

**PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF
THE ROOT PART OF PACHYSTELABREVIPES (BAKER)
BAILL ENGL(SAPOTACEAE)**

BY

EZURUIKE IKEAGWUGHICHI TIMOTHY

**DEPARTMENT OF CHEMISTRY
AHMADU BELLO UNIVERSITY, ZARIA.
NIGERIA**

MAY, 2015

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(SAPOTACEAE)**

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**A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE
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**DEPARTMENT OF CHEMISTRY,
FACULTY OF SCIENCE,
AHMADU BELLO UNIVERSITY, ZARIA.
NIGERIA**

MAY, 2015

DECLARATION

I hereby declare that this thesis titled “**Phytochemical and Antimicrobial Screening of the Root Part of *Pachystela brevipes***”(Baker) Baill Engl (*Sapotaceae*) was written by me and that it is a record of my own research work under the guidance of **Prof. G. I. Ndukwe and Dr. J. D. Habila** (my supervisors) and to the best of my knowledge, it has not been presented in any previous application for a higher degree. All quotations are indicated and sources of information are adequately acknowledged by means of references.

.....

Ezuruike Ikeagwughichi Timothy

.....

Date

CERTIFICATION

This thesis entitled “Phytochemical and Antimicrobial Screening of the Root Part of *Pachystela brevipes* (Baker) Baill Engl (Sapotaceae) by Ezuruike Ikeagwughichi Timothy meets the regulations governing the award of the degree of Master of Science in Chemistry of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

Prof. G. I. Ndukwe

Chairman, Supervisory Committee

Date

Dr. J. D. Habila

Member, Supervisory Committee

Date

Prof. V. O. Ajibola

Head of Department

Date

Dean, Postgraduate School

Date

DEDICATION

To Almighty God the giver and taker of life and who alone can Chart the course of a man in life.

ACKNOWLEDGEMENT

I want to appreciate God for watching over me and enabling me to go through this long journey beside all the life challenges I came across in the course of this work. I express my heartfelt thanks to my Supervisors Professor G. I. Ndukwe and Dr. J. D. Habila for their meticulous supervision. Sirs, your professional guidance, encouragements and stimulations were more than helpful. The human relationship established and constructive advices given are appreciated. My profound gratitude to all lecturers of this great department, may God bless you all. Very special thanks to Mr and Mrs Elijah and Rebecca Joseph for all their supports since I came to Kaduna. They gave me strength and motivation during the dark days when I was not able to see the light at the end of the tunnel. The effort you made to help me in life when it was necessary will never be forgotten. I am grateful to my family, Revd. T. U. Ezuruike, my father, Mrs Joanna Ezuruike, my mother and my siblings, Chimere, Nneoma, Chinwendu, Eberechukwu Ozioma and Chinaemenma for their prayers and encouragements all through these years, you guys are wonderful. I wish to express my deep gratitude to my colleagues in Chemistry Department, especially Ophelia A., Yerima E., Dauda Y., George A., and Mubarak D., who helped me in the lab to see that this work was a success, God will reward you people. Special thanks go to the management and students of The Incubators Secondary Academy, for being patient and supportive of me during my academic career. A special word of thanks must go to my friends Okoye E. C. Dauda Y. and Taiwo D. for standing in for me when am not around. I also gratefully acknowledge the financial support from brothers and sisters, friends and well-wishers too numerous to mention who in one way or the other rendered help to see that this work was a success, God will do so for all of you.

ABSTRACT

The root of *Pachystela brevipes*, which has some traditional medicinal applications was extracted and investigated using Petroleum ether, Chloroform, Ethyl acetate and Methanol. The results of the phytochemical analysis showed the presence of carbohydrates, cardiac glycosides, saponins, steroids/ triterpenes, flavonoids, tannins and alkaloids. The crude extracts showed zones of inhibition in the range, 16-19 mm for Petroleum ether fraction, 20-24 mm for Chloroform, 20-27 mm for Ethylacetate and 20-22 mm for Methanol, against thirteen test organisms; *Methicillin Resistant Staphylococcus aureus*, *Staphylococcus aureus*, *Escherichia coli*; *Salmonella typhi*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Candida stellatoidea*, *Candida tropicalis*, *Candida krusei*, *Proteus mirabilis*, *Proteus vulgaris* and *Streptococcus feacalis*. The minimum inhibitory concentration (MIC) of 2.5 mg/mL was recorded for the chloroform, ethylacetate and methanol fraction against the entire test organism except four; *S. pyogenes*, *C. krusei*, *P. mirabilis* and *P. vulgaris*. The Petroleum ether fraction had Minimum Inhibitory Concentration (MIC) at a concentration of 5.0 mg/mL on eleven test organisms except *S. pyogenes*, *C. krusei*, *P. mirabilis* and *P. vulgaris*. The minimum bactericidal/fungicidal concentration (MBC/MFC) determination showed that concentrations of 5-10 mg/mL of the petroleum ether, chloroform, ethylacetate and methanol fractions could completely kill the test organisms used in this work except *S. pyogenes*, *C. krusei*, *P. mirabilis* and *P. vulgaris*. Zymosterol (3 β -hydroxy-4 β -methyl-5 α -cholesta-8, 24-diene-4 α -carboxylic acid) was isolated and purified from the ethyl acetate fraction (EAF) and confirmed purely by spectral techniques (¹H NMR, ¹³C NMR and 2D NMR) as well as by comparison with the reported authentic data. This is the first time of isolating Zymosterol from the Sapotaceae family.

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ABBREVIATIONS

1D	One dimensional
2D	Two dimensional
TLC	Thin Layer Chromatography
ZI	Zone of Inhibition
MIC	Minimum Inhibition Concentration
MBC	Minimum Bactericidal Concentration
¹³ CNMR	Carbon-13 Nuclear Magnetic Resonance
¹ HNMR	Proton Nuclear Magnetic Resonance
DEPT	Distortionless Enhancement Polarization Transfer
COSY	Correlation Spectroscopy
HSQC	Heteronuclear Single Quantum Coherence
HMBC	Heteronuclear Multiple Bond Correlation
NOESY	Nuclear Overhauser Effect Spectroscopy

CHAPTER ONE

1.0 INTRODUCTION

1.1 Medicinal Plants

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food plants. They are also sometimes added to food meant for pregnant women and nursing mothers for medicinal purposes (Okwu, 1999; Okwu, 2001a). Although plants are constantly being investigated for bioactive principles, the potential of plants as sources for new drugs is still largely unexplored. Only a small percentage has been investigated phytochemically and fraction submitted to biological and/or pharmacological screening is even smaller (Hostettmann *et al.*, 1995).

In addition, the use of herbal medicine for the treatment of diseases and infections is as old as mankind. The World Health Organization (WHO) supports the use of traditional medicine provided they are proven to be efficacious and safe (WHO, 2002). In developing countries, a huge number of people lives in extreme poverty and some are suffering and dying for want of safe water and medicine and they have no alternative for primary health care. Therefore, the need to use medicinal plants as alternatives to orthodox medicines in the provision of primary health care cannot be over-emphasized. More so, herbal medicines have received much attention as sources of lead compounds since they are considered as time tested and relatively safe for both human use and environmental friendly (Fazly-Bazzaz *et al.*, 2005). They are also cheap, easily available and affordable. Many medicinal plants are claimed to be useful for wound healing in the traditional system of medicine. These plant remedies (both single plant and multi-herbal preparations) are used since ancient times even if the mechanisms of

action, toxicity and efficacy of very few of them have been evaluated. Wound healing is the process of repair that follows injury to the skin and other tissues. Following injury, an inflammatory response occurs and the cells below the dermis begin to increase protein (collagen) production.

Herbal medicine plays a very important role in the healthcare system of the developing world especially those who do not have access to orthodox medicines, an inclination supported by the World Health Organisation (WHO, 2002). Although about 40% of modern pharmaceuticals are derived from plants, none is used against viruses. In contrast, traditional healers have long used phytomedicines to prevent or cure infectious conditions. Scientists are interested in antimicrobial plant extracts because (i) all antibiotics have shelf-life; (ii) of dissatisfaction with antibiotics; (iii) of public inclination to take herbal medication; and (iv) viral diseases have no viable cure yet. The earth's resources are dwindling (Lewis, 1995) leading to critical losses of structurally diverse and potentially useful phytochemicals (Borris, 1996).

There is therefore the need to look inward within our biodiversity to search for herbal medicinal plants with the aim of validating the ethno-medicinal use and subsequently the isolation and characterization of compounds which will be added to the potential list of drugs. *Pachystela brevipes* belongs to the family *Sapotaceae* and can be widely found around the World. These plants have been used as building material, as food, because the eatable fruits, as well as remedies in folk medicine. Some biological activities have been reported to species of this genus such as antioxidant, anti-inflammatory, antibacterial and antifungal. However, the real potential of this genus as source of new drugs or phytomedicines remains unknown.

1.2 HERBAL MEDICINE/HERBALISM

Medical herbalism, or simply, herbalism or herbology, is “the study of herbs and their medicinal uses”. This definition can be extended to include the cultivation, collection, or dispensing of aromatic plants, especially those considered to have medicinal properties. Other terms substituted for medical herbalism, include: herbal or botanical medicine, or phytotherapy, previously defined as “the use of plant materials to prevent and treat ill health or promote wellness” (Ameh *et al.*, 2010). It had been noted that the practice dates to antiquity, and that the primacy of herbalism in medicine is evident from the large number of modern drugs that owe their origin to ethnobotanical remedies. It was further noted that while plants synthesize a large variety of secondary metabolites in response to various ecophysiological stimuli, most of such metabolites originate from a relatively few biosynthetic pathways that include the pathways for alkaloids; terpenes/ terpenoids/ steroids; shikimic acid/ aromatics; and polyketides. These secondary metabolites, better called phytochemicals, affect humans in ways (useful and hurtful) that require that their production, quality, distribution and use be regulated. It is, indeed, the paradoxical nature of phytochemicals that has informed the intervention of the World Health Organisation in their regulations(WHO, 1998).

The World Health Organisation (WHO) 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. Traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous system of medicine. Traditional preparations comprise medicinal plants, minerals and 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, Atharvaveda, Charak Samhita and Sushruta

Samhita. The herbal medicines / traditional medicaments have therefore been derived from rich traditions of ancient civilizations and scientific heritage (Kamboj, 2000).

Herbal medicine is still the mainstay of about 75 – 80% of the world population, mainly in the developing countries, for primary health care (Kamboj, 2000). This is primarily because of the general belief that herbal drugs are without any side effects besides being cheap and locally available (Gupta and Raina, 1998). According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times (Evans, 1994). The use of plants for healing purposes predates human history and forms the origin of much modern medicine. Many conventional drugs originated from plant sources: a century ago, most of the few effective drugs were plant based. Examples include aspirin (willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine (from the opium poppy) (Vickers and Zollman, 1999). Medical history from the beginning of time is filled with descriptions of persons who used herbs to heal the sick of the society. However, parallel to the onset of the industrial revolution we witnessed the rise of allopathic medicine. Herbal medicine was also an effective healing method, but was viewed less enthusiastically (Tirtha, 1998). Herbal products were discarded from conventional medical use in the mid-20th century, not necessarily because they were ineffective but because they were not as economically profitable as the newer synthetic drugs (Tyler, 1999). In the early 19th century, scientific methods became more advanced and preferred, and the practice of botanical healing was dismissed as quackery. In the 1960s, with concerns over the iatrogenic effects of conventional medicine and desire for more self-reliance, interest in “natural health” and the use of herbal products increased. Recognition of the rising use of herbal medicines and other non-traditional remedies led to the establishment of the office of Alternative Medicine by the National Institute of Health USA, in 1992. Worldwide, herbal medicine received a boost when the WHO encouraged developing

countries to use traditional plant medicine to fulfil needs unmet by modern systems (Winslow and Kroll, 1998).

Modern medicine has largely confined itself to the isolation or synthesis of single active ingredient for the treatment of specific disease (Bland, 1983). For a long time, plants have been the almost exclusive therapy available to humans, with the development of medicinal chemistry in the early 19th century; plants were also found to be a source of substance to be developed as drugs. Nowadays, inspite of the tremendous development of synthetic pharmaceutical chemistry and microbial fermentation, 25 % of the prescribed medicines in industrialized countries are of plant origin and some one hundred and twenty (120) plants derived compounds are used in modern therapy. In spite of these rapid developments in scientific technology, and the better understanding of the chemistry of natural products, it is fair to say that of the six thousand or so plants used in different traditional systems of medicine, only a few hundred have so far been examined in depth for their chemical constituents and physiological activity. The plant kingdom thus represents an enormous reservoir of pharmacologically valuable molecules to be discovered. The evaluation of crude drug, which eventually go into the commercial market, is of considerable importance. Physical, sensory and biomedical characteristics of drugs formed the main features of the study in earlier age than since the latter half of the 19th century; the emphasis was laid on the crude drugs, their substitutes, fluorescence analysis, preliminary phytochemical tests and adulterants, for the reason that of the commercial practices, such compiled descriptions are given in the British Pharmacopoeia, British Pharmacopoeia Commission and other Pharmacopoeias. (Sharma *et al.*, 2009).

1.3 Bioactive Natural Products from Plants

The isolation of bioactive pure compounds from medicinal plants began in the 19th century (WHO,2002). Before that time, medicine was based on herbs and potions (Patrick, 2005). The

isolation of natural bioactive compounds was the first step towards the birth of the pharmaceutical industry. Natural products isolated in the early 19th century include cocaine, (1.1) from leaves of *Erythroxylum coca* Lam. (Erythroxylaceae), morphine, (1.2) from *Papaver somniferum* Linnaeus (Papaveraceae), called the opium poppy, and quinine, (1.3) from the bark of *Cinchona pubescens* Vahl (Rubiaceae).

Since then, plants have become a biofactory of different natural substances used for different purposes, especially in natural medicine. Chinese people are among the pioneers who explored the flora for medicinal purposes. Many other societies in the world also valued plant natural products for their life-giving properties. A survey conducted by Lev and Amar showed that 85% of traditional drugs sold in Israel are derived from plants (Lev and Amar, 2000). In their article, Scott et al. mentioned that 70% of all South Africans use plant-derived traditional medicine (Scott et al., 2004). The World Health Organization estimated that approximately 80% of Africa's inhabitants use traditional medicine for primary health care (WHO, 2008). In 1999, Grabley and Thiericke reported that about 30% of drug sales in the world were based on natural products (Grabley and Thiericke, 1999). Nowadays, pharmaceutical organisations are able to produce plant-based proteins that play an important role in human body metabolism.

Many drugs available today were discovered through the isolation and analysis of the active principles from natural sources. For example, reserpine, (1.4) used in the control of hypertension, was isolated from the roots of a shrub called *Rauvolfia littoralis* ex. Pitard (Nguyen, 1990), and *Rauvolfia serpentina* (L.) Benth. ex. Kurz (Apocynaceae); (Sehrawat et al., 2002; Nguyen and Nikolaeva, 1991) salicin, (1.5) a suitable agent for fever control, was isolated from the bark of *Salix alba* Linnaeus (Salicaceae); codeine, (1.6) an important analgesic and cough suppressant, was obtained from *P. Somniferum* (Costin et al., 2007; Schulz et al., 2004) digitoxin, (1.7) used to regulate cardiac function, was isolated from

Digitalis purpurea Linnaeus (Scrophulariaceae), commonly called foxglove; taxol, (1.8) an anticancer agent, was extracted from the bark of *Taxus brevifolia* Nutt. (Taxaceae), known as the pacific yew tree (Bruice, 2004). Emetine, (1.9) which induces apoptosis in human tumour cell lines, was obtained from *Psychotria ipecacuanha* (Brot.) Standl (Rubiaceae). (Maren, et al., 2004; 2007).

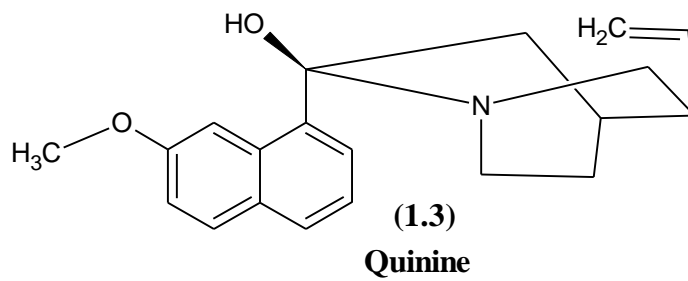
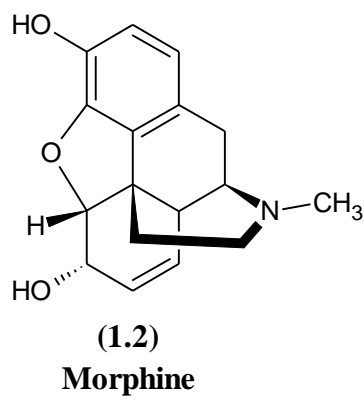
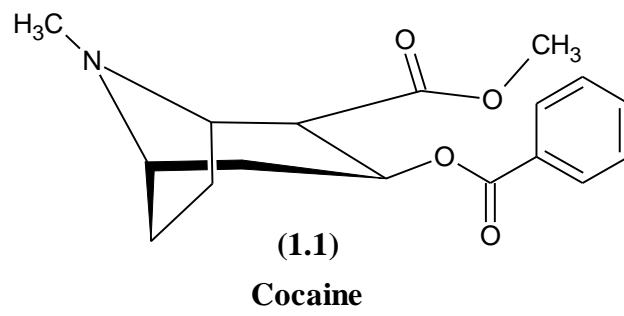
1.4 The Purpose of Isolation and Characterisation of Active Natural Products

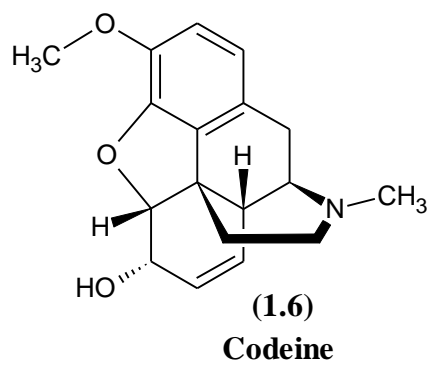
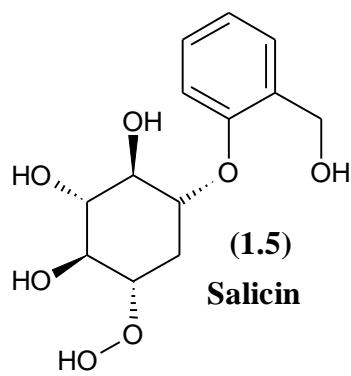
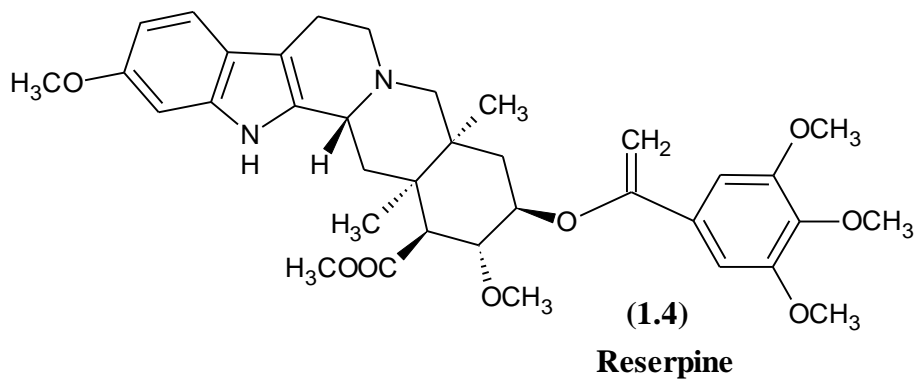
Both traditional and modern societies have used natural products for survival. (Hendrickson, 1965). The first drugs used by humans were derived from plants. (Liska, 1981). However, plants have also been used to poison humans; for example, the alkaloid coniine (1.10) produced by a poisonous plant known as *Conium maculatum* Linnaeus (Apiaceae) was ingested by Socrates, in form of hemlock in 399 BC, and was the cause of his death. Other natural compounds that can cause death include atropine (1.11) from *Atropa belladonna* Linnaeus (Solanaceae), scopolamine from *Datura stramonium* Linnaeus (Solanaceae). (Liska, 1981). In developing countries, such as those in Africa, with low living standards, pharmaceutical industries are not well developed. Yet, it is common to see people chewing leaves, flowers and bark and swallowing crude plant extracts to cure diseases.

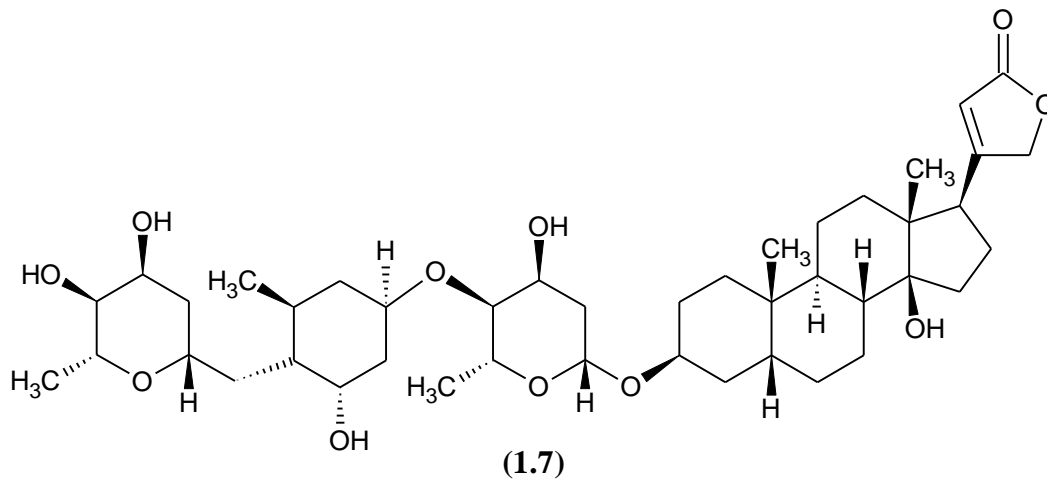
The lack of information about active ingredients contained in different parts of medicinal plants, is a big problem for users of natural products in their natural state. Also, in most cases, the necessary dose to be taken by adults and given to children is not known. Apart from the lead compound, herbal medicines contain numerous other compounds which may be biologically active and can cause side effects and toxicity (Patrick, 2005). This problem may have serious and/or fatal consequences. Therefore, the study of natural products from medicinal plants is of great importance in both developing and developed countries.

Pachystela brevipes which is used medicinally among traditional medicine practitioners for the treatment of naso-pharyngeal affections, antemetics, liver, lactation stimulants,

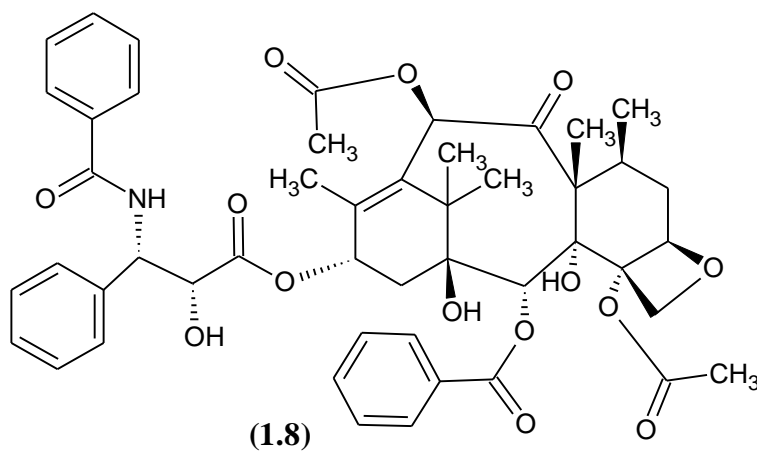
vermifuges, fabrifuges; genital stimulants/depressants dropsy, cough, pneumonia, swellings, oedema, gout; stomach troubles. (Burkill, 1985a). It is against this background that this research was initiated primarily to ascertain the medicinal potentials of this plant isolate the medicinally active ingredient(s) in it and characterize them using organic analytical techniques.



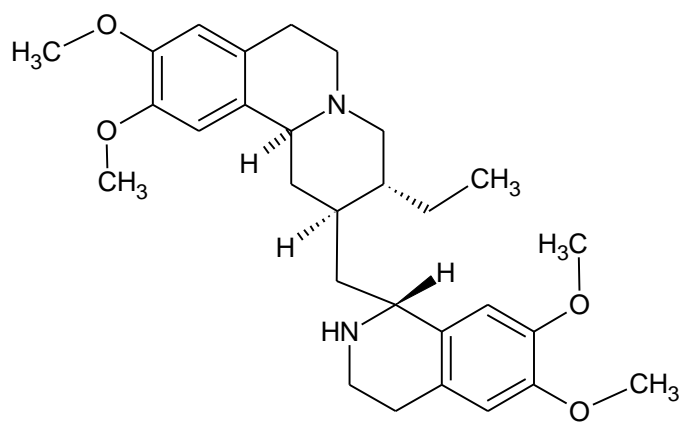




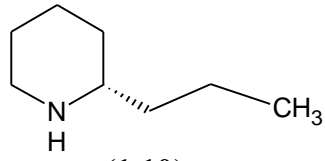
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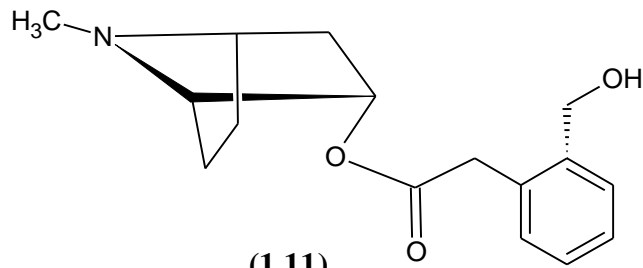
(1.8)
Taxol



(1.9)
Emetine



(1.10)
Coniine



(1.11)
Atropine

1.5 Aim of the Research

- i. The aim of the work was to authenticate or otherwise, the ethnomedicinal claims on the root part of *Pachystela brevipes* and isolate, purify and characterize some of the bioactive compound(s) that may be responsible for the claimed ethno medicinal values.

1.6 Objectives of the Research

The objectives of this research was to:

- i. Collect and identify the root part of the plant
- ii. Dry and pulverize the plant sample.
- iii. Extract the powdered plant material using different solvents based on the eluotropic series i.e. from non-polar Petroleum-ether (60-80 °C), Chloroform, Ethyl acetate and Methanol (polar).
- iv. Identify the secondary metabolites for bio-active compounds using the crude extracts of the solvents used for the extraction (Petroleum-ether, Chloroform, Ethyl acetate and Methanol).
- v. Investigate the biological or antimicrobial activities of the crude as well as purified components from the plants on some clinical isolates.
- vi. Use chromatographic techniques (Thin layer chromatography, Column chromatography and preparative thin layer chromatography) and purification to separate and purify the various components in the crude extracts, and

- vii. Use spectral techniques (^1H NMR, ^{13}C NMR and 2D NMR) for elucidation and characterization of the possible isolated compounds.

1.7 Justification of the Research

The choice of *Pachystela brevipes* as the plant of interest in this work is based on its economic relevance and also vast medicinal importance among traditional medicine practitioners in the tropical regions of Africa. Therefore, there is need for a scientific study to ascertain the medicinal potentials of this plant.

CHAPTER TWO

2.0 Literature Review

2.1 The Family *Sapotaceae*

Sapotaceae is a family of flowering plants, belonging to order *Ebenales*. The family name is coined from *zapote*, a Mexican vernacular name for one of the plants (in turn derived from the *Nahuatl* "*tzapotl*"). The name was latinised by Linnaeus as *sapota*. The family includes about 1200 species of evergreen trees and shrubs in approximately 65 genera (35-75, depending on generic definition) and is both ecologically and economically important. It is the second most valuable timber tree family in Southeast Asia. The copious latex found in their wood has been used as gutta-percha and chewing-gum.

Members of the family are found throughout the tropics, many producing edible fruits, for example shea butter and the Australian native plum (USDA, ARS, NGRP, 2003). Many also have been implicated in traditional medical practice throughout the world and some have been supported by scientific evaluation (Ndukwe *et al.*,2007).

2.2 The genus *Pachystela*

The genus *Pachystela* belongs to the family *Sapotaceae* and can be widely found around the World. These plants have been used as building material, as food, because the fruits are eatable as well as remedies in folk medicine. There is a lot of uncertainty about the genus *Pachystela* and therefore its members are continually being reclassified. Many *Pachystela* species produce edible fruits and dense hard woods for timber and firewood but there the utility ends as the woods are too heavy for furnishings (United States Department of Agriculture (USDA), 2009).

2.3 Taxonomic Classification of the Plant

i.	Botanical Name:	<i>Pachystela brevipes</i>
ii.	Kingdom:	Plantae
iii.	Sub Kingdom:	Viridaeplantae
iv.	Phylum:	Tracheophyta
v.	Sub phylum:	Euphyllophytina
vi.	Infra phylum:	Radiatopses
vii.	Division:	<i>Magnoliophyta</i>
viii.	Class:	<i>Magnoliopsida</i>
ix.	Subclass:	Asteridae
x.	Order:	<i>Ericales</i>
xi.	Super order:	Primulanae
xii.	Family:	<i>Sapotaceae</i>

- xiii. Genus: *pachystela*
xiv. Species: *pachystelabrevipes*
(Burkill,1985)

2.4 Common Names of the Plant

Nigeria: Benue : Ikpochi Malogo ,Igbo: Udara Nwa Enwe, Epira; Etuno itoki, Edo; otienne, Yoruba; osan-igbo, = star-apple of the forest, osan- odo, = star-apple of the stream, Guinea: *Basari*, Senegal: *Bambara* Kosi, Togo ; Yoruba-Nago Ahika, Dahomey; Yoruba-Nago Ahika, Ghana; Vulgar sudua, Sierra Leone; Loko Efele, Mali; Dogon Kana, Ivory Coast; Abure kerengue. (Burkill, 1985; 1994).

2.5 Habitat

Pachystela brevipes (Baker) Baill, belongs to the family of *Sapotaceae*. It is a much – branched evergreen tree with a dense, wide – spreading crown of drooping branches. It can occasionally be as much as 25 meters tall, The straight bole is up to 100cm in diameter, bearing a wide-spreading crown of drooping branches of the forest, often in lowland forest and riverine forest, damp sites, swamp forest and beside streams or other sites with permanently high water -table, at elevations up to 1,500meters, distributed from Senegal to Western Cameroons and widespread in Sudan, East Africa, South central Africa and Mozambique, where it is found at elevations from sea level up to 1,500 metres. In Tanzania it is found in areas where the mean annual rainfall is in the range of 1,500 – 2500mm. (Ken, 2012; Bouquet, 1969).

2.6 Medicinal Uses of the Plant

A root decoction is taken to treat malaria, pneumonia and as an aphrodisiac. Sap from the roots and bark is taken to treat coughs, colds, stomach complaints and hernia. A bark decoction is taken to treat stomach complaints, Oedema and swellings. The leaves are used

against hookworm infection of the small intestine. The fruit pulp is used to treat jaundice and nausea. The latex from the fruit is applied as a galactagogue. (Ken, 2012).

2.7.0 Some Plants and Isolated Compounds from *Sapotaceae* Family

2.7.1 *Chrysophyllum albidum*

African star apple (*Chrysophyllum albidum* G. Don) is a tropical edible fruit tree. It belongs to the family of *Sapotaceae* which has up to 800 species and make up almost half of the order (Ehiagbonare *et al.*, 2008). It is primarily a forest tree species and its natural occurrences have been reported in diverse ecozones in Nigeria, Uganda, Niger Republic, Cameroon and Cote d'Ivoire (Bada, 1997). The plant often grows to a height of 36 metres though it may be smaller. The African star apple fruit is a large berry containing 4 to 5 flattened seeds or sometimes fewer due to seed abortion. The plant has in recent times become a crop of commercial value in Nigeria (Oboh *et al.*, 2009). The fleshy pulp of the fruits is eaten especially as snack and its fruit has been found to have higher contents of ascorbic acid than

oranges and guava (Amusa *et al.*, 2003). It is also reported as an excellent source of vitamins, iron, and flavours to diets (Adisa, 2000). The seeds are also used for local games or discarded (Bada, 1997). *C. albidum* fruit is common in both urban and rural centres especially during the months of December to April. The fruits are not usually harvested from the trees, but left to drop naturally to the forest floor where they are picked up (Amusa *et al.*, 2003).

2.7.2 Medicinal Uses of *Chrysophyllum albidum*

The roots and leaves of *C. albidum* have been widely used for medicinal purposes. In addition, its seeds are a source of oil, which is used for diverse purposes. (Ugbogu and Akukwe, 2008) The fruits also contain 90% anacardic acid, which is used industrially in protecting wood and as source of resin, while several other components of the tree including the roots and leaves are used as a remedy for yellow fever and malaria. The leaves

are used as emollients and for the treatment of skin eruptions, diarrhea and stomach ache, which are as a result of infections and inflammatory reactions (Adisa, 2000). The people of south western Nigeria have been using *C. albidum* leaves for the management of infections / ailments since prehistoric times, although scientific evidence for its antimicrobial effect is still lacking. Studies have been carried out on seeds of *C. albidum*; for example seed storage and its food value, physical properties of the seed, use of the seed shell for the removal of metal ions and antimicrobial effect of the oil seed against some local clinical bacteria isolates (Amusa *et al.*, 2003; Oyelade *et al.*, 2005; Ugbogu and Akukwe, 2009; Oboh *et al.*, 2009). Studies have also examined its anti-nociceptive, anti-inflammatory and antioxidant activities of eleagnine: an alkaloid isolated from the seed (Idowu *et al.*, 2006).

2.7.3 *Madhuca longifolia* (Koenig) J.

The species is distributed in northern, central and southern part of peninsular India, Sri Lanka and Burma. The tree grows on a wide variety of soils but thrives best on sandy soil. It also grows on shallow, clayey and calcareous soils. It is found up to an altitude of 1200m, mean annual maximum temperature of 28-50°C, minimum 2-12°C; annual rainfall from 550-1500mm. It is not frost-hardy (Kirtikar and Basu, 2001).

2.7.4 Ethnomedical Uses of Bark of *Madhuca longifolia*

A cup of infusion of bark is taken orally twice a day by some West African tribes for the treatment of diarrhoea. Besides, the stem bark is used in chronic tonsillitis, leprosy and fever (Kirtikar and Basu, 2001). It is commonly used for the treatment of snakebite as antidote for southern part of Tamilnadu, India (Ramar *et al.*, 2008). Decoction of stem bark is used to cure skin disease, (Ayyanar and Ignacimuttu, 2005; Joseph and Siddha, 2008). Powdered bark is employed for the treatment of scabies, *Madhuca longifolia* leaves are expectorant and also used for chronic bronchitis and Cushing's disease (Prajapati *et al.*, 2008). The leaves are applied as a poultice to relieve eczema, as an antidiabetic activity, Itch, Swellings, Fractures

and Snake-bite poisoning (Kumar *et al.*, 2011). In 2010, the plant was reported as a wound healing plant (Smitha *et al.*, 2010) as an antibacterial activity, Rheumatism, Bleeding and Spongy gums (Tambekar and Khanate, 2010). Roy *et al.* also reported that the plant has an antioxidant activity and anti-ulcer activity (Roy *et al.*, 2010) and rheumatism, Ulcer and Tonsillitis was reported by Prashanth *et al.* (Prashanth *et al.*, 2010). In addition, the plant was found effective in the treatment of Skin diseases, epilepsy, Pneumonia and piles (Khare, 2007). Astringent, Stimulant, Emollient, Demulcent, Rheumatism, Piles, Nutritive and Antiepileptic activity was equally reported about the plant (Khond *et al.*, 2009). Thereafter, Chandra in 2008 reported the presence of an analgesic activity (Chandra, 2008) and Gaikwad also investigated the presence of an anti-inflammatory activity (Gaikwad, *et al.*, 2009). Some of the compounds isolated from this plant include; β -D-glucose of β -sitosterol (2.1) oleanolic acid, 3β -carprylate, (2.2) oleanolic acid palmitate, (2.3) stigmasterol, (2.4) xanthophyll, (2.5) protabasic acid (2.6).

2.7.5 *Mimusops elengi*

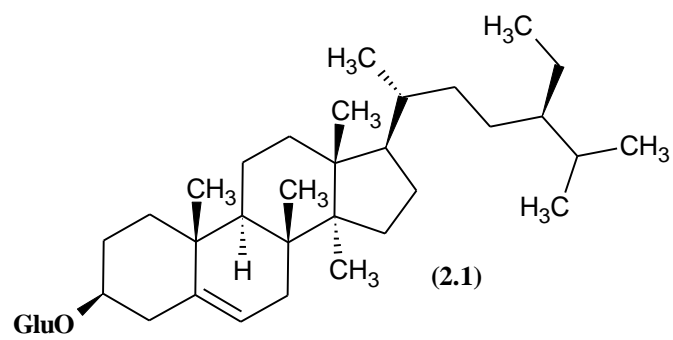
Phytochemical investigation of the plant reveals that the bark decoction is used for the treatment of stomach disorder, anthelmintic, biliousness and diseases of the gums and teeth. The flower is also used in the treatment of the bowels complaints, disease of blood, liver complaints, and diseases of the nose, headache and their smoke is good in asthma. The fruit is astringent to the bowel, good for the teeth, causes flatulence. The seed fix loose teeth and used as a cure troubles in the head. The root is aphrodisiac, diuretic, astringent to the bowel, good for gonorrhoea and used as a gargle which cures relaxation of the gums. The flowers, which appear twice a year, are somewhat fragrant and powerfully aromatic. (Kirthikar and Basu, 1999; Rajkumara *et al.*, 2012; Singh, 2003). Below are some of the isolated compounds from *Mimusops elengi*;

2.7.6 *Pouteria* species

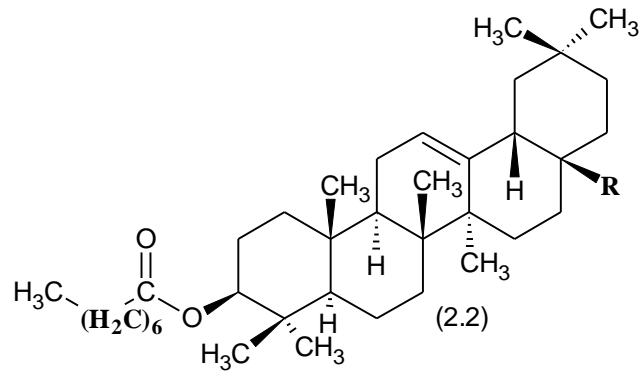
The genus *Pouteria* is among the 57 genera of *Sapotaceae*. Commonly, this genus is known as *Pouteria* trees. Phytochemical investigation reveals the presence of Triterpenes and flavonoids as the abundant principles of this genus. The triterpenes isolated are usually long chain or acetate esters (Cíntia *et al.*, 2009).

2.7.7 Biological Activities of *Pouteria* Species

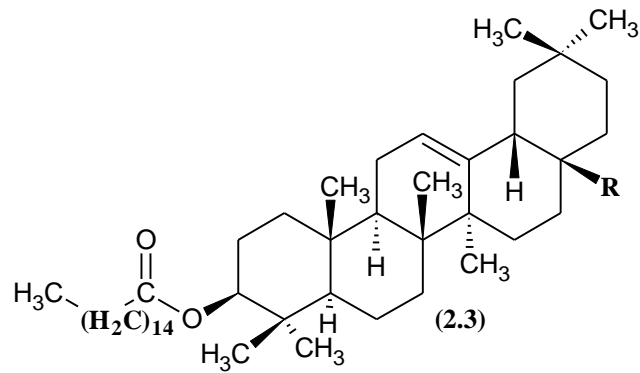
Many *Pouteria* species have been used in folk medicine for ailments ranging from fevers, diabetes, yaws, inflammation, ulcers, nausea, back pain, to promote lactation (Cíntia, *etal.*, 2009; Ma, *et al.*, 2004; Perfeito, *et al.*, 2005; Manosroi *et al.*, 2005). Scientific data is gradually growing to support these claims: antioxidant and free radical scavenging activities have been reported to be present in the more polar extracts and fractions (Rice-Evans *et al.*, 1996). The compounds present in the genus represent several activities ranging from anti-malarial, anti-cancer, and anti-inflammatory among a host of many others (Ying *et al.*, 1991; Pelzer *et al.*, 1998; Nijveldt *et al.*, 2001; Hodges *et al.*, 2003; Cushnie and Lamb, 2005; Fontanay *et al.*, 2008). Some of the compounds isolated from *Pouteria* species includes; Lupeol (2.12), Teraxasterol (2.13), Pseudotaraxasterol (2.14), Cycloartenol (2.15), Lanosterol (2.16), Lanosta-7, 24-diene, (2.17), Fridelinol (2.18), Dammerenediol (2.19), Spinasterol (2.20) etc. as reported by (Cíntia *et al.*, 2009).



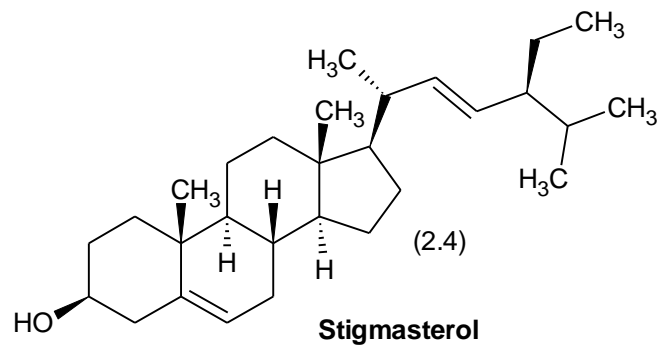
B-D-Glucoside of B-Sitosterol



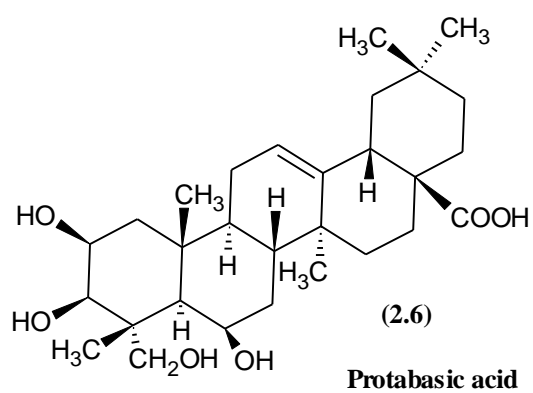
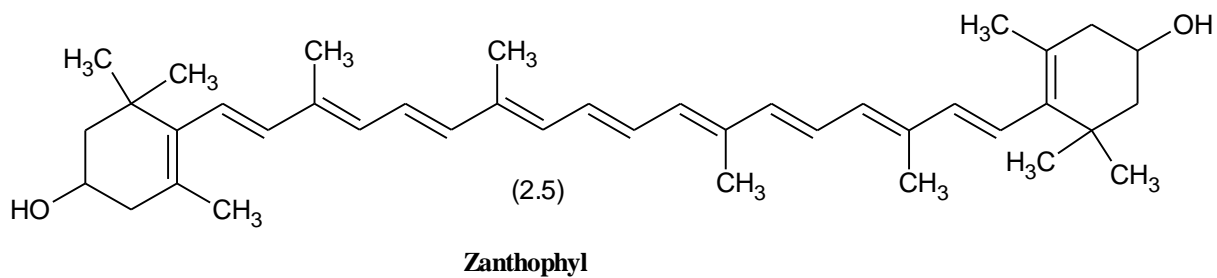
Oleanolic acid 3B-Carprylate R=COOH



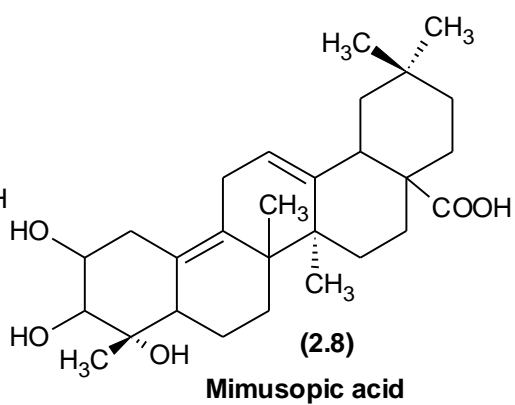
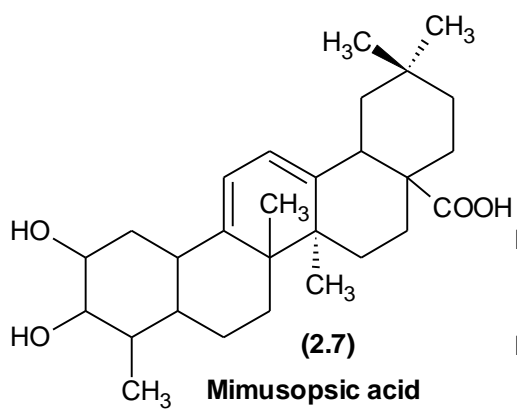
Oleanolic acid Palmitate R=COOH

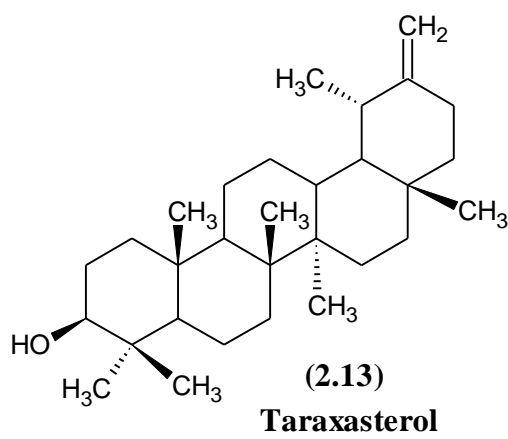
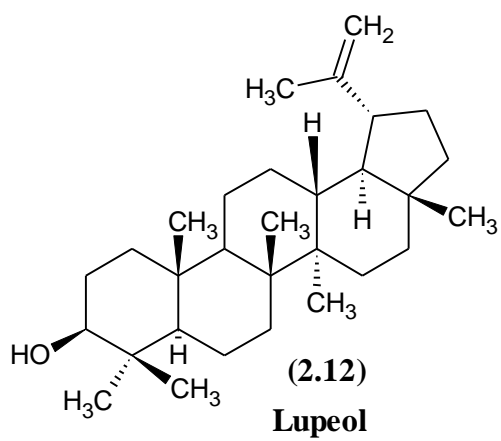
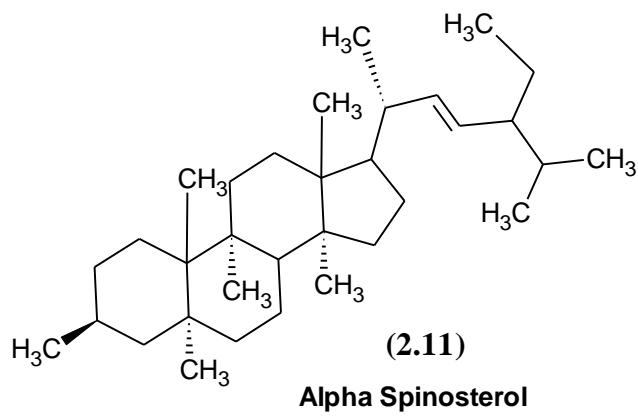
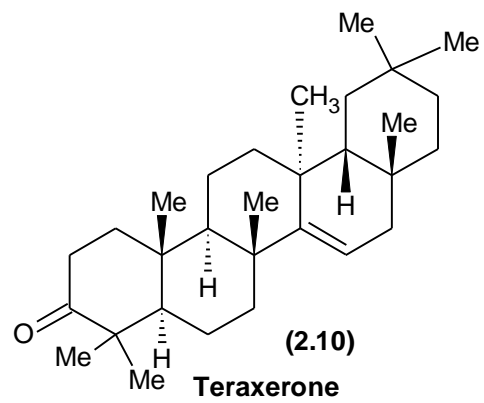
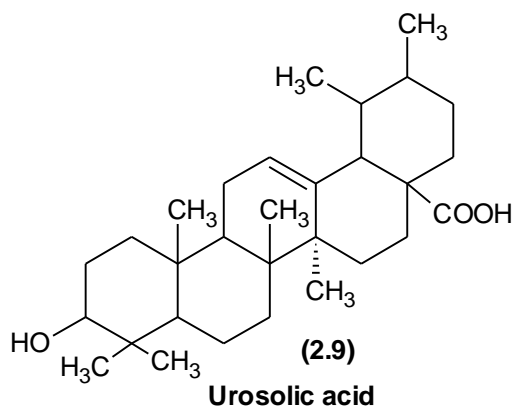


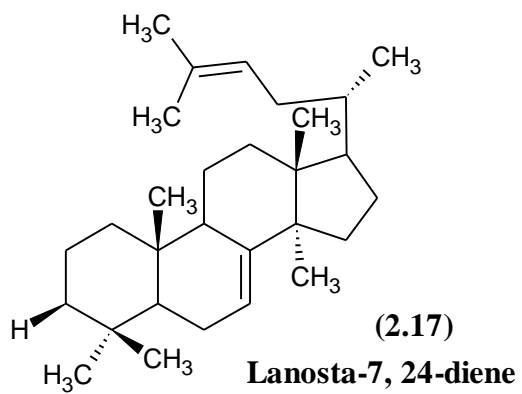
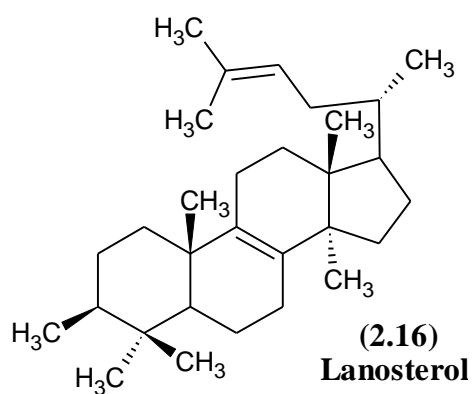
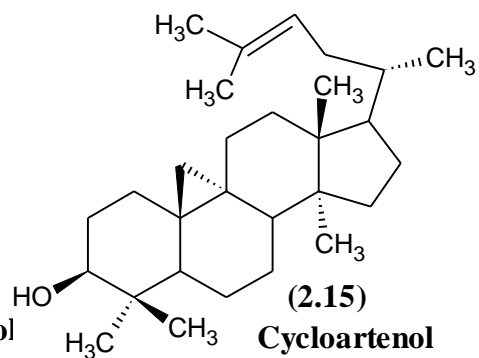
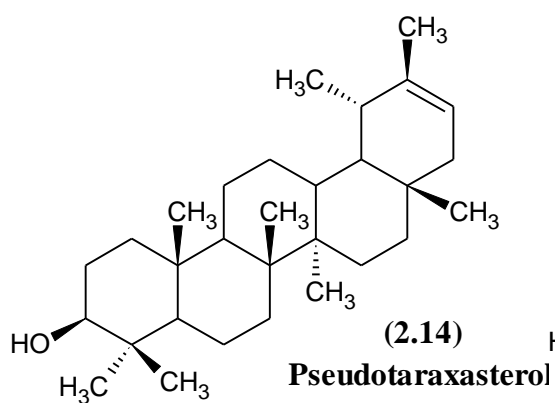
Stigmasterol

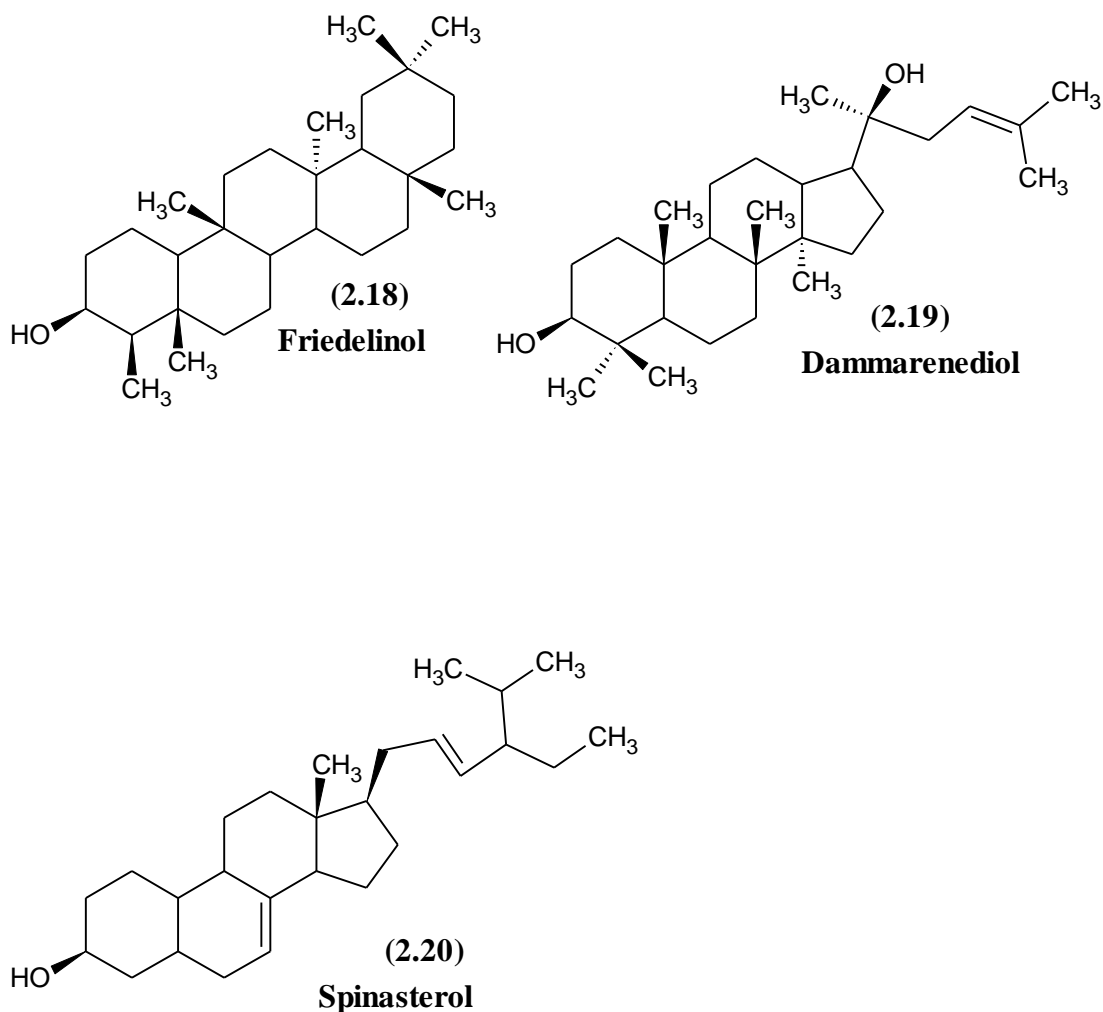


Isolated Compounds from *Mimusops elengi*









2.8 TEST ORGANISMS

2.8.1 *Staphylococcus* "Gram - Positive"

The genus *Staphylococcus* consists of cluster-forming, gram-positive cocci. *Staphylococcus* derived from Greek *staphyle* "bunch of grapes" and *kokkos* "grain or berry" the main pathogen within the genus. *Staphylococcus aureus* is the cause of a wide range of major and minor infections in man and animals. Currently, there are some 27 different species of *Staphylococci*. These fall into two main groups on the basis of their capability to clot blood

plasma by action of the enzyme coagulase. *Staphylococcus aureus* is by far the most important coagulase positive species. The coagulase-negative *Staphylococci* are skin commensals, which are now recognized as important opportunistic pathogens that can cause infections associated with prosthesis, catheters and implants (*Staphylococcus epidermidis*) and urinary tract infections (*Staphylococcus saprophyticus*). It has not been common practice for medical microbiologists to identify coagulase-negative *Staphylococci* to the species level. However, methods are now available that facilitate identification and the importance of other recently recognized coagulase-negative species is being assessed. (Pfaller and Herwaldt, 1988).

Staphylococcus aureus which is present in the nose and on the skin of a variable proportion of healthy people is an opportunistic pathogen, in that, it causes infection most commonly at sites of lowered host resistance, such as damaged skin or mucous membranes or haematomas in the cancellous tissues of a long bone (Jones *et al.*, 1990; Zakari, 2009).

2.8.2 Penicillin-Sensitive Strains

These are almost sensitive to all anti staphylococcal antibiotics like penicillin, cephalosporin, erythromycin, lincosamides and fusidic acid (Eastman and Adams, 1983).

2.8.3 β -Lactamase (penicillinase) Producing Strains

These are resistant to all methicillin, flucloxacillin and nafcillin. Most strains of *Staphylococcus aureus* isolated from patients belong to this group (Eastman and Adams, 1983).

2.8.4 Antibiotic-Resistant Strains

All these strains produce β -lactamase and are additionally resistant to one or more of the anti-staphylococcal antibiotics, most frequently to tetracycline and erythromycin, less commonly to lincosamides, fusidic acid and amino glycosides. Resistant strains of this kind are in a

minority and are almost always isolated from hospital contracted infections (Eastman and Adams, 1983).

2.8.5Methicillin-Resistant Strains (MRSA): Although these strains are usually detected in tests of methicillin, they are also resistant to flucloxacillin and virtually all β -lactam agents, and are believed to have epidemiological significance as a group. They require treatment with vancomycin and teicoplanin (Greenwood and Peutherer., 1993).

2.8.6Streptococcus(Gram - Positive)

Streptococci are Gram - positive bacteria arranged in chains of varying length each cell is approximately 1.0 μ m in diameter, non - motile, non- sporing and may be capsulate.

Streptococci form part of the normal flora of man and animals. They inhabit various sites notably the upper respiratory tract, and usually live harmlessly as commensals. Some species, of which or*Streptococcipyogenes* is the most important, may adopt a more aggressive role. This organism can cause a wide spectrum of diseases, including tonsillitis, erysipelas, impetigo, scarlet fever, and septicaemia, which range in severity from the trivial to the life threatening or *Streptococcipyogenes* is also involved in the aetiology of rheumatic fever and acute glomerulonephritis. Other pathogenic species includes *Streptococciagalactiae* an important cause of neonatal infection and *Streptococcus pneumoniae*, commonly called *Pneumococcus* which primarily causes disease of the middle ear, paranasal sinuses mastoids and the lung parenchyma but may spread to other sites, such as the joints, peritoneum, endocardium and biliary tract, in particular the meninges

2.8.7Klebsiella pneumonia (Gram-Negative)

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium of about 1-2 μ m long and 0.5-0.8 μ m wide, found

in the normal flora of the mouth, skin, and intestines (Ryan and Ray, 2004). It tends to be slightly shorter and thicker than other enterobacteria. In recent years, *klebsiellae* have become important pathogens in nosocomial infections (Postgate , 1998). The Danish scientist Hans Christian Gram (1853–1938), developed the technique now known as Gram staining in 1884 to discriminate between *K. pneumoniae* and *Streptococcus pneumoniae*(Postgate, 1998).*Klebsiella* was named after the German bacteriologist Edwin Klebs (1834–1913). Multiple-resistant *Klebsiella pneumoniae* have been killed *in vivo* via intraperitoneal, intravenous or intranasal administration of phages in laboratory tests (Podschun and Ullman, 1998). The capsular material is produced in greater amount in media of carbohydrates. A useful characteristic of all strains of *Klebsiella pneumoniae* species, *aerogenes* and *pneumonia* is the ability to form gas within four (4) days from starch, which practically no other member of the enterobacteriaceae is capable of doing. As with many bacteria, the recommended treatment has changed as the organism has developed resistances. *Klebsiella* organisms are often resistant to multiple antibiotics. Current evidence implicates a plasmid as the source of the resistant genes. *Klebsiella* with the ability to produce extended-spectrum beta-lactamases ESBL are resistant to many classes of antibiotics. The most frequent resistances include resistance to aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and sulfamethoxazole-trimethoprim (Podschun and Ullman, 1998).

2.8.8 Salmonella (Gram – Negative)

Salmonellae are widely distributed in nature. All vertebrates appear capable of harbouring *Salmonellae* in their gut. It has also been isolated in a wide range of arthropods such as flies, cockroaches, fleas, and ticks.*Salmonella typhi* and *Salmonella paratyphi* A, B, and C are primary human pathogens which are rarely isolated from other animals. *Salmonella paratyphi*, while essentially a human pathogen, is occasionally; isolated from cattle, pigs,

poultry, and other animals. The cycle of transmission in these hosts has not been fully demonstrated (Christie, 1987). The ability of *Salmonellae* to enter and survive within macrophages and other cells particularly in the liver and kidney occasionally leads to persistent infection and the chronic carrier state where sufferers from *Salmonella* infection continue to excrete the organism in their stools for days or weeks after complete clinical recovery.

In the treatment and clinical management of enteric fever due to *Salmonellae* the problem of bone marrow toxicity and widespread emergence of chloramphenicol resistance in *Salmonella typhi* in many parts of the world prompted the search for alternative drugs and among these are ampicillin and cotrimoxazole and they have been shown to be comparable in efficacy to chloramphenicol (Levine *et al.*, 1989).

2.8.9 *Escherichia coli* (gram-negative)

Escherichia coli are a widespread intestinal parasite of mammals and birds. It is always present whenever there is faecal contamination but it does not lead an independent existence outside the body. Some strains are pathogens in man and animals and cause some septic infection and diarrhoea. It is the most common type of acute urinary track infection. *Escherichia coli* also cause neonatal meningitis and septicaemia as well as sepsis in operation wounds and abscesses in a variety of organs (Greenwood and Peutherer., 1993).

E. coli that causes urinary track infection often originates in the gut of the patient. It is naturally sensitive to many antibiotics, although moderately resistant to benzyl penicillin. It is sensitive to ampicillin, tetracycline, chloramphenicol, streptomycin, gentamycin sulphonamides and so on. Many strains however, have acquired plasmids conferring resistance to one or more of these drugs. Extra intestinal *E. coli* infections are treated with specific antimicrobial therapy which preferably requires the result of laboratory test for sensitivity guide.

2.9 FUNGI

Fungi constitute large diverse group of heterotrophic organisms which exist as saprophytes, parasites or commensal. Most are found as saprophytes in the soil and on decaying plant material. About 180 of the 250,000 known fungi species are recognized as capable of causing diseases (mycosis) in man and animals (Rippo, 1988). Most of these are mould fungi but

there are number of pathogenic yeast fungi and many are dimorphic. Dimorphic fungi usually assume the mould form when growing as saprophytes in nature and the yeast form when causing infection. In the laboratory, the tissue form can be induced by culture at 37^oc on rich media such as blood agar, whereas the mould form develops when incubated at a lower temperature (22-27^oC) on a less rich medium such as Sabouraud's agar (Rippo, 1988).

Yeast infections affecting skin and nails and mucous membranes of the mouth and vagina, are usually caused by *Candida albicans* which are found as commensal of man. Yeast infections are generally endogenous in origin but can be transmitted sexually.

There are relatively few therapeutically useful antifungal agents that are available. This is due mainly to the fact that fungi and man are both eukaryotes and most substances that kill or inhibit fungal pathogens are also toxic to the host. Most superficial *Candida* infections respond well to topical therapy with nystatin amphotencin B. or azoles (Rippo, 1988).

Superficial *Candida* infections occasionally occur on the penis after intercourse with females with vaginal thrush. The yeast may also infect the outer ear(Roberts *et al.*, 1984)

In oral *candidiosis*, nystatin, amphotericinB and miconazole may be effective in lozenge or gel form. Most cases of vaginal candidiosis can be treated successfully with a single application of an azoles derivative with oral therapy using fluconazole or itraconazole (Odds, 1988; Zakari, 2009).

CHAPTER THREE

3.0 METHODOLOGY

3.1 APPARATUS, SOLVENTS AND REAGENTS

3.1.1 Apparatus

Soxhlet Extractor

Rotary Evaporator

Separating Funnel

Top-loading balance

Oven

3.1.2 Solvents for Extraction

All the solvents were distilled before use and they include;

Petroleum Ether (Sigma Aldrich Chemicals)

Chloroform (Sigma Aldrich Chemicals)

Ethyl acetate (Sigma Aldrich Chemicals)

Methanol (Sigma Aldrich Chemicals)

3.1.3 Reagents for Phytochemical Test

Ethanol

Iron (III) chloride

Potassium hydroxide

Sodium hydroxide

Hydrochloric acid

Sulphuric acid

Acetic acid

Magnesium metal

Acetic anhydride

Wagner's Reagent (prepared by dissolving 5.0g of KI and 3.18g of I₂ in 250ml of distilled water).

Molisch's Reagent: (prepared by dissolving 2.50g of 1-naphthol in 25ml of dilute ethanol).

Meyer's Reagent: (prepared by dissolving 4.0g of KI in distilled water; 1.36g of HgCl₂ was also dissolved in distilled water and the two mixed and made up to 100ml volumetric flask).

Fehling's Solution A: (prepared by dissolving 7.0g of CuSO₄ in 100ml of distilled water and addition of a few drops of Conc. H₂SO₄)

Fehling's Solution B: (prepared by dissolving 35.2g sodium potassium tartrate and 15.4g NaOH in 100mls distilled water)

Dragendorff's Reagent:(prepared by dissolving 1.7 g bismuth nitrate and 20 g tartaric acid in 80 ml of water and 16 g potassium iodide in 40 ml of water)

3.1.4 Material for Bioassay Analysis

Petri-dishes

Bunsen burner

Autoclave

Incubator

Syringes

Test tubes

Wire loop

Distilled water

Ruler

Cotton wool

Silica gel (SiO₂.xH₂O)

Nutrient Broth (CM0001 Oxoid)

Mueller-Hinton Agar (MHA)

Dimethyl sulphuroxide (DMSO)

Saline solution

3.1.5 Test Bacteria

Gram-Positive Bacteria

Bacillus Subtilis

Corynebacterium ulcerans

Streptococcus pneumoniae

Streptococcus faecalis

Streptococcus pyogenes

Staphylococcus aureus

Gram-Negative Bacteria

Klebsiella pneumoniae

Proteus mirabilis

Pseudomonas aeruginosa

Salmonella typhi

Escherichia coli

Test Fungi

Candida albicans

Candida tropicalis

Candida krusei

3.1.6 Materials for Chromatography

TLC plates

Silica gel

Capillary tubes

Beakers

Syringes

Glass funnel

Chromatographic tank

Whatman filter paper no. 2

3.2.0 Plant Materials

3.2.1 Collection of Plant

The root and leaves of the plant were collected from Okpokwu L.G.A. of Benue State, Nigeria, in March 2013. Identification and authentication was made by Mallam Umar Gallah of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria. A voucher specimen of number 7106, was deposited there.

The plant material was dried indoors in a well-ventilated room for 3 weeks and then size reduced in a wooden mortar. The pulverised plant material was packaged and labelled appropriately.

3.2.2 Extraction Procedure

About 1000g of the plant material was extracted with redistilled methanol using soxhlet extraction and cold maceration methods as described by Harbone, 1984. The extract was then concentrated *in-vacuo* using a rotatory evaporator at about 40⁰C.

3.3.0 Phytochemical Screening

Chemical tests were carried out on the plant extracts to identify the secondary metabolites present using standard procedures as described by Sofowora, 1982; 1993a; Edeoga *et al.*, 2005.

3.3.1 Test for Alkaloids

General Test

Stir the crude extract (0.2g) with 1% hydrochloric acid (5ml) on a water bath. To the filtrate (1ml) was added, 2-3 drops of the following reagents were added:

Wagner reagent solution of iodine in potassium iodide,

Mayer's reagent (potassium mercuric iodide solution)

Dragendroff's reagent (potassium bismuth iodide solution)

The presence of precipitate in most or all of the above reagents indicates the presence of alkaloids (Trease and Evans, 1989), that is

Light brown to brownish precipitate- Wagner's reagent

White or creamy white precipitate - Mayer's reagent

Orange or orange-yellow precipitate - Drangendroff's reagent

3.3.2 Test for Cardiac Glycosides

The extract (1.0g) was dissolved in water. Lead subacetate solution (1ml) was added and filtered. The filtrate was divided into two for the following tests:-

Keller-killiani Test (for deoxysugars)

To the first portion of the filtrate was added chloroform, the chloroform layer (lower) was separated and evaporated to dryness on water bath. The residue was dissolved in 3.5% ferric chloride (3ml) solution in glacial acetic acid. The mixture was allowed to stand for a minute before it was transferred to a test tube. Concentrated sulphuric acid (1.5ml) was added down the side of the test tube to form a lower layer on standing, and a brown colour was observed at the interface of the two liquids while a pale green colour was observed (Brain and Turner, 1975).

Salkowski's Test

Chloroform (2 ml) was added to 1 ml of the extract H_2SO_4 was carefully added. A reddish brown colour at the interface was observed.

3.3.3 Test for Flavonoids

Shinoda's Test

The extract (0.2g) was diluted with ethanol. Some pieces of magnesium filing were added followed by five drops of concentrated hydrochloric acid. A pink colour was observed (Silva *et al.*, 1998).

Sodium Hydroxide Test

The extract (0.2g) was dissolved in water (2ml) and filtered. 10% aqueous sodium hydroxide (2ml) solution was added. A Yellow solution indicates the presence of flavonoids. On addition of dilute hydrochloric acid, the solution become colourless, (Brain and Turner, 1975).

Ferric Chloride Test

The extract (0.1g) was boiled with water and filtered. To the filtrate (2ml), 2 drops of 10% ferric chloride solution was added. A green colour was observed.

3.3.4 Test for Carbohydrate

Molisch's Test

Few drops of Molisch's reagent was added to a little quantity of the extract in a test tube and a small quantity of concentrated sulphuric acid was allowed to run down the side of the test tube to form a lower layer. The formation of a purple colour was observed at the interface of the two layers (Trease and Evans 1989).

Fehlings Test

To 2ml of the extract, 5ml of a mixture of fehling's solution A and B in the ratio of 1:1 was added and the mixture boiled for few minutes. A brick red precipitate was observed (Trease and Evans, 1989).

3.3.5 Test for Anthraquinone Glycosides.

To the extract (0.2g) was added benzene (10ml) and shaken for 5 minutes. 10% ammonia (5ml) solution was added and shaken. The presence of a pink, red or violet colour in the ammoniacal (lower layer) phase indicates the presence of free anthraquinones.

For the anthraquinone glycosides, the extract (0.2g) was boiled with dilute sulphuric acid (10ml) and filtered while hot. The filtrate was shaken with benzene (5ml), the benzene layer was separated and to it, ammonia solution (10 %, 3ml) was added and mixture shaken. The layers were allowed to separate. A violet colouration was observed at the lower layer which shows the presence of anthraquinone glycosides (Sofowora, 1993a).

3.3.6 Test for Saponins

Frothing Test

Small quantity of the extract was dissolved in 10ml of distilled water. This was shaken vigorously for 30 seconds and was allowed to stand for 30 minutes. A honey comb froth formed for more than 30 minutes indicates saponin (Trease and Evans, 1989).

3.3.7 Test for Steroids and Terpenoids

Salkowski's Test for Steroids: the extract (0.5g) was dissolved in chloroform and concentrated sulphuric acid (2ml) was carefully added down the side of the test tube to form a lower layer. A reddish brown colour at the interface was observed (Sofowora, 1993).

Liebermann - Burchard's Test

The extract (0.5g) was dissolved in chloroform (2ml) and filtered into a clean, dry test tube. Acetic anhydride (2ml) was added to the filtrate and shaken. Few drops of concentrated sulphuric acid were added carefully down the side of the tube to form a lower layer. A brownish-red ring at the zone of contact of the two liquids and the upper layer turning green denotes the presence of sterols and terpenes.

3.3.8 Test for Tannins

Ferric Chloride Test

The extract (0.2g) was stirred with water (5ml) and filtered. To the filtrate (2ml) in a test tube, two drops of ferric chloride solution were added. A greenish black precipitate indicate the presence of condensed tannins, while a bluish black precipitate observed indicates the presence of hydrolysable tannins(Trease and Evans, 1989).

Lead Subacetate Test

To the aqueous extract (1ml), three drops of lead sub acetate solution was added. A coloured precipitate was observed (Trease and Evans, 2002).

3.4.0 Antimicrobial Screening

3.4.1 Test Organisms

The organisms for this research viz *Methiicillin Resistant Staphylococcus Aureus*,(MRSA),*Staphylococcus aureus*; *Streptococcus pyrogenes*; *Escherichia coli*; *Salmonella typhi*; *Shigella dysenterea*; *Pseudomonas aeruginosa*; *Klebsiella pneumoniae*,*Candida krusei*, *Candida stellatoidea*, *Candida tropicalis*, *Streptococcus feacalis*, *Proteus vulgaris*, *Proteus mirabilis*were obtained from the Department of Medical

Microbiology, Ahmadu Bello University, Teaching Hospital Zaria. All the test organisms were checked for purity and maintained in agar slants.

3.4.2 Cultivation and Standardization of Test Organisms

A loop full of each of the test organism was taken from the agar slant and sub cultured into test tubes containing sterile nutrient agar and sabouraud dextrose medium (20 ml) for the fungi. The test tubes were then incubated for 24 hours at 37⁰C for two days. The broth culture was standardized using sterile normal saline to obtain a density of 10⁶ cfu/ml for bacteria. A sporulated test fungal spores was harvested with 0.05% Tween80 in sterile Normal saline and standardized to 10⁶ spores/ml (NCCLS, 1990; 1993).

3.4.3 Preparation of Culture Media

The prescribed quantities of the dehydrated bacteriological culture media was weighed and hydrated with distilled water according to the manufacturer's specification. Where necessary, gentle heat was applied to aid dissolution and the resultant suspensions were dispensed into clean bottles and sterilized at 121⁰C for 15 minutes in an Adelphi bench autoclave(NCCLS, 1990; 1993).

3.4.4 Determination of Zone Of Inhibition

The antimicrobial activities of the plant extracts were determined using some pathogenic microbes. The microbes were obtained from the department of Medical Microbiology, A. B. U. Teaching hospital Zaria. The extract (0.1)g was weighed and dissolved in 10mls of Dimethylsulphoxide (DMSO) to obtain a concentration of 10mg/ml. Mueller Hinton agar was the medium used as the growth medium for the microbes. The medium was prepared according to the manufacturer's instructions, sterilized at 121⁰C for 15 minutes, poured into sterile Petri dishes and was allowed to cool and solidify (Baker and Thornsberg, 1983;

NCCLS, 1990). Diffusion method was used for screening the extracts. The sterilized medium was seeded with 0.1ml of the standard inocula of the test microbe, the inocula was spread evenly over the surface of the medium by the use of sterile swab. By the use of a standard cork borer of 6mm in diameters a well was cut at the centre of each inoculated medium. 0.1ml of the solution of the extract of the concentration of 10mg/ml was then introduced into each well on the inoculated medium. Incubation was made at 37⁰C for 24hours, after which each plate of the medium was observed for Zone of inhibition. The zone was measured with a transparent ruler and the result recorded in millimetres.(Bauer *et al*; 1966; Barry and Thornsberry, 1985).

3.4.5 Determination of Minimum Inhibitory Concentration

The minimum Inhibition Concentration of the extract was carried using broth dilution method. Mueller Hinton broth was prepared; 10mls was dispersed into test tube and was sterilized at 37⁰C for 15minutes, the broth was allowed to cool. Mc-farland's standard turbidity scale number 0.5 was prepared to give turbid solution. Normal saline was prepared, 10mls was dispensed into sterile test tube and the test microbe was inoculated and incubation was made at 37⁰C for 6hours. Dilution of the test microbe in the normal saline was made until the turbidity marched that of the Mc-farland's scale by visual comparison at this point the test microbe has a concentration of about 1.5×10^8 cfu/ml. Two fold serial dilution of the extract in the sterilized broth was made to obtain the concentration of 10, 5, 2.5, 1.25 and 0.625mg/ml. The initial concentration was obtained by dissolving 0.1g of the extract in 10mls of the sterile broth. Having obtained the different concentrations of the extract in the sterile broth, 0.1ml of the test microbe in the normal saline was then inoculated into the different concentrations, incubation was made at 37⁰C for 24hours, 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 minimum inhibition concentration.(Bauer *et al*; 1966; Barry and Thornsberry, 1985).

3.4.6 Determination of Minimum Bactericidal/Fungicidal Concentration

(MBC/MFC)

Minimum bactericidal concentration /fungicidal concentration were carried out to determine whether the test microbes were killed or only their growth was inhibited. Mueller Hinton agar was prepared, sterilized at 121⁰C for 15minutes, transferred into sterile Petri dishes and was allowed to cool and solidify (NCCLS, 1993; Cheesbrough, 2002).The content of the MIC in the serial dilution were then sub cultured onto the prepared medium, incubation was made at 37⁰C for 24 hours, after which the plates of the medium was observed for colony growth.The MBC/MFC were the plates with lowest concentration of the extract without colony growth.(Bauer *et al*; 1966; Barry and Thornsberry, 1985).

3.5.1 Partition of Crude Extracts

The methanol extract used in the *in vitro* studies, was defatted with n-hexane and partitioned into chloroform and ethyl acetate. This was done by first dissolving the methanol extract in distilled water (200ml). The mixture was transferred into a 500ml separatory funnel and extracted with several 150ml portions of chloroform and ethyl acetate separately. The extracts collected were concentrated *in vacuo* and allowed to dry.

3.5.2 Thin Layer Chromatography (TLC)

The ethyl acetate extract was subjected to thin layer chromatographic analysis to find the solvent system that will give better separation of the components therein. This was achieved using pre-coated TLC plates. A micro quantity of the sample solution was spotted on TLC pre-coated (MERCK) F254 plates, and developed with various ratios of organic solvents

(petroleum spirit, ethyl acetate, methanol, chloroform) taking into consideration of their polarity. The solvent system, Hexane / Ethyl acetate (8:2 and 7:3) which separated the components to high degree of resolution was considered. The plates were visualized under visible and UV-light (366 and 254 nm). Visualization was also achieved by spraying the plates with 10% sulphuric acid and heated at 100 °C for 2-5 minutes.

3.5.3 Column Chromatography of Ethyl acetate Extract

The separation of the ethyl acetate extract into different chemical components was carried out in two stages using column chromatographic technique with silica gel as the stationary phase. A glass column of 100 cm long and internal diameter of 5 cm was used, 2.0g of silica gel (60 – 120) mesh was activated in an oven at 110 °C for 1 hour. It was allowed to cool at room temperature in a desiccator. Slurry of silica gel was made with the solvent system and packed into the column. The system was preconditioned by passing the solvent continuously for 30 minutes.

Ethyl acetate extract (8.0 g) was dissolved in minimum volume of the solvent system and mixed with 10.0g of silica gel. This was then loaded on the top of the column and after draining the solvent to the level of the gel bed, 3.0g of silica gel was added to protect the surface of the column when eluting the column.

Fractions were collected using conical flask at a flow rate of 50cm³ per hour until the issuing eluent was clear of extract. All the fractions collected were concentrated *in vacuo* using rotary evaporator, spotted on TLC plates and developed with the same solvent system. Similar fractions were pooled together and allowed to evaporate at room temperature under laboratory condition. TLC showed that fractions 27-31 were similar and so combined together and finally subjected to preparative thin layer chromatography (PTLC) technique for further purification. The purified sample obtained was crystalline and was labelled IKE 1.

3.5.4 Isolation and Purification of Compound

A small quantity of Ethyl acetate fraction (EAF) was dissolved in ethyl acetate and the solution was spotted on TLC plates. The plates were developed using several solvent systems; the solvent systems of Hexane / Ethyl acetate (8:2 and 7:3) gave better separation of the components, and were used in the TLC monitoring of the Column Chromatography. The ethyl acetate fraction (EAF) (12 g) was subjected to column chromatography on a silica gel (60 – 120 mesh) with gradient elution using Hexane and Ethyl acetate (Jain and Bari, 2010). Eluents were collected in 25ml aliquots and TLC was used to monitor the fractions.

A total of 97 collections were made and pooled into 7 major fractions, based on their TLC profiles. Fraction 3 indicated significant proportion of the compound of interest and was further subjected to purification by preparative TLC using the solvent system Hexane / Ethyl acetate (8:2 and 7:3). A single homogenous spot was obtained on TLC with two different solvent systems Hexane / Ethyl acetate (8:2). This compound, coded (IKE1), appeared as white crystalline and was subjected to spectral analysis.

3.6.0 Spectroscopic Analysis/Measurement

The structure of compound IKE 1 was determined using NMR spectroscopy which includes; ^1H NMR, ^{13}C NMR and 2D NMR. The NMR spectra were recorded on a Bruker-Avance (100MHz-400MHz) in deuterated chloroform with TMS as internal standard at the University of Kwazulu Natal, Westville Campus, Durban.

3.7.0 Antimicrobial Activity of IKE 1

The antimicrobial screening of the compound was carried out on the following twelve clinical microbes; *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium ulcerans*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi*, *Streptococcus faecalis*, *Pseudomonas aureginosa*, *Candida albicans*, *Candida krusei*, and *Proteus mirabilis* to determine their activities based on the traditional uses of this plant. The cork and bore

diffusion method (Bauer *et al*; 1966; Barry and Thornsberry, 1985) was used in the antimicrobial screening.

CHAPTER FOUR

4.0 RESULTS

4.1 EXTRACTION

The pulverized root part of *Pachystela brevipes*(1000 g) after extraction and defatting with petroleum ether and methanol gave a brown residue weighing 40g for methanol and 12 g for petroleum ether. Partitioning of the methanol extract (22 g) of the root part of *Pachystela brevipes* gave an amber coloured residue of ethyl acetate 8.4 g and chloroform 7.5 g (Table 4.1).

4.2 Phytochemical Screening of the Extracts

The petroleum ether extract, chloroform extracts of the root part of *Pachystela brevipes* was carried out along with the ethyl acetate and methanol fractions. The methanol extract was found to contain mainly carbohydrates, saponins, steroid/triterpenes tannins and cardiac glycosides as summarized in table 4.2.

4.3 Antimicrobial Activity

Table 4.3 shows the zones of inhibition for the petroleum ether, chloroform, ethyl acetate and methanol extracts of the root part of *Pachystela brevipes* on *Methicillin resist.staph. aureus*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Streptococcus pyogenes*, *Corynebacterium ulcerans*, *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Candida tropicalis*, *Candida stellatoidea*, *Candida krusei*, *Proteus mirabilis* and *Proteus vulgaris*. Eleven of them showed sensitivity to the extracts. The table also shows that ethyl acetate extract showed the same activity to the microbes when compared with pet-ether, chloroform and methanol extracts.

Table 4.1: Extractive Values of Methanol Extract of the Root Part of *Pachystela brevipes*

Extract	Colour	Weight (g)	Percentage yield (%)
Petroleum ether	Light brown	12.00	1.20
Chloroform	Dark brown	7.50	0.75
Ethyl acetate	Amber	8.40	0.84
Methanol	Brown	40.00	4.00

Table 4.2: Phytochemical Constituents of the Root Part of *Pachystela Brevipes*

CONSTITUENTS	TEST	OBSERVATION	INFERENCE			
			Pet Ether	CH Cl ₃	Ethyl acetate	CH ₃ OH
Carbohydrate	a.Molisch test	Purple colour at interphase	+	+	+	+
	b.Fehling test	Brick red precipitate	+	+	+	+
		No precipitate	+	+	+	+
Anthraquinones	a.Borntrager's test	No change	-	-	-	-
	b.Combined Anthraquinone	No change	-	-	-	-
Saponin	a.Frothing test	Frothing which persist > 30 min	-	-	-	+
Steroid and triterpenes	a.Lieberman Burchard's test	Red colour ring	-	-	+	+
	b.Salkowski test	Red colour at interphase	-	-	+	+
Tannins	a.Lead subacetate	Yellow coloration	-	-	-	+
	b.FeCl ₃ test	Greenish black coloration	-	-	-	+
Alkaloids	a.Meyer's test	No precipitate	-	+	-	-
	b.Dragendoff's test	No precipitate	-	+	-	-
	c.Wagner	No precipitate	-	+	-	-

Cardiac glycosides	a.Kella-killiani test	brown ring colour	+	++	+	+
	b.Salkowski test	reddish-brown colour	+	++	+	+
Flavonoid	a.Sodium hydroxide	Yellow coloration	-	-	-	-
	b.FeCl ₃ test	Yellow coloration	-	-	-	-
	c.Shinoda's Test	A red coloration	-	-	-	-

Key: EtOAc = ethyl acetate extract, CHCl₃ = Chloroform, CH₃OH = Methanol,- = absent,

+ = present, ++ = more present.

Table 4.3: Zones of Inhibition of the Extracts Against the Test Microorganism (mm)

Test organisms	Petroleum ether extract	Chloroform extract	Ethylacetate extract	Methanol extract	Ciprofloxacin 5µg/disc	Fluconazole 5µg/disc
<i>Mettricillin resist.staph. aureus</i>	17	20	23	21	35	-
<i>Staphylococcus aureus</i>	19	21	21	20	34	-
<i>Streptococcus faecalis</i>	18	21	20	20	38	-
<i>Streptococcus pyogenes</i>	-	-	-	-	37	-
<i>Corynebacterium ulcerans</i>	16	20	22	21	-	-

<i>Escherichia coli</i>	19	22	24	20	35	-
<i>Salmonella typhi</i>	18	20	22	20	40	-
<i>Shigella dysenteriea</i>	19	23	25	22	39	-
<i>Pseudomonas aeruginosa</i>	17	20	22	21	37	-
<i>Klebsiella pneumonia</i>	19	24	27	22	42	-
<i>Candida tropicalis</i>	16	20	20	20	-	29
<i>Candida stellatoidea</i>	18	22	24	20	-	30
<i>Candida krusei</i>	-	-	-	-	-	34
<i>Protus mirabilis</i>	-	-	-	-	32	-
<i>Proteus vulgaris</i>	-	-	-	-	-	-

Table 4.4: Minimum Inhibitory Concentration (mg/ml) of the Crude Extracts Against the Test Organism

Table 4.4 shows the MIC of the petroleum ether, chloroform, ethyl acetate and methanol extracts of the root part of *Pachystela brevipes* on the microbes. From table 4.4, it shows that

the chloroform, ethyl acetate and methanol fraction of the root part of *Pachystela brevipes* has a higher activity against most of the micro-organisms. While petroleum ether fraction of same plant showed less activity.

Table 4.5: Minimum Bactericidal/Fungicidal Concentration (mg/ml) of the Crude Extracts Against the Test Organism

Table 4.5 shows the MBC/MFC of the petroleum ether, chloroform, ethyl acetate and methanol extracts of the root part of *Pachystela brevipes* on the microbes.

4.4 Antimicrobial Activity of the Pure Compound

Table 4.6 shows the zones of inhibition of the pure compound of the root part of *Pachystela brevipes* on *Staphylococcus aureus*, *Streptococcus pyogens*, *Corynebacterium ulcerans*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi*, *Streptococcus feacalis*, *Pseudomonas aureginosa*, *Candida albicans*, *Candida krusei*, and *Proteus mirabilis*. Nine of them showed sensitivity to the isolated compound, except three; *Streptococcus pyogens*, *Proteus mirabilis* and *Candida krusei*.

Table 4.4: Minimum Inhibitory Concentration (mg/ml) of the Crude Extracts Against the Test Organism

Test	Pet ether	Chloroform	Ethyl	Methanol
------	-----------	------------	-------	----------

organisms	acetete			
	5.00	2.50	2.50	2.50
<i>M.R.S. aureus</i>				
<i>S. aureus</i>	5.00	2.50	2.50	2.50
<i>S. feacalis</i>	5.00	2.50	2.50	2.50
<i>S. pyogenes</i>	-	-	-	-
<i>C. ulcerans</i>	5.00	2.50	2.50	2.50
<i>E. coli</i>	5.00	2.50	2.50	2.50
<i>S. typhi</i>	5.00	2.50	2.50	2.50
<i>S.dysenteriea</i>	5.00	2.50	2.50	2.50
<i>Paeroginosa</i>	5.00	2.50	2.50	2.50
<i>K.pneumonia</i>	5.00	2.50	2.50	2.50
<i>C. tropicalis</i>	5.00	2.50	2.50	2.50
<i>C.stellatoidea</i>	5.00	2.50	2.50	2.50
<i>C. krusei</i>	-	-	-	-
<i>P. mirabilis</i>	-	-	-	-
<i>P. vulgaris</i>	-	-	-	-

Table 4.5: Minimum Bactericidal/Fungicidal concentration (mg/ml) of the Crude Extracts Against the Test Organism

Test organisms	Pet ether	Chloroform	Ethyl acetete	Methanol
-----------------------	------------------	-------------------	----------------------	-----------------

<i>M.R.S.</i>	10.00	10.00	5.00	5.00
<i>aureus</i>				
<i>S. aureus</i>	10.00	5.00	5.00	10.00
<i>S. feacalis</i>	10.00	10.00	10.00	10.00
<i>S. pyogenes</i>	-	-	-	-
<i>C. ulcerans</i>	10.00	10.00	5.00	5.00
<i>E. coli</i>	10.00	5.00	5.00	10.00
<i>S. typhi</i>	10.00	10.00	5.00	10.00
<i>S.dysenteriea</i>	10.00	5.00	5.00	5.00
<i>Paeroginosa</i>	10.00	10.00	5.00	10.00
<i>K.pneumonia</i>	10.00	5.00	5.00	5.00
<i>C. tropicalis</i>	10.00	10.00	10.00	10.00
<i>C.stellatoidea</i>	10.00	5.00	5.00	10.00
<i>C. krusei</i>	-	-	-	-
<i>P. mirabilis</i>	-	-	-	-
<i>P. vulgaris</i>	-	-	-	-

Table 4.6: Zone of Inhibition of the Test Drugs Against the Microbes

Test organisms	Pure component	Ciprofloxacin	Fluconazole
<i>Staphylococcus aureus</i>	23	37	-
<i>Streptococcus pyogenes</i>	-	32	-
<i>Streptococcus faecalis</i>	24	35	-
<i>Corynebacterium nulcerans</i>	22	32	-
<i>Bacillus subtilis</i>	29	42	-
<i>Escherichia coli</i>	26	37	-
<i>Klebsiella pneumonia</i>	28	42	-
<i>Salmonella typhi</i>	24	37	-
<i>Protus mirabilis</i>	-	32	-
<i>Pseudomonas aeruginosa</i>	22	-	-
<i>Candida albican</i>	24	-	35
<i>Candida krusei</i>	-	-	34

Table 4.7: Minimum Inhibitory Concentration (MIC) and Bactericidal/Fungicidal Concentration (MBC/MFC) (mg/ml) of the Pure Extract Against the Test Microorganism

Test organisms	MIC	MBC
<i>Staphylococcus aureus</i>	10.00	20.00
	-	-
<i>Streptococcus pyogenes</i>	10.00	20.00
<i>Streptococcus faecalis</i>	10.00	40.00
<i>Corynebacterium ulcerans</i>	5.00	10.00
<i>Bacillus subtilis</i>	10.00	20.00
<i>Escherichia coli</i>	5.00	10.00
<i>Klebsiella pneumonia</i>	10.00	20.00
<i>Salmonella typhi</i>		

	-	-
<i>Protus mirabilis</i>	10.00	40.00
<i>Pseudomonas aeruginosa</i>	10.00	20.00
<i>Candida albican</i>	-	-
<i>Candida krusei</i>	-	-

4.5: Spectral Analysis

Figure 4.1: Proton NMR of IKE1

This shows the result of proton NMR of the isolated compound which indicates the number of proton signals present in the spectra.

Figure 4.2 Carbon-13 NMR of IKE1

This is the result of the carbon-13 NMR of the isolated compound which indicates the number of carbon signals present in the spectra.

Figure 4.3: DEPT of IKE1

This result shows the spectra for the Distortionless Enhancement by Polarisation Transfer (DEPT) which is used to determine the presence of the primary (CH_3), secondary (CH_2), and tertiary (CH) carbon atoms.

Figure 4.4: COSY of IKE1

This is the result of Correlation Spectroscopy (COSY) of the isolated compound which shows coupling between sets of protons.

Figure 4.5: HMBC of IKE1

This shows the Heteronuclear Multiple-bond Correlation Spectroscopy (HMBC) which detects heteronuclear correlations over longer ranges of about 2-4 bonds.

Figure 4.6: HSQC of IKE1

It shows the Heteronuclear Single-Quantum Correlation Spectroscopy (HSQC) which detects correlations between nuclei of two different types which are separated by one bond.

Figure 4.7: NOESY of IKE1

This presents the Nuclear Overhauser Effect Spectroscopy (NOESY) which is useful for determining which signals arise from protons that are close to each other in space even if they are not bonded. NOESY also detects chemical and conformational exchange.

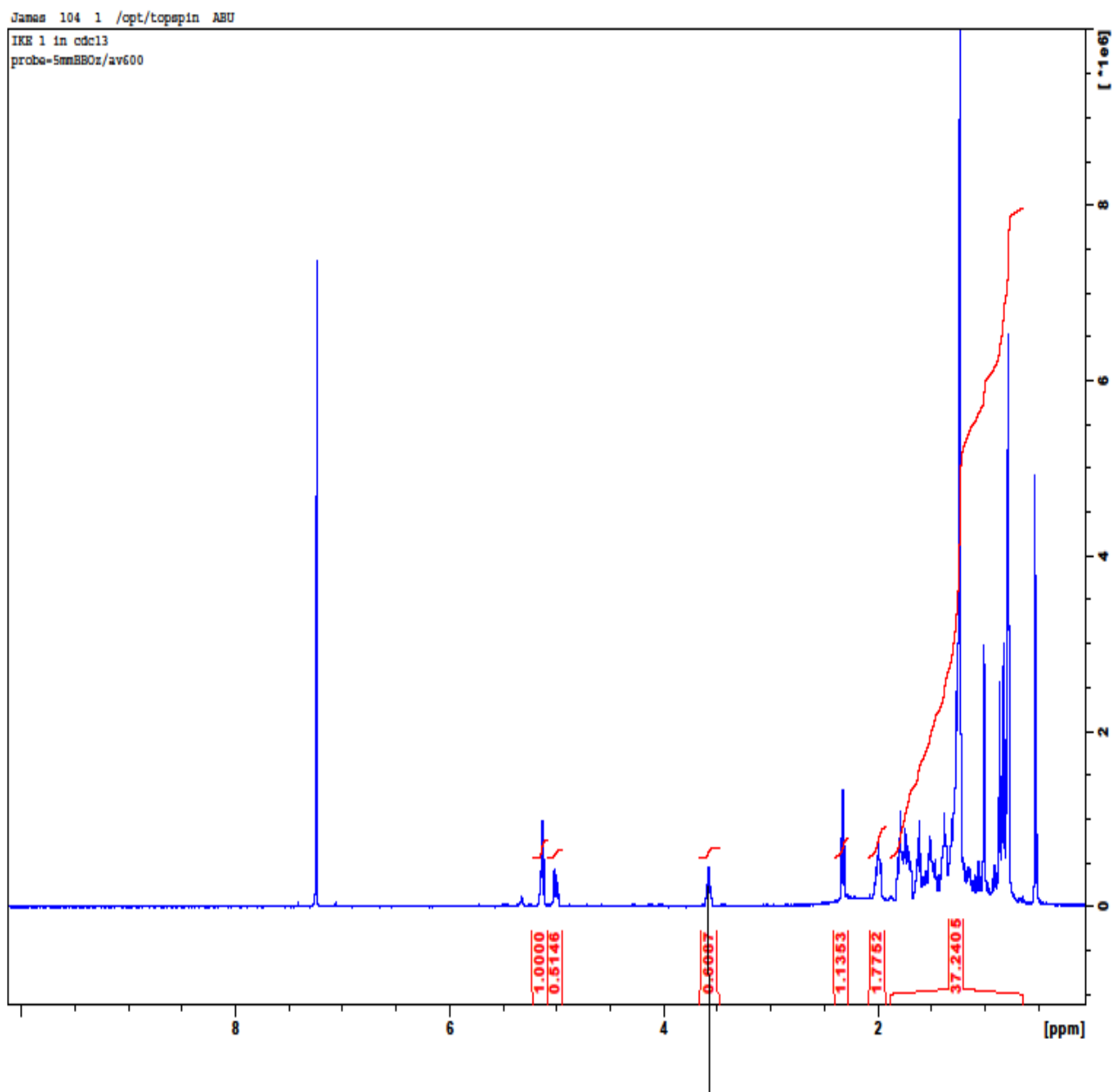


Figure 4.1: Proton NMR of IKE1

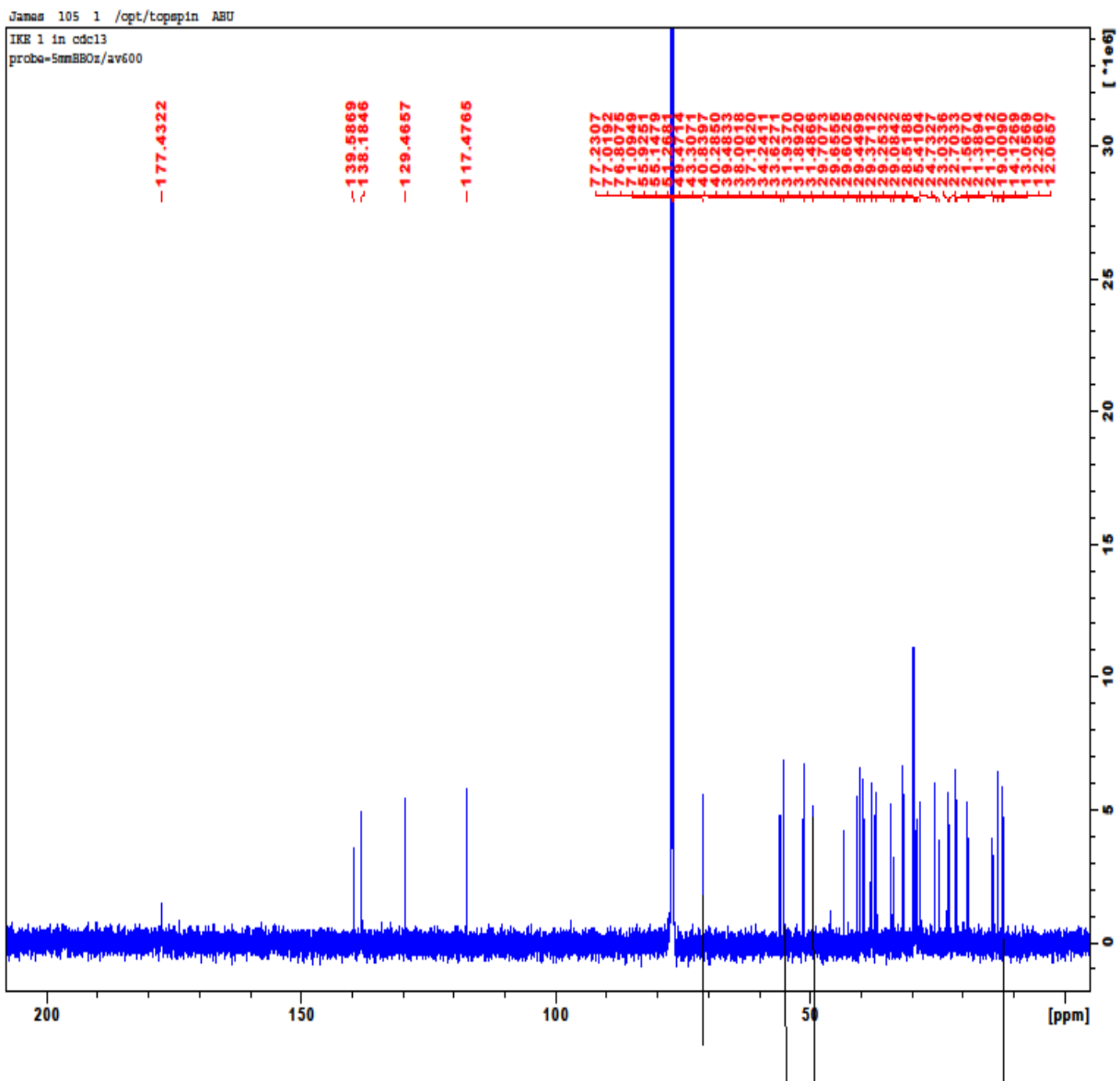


Figure 4.2 Carbon-13 NMR of IKE1

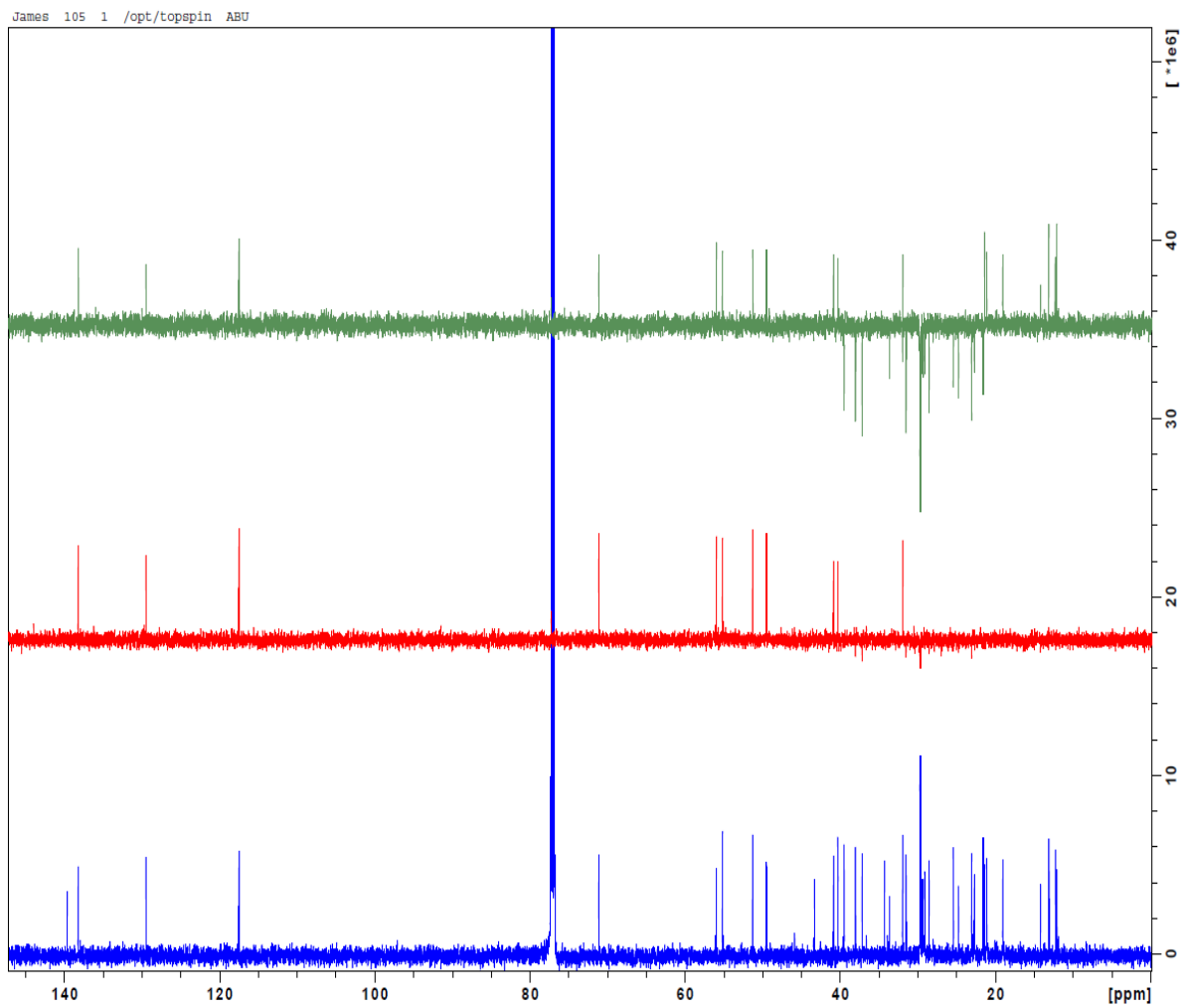


Figure 4.3: DEPT of IKE1

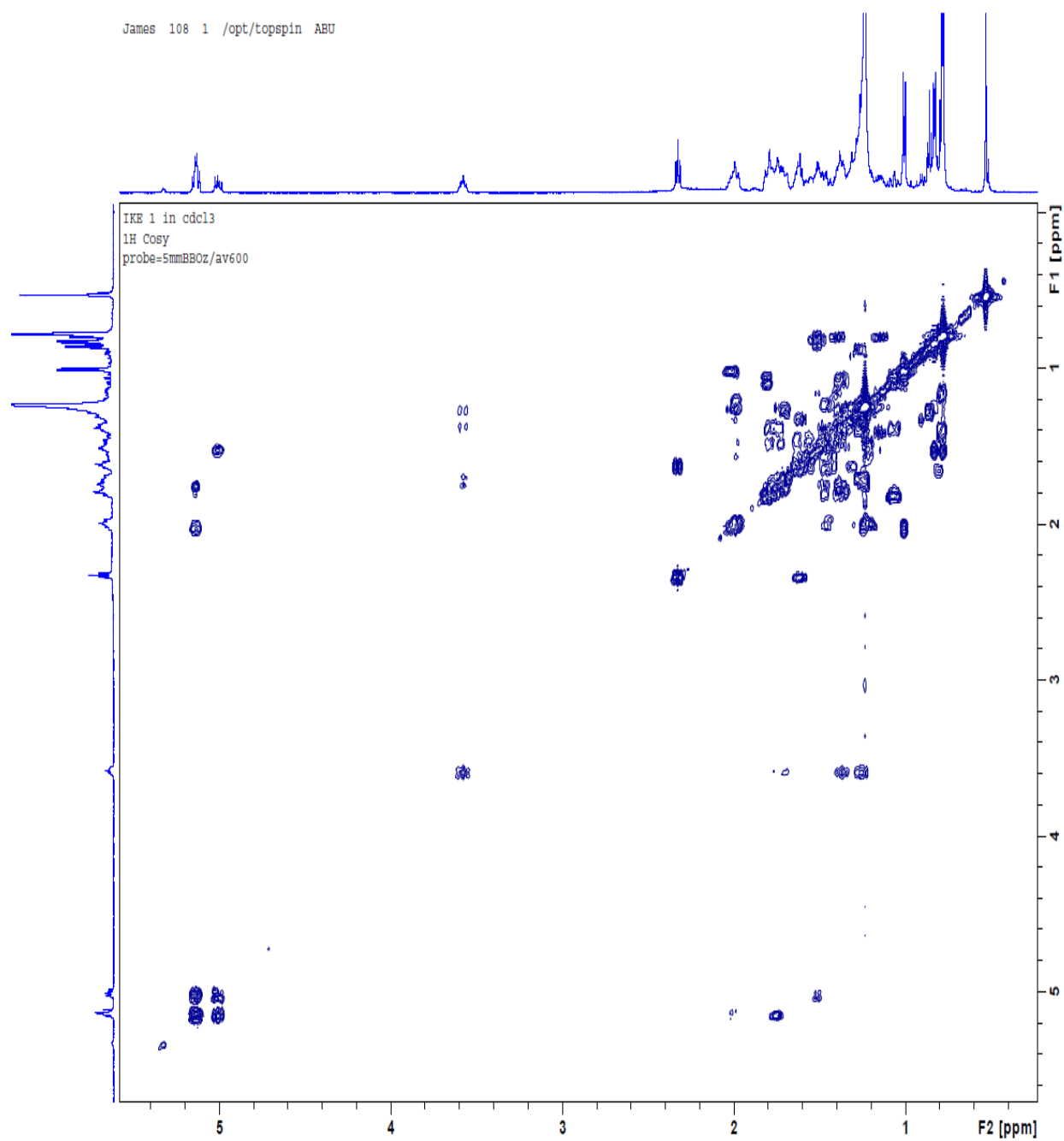


Figure 4.4: COSY of IKE1

James 111 1 /opt/topspin ABU

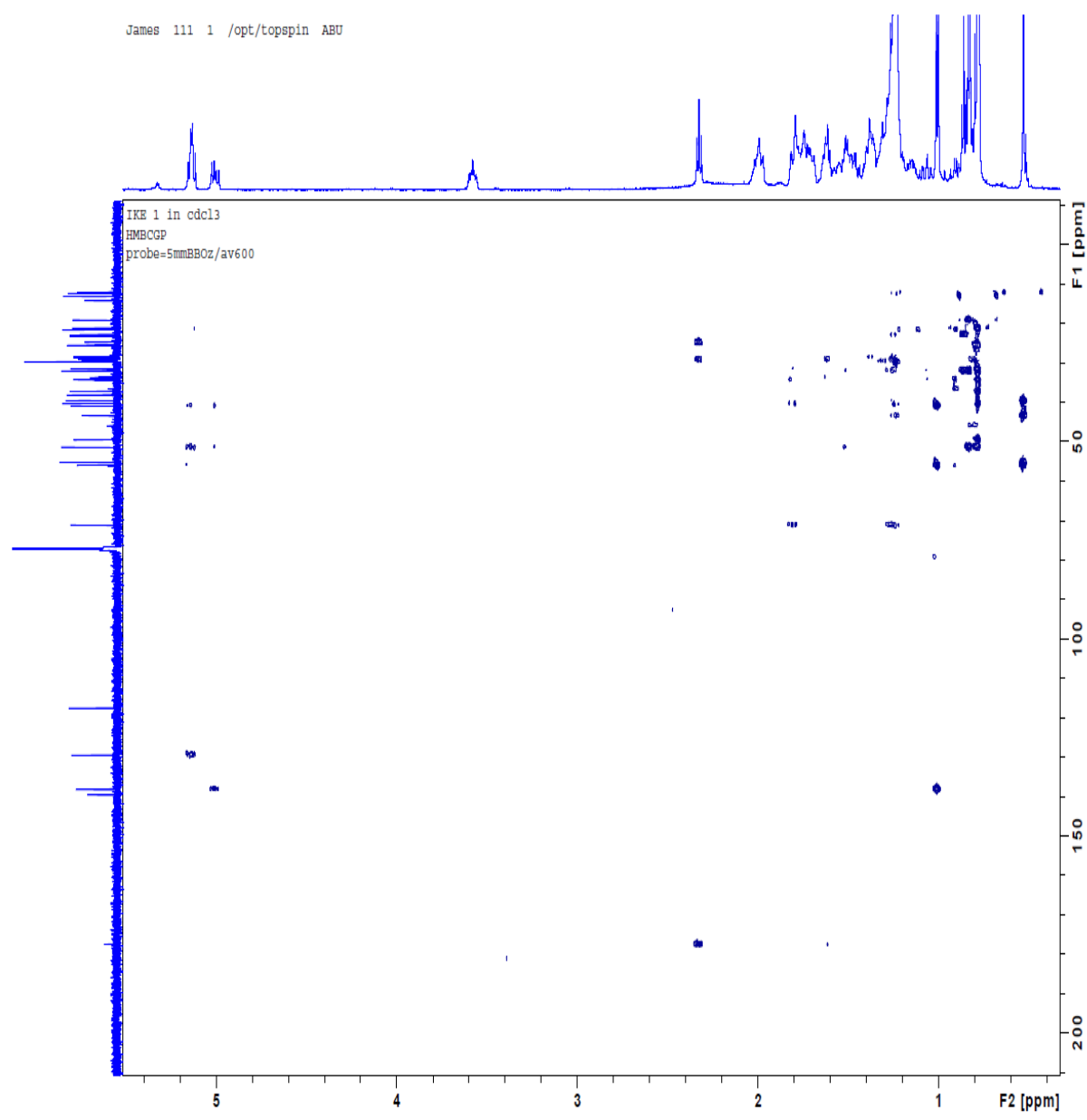


Figure 4.5: HMBC of IKE1

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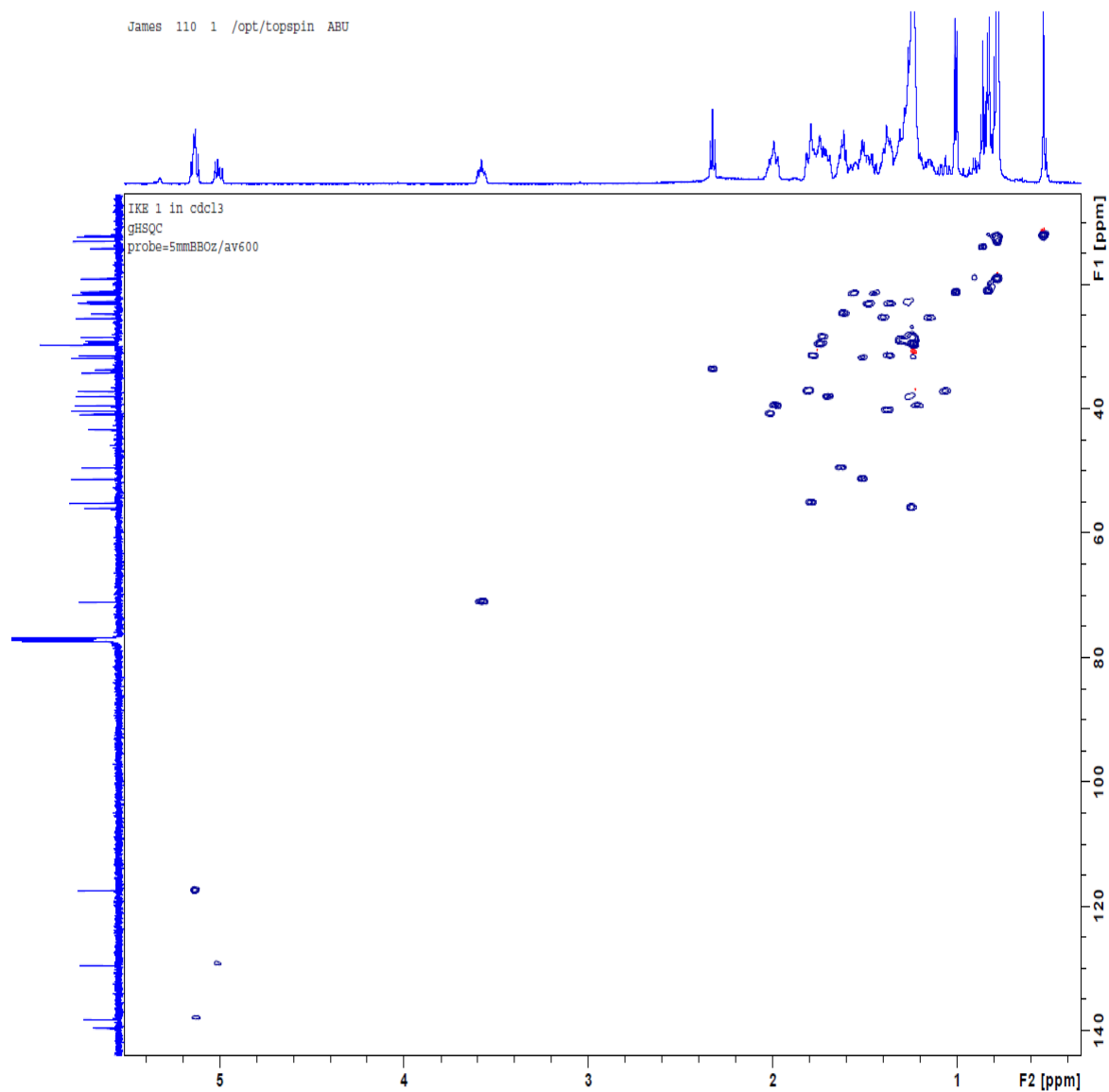


Figure 4.6: HSQC of IKE1

James 109 1 /opt/topspin ABU

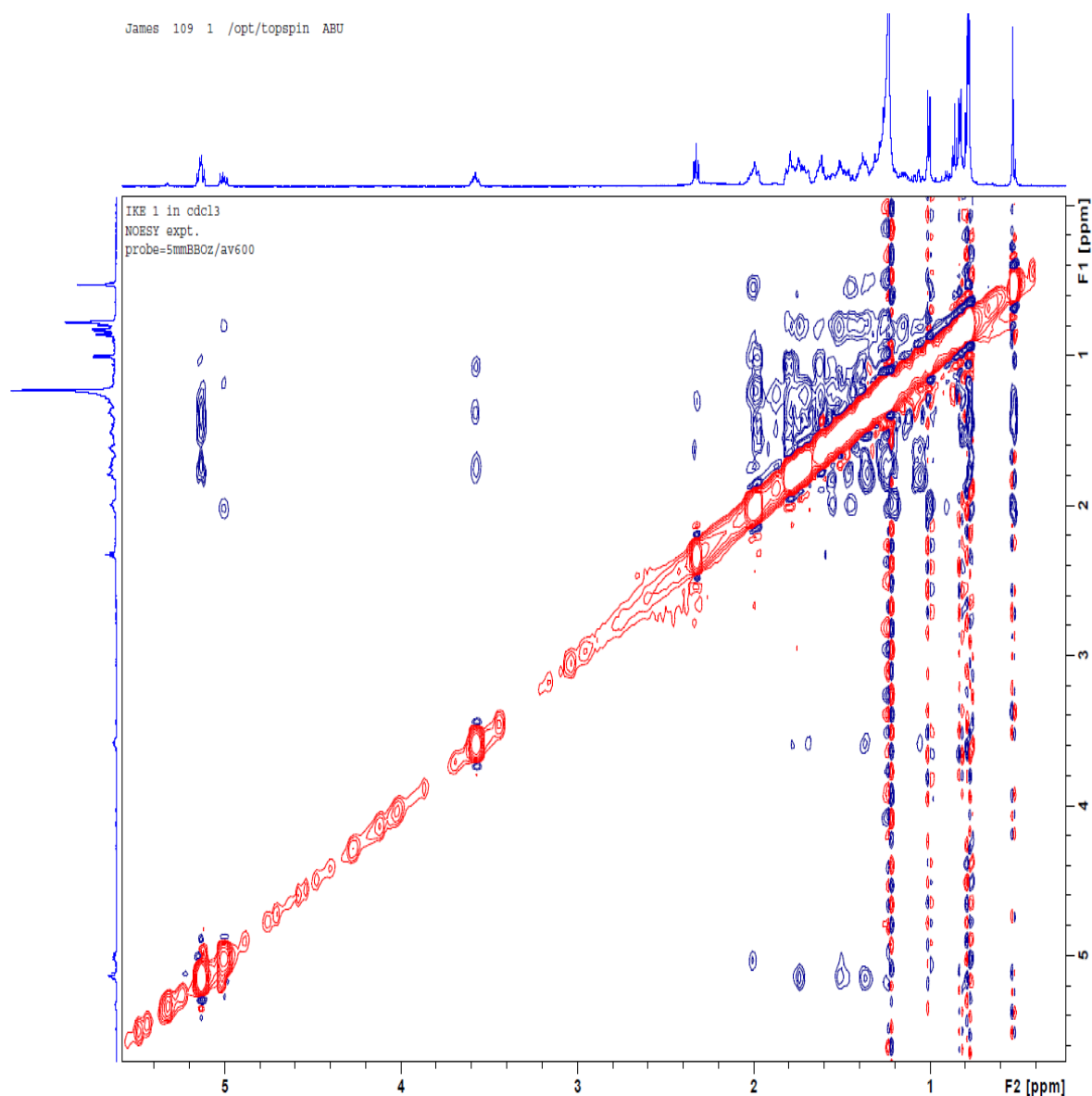
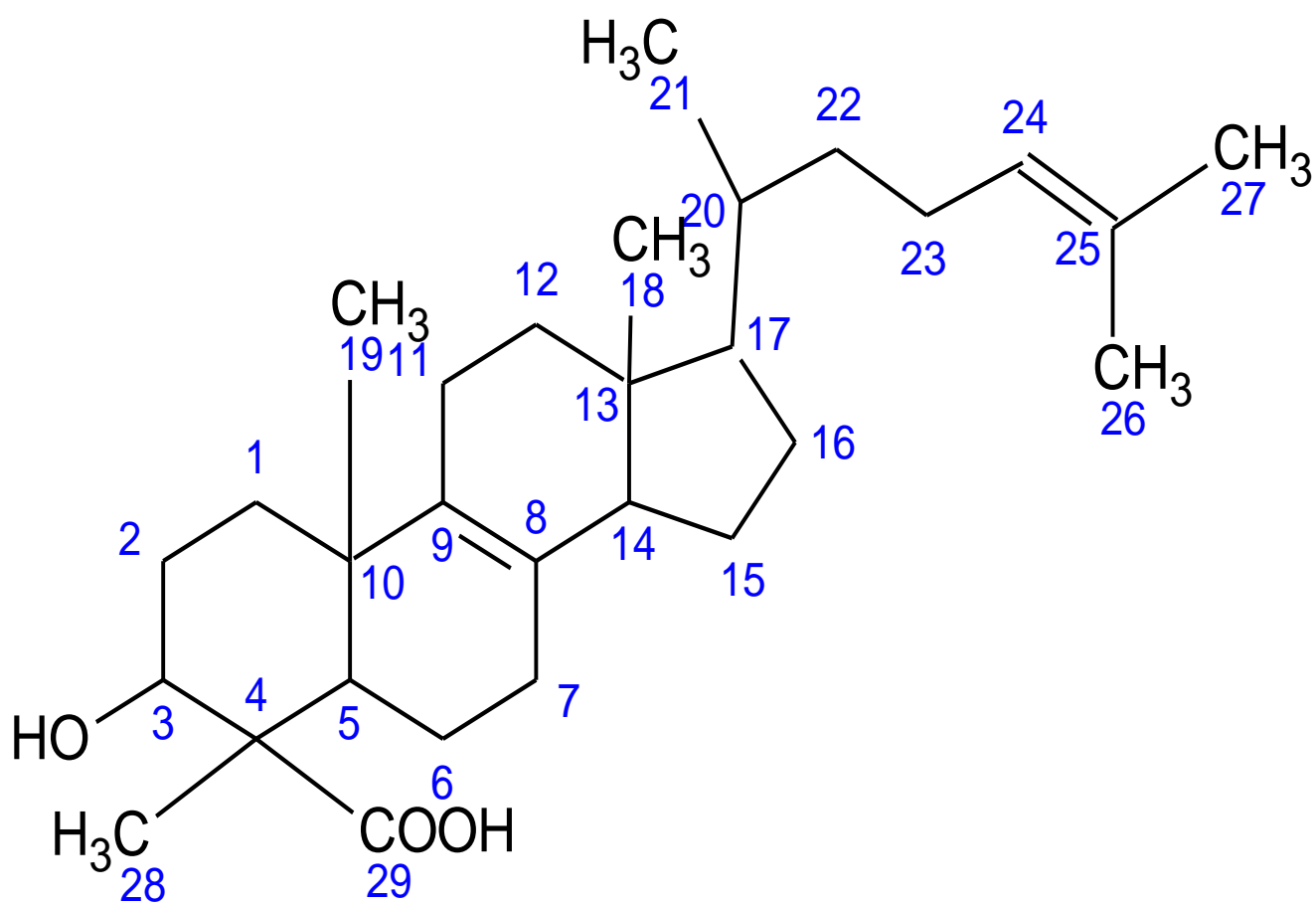


Figure 4.7: NOESY of IKE1

Table 4.8: ^{13}C NMR and ^1H NMR assignment of IKE1

Position	Zymosterol Literature value Ulrike <i>et al.</i> , 1981	Zymosterol Experimental value	Zymosterol Experimental value.
C ₁	35.10	37.16	
C ₂	31.50	31.48	
C ₃	70.90	71.09	3.50
C ₄	38.20	43.30	
C ₅	40.70	31.93	
C ₆	25.50	51.26	
C ₇	27.10	31.89	
C ₈	128.00	117.48	
C ₉	134.80	139.59	5.01
C ₁₀	35.60	34.24	
C ₁₁	22.80	21.10	
C ₁₂	36.90	39.48	
C ₁₃	42.00	43.30	
C ₁₄	51.80	55.93	
C ₁₅	23.70	24.73	
C ₁₆	28.70	28.52	
C ₁₇	54.70	55.15	
C ₁₈	11.20	12.06	
C ₁₉	17.80	19.00	
C ₂₀	36.00	40.28	
C ₂₁	18.60	21.39	
C ₂₂	36.00	39.48	
C ₂₃	24.70	31.93	

C ₂₄	125.00	138.18	5.98
C ₂₅	130.60	129.47	5.21
C ₂₆	17.60	22.70	
C ₂₇	25.70	23.03	
C ₂₈		14.12	
C ₂₉	Acetate 170.66	177.43	



Name: 3 β -hydroxy-4 β -methyl-5 α -cholesta-8, 24-diene-4 α -carboxylic acid

Or 3 β -hydroxy-30-norlanosta-8, 24-dien-29-oic acid

IKE1-(C₂₉H₄₆O₃, 442.7 g/mol)

Figure 8: IKE 1: Zymosterol

CHAPTER FIVE

5.0 DISCUSSION

5.1 Phytochemical screening

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowra, 1993c). Such as carbohydrates, tannins, saponins, cardiac glycosides, steroid, triterpenes and alkaloids. The presence of these could account for high antimicrobial activity demonstrated by the plant. Tannins, saponins and alkaloids have been reported to have pronounced physiological effect particularly on the nervous system (Simkin *et al.*, 2008).

The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation (Just *et al.*, 1998). Saponins have the ability of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of

foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Okwu, 2004; Sodipo *et al.*, 2000).

Steroid and Triterpenes have been reported to have antibacterial properties (Raquel, 2007), and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001b). Triterpenes have important biological roles, for instance the triterpene lanosterol is the precursor from which steroid hormones are made in nature and the tetraterpene β -carotene is a major dietary source of vitamin-A McMurry, 1992, Waterman and Grundon 1983 reported that triterpenes have some chemotaxonomic properties (Harborne, 1983).

Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is cytotoxicity (Nobori *et al.*, 1994), analgesic (Antherden, 1969; Harborne, 1973), antispasmodic and antibacterial (Okwu *et al.*, 2004; Stray, 1998); properties of alkaloids. Glycosides are known to lower the blood pressure according to many reports (Nyarko *et al.*, 1990).

The presence of these phytochemicals in *Pachystela brevipes* extracts suggests that the plant is pharmacologically active, supporting the claim by the traditional healers proving the plant to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

5.2 Antimicrobial Screening of the crude extracts

The antimicrobial and sensitivity test of the crude extracts of the root parts of *Pachystela brevipes* were carried out using fifteen pathogens namely:- *Staphylococcus aureus*, *Streptococcus faecalis*, *Streptococcus pyogenes*, *Mettricillin resist. staph. aureus*, *Corynebacterium ulcerans*, *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Candida tropicalis*, *Candida stellatoidea*,

Candida krusei, *Protus mirabilis*, and *Proteus vulgaris* . The results obtained are shown in tables 4.3 - 4.6.

The result of the antimicrobial tests (Table 4.4) showed that all the extracts had significant activity against the micro-organisms with zones of inhibition ranging between 16 and 27 mm. The ethyl acetate extract was observed to be the most active with the zone of inhibition of 27 mm against *K. pneumoniae*, it also exhibited the highest activity against the fungus *C. stellatoidea* with a zone of inhibition (ZI) of 24 mm. When compared with pet ether, chloroform and methanol extracts which had a zones of inhibition of 18 mm, 22 mm, and 20 mm respectively against the fungus *C. stellatoidea*. A range of 20-24 mm ZI was observed for *Mettricillin resistant staphylococcus aureus*, *Staphylococcus aureus*, *Escherichia coli*; *Salmonella typhi*, *Shigella dysenterea*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* *Candida stellatoidea*, *Candida tropicalis* and *Streptococcus feacalis* which showed zones of inhibition in all the plant extracts except *Proteus vulgaris*, *Proteus mirabilis*, *Candida krusei* and *Streptococcus pyogenes* which showed no zone of inhibition in all the four extracts of the plant.

The result of the determination of minimum inhibition concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) shown in Tables 4.5 and 4.6 shows that all the plant extracts inhibited and completely kill *Mettricillin resistant staphylococcus aureus*, *Staphylococcus aureus*, *Escherichia coli*; *Salmonella typhi*, *Shigella dysenterea*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* *Candida stellatoidea*, *Candida tropicalis* and *Streptococcus feacalis* at various concentrations; pet ether (MIC = 5.0 mg/mL, MBC = 10.0 mg/mL), chloroform (MIC = 2.50 mg/mL, MBC = 5.0 – 10.0 mg/mL), ethyl acetate (MIC = 2.50 mg/mL, MBC = 5.0 – 10mg/ML), methanol (MIC = 2.50 mg/mL, MBC = 5.0 – 10 mg/mL) respectively. From the results, the ethyl acetate fraction was the most active while Pet ether fraction was the least active.

This shows that this extract has antifungal effect at the highest concentration against the test fungi used in this research.

The result also shows that the extracts can be used to treat infections caused by these test bacteria and fungi. For example, infections caused by *Salmonella typhi* and *Escherichia coli*, such as typhoid fever (enteric fever), food poisoning, gastro-enteritis, urinary tract infections and other infections in which ciprofloxacin is used for treatment.

The broad spectrum exhibited by the root part of the plant *Pachystela brevipes*, as shown in the antimicrobial results lend credence to the traditional uses of the plant in folk medicine. The plant contains anti-pathogenic substances which can be isolated and their toxicities studied to ensure their safe usage, and to ascertain that they are not toxic to humans.

5.3 Antimicrobial screening of the isolated compound

The antimicrobial and sensitivity test of the pure extract of the root part of *Pachystela brevipes* were carried out using twelve pathogens namely; *Staphylococcus aureus*, *Streptococcus faecalis*, *Streptococcus pyogenes*, *Corynebacterium ulcerans*, *Bacillus Subtilis*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Candida albicans*, *Candida krusei*, and *Proteus mirabilis*. The result of the antimicrobial tests (Table 4.7) showed that the isolated extract of the root part of *Pachystela brevipes* had significant activity against the micro-organisms with zones of inhibition ranging between 22 and 29 mm. The isolated extract was observed to have the highest zone of inhibition of 29 mm against *B. subtilis* followed by *K. pneumonia* which had a Zone of inhibition of 28 mm. It also exhibited a zone of 24 mm against the fungus *C. albicans*. A range of 22 – 26 mm zone of inhibition was observed for *Staphylococcus aureus*, *Streptococcus faecalis*, *Corynebacterium ulcerans*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Candida albicans*, which showed zones of inhibition in all the plant extracts except *Candida*

krusei, *Streptococcus pyogenes* and *Proteus mirabilis* which showed no zone of inhibition in the isolated extract of the plant.

The result of the determination of minimum inhibition concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) shown in Tables 4.7 shows that the isolated compound inhibited and completely kill the test organisms at various concentrations; *Staphylococcus aureus* (MIC = 10 mg/ml, MBC = 20 mg/ml), *Streptococcus feacalis*, (MIC = 10 mg/mL, MBC = 20 mg/mL), *Corynebacterium ulcerans* (MIC = 10 mg/mL, MBC = 40 mg/mL) *Bacillus Subtilis* (MIC = 5 mg/mL, MBC = 10 mg/mL), *Escherichia coli* (MIC = 10 mg/mL, MBC = 20 mg/mL), *Salmonella typhi* (MIC = 10mg/mL, MBC = 20 mg/mL), *Pseudomonas aeruginosa* (MIC = 10mg/mL, MBC = 40 mg/mL), *Klebsiella pneumonia* (MIC = 5 mg/mL, MBC = 10 mg/mL), and *Candida albicans* (MIC = 10 mg/mL, MBC = 20 mg/mL) respectively.

This implies that the isolated compound has a high activity and would therefore kill the organisms. It also means that the isolated compound would be very effective in curing diseases caused by any of these organisms. The plant is to some extent fungicidal in action.

5.4 SPECTROSCOPY

Norlanosta dien oic acid (IKE 1); this was a white crystalline substance. The NMR analyses (1D and 2D) confirmed that the compound was 3 β -hydroxy-30-norlanosta-8, 24-dien-29oic acid or 3 β -hydroxy-4 β -methyl-5 α -cholesta-8, 24-diene-4 α -carboxylic acid. The proton decoupled ¹³C-NMR spectrum showed 29 carbon atoms (Table 4.9). The Distortionless Enhancement Polarization Transfer (DEPT).subpectrum indicated 10 methine (CH) carbon, eight methylene (CH₂) and six methyl (CH₃) groups. Quaternary carbon atoms do not contain attached protons hence do not appear in DEPT subpectrum. They may be identified as the

signals which are additionally in the proton broadband decoupled ^{13}C -NMR spectra. Therefore five (5) quaternary carbons were identified.

From the ^1H NMR spectra, it showed the presence of one oxymethine proton at δ 3.78 (multiplet). It also showed the presence of olefinic protons at δ 5.01, 5.19 and 5.21 (multiplet). The signal from δ 0.35-2.30 (multiplet) are due to the presence of overlapping methyl, methylene and methine protons respectively.

From the spectral analysis of IKE1, the ^{13}C NMR shows the presence of twenty nine carbon atoms (table 4.8, figure 4.2). The chemical shift at δ 71.09 indicated the presence of an oxymethine carbon. The signal at δ 139.59, 117.48 138.18 and 129.47 respectively indicates the presence of olefinic carbons. The signals from δ 21.10, 31.48, 37.16, 31.89, 39.48, 24.73, 28.52, 39.48 and 31.93 are probably due to CH_2 groups, while the CH_3 methyl groups are indicated by the following signals δ 12.06, 19.00, 21.39, 22.70, 23.03 and 14.12. The chemical shift signal at δ 177.43 indicated the presence of a carboxylic acid respectively.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The root part of *pachystela brevipes* collected from Okpokwu in Benue State, was properly identified at the Herbarium unit of department of Biological science A.B.U. Zaria with a Voucher number 7106. The root was subjected to air drying, pulverization and extraction. The crude extracts obtained were further subjected to preliminary phytochemical screening and then antimicrobial evaluation. Ethyl acetate being the most active of all was subjected to chromatographic separations where compound IKE 1 was obtained, which after undergoing some spectroscopic analysis revealed to be a steroid called Zymosterol.

6.2 Conclusion

This research into the use of *Pachystela brevipes* for the treatment of infectious diseases has achieved the stated aim. Although Zymosterol must have been isolated from different family of plants previously, this is the first report of its isolation from the *Sapotaceae* family. This work will therefore add to the global database of natural products.

Based on the findings in this research work, it can be stated that the use of *Pachystela brevipes* in the treatment of infectious diseases is justified.

6.3 Recommendation for further work

3 β -hydroxy-3 α -norlanosta-8, 24-dien-29-oic acid or 3 β -hydroxy-4 β -methyl-5 α -cholesta-8, 24-diene-4 α -carboxylic acid was isolated from the root part of *Pachystela brevipes*. Its bioactivity is yet to be fully established and there is the need for further work to be carried out in order to achieve this and possibly integrate it into orthodox chemotherapeutic preparations.

We wish to recommend that further work should be extended to the plant leaves, stem bark, and the fruits to also justify the general use of the plant as medicinal plant.

Also there is the need to further isolate, elucidate and utilize the various components in this root part which were not yet isolated and established in the course of this work and

Other biological and pharmacological studies should be carried out on IKE 1

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