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

BY

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ZARIA.

JANUARY, 2015.

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BY

YAHAYA IBRAHIM B.Sc ABU (2010)

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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

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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.

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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

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ABSTRACT

*Acacia niloticа*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.

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ACRONYMS AND ABREVIATIONS

Acronyms	Meanings
ATM	African Traditional Medicine
CFU	Colony formed per unit
^{13}CNMR	Carbon- 13 Nuclear Magnetic Resonance
CY2	Compound isolated
DMSO	Dimethylsulfoxide
Eg	Example
Etc	Et cetera
et al	And co-workers
EtOAc	Ethyl acetate
H_2SO_4	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
^{1}HNMR	Proton Nuclear Magnetic Resonance
TLC	Thin Layer Chromatography
TM	Traditional Medicine
WHO	World Health Organisation

Chapter one

1.0 Introduction

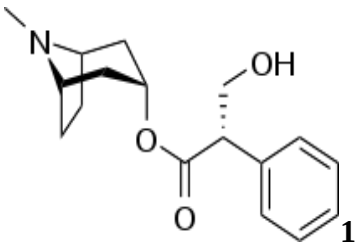
1.1 Plants and Medicine

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 an essential role in health care, with about 80% of the world's inhabitants relying mainly on 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).

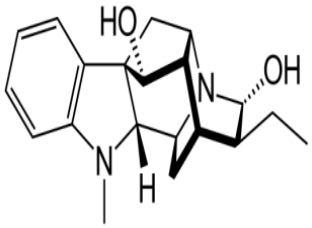
Whole plants or plant parts are used in traditional medicinal system where the synergistic effects of many constituents are evaluated on the whole human body inflicted with diseases (Rao, 2001). New drugs formulated on basis of organic chemistry and biochemistry-focuses

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).

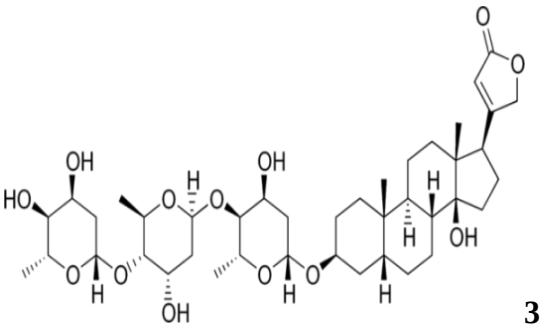
Many of the medicinal plants recorded inBurkill's (1966) dictionaries were reinvestigated recently using modern equipments and techniques to analyze their chemistry, to isolate new compounds and to formulate new drugs. An analysis of drugs approved by the Food and Drug Administration in the United States 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.



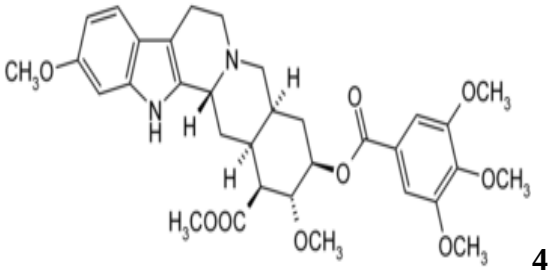
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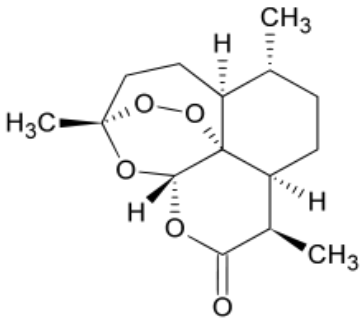
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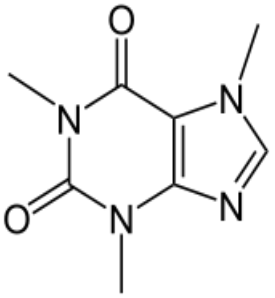


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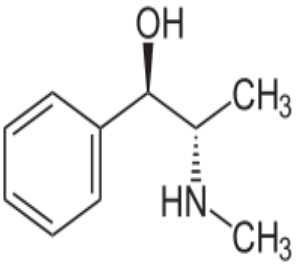


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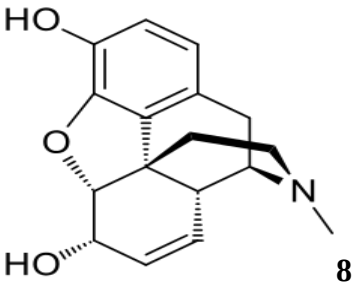
Fig 1(1-5): Some phytochemical constituents isolated from Plants (Evans, 2009).



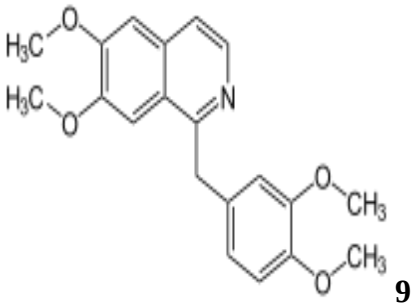
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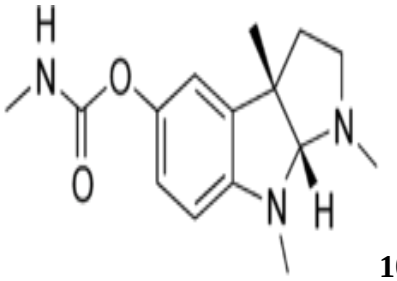
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Fig 2(6-10): Somephytochemical constituents isolated from Plants(Evans, 2009).

1.2Traditional 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.

1.3 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.

1.4Traditional 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).

1.5 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.

1.5.1 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).

1.5.2 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.

1.5.3 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 250’000- 500,000 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 phytomedicineundoubtlessly one of mankind's oldest professions (Mahesh and 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).

1.5.4 Statement of research problem

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

1.5.5 Justification

About 80% of the world's inhabitants are relying mainly on traditional medicines for their 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).

1.6.1 Hypothesis

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

1.6.2General 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.

1.6.3Objectives

- 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

2.0 Literature review

2.1 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).

2.2Economic 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).

2.3 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-dihydrobenzoic acids, 7,8.3',4'-tetrahydroxy-4-methoxyflavan-3-ol, 3,4-dihydroxybenzoic acids, 7,8,3'- trihydroxy-3,4'-dimethoxyflavone, and 7,3',4'-trihydroxyflavone have been isolated and characterised from *Acacia* species. Stigmasterol, β-sitosterol, β-sitosterol-3-O-β-D-glucoside, stigmasterol-3-O-β-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-7,8,3',4'-tetrahydroxydihydroflavonol, 3,3-hydroxyl-4-methoxybenzoic acid methyl ester, 3,7,8,3',4'-pentahydroxyflavone, 7,8,3',4'-tetrahydroxy-3-methoxyflavone, 3,4-dihydroxybenzoic acid ethyl ester, 3,4,2',3',4'-pentahydroxy-trans-chalcone, 3,7,8,3'-tetrahydroxy-4-methoxyflavone were isolated from *A.confusa*. Gallate acid methyl ester, gallic acid, naringenin, (+)-catechin, 2-O-β-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-3β-ol, methyl-2-hydroxy-4-hydroxy-3,6-dimethybenzoate, β-sitosterol, β-O-glucoside and linoleic acids were

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-gallate, β-catechin, 8-methoxyflavone-3,4-diones, 5-hydroxy-pipecolic acids, acacatechin, epicatechol, 7,8,4-trihydroxy-3-methoxyflavone, quercetagetin, quercetin, rutin, afzelichin, 4-methoxyglucoronic acids, β-sitosterol, D-galactose, erythrodiol, α-sitosterol-3-O-D-glucoside, sitosterol, β-amyrin, catechol, 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).

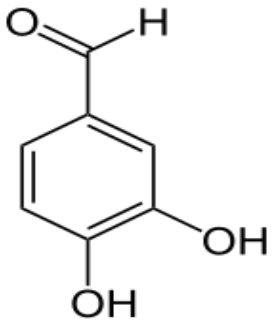
2.4Description of *A. nilotica*

*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

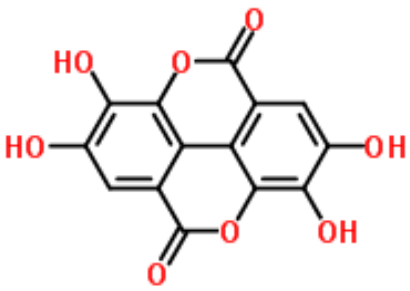
brown depending on the quantity of tannins in the sample. The lighter, more highly valued gums are soluble in water and very viscous; the tannins in the darker gum reduce the solubility. The gum has a moisture content of about 13% and is slightly dextrorotary (New, 1984).

2.5 Chemistry of *A. nilotica*

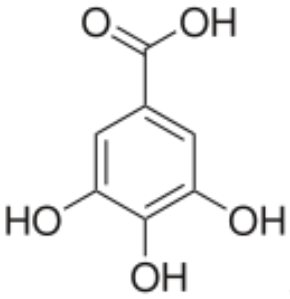
The plant contains chemical compounds like diester, pentacosanedioic acid dihexadecyl ester and is alcohol, heptacosane 1, 2, 3-triol (Banso, 2009). The seeds contain high percentage of phenolic constituents consisting of m-digallic acid (5), gallic acid (3), protocatechuic (1) and ellagic acids (2), 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, β-amyrin (7) and β-sitosterol (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.



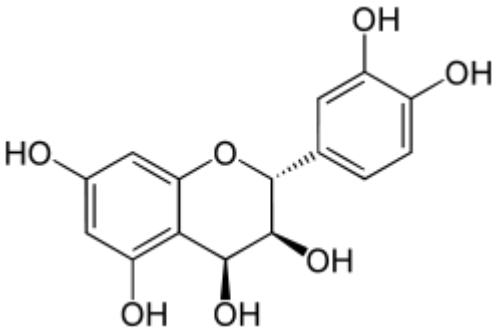
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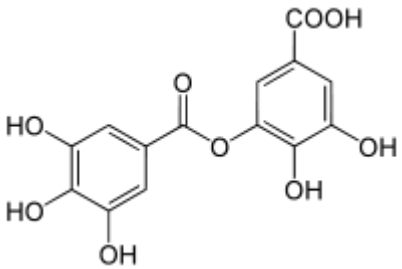
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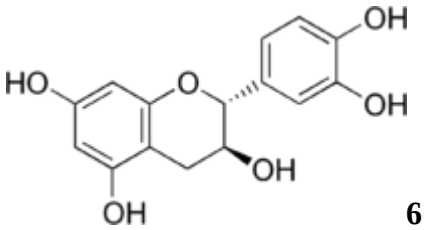


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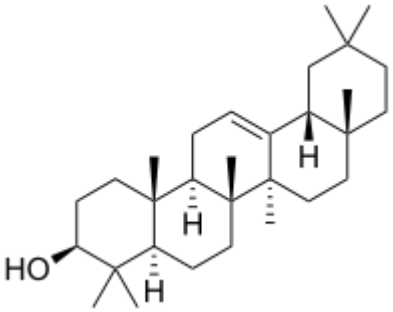


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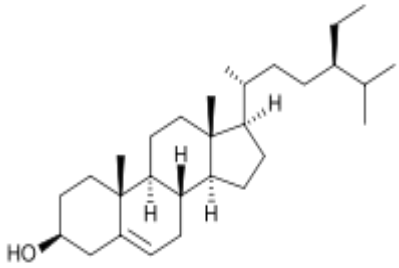
Fig 3(1-5): Somephytochemical constituents*A. nilotica*(Evans, 2009).



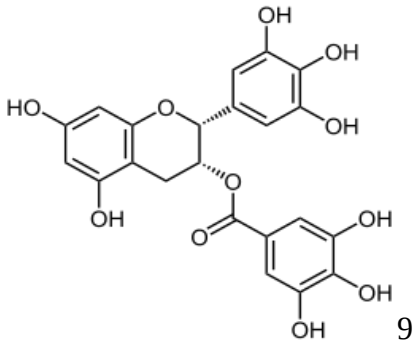
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9

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

2.6 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



Plate II. *Acacia nilotica* fruits showing the pods

CHAPTER THREE

3.0 Materials and methods

3.1 Equipments used

Oven

Mortar and pestle

Soxhlet apparatus

Water bath

Autoclave

Test tubes

Petri dishes

Sterile swab

Glass column

Iodine chamber

Dessicator

3.2Collection and identification of plant materials

The plant materials were collected from the staff quaters, Ahmadu Bello University Zaria main campus in the month of May 2013. The plant was identified and authenticated by the herbarium keeper, Mallam Musa at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria and was assigned a voucher number 901457.

3.3 Preparation of plant material

The podswere crushed and seeds were removed.The crushed pods were air dried for 3 days in the laboratory after which they were oven dried at 40-50^{0}C for 4hours so as to remove all moisture content,it was then ground into powder using pestle and mortar.

3.4 Extraction of plant materials

The powdered plant (500 g) was extracted on a Soxhletapparatus with hexane (2.5 L). The filtrate was concentrated to dryness on a water bath and kept safe in a dessicator.

3.5 Chromatographic studies.

TLC plates (Pre-coated silica on aluminium back), Merck (Germany).

Silica gel for column chromatography (60-120 mesh), Merck (Germany).

3.5.1 Solvents used

Hexane (Sigma Aldrich)

Ethyl acetate (Sigma Aldrich)

Dimethyl sulfoxide (DMSO)

Normal saline

3.5.2 TLC solvent systems

Hexane- Ethyl acetate (9:1), (8:2) and (7:3)

3.5.3 TLC spray reagents

Anisaldehyde spray:- Alcohol (90 ml) + Conc H_2SO_4 (5 ml) + Anisaldehyde (5ml)

3.5.4TLC profiling of crude extract

The hexane extract was spotted on three different TLC plates and developed using hexane: ethyl acetate (9:1), (8:2) and (7:3) respectively. The plates were sprayed with anisaldehyde/ sulphuric acid as detecting reagent followed by heating at 110^{o}C for 5min. Chromatograms were viewed immediately (Paschal *et al.*, 2002).

3.5.5Column Chromatography of hexane extract

This was aimed at isolating a pure compound present in hexane extract of *A.nilotica* pod. The eluting solvents used were 100% Hexane, 95:5 (Hexane: Ethyl acetate), 90:10 (Hexane: Ethyl acetate) and 80:20 (Hexane:Ethyl acetate).

The procedure was carried out following Brain and Turner (1975) as outlined below;

100 g of silica gel was mixed with twice its volume (n-hexane) in a beaker and stir with rod.

One quarter of the column was filled with the initial solvent system with the tap open, pouring the slurry gradually and continuously into the column using funnel and the tap closed when the solvent reached above the bed.

The column was allowed to stabilize so as to ensure the absence of air bubbles.

Hexane extract (2g) was preabsorbed in silica gel and loaded into the column.

Hexane (100 %) was used as the initial eluting solvent which was gradually increased in polarity by addition of ethyl acetate

Continuous elution was carried out and 54fractions were collected in beakers and monitored using TLC (Merck F_{254}) Hexane: Ethyl acetate (8:2) as the solvent system.

Further purification was carried out on a smaller column where fractions 20-23 with similar profile weighing 270mg were pooledtogether, coded (B) and chromatographed over 25g of silica gel. Hexane-ethyl acetate (9:1) was used as the eluent.

3.5.5.1Determination of Melting Point of Compound CY2

This was determined using the stuart melting point apparatus with model number 5315. This was carried out by collecting small quantity of the compound in a capillary tube and the capillary tube along with a thermometer was placed in the melting point apparatus. This was observed until the compound melted completely before reading from the thermometer was recorded.

3.5.5.2 Spectroscopic analysis

Nuclear Magnetic Resonance: Nuclear Magnetic Resonance (NMR) Spectroscopy conducted were 1D (^{1}H and ^{13}C). Spectra were obtained on ARK-400 MHz (Brucker/ TOPSPIN) at University of Pretoria South Africa.

3.5.5.3Antimicrobial studies

Test organisms

The antimicrobial activities of the hexane crude extract and the isolated compound (CY2) was determined using, eight microbes (*Staphylococcusaureus, Streptococcuspyrogenes,Corynebacteriumulcerans, Eschericiacoli, Shigeliadysenteriae, Salmonella typhi, Candida albicans*and *Candida krusei*)obtained during the month of July 2013 from Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital,Zaria.

The experiment was carried out using agar well diffusion method as described by (Azoro, 2002) in the Microbiology Unit of National Institute of Leather Science and Technology (NILEST) Zaria.

3.5.5.4Preparation of Stock Solution

The initial stock solution was prepared by weighing 0.2g of the crude extract and 0.4mg of the isolated compound (CY2), each dissolved in 10ml of DMSO to obtain a concentration of 20mg/ml of the crude and 40µg/ml for the isolated compound.

3.5.5Media for Microbial Growth

Mueller Hinton agar was used as the growth medium for the microbes. The agar (20g) was prepared by mixing in distilled water and sterilized in an autoclave for 15 minutes at 121^{o}C. The sterilized medium was then transferred into sterile Petri dishes and allowed to solidify. Diffusion method was used for screening the extract. The sterilized medium was seeded with 0.1ml of the standard inoculums of the test microbes and spread evenly over the surface of the media with a sterile swab. By the use of a standard cork borer, a well of 6mm in diameterwas cut at the centre of each inoculated medium.

3.6Zones of inhibition

0.1ml of solution of the extract from the concentration of 20mg/ml and40µg/ml was then introduced into each well on the medium. The inoculated medium was then incubated at 37^{0}C for 24hrs, after which the medium was observed for the zones of inhibition of growth. The zones of inhibition were measured with a transparent ruler and the result recorded in millimetre (Azoro, 2002).

3.6.1 Minimum Inhibitory Concentration (MIC)

The minimum inhibition concentration (MIC)was determined using broth dilution technique. Mueller Hinton broth preparation; 10ml was dispensed into test tubes and was sterilized at

121^0C for 15min. The broth was allowed to cool.Mc-farland's turbidity standard scale number 0.5 was prepared to give turbidity to the broth solution, according to the manufacturer's specification.

Normal saline (NS) was prepared and 10ml was dispensed into the sterile test tubes, the test microbes were inoculated into the test tubes and incubated at 37^0C for 6hrs. Dilution of the microbes was done continuously in the NS until the turbidity reached that of the Mc-farland standard by visual comparison. At this point the test microbes have a concentration of 1.5×10^8cfu/ml.

Two fold serial dilutions of the extract in the broth were made to obtain the concentrations of 40µg/ml, 20µg/ml, 10µg/ml, 5µg/ml, and 2.5µg/ml. The initial concentration was obtained by dissolving 0.4mg of the extract in 10mls of the sterile broth.

Having obtained the different concentration of the extract in the sterile broth, 0.1ml of the test microbes in the NS was inoculated into the different concentrations, incubation was made at 37^0Cfor 24hrs, after which each test tube was observed for turbidity (growth). The lowest concentration of the extract in the broth which shows no turbidity was recorded as the MIC (Azoro, 2002).

3.6.2 Minimum Bactericidal Concentration/Minimum Fungal Concentration (MBC/MFC)

The MBC/MFC was carried out to determine whether the test microbes were killed or if only growth was inhibited. Mueller Hinton agar was prepared, sterilized at 121^0C for 15min, poured into the Petri dishes and allowed to cool and solidify.

The content of the MIC in the serial dilution was sub-cultured onto the prepared medium and incubation was done at 37^0C for 24hrs, after which the plates of the medium were observed for colony growth. The plates with the lowest concentration of compound/extract without

colony growth indicate the MBC/MFC. Where MBC is for bacterial growth and MFC is for fungal growth (Azoro, 2002).

Chapter four

4.0 Results

4.1Extraction

The ground material (500 g) extracted with hexane using soxhlet apparatus and yielded 3 g of crude extract(0.6%w/w).

4.2TLC profile of the hexane extract

The TLC of *A.nilotica* pods hexane extract carried with hexane-ethyl acetate (9:1 and 8:2) showed separations shown in the chromatograms below.

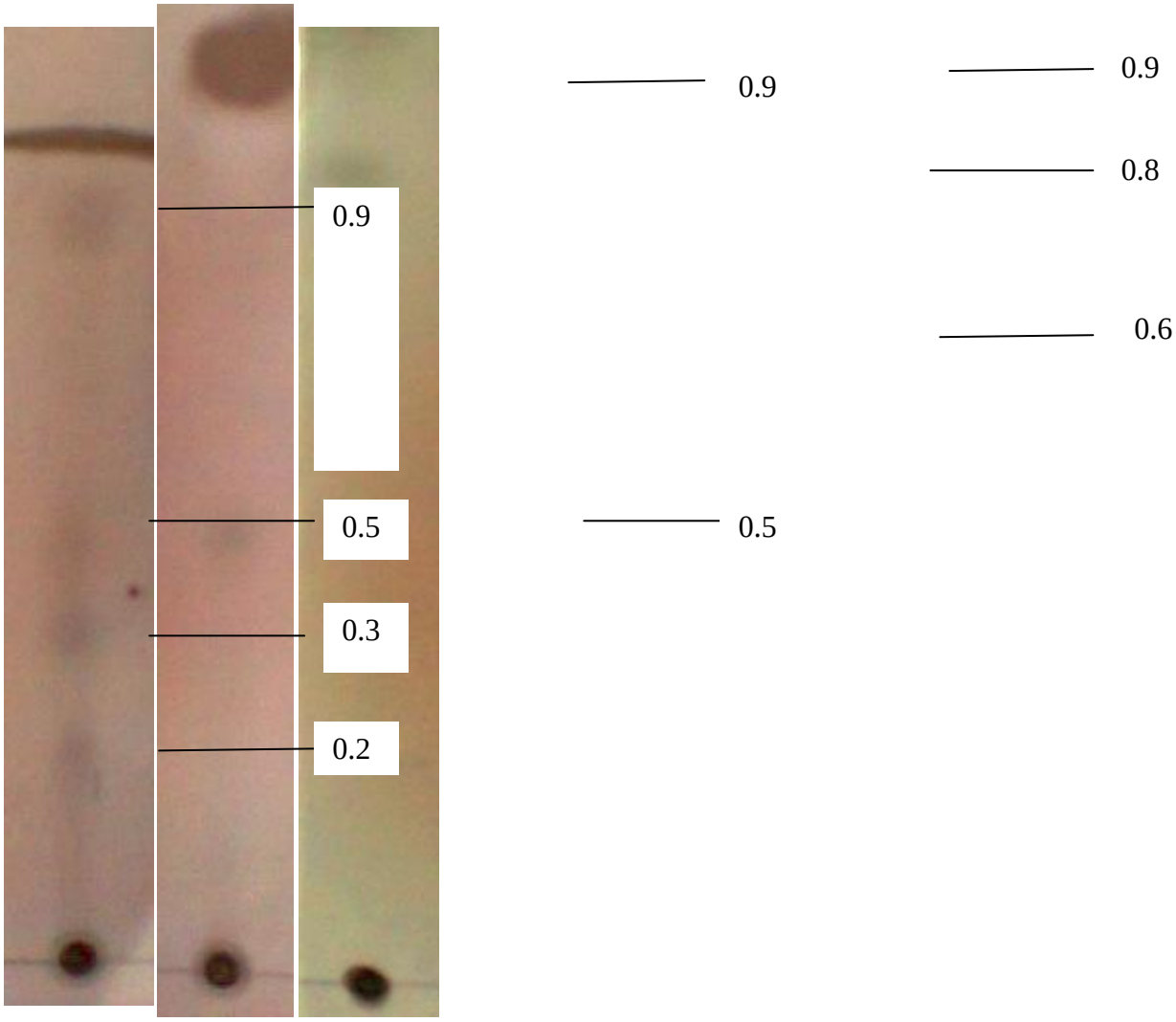


Plate III: TLC Chromatograms of hexane crude extract of *Acacia nilotica* pod using solvent system hexane: ethyl acetate (9:1), (8:2), and (7:3) sprayed with Anisaldehyde/ sulphuric acid showing the various spots.



Plate IV: Fractions 20-23 with similar profile pooled together and coded as B developed in Hex: EtOAc (8:2), viewed in an iodine chamber.



Plate V: Chromatogram of fractions 20-23 pooled together and redeveloped on a single plate with (Hexane-ethylacetate 9:1), sprayed with Anisaldehyde/ sulphuric acid coded as C



Plate VI: Chromatogram of C purified on a smaller column showing single spot on fractions 2-4, sprayed with anisaldehyde/ sulphuric acid coded as CY2

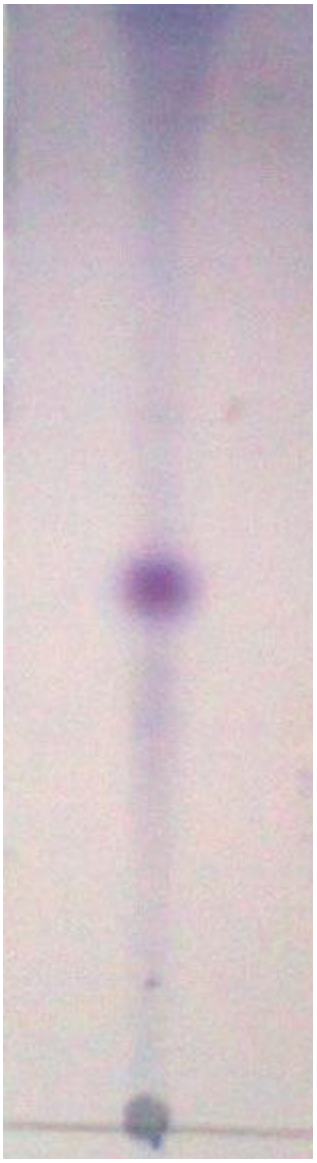


Plate VII: Fractions 2-4 of CY2 combined and developed in Hex: EtOAc (8:2), showing a single spot sprayed with anisaldehyde/H_2SO_4

4.4Melting point of isolated compound CY2

Compound CY2 existed as a white amorphous powder (160mg) which has a melting of63-64^{0}C.

4.5 Spectroscopic properties of Compound CY2

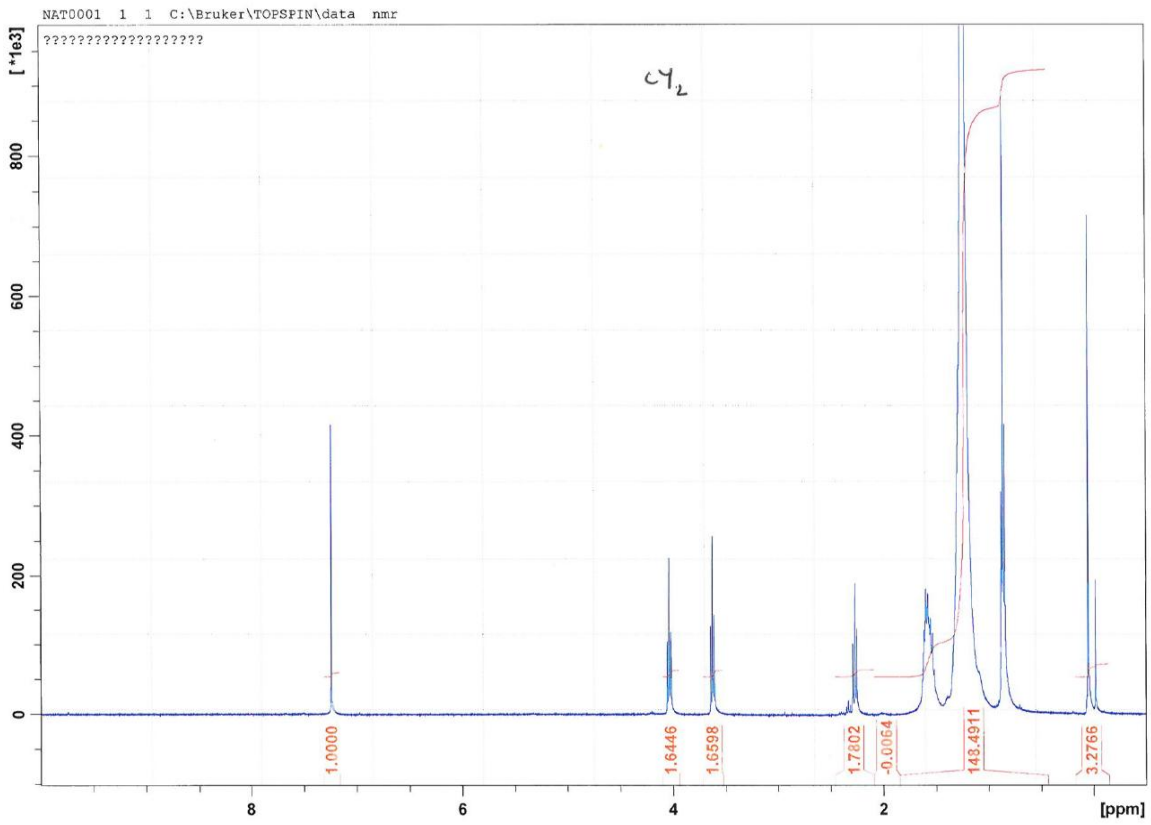


Fig. 5: ^{1}HNMR Spectrum of compound CY2 in $CDCl_3$ showing integration

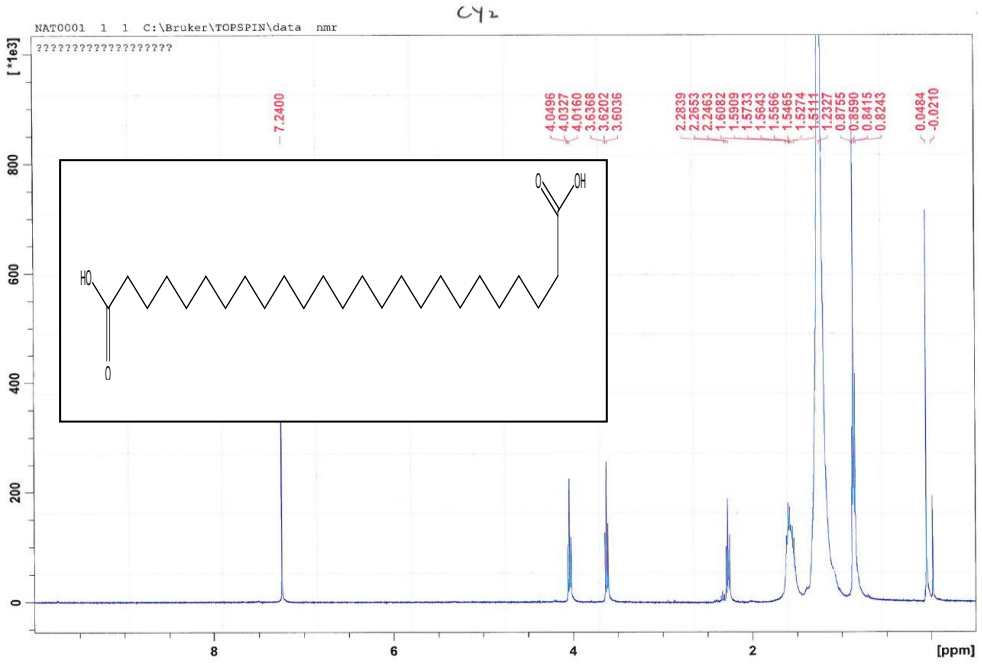


Fig 6: ^{1}HNMR spectrum of compound CY2 (EXPANDED) in $CDCl_3$

The spectrum showed multiplet at 4.04 for methylene protons attached to methoxy bearing protons, triplet at 2.28 for methylene protons attached to ester carbonyl, multiplet at 1.60 for methylene protons, a singlet at 1.23 for methylene proton and a triplet at 0.87 for two methyl groups.

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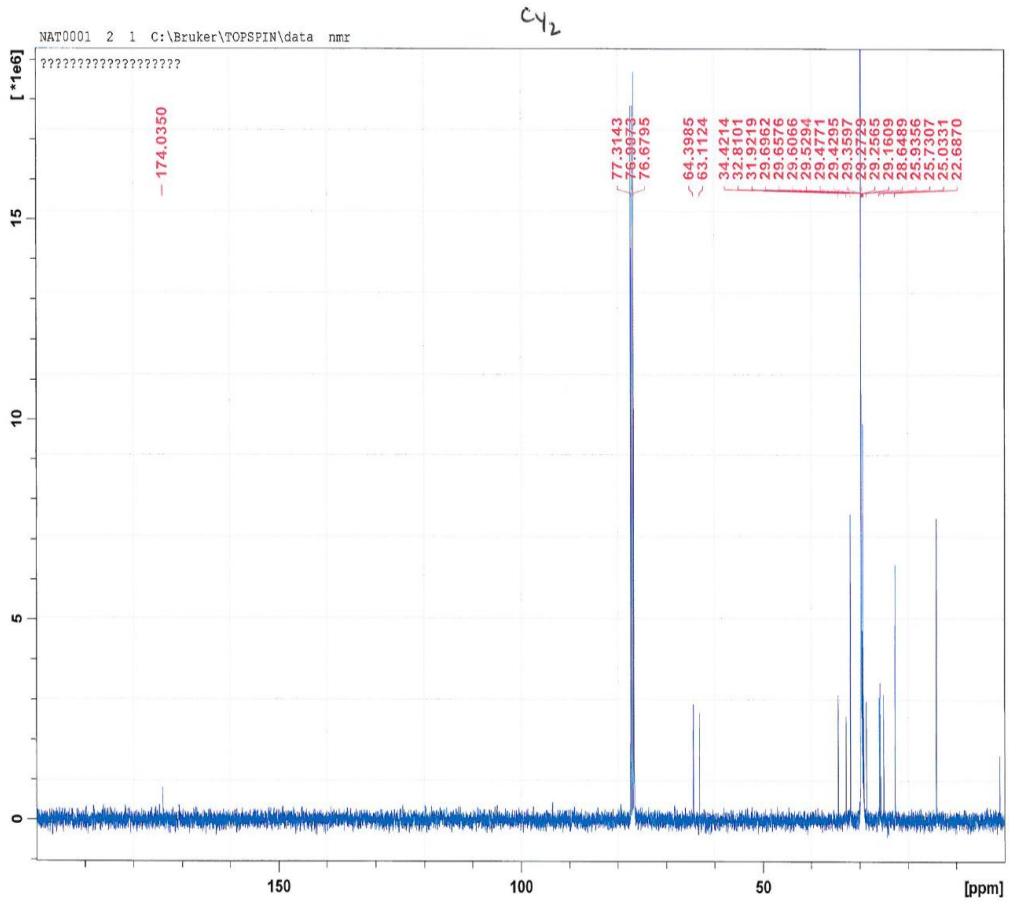


Fig 7: ^{13}CNMR spectrum of compound CY2 showing chemical shifts

The spectrum showed signals at δ 174.0 carbonyl carbon of the carboxylic acid group, it also showed signals at δ 64.39 for methylene carbon atom attached to carbonyl carbon atom, δ 31.92 and 22.68 for methylene carbon atoms.

Table 1: ^{1}HNMR spectral data of compound CY2 and literature reported in ppm (in $CDCl_3$)

H/C- Position	*Pentacosane-dioic acid	CY2
1	4.06	4.04
2	2.29	2.28
3	1.61	1.60
4	1.26	1.23
5	0.89	0.87

*Rohini*et al.*, (2006).

Table 2: ^{13}CNMR spectral data of compound CY2 and literature reported in ppm

(In $CDCl_3$)

H/C-Position	Pentacosane-dioic acid	CY2
1	173.50	174.00
2	64.39	64.39
3	31.93	31.92
4	29.69	29.69
5	28.71	28.64
6	25.94	25.93
7	22.71	22.68

*Rohini*et al.*, (2006).

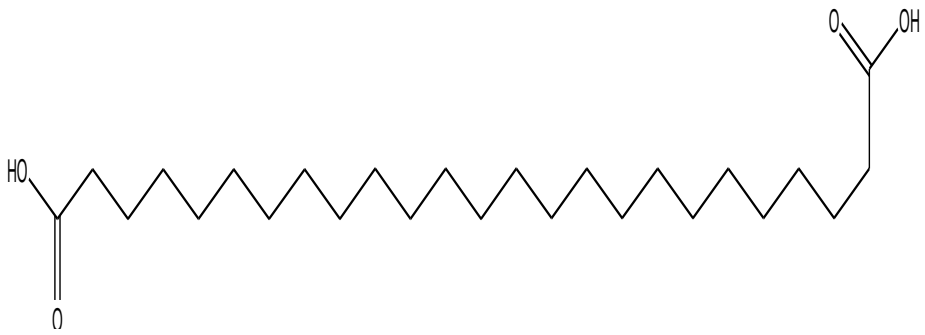


Fig. 8: Proposed structure of compound CY2

4.5 Results of the Antimicrobial studies

Table 3: Zones of inhibition of the extract and compound against the test microbes (mm^2)

The hexane extract and Compound CY2 from *A.niloticapod*were observed to show zones of inhibition between 20 mmand 31 mmfor all the bacteria strains except *C. ulceran*which showed resistance to both the crude extract and compound CY2 but was susceptible to Ciprofloxacin which is the standard antibacterial drug used. One of the fungi strain *C. albicans*was also susceptible to the crude extract and Compound CY2, showing zone of inhibition of 20 mmfor the crude and 25 mm for Compound CY2. The second fungi strain, *C. krusei* was resistant to both the crude extract and Compound CY2, compared to the standardFluconazole. It is observed that the zones of inhibition of the isolated compound are higher than that of the crude extract for all organisms that showed susceptibility (Table)

Test organisms	**C. extract**	**CY2**	**Ciprofloxacin**	**Fluconazole**
Staphylococcus aureus	24	28	36	0
Streptococcus pyrogenes	21	25	37	0
Corynebactoriumulceran	0	0	36	0
Escherichia coli	22	25	35	0
Shigelladysenteria	27	31	39	0
Salmonella typhi	20	23	42	0
Candida albicans	20	25	0	37
Candida krusei	0	0	0	34

Table 4: Minimum inhibitory concentration (MIC) of the compound and crude extract against the test microbes

The minimum inhibitory concentration of the crude extract was observed to be 5 mg/ml for *S. aureus, S. pyrogenes, S. typhi, E. coli* and 10 mg/ml for *S. dysenteriae,* while *C. ulceran* showed no activity. The MIC in compound CY2 was observed to be 10 µg/ml for *S. pyrogenes, S. typhi, E. coli and* 5µg/ml for *S. aureus, S. dysenteriae*respectively. Also the crude extract MIC for *C. albicans* was 5mg/ml while that of compound CY2 was observed to be 10 µg/ml, but there was no activity observed in *C. krusei*.

Test organisms	**Crude extract (mg/ml)**					**CY2 (µg/ml)**				
	20	**20**	**5**	**2.5**	**1.25**	**40**	**20**	**10**	**5**	**2.5**
S. aureus	-	-	¥	+	++	-	-	-	¥	+
S. pyrogenes	-	-	¥	+	++	-	-	¥	+	++
C. ulceran										
E. coli	-	-	¥	+	++	-	-	¥	+	++
S. dysenteriae	-	-	-	¥	+	-	-	-	¥	+
S. typhi	-	-	¥	+	++	-	-	¥	+	++
C. albicans	-	-	¥	+	++	-	-	¥	+	++
C. krusei										

Key: **No growth (-)**,**MIC (¥)**,**Light growth(+)**, **Moderate growth(**++**)**

Table 5: Minimum bactericidal/ fungal concentration of the crude extract and compound against the test organisms

The MBC of the crude extract for *S. pyrogenes*, *E. coli* and *S. typhi*was observed to be 20 mg/ml while that of *S. aureus* and *S. dysenteriae*was 10 mg/ml. The MBC of CY2 for *S. aureus* and *S. dysenteriae*was 10µg/ml, and that of *S. pyrogenes* and *E. coli* was 20 µg/ml. No activity was observed in *C. ulceran.* The MFC for *C. albicans* was observed to be 20 mg/ml and 20 µg/ml for the crude extract and CY2. There was no activity observed in *C. krusei*.

Test organisms	**Crude extract (mg/ml)**					**CY2 (µg/ml)**				
	20	10	5	2.5	1.25	40	20	10	5	2.5
S. aureus	-	¥	+	++	+++	-	-	¥	+	++
S. pyrogenes	¥	+	++	+++	++++	-	¥	+	+ +	+++
C. ulceran										
E. coli	¥	+	++	+++	++++	-	¥	+	++	+++
S. dysenteriae	-	¥	+	++	+++	-	-	¥	+	++
S. typhi	¥	+	++	+++	++++	¥	+	++	+++	++++
C. albicans	¥	+	++	+++	++++	-	¥	+	++	+++
C. krusei										

Keys: **no colony growth (-), MBC (¥), scanty colony growth (+), moderate colony growth (++), heavy colony growth (+++), very heavy colony growth (++++).**

Chapter five

5.0 Discussion

Plant-based traditional medicine system continues to play an essential role in health care system, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care. *A. niloticai*s a multipurpose plant belonging to the family Mimosaceae. It is commonly known as Prickly acacia in English and *Bagaruwa* in Hausa. The various parts of this plant have different uses in different localities. The pod is traditionally used in treating stomach pain, sore throat, foot and mouth disease, skin infection and dysentery in the northern part of Nigeria.

The TLC profile of the crude extract using hexane-ethyl acetate (9:1) as solvent system and anisaldehyde/ sulphuric acid as spray reagent showed spot which is purple in colour suggesting the presence of terpenpoid.Compound CY2 existed as a white amorphous powder (160mg) which melted between 63-64^{0}C.

The ^{1}HNMR spectrum of compound CY2 showed the presence of multiplet at 4.04 for methylene protons attached to methoxy bearing protons, triplet at 2.28 for methylene protons attached to carbonyl group, multiplet at 1.60 for methylene protons, singlet at 1.23 for methylene proton and a triplet at 0.87 for two methyl groups.

The ^{13}CNMR spectrum of compound CY2 shows signal at δ 174.0 for carbonyl carbon of the carboxylic acid group, it also showed signals at δ 64.39 for methylene carbon atom attached to carbonyl carbon atom, δ 31.92 and 22.68 for methylene carbon atoms.Thus, the structure of CY2 was assigned as the known compound **Pentacosane-dioic acid**. The spectral data are consistent to the reported literature values (Rohini*et al.*, 2006) which is a compound with 25 carbon atoms though 24 carbons reflected on the spectra. This might be as a result of degenerate i.e. two carbons atoms resonating in the same chemical shift or overlapping one another. The compound also has two carboxylic groups at the terminal ends; these two functional groups might be responsible for its antimicrobial activity.

The antimicrobial activity of the hexane extract as well as compoundCY2 from *A.nilotica*pod are shown in table 3 and 4. The plant extract exhibited activity against most of the test microorganisms but the isolated compound CY2 showed a higher activity. This might be attributed to the fact that other compounds present in the crude extract were hindering or interfering with the activity of the pure compound.The wider zones exhibited by the control drug (Ciprofloxacin) indicated that it has a higher antibacterial activity as compared to the plant extracts and compound CY2. The results from the MIC and MBC also revealed that compound CY2 had double the strength of the hexane extracts. Although no activity has so far been reported on this compound, it was observed that the compound had a better antimicrobial activity as compared to the crude extract. The compound showed higher antibacterial activity against *S.dysenteriae*and *S. aureus*with inhibition zone of 31mm and 28mm, compared to the crude which has inhibitory zone of 27mm and 24mm.This result is in line with the findings of (Banso, 2009). *S. pyrogenes* and *E. coli* both showed inhibition at 25mm for compound CY2 but exhibited a lower activity of 21mmand 22mm for the crude extract. *S. typhi* showed the least zone of inhibition with inhibitory zone of 23mm for the isolated compound and 20mm for the crude extract.*C. ulceran*showed no activity in both crude extract and compound CY2.Also the isolated compound as well as the crude extract was tested on two fungi strains, *C. albican*was susceptible to the isolated compound with zone of inhibition of 25mmand 20mmfor the crude extract.*C. kiruse*ishowed no activity against the extract and compound thereby showing no zone of inhibition. Fluconazole which is the control drug used for the antifungal activity showed a higher zone of inhibition of 37mm.It is quite obvious that from literature, most antimicrobial studies carried out are on high polar solvents which include methanol, ethanol and ethyl acetate. Much has not been done on the less polar solvent e.g hexane.

The antifungal and antibacterial studies of *A. nilotica* and *A. catachu* stem bark extracts also reported that methanolic extracts were more active against the microbes than the hexane extracts (Saini*et al*., 2008). This can be supported by the claims that methanol is an ideal solvent for the extraction and isolation of phytochemicals with antimicrobial activities (Bhawna and Bharti, 2010; Poovendron*et al*., 2011). Therefore, the efficacy of the methanol extracts over the hexane extracts can be attributed to the presence of phenolic compounds. Phenolic compounds that were observed present in both extracts may also be responsible for their antimicrobial activities as previous reports shows that phenolic compounds exhibit antimicrobial activities against disease causing microorganisms (Ismail and Abdulsamad, 2010; Nyananyo and Akada, 2011; Hussein,2012). Plants containing tannins have also been reported to utilise traditionally in the treatment of diarrhoea and dysentery while saponins were also reported to have the natural tendency to ward off microbes (Adaramola*et al*., 2012).

Furthermore, the MIC of *S. aureus*and *S. dysenteriae*for compound CY2 was exhibited at 5µg/ml.*S. pyrogenes, E. coli, S. typhi and C. albicans*showed the MIC/MFC at 5µg/ml for compound CY2 and 10mg/ml for the crude extract.While all the test microbes showed a 5mg/ml MIC against the crude extract except *S. dysenteriae*which had a MIC of 2.5mg/ml. It was also confirmed that *A. nilotica*demonstrates highest activity against three bacterial (*E. coli, S. aureus*and *S.typhi*) and two fungal strain (*C.albicans*and *A.niger*) (Kalaivani*et al*., 2010b).

The MBC/MFC of *S. aureus and S.dysenteriae* for both crude extract and compound CY2 was observed at 10mg/ml and 10µg/ml respectively. 40µg/ml and 20mg/ml was observed in the case of *S. typhi* while other organisms exhibited MBC/MFC at 20µg/ml and 20mg/ml for both isolated compound and crude extract accordingly. From the result above, it is observed that Pentacosane -dioc acid exhibited MIC at 5µg/ml against *S. aureus*and *S. dysenteriae*. It

also exhibited MBC at 10µg/ml against *S. aureus*and *S. dysenteriae* compared to other organisms. It can be concluded that compound CY2 had a better antibacterial and fungal activity compared to the hexane crude extract.*C. ulceran* and *C. krusei* were resistant to the extract and isolated compound, this might be as a result of one of the mechanisms of drug resistance which include; Production or possession of plasma which membrane translocate efflux which pump which pumps out drug from the system of the microbe, the microbes inactivate drugs by modification of its chemical structure, they can modify the target cellular structure of the drug in there system. E.g., Hydrolysis of β- lactone ring of penicillin enzymepenicillinan, they can modify their membrane to become impermeable to the drug, they can also by-pass the pathway that is been inhibited by the drug i.e they can use an alternative pathway (Willey *et al.,* 2008).

Chapter six

6.0 SUMMARY CONCLUSION AND RECOMMENDATION

6.1 Summary

The growth and well-being of plants is the only basis for an earthly paradise to man. For thousands of years, plants have been exploited by man for reasons which include shelter, food, clothing and most importantly medicine.Traditionally, plants have been used in treating different kinds of ailments of which different constituents from these plants are responsible for its pharmacological activity. The overall aim of this work is to provide the scientific basis for the use of *A. nilotica* pod in the treatment of some bacteria and fungi diseases. This work was focused on:

Isolation of a compound from*A.nilotica*pod by chromatographic means.

Establishing the antimicrobial property of the crude extract as well as isolated compound from *A. nilotica* pod.

To achieve these aims, the pods of *A. nilotica*was collected and investigated in the laboratory. The n-hexane extract was investigated chromatographically and a compound isolated, structure elucidation of the compound was done. The result of the spectroscopic analysis (^{1}HNMR and ^{13}CNMR) revealed that the compound isolated is a Pentacosane-dioic acid. The chemical shift of this compound is in close agreement with a reported literature by (Rohini*et al.,* 2006). The n-hexane extract as well as the isolated compound was also subjected to antimicrobial study and it was observed that the isolated compound had a higher activity, with the MIC at 5µg/ml and the MBC/MFC at 10µg/ml.

6.2 Conclusion

*A. nilotica*is an imperative multipurpose plant that has been used broadly for the treatment of various diseases (Singh *et al*., 2009b). Popularly known as *Bagaruwa* by the Hausa's in northern part of Nigeria, it has been used for the treatment of foot and mouth disease, dysentery, stomach pain, sore throat among others. This research has proved that n-hexane extract also contains phytochemicals that are good sources of antimicrobial agents of which Pentacosane-dioic acid is a potential candidate as compared to previous works that are focused on the polar extracts. It is also important to note that this is the first time the activity is being reported for this compound.

6.3 Recommendation

*A. nilotica*has proved to be a useful plant and has been used traditionally for long. Most of the scientific research on this plant has been done using polar solvents and on the roots, stem bark and leaves of the plant. So far, just one compound has been reported to have been isolated from the pod of this plant using n-hexane. There is a need to investigate the potentials of the pods of this plant and efforts should also be made towards isolating more compounds which might be a lead to discovering a new drug. More biological activities can also be conducted or established on this compound so as to exploit its full potentials.

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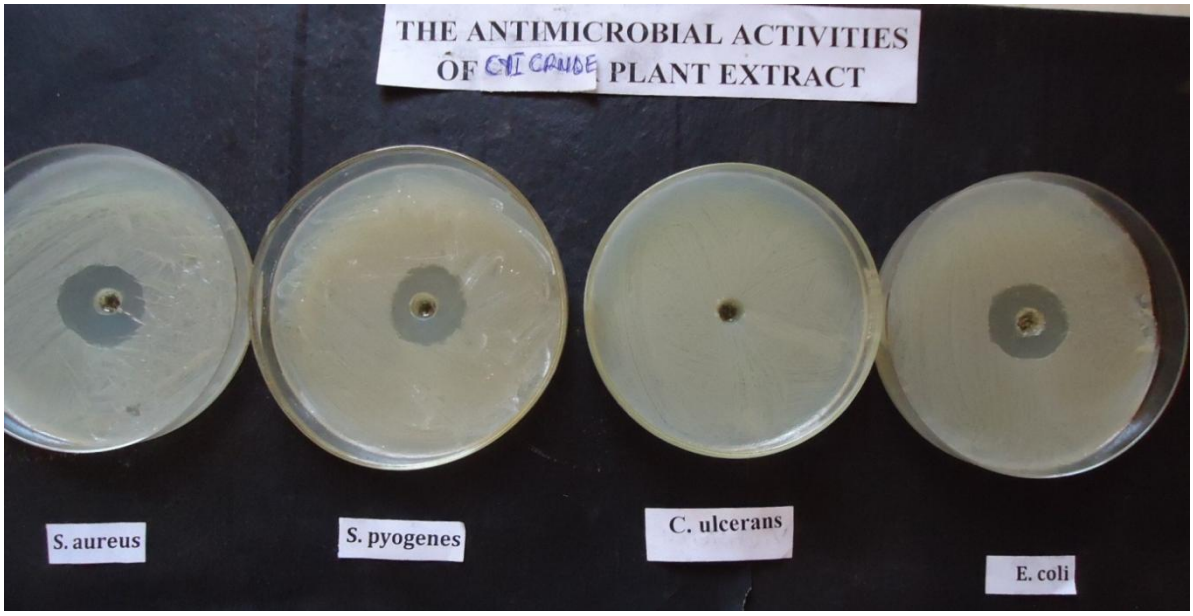
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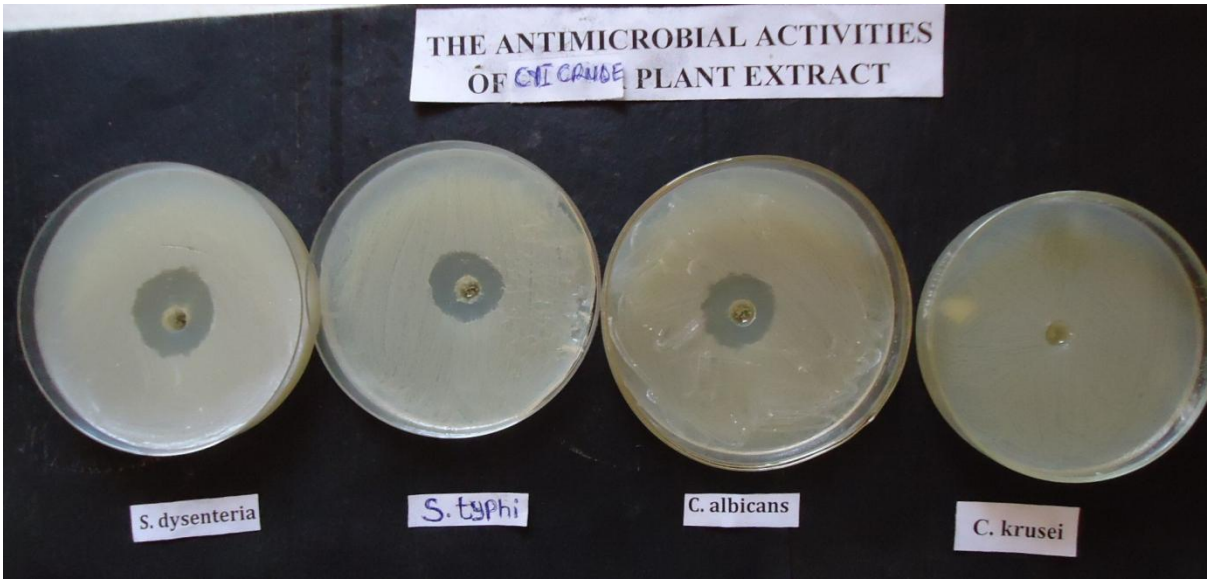
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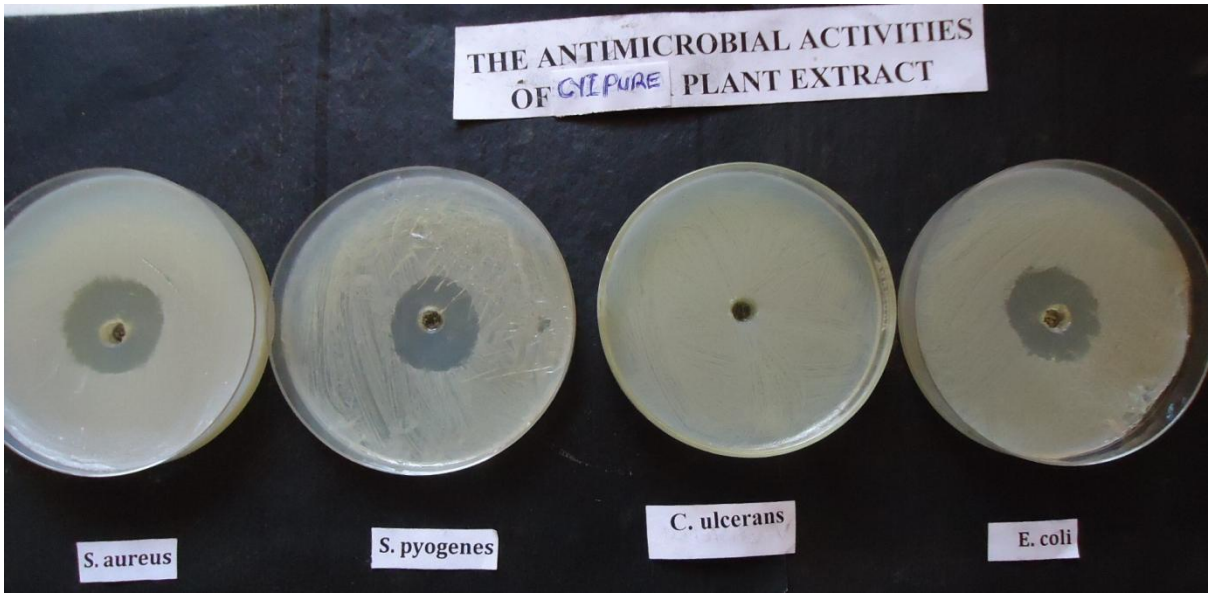
Appendix



Appendix I: Petri dishes showing zones of inhibition of the extract against the test microbes



Appendix II: Petri dishes showing zones of inhibition of the extract against the test microbes



Appendix III: Petri dishes showing zones of inhibition of CY2 against the test microbes



Appendix IV: Petri dishes showing zones of inhibition of CY2 against the test microbes