PHARMACOGNOSTIC AND ANTIMICROBIAL STUDIES ON THE STEM-BARK OF *FICUS KAMERUNENSIS* WARB. (MORACEAE)

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NOVEMBER, 2015

DECLARATION

I declare that the work in this Dissertation entitled "**Pharmacognostic and Antimicrobial Studies on the Stem-bark of *Ficus kamerunensis* Warb. (Moraceae) "** has been carried out by me in the Department of Phamarcongosy and Drug Development. The information derived from the literatures has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this in any other Institution.

_____ _____

Uwaisu Iliyasu Date

CERTIFICATION

This dissertation entitled "**PHARMACOGNOSTIC AND ANTIMICROBIAL STUDIES ON THE STEM-BARK OF *FICUS KAMERUNENSIS* WARB. (MORACEAE)"** written by Uwaisu ILIYASU meets the regulation governing the award of Master of Science in Pharmacognosy of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research work is dedicated in the fond memory of my beloved Father, late **ALHAJI ILIYASU ADAMU KAJURU** (The Chief Imam of Kajuru). May his gentle soul rest in Jannatul Firdaus, amin.

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ABSTRACT

Ficus kamerunensis is an epiphytic shrub or tree growing up to 20 m high. The plant used in ethnomedicine to treat microbial infections such as sexually transmitted infections. Pharmacognostic studies were conducted on the fresh and dried whole stem-bark of *F. kamerunensis.* The quantitative physical standards were carried out. Preliminary phytochemical screenings of the powdered stem bark of the plant were conducted using appropriate chemical reagents. The powdered stem bark (1 kg) of the plant was extracted using cold maceration technique with 3 L of 95% methanol in a glass jar for 3 days (72 hours) at room temperature, then partitioned with diethylether, ethyl acetate and n-butanol. Thin-layer chromatography was carried out using diethyl ether fraction to obtain the best solvent system to be used for column chromatography. A combination of column chromatography and preparative thin layer Chromatography using Column chromatography were carried out using the best solvent system obtained (Hexane: Ethyl acetate 7:3) for compound isolation. The structure of the isolated compound was determined by the analysis of ^{1}H NMR, ^{13}C NMR, and 2D NMR spectral data, as well as comparison with reported data. Antimicrobial evaluations of the stem-bark of the plant were carried out for diethyl ether, ethyl acetate, n-butanol and 95% methanol extract at different concentrations (50, 25, 12.5, 6.25 and 3.125 mg/ml) and tested against four Gram-positive bacteria—*Staphylococcus aureus, Streptococcus pyrogenes, MRSA,* and *Neisseria gonorrheae*, and four Gram-negative bacteria—*Corynebacterium ulcerans, E. coli, Pseudomonas aeroginosa* and *Proteusmirabilis* as well as four fungi— *Candida albicans, Candida krusei, Candida stellatoidea* and *Candida tropicalis.* Mc-farland's turbidity standard scale (number 0.5) was used to standardize the test micro organisms. Agar Diffusion method was used to screen the extracts while the minimum inhibitory concentrations (MIC) as well as minimum bactericidal and fungicidal concentrations (MBC/MFC) were determined using broth dilution method.The acute

toxicity studies (LD_{50}) was carried out using Lorke method. Phytochemical screening of the powdered stem-bark of *Ficus kamerunensis* reveals the presence of carbohydrates, anthraquinones, saponins, cardiac glycosides, terpenoids, flavonoids, tannins and alkaloids. Physicochemical studies showed moisture content, total ash acid insoluble ash, water soluble ash, alcohol extractive and water extractive values of 11.7, 8.8 1.7, 3.7, 10.4 and 18.4 % respectively. Antimicrobial evaluation shows that the extracts have activity against *E. coli*, *Candida albican*, *Candida stellatodea*, *Corynebacterium ulcerans*, *MRSA*, *Neisseria gonorrhea*, *Pseudomonas aeroginosa Staphylococcus aureus* while *Candida tropicalis*, *Candida krusei*, *Proteus mirabilis* and *Streptococcus pyrogens* were resistant to the tested extracts. Known compound (pentacyclic triterpene) α-amyrin acetate was isolated and characterized from the diethyl ether fraction of the methanol extract of the plant material.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ACRONYMS AND ABBREVIATIONS

Acronym	Meaning
ABUTH	Ahmadu Bello University Teaching Hospital
$AlCl_3$	Aluminium Chloride
$CDCl_3$	Deuterated Chloroform
CF	Column Fraction
CP	Ciprofloxacin
COSY	Correlation Spectroscopy
DE	Diethyl ether
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethyl Sulfoxide
EA	Ethyl acetate
e.t.c.	Etcetera
et al	and coworkers
FC	Fluconazole
F.A.W.	Formalin: Acetic acid: Water
$FeCl_3$	Ferric Chloride
HCl	Hydrochloric Acid
H_2S0_4	Tetra Oxosulphate vi acid
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Correlation

i.p	Intra Peritoneal
LD_{50}	Median Lethal Dose
NB	n-Butanol
NILEST	National Institute of Leather Science and Technology
ME	Methanol
MDRS	Multi Drug Resistant Strains
ml	milli litre
mm	milli mitre
MRSA	*Methicillin Resistant Staphylococcus Aureus*
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
R_f	Retention Factor
STI	Sexually Transmitted Infection
T.S	Transverse Section
L.S	Longitudinal Section
TLC	Thin Layer Chromatography
USEPA	United States Environmental Protection Agency
USDA	United State Department of Agriculture
v/v	Volume by Volume
w/w	Weight by Weight
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

The World Health Organization (WHO) estimates that up to 80% of the population in Africa make use of traditional medicine as well as about 65% of the world's population (Fabricant and Farnsworth, 2011). Plants used in traditional medicine, also called phytomedicine are plant-derived medicines that contain chemicals, more usually, mixtures of chemical compounds that act individually or in combination on the human body to prevent disorders and to restore or maintain health (Van- Wyk, *et al*,1997). About 3.4 billion people in the developing world depend on plant based traditional medicines. This represents about 88 percent of the world's inhabitants who relied mainly on traditional medicine for their primary health care (WHO, 1999). Owing to poverty, unawareness and unavailability of contemporary health facilities, most people, especially rural people are still compelled to practise traditional medications for the treatment of their day to day illnesses (Khan, *et. al*, 1993).

Natural products (secondary metabolites) have been the most successful source of potential drug leads. However, their recent implementation in drug discovery and development efforts have somewhat demonstrated a decline in interest (Mishra *et.al,* 2012). Nevertheless, natural products continue to provide unique structural diversity in comparison to standard combinatorial chemistry, which presents opportunities for discovering mainly novel low molecular weight lead compounds. Since less than 10% of the world's biodiversity has been evaluated for potential biological activity, many more useful natural lead compounds await discovery with the challenge being how to access this natural chemical diversity (Cragg and Newman, 2005).

1.1 Pharmacognosy

According to American Society of Pharmacognosy "Pharmacognosy is the study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs substances of natural origin as well as the search for new drugs from natural sources" (Tyler, 1999). As practised today, pharmacognosy includes the extensive study of natural products from plants, bacteria, fungi and marine organisms,botanical dietary supplements as well as herbal remedies (Cardellina, 2002). Pharmacognosy can also be defined as "the scientific and systematic study of physical, chemical, structural and biological features of crude drugs as well as their history, method of cultivation, collection and preparation for commercial purposes"(Gokhale *et al*., 2002). It is the science which provides infrastructure for the evolution of novel medicines. It is a long-established pharmaceutical science which has played an alternative role in finding, characterization, standardization and manufacturing of plant material as well as phytomedicines regarding their macroscopic, microscopic and biochemical characteristics (Kaplan, 2001; Kinghorn, 2004; Gokhale *et al*.,2002). Pharmacognosy is the scientific study of crude drugs originated from three different natural sources namely plants, animals and minerals. It is estimated that 90% of the crude drugs are originated from plant sources while the remaining are from other two sources (Joyce *et al*., 2002).

Plants have been considered as potential source of medicines for curing various ailments and disorders since the dawn of civilization and led to the establishment of the conventional knowledge of plants all around the sphere. Initially these medicines were utilized in the form of crude drugs, poultices, teas, tinctures, powders, and other herbal formulations. The particular plants to be used and the methods of application for a specific ailment were passed down through verbal communication (Ahmad *et al.*, 1998; Balick and Cox, 1997; Samuelsson, 2004). Plants containing inborn potentially active ingredients used to cure

disease or relieve pain are called medicinal plants (Okigbo *et al.,* 2008). Plants play a therapeutic and restorative role in protecting human beings from the adverse effects of diseases and other complications, thus considered to have a beneficial role in healthcare system. That is the reason that large proportion of population of the developing countries still rely on herbal medicines. Despite their importance, medicinal plants are seldom handled within an organized manner and most are of them are exploited with little or no respect for the future (Srivastava *et al.,* 1996; Nair *et al.,* 2012).

Plants are the natural and most easy accessible source of therapeutically active biological principles, thus there is a need to screen out plants for development of new drugs. For this purpose plants have been assayed widely but still large number of them have not arrived to the conventional health care system (Esimone *et al*, 2003; Bhattarai *et al*., 2006).

Pharmacognosy is also considered as a good example of a modern discipline that could serve to arouse the interesting medicinal sciences. Increased interest in the study of natural products in drug development, as well as rapidly altering investigation strategies are the driving forces, modernizing the pharmacognosy. Pharmacognosy, now a days focuses on finding novel and unique molecules and revealing unknown targets by studying such molecules in nature. It is now well understood that pharmacognosy is one of several scientific disciplines that have strategic position in connecting biology with chemistry and even medicine. New and improved strategies regarding the selection of organism, bioassays techniques, isolation procedures, and structure elucidation are constantly devoloped based on the latest advancements in pharmacognosy (Bruhn and Bohlin, 2004). Pharmacognosy provide basis for the study of secondary metabolites (natural product molecules) which are beneficial for their ecological, medicinal, gustatory or other functional properties. The natural species which are the basis for medicinally important compounds are of the origin of biological kingdoms, particularly marine invertebrates, plants, fungi, and bacteria.

The field of Pharmacognosy is not limited to special area and is constantly being re-invigorated by input from time to time by new developments in scientific fields and technologies. This is the reason that now a days Pharmacognosy is a good option for those who like to work at the interface of many diverse but harmonizing branches of science that relate to the natural world (Kinghorn, *et. al* 2004; Samuelsson, 2004).

Pharmacognostic study of crude drugs involves five customary parameters i.e. the botanical, organoleptic, physical, chemical, biological, pharmacological and microbiological parameters. These parameters used to disseminate the unique features of crude drugs in three different stages namely identification, isolation of compound or active principles and screening for biological activities.

Now a days, due to advancement of modern and new sophisticated methods, plant scientists are taking more intrest in exploring new drugs from natural and biologically active compounds of the plants ,which could be serve as inexhaustible resources for pharmaceutical industries (Yakubu *et al.,* 2007).Therefore, search for new drugs from microorganisms, fungi, plants and animals must be persistent and these can be the sources of innovative and prevailing restorative agents for newer, safer and accessible drugs (Lindequist *et al.,* 2005).

1.2 Pharmacognostic study

In the last few decades there has been an enormous development in the field of herbal medicine. It gets commercializing in developing and developed countries due to its natural derivation and less significant side effects. Herbal drugs play a significant role in health care programs, especially in developing countries (Mulla and Swamy, 2010). Because of the popularity, herbal praparations for various ailments are now being prepared on a large scale in mechanical units, where availability of good quality and authentic raw materials, availability of standards, appropriate standardization procedure of drugs and formulations,

quality control parameter etc are some of the problems faced by the manufacturer. Due to these discrepancies, it is now necessary to make efforts for the standardization of the plants materials to be used as medicine. This standardization procedure can be achieved by stepwise pharmacognostic assessment (Ali *et al.,* 2011; Agrawal *et. al*, 2007). Despite the modern techniques, standardization and authentication of plant drugs by pharmacognostic procedures is more trustworthy. The morphological and anatomical description of a medicinal plant is the first step towards standardization of plant materials and should be carried out before the commencement of any experimental procedure for the detection of adulterations and impurities (WHO, 1996).

1.3 Medicinal plants

Medicinal plants have been the mainstay of traditional herbal medicine amongst rural dwellers worldwide since antiquity to date. One of the ancient authors who described medicinal natural product of plant and animal origins, listed approximately 400 different plant species for medicinal purposes. Natural Product have been an integral part of the ancient traditional medical system, e.g Chinese, Ayurvedic and Egyptian (Sarker and Nahar, 2007).

Medicinal plants are those plants that possess medicinal properties to cure various ailments. These plants are untapped rich sources of biologically active compounds (phytochemicals) vital to human health. The phytochemicals, usually referred to as secondary metabolites of the plants, have no nutritive value in plants. These are the compound that possess the diverse medicinal properties of the plants. The common phytochemicals include saponins, alkaloids, tannins, terpenoids, and flavonoids among others. These compounds are widely distributed in the plant kingdom and are usually found at varying concentrations in different parts of plants (Kee *et al.,* 2007).

Medicinal plants are increasingly gaining acceptance even amongst the literates in urban settlements, probably due to increasing inefficacy of many orthodox drugs used for control of infections such as typhoid fever, gonorrhea and tuberculoses as well as increase in resistance by several bacteria to various antibiotics and increasing cost of prescription drugs, for maintanance of personal health (Levy, 1998 ; Van den Bogaard and Stobberingh 2000 ; Smolinski *et. al*, 2003). Medicinal plants as primary sources, contribute more than 50% of all drugs currentlyin clinical use and higher plants contribute no less than 25% of the total (Van-Wyk *et al.*, 1997). Well known examples of plant-derived drugs include aspirin, morphine, cocaine, reserpine, berberine, atropine, taxol, vincristine and so forth. Traditional healers play an integral role in providing leads to the discovery of new bioactive plant-derived compound. The process of selecting plants with desired compounds and or bioactivity would be very difficult and time consuming without assistance from the traditional healers (Rajadurai *et al*. 2009; Van-Wyk *et al.*, 1997).

1.4 Phytochemistry

Phytochemistry essentially deals with the enormous different types of organic substances that are not only elaborated but also accumulated by plants. The detailed phytochemical study of an unknown plant may be accomplished right from elucidation of chemical structures of pure constituents to the elaborated study of their biological characteristics (Ashutosh, 2007).

The biosynthesis and breakdown of proteins, fats, nucleic acids and carbohydrates, which are essential to all living organisms, is known as primary metabolism with the compounds involved in the pathways known as “primary metabolites” (Dewick *et. al* 2008). Secondary metabolites are generally not essential for the growth, development or reproduction of an organism and are produced either as a result of the organism adapting to its surrounding environment or are produced to act as a possible defense mechanism against predators to assist in the survival of the organism (Colegate and Molyneux, 2006). The biosynthesis of

secondary metabolites is derived from the fundamental processes of photosynthesis, glycolysis and the Krebs cycle to afford biosynthetic intermediates which, ultimately, results in the formation of secondary metabolites also known as natural products (Dewick *et. al*, 2008). It can be seen that although the number of building blocks are limited, the formation of novel secondary metabolites is infinite. The most important building blocks employed in the biosynthesis of secondary metabolites are those derived from the intermediates: Acetyl coenzyme A (acetyl-CoA), shikimic acid, mevalonic acid and 1-deoxyxylulose-5-phosphate. They are involved in countless biosynthetic pathways, involving numerous different mechanisms and reactions (e.g. alkylation, decarboxylation, aldol, Claisen and Schiff base formation (Dewick *et. al*, 2008). It is hypothesized that secondary metabolism utilizes amino acids and the acetate and shikimate pathways to produce "shunt metabolites" (intermediates) that have adopted an alternate biosynthetic route, leading to the biosynthesis of secondary metabolites. Modifications in the biosynthetic pathways may be due to natural causes (e.g. viruses or environmental changes) or un-natural causes (e.g. chemical or radiation) in an effort to adapt or provide longevity for the organism (Sarker *et.al*, 1995).

1.5 Antimicrobial activities

Herbal drugs are now considered as an alternative in such situations (Sofowora, 1993). Now it is of great importance to explore effective treatments of microbes. Researchers aretherefore taking much attention in folk medicine in search of better drugs against microbial infections (Srinivasan *et al.,* 2000).

1.6 Antimicrobial Agents of plant origin

A number of plants contain compound that have antimicrobial property (Khan *et al.,* 2003). Compounds such as emetine, berberine and qunine which are derived from plants are very effective for the infectious microbes (Iwu *et al.,* 1999). Plants extracts from more than 157 plant families have been described which have potential antimicrobial properties (Narayana

et., al., 2011). Below are some examples of plants which have proven antimicrobial effects (Mahmood and Muhammad, 2013).

Table 1.1: Some medicinal plants with antimicrobial activities

Plant Name	Part used	Solvent	Organism	Reference
Balanites aegyptiaca and Moringa oleifera	Leaves	Ethanol Water and Acetone	*Salmonella typhi*	Doughari, 2006.
Allium sativum Zingiber officinale and Citrus aurantifolia	Fruits and Rhizome	Water and Ethanol	*Bacillus spp., Staphylococcus aureus, Escherichia coli, Salmonella spp.*	Onyeagba *et al.*, 2004.
Ximenia americana	Leaves	Ethanol	*Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus subtilis, Staphylococcus aureus, Candida albicans*	Ogunleye and Ibitoye, 2003.

Syzyium aromaticum, Cinnamomum cassia, Salvia officinalis, Thymus vulgaris and Rosmarinus officinalis	Seeds, bark and Leaves	Water, Methanol and Ethanol	*Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis*	Abu-shanab *et al.*, 2004.
Parthenium hysterophorus, Stevia rebaudiana and Ginkgo biloba	Leaves	Methanol, ethanol and dichloro-methane	*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis, Enterococcus, and Staphylococcus aureus*	Fazal *et al.*, 2011.

1.7 Statement of the Research Problem

Natural products continue to provide unique structural diversity in comparison to chemistry, which presents opportunities for discovering mainly novel low molecular weight lead compounds. Since less than 10% of the world's biodiversity has been evaluated for potential biological activity, many more useful natural lead compounds await discovery with the challenge being how to access this natural chemical diversity (Cragg and Newman 2005).

Current problems associated with the use of antibiotics, increased prevalence of multiple-drug resistant (MDR) strains of a number of pathogenic bacteria such as methicillin resistant *Staphylococcus aureus*, *Helicobacter pylori*, and *Klebsiela pneumonia* has revived the interest in plants with antimicrobial properties (Voravuthikunchai and Kitpipit, 2003).

Microbial infections are major global causes of acute illness, infertility, long term disability and death of men, women and infants (WHO, 2013).

Sexually transmitted infections (STIs) are among the most common and unpleasant infectious disease in young adult in both industrialized and developing countries. In Africa, each year some 340 million new cases of STIs occur. Nigeria is among the countries with high prevalence according to WHO report (WHO, 2013).

1.8 Justification of the research

Overtime, inappropriate use of antimicrobial drugs has resulted in development of multi-drug resistance by most microorganisms. Such drugs are also associated with varied adverse effects including hyper sensitivity reaction, allergic reaction and immune suppression (Ahmad *et al*, 1998).

The global emergence of multi-drugresistant (MDR) bacteria significantly causing treatment failure due to increasingly limiting the effectiveness of current drugs

(Hancock, 2005).This situation forced scientists to search for new antimicrobial substances. There is a constant need for new and effective therapeutic agents (Bhavnani and Ballow, 2000). Therefore, there is a need to develop alternative antimicrobial drug for the treatment of infectious diseases from medicinal plants (Cordell, 2000).

*Ficus kamerunensis*is considered for the current study being one out many medicinal plants that is use by the traditional medical practioners for the treatment of microbial infections but yet to be proven scientifically.

1.9 Hypothesis

Ficus kamerunensis contains bioactive compounds which are responsible for its antimicrobial properties.

1.10 Aim

The aim of this research work is to provide scientific bases in the use of *F. kamerunensis* for the management of bacterial infections by the traditional medical practioners and herbalist as well as the pharmacognostic standards for monograph.

1.11 Objectives

1. To evaluate the pharmacognostic features of the stem-bark of *F. kamerunensis*
2. To evaluate the chemical constituent(s) of the stem-bark of the plant
3. To evaluate the methanolic extract, partitioned fractions and major column fractions of *F. kamerunensis* for antimicrobial activities.
4. To evaluate the acute toxicity of the plant.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of Moraceae Family

The Moraceae family consists of about 50 genera and nearly 1400 species including important groups such as Artocarpus, Morus and Ficus (Venkataraman, 1972).They are usually woody plants with alternate leaves, conspicuous and milky latex. The flowers are unisexual, with or without sepals, 1-4 stamens and one independent ovule in a uniocular ovarium.They are arranged in many kinds of inflorescence, such racemens in Scocea and synconia in Ficus, and monoecy, androecy and gymnodioecy seem to have evolved multiple times in the history of the family from ancestral dioecy (Datwyler and Weiblem, 2004).Moraceae exhibit an amazing diversity of morphological and life history traits, particularly inflorescence architectures, breeding systems, and pollination syndromes (Berg, 1998). Recent studies of Moraceae using DNA sequences have reconstructed the evolutionary history of the family to clarify relationships among genera, to identify the closest relatives of the figs (Datwyler and Weiblen 2004), and to examine biogeography (Zerega *et al*. 2005).

2.2 Description of the Genus Ficus

Ficus is a genus of about 800 species and 2000 varieties of woody trees, shrubs and vines in the family Moraceae occurring in most tropical and subtropical forests worldwide (Hamed, 2011). Many Ficus species consists of numerous varieties, significant genetic diversity, outstanding pharmacological activities which are of remarkable commercial importance (Woodland, 1992). Collectively they are regarded as figs, and produced sap, leaves and flower buds (Ijeh and Ukweni, 2007). Most of

them are found growing very well on rocks, bush, and swamps and on hard surfaces (Abbiw, 1990). They are mainly trees ranging from 21–50 m high (Aluka, 2008). They produce flowers between August and February of each year, and the flowers are borne inside the plant, which is a unique chacteristic among the figs and they are propagated by seeds (Bouquet, 1969). A number of ficus species are used as food and are utilized for their medicinal properties especially amongst people where these species grow (Lansky *et.al,* 2008).In Africa, the plants are distributed in South Africa, Mozambique, Zimbabwe, Botswana, Nigeria, and Sudan (Berg, 1992). In Nigeria there are over 45 different species of *Ficus* were reported by Keay and Onochie (1964).

2.3 Nutritional Properties of Ficus

Fig is very nourishing and is used in industrial products in food industries. It is rich in vitamins, mineral elements, water, and fats. Figs are amongst the highest plant sources of calcium and fiber. According to USDA data for the Mission variety, dried figs are richest in fiber, copper, manganese, magnesium, potassium, calcium, and vitamin K, relative to human needs. Many other nutrients are also present in smaller quantities (Vinson, 2009).

2.4 Some Medicinal properties and uses of the Genus Ficus

Figs are reported to posses' laxative effects and antioxidant properties. They are good source of flavonoids and polyphenols (Vinson, 2009). Figs are also reported to be used in various disorders, such as gastrointestinal, respiratory, inflammatory, cardiovascular disorders so also ulcerative diseases, and cancers (Rubnov *et.al,* 2005). Specifically, the roots are used in treatment of ringworms where as the fruits used as antipyretic, purgative, aphrodisiac and have shown to be useful in inflammations and paralysis (Kirtikar and Basu 1996).They have been reported to have antioxidant, antiviral,

antibacterial, hypoglycemic, cancer suppressive and antihelminthic effects (Solomon *et al*, 2011).

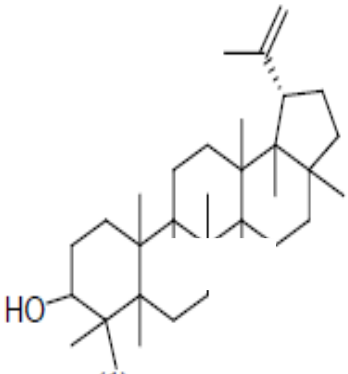
Antimicrobial activities of some members of the genus have been reported, example, the ethanol leaf extract of *Ficus exasperate* is reported to inhibit the growth of *E. coli* at a dose of 300 mg/ml while that of *S. albus* was at 700 mg/ml (Odunbaku *et al.*, 2008), the methanol extract of the of the stem-bark of *Ficus ingens* demonstrated antimicrobial activity against *S. aureus* at a dose of 5mg ml (Aliyu *et.al*, 2008), the ethanol extract of the stem-bark of *Ficus syncomorus*is reported to inhibit the growth of *H. pylori* at a dose of 200 mg/ml (Njume *et.al*, 2009), ethanol extract of the stem-bark of *Ficus sur* inhibit the growth of *E. coli, S.aureus, B. subtilis* and *Pseudomonas aeroginosa* at a dose of 2 mg/ml (Solomon *et.al*, 2011).

2.5 Phytochemical Constituents of the Genus Ficus

Chemically, the ficus contains mainly glycosides such as saponins, flavonoids, anthraquinones as well as alkaloids and tannins (Ukwubile, 2010). Some bioactive compounds such as arabinose, β-amyrins, β-carotenes, β-setosterols and xanthotoxol (Gilani *et.al*,2008). Previous phytochemical studies of Ficus resulted in the isolation of flavonoids, coumarins, alkaloids, steroids, triterpenes, ceramides and salicylic acids (Kamga *et. al*, 2010). The following compounds were isolated from the genus Ficus:

Lupeol (leaf) (1); psoralen (root) (2); ficusin (leaf) (3); bergapten (leaf) (4); umbeliferone (leaf) (5); guaiazulene (root) (6); scopoletin (leaf) (7); xanthotoxin (leaf) (8); β- sitosterol (leaf) (9); 7-hydroxy coumarin (leaf) (10); rutin (leaf) (11); imperatorin (leaf) (12); o-phenylphenol (fruit) (13); guaiacol (root) (14); dopamine (fruit) (15); cadalene (leaf) (16); ρ- cymene(fruit) (17); angelicin (fruit) (18); adrenaline

(fruit) (19); lutein (leaf) (20); quercitin (leaf) (21); raffinose (fruit) (22); rhamnose (fruit) (23); serotonin (root) (24) (Anshul, *et. al*, 2012).



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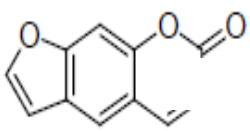

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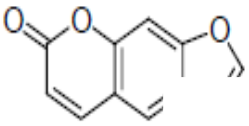

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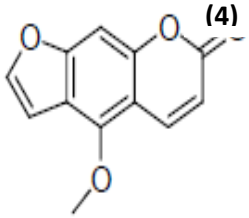

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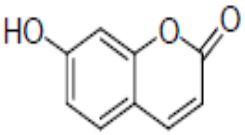

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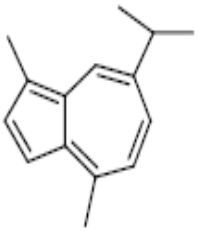

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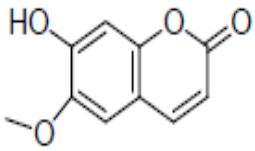

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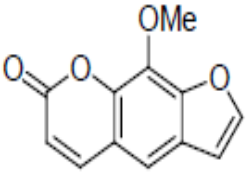

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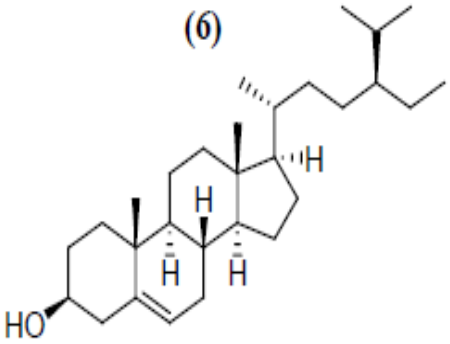

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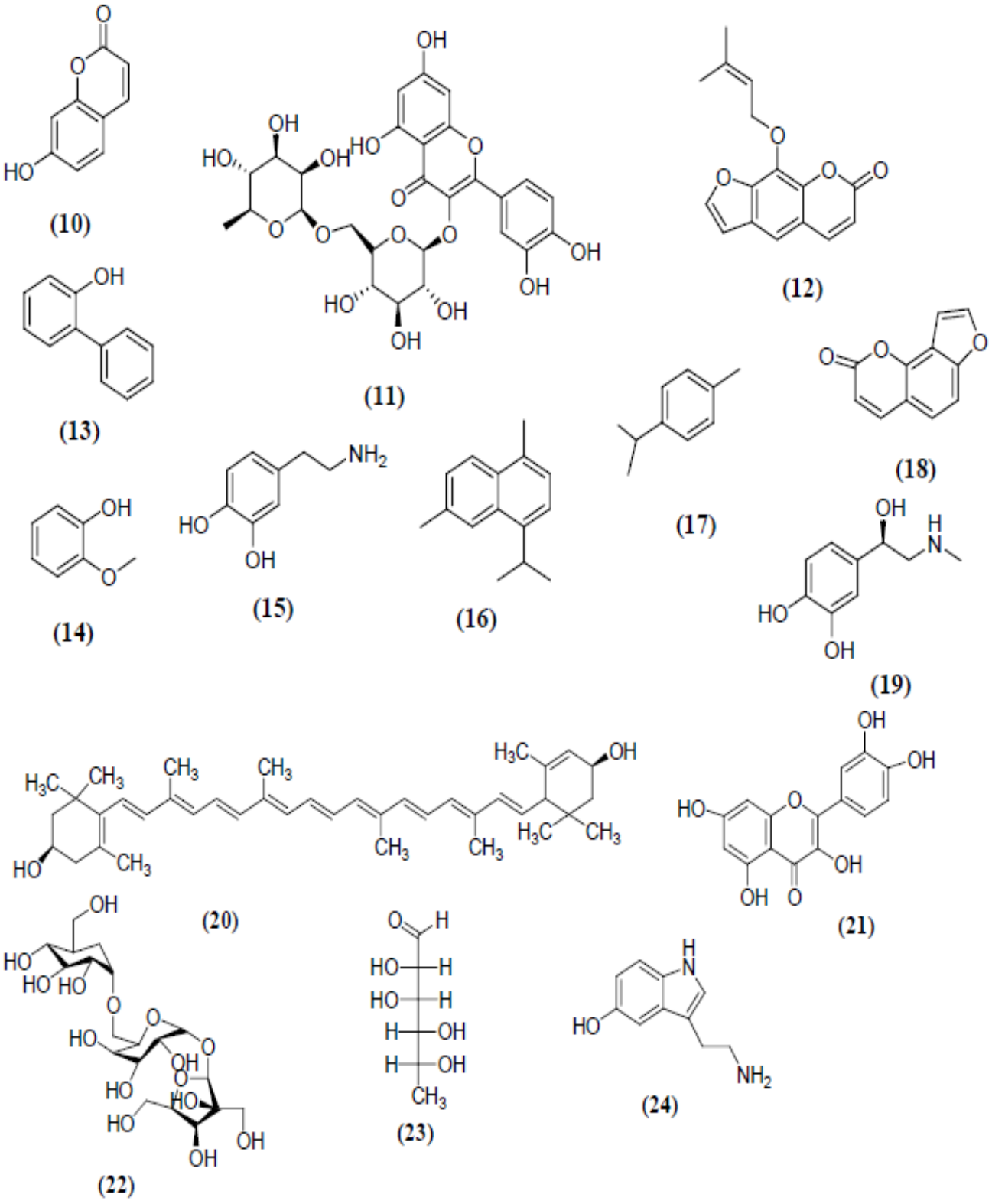

Figure 1: Chemical structures of some Compounds isolated from the Genus Ficus

2.6 Description of the *Ficus kamerunensis* species

2.6.1 Botanical Description of *Ficus kamerunensis*

Ficus kamerunensis (plate 1) is an epiphytic shrub, strangling, sometimes lianescent, or tree growing up to 20 m high. The plant is commonly available in the dense humid evergreen forest from Serra Leone to Western Cameroon and extending into Democratic Republic of Congo (Formerly known as Zaire).The major characteristic feature of this plant is exudation of a copious white latex from its stem-bark (Hutchinson and Dalziel, 1957).

2.6.2 Scientific Classification of *Ficus kamerunensis*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Rosales

Family: Moraceae

Genus: Ficus

Species: *Ficus kamerunensis*

Plate I: Picture of the *Ficus kamerunensis* in its natural habitat.

Plate II: Trunk of *F. kamerunensis*

Plate III: Branch bearing fruits of *F. kamerunensis*

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 List of Chemicals and Reagents

- Acetic acid (JHD, AR; Lobal Chem, India)
- Aluminium Chloride (Sigma-Aldrich, St. Lous, MO, USA)
- Anisaldehyde (Sigma-Aldrich, St. Lous, MO, USA)
- Chloral hydrate (Sigma-Aldrich, St. Lous, MO, USA)
- Chloroform (Sigma-Aldrich, St. Lous, MO, USA)
- Diethyl ether(JHD,AR; Lobal Chem,India)
- Ethanol (Park Scientific Ltd, Northampton, UK)
- Ethylacetate (Qualikems fine chem., Nandesari, India)
- Fast green (BDH Laboratory Chemicals Division, POOLE. England)
- Ferric Chloride (Sigma-Aldrich, St. Lous, MO, USA)
- Formalin (Sigma-Aldrich, St. Lous, MO, USA)
- Glycerol (Sigma-Aldrich, St. Lous, MO, USA)
- Hydrochloric acid (JHD, AR; Lobal Chem, India)
- Methanol (Merck-Germany)
- Methylene blue (BDH Laboratory Chemicals Division, POOLE. England)
- n-Butanol (Loba Chem, India)
- n-Hexane (JHD, AR; Lobal Chem, India)
- Paraffin wax (BDH Laboratory Chemicals Division, POOLE. England)
- Phloroglucinol (Sigma-Aldrich, St. Lous, MO, USA)
- Safranin (BDH Laboratory Chemicals Division, POOLE. England)
- Silica gel-60 for column (60-120µm in size)

- Sodium Hypochlorite (Sigma-Aldrich, St. Lous, MO, USA)
- Sudan Red Solution (BDH Laboratory Chemicals Division, POOLE. England)
- Sulphuric acid (JHD, AR; Lobal Chem, India)
- Sulphuric acid (Sigma-Aldrich, St. Lous, MO, USA)
- TLC silica gel 60 F_{254} pre-coated plates (Merk-Germany)
- Vanillin (Sigma-Aldrich, St. Lous, MO, USA)
- Xylene (Sigma-Aldrich, St. Lous, MO, USA)
- Zinc ChlorideSolution (JHD, AR; Lobal Chem, India)

3.2 List of Equipments

- Camera Lucida
- Compound microscope (Fisher Scientific, UK)
- Dessicator
- Disposable syringes (1ml, 5ml, and 10ml).
- Glass column (75 by 3.5 cm)
- Glass Slides and Cover slips (Fisher Scientific, UK)
- Laboratory glass wares (Funnel, Conical flask, Beakers, Measuring cylinder)
- Mechanical shaker (Stuart Scientific Flask Shaker, Great Britain)
- Melting point Apparatus (Gallencamp, USA)
- Metallic cages and feeding bottles for mice
- Microtome (C 740527, Cambridge Instrument Company Ltd, London and Cambridge, England)
- Slide dryer (Hospital and Lab. Supply Ltd, London, UK)
- Stage Micrometer and Ocular Lens (Graticules Ltd, Ton bridge, Kent. England)
- TLC tanks (Uni kit® TLC Chromatank® , Shandon Germany)

- Water bath (HHS, Mc Donald Scientific International,)

3.3 Collection and Identification of *F. Kamerunensis*

Sample of *F. kamerunensis* including the aerial parts and stem-bark were collected by a local plant collector, Mal. Maiwada from Ahmadu Bello University Samaru campus behind A.B.U press limited, Samaru, Sabon Gari Local Government Area, Kaduna State, Nigeria in September, 2013. The plant was identified by Mal. Musa Muhammad of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria and a specimen voucher number (900308) was deposited in the Herbarium.

3.4 Preparation of Stem-bark of *F. kamerunensis*

Sufficient quantity of stem-bark of *F. kamerunensis* was obtained by making longitudinal and transverse incisions through the outer layer of the stem of the plant followed by peeling. The bark was dusted, cleaned and all foreign matter removed, it was then air-died and comminuted to powder form, stored in an air-tight container for futher use.

3.5 Pharmacognostic Studies on *F. kamerunensis*

Pharmacognostic studies were conducted on the fresh and dried whole stem-bark of *F. kamerunensis*

3.5.1 Macroscopic Examination on the dried stem-bark of *Ficus kamerunensis*

Macroscopic observations were conducted on the whole dried stem-bark of the plant and these include; colour, odour, taste, texture, shape and fracture (Brain and Turner, 1975).

(a) Colour The colour of the dried stem-bark was determined under diffused daylight result was recorded.

(b) Odour A piece of the dried stem-bark was placed on the palm of the left hand and air was blown using the right hand and perceived the odour using the organ of smelling, the strength of the odour was determined.

(c) Taste A piece of the dried stem-bark was tasted using the organ of taste (tongue) and the taste was determined, noted and result was then recorded.

(d) Surface texture The surface characteristic of the dried sample of the stem-bak was determined by feeling of its outer and inner surfaces.

(e) **Size** a graduated ruler in centimetres/millimetres was used for the measurement of thickness of a sample of the dried stem-bark.

3.5.2 Microscopical examination of the Stem-bark of *F. kamerunensis*

Anatomical transverse section (T.S) and Longitudinal section (L.S) of the stem-bark samples were made, examined under microscope and described using the terms outlined in Dutta (2003). This procedure was carried out according to Feder and Brien (1968) as outlined below.

a) **Fixation;** the fresh Stem-bark of *F. kamerunensis* were picked off directly from the tree and dipped immediately into the fixative, Fomaline Acetic Acid (70% ethanol 40%, formalin and glacial acetic acid) at the ratio 45:1:1, and was allowed to stand for 24 hours.

b) **Dehydration;** the sample above was transferred in to 30%, 50%, 70%, 95% and 100% ethanol. This was carried out in each graded alcohol for 2 hours each.

c) **Clearing;** the sample was transferred in to chloroform: ethanol (1:3), chloroform: ethanol (1:1), chloroform: ethanol (3:1), and 100% Chloroform. This was also carried out after every 2 hours each.

d) **Infiltration and Embedding;** chips of paraffin wax was added slowly in to the Leaf sample and this was left to stand for 24 hours before transferring in to an oven at 60^{o}C.

After melting, the paraffin containing the sample was transferred in to the embedding box and allowed to solidify. This was then trimmed and mounted on the microtome to get the transverse or cross section of the leaf. The transverse section was then transferred on to slides.

e) **Staining;** the transverse section of sample was dewaxed in xylene, by changing twice for 5 minutes each. This was then hydrated in 95%, 70%, 50%, and 30% ethanol for 2 minutes each. The transverse section was then transferred in to safranin and left to stand for 30 minutes before washing with water. It was then transferred in to 0.5% HCl in 70% ethanol shortly before dipping in to fast green for 2 minutes and washed with water. The transverse section was then further dehydrated in 30%, 50%, 70%, 95% and 100% ethanol for 2 minutes each and cleared in xylene for another 2 minutes. Gum (Balsam) was sprayed along the transverse section and cover silp was placed.

3.5.3 Determination of Physico-Chemical Parameters of the Powdered Stem-Bark of *F. kamerunensis*

The quantitative physical standard (solvent extractive values, Ash residues, moisture content) were determined as described in the British Pharmacopoeia (2012).

(a) Moisture Content

The loss on drying method was adopted for this procedure. The powdered stem bark of the plant (3g) was weighed in a crucible. It was then heated for 1hour in an oven maintained at 105^0 C, cooled in a dessicator and re-weighed. The procedure was repeated until no further loss in weight was obtained. The moisture content was determined in percentage as:

$$\text{Moisture Content (\%)} = \frac{Weight\ of\ Water\ Lost}{Original\ Weight\ of\ Sample} \times 100$$

(b) Ash Values

(i) Total Ash

The total ash of the powdered stem bark of the *F. kamerunensis* was determined by weighing 2 g of the powdered sample in a crucible. This was heated gently at 450^0 C. Heating was done until all the carbon was removed. The total ash value was calculated in percentage as:

$$\text{Total Ash Value (\%)} = \frac{Weight\ of\ Residual\ Ash}{Weight\ of\ Sample} \times 100$$

(ii) Acid Insoluble Ash

Dilute HCl (25 ml) was added to the ash obtained in (i) above. This was boiled for 5 minutes and the insoluble matter was collected on ashless filter paper. The beaker containing the acid and crucible were washed in hot water and the washings were passed through the filter. The washing was continued until the residue was free of acid. The residue and filter were dried gently in an oven and ignited in a tarred crucible. It was allowed to cool and weighed. The acid insoluble ash was calculated in percentage as:

$$\text{Acid Insoluble Ash (\%)} = \frac{Weight\ of\ Residual\ Ash}{Initial\ Weight\ of\ Sample} \times 100$$

(iii) Water Soluble Ash

The water soluble ash value of the powdered stem bark of theplant was determined separately. The ash obtained following method (i) above was used. The procedure used to obtain the water insoluble ash was the same as in (ii) above except that water was used instead of dilute HCl. The water insoluble ash was determined in percentage as:

$$\text{Water ash (\%)} = \frac{Weight\ of\ Initial\ ash - Weight\ of\ Residual\ \ Ash}{Initial\ Weight\ of Sample} \times 100$$

(c) Solvent Extractive Values

(i) Alcohol Soluble Extractive Value

Powdered drug (5 g) was macerated with 100 ml of alcohol in a stoppered flask, shaking was done with the aid of a mechanical shaker during first 6 hr and allowed to stand for 18 hr. It was filtered after 24 hr. 20 ml of the filtrate was evaporated in a tarred evaporating dish at 105°C in an oven and weighed. Alcohol soluble extractive values were calculated (in percentage) with reference to the initial weights of the extract, five determinations were recorded and the average determined.

Alcohol Extractive Value (%) = $\frac{Weight\ of\ residue}{Weight\ of\ Sample} \times 100$

(ii) Water Soluble Extractive Value

The same procedure above was repeated in determining the water soluble extractive value, using water as the solvent. The water soluble extractive value was determined as:

Water Soluble Extractive Value (%) = $\frac{Weight\ of\ Residue}{Weight\ of\ Sample} \times 100$

3.6 Preliminary Phytochemical Screening of the Stem-Bark of *F. kamerunensis*

The methods used for the preliminary phytochemical screening of the powdered stem bark of *F. kamerunensis* are as follows:

3.6.1 Tests for carbohydrates

Extraction: powdered plant material (5g) was boiled in 50ml distilled water on water bath for 5 minutes. The mixture was filtered while hot and allowed to cool to room temperature. The filterate was then used for the following tests as described by (Evans, 2002).

(a) Molisch's (General) Test for Carbohydrates: To 1 ml of the filterate, 1 ml ofMolisch's reagent was added in a test tube, followed by 1 ml of concentrated sulphuric acid down the test tube. A reddish coloured at the interfacial ring indicates the presence of carbohydrate (Evans, 2002).

(b) Barfoed's Test for Monosacchrides: To 1 ml of the filtrate, 1ml of Barfoed reagent was added and the mixture heated on water bath for 5 minutes. A red precipitate indicates the presence of monosaccharide (Evans, 2002).

(c)Fehling Test; To 2 ml of the filtrate 5 ml equal mixture of Fehling solutions A and B were added and the mixture boiled for 5 minutes. Brick red coloured precipitates indicate the presence of reducing sugar (Evans, 2002).

(d) Selivanoff's Test for Ketose Sugars: To 1 ml of the filtrate, some crystals of resorcinol were added, followed by an equal volume of concentrated HCl and the mixture was placed on water bath for 5 minutes. A rose colour indicates the presence of ketose sugar (Evans, 2002).

3.6.2 Tests for Anthraquinones

(a) Test for free Anthraquinones (Bontrager's Test): Chloroform 5 ml was added to 0.5g of powdered drug in a dry test tube and shaken for 5 minutes. The mixture was filtered and shaken with an equal volume of 10% ammonium solution.A bright pink color in the upper aqueous layer indicates the presence of anthraquinones (Evans, 2002).

(b) Modified Bontrager's Test: The powdered 1g drug was boiled in 5 ml 10% HCl for 5 minutes on a water bath. The mixture was filtered while hot and then allowed to cool to room temperature. The filtrate was extracted with 5 ml benzene in a separating funnel. The upper benzene layer was pipette-off intoa test tube and shaken with half its volume of 10% ammonium hydroxide. If the lower layer becomes rose pink or cherry red, it indicates the presence of anthraquinone derivatives in its free or combined form (Evans, 2002).

3.6.3 Tests for Cardiac glycosides

Extraction: The powdered drug (2g) was boiled with 10 ml of 95% alcohol on a water bath for 5 minutes, it was cooled and filtered. Strong lead sub acetate solution (3 ml) was added to the filtrate and the mixture filtered and the filtrate divided into two portions. To the first portion, 10 ml of chloroform was added in separating funnel and the mixture shaken for 5 minutes. The lower chloroform layer was run off into a beaker and divided into two portions.

(a) Keller-Killiani Test: The first portion of the second filtrate was transferred into an evaporating dish and evaporated to dryness on a water bath. The residue was dissolve in 1 ml glacial acetic acid containing traces of $FeCl_3$ solution and then transferred into a dry test-tube.The test tube was held at 45 angle and 2 ml concentrated $H_2S0_4$was run down the side of the test tube to form a lower layer. A purple-brown ring at the interface indicates the presence of deoxysugars; and a pale green colour in the upper acetic acid layer is due to the presence of steroid, which indicates the presence of cardiac glycoside (Evans, 2002).

(b) Legal Test: The second portion of the second filtrate was transferred into an evaporating dish then evaporated to dryness on a water bath. The residue was dissolved in pyridine and a drop eachof 2% sodium nitroprusside and 20% sodium hydroxide solutions were added. Production of deep red colour indicates the presence of cardenolides (Evans, 2002).

(c) Kedde Test: To the second portion of the first filtrate 1ml of 2% solution of 3, 5-dinitrobenzoic acid in 95% alcohol was added. The solution was made alkaline by the addition of 5% Na0H; the presence of purple- blue colour indicates the presence of cardinolides (Evans, 2002).

3.6.4 Test for Saponin Glycoside

Frothing Test: Distilled water (5 ml) was added to (0.5g) of the powdered drug in a test tube and shaken vigorously for 30 seconds. The test tube was allowed to stand in a vertical position and observed for a period of 30 minutes. A honey comb froth that persisted for 15 minutes indicates the presence of saponins (Sofowora, 1993).

3.6.5 Tests for Steroids and/or Triterpenoids

Extraction: The powdered drug (2g) was boiled with 10 ml of 80% alcohol on a water bath for 5 minutes. It was cooled, filtered and transferred into an evaporating dish. The filterate was evaporated to dryness on a water bath and 5 ml of chloroform was added to the residue. The extract was divided into two and used for the following tests;

(a) Libermann- Burchard Test: To first portion of the filtrate 2 ml of acetic anhydride was added and mixed gently. 1 ml concentrated H_2S0_4 was added down the side of the test tube. A blue- green color in the upper layer and a reddish ring indicates the presence of steroids; while a red, pink or purple color indicates the presence of triterpenes (Harbone, 1973).

(b) Salkowski Test: To the second portion of the filtrate 1 ml of concentrated $H_2S0_4$was added down the side of the test tube.A cherry red colour at the interface indicates the presence of unsaturated sterols (Sofowora, 1993).

3.6.6 Tests for Flavonoids

Extraction: The powdered drug (3g) was boiled with 20 ml of 70% aqueous ethanol on a water bath for 5 minutes and filtered while hot. Filtrate was allowed to cool to room temperature, divided into 3 portions and used for the following tests:

(a)Ferric Chloride Test: To the third portion of the filtrate, 3 drops of ferric chloride solution was added. A greenish black colour indicates the presence of phenolic nucleus (Evans, 2002).

(b) Sodium hydroxide Test: To the second portion of the filtrate 2 ml of 10% sodium hydroxide solution was added. A yellow solution indicates the presence of flavonoid which on addition of dilute HCl becomes colorless (Evans, 2002).

(c) Shinoda's Test: To the first portion of the filterate, some magnesium chips and 5 drops of concentrated HCl were added; production of a pink or red colour indicates the presence of flavonoids (Silver *et.al.*, 1998).

3.6.7 Tests for Tannins

Extraction: The powdered drug (3g) was boiled with 10 ml of distilled water on a water bath.It was filtered while hot and the filtrate cooled and divided into 3 portions and used for the following tests;

(a)Ferric Chloride Test: To the first portion in a test tube, 1 ml of ferric chloride solution was added. A green or dark green precipitate indicates the presence of condensed tannins while a blue or blue – black precipitate indicates the presence of hydrolysable tannins (Evans, 2002).

(b) Lead sub-acetate Test: To the second portion of the filtrate, 3 drops of lead sub-acetate solution was added, a coloured precipitate indicates the presence of tannins (Evans, 2002).

(c) Bromine water Test: To the third portion of the filtrate, 3 drops of bromine water were added. A buff coloured precipitate indicates the presence of condensed tannins (Abubakar, 1993).

3.6.8 Test for Alkaloids

Extraction: The drug (5g) was boiled in 50ml of 1% H_2S0_4 in 50% ethanol. It was cooled, filtered and the filterate transferred into a separating funnel. Concentrated ammonia solution was added to the filtrate until the solution was alkaline; 20ml of chloform was added. The mixture was shaken gently and allowed to separate. The lower chloroform layer was run off into another separating funnel and extracted with 20 ml dilute H_2S0_4. The acidic extract was divided into 5 portions and used for the following tests:

(a) Mayer's Test: To the first portion of the filtrate 1ml of Mayer's reagent was added drop by drop. A cream coloured precipitate indicates the presence of alkaloids (Evans, 2002).

(b) Dragendoff's Test: To the second portion of the filtrate, 1 ml of Dragendoff's reagent was added drop by drop. An orange-red precipitate indicates the presence of alkaloids (Evans, 2002).

(c) Wagner's Test: To the third portion of the filtrate, 1ml of Wagner's reagent was added drop by drop. A reddish brown precipitate indicates the presence of alkaloids (Evans, 2002).

(d) 1% Picric acid Test: To the fourth portion of the filtrate 1 ml of picric acid solution was added drop by drop. A yellow precipitate indicates the presence of alkaloids (Evans, 2002).

(e) 10% Tannic Acid Test: To the fifth portion of the filtrate 1ml of 10% Tannic acid solution was added drop by drop. Appearance of buff coloured precipitate indicates the presence of alkaloids (Evans, 2002).

3.7 Phytochemical Analysis of the Stem-bark of *F. kamerunensis*

3.7.1 Extraction of the Stem-bark of *F. kamerunensis*

The powdered stem bark (1 kg) of *F. kamerunensis* was extracted using cold maceration technique with 3 L of 95% methanol in a glass jar for 3 days (72 hours) at room temperature. The extract was filtered; the filtrate was then concentrated, evaporated to dryness on water bath and stored properly in a dessicator for further use.

3.7.2 Fractionation of the Methanol Extract of the Stem-bark of *F. kamerunensis*

The methanol extract (80 g) was suspended in 500 ml of water and filtered; the filtrate was transferred into a separating funnel and partitioned twice with 300 ml of diethyl ether. The diethyl ether fraction (EXT1) was collected into a clean and dried conical flask on equilibration. The aqueous portion was then partitioned twice with 300 ml of ethyl acetate, on equilibration; the ethyl acetate fraction (EXT 2) was collected in a clean and dried conical flask. The aqueous portion was furtherpartitioned twice with 300 ml n-butanol (saturated with water) and on equilibration both the n-butanol fraction (EXT3) and the aqueous fraction (EXT4) were collected into separate clean and dried conical flasks (See Fig. 3.1).The partitioned fractions (diethylether, ethyl acetate, n-butanol and aqueous) were concentrated over a water bath, transferred into sample battles, labelled appropriately and stored for further use.

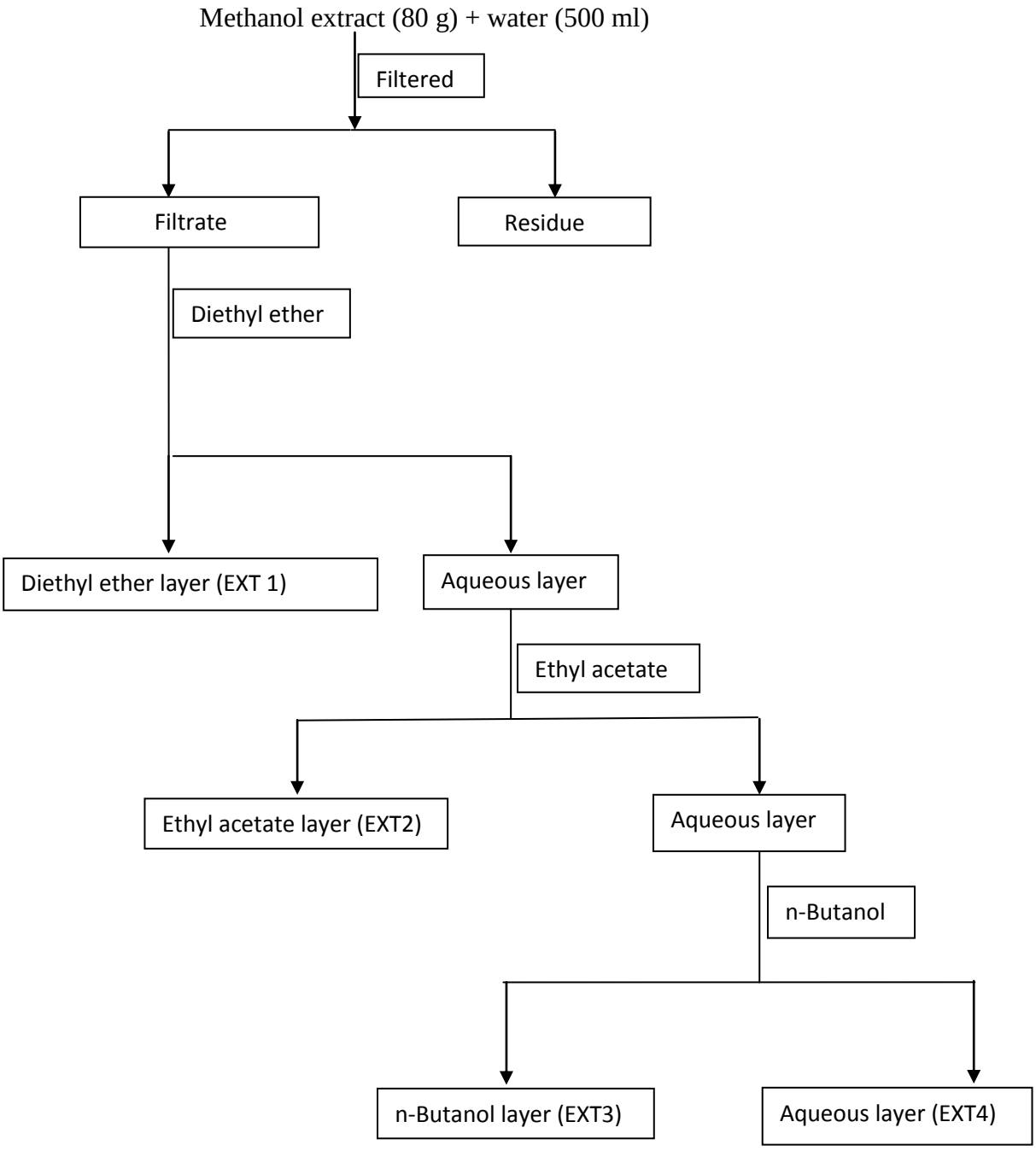

Figure 2: Fractionation Chart of methanol extractof the powdered Stem-bark *F. kamerunensis*.

3.8 Thin Layer Chromatography (TLC) of theDiethyl ether (EXT1) Fraction

3.8.1 Preparation of solvent system for TLC

The chromatographic tank was washed and dried. Different solvent systems were prepared each for the different fractions obtained above.These solvents systems each in separate tank were allowed to stand and saturate the tank before the development of the TLC plates.

The solvent systems used in the separation are given in the table 3.1 below:

Table 3.1: Solveent Systems for the TLC Analysis of Diethyl ether (EXT1) Fraction

S/NO	SOLVENT SYSTEM	RATIO
1	Hexane:Ethyl acetate	1:9, 1:4, 3:7 & 2:3
2	Chloroform: Ethyl acetate	1:9, 1:4, 3:7 & 1:3
3	Chloroform:Methanol	9:1 & 4:1
4	Ethyl acetate: Methanol	4:1& 7:3
5	Ethyl acetate: Methanol:Water	10:1.5:1
6	Hexane:Ethyl acetate: Methanol:Water	15:8:4:1
7	Benzene:Acetic acid:Water	8:1:1 & 6:1:1

3.8.2 Spotting of the Sample

The pre-coated plates (2.5 x 10cm) were spotted 1.5cm from the origin using a microcapillary tube, with the concentrated portions of the fractions, this was allowed to dry and developed in the solvent saturated tank containing the solvent system number 1, in table 3.1, until solvent front reached about 8cm of the plate. It was then removed, solvent front marked and air dried.Same process as above was carried out using diethyl ether (EXT1) fraction using the solvent systems outlined in table 3.1. The developed plates were sprayed with 10% H_2S0_4 and P-anisaldehyde|H_2S0_4 spraying reagents

3.8.3 Documentation

Pictures of the plates were taken immediately after they were developed and R_f values were calculated for each spot in the plate using the formulae,

$$R_f = \frac{\text{Distance moved by the spot}}{\text{Distance moved by the solvent}}$$

The best solvent system obtained for the least polar fraction was further used for column chromatography.

3.9 Column Chromatography of the Diethyl ether Fraction (EXT1)

Column chromatography was carried out using the best solvent system obtained (Hexane: Ethyl acetate 7:3) for the diethyl ether fraction from the above TLC. A glass column of dimension 75 by 3.5 cm was wetpacked with (80g) silica gel-60(60-120µm in size); the side of the column was taped gently with a glass rod to even the compaction of the particles. Sample (2g) of the crude diethyl ether extract was pre-adsorbed on dried silica gel and introduced into the column and allowed to stabilize over night before elution

began. The column was eluted in a gradient profile, starting with n-hexane (100%) and ethyl acetate was added gradiently from 0 to 50%.and fractions collected were monitored on TLC plate (Silica gel-60 F_{254} aluminiun barking from Merck, Germany) using hexane: ethyl acetate (9.5: 0.5).

3.9.1 Purification of the EXT1

Combined fraction B (200 mg) was packed on column (1.0 by 45 cm glass column), packed with 10 g silca gel-60(60-120µm in size); the column was eluted with hexane: ethyl acetate (9:1) and fractions collected were monitored on TLC plate (Silica gel-60 F_{254} aluminiun barking from Merck, Germany) using hexane: ethyl acetate (9.5: 0.5). Further purification was carried out using preparative TLC with a glass precoated silica gel of 20 X 20 size and thickness. Here 100ml of a solvent system (hexane: ethyl acetate 9:0.5) was prepared and transferred into a chromatographic tank and allowed to saturate. The preparative TLC plate was streak with the Fraction from the origin, it was air- dried and developed in the tank until it reaches about 18 cm, and it was gently removed and allowed to dry. A glass cutter was used to cut two egdes of the developed plate (about 2 cm each), sprayed with 10% H_2S0_4 and spots were visualized using air blower to monitor the pattern of the spots moved. The area covered by the prominent spot was traced carefully with a syring needle using the two sides cut and sprayed plate above as a guide. Thereafter, the traced area was subsequently scraped and extracted with ethyl acetate, then filtered and concentrated.

3.10 Analysis of the Isolated Compound

3.10.1 Physicochemical Analysis

Physiochemical studies conducted on the isolated compound include; colour, solubility in different solvents (hexane, chloroform, ethylacetate, acetone and methanol), melting point

determination and TLC sprayed with the following reagents; Libermann- Burchard, $FeCl_3$ and Dragendorf's spraying reagent.

3.10.2 Structure Elucidation of the isolated compounds

The compound isolated was coded UIK and 20mg of it was sent to the Chemistry Department of KwaZulu Natal University, South Africa for Nuclear Magnetic Resonance (NMR) Spectroscopic analysis including one dimemsion (1D ^{1}HNMR, ^{13}CNMR, DEPT-90 and DEPT-135) and (2D ^{1}H-HCOSY, NOESY, HSQC, and HMBC).

3.11 Antimicrobial Studies of *F. kamerunensis*

3.11.1 Collection of Clinical Isolates

Twelve (12) microbial clinical isolates were collected from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH) Zaria. The clinical isolates used include four (4) each of Gram Positive, Gram Negative bacteria and Fungi. These are;

- *Methicillin Resistant Staphylococcus aureus (MRSA),*
- *Staphylococcus aureus,*
- *Streptococus pyrogens*
- *Corynebacterium ulcerans*
- *Escherichia coli*
- *Proteus mirabilis*
- *Neisseria gonorrhoea*
- *Pseudomonas aeruginosa*
- *Candida albicans*
- *Candida krusei*
- *Candida stellatoidea*

- *Candida tropicalis*

3.11.2 Preparation of Stock Solution

The extract (0.5g) each of the column fraction, diethyl ether extract, ethyl acetate extract, n-butanol extract, and methanol Extract of the stem bark of *F. kamerunensis* were weighed and dissolved in 10ml of DMSO seperately to obtain a concentration of 50mg/ml as an initial concentration of the extracts used for the antimicrobial studies. From the stock solution, two fold serial dilutions were made to obtain 50, 25, 12.5, 6.25 and 3.125 mg/ml concentration of each of the extracts. Standard antibiotics Ciprofloxacin and Fluconazole at a concentration of 5 µg/ml were used as the positive control drug for both antibacterial and antifungal respectively.

3.11.3 Mc-Farland Barium Sulphate Turbidity Standard

This was prepared by dissolving 0.05 ml of 1% Barium sulphate in 9.95 ml 1% sulphuric acid using normal saline. This corresponds to number 0.5 Mcfarland standard scale, while the stardized bacteria culture is 1.5×10^8 CFU/ml.

3.11.4 Preparation of Culture Media

The experiment was carried out using agar well diffusion method as described by Azoro (2002) and Mobasher *et. al* (2005) in the microbiology units of National Institute of Leather Science and Technology (NILEST) Zaria, Kaduna State, Nigeria.

Culture media for both antibacterial and antifungal were prepared using Muller Hinton agar by dissolving (38g) of the Muller Hinton agar in (1 litre) of distilled water, it was boiled and dissolved completely, then sterilized at 121^0C for 15 minutes and poured into sterilized Petri dishes and were allowed to cooled and solidified.

The sterilized medium was seeded with 0.1ml of the standard inocula (1.5×10^8 CFU/ml). of the test pathogenic microbes, the inocula was spread evenly over the surface of the medium with a sterile swab.

By the used of standard cork-borer of 6mm in diameters, a well was cut at the centre of each inoculated medium then, (0.1ml) of the solution of the extract of the concentration of (50, 25, 12.5, 6.25 and 3.125 mg/ml) were then introduced into each well on the inoculated media.

Incubation of the media was made at 37^0C for 24hours, after which the plates were observed for the zone of inhibition of growth, the zones were measured with a transparent ruler and the result recorded in millimeters.

3.11.5 Determination of Zones of Inhibition

Solution of 0.5 ml of the 50 mg/ml of each of the extract was introduced in to each of the wells made on the media. All the media were then inoculated at 37^oCfor 24 hours and the zone of inhibition was measured using a transparent ruler and the result was recorded in millimeter and the results were recorded.

3.11.6 Determination of Minimum Inhibition Concentration (MIC)

The minimum inhibitory concentration determination of the extracts was carried out using the broth dilution method.

Equal volume of the concentrations (50, 25, 12.5, 6.25 and 3.125 mg/ml) were incorporated in nutrient broth in 1:1 and (0.1ml) of the standardized suspension of the test organisms (1.5×10^8 CFU/ml) was added in to each of the test-tube. The tubes were then incubated at 37^0c for 24 hours. Tubes containing broth and extract without inocula were incubated to serve as positive control while a tube containing broth and inocula serves as negative control for comparison. The presence of growth (turbid solution) or absence of growth (clear solution) at the end of incubation period was recorded. The highest dilution (least concentration) of the extract showing no detectable considered the minimum inhibitory concentration (MIC).

3.11.7 Determination of Minimum Bactericidal and Fungicidal Concentration (MBC and MFC)

Minimum bactericidal concentration and Fungicidal concentrations were determined by sub-culturing 0.1ml from the above MIC test dilutions that showed visible growth (turbidity) and all others in which there was no detectable growth on a fresh exract free solid medium and incubated at 37^0c for 24 hours. The least concentration that shows no single bacteria colony was considered as the minimum bactericidal concentration (MBC).

3.12 Biological Studies of *F. kamerunensis*

The Biological studies conducted include LD_{50} to establish the safety margin of the plant. Antimicrobial studies was conducted on the column fractions (C and D), diethyl ether extract, ethyl acetate extract, n-butanol extract, and methanol extract.

3.12.1 Median Lethal Dose (LD_{50}) Determination of Methanol Extract of *F.kamerunensis*

The LD_{50} was determined using the method of Lorke, (1983). The study was conducted in two phases using a total of 13 mice. In the first phase, nine mice were grouped into three groups of three mice each. Methanol extract (10g) of *F.kamerunensis* was dissolved in 10 ml of distilled water serial dilution of 10, 100 and 1000 mg/ml of the extract were prepared.In the first phase, nine mice were grouped into three groups of three mice each. The groups received the methanol extract at a dose of 10, 100 and 1000 $mgkg^{-1}$ body weight intraperitonially (*i.p.)* and observed for any physical changes before allowed to stand for 24 hours and the number of death was recorded for each group. The second phase involved four groups of one animal each and were administered the methanol extract at the doses of 140, 225, 370 and 600mg/kg body weight respectively which were calculated based on the result obtained in the first phase. Signs of toxicity and lethality

were observed for 24 hours. The LD_{50} was calculated as the geometric mean of the lowest lethal dose that caused death and the highest non-lethal dose that did not cause death.

$$\mathbf{LD_{50}} = \sqrt{\boldsymbol{minimum\ dose\ of\ death} \times \boldsymbol{minimum\ dose\ of\ survival}}$$

3.13 Statistical Analysis

Data were expressed as Mean ± Standard Error of Mean.

CHAPTER FOUR

4.0 RESULTS

4.1 Pharmacognostic Studies on *F. kamerunensis*

4.1.1 Macroscopical Features of the Stem-bark of *F. kamerunensis*

Bark (plate iv) is dark-brown in colour, inner surface is light-brown, shape is concave, taste is mucillaginous without any characteristic odour, soft surface, 0.5-1.8 cm thick and fracture is fibrous. The organoleptic features are summarized as shown in (Table 4.1)

Table 4.1: Macroscopic features of the stem-bark of *F. kamerunensis*

MORPHOLOGICAL CHARACTER		OBSERVATION
Colour	:	dark-brown
Odour	:	Non-characteristic
Shape	:	Concave
Taste	:	Mucillaginous
Fracture	:	Fibrous
Size	:	0.5-1.8 cm

Plate III: The Stem-bark of *F. kamerunensis*

4.1.2 Microscopical Features of the Stem-bark of *F. kamerunensis*

A transverse section of the Stem-bark of *Ficus kamerunensis* showed the outermost zone with cork tissue composing of three rows of thick-walled rectangular cells. The cortex was fairly wide and composed of several rows of cells. A wide continuous band of sclereids was present at the periphery of the cortex. The cortical parenchyma cells were thin-walled and more or less cubical to oblong. The inner bark was thin and consists of radial segments of phloem alternating with two seriate medullary rays.

4.3 Physicochemical constants of Powdered Stem-bark of *F. kameunensis*

4.3.1 Moisture Content (loss on drying) of the Powdered Stem-bark of *F. kamerunensis*

The result of moisture contents using loss on drying method was determined to be 10.5%

4.3.2 Ash values of powdered stem-bark of *F. kamerunensis*

The percentage yield of total ash, acid insoluble and water soluble matter were determined to be 8.5%, 1.5% and 3.5% respectively.

4.3.3 Extractive Values of Powdered Stem-Bark of *F. kamerunensis*

The ethanol and aqueous extractive values of the powdered stem bark of *F. kamerunensis* were obtained as 10.5% and 18.5% respectively.

The results of physical constants determination are summarized in the table below:

Table 4.2: Physicochemical Constants of the Powdered Stem-bark of *F. kamerunensis*

Parameter	**Values (% w/w)***	**± SEM**
Moisture content	11.7	0.3391
Total ash values	8.8	0.2000
Acid insoluble ash	1.7	0.1225
Water soluble ash	3.7	0.1225
Ethanol extractive value	10.4	0.1000
Water extractive value	18.4	0.1000

*Average mean of five determinations.

4.4 Preliminary Phytochemical screening of the Stem-Bark of *Ficus kamerunensis*

The preliminary phytochemical screening of the powdered stem bark of the plant indicated the presence of carbohydrates, flavonoids,triterpenes and steroids, cardiac glycosides, saponins and Tannins, (Table 4.3), TLC profiles of the extracts were obtained to conform the findings of the preliminary screening for the phytochemicals.

Table 4.3: Results of Preliminary Phytochemical screening of the Stem-bark of *F. kamerunensis*

Constituent Tested	Observation	Result
Carbohydrates:		
(a) Molich's test	A reddish coloured at the interfacial ring	Carbohydrate present
(b) Barfoed's test	A red coloured precipitate	Monosaccharide present
(c)Fehling test	A brick red coloured precipitate	Reducing sugar present
(d)Selivanoff's test	A rose colour solution	Ketose sugar present
Anthraquinones		
(a) Bontrager's test	A bright pink colour in the upper aqueous layer	Anthraquinones present
(b)Modified Bontrager's test	A rose pink at the lower layer	Anthraquinone derivativ in its free or combined Form present
Cardiac glycosides		
(a) Keller-Killiani test	A purple-brown ring at the interface and a pale green color in the upper acetic acid layer	Deoxysugars and Steroid indicates presence of cardiac glycoside
(b) Legal test	A deep red colour	Cardenolides present
(c) Kedde test	A purple- blue colour	Cardenolides present
Saponin Glycosides		
Frothing test	A honey comb froth that persisted for 15 minutes	Saponin Glycoside present
Steroids/ Triterpenoids		
(a) Libermann-Burchard test	A purple colour observed	Triterpenes present
(b) Salkowski test	A cherry red colour at the interface	Unsaturated Sterols present

Flavonoids		
(a) Ferric Chloride test	A greenish black colour	Phenolic nucleus present
(b) Sodium hydroxide test	A yellow solution	Flavonoid present
(c) Shinoda's test	A pink colour	Flavonoid present
Tannins		
(a) Ferric Chloride test	A green precipitate	Condensed tannins present
(b) Lead sub-acetate test	A coloured precipitate	Tannins present.
(c) Bromine water test	A buff coloured precipitate	Condensed tannins present
Alkaloids		
(a) Mayer's test	A cream colored precipitate	Alkaloid present
(b) Dragendoff's test	An orange-red precipitate	Alkaloid present
(c) Wagner's test	A reddish brown precipitate	Alkaloid present
(d) 1% Picric acid test	A yellow precipitate	Alkaloid present
(e) 10% Tannic Acid test	A buff coloured precipitate	Alkaloid present

4.5 Phytochemical Analysis of the powdered stem-bark of *Ficus kamerunensis*

4.5.1 Extraction of the powdered stem-bark of *Ficus kamerunensis*

The methanol extracts yielded 101.51g (10.15%w/w) from the cold maceration of the powdered plant material.

4.5.2 Fractionation of the methanol extract of Stem-bark of *F. kamerunensis*

Four (4) fractions (labelled EXT1 to 4) were obtained after successive fractionation with the three solvents, these are; diethyl ether (EXT 1), ethyl acetate (EXT 2), n-butanol (EXT 3) and aqueous (EXT4). The extracts were concentrated on water bath and evaporated to dryness to give a mean yield of 0.24, 0.46, 1.57 and 6.54% respectively.

4.5.3 Thin Layer Chromatography of the diethyl ether fraction (EXT1) of *F. kamerunensis*

Technique	-	Ascending technique
Sample	-	EXT 1
Adsorbent	-	Pre-coated Silica gel plates
Mobile phase	-	The solvent systems in table 3.1
Visualization	-	P-anisaldehyde|H_2S0_4 spraying reagent and heating at 105◦C for 10 minutes
Colour of Spot In daylight	-	Colourless
Colour after Spray	-	See table 4.4
R_f-values of Spots	-	See table 4.4

Table 4.4: TLC Profile of thediethyl etherfraction (EXT1)

SOLVENT SYSTEM	No.of Spots	Colour After Spray	R_f values
Hexane:Ethyl acetate (7:3)	4	Purple, Brown,Brown and Pink	0.46, 0.57, 0.73 and 0.88
Chloroform: Ethyl acetate (7:3)	4	Purple, Brown, Brown and Pink	0.18, 0.25, 0.80 and 0.96
Chloroform:Methanol (9:1)	3	Purple, Brown and Brown	0.72, 0.80 and 0.88
Ethyl acetate: Methanol (4:1)	2	Purple and Pink	0.80 and 0.91
Ethyl acetate: Methanol:Water (10:1.5:1)	4	Purple, Purple, Black and Pink	0.31, 0.43, 0.78 and 0.88
Hexane:Ethylacetate:Methanol:Water (15:8:4:1)	4	Purple, Brown, Pink and Pink	0.23, 0.61, 0.77 and 0.89
Benzene:Acetic acid:Water (8:1:1)	3	Blue,Purple and Pink	0.08, 0.58 and 0.70

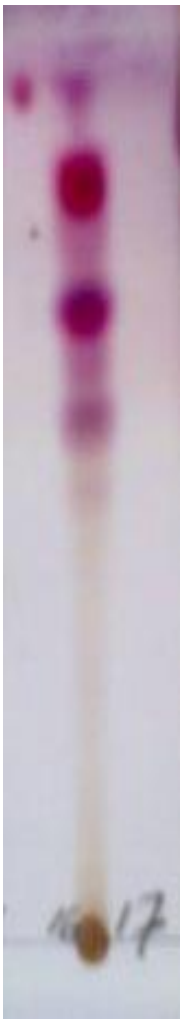

Plate IV: Chromatogram for the EXT 1 Using hexane: ethyl aceatate (7:3) sprayed with P-anisaldehyde and heated at 105°Cfor 10 minutes.

4.5.4 Column Chromatography for the Isolation of Compound from EXT 1

A total of 110 fractions each of 20ml volumes were collected. Factions that gave similar TLC profiles were pooled to give nine (A-I) combined fractions. Combined fraction B gave three spots on TLC that were closed to each other and a higher quantity (200mg); white amorphous powder. Other combined fractions gave smaller quantities with two to four spots also, but white-yellowish fatty substances which are later screened for antimicrobial activity (Table 4.5 and Plate VI)

The combined fraction B was re-chromatographed using silica gel column and a total of 25 fractions each of 20ml were collected. Fraction 15 gave three spots with one prominent spot at the middle with two other spots above and below the prominent one with relative distance in between them (Plate VII and VIII). Preparative TLC was carried out and a single prominent spot (Plate IX) was obtained, it was a white, amorphous powder (32mg) with $R_f$value of 0.64 (hexane: ethyl acetate 9.5:0.5).

Table 4.5 Fractions obtained from Column Chromatography of EXT 1

Code	Fractions	Eluting Solvent	Colour of spots	No. of spots	R_f values	Quantity (mg)
A	1-60	100% H	Nil	Nil	Nil	Nil
B	61-66	H:EA (9:1)	Pink & Brown	2	0.62 & 0.74	200
C	67-75	H:EA (9:1)	Green,Purple Brown & Pink	4	0.43, 0.49, 0.63 & 0.74	40
D	76-80	H:EA (8.5:1.5)	Green, Purple Brown & Pink	4	0.45, 0.55, 0.69 & 0.79	37
E	81-88	H:EA (8.5:1.5)	Green, Purple Brown & Pink,	4	0.40, 054, 0.72 & 0.92	40
F	89-91	H:EA (8:2)	Purple, Brown & Pink	3	0.50, 0.60 & 0.76	20
G	92-94	H:EA (8:2)	Purple & Brown	2	0.58 & 0.74	13
H	95-102	H:EA (7:3)	Brown & Pink	2	0.54 & 0.70	15
I	103-110	H:EA (7:3)	Green, Brown	2	0.56 & 0.70	10

Key: H □ Hexane and EA □ Ethyl acetate

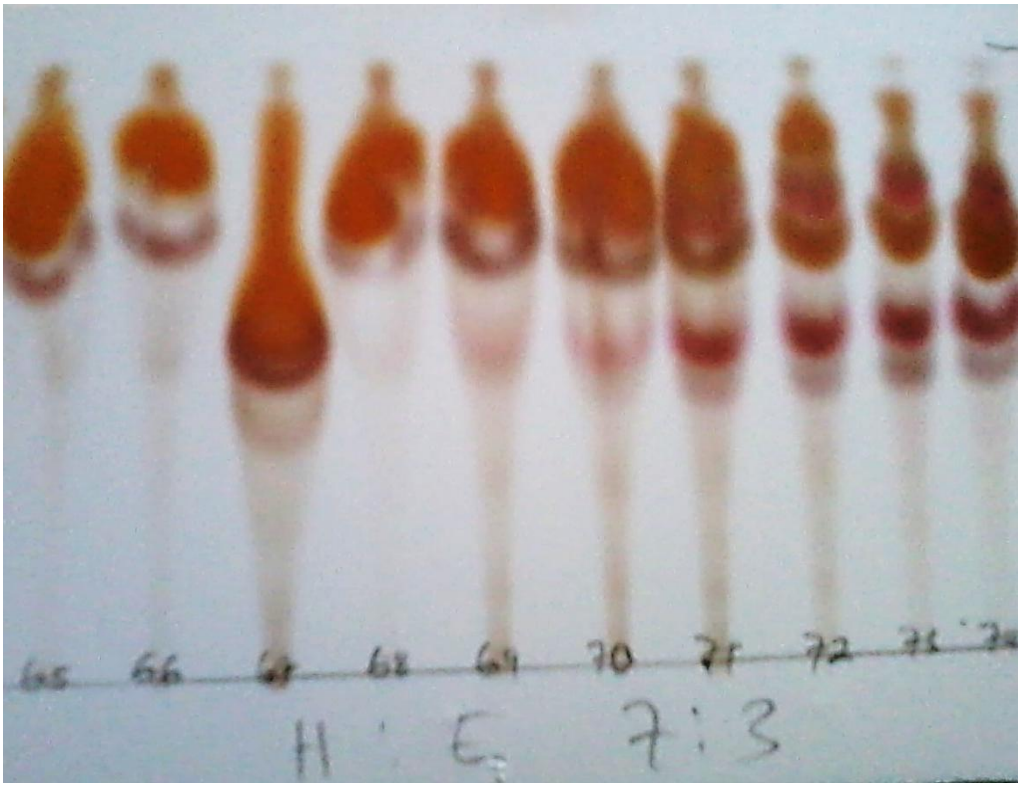

Plate: V Chromatogram of the major Column fractionUsing hexane: ethyl aceatate (7:3) sprayed with H_2S0_4in Methanol and heated at 105°C for 10 minutes.

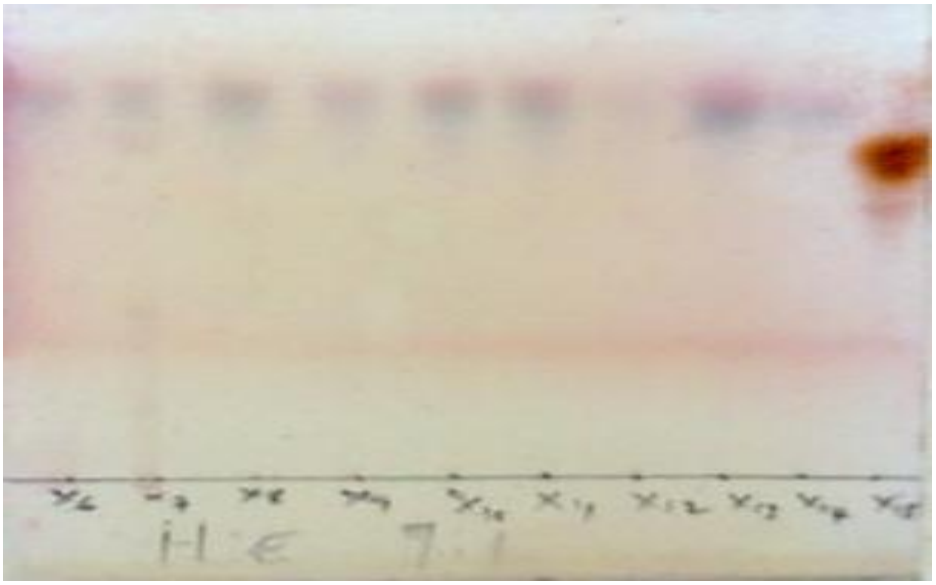

Plate VI: Chromatogram for the second Column fractionsUsing hexane: ethyl aceatate (9:1) sprayed with H_2S0_4in Methanol and heated at 105°C for 10 minutes.

Plate VII: Chromatogram for the Isolated Compound before purificationUsing hexane: ethyl aceatate (9.5:0.5) sprayed with H_2S0_4 in Methanol and heated at 105°C for 10 minutes.

PlateVIII: Chromatogram of the pure Isolated Compound Using hexane: ethyl aceatate (9.5:0.5) sprayed with H_2S0_4in Methanol and heated at 105°C for 10 minutes.

4.5.5 Preliminary Studies on The isolated Compound

Color: White amorphous powder

R_f values: 0.64 (hexane-ethyl acetate 9.5:0.5)

Solubility: Soluble in chloroform and ethyl acetate, partially soluble in hexane and Insoluble in ethanol and acetone

Melting point: 220-222^{0}C

Spaying Reagents:

Libermann- Burchard: pink coloured spot was observed

$FeCl_3$: No blue or green coloured spot observed

Dragendoff's: No orange-red coloured spot observed

4.5.6 Structure Elucidation of the isolated compound

The proton (^{1}H) NMR Spectra of the UIK sample indicated the presence of many methyl protons in the region δ 0.88 to 1.24 ppm; methylene protons in the region δ 1.5 to 2.8 ppm; acetate de-shielded methyl proton at δ 2.05 ppm and hydroxymethine proton appeared as doublet of doublets at δ 3.24 ppm, the compound also indicated the presence of two methine protons; proton at δ 5.12 ppm as a triplet suggesting the presence of a trisubstituted olefinic bond, the other is oxygenated at δ 4.44 ppm on position-3 thus, suggesting triterpenoid or steroid acetate as shown in (Appendix VIII).

The carbon 13 NMR spectra (Appedix IX) indicated the presence of 32 carbon peaks; with a carbonyl carbon (δ 171.04 ppm) and one double bond (δ 124.33 ppm, δ 139.64 ppm). The DEPT 90 and 135 spectra (Appendix X middle and upper spectra respectively) indicated the presence of eight methyls, an acetyl group, nine methylenes, seven methines, and six quaternary carbon signals (Table 4.6).

Table 4.6: DEPT 90 and 135 showing Chemical Shift Values (δ in ppm) for the different types of carbon

Methyl	Methylene	Methine	Quaternary
16.66	18.33	39.99	33.33
16.74	23.61	40.04	33.75
16.87	26.66	48.83	37.72
17.40	28.10	55.27	38.33
21.40	28.33	59.58	42.08
23.74	31.66	80.93	139.64
28.10	33.33	124.33	
28.75	38.47		
	41.66		

In proton – proton correlation spectroscopy (^{1}H- ^{1}H COSY) experiment, the spectrum (Appendix XI) depicts a mirror image about the diagonal and as such any one side of the diagonal can be use to establish the correlation. The proton COSY shows strong correlation between proton at δ 5.12 with 1.45 and 1.84 ppm as well as proton at δ 4.44 with 1.08 and 1.62 ppm as presented in table 4.10.

The proton - proton Nuclear Overhauser Effect Specroscopy (^{1}H- ^{1}H NOESY) is a two dimensional NMR analysis which display protons that coupled with one another through space. Coupled protons that gave cross peaks about the diagonal displayed spins that are spatially close. The compound shows coupling of proton at δ 4.40 with that at 0.88, 1.04 and 1.68 ppm. So also, proton at δ 5.12 coupled with 0.76, 1.45 and 1.85 ppm as shown in (Appendix XII).

Table 4.7: COSY and NOESY Correlations for the Compound UIK

COSY		**NOESY**	
Proton (δ ppm)	**Correlation (ppm)**	**Proton (δ ppm)**	**Correlation (ppm)**
5.12	1.45 and 1.84	5.12	0.76, 1.45 and 1.84
4.44	1.08 and 1.62	4.40	0.88,1.04 and 1.68

Heteronuclear Multiple Bond Coherence (HMBC) displays the coupling of carbon to proton in more than one bond (2 to 4 bonds). The HMBC experiment (Appendix XIII) of the compound depicts the multiple bonds relationship between the neighbouring carbon and hydrogen atoms as shown in (Table 4.8).

Heteronuclear Single Quantum Correlation (HSQC) analysis is similar to HMBC except that the spectra give the coupling between carbon and protons that are attached to it directly. The HSQC experiment of the compound (Appendix XIV) gives the position of each carbon atom in relation to the hydrogen atom(s) that are directly attached to it (Table 4.8).

Table 4.8: HMBC and HSQC showing Coupling between Carbons and protons of Compound UIK

HMBC		HSQC	
δ ^{1}H (ppm)	**δ ^{13}C (ppm)**	**δ ^{1}H (ppm)**	**δ ^{13}C (ppm)**
5.12	60, 47, 42 and 24	5.12	124
4.44	171 and 16.87	4.44	80.98
2.06	21	2.04	21.40
2.02	171	2.00	28.11
1.97	35	1.92	21.60
1.85	140 and 124	1.84	26.66
1.94	42, 23 and 21	1.45	40.04
1.62	80	1.44	17.40
1.61	80 and 24	1.43	28.33
1.60	80 and 55	1.40	28.75
1.59	80 and 35	0.96	21.40
1.50	40 and 24	0.82	48.08
1.25	17	0.76	16.87
1.19	24		
1.10	42		
1.09	42, 40 and 26		
1.0	47, 42, 40 and 33		
0.97	55, 47, 42, 40 and 16		
0.88	40 and 32		
0.82	80, 55, 47, 37, 31, 23 and 16		
0.81	80, 55, 47, 37, 27 and 16		
0.79	60, 42, 40, 37, 35, 34 and 27		
0.76	16		

4.7 Antimicrobial Studies of *F. kamerunensis*

The Methanol extract (ME), partition fractions: Ethyl acetate (EA), Diethyl ether (DE), n-Butanol (NB) and Column fraction (CF) showed antibacterial and antifungal activities against the test organisms with zones of inhibition ranged from 18- 31mm compared to Control Ciprofloxacin (CP) and Fluconazole (FC). The EA extract had the highest zones of inhibition, followed by the CF, DE, and then (NB) while the ME had the least zone of inhibition. The results are shown in (Tables 4.10).

Table 4.10: Zones of Inhibition of the extracts in (mm)

Test organism	EA	CF	DE	NB	ME	CP	FC
M R S A	30	27	26	22	21	0	0
S. aureus	30	28	26	23	20	37	0
S. pyrogens	0	0	0	0	0	35	0
C. ulcerans	29	26	27	24	18	35	0
E. coli	29	27	26	22	20	32	0
P. mirabilis	0	0	0	0	0	34	0
N.gonorrhea	31	28	25	23	21	0	0
P. aeruginosa	28	27	26	22	19	32	0
C. albicans	29	26	25	24	20	0	32
C. krusei	0	0	0	0	0	0	35
C. stellatoidea	28	27	26	23	20	0	37
C. tropicalis	0	0	0	0	0	0	34

4.7.1: Minimum Inhibition Concentrations of the Plant Extracts and the Column Fraction

The minimum inhibition concentrations (MIC) against all the microbes were determined to be 6.25mg/ml for EA and CF and 12.5mg/ml for DE, NB and ME extracts. However, the MIC for CF against *Corynebacterium ulcerans* and *candida albicans* was found to be 12.5mg/ml while ME shows highest value of 25mg/ml against *Corynebacterium ulcerans* and *Pseudomonas aeruginosa*.The results of minimum inhibitory concentrations of the extract against each isolates are summarized in (table 4.11)

Table 4.11: Minimum Inhibitory Concentration (MIC) of the Extract and the column fraction

Test organism	EA (mg/ml)					CF (mg/ml)					DE(mg/ml)					NB (mg/ml)					ME (mg/ml)				
	50	*25*	*12.5*	*6.25*	*3.12*	*50*	*25*	*12.5*	*6.25*	*3.12*	*50*	*25*	*12.5*	*6.25*	*3.12*	*50*	*25*	*12.5*	*6.25*	*3.12*	*50*	*25*	*12.5*	*6.25*	*3.12*
M R S A	_	_	_	*	+	_	_	_	*	+	_	_	*	+	++	_	_	*	+	++	_	_	*	+	++
S. aureus	_	_	_	*	+	_	_	_	*	+	_	_	*	+	++	_	_	*	+	++	_	_	*	+	++
C. ulcerans	_	_	_	*	+	_	_	*	+	++	_	_	_	*	+	_	_	*	+	++	_	*	+	++	+++
E. coli	_	_	_	*	+	_	_	_	*	+	_	_	*	+	++	_	_	*	+	++	_	_	*	+	++
N.gonorrhea	_	_	_	*	+	_	_	_	*	+	_	_	*	+	++	_	_	*	+	++	_	_	*	+	++
P. aeruginosa	_	_	_	*	+	_	_	_	*	+	_	_	*	+	++	_	_	*	+	++	_	*	+	++	+++
C. albicans	_	_	_	*	+	_	_	*	+	++	_	_	*	+	++	_	_	*	+	++	_	_	*	+	++
C. stellatoidea	_	_	_	*	+	_	_	_	*	+	_	_	*	+	++	_	_	*	+	++	_	_	*	+	++

KEY: _ = No turbidity (No growth), *** = MIC**, + =Turbid (Light growth), ++ = (Moderate turbid), +++ = (High turbidity).

4.7.2 Minimum Bactericidal/Fungicidal Concentration of the plant extracts and the column fraction (MBC/MFC)

The minimum bactericidal concentration (MBC) for the EA and CF was determined to be 12.5mg/ml while for the DE, NB and ME it was found to be 25mg/ml against all the test microbes. However, the value obtained for ME against *C. ulcerans, P. aeruginosa* and *C. albicans* was 50mg/ml the results were all summarized in table 4.12.

Table 4.12: Minimum Bactericidal/Fungicidal Concentration of the Extract and the column fraction

Test organism	EA (mg/ml)			CF (mg/ml)			DE(mg/ml)			NB (mg/ml)			ME (mg/ml)			
	25	*12.5*	*6.25*	*25*	*12.5*	*6.25*	*25*	*12.5*	*6.25*	*25*	*12.5*	*6.25*	*50*	*25*	*12.5*	*6.25*
M R S A	_	*	+	_	*	+	*	+	++	*	+	++	_	*	+	++
S. aureus	_	*	+	_	*	+	*	+	++	*	+	++	_	*	+	++
C. ulcerans	_	*	+	*	+	++	*	+	++	*	+	++	*	+	++	++
E. coli	_	*	+	*	+	++	*	+	++	*	+	++	_	*	+	++
N.gonorrhea	_	*	+	_	*	+	*	+	++	*	+	++	_	*	+	++
P. aeruginosa	_	*	+	_	*	+	*	+	++	*	+	++	*	+	++	+++
C. albicans	_	*	+	*	+	++	*	+	++	*	+	++	_	*	+	++
C. stellatoidea	_	*	+	_	*	+	*	+	++	*	+	++	_	*	+	++

KEY: - = (No Colony growth), * = (MIC/MFC), + =Scanty colonies growth (Light growth), ++= (Moderate turbid colonies growth), +++ = (Heavy colonies growth).

4.6 Median Lethal Dose (LD_{50}) of the Methanol Extract of *F.kamerunensis*

The median lethal dose of the methanol extract of *F. kamerunensis* was determined intraperitonially (*i.p.*) in mice and the LD_{50}value was found to be 288.50mg/kg. The results of median lethal dose (LD_{50}) of both first and second phases are given in (table 4.9) below:

Table 4.9: Determination of LD_{50} of *F. kamerunensis* of Ethanol Extracts in Mice

Doses $mgKg^{-1}$	No of dead Animals / No of Animals Used
First Phase	
10	0/3
100	0/3
1000	3/3
Second Phase	
140	0/1
225	0/1
370	1/1
600	1/1

CHAPTER FIVE

5.0 DISCUSSION

Pharmacognostic evaluation of a plant or plant parts is considered to be an important step that provides valuable information in terms of its morphological, microscopical and physical characteristics. The macroscopic, microscopic and physical evaluation of the fresh and dried plant materials revealed some features which are of importance and can be applied in establishing the identity of the plant parts in its fresh form and dried form. This is important in the study of crude drugs since the first step in the study of crude drug is establishing the correct identity of the plant.

Macroscopically, the bark is dark-brown in colour, inner surface is light-brown, shape is concave, taste is mucillaginous without any characteristic odour, soft surface, 0.5-1.8 cm thick and fracture is fibrous.

Microscopically, the transverse section of bark of *Ficus kamerunensis* showed the outermost zone with cork tissue composing of three rows of thick-walled rectangular cells. The cortex was fairly wide and composed of several rows of cells. A wide continuous band of sclereids was present at the periphery of the cortex. The cortical parenchyma cells were thin-walled and more or less cubical to oblong. The inner bark was thin and consists of radial segments of phloem alternating with two seriate medullary rays. The macroscopic and microscopic features of the plant were in conformity with those of some plant members of the genus ficus such as *Ficus bengalensis* Linn as described by (Vikas and Vijay, 2010).

The phytochemical screening of the powdered stem bark of the *Ficus kamerunensis* reveals the presence of carbohydrate, anthraquinones, saponins, cardiac glycosides, triterpenoids, flavonoids, tannins and alkaloids. This is consistent with some members of the genus as in the bark of *F. racemosa* as reported by (Poongothai *et al*., 2011).

Results of physicochemical parameters showed the presence of moisture content of (11.7%). It is very essential to control moisture content since higher moisture content in plant material may lead to its deterioration and may therefore result in biodegradation of active constituents (Prasad *et al*., 2013). The ash values indicate inorganic residues obtained when vegetable drugs are incinerated. Total ash in a plant material includes both physiological as well as non physiological ash while acid insoluble ash is a part of total ash that is soluble in dilute and is indicative of silica present especially in sand and siliceous earth where as water soluble ash is the water soluble portion of the total ash (Prasad *et al*., 2012).

It was observed from the result that the plant material showed the presence of lesser quantity of acid insoluble ash (1.7%) compared to the water soluble ash (3.7%). It was observed that there was a reduction in extractive values with decrease in extractive solvent polarity. The alcohol and water soluble value investigated using the plant powdered material revealed water to have high extractive yield (18.4%) than alcohol which implies more constituents of the plant to be soluble in water than in alcohol (10.4%). Alcohol and water soluble extractive values can be used to detect exhausted and already utilized drugs which could be fraudulently used as substitute or adulterants (Elujoba, 1999; Khan *et al*., 1993).

Considering the above finding which is in line with other research work conducted, despite the higher extractive value of water, being a universal solvent and its use primarily as solvent by traditional healers, alcohol is still given preference in terms of

choice of solvent when it comes to medicinal plant researches. The choice of solvent in a research involving plants depend on so many factors among which include the diversity of different phyto compounds to be extracted and also what is intended with the extract.

Despite the higher yield of water observed over alcohol, ethanol was chosen as the solvent of extraction in this study. This is due to higher activities of ethanol extracts as compared to aqueous extract (Tiwari *et al.,* 2011). The yield obtained from the extraction of (1kg) powdered material of the plant was 101.15g which is roughly the alcohol extractive value (10.15%).The fractionation of the methanol extract gave a low yield with diethyl ether (2.4g i.e 0.24%), followed by ethyl acetate (4.6g i.e 0.46%) and for the n-butanol which is more polar yielded (15.7g i.e 1.57%) while the aqueous gave 65.37g i.e 6.54%. This shows the richness of the drug in polar compound (Umar, 2010). The structural elucidation of the isolated compound from the diethyl ether fraction of the methanol extract of the powdered stem bark of *F.kamerunensis* was successfully carried out with the use of nuclear magnetic resonance (NMR) Spectroscopy involving (1D ^{1}HNMR and ^{13}CNMR, DEPT 90 and 135 as well as 2D COSY, NOESY, HMBC and HSQC). The use of data from literatures in this elucidation has extensively been appreciated.

The compound UIK was obtained after purification using preparative TLC from rechromagraphed fraction 15 (f_{15}) of the main combained fraction (B). Preliminary studies on the the compound shows that it was a solid white amorphous powder, with a melting point of 220-222^{0}C wich is close to the value 224-226^{0}C reported for acetate-α-amyrin by (Sissay and Abeba, 2005). The compound shows positive reaction to the Libermann-Bucchard sparaying reagent (pink coloured spot) suggesting steroidal or triterpinoidal nucleus.

The proton (^{1}H NMR) of the UIK sample indicated the presence of many methyl protons in the region δ 0.88 to 1.24 ppm; methylene protons in the region δ 1.5 to 2.8 ppm; acetate de-shielded methyl proton at δ 2.05 ppm and hydroxymethine proton appeared as doublet of doublets at δ 3.24 ppm, the compound also indicated the presence of two methine protons; proton at δ 5.12 ppm as a triplet suggesting the presence of a trisubstituted olefinic bondthe other is oxygenated at δ 4.44 ppm on position-3 thus, suggesting triterpenoid or steroid acetate (Sissay and Abeba, 2005).

The carbon 13 NMR spectra indicated the presence of 32 carbon peaks; with a carbonyl carbon (δ 171.04 ppm) and one double bond (δ 124.33 ppm, δ 139.64 ppm). The DEPT 90 and 135 spectra indicated the presence ofeight methyls, an acetyl group, nine methylenes, seven methines, and six quaternary carbon signals.

The ^{1}H and ^{13}C NMR values for all the protons and carbons were assigned on the basis of COSY, HMQC and HMBC correlations.

A search in literature found that the physical and spectral data of the UIK are consistent with the reported literature values (Sissay and Abeba, 2005) which was supportive to the structure of a ursane pentacyclic triterpene skeleton having a hydroxyl group at C-3 position with a double bond at C-12 as well as a carbonyl carbon.Thus, the structure of the UIK was assigned as the known compound **α-amyrinacetate** (Table 5.1 and Fig**.** II)

Table 5.1: Comparative analysis of ^{1}H and ^{13}C NMR data of literature Values and compound UIK.

S/N	$^{1}H^{*}$	$^{13}C^{*}$	$^{1}H^{**}$	$^{13}C^{**}$	Carbon type
1		38.80	0.88	38.47	CH_2
2		28.50		28.10	CH_2
3	4.5 (dd, 1H)	81.30	4.44(dd, 1H)	80.98	CH
4		38.10		38.33	C
5		55.60	1.32 (d, 1H)	55.27	CH
6		18.60		18.33	CH_2
7		33.20		33.33	CH_2
8		37.20		37.72	C
9		48.00		48.83	CH
10		34.10		33.75	C
11		23.60		23.61	CH_2
12	5.15 (t, 1H)	124.70	5.12 (t, 1H)	124.33	CH
13		140.00		139.64	C
14		42.40		42.08	C
15		28.50		28.33	CH_2
16		27.00		26.66	CH_2
17		33.20		33.33	C
18		59.40		59.58	CH
19		40.00		40.04	CH
20		40.00		39.99	CH
21		31.60		31.66	CH_2
22		41.90		41.66	CH_3
23		28.40	1.24 (s, 3H)	28.10	CH_3
24		16.10	0.88 (s, 3H)	16.66	CH_3
25		17.10	1.44 (s, 3H)	17.40	CH_3
26		17.20	0.76 (s, 3H)	16.87	CH_3
27		23.60	1.94 (s, 3H)	23.74	CH_3
28		28.40	0.88 (s, 3H)	28.75	CH_3
29		17.90	1.0 (s, 3H)	16.74	CH_3
30		21.70	0.96 (s, 3H)	21.40	CH_3
1^{1}		171.40		171.04	$\underline{C}OO$
2^{1}	2.05 (s, 3H)	21.70	2.06 (s, 3H)	21.60	$\underline{C}H_3C00$

*(Sissay and Abeba, 2005) ** (Compound UIK)

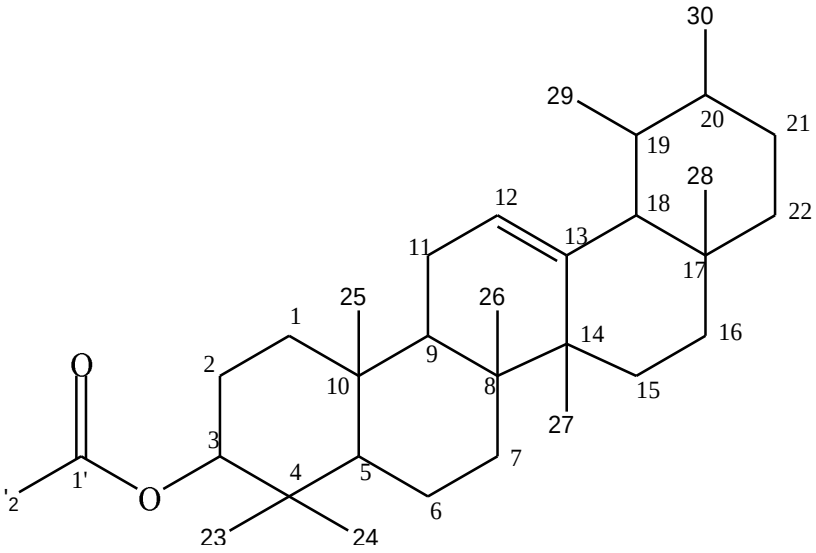

Figure 3: Chemical structure of the UIK Compound (α-amyrin acetate) Isolated from the diethyl ether fraction of the methanol extract of the powdered stem-bark of *F. kamerunensis*

The acute toxicity carried out on the methanol extract of *F.kamerunensis* was found to be 288.5mg/kg body weight in mice when administered intraperitonially (Lorke, 1983). The result of the toxicity studies at the value obtained above shows that the plant material used for the studies is toxic which falls within range (>500 mg/kg) of the categories of toxicity classification for terrestrial organisms (USEPA, 2013).

The extract, partion fraction and the major column fraction of the powdered stem bark of *F.kamerunensis* significantly inhibited the growth of the test microorganism with the zones of inhibition ranged from 18- 31 mm. The antimicrobial activity of other members of the genus ficus has been reported; leaves of *F.abutilifolia* (Ukwubile 2010), *F. exasperate* (Odunbaku *et al., 2008), F.ingens* (Aliyu *et.al*, 2008), *F.syncomorus* (Njume *et.al*, 2009) etc. Research reports indicates that the the ability of the plant extract to inhibit the growth of microorganism may be as a result of the bioactive compounds such as flavonoids, saponin, alkaloids, tannins and phenols(Osuagwu *et al*., 2007). The above literatures support the present studies of the antimicrobial property of the plant, which shows presence of these bioactive compounds.

Antibacterial activity was expressed as diameter zone of inhibition (Table 4.11). A zone of observable inhibition of growth of each micro-organism served as a criterion for declaring an extract sensitive and was indicated by a clear zone around the well. The diameter of zone of inhibition of extract against test microorganisms (in mm) was highest for the ethyl acetate, followed by column fraction, diethyl ether then n-butanol and the least was methanol extract.The zones of inhibition for the extracts and column fraction are in the ranges of 18-31mm in diameter against the various test microbes. A

wider dimension for the zones of inhibition of 31mm and 29mm were observed for ethyl acetate against *N.gonorrhoae* and *E.coli* respectively which strogly support the ethnomedical usage of the plant against sexually transmitted infections (STIs). The sensitivity of the test microbes to the extracts and column fraction were within similar ranges, however, *S.pyrogens, P.mirabilis, C.krusei* and *C.tropicalis* were insensitive to the entire extract and column fraction compared to Control Ciprofloxacin (CP) and Fluconazole (FC).

Minimum Inhibitory Concentration (MIC) is defined as the highest dilution or least concentration of a sample that inhibit the growth of microorganisms. Determination of the MIC is important in diagnostic laboratories because it helps in confirming resistance of microorganism to an antimicrobial agent and it monitors the activity of new antimicrobial agents. The MBC and MFC were determined by subculturing the test dilution (used in MIC) on to a fresh solid medium and incubated further for 24 hours. The concentrations of the plant extracts that completely killed the bacteria and fungi were taken as MBC and MFC respectively. Moreover, it was noted that most of the antimicrobial properties in different extract shows, MBC value that is almost two fold higher than there corresponding MICs (Omar *et.al*, 2010). The above literature supports the results obtained for the MIC, MBC and MFC. The MIC for the ethyl acetate fraction and Column fraction was (6.25mg/ml) while there MBCs and MFCs was (12.5mg/ml). Moreover, the MICs for the diethyl ether, n-butanol and methanol extracts were (12.5mg/ml) while there MBCs and MFCs was (25mg/ml). However, methanol extract shows highest MBC and MFC value of (50mg/ml) against *C.ulcerans, p.aeruginosa* and *C.albicans.*

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

- Pharmacognostic evaluation of the plant *Ficus kamerunensis* shows that, macroscopically, the bark is dark-brown in colour, inner surface is light-brown, shape is concave, taste is mucillaginous without any characteristic odour, soft surface, 0.5-1.8 cm thick and fracture is fibrous.
- Microscopically, the transverse section of the stem-bark of the plant showed the outermost zone with cork tissue composing of three rows of thick-walled rectangular cells. The cortex was fairly wide and composed of several rows of cells. A wide continuous band of sclereids was present at the periphery of the cortex. The cortical parenchyma cells were thin-walled and more or less cubical to oblong. The inner bark was thin and consists of radial segments of phloem alternating with two seriate medullary rays.
- Phytochemical screening of the powdered stem -bark reveals the presence of carbohydrate, anthraquinones, saponins, cardiac glycosides, triterpenoids, flavonoids, tannins and alkaloids.
- Physicochemical parameters studied showed the moisture content to be (11.7%). Acid insoluble ash (1.7%) and water soluble ash (3.7%). The alcohol and water soluble value investigated using the ground plant powdered material revealed (18.4%) and (10.4%) respectively.
- The yield obtained from the extraction of (1kg) powdered material of the plant was 101.15g that is (10.15%).The fractionation of the methanol extract gave

0.24% yield with diethyl ether, ethyl acetate 0.46% n-butanol 1.57% and 6.54% for the aqueous.

- The compound UIK was isolated after purification using preparative TLC from rechromagraphed fraction 15 (f_{15}) of the combained fraction (B). The compound UIK is white amorphous powder, with a melting point of 215-218^{0}C. It gives pink colour with Libermann-Bucchard sparying reagent.
- The structure of the UIK compound isolated was assigned as the known compound α-amyrin acetate and is reported for the first time from the plant to the best of my knowledge.
- The acute toxicity of the methanol extract of *F.kamerunensis* was found to be 288.5mg/kg body weight in mice when administered intraperitonially (*ip*).
- The diameter of zone of inhibition of extracts against test microorganisms (in mm) was highest for the ethyl acetate, followed by column fraction, diethyl ether then n-butanol and the least was methanol extract.The zones of inhibition for the extracts and column fraction are in the range of 18-31 mm. however, *S.pyrogens, P.mirabilis, C.krusei* and *C.tropicalis* were resistant to the entire extract and column fraction.
- The MIC for the ethyl acetate fraction and column fraction was (6.25 mg/ml) while MBCs and MFCs are 12.5mg/ml. Moreover, the MICs for the diethyl ether, n-butanol and methanol extracts were (12.5mg/ml) while the MBCs and MFCs was (25mg/ml). However, methanol extract shows highest MBC and MFC value of (50mg/ml) against *C.ulcerans, P.aeruginosa* and *C.albicans.* This report on the antimicrobial activity of the stem-bark of the plant is for the first time to the best of my knowledge.

6.2 Conclusion

The pharmacognostic and antimicrobial studies of the stem-bark of *F.kamerunensis* were successefully carried out and reported for the first time to the best of my knowledge.The macroscopic and microscopic features of the plant were found to be consistent with other members of the genus Ficus.The evaluated antimicrobial property of the plant supports the ethnomedical usage of the plant for the treatment of sexually transmitted infections. A known compound **α-amyrin acetate** was isolated and also reported for the first time from the stem-bark of the plant.

6.3 Recommendation

- Acute and chronic toxicity studies should be extensibly carried out on the different parts of the plant so that proper recommendation on how the plant should be used or otherwise will be properly guided.
- Other fractions (ethyl acetate and n-butanol fractions) should be explored for possible isolation and elucidation of structure of other chemical compounds.
- Activity (Bioassay) guided isolation should be carried out specifically on the ethyl acetate fraction for antinimicrobial property so that a pure compound with good antimicrobial activity may be isolated and its elucidated.

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APPENDICES

APPENDIX I

Determination of some physical Parameters of the powdered Whole Plant of *F.kamerunesis*

a) **Determination of moisture content of powdered whole plant of *F.kamerunesis***

2g of the powdered plant material was used

Description	1	2	3	4	5
Constant weight of crucible (g)	50.71	46.73	50.30	40.15	40.23
Weight of crucible + powder before heating(g)	52.71	48.73	52.30	42.15	42.23
Wt of crucible + content after heating to constant weight (g)	52.50	48.50	52.05	42.39	41.99
Loss in weight (g)	0.21	0.23	0.25	0.24	0.24
Loss on drying (%)	10.5	11.5	12.5	12.0	12.0
Average mean (%)	11.7				

Sample calculation

% Loss on drying= [(wt of crucible and content before heating- wt of crucible and content after heating) ÷ 2g] ×100

= [(52.71-52.50) ÷ 2] × 100

=10.5%$^{w}/_{w}$

APPENDIX II

b) **Determination of Ash Value of powdered whole plant of *F.kamerunesis***

2g of the powdered plant material was used

Description	1	2	3	4	5
Constant weight of crucible (g)	39.25	37.42	38.27	39.25	38.26
Weight of crucible and content (g)	41.25	39.42	40.27	41.25	40.26
Weight of crucible and Ash (g)	41.08	39.25	40.09	41.08	40.07
Weight of Ash (g)	0.17	0.17	0.18	0.17	0.19
Ash Value (%)	8.5	8.5	9.0	8.5	9.5
Average mean (%)			8.8		

Sample calculation

$$\text{Ash value} = \frac{\text{weight of Ash}}{\text{Initial weight of drug}} \times 100$$

$$\text{Ash Value} = \frac{0.17 \times 100}{2\ g} = 8.5\ \%w/w$$

APPENDIX III

(c) **Determination of Acid insoluble Ash of powdered whole plant of *F.kamerunesis***

2g of the powdered plant material was used

Description	1	2	3	4	5
Constant weight of crucible (g)	39.29	37.42	38.27	38.26	39.25
Weight of crucible and Acid in. ash (g)	39.32	37.45	38.31	38.29	39.29
Weight of Acid insoluble ash (g)	0.03	0.03	0.04	0.03	0.04
Acid Insoluble Ash Value (%)	1.5	1.5	2.0	1.5	2.0
Average mean (%)			1.7		

Sample calculation

$$\text{Acid Insoluble Ash value} = \frac{\text{weight of acid insoluble ash}}{\text{Initial weight of drug}} \times 100$$

Acid insoluble ash = 0.03/2g = 1.5 %w/w

APPENDIX IV

(d) **Determination of water soluble Ash of powdered whole plant of *F.kamerunesis***

Description	1	2	3	4	5
Constant weight of crucible (g)	39.25	38.26	39.29	37.42	38.42
Weight of crucible and Ash (g)	39.65	38.66	39.69	37.82	38.82
Weight of Ash (g)	0.40	0.40	0.40	0.40	0.40
Weight of water Insoluble ash (g)	0.32	0.32	0.33	0.33	0.33
Weight of water soluble ash (g)	0.08	0.08	0.07	0.07	0.07
Water soluble Ash Value (%)	4.0	4.0	3.5	3.5	3.5
Average mean (%)			3.7		

Sample calculation

$$\text{Water soluble Ash value} = \frac{\text{Wt of total ash - Wt of Water Insoluble Ash}}{\text{Initial weight of drug}} \times 100$$

$$\text{Water soluble ash Value} = \frac{(0.4 - 0.33) \times 100}{2\ \text{g}} = 3.5\ \%\text{w/w}$$

APPENDIX V

Determination of Extractive values of powdered whole plant of *F.kamerunesis*

(a) Water –Soluble Extractive value

5 g of the powder was used in 100 ml of water.

Description	1	2	3	4	5
Constant weight of dish (g)	239.84	230.0	200.0	225.3	230
Weight of crucible + content + heating (g)	240.765	230.925	200.9	226.225	230.925
Water extractive content (g)	0.925	0.925	0.9	.925	0.925
water extractive Value (%)	18.5	18.5	18.0	18.5	18.5
Average mean (%)			18.4		

Sample calculation

Water extractive value = Wt of dish & content after heat (g) - Const wt. of dish (g)/Initial weight of Drug ×100

= (0.925÷5) ×100 = 18.5%$^{w}/_{w}$

APPENDIX VI

(b) **Alcohol – Soluble Extractive value**

5 g of the powdered whole plant of *F.kamerunesis* was used in 100 ml of 90% ethanol

Description	1	2	3	4	5
Constant weight of dish (g)	123.18	123.97	123.175	122.30	124.50
Weight of dish + content + heating (g)	123.705	124.495	123.675	122.825	125.025
Alcohol extractive content (g)	0.525	0.525	0.500	0.525	0.525
Alcohol extractive Value (%)	10.5	10.5	10.0	10.5	10.5
Average mean (%)			10.40		

Sample calculation

Alcohol extractive value = {Wt of dish + content after heat (g) - Constant wt. of dish (g)}÷initial weight of drug X 100

= [(123.705-123.18) ÷5] ×100 = 10.50 w/w

APPENDIX VII

Determination of Acute toxicity of extract of *F. kamerunesis* using Lorke's Method in mice

Phase I

Group	Dose $mgKg^{-1}$	No. Of death/ No. Of Survival
1	10	0/3
2	100	0/3
3	1000	3/3

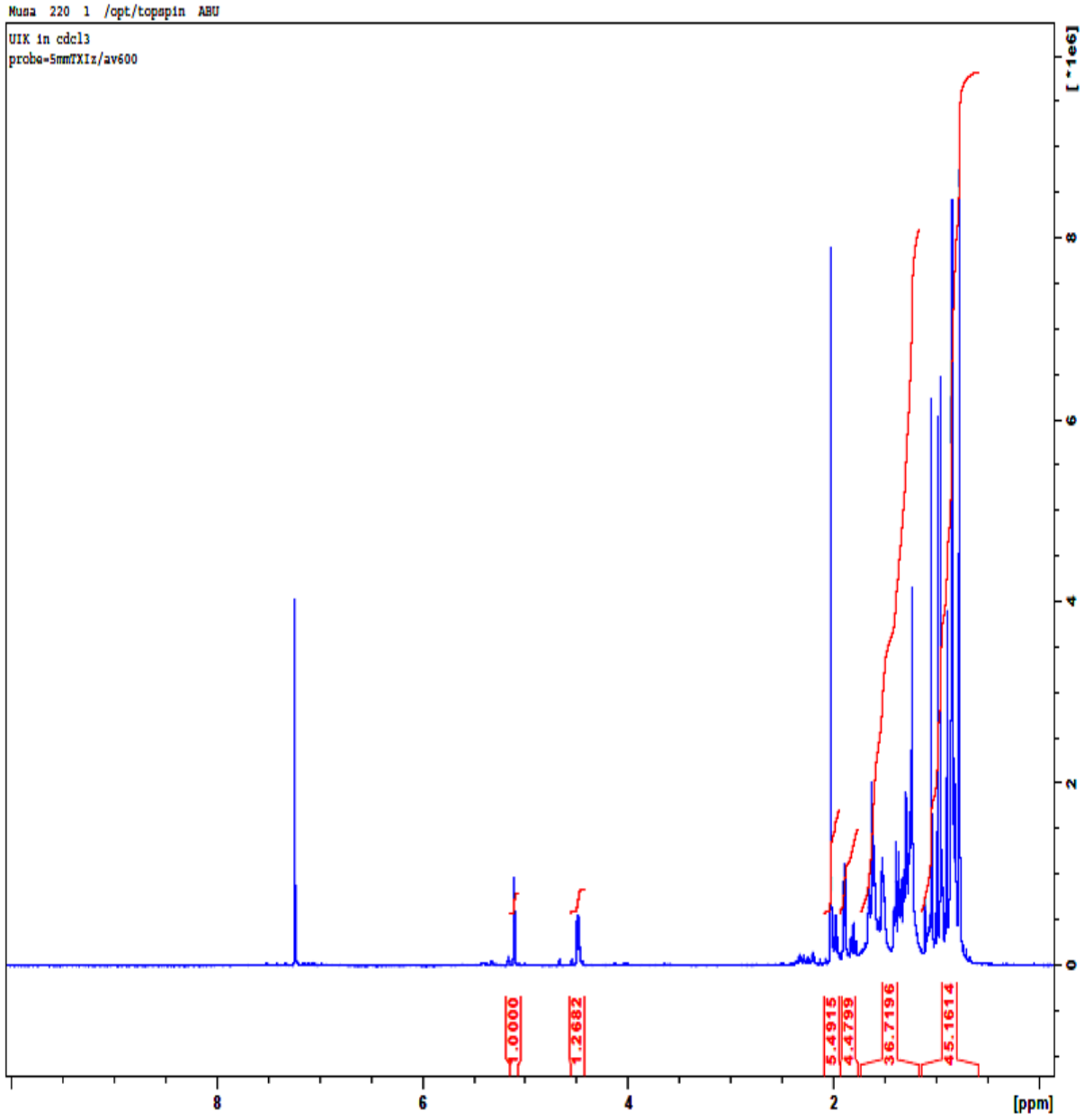
Phase II

Group	Dose $mgKg^{-1}$	No. Of death/ No. Of Survival
1	140	0/1
2	225	0/1
3	370	1/1
4	600	1/1

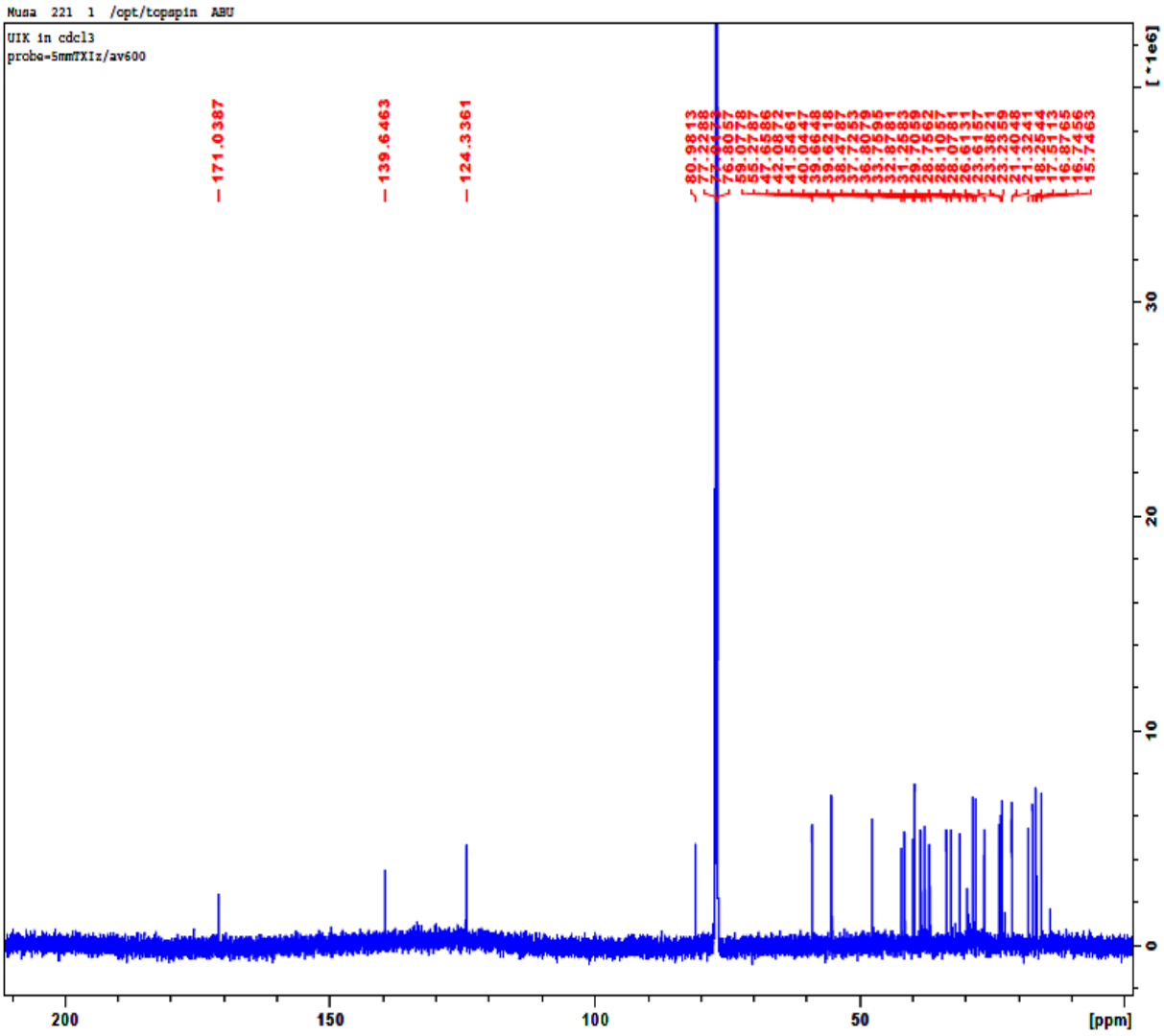
$LD_{50} = \sqrt{\text{Minimum toxic dose x Maximum tolerated dose}}$

$LD_{50} = \sqrt{225} \times 370 = 288.50 mg/kg$ i.p

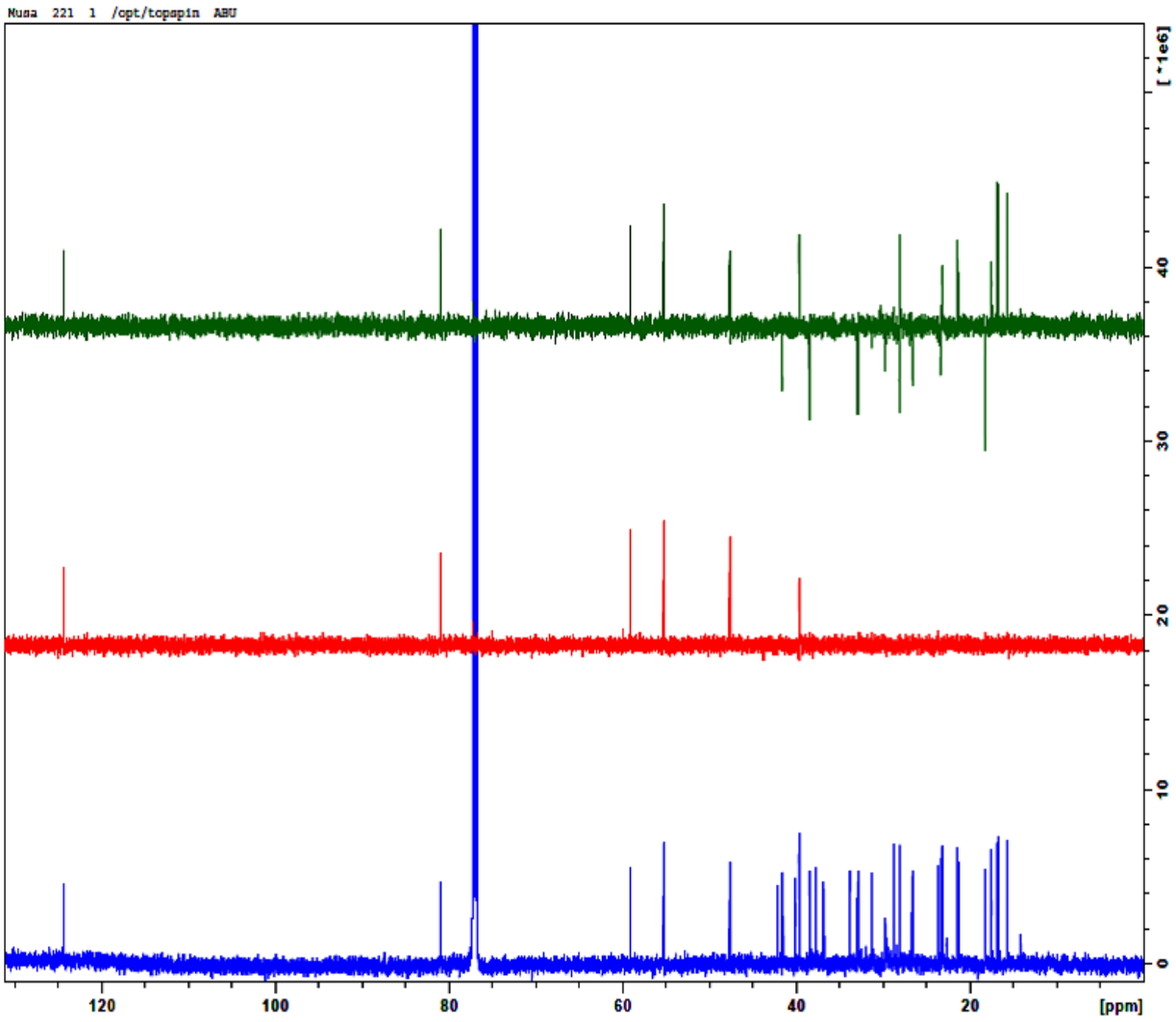
APPENDIX VIII: ^{1}H-NMR for Compound UIK



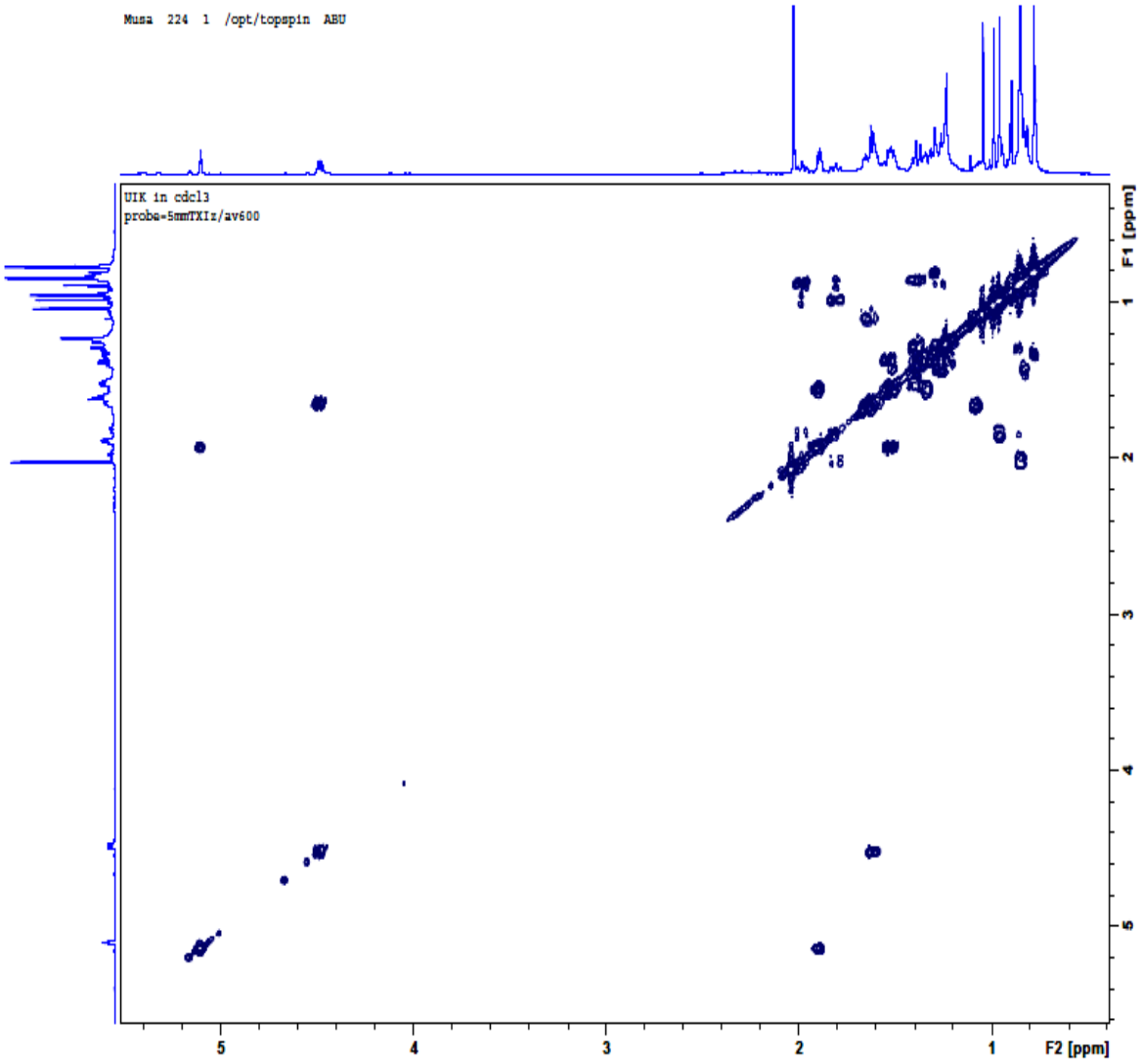
Appendix IX: ^{13}C-NMR for Compound UIK



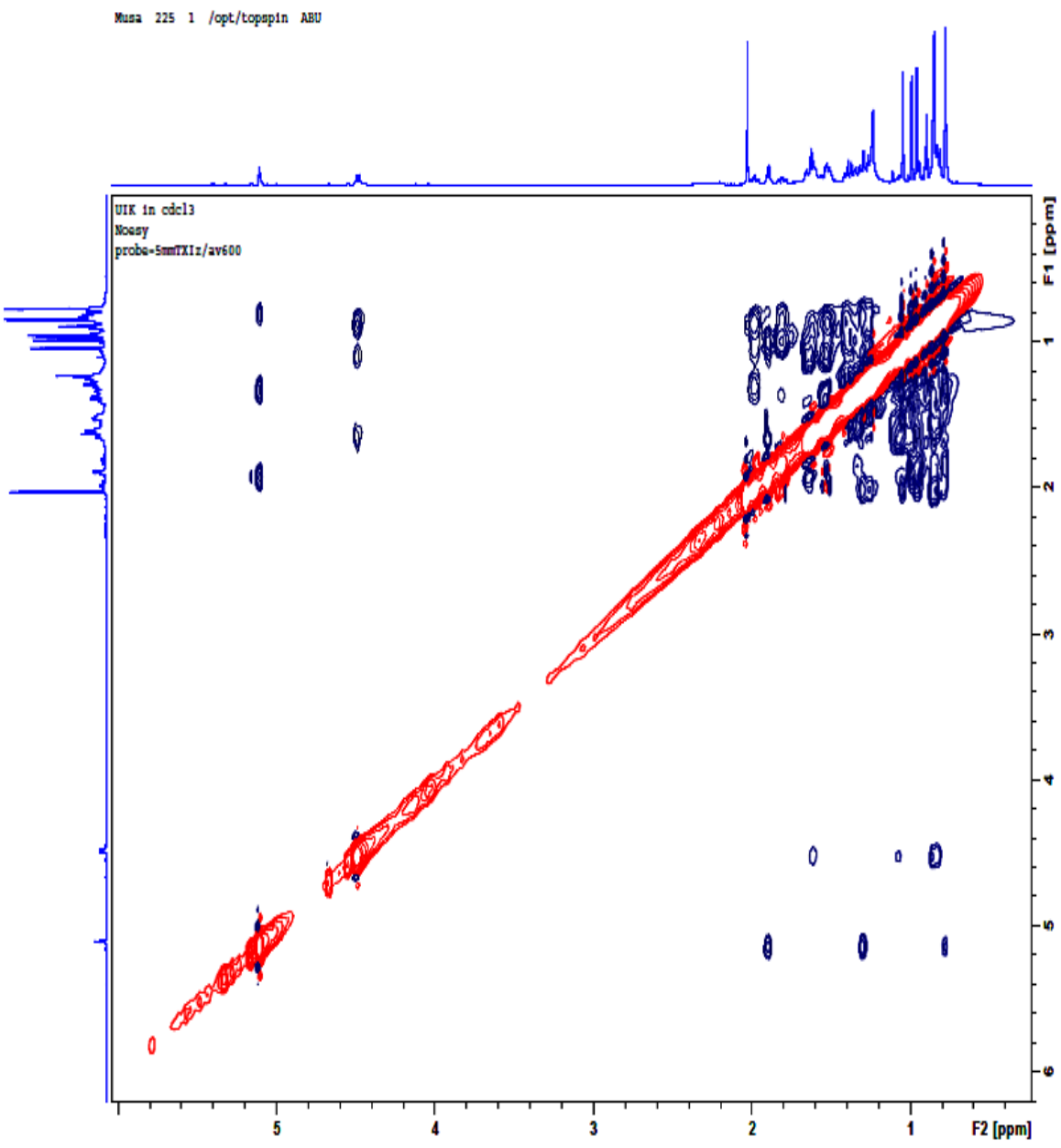
Appendix X: DEPT 90 and 135 for Compound UIK



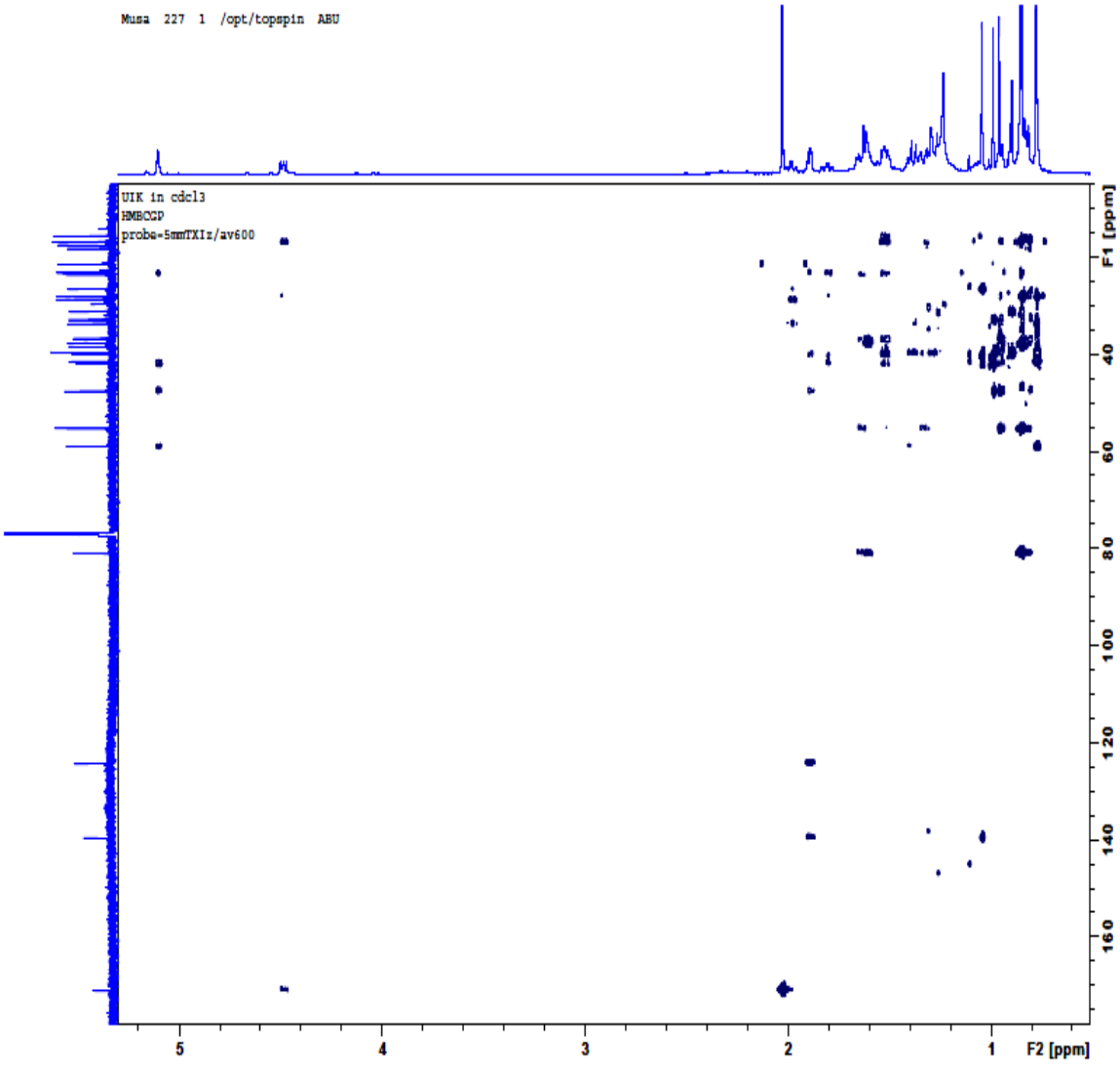
Appendix XI: ^{1}H-^{1}H COSY Spectra for Compound UIK



Appendix XII: ^{1}H-^{1}H NOESY Spectra for Compound UIK


Musa 225 1 /opt/topspin ABU
UIK in cdcl3
Noesy
probe=5mmTXIz/av600
F1 [ppm]
F2 [ppm]
1
2
3
4
5
6

Appendix XIII: HMBC Spectra for Compound UIK



Appendix IV: HSQC Spectra for Compound UIK