tahHydrWoxKy-\traOns - FKDOFRQH-te tra hy dr ox y-4 -m et ho xy¶flavone were isolated from *A.confusa*. Gallate acid methyl ester, gallic acid, naringenin, (+)-catechin, 2-O-ȕ-arabinofuranosyl and octyl-2- hydroxyphenylcarbanate (nilobamate) was isolated from the flowers and seed-pods of *A. nilotica.* Pentacyclicterpenoids and triterpenoids such as (2OX) 3-oxolupane-30al, (2OS) 3- oxolupane-30al, 30-hydroxylup-20(29)-en-3-one, 30-hydroxylup-20(29)-en- -ȕol, methyl-2- hydroxy-4-hydroxy-3,6-GLPHWK\E-HVQL]WRRDV-WWOH-Hgl uUc oRsiȕOde an d lȕinoleic acids were

HVWHU

isolated from stem bark of *A. mellifera.*Species such as *A. ehrenbergiana, A. polyacantha, A.senegal* and *A*. *tortilis* have been reported to contain apigenin, gallic acid, methyl gallate, flavan-3-ol-gallate, gallocatechin-3-JDOOD-caWteHch in, 8-ȕmethoxyflavone-3,4-diones, 5- hydroxy-pipecolic acids, acacatechin, epicatechol, 7,8,4-trihydroxy-3-methoxyflavone,

quercetagetin, quercetin, rutin, afzelichin, 4-PHWKR[\JOXFRU-siRtosQterLol,FD - DFLGV

JDODFWRVH

-sHitoUste\roWl-3K-OU-DR-JGOLXRFOR V LGĮH -amVyrLinW,

RcaVteWchoHl,URO

gallocatechol, apigenin-6,8-Bis-C-glucoside, 4-hydroxypipecolic acids, betuline and octacosan-1-ol among other secondary metabolites (Muhaisen*et al.,* 2002; Bala, 2006; Valentine *et al*., 2012; Sakina*et al*., 2012).

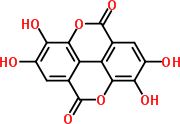
* 1. **Description of *A. nilotica***

*Acacia nilotica*is a single stemmed plant which grows to about 15-18 m in height and 2-3 m in diameter. The Pods are 7-15 cm long, green and tomentose (when immature) or greenish black (when mature), indehiscent, deeply constricted between the seed giving a necklace appearance. The Seeds are 8-12 per pod, compressed, ovoid, dark brown shining with hard testa (Iman *et al*., 2007). The leaves are bipinnate, pinnate 3-10 pairs, 1.3- 3.8 cm long, leaflets 10-20 pairs, and 2-5mm long (Beniwal*et al*., 1992). The flowers are globular heads, 1.2-1.5 cm in diameter of a bright golden yellow colour, develop either in axillary or whorly pattern on peduncles 2-3 cm long located at the end of branches (Bargal and Bargali, 2009). The stems are usually dark to black coloured, deep longitudinal fissured, grey-pinkish slash, exuding a reddish low quality gum (Brenan, 1983). The bark is a tinge of orange and/or green (young tree), but older trees have dark, rough bark and tend to lose their thorns (Khan *et al*., 2009). The thorns are thin, straight, light grey exist in axillary pairs (usually 3-12), 5-7.5 cm long in young trees. The root generally has a brown colour in older plant and whitish in younger regions. The gum varies in colour from very pale yellowish brown to dark reddish

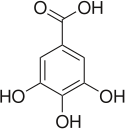
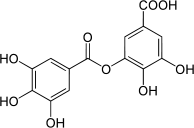
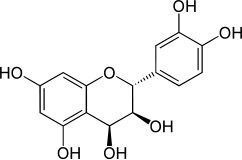
brown depending on the quantity of tannins in the sample. The lighter, more highly valued gums are soluble in water and very viscous; the tannins in the darker gum reduce the solubility. The gum has a moisture content of about 13% and is slightly dextrorotary (New, 1984).

* 1. **Chemistry of *A. nilotica***

The plant contains chemical compounds like diester, pentacosanedioic acid dihexadecyl ester and is alcohol, heptacosane 1, 2, 3-triol (Banso, 2009). The seeds contain high percentage of phenolic constituents consisting of m-digallic acid (5), gallic acid (3), protocatechuic (1) and ellagic acids (2), leucocyanidin (4), oligomer 3,4,7- trihydroxyflavan 3,4-diol and 3,4,5,7- tetrahydroxy flavan-3-ol and epicatechol (6). The mature seed also contains crude protein, crude fibre, crude fat, carbohydrates, potassium, phosphorus, magnesium, iron and manganese occurred in high concentrations and it is richer source of cystine, methionine, threonine, lysine and tryptophan. The fruit contains mucilage and saponins (Pande, 1981; Siddhuraju*et al*., 1996). Pods containgallic acid and condensed tannins. Leavescontainapigenin, 6-8-bis-D-glucoside, rutin, 8% digestive protein (12.4% crudeprotein). Relative levels of tannin in different parts of plant is, deseeded pods (50%), pods (5.4%), leaves (7.6%), bark (13.5%) and twigs (15.8%). Bark contains tannin (12-20%), terpenoids, saponins, glycosides, Phlobetannin, gallic acid, protocatechuic acid pyrocatechol, catechin, epigallocatechin-5,7-digallate,andNilobate (9) (Chaubal, 2006). Root contains octaconsanol, betulin, ȕ-amyrin (7) DQG-s itosȕterol (8). Gum is composed of galactoaraban which gives on hydrolysis L-arabinose, D-galactose, L-rhamnose, D-glucuronic acid and 4- O-methyl- D-glucuronic acid.



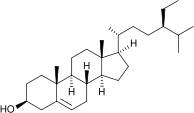
## 1 2

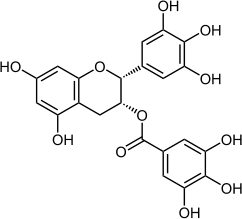
 **3 4**

5

**Fig 3(1-5):** Somephytochemical constituents*A. nilotica*(Evans, 2009).

## 6 7

**8**

 9

**Fig 4(7-9):**Somephytochemical constituents from *A. nilotica*(Evans, 2009).

* 1. **Some economic importance of *A. nilotica***

*Acacia nilotica*is a pioneer species*;* it isrelatively high in bioactive secondary compounds and is important for a variety of functions. It is economically used as a source of tannins, gums, timber, fuel and fodder (Gupta, 1970; Mahgoub, 1979). The pods (fruits) of *A. nilotica* provide a multitude of uses for the people including traditional medicinal application (Agunu*et al*., 2005). Prickly acacia plant is therapeutically used as anti-cancer, Anti tumours, Antiscorbutic, Astringent, Anti-oxidant, Natriuretic, Antispasmodial, Diuretic, Intestinal pains, Diarrhoea, Nerve stimulant, Cold, Congestion, Coughs, Dysentery, Fever, Hemorrhages, Leucorrhoea, Ophthalmia and Sclerosis (Saini, 2008). Seeds have Antimalarial, Antidiabetic, Antihypertensive and Antispasmodic activities. The leaves and pods are an excellent fodder with anti inflammatory properties, rich in protein. The pods have Molluscicidal and Algicidal properties. Bark is used in the treatment of Hemorrhages, cold, Diarrhoea, tuberculosis and leprosy. Root is used as an aphrodisiac and the flowers for treating syphilis lesions. Gum obtain from the tree is pharmaceutically used as suspending and emulsifying agent and in preparation of many formulations.



**PlateI:** *Acacia nilotica*tree in its Natural Habitat