ISOLATION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM STEMBARK EXTRACT OF *Uapaca pilosa*Hutch

BY

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NIGERIA.

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BY

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DEPARTMENT OF CHEMISTRY,
FACULTY OF SCIENCE
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

SEPTEMBER, 2016

DECLARATION

I declare that the work in this dissertation entitled **'Isolation and Characterization of Bioactive Compounds from Stembark Extract of *Uapaca pilosa* (Hutch.)'** was carried out by me in the Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria. The information derived from literature has been duly acknowledged in the text and list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Ayodele Joshua ATIBIOKE	**.....**	**.....**
Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled **'Isolation and Characterization of Bioactive compounds from Stembark Extract of *Uapaca pilosa* (Hutch.)'** by Ayodele Joshua ATIBIOKE, meets the regulations governing the award of Master degree in Organic Chemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Professor I. G. Ndukwe **.....** **.....**
Chairman, Supervisory Committee Signature Date

Professor M. S. Sallau **.....** **.....**
Member Supervisory, Committee Signature Date

Professor V.O. Ajibola **.....** **.....**
Head of Department Signature Date

Professor Kabir Bala **….....** **.....**
Dean School of Postgraduate Studies Signature Date

DEDICATION

This work is dedicated to the Almighty God and to the entire family of Mr and Mrs Clement O. Atibioke.

ACKNOWLEDGEMENTS

My greatest gratitude goes to the Almighty and Omnipotent God for the gift of life, good health and the privilege to undertake this study. I also appreciate my supervisors; namelyProf. I. G. Ndukwe and Prof. M. S. Sallau for their time, support and mentorship throughout the period of this research. Sirs I will forever be grateful. I owe my profound gratitude to Dr J. D. Habila for his advice, guide and through whom the NMR spectroscopic analyses were carried out. Mallam Kabir of the Department of Pharmacognosy, and Mallam Shittu of the Department of Microbiology, all of Ahmadu Bello University, Zaria.For their assistance in the phytochemical and antimicrobial screening during the course of this research. I will be ungrateful if I forget to appreciate both academic and non-academic staff of Chemistry Department Ahmadu Bello University. I also appreciate my colleagues J. Achika, T. Nasir, M. Danmbata, Adebiyi Philip, B. Okoli, Kay Odeja, R. Obansa, B. Olukotun, J. Jephtha, C. Nweze, M. Gazali and S. Lukman you guys are the best. I will like to thank my friends; Ajayi Rita, Abodunrin Oluwatosin, Osumeje Blessing, Bukola Rosiji, Babachani Zainab, Onuche Ugbede-ojo, Chicharito, Ismaheel, Late Amodu Daniel and others for their friendly advice. I cannot but thank my family; Mr. Atibioke Oluwafemi Clement and Mrs. Victoria Silifat Atibioke, my siblings; Adeyemi Balogun, Adewale, Olajide, Oluwafunmilayo, Adejoke Momoh and Oyindamola for their financial and moral support. My gratitude goes to all my friends and well-wishers even though I cannot mention all your names but you are all dear to me.

ABSTRACT

Uapaca pilosa(Hutch.) a plant used in some parts of Africa in the treatment of dysentery, menstrual pain, fever, constipation, erectile dysfunction, skin infections, female sterility, pile, rheumatism, emetic, tooth-troubles and fatigue. The dried plant was extracted, the extract was subjected tophytochemical investigation using standard method revealed the presence of alkaloids, flavonoids, anthraquinones, tannins, saponins, steroids, terpenoids and glycocides. Extensive silica gel column chromatography of the ethylacetate fraction of the stem bark extract, the most active of all the fractions, led to the isolation of two compounds GF1 and GF2. Their identities were determined by analysis of their spectral data using FTIR, 1D and 2D NMR. The structures of the compounds were supported by comparing their spectral data with the literature. GF1 was found to be betulin while GF2 was found to be beta-sitosterol. The antimicrobial screening of the crude extract and fractions using agar well diffusion methodshowed activity against *Staphylococcus aureus, Shigella dysenteriae, Salmonella typhii, Bacillus subtilis* and *Escherichia coli*. The Zone of Inhibition of the plant extract against selected microorganisms ranges from 13mm to 17mm against *Staphylococcus aureus,* 10mm to 14mm against *Bacillus subtilis,* 12mm to 15mm against *Shigella dysenteriae,* 15mm to 18mm against *Escherichia coli*and 10mm to 11mm against*Salmonella typhii*. The MIC and MBC for the extract, fractions and isolated compounds were also determined. The range of Minimum Inhibitory concentration is between 6.25 mg/mL to 25 mg/mL for *Staphylococcus aureus*, 25 mg/mL for *Shigella dysenteriae,* 6.25 mg/mL for *Bacillus subtilis*and 12.50 mg/mL for *Escherichia coli* while the Minimum Bactericidal Concentration range between 12.50 mg/mL for *Staphylococcus aureus*, 50 mg/mL for *Shigella dysenteriae*, 12.50 mg/mL for *Bacillus subtilis* and 25 mg/mL for *Escherichia coli*. This study on the stem bark

extract from *Uapaca pilosa*, used traditionally in some parts of Africa as a medicinal plant for the treatment of various ailments has confirmed that it has antimicrobial activity against the microbes that cause some of these diseases.

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CHAPTER ONE

1.0 INTRODUCTION

Over the years the world traditional medicine has been known to take its source from higher plants and their extracts in the treatment of diseases and infections (Sofowora, 1983). Until 19th century, when the development of chemistry and synthetic organic chemistry started, medicinal plants were the sources of active materials used in healing and curing human diseases. Before the advent of modern methods of producing drugs, medicinal plants such as *Allium sativum*, *Azadirchata indica* and *Citrus limonum* were used in treating both malaria and typhoid fever. Also some plant leaves were used in treating skin rashes and to heal wounds. Likewise, modern pharmaceuticals rely heavily on these medicinal plants for their raw materials such as cocoa leaves and opium plant from papaver species for analgesics. The active principles of plants differ from one plant to another due to the diversity in biological activities (Sofowora, 1983; Kubmarawa *et al.*, 2007; Krishnaiah *et al.*, 2009).

Traditional medicinal practice has been established for centuries in many parts of the world. Numerous plants and herbs are used globally by traditional medicine practitioners. The practice is known to vary from one country to another (Sofowora, 1984). Extracts from the various plant parts (leaves, stem bark and roots) of various higher plants are used in herbal medicine production (Sofowora, 1983, 1984, 1993). Plants` extracts are given singly or as concoctions for the treatment of various ailments. In actual sense more than 75% of the world population depend on these various forms of concoctions and herbal decoctions for the treatment of infections (Robenson and Zhang, 2011). Phytochemical constituents are the basic raw materials source for the establishment of pharmaceutical industries (Mothana and Lindequist, 2005; Wojdylo *et*

al., 2007). The constituents present in the plant play vital roles in the crude drugs identity. Phytochemical screening is very important in identifying new sources of therapeutically and pharmacologically important compounds like alkaloids, anthraquinones, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids (Akindele and Adeyemi, 2007).

Some plants such as *Aloe vera, Alliium sativum, Maranta arundinacea, Pimpinella anisum* and *Arnica montana* widely distributed in Africa, Asia and Southern part of North America have been reported to be the basis of treatment in human diseases and also as useful components in the development of new active components (Boudreau and Beland, 2006; Bunyapraphatsara*et al*., 1996; Alan *et al.*, 1995). The World Health Organization (WHO) estimates that 80 % of the world's population relies mainly on herbal medicine for primary healthcare (Hong *et al*., 2010). In China, traditional medicine is largely based on around 5000 plants which were used in treating 40 % of urban patients and 90 % of rural patients (Abdel-Azim *et al*., 2011). In industrialized countries, plants have contributed more than 7000 compounds used in the pharmaceutical industries including ingredients in heart drugs, laxatives, anti-cancer agents, hormones, contraceptives, diuretics, antibiotics, decongestants, analgesics, ulcer treatments and anti-parasitic compounds (Simo, 2012). About 25 % of all prescription drugs dispensed by Western pharmacists is likely to contain ingredients derived from plants (Simo, 2012). These include: *Laevodopamine* from tropical legume *Mucuna deeringiana*, used for treating Parkinson disease (dos Santos *et al*., 2012). Picrotoxin derived from *Anaminta cocculus*, a tropical climbing plant from south East Asia, is used as a nervous system stimulant and in cases of barbiturate poisoning (Abebe and Haramaya, 2013). Reserpine, extracted from the root of the serpent-root, *Rauwolfia*

serpentine, is used for lowering blood pressure, as a tranquilizer and in India as a remedy for snake bites (Unnikrishnan, 2004). Eucalyptol obtained from species of eucalyptus, is a well-known antiseptic used in throat medicines, cough syrups, ointments, liniments, as inhalant for bronchitis and asthma. Eucalyptus is used throughout the world and is regarded as a universally available product (Eschler *et al*., 2000). Cultivation has replaced wild collection for the supply of some essential drugs used in modern medicine. The Madagascar rosy periwinkle (*Cathrathus roseus*) is widely cultivated in Spain and Texas for its alkaloids vinblastine and vinscristine, which are used for treating childhood leukaemia and hoolgkin disease (Bauer *et al.*, 1996).

The best known example is probably aspirin, chemically related to a compound that was first extracted from the leaves and bark of willow tree, *Salix alba* and a herb meadow sweet, *Filipino dula malaria*. The anti-malarial drug quinine, extracted from the bark of a South American tree, *Cinchona ledgeriana*, was first brought to Europe (where malaria was widespread) in early 17^{th} century by Jesuit priests (Fruhstorfer *et al*, 2001). It was once remarked that Oliver Cromwell died of malaria because he refused to be treated with a "Jesuit" medicine. Synthetic guanine has now been developed for drug use, but the bark is still in use to treat certain heart arrhythmias and commercially sold as a bitter flavouring agent well known in tonic water (Fruhstorfer *et al*, 2001).

Also, the bark of yohimbe, *Pausinnystalia yohimbe* is used extensively in traditional healthcare system in West Africa (Robber and Tyler, 1999).

1.1 Statement of the Research Problem

About half of the number of death recorded in the tropical countries are largely due to infectious diseases (Iwu *et al.*, 1999). This can be linked to the increasing bacterial

resistance to antibacterial drugs (Ojiako, 2014). Hence there is need to develop a more convenient and very active therapeutic antimicrobial agents.

1.2 Aim of the Research

The aim of this research work is to isolate and characterise bioactive components present in the plant.

1.3 Objectives of the Research

i. phytochemical screening of crude plant extracts,

ii. antimicrobial screening of the crude extract of the plant,

iii. isolation and identification of phytochemicals present in the extracts and

iv. antimicrobial screening of isolated/identified compounds.

1.4 Justification of the Research

Uapaca pilosa (Hutch) has been used in many tropical communities in traditional medicine for the treatment of protozoa, bacteria and fungi infections. To the best of our knowledge, the phytochemistry and antimicrobial activity studies of *Uapaca pilosa* have not been studied. Hence there is need to validate the ethnomedicinal uses of the plant.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The *Euphorbiaceae* Family

Family *Euphorbiaceae* is one of the largest families of flowering plants, composed of over 300 genera and 8,000 species (Webster, 1994). According to researchers, the family is very diverse in range, composed of all sorts of plants ranging from large woody trees through climbing lianas to simple weeds that grow prostrate to the ground. Members are widely distributed all around the world constituting both old world and new world plants some of which are yet to be identified. Many family members are inhabitants of tropical climates surviving hot dry desert conditions while others are rainforest trees and herbs (Webster, 1994). The family is divided into five subfamilies which include *Acalyphoideae, Crotonoideae*, *Euphorbioideae, Phyllanthoideae* and *Oldfieldoiideae* (Webster, 1975). According to this classification, the first three subfamilies are characterized by one ovule per locule (uni-ovulate) while the last two have two ovules (bi-ovulate). Phytochemical and molecular phylogenetic studies eventually accumulated evidence pointing to non-monophyly of *Euphorbiaceae* (Mwine and Van Damme, 2011; Tokuoka and Tobe, 1995). This culminated into partitioning of the traditional *Euphorbiaceae* into five families, where only uni-ovulate subfamilies constituted family *Euphorbiaceaesensu lato*, others being upgraded with additions or subtractions into their own families (Webster, 1994) and was validated by the APG II group (Wurdack *et al*., 2005). The new classification left family *Euphorbiaceaesensu lato* with five subfamilies, 49 tribes, 317 genera and about 8,000 species (Webster, 1994).

2.2 The *Uapaca* genus

Uapaca is an evergreen, dioecious, small to medium-sized tree under *Euphorbiaceae* family and belongs to the order malpighiales. The plant is distributed in tropical Africa and Madagascar. Other member of the family include *Uapaca togoensis, Uapaca guinessis, Uapaca bail, Uapaca paludosa, Uapaca le-testuana, Uapaca lebrunii, Uapaca littoralis,* and *Uapaca macrostipulata* (Breteler, 2012). *Uapaca* species are part of ethno medicinal plants used by Africans in treating various diseases. Some of the species in the genus are represented in West Africa (Hutchinson and Dalziel, 1958; Heywood, 1978; Van Damme, 2001). Two of the species *Uapaca stipularis* and *Uapaca kirkiana* are commonly used in folkloric medicine, which is a practice by lay people in the West African sub region (Oliver, 1960). *Uapaca heudelotii* is ethnomedicinally reported to treat skin infections, female sterility, as an emetic, pile and gargle for tooth-troubles. It also serves as a source of highly-priced hard timber (Dalziel, 1937; Burkill, 1985). *Uapaca togoensis* is reported in the treatment of female infertility, as a restorative wash against fatigue and for making charcoal (Irvine, 1948). The wood of *Uapaca staudtii* is termite-proof, difficult to work upon because of its chemical composition and strength; it is used for making furniture, railway sleepers and barrel staves (Dalziel, 1937). *Uapaca paludosa* and *Uapaca vanhouttei* have also been reported for making charcoal and are used as firewood (Dalziel, 1937).

2.3 Uapaca pilosa

Uapaca pilosa (Hutch.) is an indigenous tropical African plant, which can be found in Madagascar, Congo, Nigeria and other Africa countries. It has well branched broad leaves, thick stem and edible fruit. To the best of our search of the literature, the

phytochemistry and antimicrobial screening of any part *Uapaca pilosa* have not been studied.

2.4 Taxonomy of the Plant

Name: *Uapaca pilosa (*Hutch.)

Family: *Euphorbiaceae*

Genus: *Uapaca*

Species: *pilosa*

Idoma name: Obloblo



Plate 2.1: Uapaca pilosa



Plate 2.2: Uapaca pilosa showing the plant fruit.

2.5 Traditional Uses of *Uapaca pilosa*

In both Central and West Africa, stem bark of *Uapaca pilosa* is given as traditional remedies against malaria and associated symptoms, wounds, boils, rheumatism, skin diseases and toothache (Bouquet, 1969;Betti, 2004). The root is used in the treatment of erectile dysfunction, pile and menstrual pain (Betti, 2004).

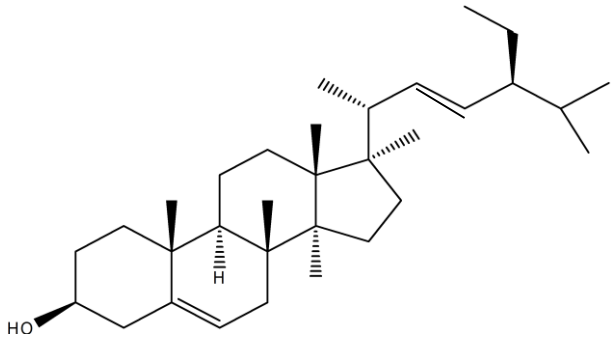
2.6 Medicinal Importance of Other *Uapaca* Species

There are limited reports on the medicinal and pharmacological uses of *Uapaca* species. However, documentation on therapeutic uses of herbal *Uapaca heudelotii* is medicinally useful for treating skin infections, female sterility and pile. Also used in the treatment of rheumatism, as an emetic and tooth-troubles (Dalziel, 1937; Edeoga, 2005). *Uapaca togoensis* is used for treating female infertility and as a restorative wash against fatigue (Irvine, 1948).

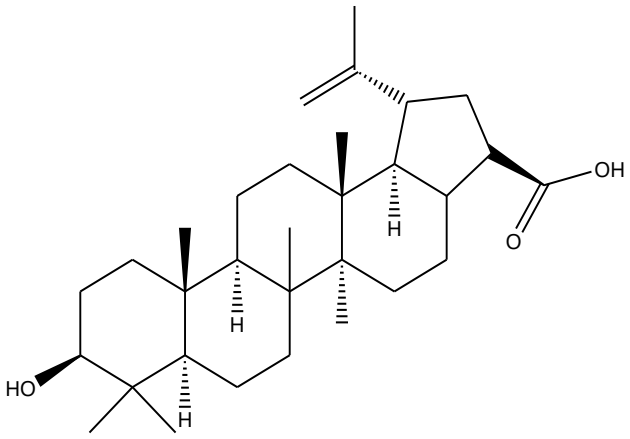
2.7 Some Compounds Isolated from *Uapaca* Species

A new Betulin derivative Samviterin **(13)** together with other known compounds beta-sitosterol **(1)**, stigmasterol **(2)**, betulonic acid **(7)**, betulin **(8)**, lupeol **(4)**, betulinic acid **(3)**, squalene **(5)**, magaric acid **(6)**, palmitic acid **(9)**, stearic acid **(10)**, methylpalmitate **(11)** and pentadecanoic acid **(12)** were isolated from DCM extract of *U. paludosa* trunk bark and they had very potent antiplasmodial activity against *Plasmodium falciparum*(Banzouzi *et al.*, 2015

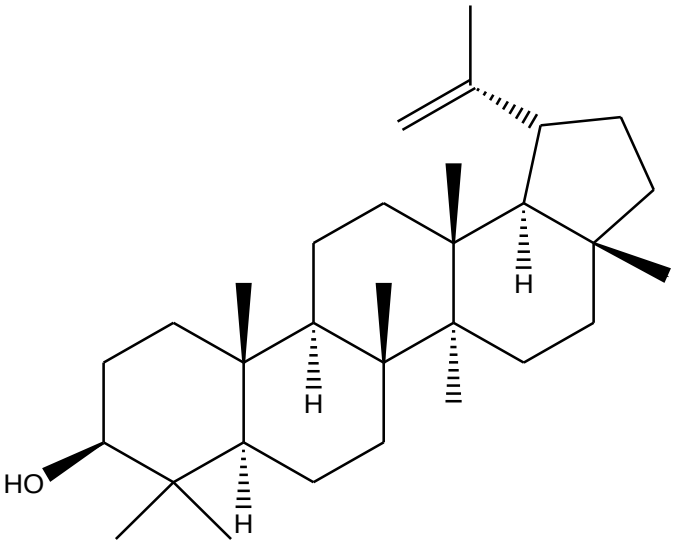
Beta-sitosterol **(1)**



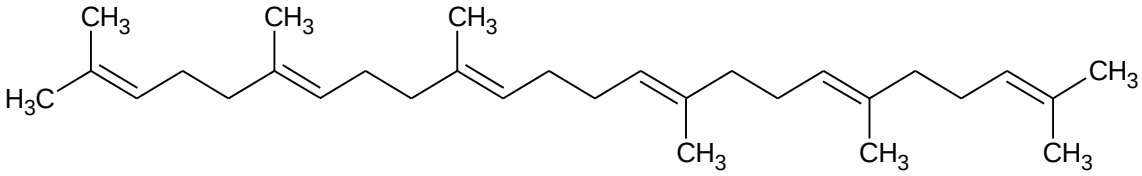
Stigmasterol **(2)**



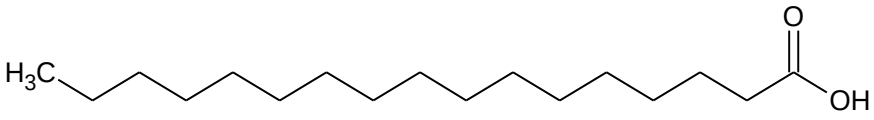
Betulinic acid **(3)**



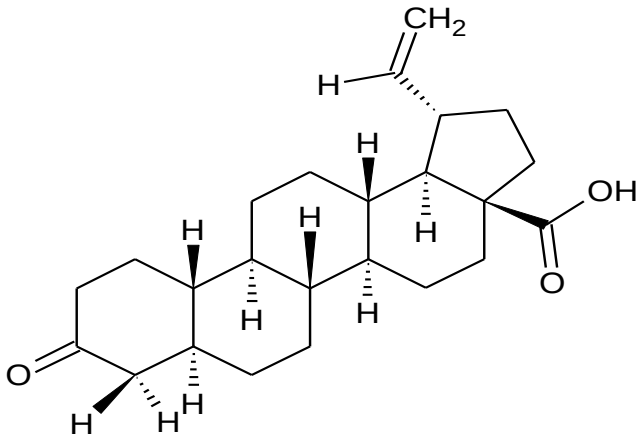
Lupeol **(4)**



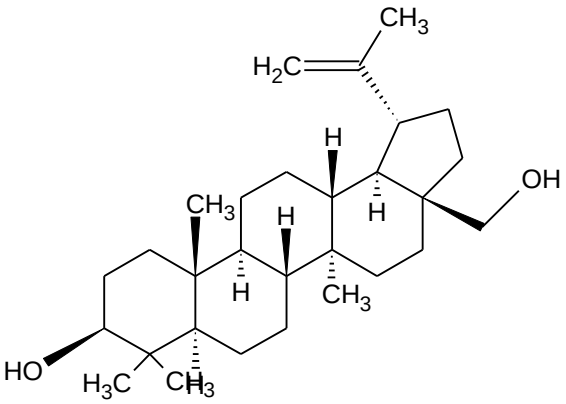
Squalene **(5)**



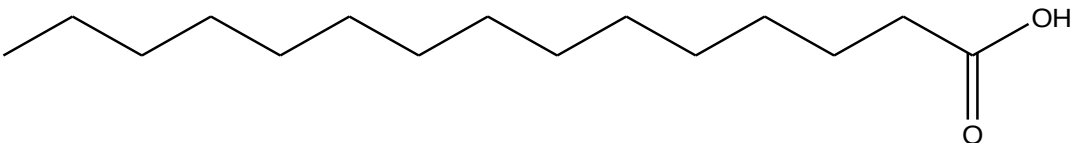
Magaric acid **(6)**



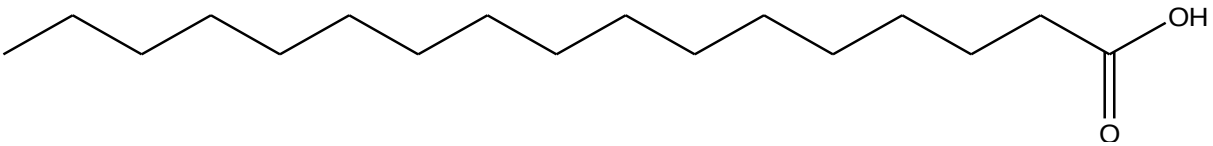
Betulonic acid **(7)**



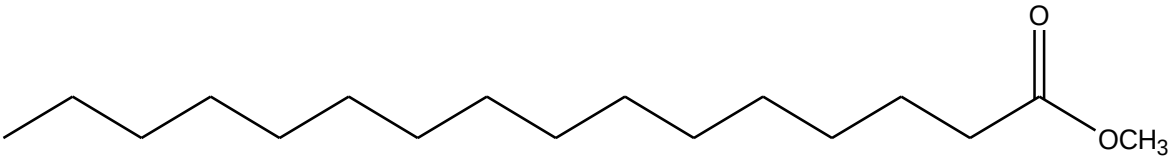
Betulin **(8)**



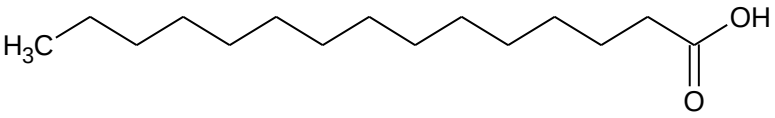
Palmitic acid **(9)**



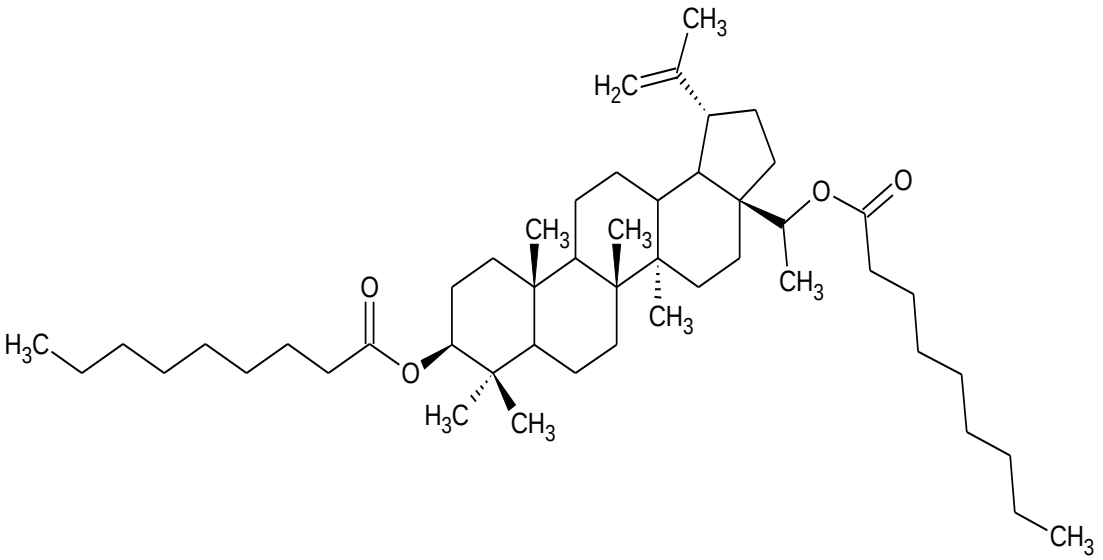
Stearic acid **(10)**



Methylpalmitate **(11)**



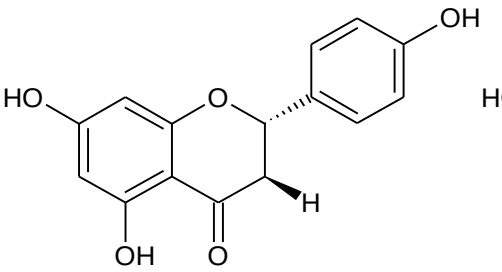
Pentadecanoic acid **(12)**

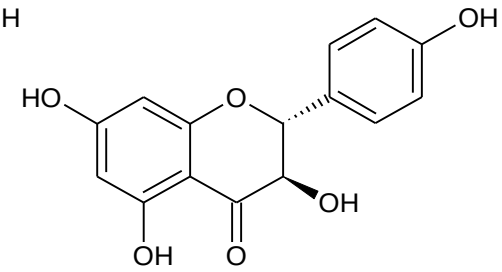


Samvisterin **(13)**

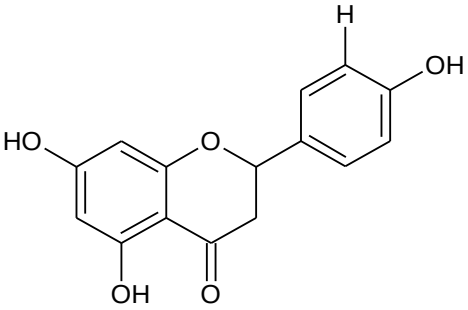
2.8 Some Compounds Isolated from *Euphorbeceae* Family

Most of isolated compounds from *Euphorbeceae* were mainly alkaloids, flavonoids and terpenoids which have been reported to show promising medicinal properties. Naringenin (**14**), aromadendrin (**15**), apigenin (**16**) and4'-O-methoxyluteolin-7-O-rhamnoglucoside (**17**) were isolated from *Euphorbia cuneate* (Sannomiya *et al*., 2005; Galati *et al*., 2000), jatropham (**18**), jatrophatrione (**19**) and acetylaleuritolic acid (**20**) were isolated from *Jatropha macrorhiza* (Wiedhopf *et al*., 1973; Jolad *et al.*, 1977),15-O-acetyl-3-O-propionyl-5-Obutanoyl-7-O-nicotinoylmyrsinol (**21**) and 15-O-acetyl-3,5-O-dibutanoyl-7-O-nicotinoylmyrsinol (**22**) were isolated from the aerial parts of *Euphorbia marschalliana* (Jassbi *et al*., 2004), isolation of Curcasone-A (**23**), Podocarpane (**27**) and Nobiletin (**29**) from *Jatropha curcas* (Demissie and Lele, 2013), also the isolation of compounds triterpene-I (**25**) triterpene-II (**26**) and Jatropholone-A (**24**) from *Jatropha gossypifolia* has been reported (Grode*et al*., 1983). Xavier and Angelo, (1995) reported the isolation of Vitexin (**28**) from leaves part of *Jatropha pohliana*, Kaemferol (**30**) isolated from *Jatropha variegeta* was also reported (Kanth*et al*., 2011).

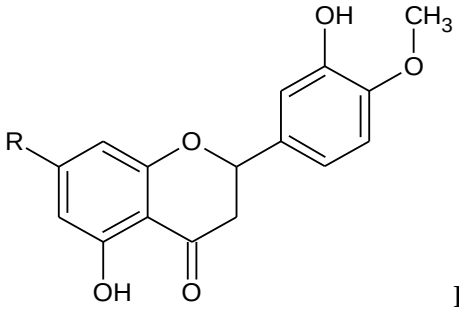




Naringenin **(14)**Aromadendrin **(15)**

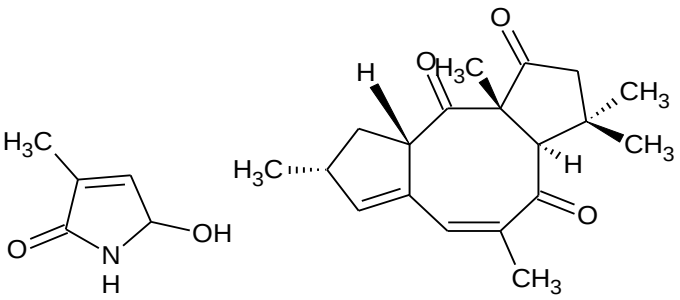


Apigenin **(16)**

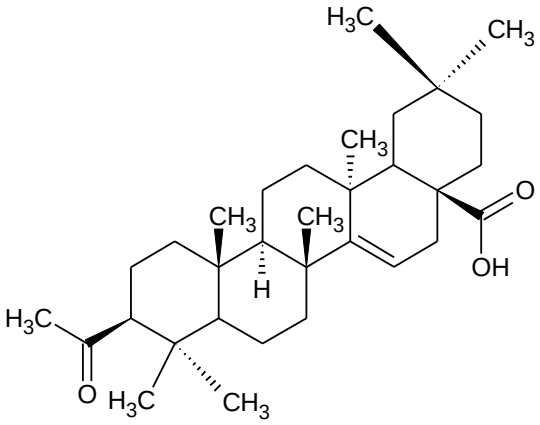


R=

4`-O-methoxyluteolin-7-O-rhamnoglucoside **(17)**



Jatropham **(18)**Jatrophatrione **(19)**



Acetylaleuritolic acid **(20)**

15-O-acetyl-3-O-propionyl-5-O-butanoyl-7-O-nicotinoylmyrsinol **(21)**

15-O-acetyl-3,5-O-dibutanoyl-7-O-nicotinoylmyrsinol **(22)**

Curcasone-A **(23)**

Jatropholone-A **(24)**

Triterpene-I **(25)**

Triterpene-II **(26)**

Podocarpane **(27)**

Vitexin **(28)**

Nobiletin **(29)**

Kaemferol **(30)**

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1.0 Materials

3.1.1 Solvents

Solvents used were of general purpose grade and were distilled before used. The solvents used are:

n-hexane

Chloroform

Ethyl acetate

Methanol

3.1.2 Equipment

The equipment used includes the following:

Analytical balance (AND Instrument, GR-200-EC)

Top loading balance (Denver Instrument, XP 600×0.02 g)

Melting point apparatus (Ernst Leitzwetzlar)

Rotary evaporator

Oven

Vacuum pump

FTIR Agilent Technologies Carey 630 FTIR spectrometer

UV lamp 254-326 nm spectrophotometer (Hitachi U-3200)

NMR Bruker AVANCE spectrometer III (600 MHz)

3.1.3 Reagents

1% Hydrochloric acid

10% Ammonium solution

10% Sodium hydroxide solution

Ferric chloride solution

Fehling solution

Conc. sulphuric acid

Chloroform

Dragendorff's reagent

Molisch's reagent

Mayer's reagent

Anhydrous acetic acid

Glacial acetic acid

95% Ethanol

3.1.4 Antibacterial Studies

3.1.4.1 Microbiological Media

Nutrient broth

Nutrient agar and

Sabouroud dextrose agar

Muller Hinton Agar

3.1.4.2 Test Organisms

Staphylococcus aureus

Bacillus subtilis

Candida albicans

Candida krusei

Salmonella typhii

Shigella dysenteriae

Escherichia coli

3.2.0 Methods

3.2.1 Collection of Plant Material.

The plant was collected fresh from Otukpo, Benue State, Nigeria in February, 2015.The plant was authenticated by Mr Namadi Sanusi at the Biological Science Department, Ahmadu Bello University, Zaria Nigeria. A voucher specimen with Herbarium number 1279 was deposited in the Herbarium. The stem bark was separated, air-dried and pulverized using wooden mortar and pestle.

3.2.2 Extraction of Plant Material.

The pulverized plant material (1.5 kg), was extracted exhaustively with methanol using cold maceration method for one week. The extract was filtered and concentrated *in vacuo* at 40^{o}C using a rotary evaporator. The concentrated methanol extract was reconstituted in water and partitioned with hexane, chloroform and ethyl acetate. The resulting fractions were concentrated *in vacuo* at 40˚C using rotary evaporator.

3.2.3 Preliminary Phytochemical Screening

Extracts obtained from the concentrated fractions were subjected to various phytochemical tests to identify the constituent secondary metabolites using standard methods as described by (Silva *et al*., 1998; Cannell, 2000).

3.2.3.1. Test for Reducing Sugars (Molischs test)

The crude methanol extract and other three fractions (2 g each) were independently and respectively dissolved in 5 ml of distilled water and heated on a water bath and the solutions were filtered accordingly. To a portion of the filtrate, four drops of Molisch's reagent was added. Concentrated sulphuric acid (3ml) was carefully added to the mixture from the side of the test tube to form a lower layer. A purple colouration indicates presence of reducing sugars.

3.2.3.2 Test for Tannins (Ferric Chloride test)

Crudemethanol extract, ethyl acetate, n-hexane and chloroform fractions (0.3 g) each was boiled with water and filtered, 2 drops of ferric chloride was added to the filtrate. Formation of a green precipitate was observed in three of the four sample (methanol, ehthylacetate and chloroform) and not in n-hexane fraction which indicates absence of phenolic OH groups in hexane fraction.

3.2.3.3 Test for Flavonoids (Shinoda test).

3.2.3.3.1 Magnessium Chips test

A portion of the extracts (0.2 g) were separately dissolved in 95% Ethanol, 3 pieces of magnesium chips was then added, this was followed by the addition of 2 - 3 drops of concentrated hydrochloric acid. Appearance of an orange colour indicates the presence of flavonoids in methanol and ethyl acetate extracts and no colouration was observed in chloroform and hexane fractions.

3.2.3.3.2 Sodium Hydroxide test

The respective extracts (1g) were dissolved in 10 % aqueous sodium hydroxide solution and filtered to give yellow colour, a change in colour from yellow to colourless on addition of dilute HCl was observed in methanol and ethyl acetate extracts which indicate the presence of flavonoids.

3.2.3.4 Test for Anthraquinones

3.2.3.4.1 Free Anthraquinones

Small quantity of the extracts were shaken with 10 ml of benzene, the contents were filtered and subjected to the following test independently and respectively. 10% ammonia solution (5 ml) was added to the filtrate, the mixture was shaken. No colour change was observed in the ammoniacal layer (Lower phase) that indicates the absence of free anthraquinone in all the extracts.

3.2.3.4.2 Combined Anthraquinones

The extract was boiled with 10 ml of aqueous sulphuric acid, filtered hot and subjected to the following test independently and respectively. The filtrate was shaken with 5 ml benzene, the benzene layer was separated and to half its own volume, 10% NH_4OH was added. A pink colouration was observed in the ammonia phase (lower phase) which indicates the presence of combined anthraquinone or anthraquinone derivative in methanol, ethyl acetate and n-hexane extracts.

3.2.3.5 Test for Saponnins (Frothing test)

Each extract (0.5g) was shaken with water in a test tube. Frothing which persisted for 15 minutes was observed in the methanol extract only which indicate the presence of saponnins.

3.2.3.6 Test for Glycoside ($FeCl_3$ test)

Each extract (0.5g) was subjected to the following test independently and respectively. Conc. H_2SO_4 (5 ml) was added and boiled for 15 min. This was then cooled and neutralized with 20% KOH. The solution was divided into two portions. Three drops of ferric chloride solution was added to one of the portions, and a green to black precipitate was observed.

3.2.3.7 Test for cardiac glycoside (kella-killani test)

The extracts (0.5g) which were separately and respectively dissolved in glacial acetic acid (5 ml) containing traces of ferric chloride in a test tube was held at an angle of 45˚ and concentrated sulphuric acid (1 ml) was added carefully down the side. A purple ring colour at the interface was taken as indication of the presence of cardiac glycosides in all the samples.

3.2.3.8 Test for Steroids/Triterpenes

3.2.3.8.1 Liebermann-Buchard test

The extracts were separately and respectively dissolved in chloroform and a few drops of acetic anhydride were added followed by concentrated sulphuric acid. The mixture was carefully mixed and a blue colour that changed with time were observed in all the extracts which indicates the presence of steroids/triterpenoids.

3.2.3.8.2 Salkowski test

A little quantity of the extracts were separately and respectively dissolved in chloroform (1 ml) and to it concentrated sulphuric acid (1 ml) was added down the test tube to form two phases. Formation of yellow colouration were observed in all the four extracts. Which indicate the presence of sterol in all the extracts

3.2.3.9. Test for Alkaloids

The extracts (0.5g) were separately and respectively stirred with 1% aqueous hydrochloric acid (5 ml) on a water bath and filtered. The filtrate (3 ml) was divided into three. To the first portion three drops of freshly prepared Dragendoff's reagent was added and an orange to brownish precipitate were observed in all the four extracts. To the second portion 1 drop of Meyer's reagent was added and white to yellowish colour precipitate were observed in all the four extracts. To the third portion 1 drop of Wagner's reagent was added, a reddish- brown precipitate was formed in all the samples.

3.2.4.0 Antimicrobial Studies of Extracts and Isolated components

The antimicrobial activities of all the extracts, isolated compounds and control drugs (Amoxillin and Fluoconazole) were determined using clinical isolates *in vitro*. The agar well diffusion method of Nostro *et al.* (2000) was used in the antimicrobial screening of the extract while disc dilution was used for the positive control due to non-availability of powder form of the drug.

3.2.4.1 Preparation of the Plants Extracts for antimicrobial screening

Stock solution of the plant extracts were prepared by dissolving 0.5g of the n-hexane, chloroform, ethylaceate and methanolic extracts in 10 ml of dimethylsulphoxide (DMSO) to obtain a concentration of 50 mg/ mL. From the stock solution, serial dilution of the extract of concentrations of 50 mg/ mL, 25 mg/ mL, 12.5 mg/ mL and 6.25 mg/ mL were obtained for the extracts.

3.2.4.2 Preparation of culture media

They were prepared by suspend 38 g of the medium in one litre of distilled water with frequent agitation and boiled to aid dissolution, autoclaved at 121 $^{\circ}$C for 15 minutes. Cooled to 45 ^{0}C and 25 ml of the sterilized media were asceptically dispensed into sterilized petri dishes. The media were covered and allowed to solidify.

3.2.4.3 Susceptibility Test

The antimicrobial screening was carried out using agar well diffusion method as described by Nostro *et al*., (2000). The antimicrobial activities of the n-hexane, chloroform, ethyl acetate fractions and methanol extract of the stem bark of *Uapaca pilosa* were determined using stock concentration of 100 mg/mL. 0.1 ml standard inoculums of the test organisms were uniformly streaked unto freshly prepared Mueller Hinton Agar plates with the aid of a sterile swab stick. Using a sterile cork borer (6 mm diameter) 5 appropriately labelled wells were punched into each agar plate. Thereafter, 0.2 mL of the appropriate extract concentration was placed in each well and allowed to diffuse into the agar. An extra plate was also streaked with the inocula and amoxillin as standard (5 µg/disc) was placed in it. The plates were incubated at 37 $^{\circ}$C for 24 hours. While for the fungus, Sabouraud dextrose agar was used at an incubation period of 72 hours at 25 $^{\circ}$C. The antimicrobial activities were expressed as diameter of zones of inhibition produced by the plant extracts and reported in millimeter (mm).

3.2.4.4 Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of the extract was carried out on the microorganisms that were sensitive to the extract and was done using broth dilution method. Nutrient broth was prepared according to the manufacturer's instructions as recommended by

NCCLS (Nostro*et al*., 2000). 10ml was dispensed into test tubes and was sterilized at 121^{o}C for 15 minutes, and the broth was allowed to cool. Minimum inhibition McFarland turbidity standard scale number 0.5 was prepared to give turbid solution. Normal saline was prepared and was dispensed into test tube and the test microorganism was then inoculated into the normal saline, incubation was at 37^{o}C for 6hrs, dilution of the microorganism in the normal saline was performed until the turbidity marched that of the McFarland by visual comparison at this point the microorganism had a concentration of about 1.5 X 108 cfu/ml. Two-fold serial dilution of the extract in the broth was performed to obtain the concentrations of 10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml and 0.625mg/ml. The initial concentration was obtained by dissolving the extract (0.1g) in the extracts in the broth (10mls), 0.1ml of the standard inoculated into the different concentrations of the extracts in the broth, was then inoculated at 37^{o}C for 24hrs after which the test tubes were observed for turbidity (growth). The lowest concentrations of the extract which shows no turbidity was recorded as the minimum inhibitory concentrations.

3.2.4.5 Minimum bactericidal concentration (MBC)

Minimum bactericidal concentration of the extracts were carried out to check whether the test microbes were killed or only their growth was inhibited. Mueller Hinton and Sabouraud dextrose agars were prepared according to the manufacturer's instruction, boiled to dissolve and were sterilized at 121^{o}C for 15 minutes, the media were cool to 45^{o}C and the medium (20ml) was poured in to sterile Petri dishes, the plates were covered and allowed to cool and solidify. The contents of the MIC in the serial dilution was inoculated on to the media, the media were incubated at 37^{o}C for 24hrs for the bacteria and at 30^{o}C for 1-7 days for fungi, after which the plate were observed for

colonies growth. The MBC/MFC were the plate with lowest concentrations of the extract without colony growth.

3.2.4.6 Minimum fungicidal concentration (MFC)

The minimum fungicidal concentration were determined according to Bauer et al., 1996. The content of the MIC in serial dilution was sub-cultured onto the prepared medium and incubation at 25 ^{0}C for 24 hours. Thereafter each plate of the medium was observed for the growth of colony. The value obtain in the plate with lowest concentration of the extracts without colony growth was recorded as the MFC.

3.3.0 Column Chromatography

The following column conditions were employed in running the column chromatography.

(a) Technique - gradient elution.

(b) Column - A glass column of dimensions 75 by 3.5 cm was used.

(c) Stationary phase - Silica gel of 60 – 200 mesh size.

(d) Column packing – This was done by the wet slurry method.

(e) Sample loading – The sample was loaded by the dry loading method (Cannell, 1998). The sample was dissolved in minimum amount of suitable organic solvent, mixed with small quantity of silica gel, dried, triturated and then loaded on top of the previously packed column.

Solvents System of Elution: Various solvent systems comprising 100 % hexane, hexane/ethyl acetate mixtures (97.5: 2.5, 95: 5, 90: 10, 80: 20, 70: 30, 60: 40, 50: 50, 40; 60, 30: 70, 20: 80, and 10: 90 %) ethyl acetate 100 % and methanol 100 % were used in eluting the column.

3.3.1 Chromatographic Separation

3.3.1.1 Column Chromatography of ethyl acetate Fraction of *Uapaca pilosa*

The ethyl acetate fraction (12.6 g) of *U. pilosa* was chromatographed on silica gel packed column of dimension 75 by 3.5 cm the column was eluted continuously using 100% n-Hexane and later n-Hexane: Ethyl acetate mixtures. Sixty one fractions of 100 ml were collected. The sixty one fractions were pooled together based on similarities in their TLC profile to give 10 sub-fractions. Sub-fraction 1 gave 3 spots, Sub-fraction 2 gave two spots and were labelled SF_1 and SF_2 respectively.

3.3.1.2 Preparative Thin Layer Chromatography of the Sub-fractions (SF_1 and SF_2)

Sub-fractions SF_1 and SF_2 were subjected to preparative Thin Layer Chromatography using aluminium plate coated with silica gel. One out of the three spots was removed from SF_1, another out of the spots was removed from SF_2 as pure compounds and labelled compound GF_1(50 mg) and GF_2 (20 mg) respectively the spots that was removed are those that are more pronounced on the plate. In the PTLC of SF1 hexane 90 % and ethyl acetate 10 % was used to develop the plate, while hexane 70 % and ethyl acetate 30 % was used to develop the PTLC of SF2 subfraction. The isolated compounds GF1 and GF2 were subjected to spectroscopic analysis to elucidate their chemical structures.

3.4 Melting Point Determination

The melting points of the isolated compounds were determined using the Ernst Leitzwetzlar melting point apparatus in the Department of Chemistry, A.B.U, Zaria.

3.5 Spectral Analysis

The compounds were subjected to NMR analyses measured on a Bruker Advance (600 MHZ) spectrophotometer. Chemical shift values (δ) were reported in parts per million (ppm) relative to TMS standard and coupling constants are given in Hz. The NMR solvent used for the NMR analysis was deuterated chloroform ($CDCl_3$).

CHAPTER FOUR

4.0 RESULTS

4.1 Result of Extraction of the Stembark of *Uapaca pilosa*

The results of solvent extraction of stem bark from *Uapaca pilosa* showing composition of crude methanol extract, n-hexane, chloroform and ethyl acetate fractions are as presented in Table 4.1.

4.2. Result of Phytochemical screening

The results of the phytochemical screening of the stem bark extracts from *Uapaca pilosa* are as shown in Table 4.2.

4.3 Result of antimicrobial activity of the plant extracts

The results of the antimicrobial susceptibility tests, expressed in terms of diameter of zones of inhibition are shown in Table 4.3-4.5

4.4 Result of Chromatographic Separation

4.4.1 Thin Layer Chromatography of ethyl acetate extract of *Uapaca pilosa*

Thin layer chromatography (TLC) was carried out on the ethyl acetate fraction, and the results are as shown in Table 4.6.

4.5 Column Chromatography of Ethyl acetate fraction

Column chromatography of ethyl acetate fraction (12.6 g) was carried out and the results are shown in Table 4.7.

Table 4.1 Extraction of Stem Bark of *Uapaca pilosa*

Solvent	Mass of extracts (g)	Percentage yield (%)
Hexane	16.35	1.09
Chloroform	8.62	0.58
Ethyl acetate	25.60	1.71
Methanol	270.25	18.01

Table 4.2: Result of the phytochemical screening of the plant extracts

Phytochemical	Methanol	Ethyl acetate	Chloroform	n-hexane
Alkaloids	+	+	-	+
Anthraquinones	+	+	-	+
Reducing sugar	+	+	+	-
Flavonoids	+	+	-	-
Glycosides	+	+	+	+
Saponins	+	-	-	-
Tannins	+	+	+	-
Steroids	+	+	+	+
Triterpenes	+	+	+	+

Key: + = present, - = absent

Table 4.3: Zone of Inhibition (mm) of the extracts and standard drugs (µg/mL)

Test organism	Average Zone of Inhibition (mm)				Amoxillin 5 µg/disc	Fluconazole 5 µg/disc
	Methanol	Ethyl acetate	Chloroform	Hexane		
Staphylococcus aureus	14.00	17.00	13.00	14.00	28.00	-
Bacillus subtilis	11.00	14.00	0.00	10.00	22.00	-
Shigella dysenteriae	13.00	15.00	12.00	12.00	19.00	-
Escherichia coli	15.00	15.00	17.00	18.00	15.00	-
Salmonella typhii	10.00	10.00	0.00	11.00	20.00	-
Candida albican	0.00	0.00	0.00	0.00	-	35.00
Candida krusei	0.00	0.00	0.00	0.00	-	38.00

Key: 0 = no activity, - = not determine

Table 4.4: Minimum Inhibitory Concentration (MIC) of the plant extracts in (mg/ml)

Test organism	Methanol extract	Ethyl acetate fraction	Chloroform fraction	N-hexane fraction
S. aureus	6.25	12.50	12.50	12.50
S. typhil	0.00	0.00	0.00	0.00
S.dysentriae	25.00	25.00	0.00	0.00
B. subtilis	0.00	6.25	0.00	0.00
E. coli	12.50	12.50	12.50	12.50

Key: 0 = no activity

Table 4.5 Minimum Bactericidal Concentration (MBC) of the plant extracts in (mg/ml)

Test organism	Methanol	Ethyl acetate	Chloroform	N-hexane
S. aureus	12.50	25.00	25.00	25.00
S. typhil	0.00	0.00	0.00	0.00
S.dysentriae	50.00	50.00	0.00	0.00
B. substilis	0.00	12.50	0.00	0.00
E. coli	25.00	25.00	25.00	25.00

Key: 0 = no activity

Table 4.6: Thin layer chromatography of the ethyl acetate fraction

Spot	R_f Value	Colour in 10% H_2SO_4
1	0.80	Brown
2	0.70	Brown
3	0.60	Brown
4	0.32	Purple
5	0.16	Brown

Table 4.7: Fractions from column chromatography of ethyl acetate fraction

Fraction	Eluting solvent	Number of major spots
1	Hexane: ethyl acetate (95:05%)	3
2	Hexane: ethyl acetate (90:10%)	2
3	Hexane: ethyl acetate (80:20%)	4
4	Hexane: ethyl acetate (70:30%)	3
5	Hexane: chloroform (10:90%)	3

4.6 Result of Thin layer Chromatography analyses of GF1 and GF2

TLC analysis of GF1 using Hexane: Ethyl acetate (8:2) gave a single homogenous spot (R_f 0.66) and the TLC analysis of GF2 with Hexane: Ethyl acetate (7:3) gave a single homogenous spot (R_f 0.52). Spraying with 10% H_2SO_4 in methanol upon heating GF1 gave brown coloured spot and GF2 gave a purple coloured spot (Table 4.8).

4.7 Spectroscopic Analyses of GF1 and GF2

The results of spectroscopic analyses of compounds GF1 and GF2 are shown in Figures 4.1-4.8 and Table 4.9 for GF1 and Figures 4.9-4.15 and Table 4.10 for GF2.

Table 4.8: TLC profile of the isolated compounds (GF1 and GF2)

Compound code	Solvent system	Observed spot(s)	Colour of spot on heating	R_f value	Melting points ^0C
GF1	Hexane: Ethyl acetate (8:2)	1	Brown	0.66	255-257
GF2	Hexane: Ethyl acetate (7:3)	1	Purple	0.52	138-143

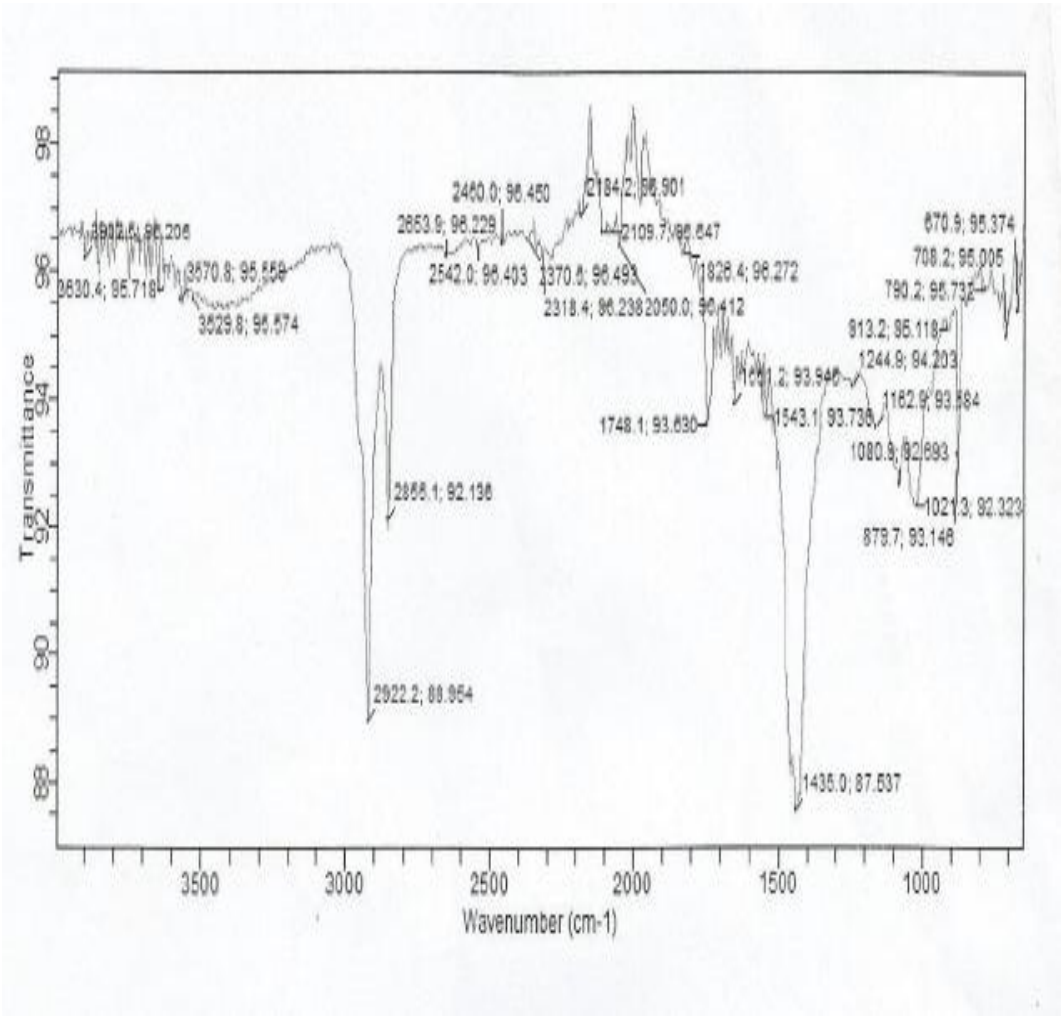


Figure 4.1: FTIR spectrum of GF1

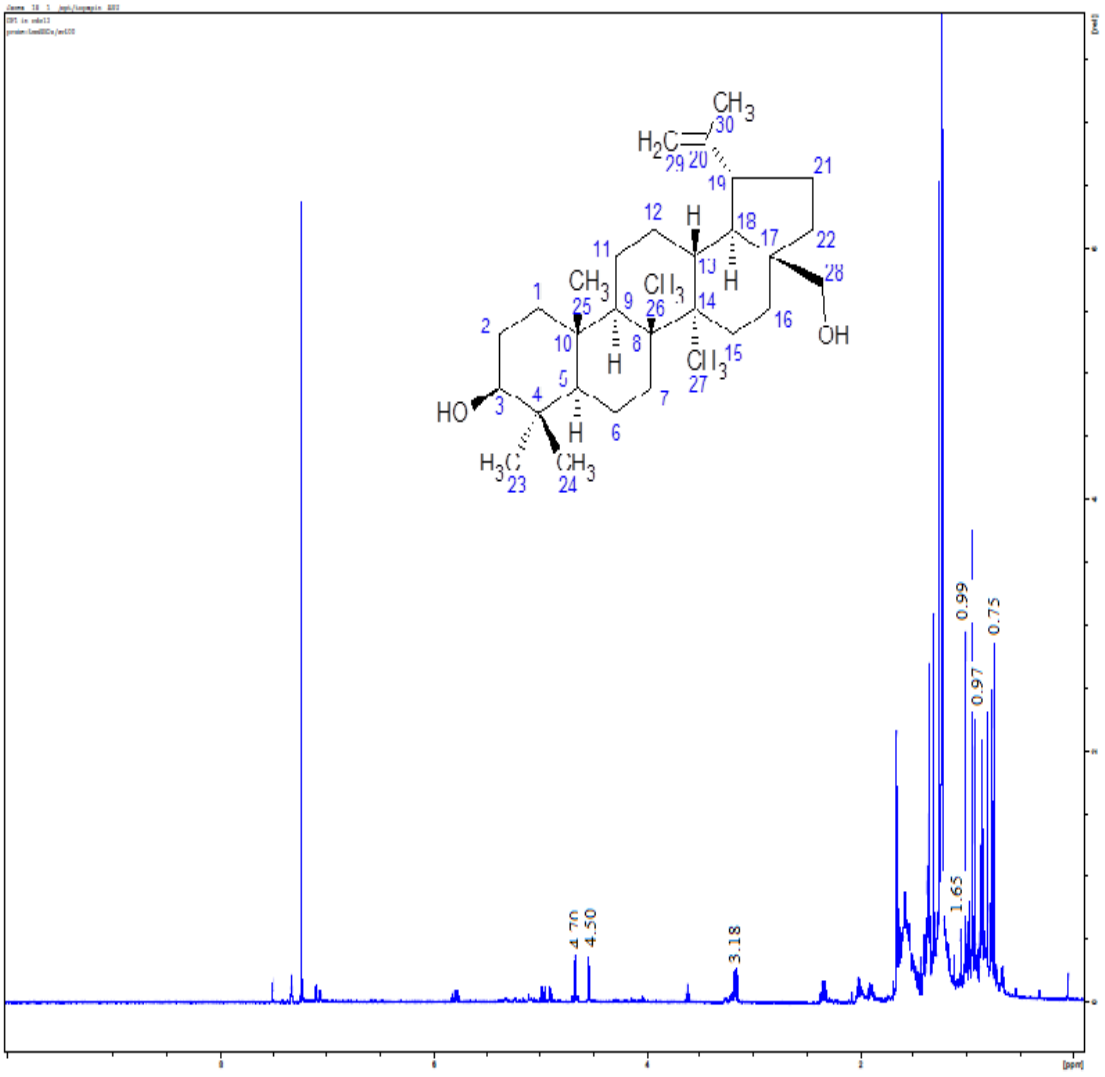


Figure 4.2: ^{1}H-NMR spectrum of GF1

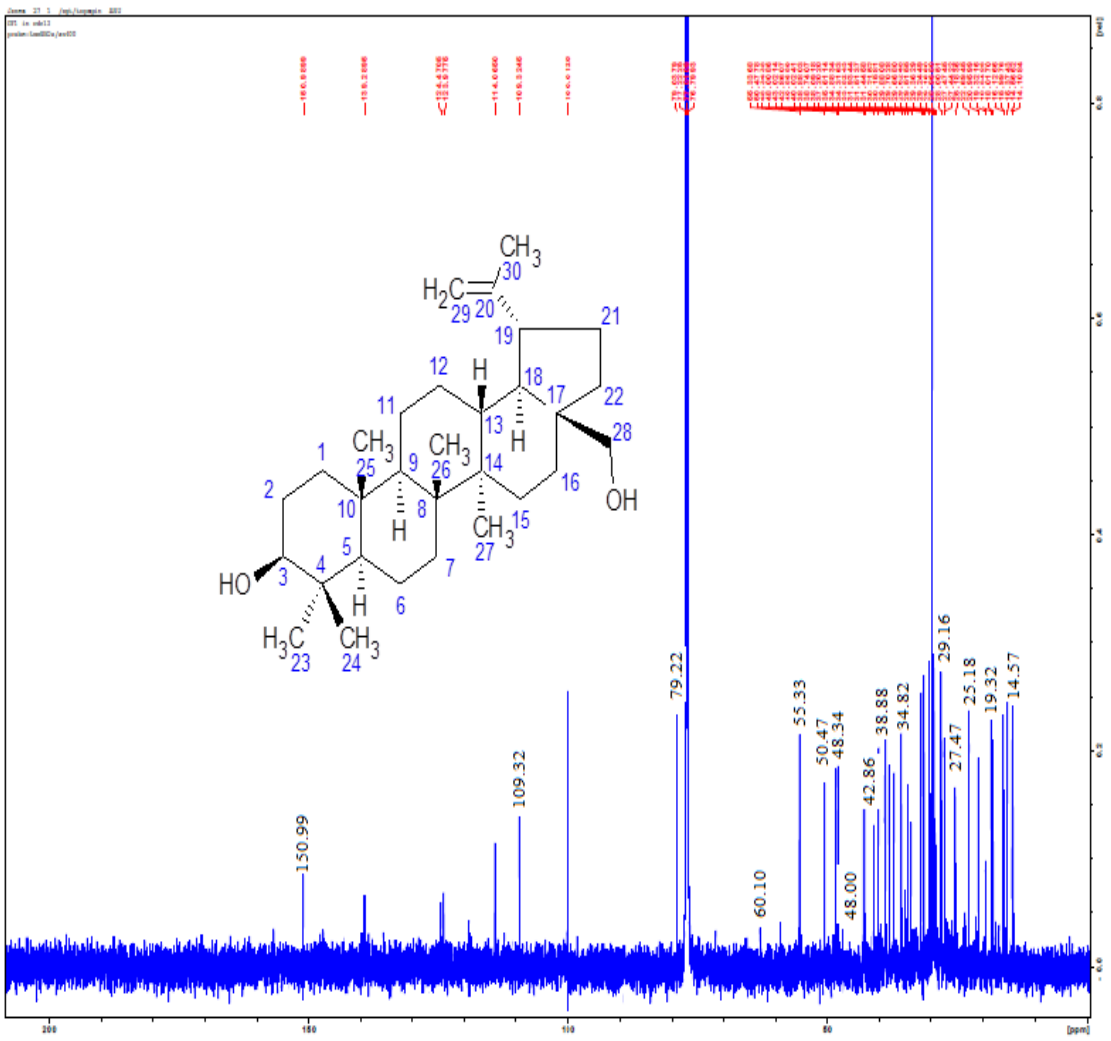


Figure 4.3: ^{13}C-NMR spectrum of GF1

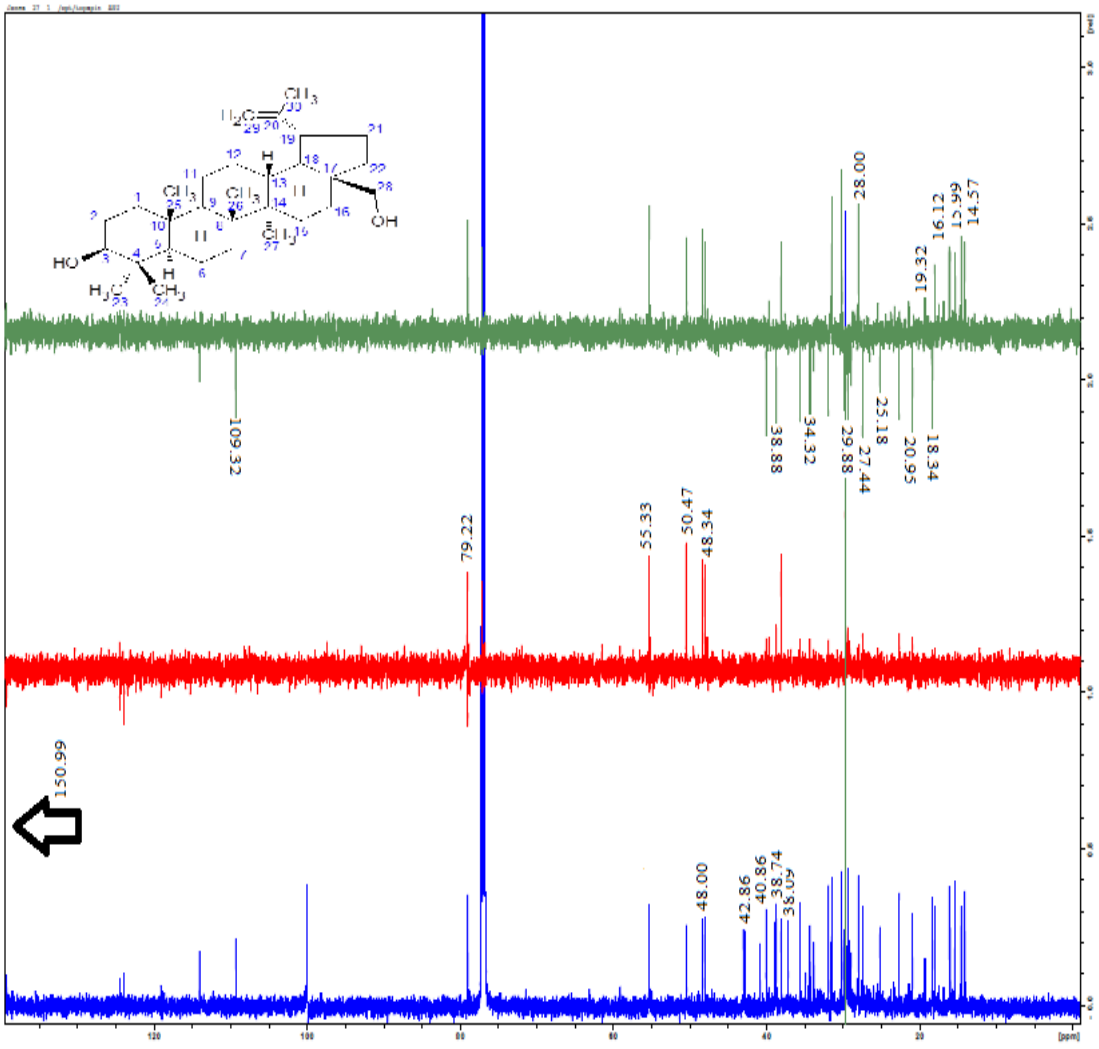


Figure 4.4 DEPT Analysis spectrum of GF1

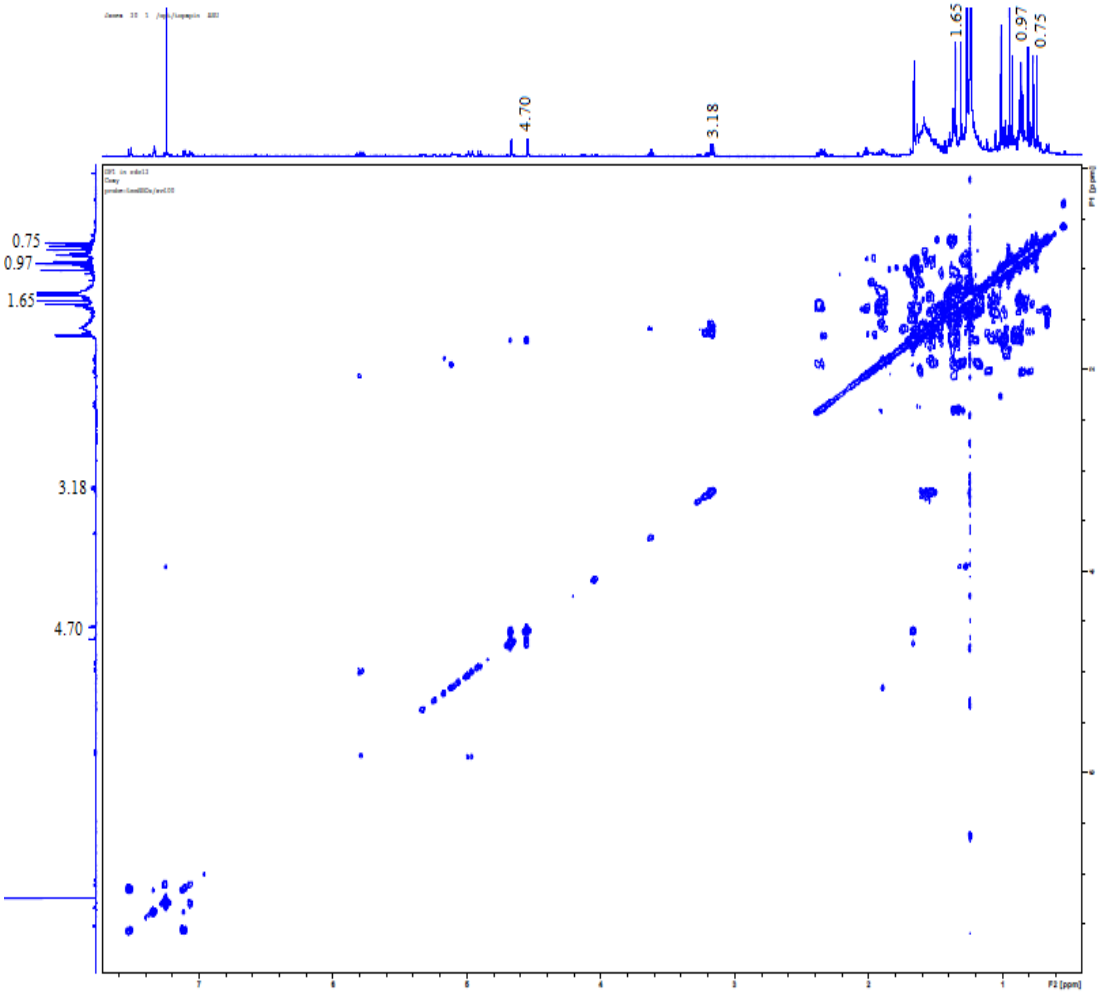


Figure 4.5: COSY Spectrum of GF1

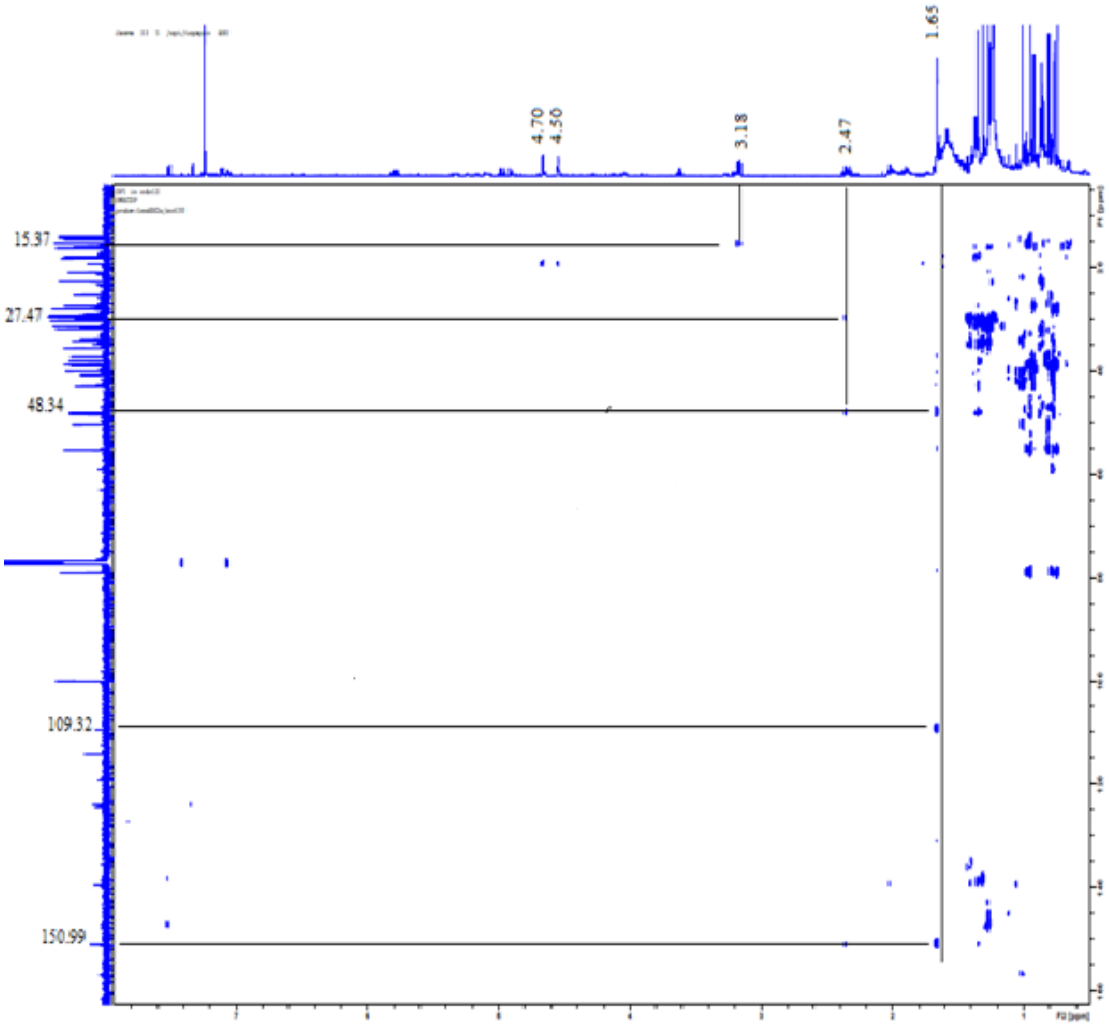


Figure 4.6: HMBC Spectrum of GF1

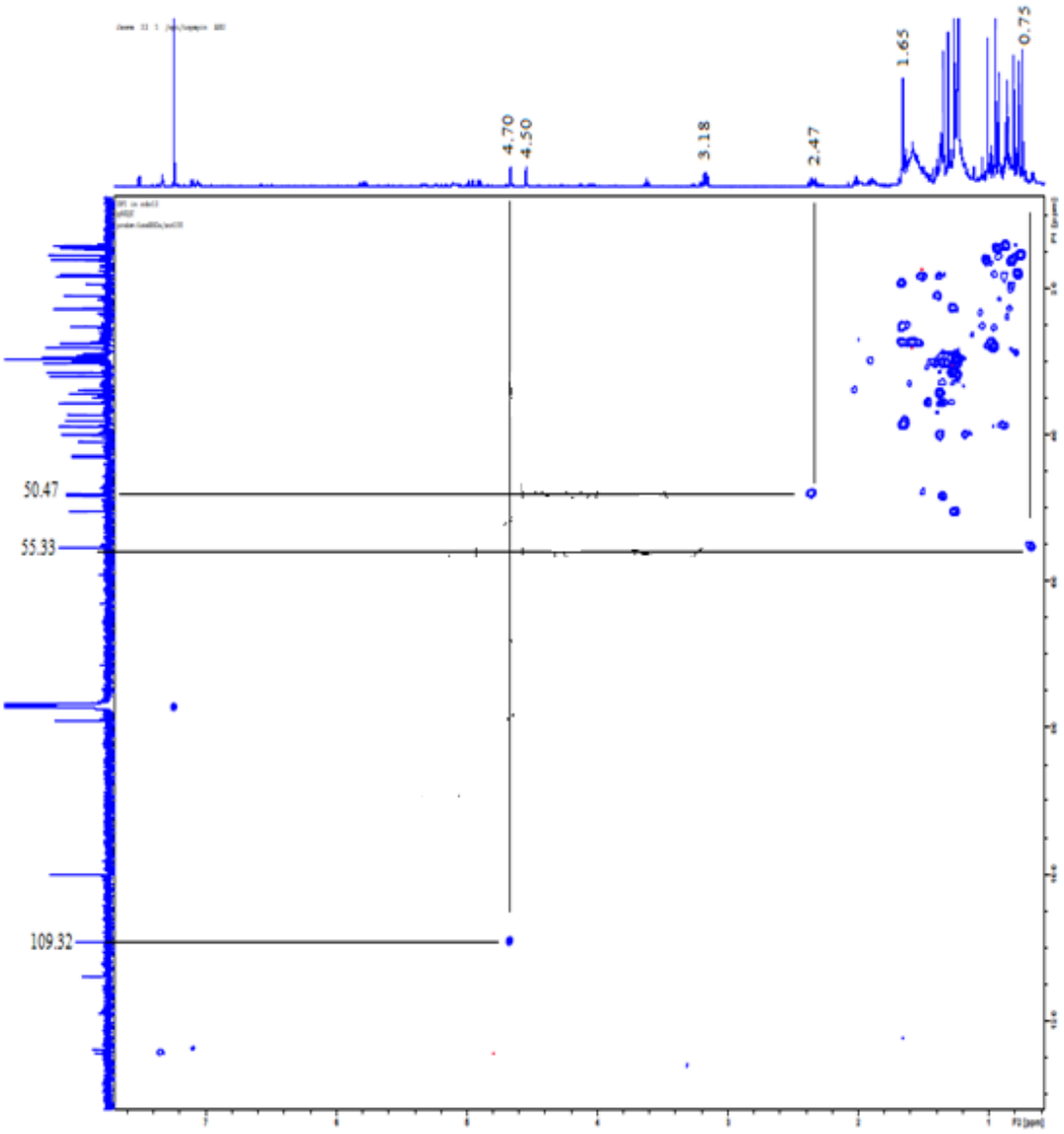


Figure 4.7: HSQC Spectrum of GF1

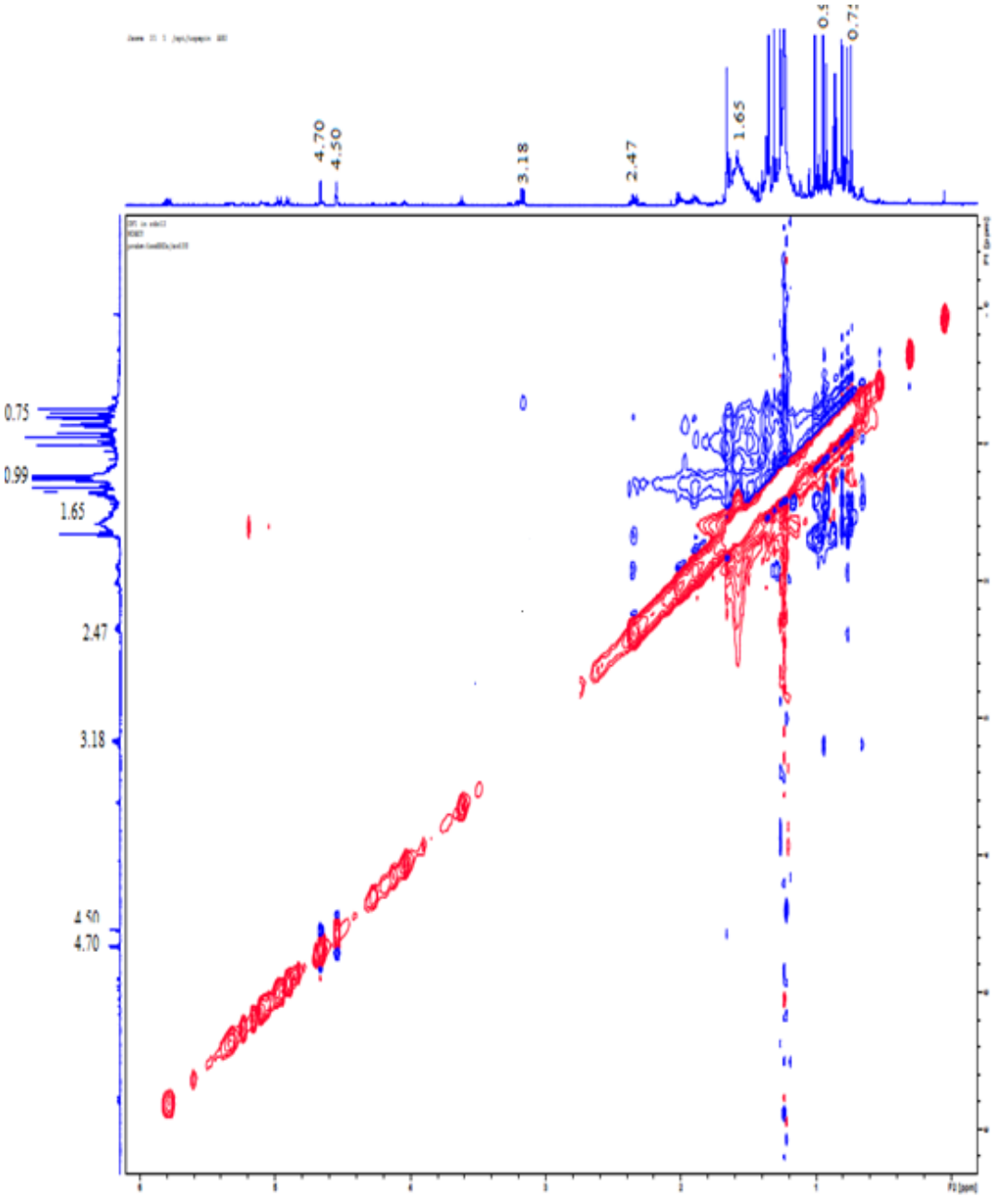


Figure 4.8 NOESY Spectrum OF GF1

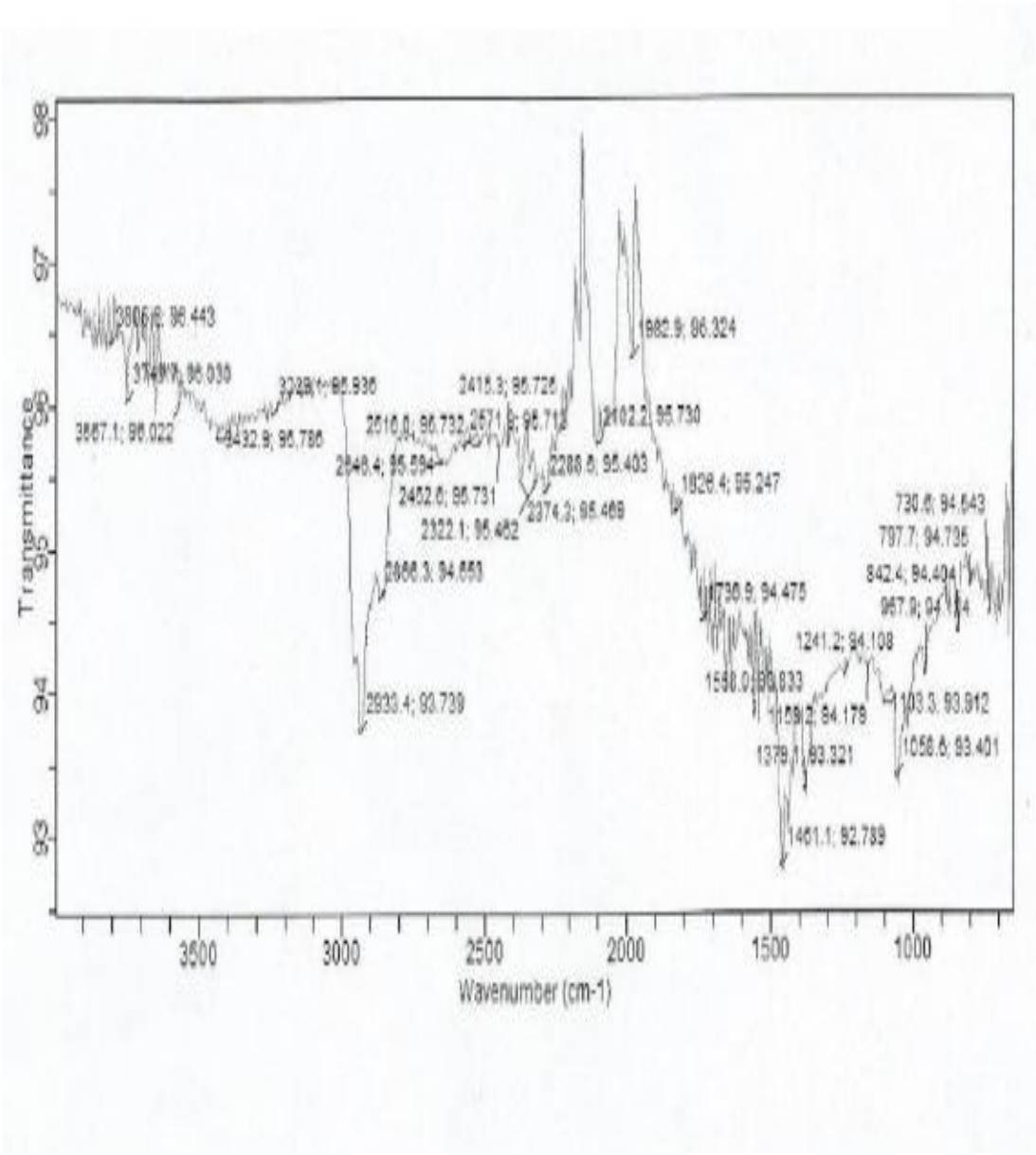


Figure 4.9: FTIR Spectrum of GF2

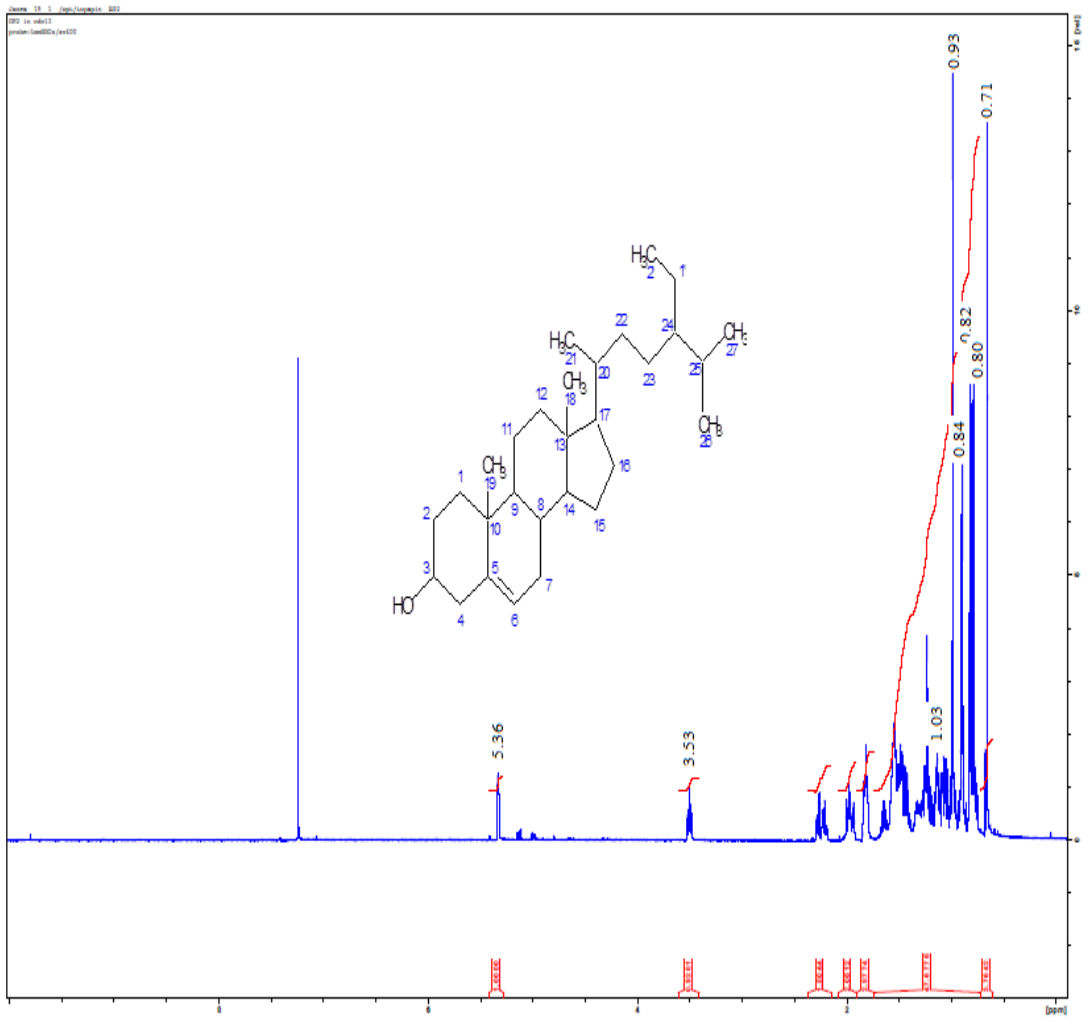


Figure 4.10: ^{1}H NMR Spectrum of GF2

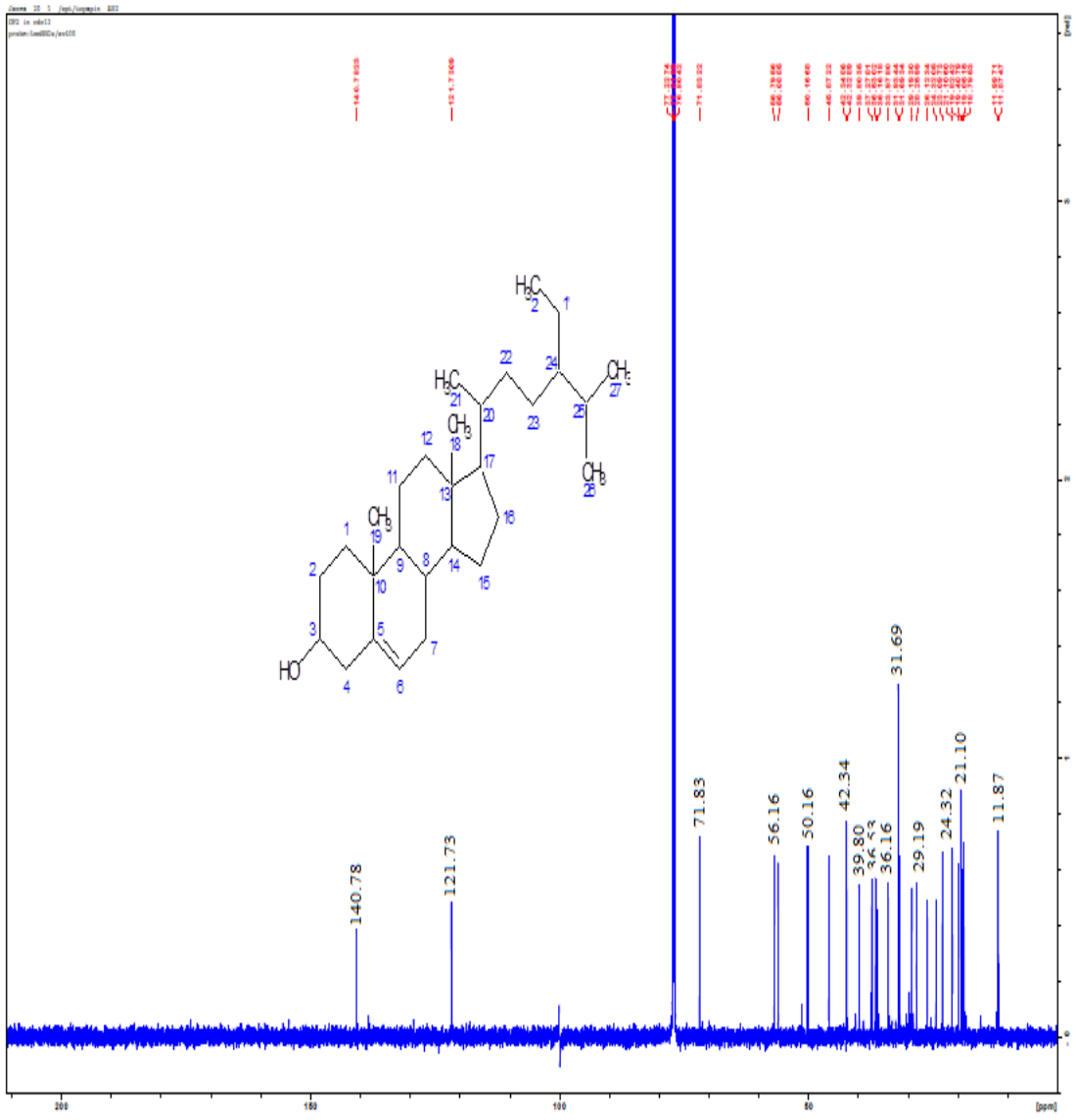


Figure 4.11 ^{13}C NMR Spectrum of GF2

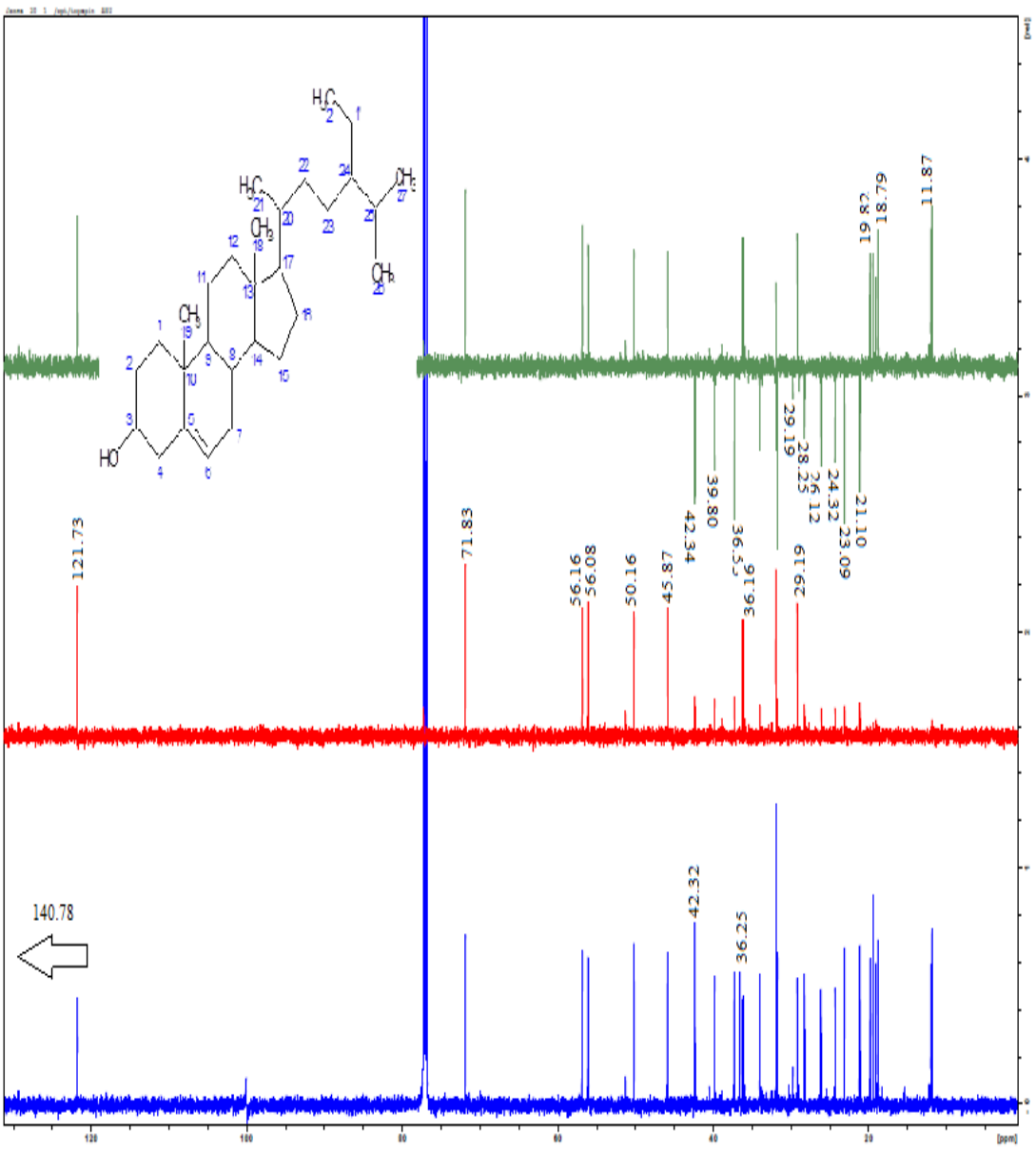


Figure 4.12: DEPT Spectrum of GF2

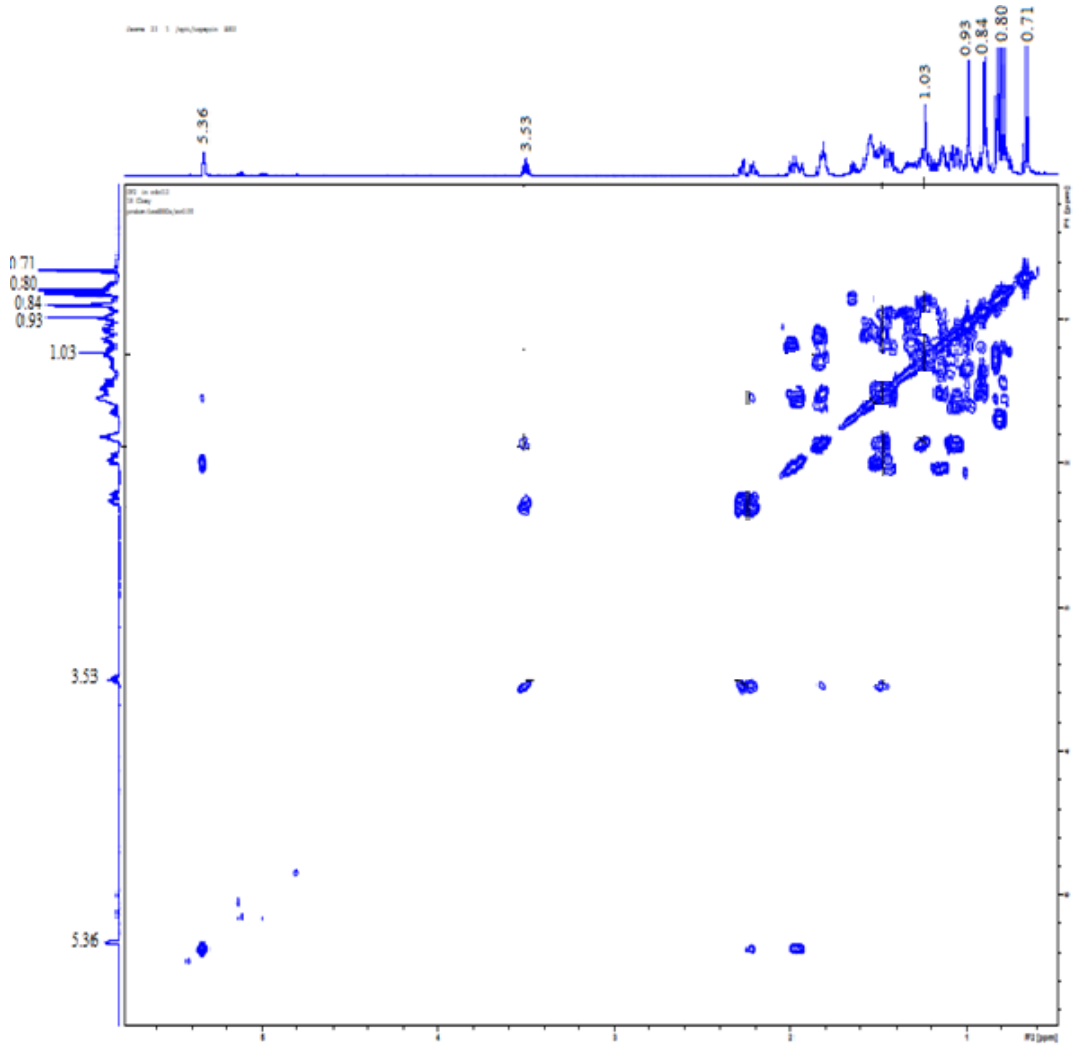


Figure 4.13: COSY Spectrum of GF2

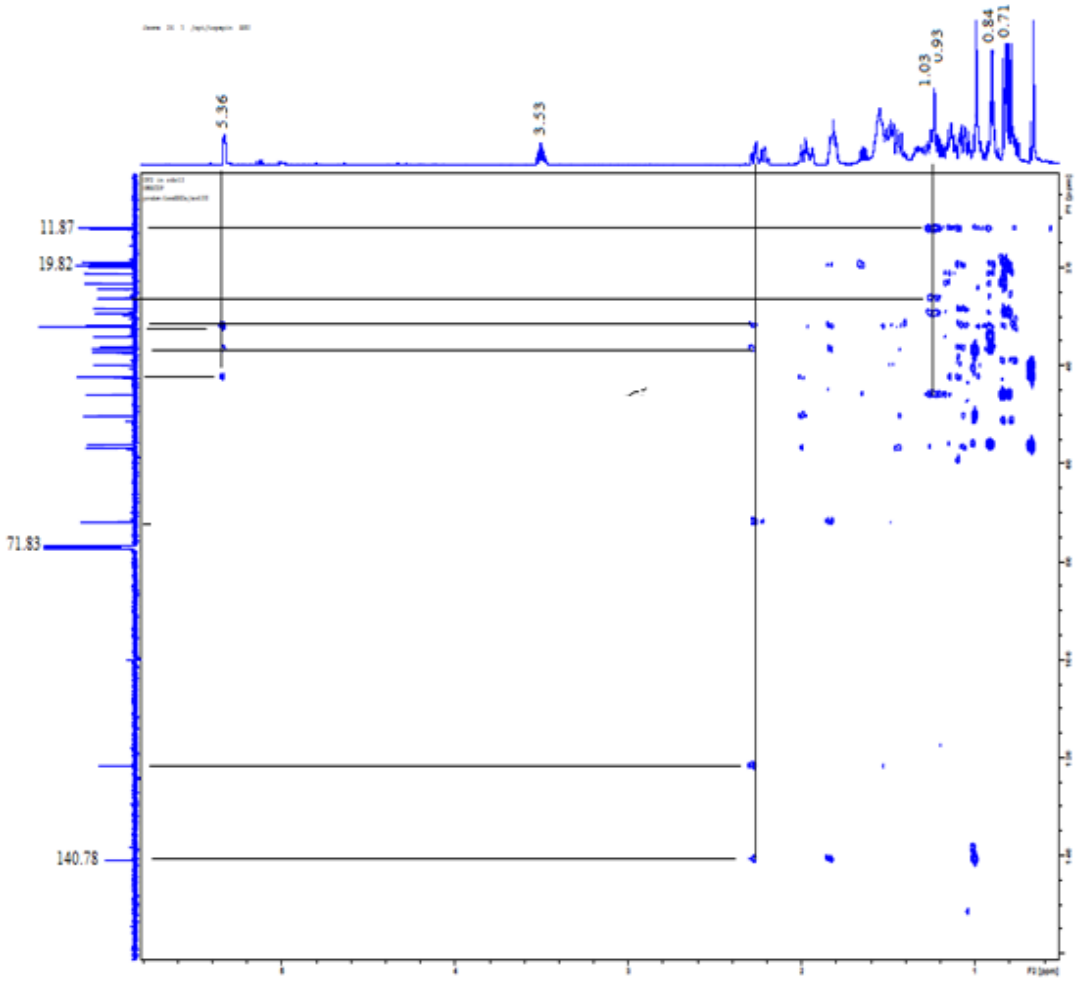


Figure 4.14: HMBC Spectrum of GF2

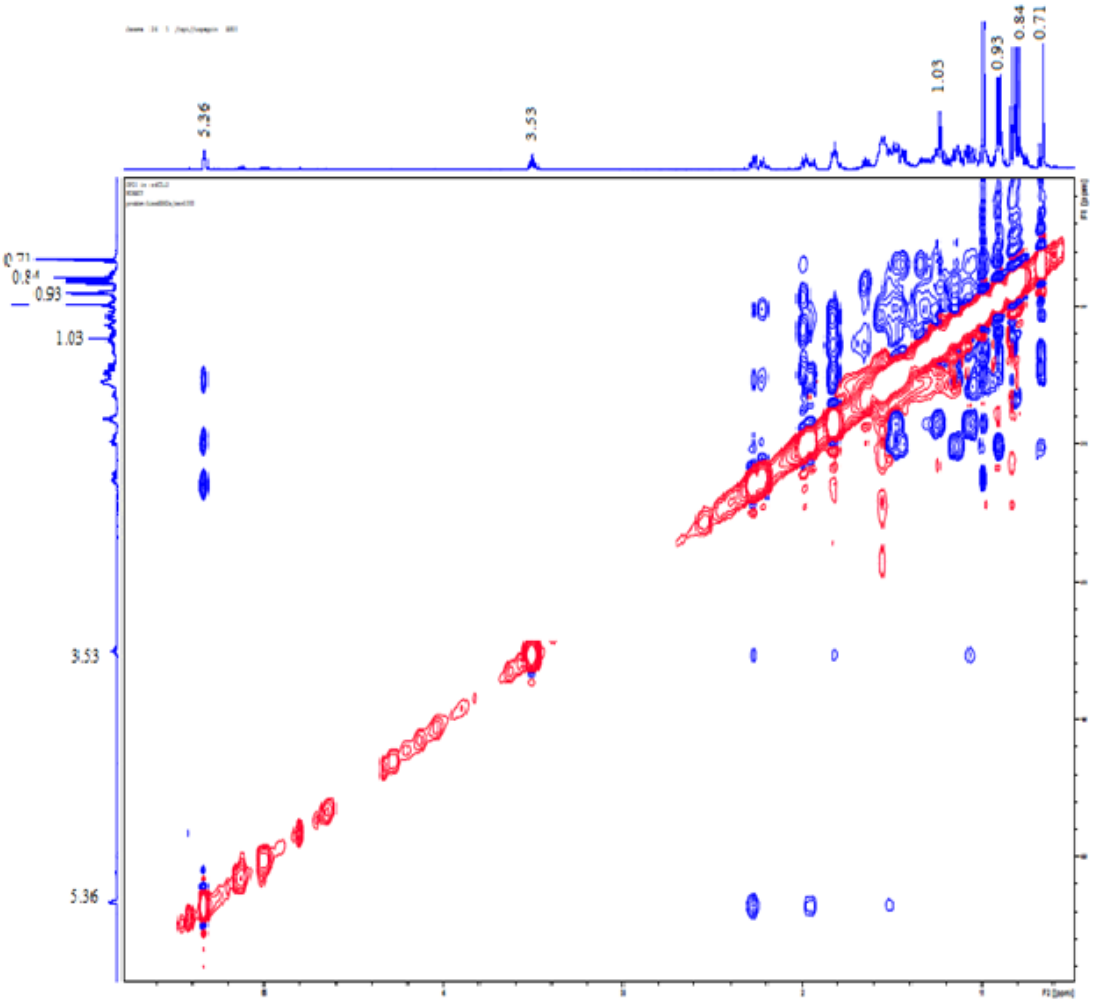


Figure 4.15: NOESY Spectrum of GF2

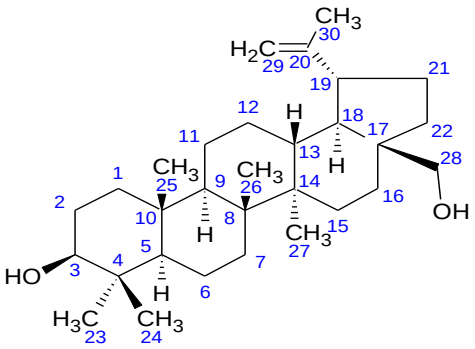


Table 4.9: Comparison between 13C NMR Data of GF1 with Literature (ppm)

Carbon position	^{13}C δ (ppm) GF1	^{13}C δ (ppm) Lit.	Remark
1	38.88	38.9	CH_2
2	27.47	27.5	CH_2
3	79.22	79.2	CH
4	38.74	38.8	C
5	55.33	55.4	CH
6	18.34	18.4	CH_2
7	34.32	34.3	CH_2
8	40.86	41.0	C
9	50.47	50.5	CH
10	38.09	37.4	C
11	20.95	20.9	CH_2
12	25.18	25.3	CH_2
13	37.20	37.2	CH
14	42.86	42.8	C
15	27.44	27.1	CH_2
16	29.16	29.2	CH_2
17	48.00	47.9	C
18	48.34	47.9	CH
19	48.34	48.8	CH
20	150.99	150.6	C
21	29.88	29.8	CH_2
22	34.32	34.1	CH_2
23	28.00	28.1	CH_3
24	15.37	15.4	CH_3
25	16.12	16.2	CH_3
26	15.99	16.1	CH_3
27	14.57	14.8	CH_3
28	60.1	60.6	CH_2
29	109.32	109.8	CH_3
30	19.32	19.2	CH_3

(Tijani *et al*., 2012)

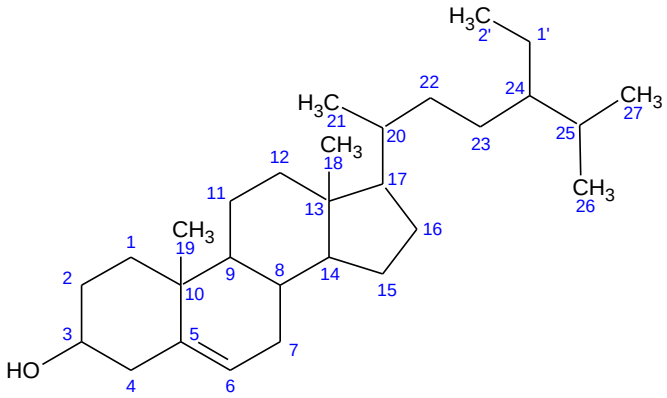


Table 4.10: Comparison between 13C NMR Data of GF2 with that Literature (ppm)

Carbon position	^{13}C δ (ppm) GF2	^{13}C δ (ppm) Lit.	Remark
1	37.28	36.87	CH_2
2	36.53	36.26	CH_2
3	71.83	73.48	CH
4	42.34	41.12	CH_2
5	140.78	140.38	C
6	121.73	121.22	CH
7	29.19	29.01	CH_2
8	31.69	31.41	CH
9	50.16	51.64	CH
10	36.53	36.25	C
11	21.10	20.61	CH_2
12	39.80	37.68	CH_2
13	42.32	41.05	C
14	56.16	56.18	CH
15	24.32	23.87	CH_2
16	28.25	27.81	CH_2
17	56.08	55.44	CH
18	11.87	11.70	CH_3
19	19.82	19.72	CH_3
20	36.16	35.49	CH
21	18.79	18.62	CH_3
22	33.97	33.36	CH_2
23	26.12	25.46	CH_2
24	45.87	45.15	CH
25	29.19	29.16	CH
26	19.05	19.10	CH_3
27	18.79	18.94	CH_3
28	23.09	22.62	CH_2
29	11.87	11.68	CH_3

(Jaju *et al*., 2010)

4.10 Antibacterial Activity of Isolated Compounds

4.10.1 Antimicrobial Activity of GF1 and GF2

The results of the antibacterial susceptibility tests, expressed in terms of diameter of zones of inhibition are shown in Table 4.11.

The Minimum Inhibitory Concentration (MIC) of GF1 and GF2 were determined using dilution method and the result are shown in Table 4.12.

The Minimum Bactericidal Concentration (MBC) of GF1 and GF2 were also determined and the result are shown in Table 4.13.

Table 4.11: Antibacterial Screening Result of GF1 and GF2 showing Zone of Inhibition (mm)

Test organisms	Zone of Inhibition (mm)	
	GF1	GF2
Staphylococcus aureus	23.00	27.00
Bacillus subtilis	18.00	27.00
Salmonella typhii	14.00	20.00
Shigella dysenteriae	18.00	28.00
Escherichia coli	20.00	24.00

Table 4.12: Minimum Inhibitory Concentration (MIC) of (GF1) and (GF2)

Test Organism	*S.aureus*	*B.subtilis*	*S.typhi*	*S.dysenteriae*	*E.coli*
GF1 (µg/mL)	6.25	12.50	12.50	6.25	6.25
GF2 (µg/mL)	6.25	6.25	12.50	6.25	12.5

Table 4.13: Minimum Bactericidal Concentration (MBC) of GF1 and GF2

Test Organism	*S.aureus*	*B.subtilis*	*S.typhi*	*S.dysenteriae*	*E.coli*
GF1 (µg/mL)	25.00	12.50	6.25	12.50	12.500
GF2 (µg/mL)	12.50	12.50	12.50	25.00	25.00

CHAPTER FIVE

5.0 DISCUSSION

5.1 Extraction of the stem bark of *Uapaca pilosa*

The plant material was identified, dried, pulverised and extracted using cold maceration method. The plant have been used traditionally as medicine in the treatment of female sterility and other ailments. The percentage yield of the extraction result as presented in Table 4.1 showed methanol has the highest yield of 270 g about 18.01 % of the total weight of the plant while chloroform has the lowest yield with recovery weight of 8.62 g representing 0.58 % of the plant.

5.2 Phytochemical Screening of the Stem bark of *Uapaca pilosa*

The crude extracts from the stembark of *Uapaca pilosa* were subjected to phytochemical screening and the results (Table 4.2) revealed the presence of all the tested secondary metabolites in methanol and ethyl acetate fraction except saponnins which was absent in ethyl acetate fraction, while alkaloids, anthraquinones, flavonoids and saponnins were absent in chloroform fraction. The results also revealed the presence of alkaloids, anthraquinones, glycosides, steroids and triterpenes were observed hexane fraction. In general, the accumulation and concentration of secondary metabolites are responsible for antibacterial activity and this varies according to plant extracts depending on their polarity (Essawi and Srours, 2000). These metabolites are known to act by different mechanisms and exert antimicrobial, antioxidants, anticancer and antituberculosis actions (Aiyegoro and Okoh., 2009).

5.3 Antimicrobial Screening of Stem bark of *Uapaca pilosa*

Antimicrobial screening showed that all the extracts of *Uapaca pilosa* exhibited moderate to good antibacterial activities. The result of Zone of inhibition (ZI) determination Table 4.3 shows inhibition which ranged from 10-18 mm (hexane fraction), 12-17 mm (chloroform fraction), 10-17 mm (ethyl acetate fraction) and 10-15 mm (methanol extract) against the entire test organism except *Candida albicans* and *Candida krusei*. The results were comparable to the drugs used as positive control (amoxillin, 15-28 mm, fluconazole; 34–38 mm). The microorganisms were not only inhibited but bactericidal at a higher concentration as shown in Table 4.5; ethyl acetate fraction and methanol extract (MBC; 12.5-50 mg/ml), chloroform fraction and hexane fraction (MBC; 25 mg/ml). The ability of the crude extracts to inhibit the growth and exert bactericidal effect on several bacterial is an indication of the antimicrobial potential of the stem bark extract, which makes the plant a good candidate for antibiotic drugs. As shown in Tables 4.3-4.5 the ethyl acetate fraction from stembark of the plant showed highest inhibitory activity compared to other extracts. The sensitivity of *E. coli* and *S. aureus* to all the extracts implies that chemical compounds in the extracts could be used to develop drugs to treat related ailments (Ramanathan *et al*., 2013). The extracts also showed good activities against *S. dysenteriae*, the bacteria responsible for bacillary dysentery. Therefore, all the extracts could serve in one way or the other as source of compounds that may be effective in the management of the ailments associated with the causative agents.

5.4 Isolation, Purification and Characterisation of Isolates from *Uapaca pilosa*

Column chromatography using silica gel as stationery phase was used for the separation of the most active extract followed by preparative thin layer chromatography led to the

isolation of 2 compounds labelled GF1 and GF2 from ethyl acetate fraction of the crude extract of *Uapaca pilosa*.

5.4.1 Isolation and Characterisation of GF1

Compound (GF1) was isolated as a white solid. Its FTIR revealed broad band at 2922cm^{-1} due to asymmetrical CH_2 stretching from the CH_2OH group, while its shoulder, 2855.1cm^{-1} can be assigned to CH_2 stretching in the first methyl ring. The 1651cm^{-1} band is due to C=C stretching and CH_2 bending in the terminal methyl group. The 1435cm^{-1} appears because of bending vibrations of the methyl and of the CH_2 groups in the rings, the band 1021.3cm^{-1} can be assigned to C-O stretching vibration in the CH_2OH group. 879.7cm^{-1} appears due to wagging vibration in the terminal group (Coates, 1996). The ^{1}H NMR spectrum (Figure 4.2) showed six methyl signals at δ_H 1.65, 0.99, 0.97, 0.96, 0.80 and 0.75 ppm. A doublet of doublets at δ_H 3.18 ppm characteristic of an α-oriented proton at C-3. Doublets for geminal protons at δ_H 4.70 and 4.59 ppm, along with the methyl signal at δ_H 1.65 ppm, suggested that compound GF1 was a lupane-type triterpenoid. A pair of oxymethylene doublets at δ_H 3.75 and 3.23 ppm, instead of a seventh methyl singlet around δ 0.8 ppm, indicated the presence of a second hydroxyl group in the molecule. The ^{13}C NMR spectrum further confirmed compound GF1 as a lupane-type triterpene derivative. A total of 30 carbon signals were observed from the spectrum. The characteristic pair of sp^2 hybridized carbon atoms comprising the double bond were observed at δ 150.9 and 109.3 ppm. Oxygenated carbon shifts were observed at δ 79.1 and 60.1 ppm respectively (Habib *et al.*, 2007; Tijani *et al.,* 2012). Based on the above spectroscopic result compound GF1 was considered to be betulin.

5.4.2 Isolation and Characterisation of GF2

Compound GF2 was isolated as white crystalline solid. On IR spectroscopic analysis, the absorption bands observed at 3567.1cm^{-1}, characteristic of phenolic O-H broad band, 2933.4cm^{-1} and 2866.3cm^{-1} for aliphatic C-H stretching. The band at 1558cm^{-1} is as a result of aromatic C=C (weak band), 1461cm^{-1}, a bending frequency for cyclic $(CH_2)n$ and 1379.1cm^{-1} for $-CH_2(CH_3)_2$. The absorption frequency at 1058cm^{-1} is typical of cycloalkane. The out of plane C-H vibration of unsaturated part was observed at 842.4cm^{-1}(Coates, 1996). The ^{1}HNMR spectrum revealed a typical steroidal nucleus with the three basic regions/environments of steroids showing signals at 0.5 ppm – 2.5 ppm and representing the methyl, methylene and methane protons overlap at 3.5 ppm. A signal which is assignable to an oxymethine proton appeared as a double doublet (dd) and an ethylenic proton showed triplet signal at 5.22 ppm (H-5). The ^{13}C-NMR spectrum of compound GF2 revealed 29 carbon signals. The signals between 11.87 ppm and 56.79 ppm are typical signals of the region of overlapping methyl, methylene and methine carbon atoms. An oxymethine carbon signal at 71.83 ppm is typical of steroids (Chaturvedula and Indra, 2012). The DEPT experiment revealed the presence of methyl carbons, eleven methylene carbons, nine methane carbons and three quaternary carbons were established from the decoupled ^{13}C spectrum. Based on the above analysis, the NMR data of compound GF2 are very similar to the data reported in the literature by Jaju *et al*., (2010) for sitosterol. Hence, compound GF2 was considered to be a beta-sitosterol (1)

The compounds isolated from the ethyl acetate fraction of the stembark extract of *Uapaca pilosa* were found to be steroids/triterpenoids. Steroids are among the most widely used class of drugs and their role in the therapy of pulmonary, inflammatory

oncological and dermatological infections has been established (Grover *et al*., 2007) and are well documented. The pharmacological and therapeutic properties such as antibacterial, antifungal, anti-inflammatory, anticancer and anti-oxidant activity of the isolated steroids have been reported (Govindappa and Poojashri, 2011). The sensitivity of *S. aureus* to the isolated compounds showed a promising activity compare to that of amoxillin drug used as the positive control indicates that the compounds can be developed to inhibit the effect of the microorganism and the plant can be used to treat diseases associated to *S. aureus*. Isolated compounds will make a tremendous impact in the treatment of diseases associated to *E. coli* due to their sensitivity to *E. coli*. Betulin, a pentacyclic lupane-type triterpenoid, isolated from the plant is known to have anti-inflammatory, antimalarial and antiretroviral properties, as well as anticancer agent by inhibition of topoisomerase (Grover *et al*., 2007). Sterols like sitosterol isolated, have been recommended for their cholesterol reducing activities and treatment of heart disease. It is also used for boosting the immune system and for preventing colon cancer as well as for gallstones, influenza, rheumatoid arthritis and chronic fatigue syndrome. Some women use it for symptoms of menopause, while some men used it for enhancing sexual activity. The use of sitosterol by athletes to reduce pain and swelling after sporting activities is well known. In foods, sitosterol is added to some margarines that are designed for use as part of cholesterol-lowering diet and for preventing heart disease (Desmond and Gribaldo, 2009). In the light of all these important and promising antimicrobial properties shown by these isolates the plant extracts provide significant potentials for the development and manufacture of new antibacterial therapies and in treatments of several diseases caused by tested microorganisms.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The plant sample *Uapaca pilosa* was collected fresh from Otukpo, Benue state, Nigeria in February, 2015. It was identified by Mr Sanusi Namadi at the Herbarium unit of Department of Biological Sciences, Ahmadu Bello University Zaria with a specimen voucher number 1279. The stem bark was subsequently subjected to air drying, pulverization and cold maceration extraction method using methanol. The methanol extract was reconstituted in water and partitioned with hexane, chloroform and ethyl acetate respectively. Extract and fractions obtained were concentrated using rotary evaporator and dried at room temperature. Crude extract and fractions obtained after extraction were subjected to preliminary phytochemical screening and antimicrobial studies. Ethyl acetate fraction showed most promising antibacterial activity against the tested organisms. Silica gel column purification and preparative thin layer chromatography of the ethyl acetate fraction led to the isolation of two pure compounds coded GF1 and GF2. The isolated compounds were identified through spectroscopic analyses such as 1D NMR (^{1}H, ^{13}C and DEPT), 2D NMR (COSY, HSQC, HMBC and NOESY) and comparison with literature. The pure compounds were revealed to be betulin (GF1) and beta-sitosterol (GF2). The isolated compounds demonstrated good antimicrobial activity against tested microorganisms.

6.2 Conclusion

The result of this study revealed the antimicrobial activities of extracts and molecules isolated from *Uapaca pilosa*. This study established that the plant offer significant potential for the development of new antibacterial therapies and treatment of diseases associated with tested microorganisms. Results from this research validates the ethnomedicinal uses of *Uapaca pilosa* for the treatment of several ailments by traditional medicine practitioners. The compounds isolated from the plant have been isolated from different plants and their antibacterial, antitumor and anti-inflammatory activity have been reported, this is the first report on their isolation from *Uapaca pilosa* (Hutch.). Therefore, this research work is an addition to the world data on natural product.

6.3 Recommendation

This study was carried out on the stembark of *Uapaca pilosa* only since the aim of research is to justify the use of this plant for the treatment of infections. The following recommendations are therefore made:

i. other parts of the plant should be screened for their antimicrobial activities so as to validate the whole plant`s antimicrobial properties,
ii. since the isolation work was made on the ethyl acetate extract only there is need to carry out work on the hexane, methanol and chloroform fractions of this plant, because they also showed some activities,
iii. structural modification of the isolated compounds for enhancement of their activities

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