PHYTOCHEMICAL INVESTIGATIONS AND ANTICANCER ACTIVITY STUDIES ON THE LEAVES OF *FICUS SYCOMORUS* LIN. (FAMILY: MORACEAE)

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APRIL, 2018

DECLARATION

I declare that the research work in this thesis titled: "PHYTOCHEMICAL INVESTIGATION AND ANTICANCER ACTIVITY STUDIES ON THE LEAVES OF *FICUS SYCOMORUS* Lin. (FAMILY: MORACEAE)*"* has been carried out by me in the Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, under the supervision of Prof. U.U. Pateh, Prof. M.I. Sule and Prof. I. Iliya. The informations derived from the literature have been duly acknowledged in the text and a list of references provided.

_____ _____

ATIKU Ibrahim Date

CERTIFICATION

This thesis entitled: "PHYTOCHEMICAL INVESTIGATION AND ANTICANCER ACTIVITY STUDIES ON THE LEAVES OF *FICUS SYCOMORUS* LIN. (FAMILY: MORACEAE)" by Atiku Ibrahim meets the regulations governing the award of a Doctor of Philosophy (PhD) degree in Ahmadu Bello University, Zaria, and is approved for its contributions to knowledge and literary presentation.

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To Allah (S.W.T.) for His Guidance and Protection.

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ABSTRACT

Ficus sycomorous (Family Moraceae) is a plant used in African traditional medicine to treat mental illness, dysentery, cough, diarrhea, tuberculosis and cancer. The aim of this research work is to isolate and characterize some compounds from the leaf of *Ficus sycomorous* and to screen the plant for anti-cancer activity. The dichloromethane (DCM) and methanol extracts were subjected to preliminary phytochemical screening using standard procedure; qualitative and quantitative antioxidant activity studies using 1, 1- diphenyl-2-picryl hydrazyl (DPPH). Flash column chromatography, column chromatography (using silica gel of mesh size 60-120), Gel filtration chromatography (using sephadex LH-20) and preparative thin layer chromatography were employed in the isolation of compounds from the DCM and methanol extracts. The two extracts were screened for anti-cancer activity using commercial absorbance assay (CellTiter 96®AQ_{ueous}One Solution Cell Proliferation Assay, Promega Corp, Madison, WI) on melanoma cancer cells)MDA-MB-435), human breast cancer cells (MDA-MB-231), human ovarian cancer cells (OVCAR3) and human colon cancer cells (HT-29). The results of phytochemical screening showed that DCM extract of *Ficus sycomorus* leaves contains terpenoids, alkaloids and cardiac glycosides but the methanol extract contains alkaloids, flavonoids, tannins, saponins, cardiac glycosides and terpenoids but coumarins and anthraquinones were found to be absent. A total of fifteen compounds were isolated in this study, of which two were new from plants origin. Squalene (A1), squalenol (A2), lupeol (A3), lupeol acetate (A4), beta amarinacetate (A5), sitosterol (A6), sitosterol-3-*O*-*β*-D-glucopyranoside (A7), α-tocopherylquinone (A8), were isolated from the DCM extract while blumenol A (A9), (6R,9R)-6,9-dihydroxy-4,7-megastimadiene-3-one (A10), (+)-ecatechin (A11), kaempferol (A12), kaempferol-3-*O*-α-L-rhamnoside (A13), quarcetin-3-*O*-β-*D*-glucopyranoside (A14) and phaophytin A (A15) were isolated from the methanol extract. The structures of these compounds were determined using nuclear magnetic resonance spectroscopy, infrared spectroscopy, mass spectrometry, electronic

circular dichroism and optical rotation measurements. Some of the isolated compounds were screened for anticancer activity using the same method as the extracts. The results of the antioxidant activity studies revealed that the DCM and methanol extracts had IC_{50} (the ability to quench 50% of the free radicals) of 42.83 µg/ml and 34.00 respectively. These results were analyzed statistically using the student's t-test and were compared with that of Vitamin C used as standard drug. The plant exhibited significant antioxidant and anti-cancer activities which could be attributed to some of the phytochemicals present in the plants. Hence, it could be useful in diseases involving oxidative stress such cancer, aging, stroke rheumatoid arthritis and heart diseases.

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Plate 4.7: Qualitative analysis of DPPH radical antioxidant studies of methanol extract 219

LIST OF ABBREVIATION

WHO = World Health Organization

TLC = Thin Layer Chromatography

PTLC = Preparative Thin Layer Chromatography

NMR = Nuclear magnetic resonance

CEE = crude ethanol extract

NCCLS = National Committee for Clinical Laboratory Standards

DCM = Dichloromethane

^{1}HNMR = Proton Magnetic Resonance

DEPT = Distortion less Enhancement by Polarization Transfer

COSY= Correlation Spectroscopy

HSQC = Homo Nuclear Single Quantum Correlation

HMBC = Hetero Nuclear Multiple Bond Correlation

ECD = Electronic Circular Dichroism

FCC = Flash Column Chromatography

HPLC = High performance liquid chromatography

GC = Gas chromatography

SPE = Solid-phase extraction

DEAE = diethylaminoethyl

DPPH = 1,1-diphenyl-2-picrylhydrazine

DMSO = Dimethyl sulfoxide

RPMI = Roswell Park Memorial Institute

CHAPTER ONE

1.0 INTRODUCTION

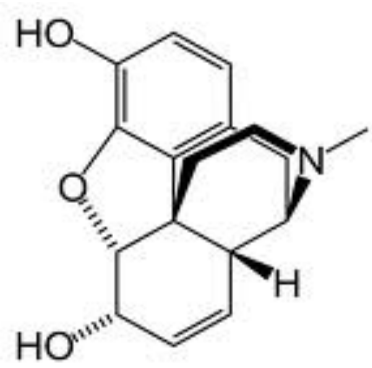
1.1 General introduction

Medicinal plants, either through systematic screening programs or by serendipity, possess an important position in the drug discovery. Many modern drugs have their origin in traditional medicine from different cultures (Abu-Dahab and Afifi, 2007).

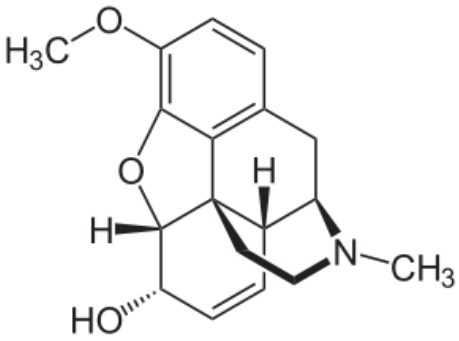
Ethnopharmacological data are obtained not only by consulting traditional healers and accumulating information on the popular medicinal use of plants, but also from literature on folk medicine (Mans *et al*., 2000). Furthermore, Elujoba (1997) noted that a plant becomes a medicinal plant only when its biological activity has been ethnobotanically reported or scientifically established. Natural products or related substances (extracts of folk medicine) accounted for 30% of the top 35 worldwide natural product-based drugs sold in 2003 (Butler, 2004). According to the World Health Organization (WHO, 2006), 80% of the world population use medicinal plants in the treatment of diseases and in African countries, this rate is much higher. It has been estimated that up to 90% of the population in developing countries rely on the use of medicinal plants to help meet their primary health care needs (WHO, 2002). The use of traditional medicine is however, not restricted to the developing countries (Jimoh, 2006). Infact over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982).

Medicinal plants have demonstrated their contributions to the treatment of diseases such as HIV/AIDS, malaria, cancer, diabetes, sickle-cell anaemia, mental disorders (Elujoba *et al*., 2005)and microbial infections (Okigbo and Mbajiuka, 2005). Iwu *et al*., (1999) reported that the primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and are more affordable treatment. Medicinal plants contain biologically active chemical substances (phytochemicals) such as saponins, tannins, essential oils, flavonoids, alkaloids and otherchemical compounds, which have preventive and curative properties. These complex chemical substances of different compositions are found as secondary plant metabolites in one or more of these plants and are useful to humanity (Sankaranarayan *et al*., 1996). Medicinal herbs have been used in one form or another under indigenous systems of medicine. Furthermore, the increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural remedies (UNESCO, 1998). Investigations into the chemical and biological activities of plants during the past two centuries have yielded compounds for the development of modern synthetic organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents (Roja and Rao, 2000). Medicinal uses of plants range from the administration of the roots, barks, stems, leaves and seeds to the use of extracts and decoctions from the plants (Ogbulie *et al*., 2004).

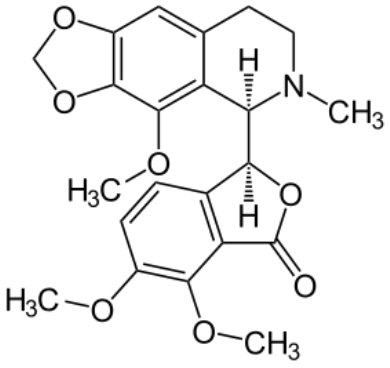
Terrestrial plants, especially higher plants have a long history of use in the treatment of human diseases. Several well known species, including licorice (G*lycyrrhiza glabra*), myrrh (*Commiphora species*) and poppy capsule latex (*Papaver somniforum*) were referred to by the first known written record on clay tablets from Mesopotamia in 2000 BC. These plants are still in use today for the treatment of various diseases and as ingredients for both orthodox and traditional medicines (Newman *et al.,* 2000). Examples are morphine (I), codeine (II), noscapine (narcotine) (III) and papaverine (IV). These compounds were isolated from *Papaver somniforum* and were developed as single chemical drugs which are still clinically in use. Hemisuccinate carbenoxolone sodium (V), a semi-synthetic derivative of glyceric acid found in Licorice is prescribed for the treatment of gastric and duodenal ulcer in various countries (Koehn and Carter, 2005).



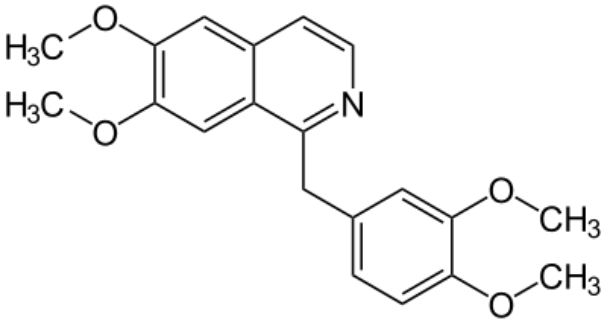
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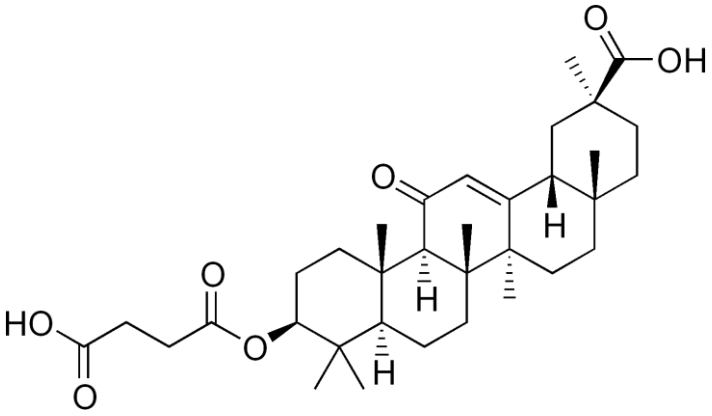
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(III)



(IV)



(V)

1.2 ANTI-CANCER AGENTS

Cancer is one of the leading causes of death around the world. Cancer is a complex multifactorial disease, which is dependent on cellular accumulation of various genetic and epigenetic events (Blagosklonny, 2005). The leading causes of death were: lung

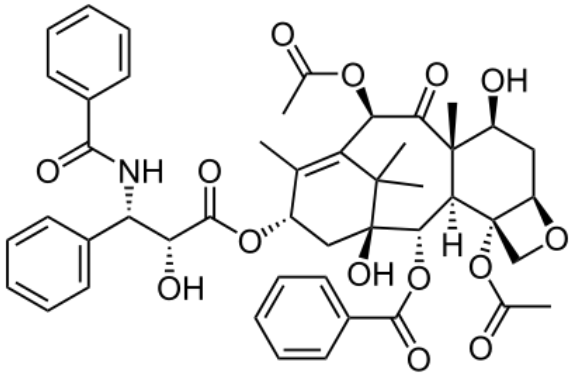
cancer (1.3 million deaths/year), stomach cancer (803,000 deaths), colorectal cancer (639,000 deaths), liver cancer (610,000 deaths), and breast cancer (519,000 deaths) (WHO, 2006). Specifically, solid tumors are the most common worldwide with higher mortality rate. Radiotherapy, chemotherapy and surgery are the key tools for cancer treatment (Fritz *et al.*, 2013).

1.2.1 Plant derived anticancer agents

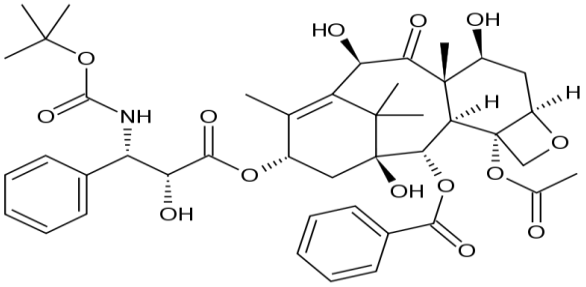
Ethnopharmacological data obtained by consulting traditional healers and by accumulating information on the popular medicinal use of plants, as well as from literatures on folk medicine revealed that about 25% of prescribed drugs in the world originate from plants and over 3000 species of plants have been reported to have anticancer properties (Rates, 2001). Medicinal plants have been used in Asian countries for the treatment of cancer and interest in this area of research has recently increased in many countries (Arulvasu *et al.*, 2010).

The plant-derived anticancer drugs, or the plant derived cancer chemotherapeutic agents were responsible for approximately one third of the total anticancer drug sales worldwide, or just under $4 billion dollars in 2007; namely, the taxanes paclitaxel (VI) and docetaxel (VII), as well as the camptothecin derivatives such as irinotecan (viii), topotecan (IX), etc (Fabricant and Farnsworth, 2001).

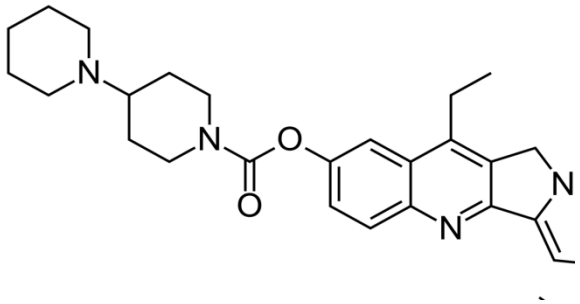
Current drug discovery from folk-medicinal plants has mainly relied on bioactivity-guided isolation methods, for example, bioactivity-guided isolation methods have led to discoveries of the important anticancer agents, paclitaxel from *Taxus brevifolia* and camptothecin from *Camptotheca acuminate.* Medicinal herbs of folk-origin are significant sources of synthetic and herbal drugs. The essential roles played by natural products in the discovery and development of effective anticancer agents, and the importance of multidisciplinary collaboration in the generation and optimization of novel molecular leads from natural product sources cannot be overemphasized (Fabricant and Farnsworth, 2001).



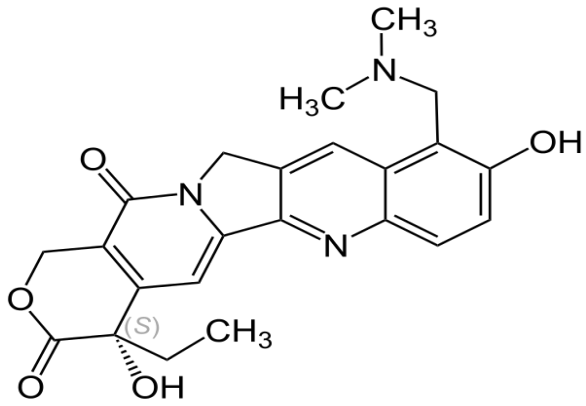
(VI)



(VII)



(VIII)



(IX)

1.3 STATEMENT OF RESEARCH PROBLEM

Cancer is one of the most dreaded diseases of the 20th century and spreading further continuously with increasing incidence in 21st century (Dashora *et al.,* 2011*).*As of 2014, cancer caused 13% of all deaths (7.4 million) worldwide. 650,000 people in Nigeria are diagnosed of cancer annually and death risk is 2 times higher than in developed countries (Parkin *et al.,* 2012).

Therefore, screening for the abundance of biodiverse components is important for research before the forests are lost to deforestation. Overall, this research may lead to new cancer chemotherapeutic agents with novel structures and/or mechanisms of action.

1.4 JUSTIFICATION

Despite the advances in anticancer drug development, cancer is still a major cause of human mortality and morbidity. Today there are powerful tools for the development of new anti-cancer agents, and phytochemicals like flavonoids, saponins and xanthones possessing potent anticancer activities are currently attracting attentions from researchers in thefield of drug discovery as anticancer drug candidates (Wu *et al.*, 2002).

Despite the significant progress in cancer therapeutics in the last decades, side effects cost and treatment schedule remain the major set back. Hence the need to discover and to develop new, alternative, or synergistic anticancer agents remains.

Cancer prevention or chemotherapy based on bioactive fractions or pure components derived from desert plants with known cancer-inhibiting properties suggests promising alternatives to current cancer therapy.

1.5 AIM AND OBJECTIVES

The main aim of this research work is to isolate and characterize some compounds from the leaves of *F. sycomorus* and to screen the plant extracts and some of the isolated compounds for anti-cancer activity.

The objectives of the research are:

1. To carry out the phytochemical screening on the leaves of *Ficus sycomorus*.
2. To investigate the antioxidant and anticancer activity of the crude extracts
3. To isolate some compound from the extracts of the leaf of the plant.
4. To elucidate the structure of the isolated compound(s).
5. To test some of the the isolated compounds for anticancer activity.

1.6 RESEARCH HYPOTHESIS

The leaves of the plant *Ficus sycomorus* contain phytochemical compounds with anticancer activity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 THE FAMILY MORACEAE

Moraceae, often called the mulberry family, is a family of flowering plants comprising of about 40 genera and over 1000 species of which over 500 species are members of the genus *Ficus* (Everett, 1968). They occur primarily in tropical and semi-tropical regions, and include a wide variety of herbs, shrubs, and trees, characterized by a milky sap (Everett, 1968; Duncan and Duncan, 1988). The Moraceae is a member of the order Urticales, class Magnoliopsida (the dicotyledons), division Magnoliophyta (flowering plants). It has been divided into five tribes: Artocarpeae, Moreae, Dorstenieae, Ficeae, and Castilleae (Rohwer *et al.,* 1993). Ficeae has only one genus, *Ficus*, with approximately about 750 species largely distributed in the tropics and subtropics (Shannon *et al.,* 2004). They can be either monoecious with bisexual inflorescences or dioecious (Weiblen, 2000). Taxonomically Ficeae is divided into two main groups (Corner, 1965; Berg and Hijman, 1989); the first group which comprises of the subgenera *Urostigma* consists of approximately 370 species, all of which are monoecious. The second group comprises of the subgenera *sycomorus*. *Sycomorus* consists of about 13 species, all of which are monoecious. Artocarpeae are represented by 12 genera and 87 species, including the economically important *Artocarpus* (jackfruit, breadfruit) (Jarrett, 1959; Berg, 1988). Castilleae include eight genera, distributed throughout the neotropics

and to a limited extent in tropical Africa (Shannon *et al*., 2004). Dorstenieae consist of eight genera (*Bosqueiopsis*, *Brosimum*, *Dorstenia*, *Helianthostylis*, *Scyphosyce*, *Trilepisium*, *Trymatococcus*, *Utsetela*) distributed in the neotropics (Berg, 1988).

2.1.1 Morphology of Moraceae Family

Flowers of the Moraceae occur in groups known as heads, spikes or catkins. Flowers are produced inside a synconium, a hollow fleshy structure. The small flowers lack petals. Male flowers consist of four sepals, which are usually leaf-like appendages, and four stamens. Female flowers consist of four sepals and a pistil with a two-chambered ovary (Everet, 1968). The fruit, developed from a single female flower, is either a fleshy drupe or a dry achene. The flowers fuse as they mature after fertilization and a multiple fruit forms. The multiple fruit consists of small drupes or achenes grouped together in a single unit, and are usually round or oval shaped. The best known fruit of the Moraceae is that of the common *Ficus* species, *Ficus carica*, which has been cultivated for thousands of years (Duncan and Duncan, 1988).

Moraceae leaves occur in a variety of shapes and sizes. For example, breadfruit, (*Artocarpus communis*), has lobed leaves that reach 61 cm in length. *Ficus carica* also has deeply lobed leaves. Other species, such as the creeping fig, *Ficus pumila*, have cordate leaves that are much smaller with entire margins. Leaves can occur singly on the stem, on alternating sides. At the base of a young leaf's petiole is a pair of stipules, but these soon falls off leaving small scars on the stem. Species of the Moraceae may be ever green, or they may have deciduous leaves that fall off at the end of the growing season (Everett, 1968).

2.2 THE GENUS *FICUS*

The genus *Ficus* consists of woody trees, shrubs, vines, epiphytes, and hemiepiphytes. They are collectively known as fig trees or figs. They are native throughout the tropics with few species extending into the semi-warm temperate zones (Sirisha *et al*., 2010).

2.2.1 Ethnomedicinal uses of *Ficus* species

The use of medicinal plants to improve health is as old as humanity. Among these plants, none may be older than the fig (Lansky *et al.,* 2008). A number of *Ficus* species are used for medicinal purposes in Ayurvedic and Traditional Chinese Medicine especially amongst people where these species grow. These uses, however, originated and are most widely found in the Middle East. In Iran, a decoction of the fruits of *Ficus carica* is taken orally for bronchitis, cystitis and nephritis (Zagari, 1992). Pharyngitis and stomatitis are treated with an oral decoction of the dried shoots (Darias *et al.*, 1986). In West Africa and Papua New Guinea, the dried leaf buds of *F. septica* are taken orally for headache and gastroenteritis. Other *ficus* species and their ethnomedicinal uses are presented in the Table 2.1.

Table 2.1 Ethnomedicinal uses of *Ficus spp* (Lansky *et al*., 2008)

Ficus species (part use)	Uses	Place
F. toxicaria (leaf, oral decoction)	Gastroenteritis	Indonesia
F. maxima(leaf and stem, Oral decoction)	Gingivitis	Honduras
F. thunbergii (leaf, oral decoction)	Lumbago, rheumatism	Japan
F. religiosa (leaf, oral decoction)	Asthma, cardiac oedema, gastritis	South Korea
F. religiosa (dried fruit, oral decoction)	Tuberculosis, fever	Bangladesh
F. carica (latex, aqueous decoction, external)	Warts	Italy
F. carica (stem latex, external)	Pain, scorpion bite	Turkey

2.3 Biological activities of *Ficus* species

A number of *Ficus* species have shown diverse biological and pharmacological activities. They have been investigated as potential repository of natural products for the treatment of various diseases including tumors, inflammatory diseases, wound healing and as anti-oxidants (Li *et al*., 2003).

2.3.1 Anti-inflammatory activity

A number of *Ficus* species have been reported by many authors to exhibit anti-inflammatory activities. For instance, in a test of anti-inflammatory activity, an aqueous extract of the root bark obtained from *Ficus elastica* significantly reduced carrageenan-induced ear oedema, compared to the positive control, indomethacin (Sackeyfio and Lugeleka, 1986). The ethanolic stem bark extract of *Ficus racemosa* inhibited cyclooxygenase-1(COX-1) (Li *et al.,* 2003) and 5-lipoxygenase (5-LOX) enzymatic activities (Li *et al.,* 2004) and prevented chemically induced renal oxidation and carcinogenesis (Khan and Sultana, 2005). Also, extracts of the leaves of *F. racemosa* demonstrated anti-inflammatory activity against carrageenan, serotonin, histamine dextran–induced rat paw oedema models, similar to the positive control, phenylbutazone (Mandal *et al.,* 2000).

2.3.2 Anticancer activity

Latex from *Ficus* species were investigated for anticancer activities as far back as the 1940‟s (Lansky *et al*., 2008). For example, fig latex was tested on bovine teat papilloma and activity compared to salicylic acid. They were equally effective in treating teat papillomatosis in cows (Hemmatzadeh *et al*., 2003). Also ethanolic extracts of *Ficus citrifolia* enhanced intracellular accumulation of daunomycin in K562/R7 leukemic cells and the cytotoxic effect of vinblastine on the growth of multi-drug resistant human

sarcoma MESSA/Dx5 cells. Thus the extract plays an adjunctive role in cancer chemotherapy (Simon *et al*., 2001).

2.3.5 Antioxidant and hypolipidaemic properties

Figs have also been shown to possess antioxidant and hypolipidaemic activities. In a study using rabbits on high cholesterol diets, an aqueous extract of the bark of *Ficus benghalensis* significantly reduced cholesterol and triglyceride levels compared with those fed with the same high cholesterol diet alone. In addition to this hypolipidaemic activity, there was a decrease in lipid peroxidation and an increase in antioxidant enzymes in rabbits given the fig extract (Shukla *et al.,* 2004). The leaves of *Ficus exasperata* also protected against the formation of excessive peroxide levels in palm oil when used during its preparation, presumably due to its antioxidant properties (Umerie *et al.,* 2004).

2.4 Phytochemistry of *Ficus* species

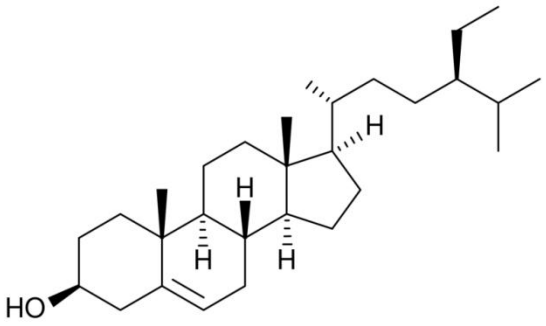
Phytochemistry is a branch of Chemistry, which studies and classifies chemical compoundsproduced in plants called phytochemicals. In a narrower sense, the term is often used to describe the large number of secondary metabolic compounds found in plants (Matsumoto*et al.,* 2008), which are known as secondary metabolites, secondary products, or natural products (Taiz and Zeiger, 2006). These plant constituents, firstly thought as functionless metabolic wastes, have important ecological functions. They protect plants against pathogens and herbivores, diseases or insect attack, they serve as attractants for pollinators, agents of competition between plants, agents of symbioses with microbes (Taiz and Zeiger, 2006), they can act as antioxidants or as signals in plants (Seigler, 1998). As a consequence these compounds exert interesting biological effects on other organisms and they can exhibit beneficialeffects for human consumers, they have significant ecological, economic and medicinal value (Hansson, 2005; Acamovic *et al*., 2005).

Several authors have isolated and identified various classes of compounds from the genus *Ficus*. They include flavonoids, alkaloids, phenolic acids, steroids, saponins, tannins, terpenoids and coumarins.

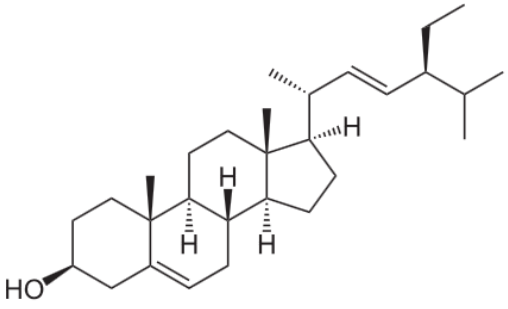
2.4.1 Steroids/Triterpenoids

Very few plant sterols have been reported in this genus. *β*-sitosterol (X) was isolated from the leaves and roots of *F. carica* and *F. septica* respectively (Wu *et al.,* 2002; Li *et al.,* 2006). Stigmasterol (XI) has been reported in the leaves and roots of *F. hirta* and *F. septica* (Wu *et al.,* 2002; Lin *et al.,* 2006). 24-methylenecycloartenol (XII), ψ-taraxasterol ester (XIII), lupeol (XIV) and baurenol (XV) have been isolated from the leaves of *F. carica* (Ahmed *et al.,* 1988; Saeed and Sabir, 2002), whereas *β*-sitosterol-3-*O*-*β*-D-glucoside (XVI) has been reported from the

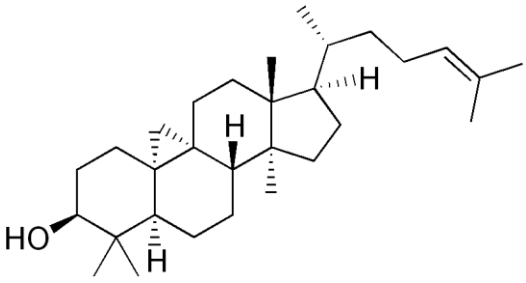
leaves of *F. septica* (Wu *et al.,* 2002). *β*-sitosterol-*β*-D-glucoside (XVI) has been isolated from the stem bark of *F. benghalensis* (Joseph and Raj, 2011).



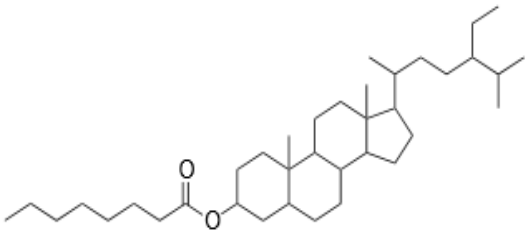
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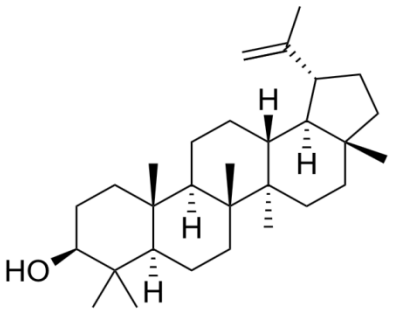
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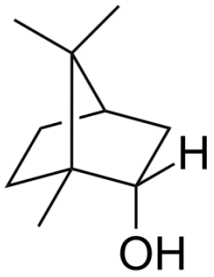
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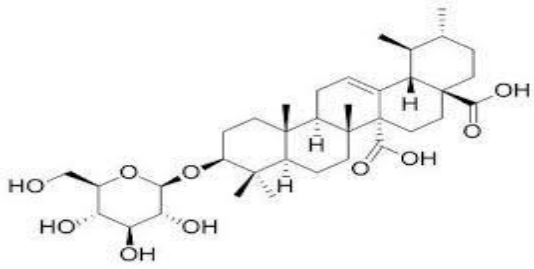
(XIII)



(XIV)



(XV)

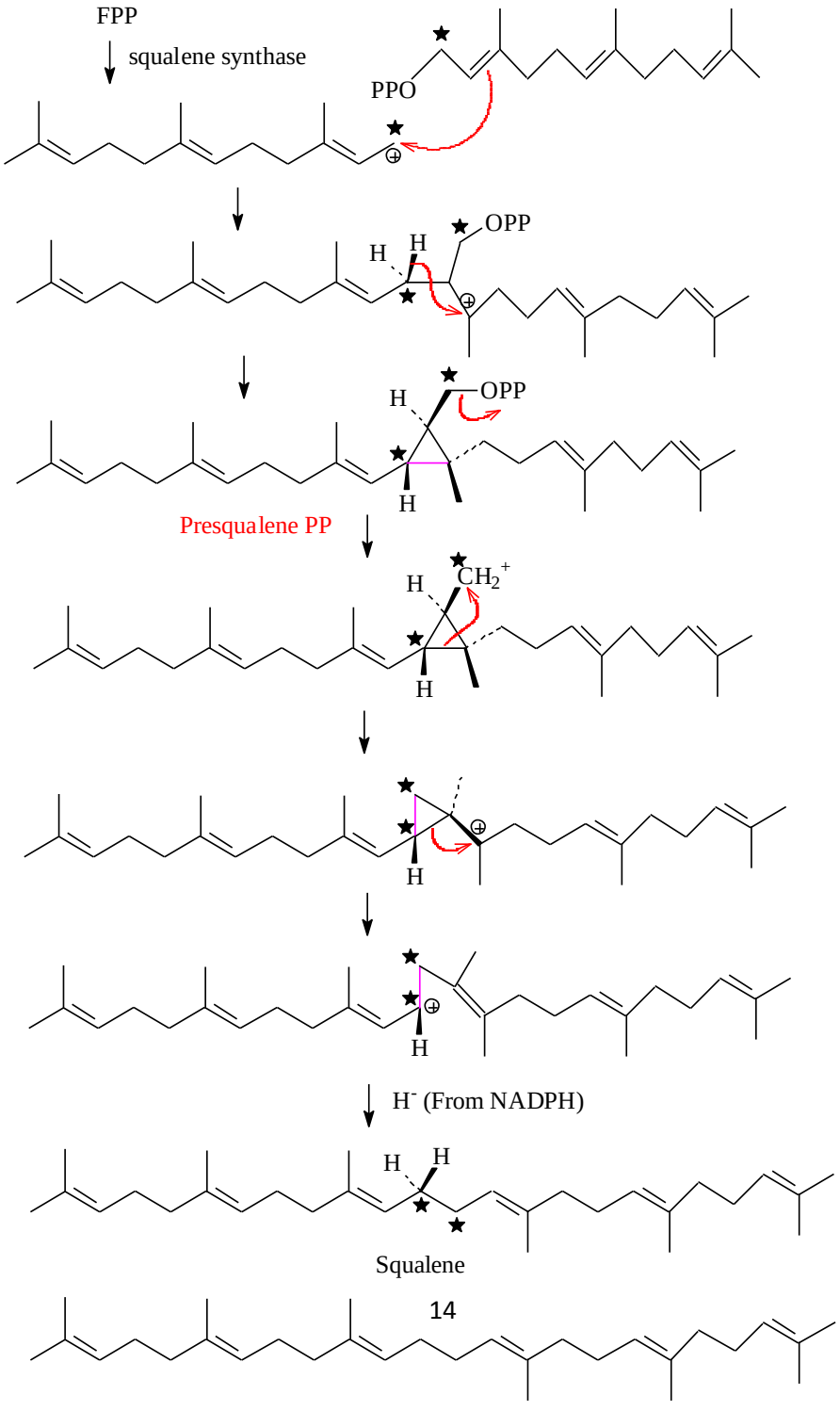


(XVI)

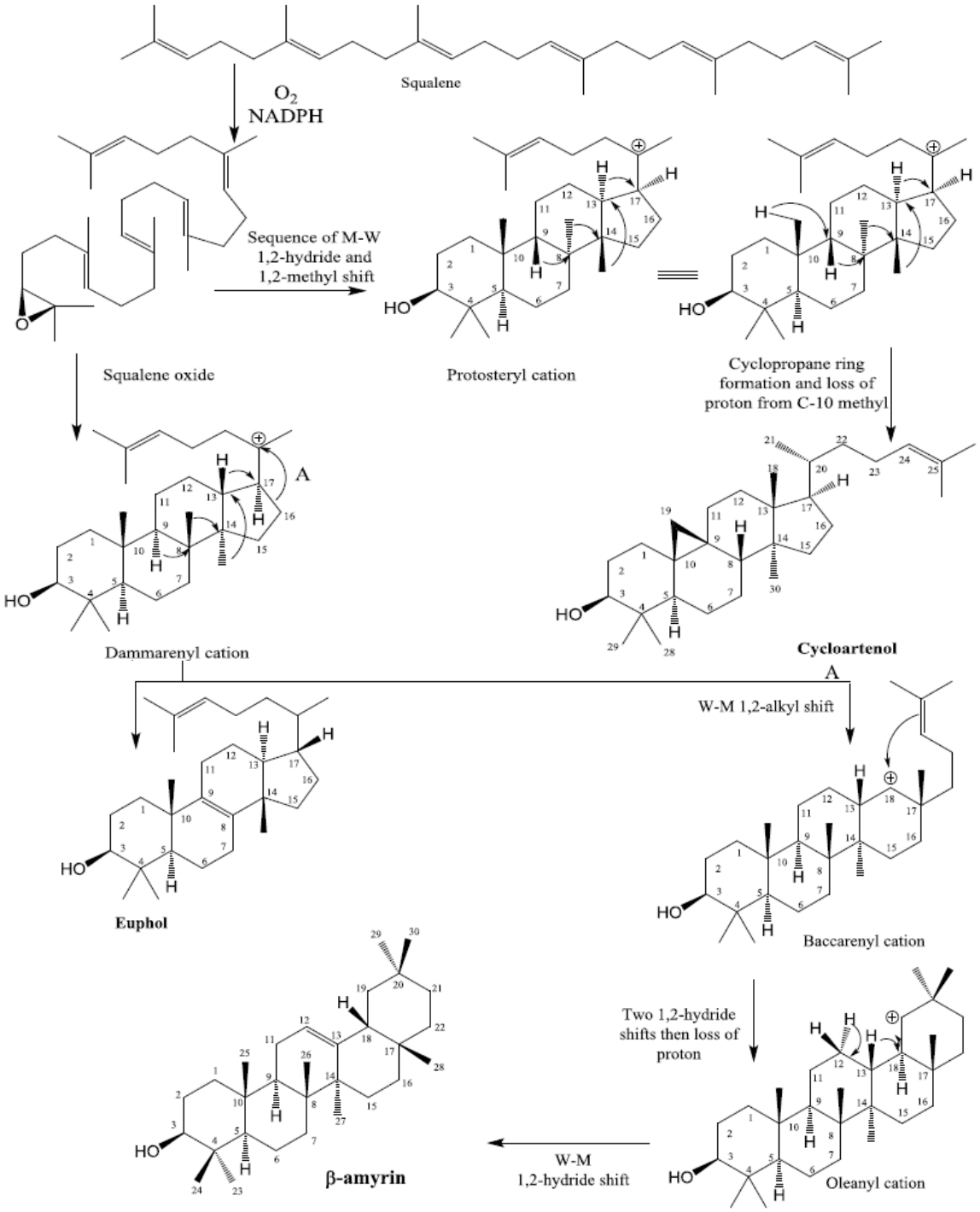
2.4.1.1 Biosynthesis of triterpenoids

A combination of two molecules of farnesyl pyrophosphate **(FPP)** that are linked tail to tail, gives the hydrocarbon **squalene** from which triterpenoids are derived (Scheme 2.1). O_2 and NADPH cofactors are required for cyclization of squalene *via* **squalene-2,3-oxide**. Folding of squalene oxide on the enzyme surface produced a series of cyclization, followed by a sequence of Wagner-Meerwein migrations of methyls and hydrides. Depending on the stereochemistry of this cation (squalene oxide), different transient cations will be produced, for example **protosteryl cation** and the **dammarenyl cation**.

These cations undergo a sequence of Wagner-Meerwein 1,2-shifts, and loss of protons to form different triterpenoid structures like cycloartenol, euphol and β-amyrin (Scheme 2.2) (Dewick, 2002).



Scheme 2.1: Biosynthesis of squalene from farnesyl pyrophosphate (Dewick, 2002)



Scheme 2.2: Biosynthesis of triterpenoides from squalene (Dewick, 2002)

2.4.2 Flavonoids

Flavonoids are a large classs of naturally occurring phenolic compounds mostly responsible for the colours of flowers and fruits (Bylka *et al*., 2004). The word flavonoid comes from the Latin word *flavus* which means yellow; however some flavonoids are red, blue, purple, or white.

Flavonoids are low molecular weight polyphenolic substances. The structure is the determiningfactor of their biological activities and it is based on the phenylpropanoid flavan nucleus (C6-C3-C6) carbon skeleton) composed of three rings assigned as A, B, and C (Figure 2.1). In fact, it is the chroman type skeleton with a phenyl substituent in the C2 or C3 position (Peterson and Dwyer 1998).

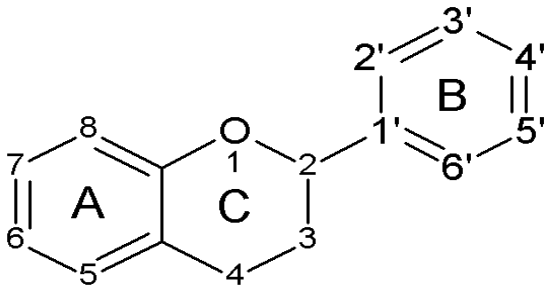


Figure 2.1 Flavonoid basic nucleus

Flavonoids are classified into groups according to the structural feature around the heterocyclic ring (bond saturation, hydroxylation and ring position) (Peterson and Dwyer, 1998).

Flavonoidscan be diversified also within each group by prenylation. Differences in the prenylated position on aromatic rings, differing lengths of prenyl chain and further modifications of the prenyl moiety (cyclisation, hydroxylation) has resulted in approximately 1,000 prenylated flavonoids occurring in plants (Yazaki*et al.,* 2009). The six major classes (Figure 2.2) of flavonoids are flavonols, flavones, flavanones, flavans (also called flavanols, catechins, leucoanthocyanidins, proanthocyanidins and tannins), isoflavonoids and anthocyanidins. Isoflavonoids differ from the common flavonoids in B ring orientation. Other flavonoid classes include dihydroflavonols, aurones, chalcones and coumarins (Peterson and Dwyer, 1998).

The possible modifications of flavan nucleus are hydroxylation (in positions 3,5,7,3',4',5'), hydrogenation, reduction, oxidation, alkylation, malonylation, sulphation, and glycosylation

(Fowler and Koffas, 2009). One or more of the hydroxyl groups can be methylated, acetylated, prenylated or sulphated (De Rijke *et al*., 2006).

Many natural flavonoids occur in the form of O-glycosides with sugar substituents bound to a hydroxyl group of the aglycone, usually located in position C3 or C7. Less frequent C-glucosides have the sugar moiety attached directly to the carbon of the aglycone usually *C*6 or *C*8. The most commonsugars are glucose, rhamnose, glucorhamnose, galactose, arabinose and lignin (Peterson and Dwyer, 1998).

The sugars can be further substituted by acyl residues such as malonate and acetate. Flavonoidglycosides from the diet are hydrolysed by animal intestinal flora to biologically active aglycones (De Rijke *et al.,* 2006). Flavonoids can be monomeric, dimeric, oligomeric or polymeric. Polymeric flavonoids (condensed tannins, non-hydrolysable tannins, proanthocyanidins) are polymers of flavan-3-ol subunits (mainly epicatechin and catechin) attached by carbon-carbon bonds, which cannot be cleaved by hydrolysis. They give characteristic colours, flavours, and astringency to plant drugs and extracts (Hagerman, 1998; Zhang and Lin, 2008). The group of prenylated flavonoids is rich in structural variety and biological activity and is characterized by the presence of isoprenoid side chain attached to the heterocyclic ring. An isoprenyl (3,3-dimethylallyl), a geranyl (*E*-3,7-dimethyl-2,6-octadienyl), a 1,1-dimethylallyl or a lavandulyl (5- methyl-2-isoprephenyl-hex-4-enyl) moiety is a part of the flavonoid structure (Chi *et al.,* 2001; Sohn *et al*., 2004).

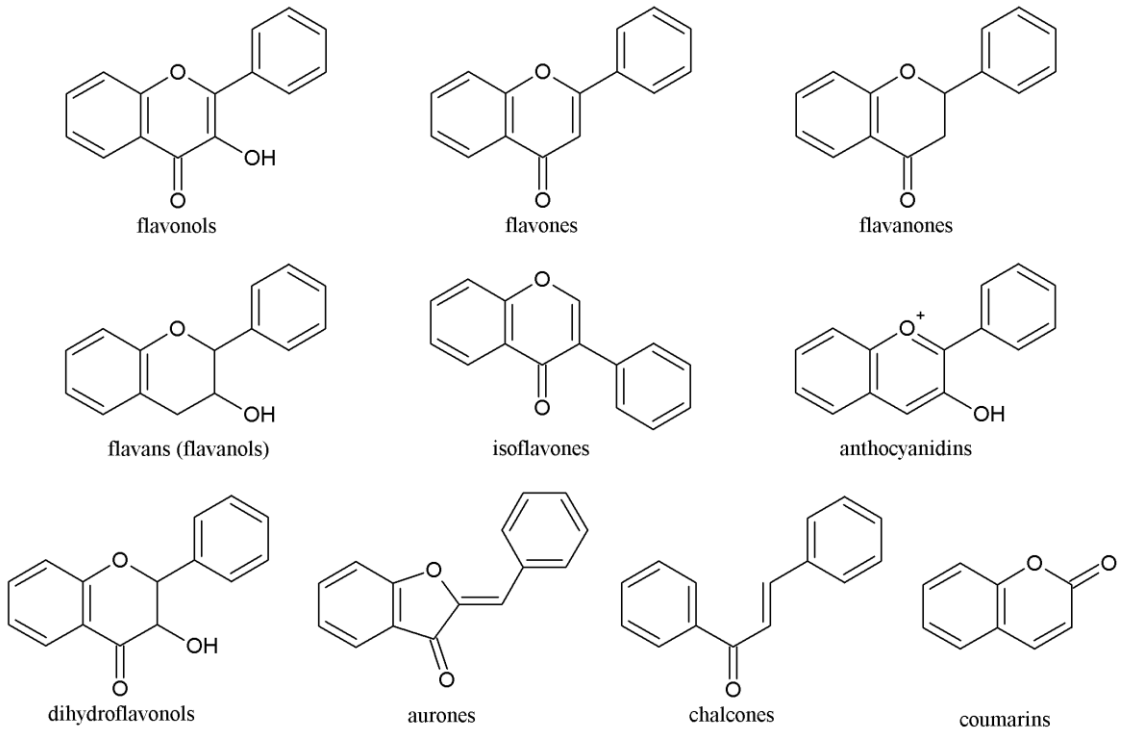
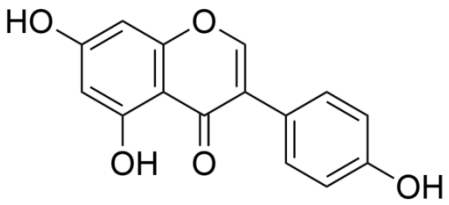
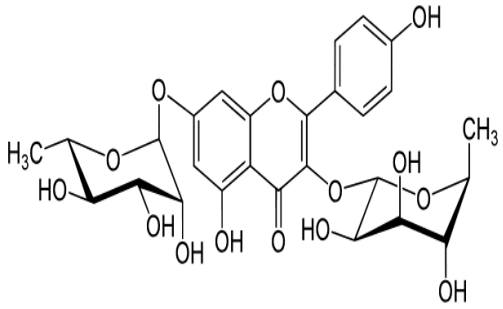


Figure 2.2: Classes of flavonoids

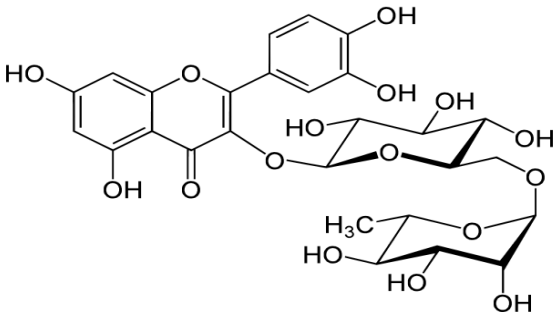
Members of the genus *Ficus* are known to contain flavonoids. Genistin (XVIII) and kaempferitrin (XIX) have been isolated from the leaves of *F. septica* (Wu *et al*., 2002). Also the leaves of *F. ruficaulis* and *F. carica* are reported to contain rutin (XX) and isoquercitrin (XXI) (El-Kholy and Shaban, 1966; Chang *et al*., 2005). Sheu *et al.,* (2005) reported the isolation of carpachromene (XXII) isoglabranin (XXIII) and norartocarpetin (XXIV) from the stem bark of *F. formosana*. Luteolin (xxv) has also been isolated from the aerial parts of *F. pumilla* (Pistelli *et al.,* 2000). The flavonoid apigenin (XXVI) has been reported in the stem bark and roots of *F. hirta* and *F. formosana* (Sheu *et al.*, 2005; Li *et al.,* 2006). Also, the root of *F. hirta* is reported to contain hesperidin (XXVII) 5-hydroxy-41, 6, 7, 8- tetramethoxy flavone (XXVIII) and 41, 5, 6, 7, 8-pentamethoxy flavone (XXIX) (Li *et al.,* 2006).



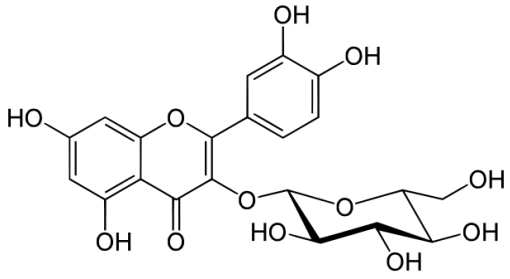
(XVIII)



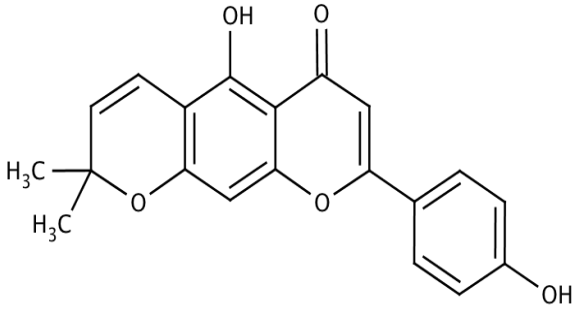
(XIX)



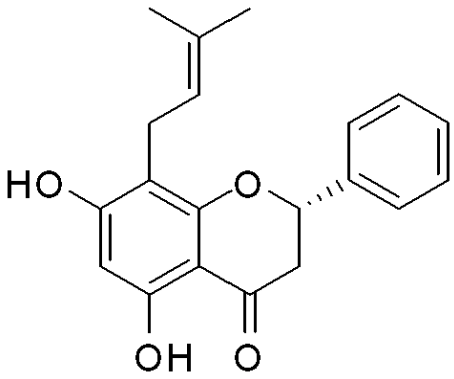
(XX)



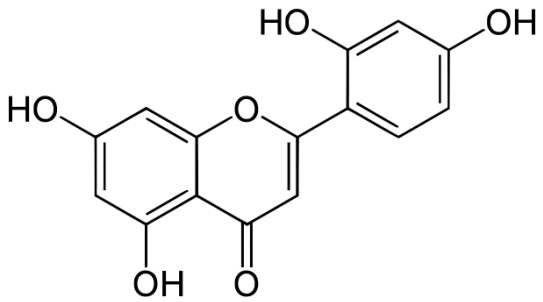
(XXI)



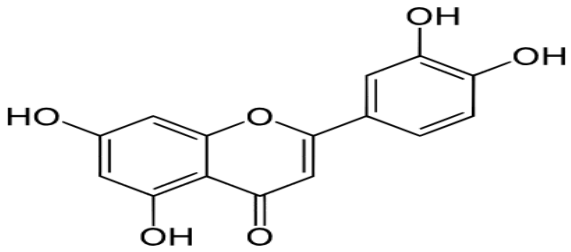
(XXII)



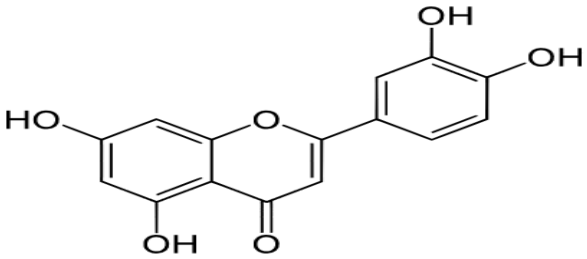
(XXIII)



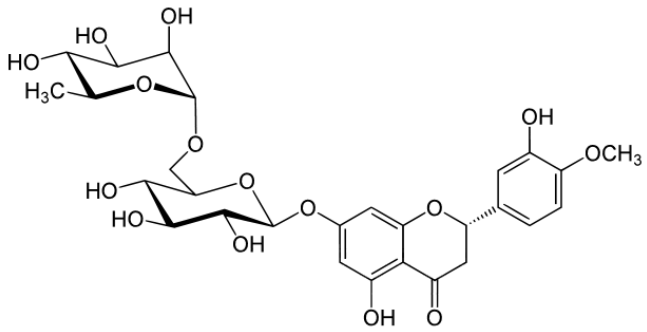
(XXIV)



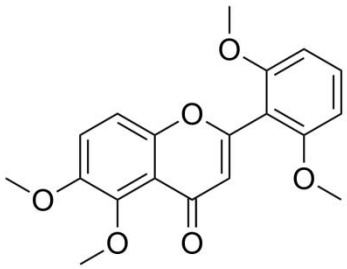
(XXV)



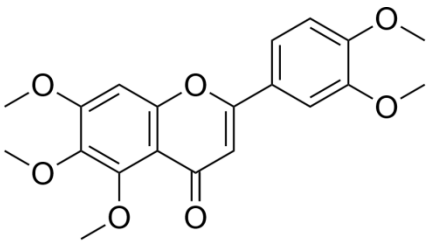
(XXVI)



(XXVII)



(XXVIII)



(XXIX)

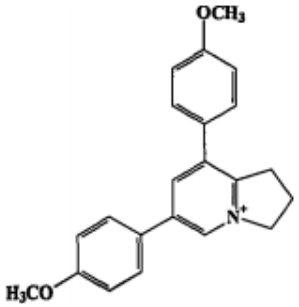
2.4.3 Alkaloids

Alkaloids are one of the most important natural products produced in plant kingdom. The alkaloids are organic nitrogenous bases found mainly in plants, but also to a lesser extent in microorganism and animals. One or more nitrogen atoms are present (usually in heterocyclic ring), typically as primary, secondary, or tertiary amines, and this usually confers basicity to the alkaloid, facilitating their isolation and purification since water-soluble salts can be formed in the presence of mineral acids. The name alkaloid is in fact derived from alkali. However, the degree of basicity varies greatly, depending on the structure of the alkaloid molecule, and the presence and location of other functional groups (Cowan, 1999).

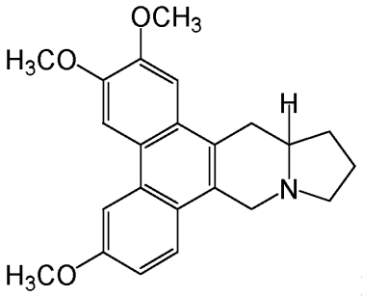
Indeed, some alkaloids are essentially neutral. Alkaloids containing quaternary amines are also found in nature. Alkaloids have traditionally been of interest due to their pronounced and various physiological activities in animals and humans. The biological activity of many alkaloids is often dependent on the amine function being transformed into a quaternary system by protonation at physiological pHs. Alkaloids are often classified according to the nature of the nitrogen-containing structure, e.g. pyrrolidine, piperidine, quinoline, isoquinoline, indole, pyrrolizidine, etc. (Cowan, 1999).

This genus is not well known for alkaloids. Few alkaloids, mostly phenanthroindolizidine alkaloids, have being isolated from the leaves and stem bark of *F. septica* and *F. hispida*. The alkaloids, ficuseptine-A (XXX) antofine (XXXI) and tylophorine (XXXII) have been

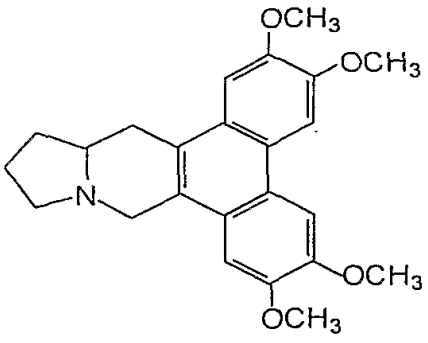
isolated from the leaves of *F. septica* whereas ficuseptine-B (XXXIII) and ficuseptine-C (XXXIV) have been reported from the stem bark. Tylocrebrine (XXXV) and isotylocrebrine (XXXVI) have been isolated from both the leaves and stem bark of *F. septica* (Baumgartner *et al.,* 1990; Wu *et al.,* 2002; Damu *et al.,* 2005). Hispidine (xxxvii) and 2-demethoxytylophorine (XXXVIII) have been reported from the stem bark and leaves of *F. hispida* respectively (Venkatachalam and Mulchandani, 1982).



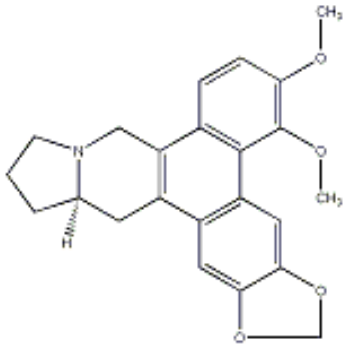
(XXX)



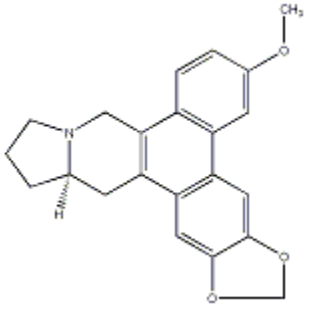
(XXXI)



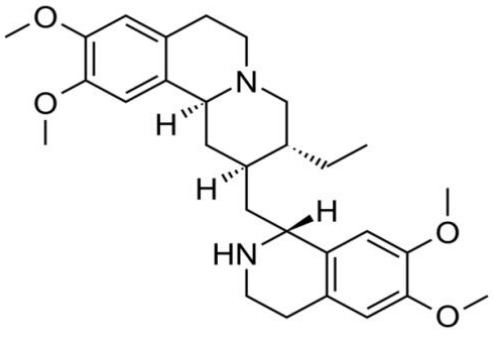
(XXXII)



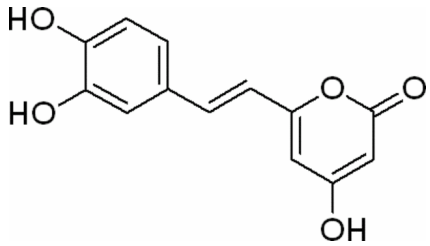
(XXXIII)



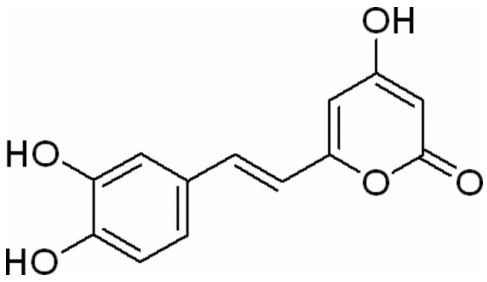
(XXXIV)



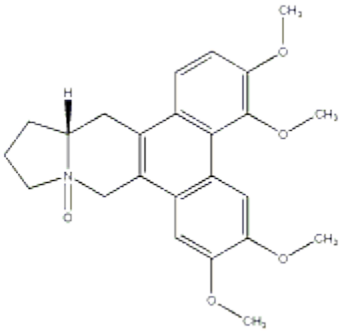
(XXXV)



(XXXVI)



(XXXVII)



(XXXVIII)

2.4.4 Tannins

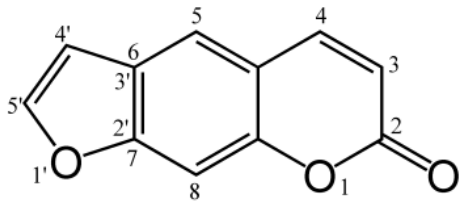
Tannins are group of phenolic substances capable of tanning leather or precipitating gelatine from solution. They occur in almost every part of the plant including bark, wood, leaves, fruits and roots (Hoste *et al.*, 2006). They occur in two forms; condensed tannins and hydrolysable tannins (Cowan, 1999). They react with proteins, forming complexes through hydrogen bonding and hydrophobic effects, as well as by covalent bond

formation. Thus, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc. (Scalbert, 1991).

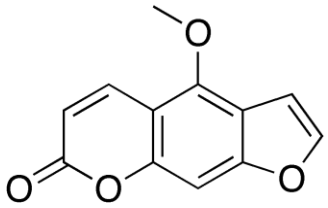
Hydrolyzable tannins have been reported to have good potential as new and safe therapeutic regimens against *H. pylori* infection *in vitro*. These compounds; tellimagrandin and tellimangrandin from a group of medicinal plants used in many countries in Asia did not affect the viability of MKN-28 cells derived from human gastric epithelium indicating that they could provide good treatment alternatives with minimal effects on the host (Funatogawa *et al*., 2004).

2.4.5 Coumarins

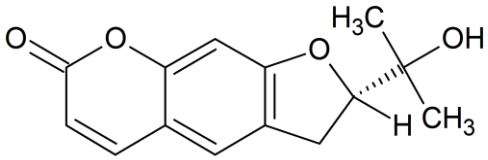
Another class of constituents commonly found in this genus are coumarins particularly, of the furanocoumarin type. Coumarins owe their class name to 'Coumarou', the vernacular name of the tonka bean (*Dipteryx odorata* Willd. Fabaceae), from which coumarin itself was isolated in 1820 (Bruneton, 1999). Coumarin is classified as a member of the benzopyrone family of compounds, all of which consist of a benzene ring joined to a pyrone ring (Ojala 2000). The benzopyrones can be subdivided into the benzo- γ -pyrones to which the coumarins belong and the benzo-γ-pyrones, of which the flavonoids are principal members. They have been isolated from the stem, leaves and roots of some *Ficus* species and are reported to be responsible for the contact dermatitis associated with *Ficus* species (Zaynoun *et al.,* 1984). The coumarins psoralen (XXXIX), bergapten (XL), marmesin (XLI), umbelliferone (XLII) and 4^1,5^1-dihydropsoralen (XLIII) have been reported in the leaves and stem bark of *F. carica* (Innocenti *et al.,* 1982). Psoralen has also been reported in the roots of *F. hirta* (Li *et al*., 2006). Chang *et al.*, (2005) isolated 5-*O*-*β*-D-glucopyranosyl-6-hydroxyangelicin (XLIV), 5-*O*-*β*-D-glucopyranosyl-8-hydroxypsoralen (XLV), 8-*O*-*β*-D-glucopyranosyl-5-hydroxypsoralen (XLVI) and 5,6-O-*β*-D-diglucopyranosylangelicin (XLVII) from the leaves of *F. ruficaulis*. Esculin (XLVIII) has also been isolated from the leaves of *F. septica* (Wu *et al.,* 2002). Bergapten (XL) and oxypeucedanin hydrate (XLIX) have been isolated from the aerial parts of *Ficus pumilla* (Pistelli *et al*., 2000). The stem bark of *F. religiosa* has also being found to contain the coumarins bergaptol (L) and bergapten (XL) (Makhija *et al*., 2010).



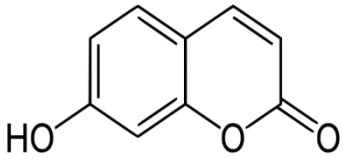
(XXXIX)



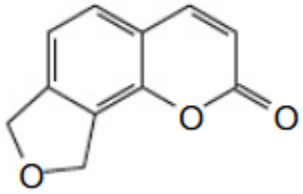
(XL)



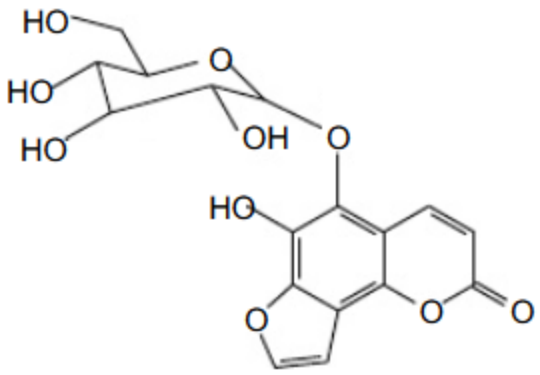
(XLI)



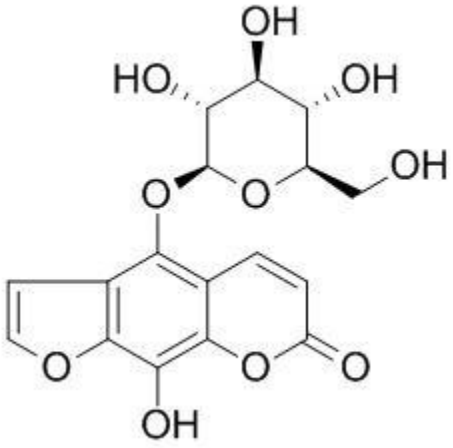
(XLII)



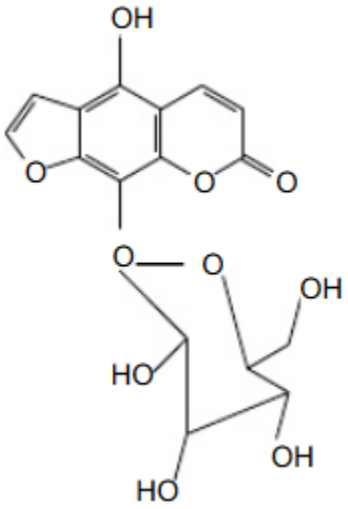
(XLIII)



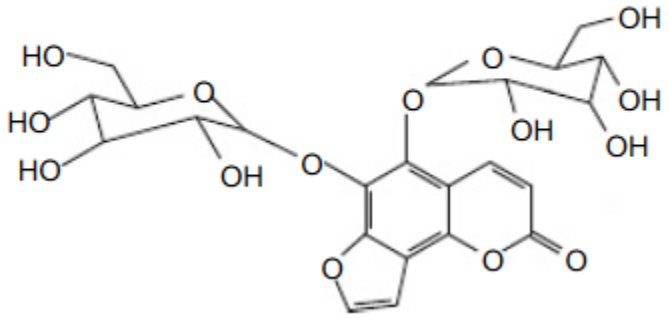
(XLIV)



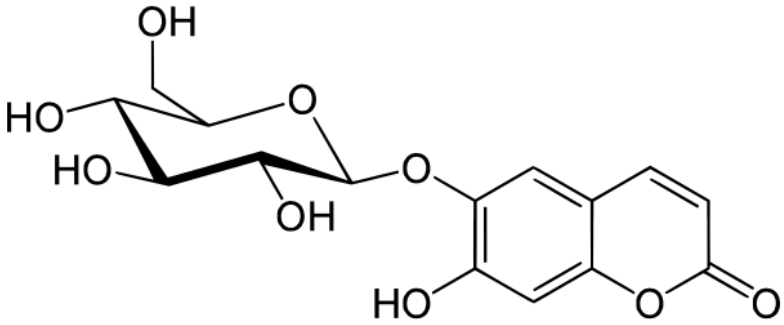
(XLV)



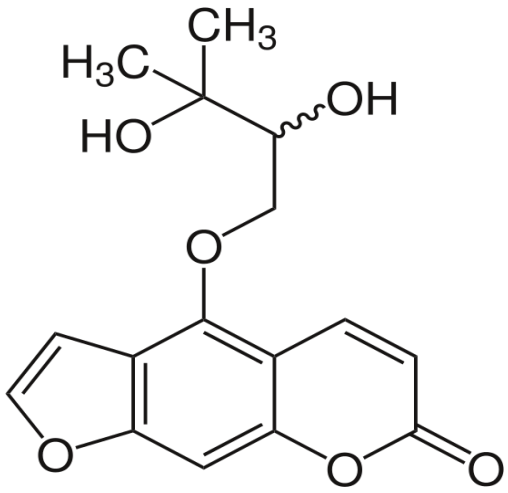
(XLVI)



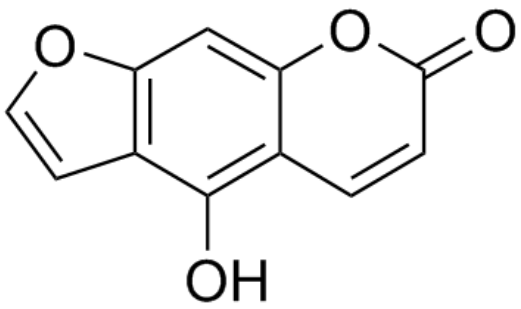
(XLVII)



(XLVIII)



(XLIX)



(L)

2.5 *FICUS SYCOMORUS* (MORACEAE)

2.5.1 Morphology of *Ficus sycomorus*

Ficus is the Latin name for fig, derived from the Persian 'fica'. In Greek 'syka' means Figure The name of the species comes from the Greek 'sykamorea' (sycamore), used in the Gospel according to St. Luke; it was the tree that Jesus cursed because it was barren (Williams *et al.,* 1980). *Ficus sycomorus* is a large, semi-deciduous spreading savannah tree, up to 21 (max. 46) m in height; it is occasionally buttressed. Its leaves are broadly (ob) ovate or elliptic, the sub base is cordate, the apex is rounded or obtuse, the margin is entirely or slightly repand-dentate (2.5-13 (max. 21) x 2-10 (max. 16 cm) and is scabrous above; the petiole is 1-5 cm long, with five to seven pairs of yellow lateral veins; the lowest pair originates at the leaf base. It can be monocious or diecious (Berg and Corner, 2005). Bark on young stems is pale green with a soft powdery covering; on older stems, grey-green, fairly smooth, with scattered grey scales and pale brown patches where scaleshave fallen off. When on the tree it produces heavy latex flow (Grison *et al*., 2002).



Plate 2.*1 Ficus sycomorus* in its natural habitat.

The plant is referred to by a number of local names: It is commonly known as *Sholla* or *Bamba* in Amharic in Ethiopia; large-fruited sycamore fig (English), Mukuyu (Shona), Mukuyukono (Shona), Muonde (Shona), Musvunguzu (Shona), Mutsvita (Shona), Umkhiwa (Ndebele), Baure (Hausa, Northern Nigeria), Tarmu (Kanuri), and Kamda (Babur/Bura), among others **(**Hyde *et al.*, 2013).

2.5.2 Distribution

The plant is widely distributedin tropical Africa stretching from Senegal to South Africa including Nigeria, Niger, Mali, Guinea, Kenya, Tanzania, Somalia, Ethiopia and IvoryCoast. In Nigeria the plant is mostly found in semi-arid regions. This savannah tree usually grows in high water table areas. It is often found along watercourses such as streams andrivers, swamps and water holes (Williams *et al*., 1980).

2.5.3 Ethnopharmacology of *Ficus sycomorus*

Ficus sycomorus has many traditional medicinal uses in the treatment of snake bites, jaundice, chest pains, dysentery, influenza, coughs and throat infections (Sofowora, 1993). Its leaves are said to be effective against jaundice and also are a much-sought fodder with fairly high nutritive

value (9% crude protein and 7mJ/kg net energy from dried material); they are valuable fodder in overstocked semi-arid areas where the trees occur naturally. Its fruits are eaten by livestock, wild animals and birds (Orwa *et al*., 2009). *Ficus sycomorus* is used in Nigeria, Niger, Mali, South Africa, Guinea, Kenya, Tanzania,Somalia, Ethiopia and Ivory Coast as extracts of fruits, leaves, root and stem bark to treatvarious ailments such as cough, diarrhea, skin infections, stomach disorders, liver disease,epilepsy, tuberculosis, lactation disorders, helminthiasis, infertility and sterility (Igbokwe *et al*., 2010; Aduom *et al*., 2012). In Palestine, the milky sap from the *F. sycomorus* stem bark is used for treating skin diseases while a decoction of the stem bark is used for problems of the digestive system. It is also used as seasoning; leaves are dried and added to cake as a condiment, eaten raw or cooked as soup (Auda, 2012).

In northern Nigeria, the stem bark of *Ficus sycomorus* is used traditionally to treat fungal diseases, jaundice and dysentery (Berg and Corner, 2005). The Hausa and Fulani tribes of northern Nigeria use the stem-bark of *F. sycomorus* to treat diabetes mellitus, fungal diseases, jaundice and dysentery (Hassan *et al*., 2007; Aduom *et al*., 2012). The parts of *Ficus sycomorus* used traditionally for the treatment of tumors and diseasesassociated or characterized by inflammation include thefruits in different stages of ripening, fresh or dry, treebark, leaves, twigs and young shoots, and also latex fromthe bark, fruit and young branches (Lansky *et al*., 2008).

2.5.4 Phytochemical constituents of *Ficus sycomorus*

The methanol leaf extract of the plant was reported to contain tannins, saponins, reducing sugars, flavon aglycones, anthraquinone glycosides, flavonoid glycosides and condensed tannins. The plant was also reported to have anthraquinone glycosides as the most potent antifungal compound in the stem bark extract (Hanssen *et al.,* 2005). The leaves of *F. sycomorus* contain calcium, phosphorus, iron, magnesium and zinc (Keay, 1989; Umar and Azare, 2006). The stem bark extract partially inhibits bacterial growth (Sandabe, 2002). Hassan *et al.,* 2007 reported that the aqueous ethanolic stem bark extract contains tannins, saponins, reducing sugars, flavon aglycones, anthraquinone glycosides, flavonoid glycosides and condensed tannins. Similarly, the aqueous extract of *F. sycomorus* stem bark was reported to contain pharmacologically active substancessuch as gallic acid, tannins, saponins, reducing sugars, alkaloids and flavones aglycones and caused no haematological, hepatic and renal toxicities (Igbokwe *et al*., 2010).

2.5.5 Biological Activities of *Ficus sycomorus*

Aduom *et al.,* (2012) established the LD50 of the methanol extract of *F. sycomorus* as 1500mg/kg. This study has further shown that the methanol stem bark extract of *F. sycomorus* at a dose of 250mg/kg bodyweight, elicited significant hypoglycemic activity in diabetic rats, $P<0.05$. The hypoglycemic effect of the methanolic stem-bark extract of *F. sycomorus* was not dosedependent. Njagi *et al.,* (2012) determined the effect of the aqueous stem bark extract on blood glucose on alloxan induced diabetic rats. The percent reduction of blood glucose levels in mice by the aqueous stem bark extract of *F. sycomorus* at the three dose levels (50, 100 and 150

mg/kg body weight) during the 1st hour were 30%, 28 % and 49%, respectively. Results indicated that all three dose levels of the extract lowered bloodglucose levels as effectively as insulin three hours after treatment.

The medicinal properties of this genus have been attributed to the presence of flavonoids, tannins, coumarins, terpenes, phytosterols, alkaloids and their glycosides (El-Sayed *et al*., 2010; Sarg *et al*., 2011). The phytochemical analysis of the methanol stem-bark extracts of *F. sycomorus* revealed the presence of flavonoids, saponins, alkaloids, reducing sugars and glycosides. Someflavonoids and glycosides have been found to stimulate β-cells regeneration, increase insulinsecretion or possess insulin-like effects (Aduom *et al*., 2012).

2.6 PHYTOCHEMICAL METHODS

The basic challenging steps in investigation of pure natural products are the isolation of individualsubstances from the plant matrix, chemical analysis and identification of the compound, as well astesting its biological activity. The phytochemical approach to the isolation and analysis of a plantmetabolite can vary according to a character of plant material, group of targeted compounds as well asavailability of methods and instruments. The isolation procedure has been described in manypublications and articles both from the general point of view (Sarker *et al.,* 2008) and from the point of particular groups of plant metabolites (Hooykaas *et al.,* 1977; Davies *et al.*, 2004).

The procedure leading from the plant material to the elucidated structure will be explained in thistheoretical part in relation to the protocols and methods used in the experimental part of this thesis.

2.6.1 Extraction

Extraction from plant material is a procedure in which different solvents with ability to extractplant components are used under specific conditions such as time and temperature. The purposeof extraction is to remove desired compounds from the plant material to the solvent. The selection ofan optimal solvent can maximize yields of target compounds and minimize the extraction of unwantedbalast. The number of possible solvents is very broad, starting with polar solvents such as water andmethanol on one side, ending with non-polar solvents such as hexane and heptane on the other side (Sarker *et al.,* 2008).

The extraction can be further improved by the use of a solvent mixture and specific extractionconditions. The chemical character and solubility of the compounds of interest are the main factorsinfluencing the choice of solvent. The quotation “similia similibus solvuntur” (like dissolves like) should be kept in mind; polar compounds can be dissolved and extracted in polar solvents and nonpolarcompounds in non-polar solvents (Sarker *et al.,* 2008).

Prior to the extraction procedure, antioxidants may be added to prevent enzymatic reactions anddecomposition of analytes. Internal (stable isotope) standards (2H, 15N, 13C) are often used

as internaltracers to control losses at each stage of extraction and purification and to increase the precision duringquantification of isolated target compounds. It may be useful to perform several trial extractions usingdifferent solvents and conditions and then compare total yield of extract or target compounds orintensity of biological activity to see which method gives the best results. The plant material used forextraction can be fresh, air-dried or freeze-dried, and should be well homogenized in mill or witha mortar and pestle to improve the efficiency of extraction. The simplest way of extraction in case ofsmall amounts of plant material (0.01-10 g) is maceration. It is recommended to repeat the extractionwith a new portion of fresh solvent. The plant material should be almost completely exhausted afterthree solvent changes. Then the collected supernatants are evaporated if necessary prior to furtherpurification (Sarker *et al.,* 2008).

2.6.2 Fractionation and purification methods

The target analytes are extracted from the plant material usually as a mixture of compounds withsimilar physical and chemical properties and they need to be separated to individuals and thenidentified. Isolation of a pure compound from crude extract or fraction is often time consuming, expensive and requires several separation steps involving different chromatographic techniques.

Generally, conventional methods such as low pressure column chromatography (using silica gel, polyamide), gel exclusion chromatography (using Sephadex LH-20) and preparative reversed-phase high pressure liquidchromatography arethe most often used to fractionate crude extract and isolate pure substances (Sarker *et al.,* 2008).

2.7 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is one of the most direct and general tools foridentifying the structure of compounds, which allows physical, chemical, electronic and structuralinformation about molecules to be obtained. The technique can provide detailed information on thetopology, dynamics and three-dimensional structure of molecules, either in solution or in the solidstate. NMR spectroscopy is also used to study molecular physics of crystals and non-crystallinematerials and in advanced medical imaging techniques, such as magnetic resonance imaging (Tyszka *et al.*, 2005; Hornak *et al*., 2009).

The NMR measurements are performed on the atomic nuclei. Structural and dynamic informationis obtainable from NMR studies of stable isotopes with non-zero spins, which contain an odd numberof protons and neutrons and have their intrinsic magnetic moment. The most commonly studiednuclei are ^{1}H and ^{13}C. NMR is a property that nuclei of certain atoms have when immersed in a staticmagnetic field and exposed to a second oscillating magnetic field after application ofan electromagnetic pulse (EMP) or pulses. The EMP causes the nuclei to absorb energy from the EMPand to radiate this energy back out at a specific resonance frequency, which depends mainly onthe nuclei under observation and the strength of the static magnetic field. Different protons in a molecule resonate at slightly different frequencies. The

resonant frequency energy of the absorptionand the intensity of the signal is proportional to the strength of the magnetic field. The NMRexperiment usually involves two sequential steps; the polarization of the magnetic nuclear spins inan applied constant magnetic field, and interruption of this alignment of the nuclear spins byemploying an electromagnetic radio-frequency pulse. Chemical shift, coupling constant and integrationof proton signals can be read from a one-dimensional spectrum and give information not only aboutthe chemical environment of the nuclei, but also the number of neighbouring NMR active nucleiwithin the molecule (Tyszka *et al.*, 2005; Hornak *et al*., 2009)

The process of identifying the compound using NMR spectroscopy often involves performing several NMR experiments to deduce the molecular structure from the magnetic properties ofthe atomic nuclei and surrounded electrons. The basic one-dimensional technique for provingthe probable structure is the recording of hydrogen spectra (^{1}H NMR spectrum). The ^{13}C spectra are alsouseful in structure determination. Frequency and intensity serve as the axis of the standard one-dimensionalspectrum and represent the characterisation of the nuclei in terms of chemical shifts andcouplings (Hornak *et al*., 2009). Two-dimensional (2D) spectra provide more information about a molecule than one-dimensional. The second dimension refers to an additionalfrequency axis, where also magnetic interactions between nuclei are considered. 2D-experiments canbe homonuclear such as COSY (correlation spectroscopy) observing proton-proton correlations, andheteronuclear providing correlation, e.g. between the ^{13}C and ^{1}H resonances. HSQC (heteronuclearsingle quantum correlation) spectrum contains a peak for each proton attached to the heteronucleuswhich is observed. HMQC (heteronuclear correlation through multiple quantum coherence) and HMBC (heteronuclear multiple bond coherence) are 2D inverse correlation techniques where the ^{13}C responses observed in ^{1}H NMR spectrum and allow the determination of connectivity between twodifferent nuclear species. HMQC is selective for direct coupling where as HMBC detects longer rangecouplings through 2-4 bonds (Martin *et al*., 1988).

2.8 CANCER

Cancer is a term, which is used to refer to a number of conditions where the cells begin to grow and reproduce in an uncontrollable way. Sometimes a cancer begins in one part of the body and then spreads to other parts of the body. This process is known as metastasis (Kleihues *et al*., 2002; Lewandowicz *et al*., 2000). Cancer is a common condition and moreover, it is deemed as a serious health problem, both in the UK and across the world.

It is estimated that 7.6 million people in the world died of cancer in 2009. In the UK cancer is responsible for 126,000 deaths per year (Cancer Research UK, 2007).

Cancer occurs when the genes in a cell become abnormal and the cell divides and grows uncontrollably (Figure 2.3). Cell division is normal, but when this process is uncontrolled a mass of tissue called a growth or tumour is formed (Cancer Research UK, 2007).

Malignant tumours are cancerous and also can spread widely to other parts of the body by entering either the blood stream or lymphatic system as well as harming nearby tissues and the organs. This rapid growth of cancerous cells is known as malignant tumour. These cells can then invade and destroy healthy tissues and organs. In addition, it can harm a number of vital organs at the same time (Merlo *et al*. 2006).

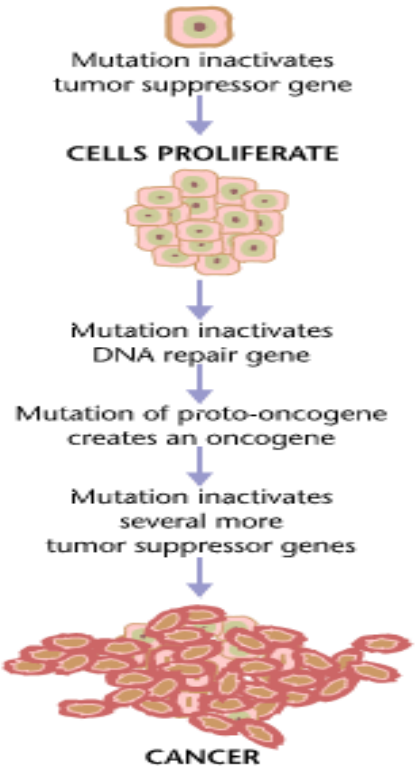


Figure 2.3: A schematic diagram showing the development of a cancer (Adapted form bio.miami.edu, January 2014).

2.8.1 Types of cancers

There are different types of cancers in the body. They include anal, bladder, breast, cervical, colon, endometrial, oesophageal, kidney, leukaemia, liver, lung, lymphoma, ovarian, pancreatic, penile, prostate, skin, stomach cancer and several others. Almost any part of the body can be infected with cancers (Roberts and Rudee, 1998). All the cells in the body have similar structures and share a majority of their functions. Cancers may be categorized based on the functions and locations of the cells from which they originate (Figure 2.3). The following terms are commonly used to distinguish tumours of different origin (Wen and Kerasi, 2008).

Carcinoma- is a tumour, which is derived from epithelial cells that line the surface of skin and organs. The digestive tract and airways are also lined with epithelial cells. This is the most common cancer type and represents about 80-90% of all cancer cases reported

Sarcoma- is a tumour, which is derived from muscle, bone, cartilage, fat and connective tissues.

Leukemia- is a cancer, which is derived from white blood cells or their precursors. The cells that form both white and red blood cells are located in the bone marrow.

Lymphoma- is a cancer of bone marrow and it is derived from cells that affect the lymphatic system.

Myeloma- is a cancer involving the white blood cells and it is responsible for the production of antibodies (B lymphocytes or B-cells) (Wen and Kerasi, 2008).

2.8.2 Causes of cancer

There are many causes of cancers and they include mutation of the genes that control cell growth, chemicals, radiations, preservatives etc (Merlo *et al*., 2006). Gene mutation is found in every cell in the body and it regulates all of its activities. Cancers are caused by damage to the DNA. The body is usually able to repair damaged DNA, but it is unable to do so in cancer cells (Goodarz *et al*., 2005). In most cases, people damage their DNA via their life style habits, which include unbalanced diet, smoking, stress, exposure to ultraviolet radiation (UV) from the sun and to substances known as carcinogens in the environment. Some carcinogens include benzene, asbestos, formaldehyde, which are specific for skin cancers and in most cases these can be prevented with care and by following health and safety regulations. Smoking causes the majority of lung cancers, but scientists have long known that tobacco contributes to a number of other forms of the disease (Roberts and Rudee, 1998). Each type of cancer is caused by different factors, which are well established, while others are uncertain and unknown (Goodarz *et al*. 2005).

2.8.3 Development of cancer

Mortality from cancer in developing countries is higher than AIDS, tuberculosis or malaria and it has been confirmed in 2008 that more than 12 million new cases of cancer were diagnosed worldwide. Out of 12 millions 7.6 million deaths have been reported. The percentage is more in developing countries i.e. 60 percent and it has been estimated that more than half of all new cases and fatalities occurred in developing countries (Stupp *et al.,* 2005). Due to poverty in development countries and poor medical infrastructure often means that cancer is a sure-fire death sentence. The rates of survival from cancer in these countries are exceptionally low. Most people do not seek medical help until their disease is advanced and incurable; it is due to lack of awareness, stigma and reliance on traditional healers. Because of the dramatic development, cancer research has given rise to a rich and complex body of knowledge. The primary step was set in the discovery of mutations in proto-oncogenes that produce oncogenes with dominant gain of function (Cyclin D1 and Cdc25A) and tumour suppressor genes with recessive loss of function (Bishop and Weinberg, 1996). This first mutation of these degenerated cells helps them to get an advantage in proliferation and progression compared to normal cells. Hanahan and Weinberg published few years ago “The Hallmarks of Cancer”. In this review they described six

different capabilities which each cell needs to degenerate in a malignant cancer cell (Bishop and Weinberg, 1996).

2.8.4 Self-sufficiency in growth signals

Self-sufficiency in growth signals was the first step which was clearly defined by cancer researchers. Normally cells required growth signals to move from G0/G1 state of cell cycle into an active proliferation state. These signals are found for example in the extracellular matrix and are transmitted into the cell by transmembrane receptors. In absence of these signals a normal cell and their receptors cannot start the proliferation machine, but many oncogenes mimic these growth signals and initiate cell cycle on their own. For example the epidermal growth factor receptor (EGFR) is upregulated in stomach, brain and breast tumours. This liberation from dependence on exogenously derived signals disrupts a critically important homeostatic mechanism that normally operates to ensure a proper behaviour of various cell types within a tissue (Hanahan and Weiblen, 2000).

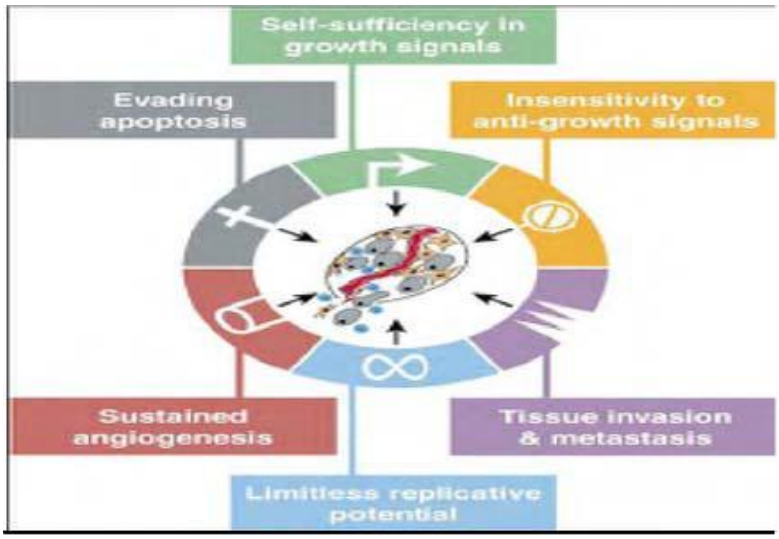


Figure 2.4 self-sufficiency in cancer growth signals

2.9 Cancer cell lines

A cell line refers to cells derived from a single cell type that have been adapted to grow continuously in the laboratory and are used in research (Cancer.org, 2006).

2.9.1 Hepatocellular carcinoma (Hep3B) cell line

The Hep3b cell line is a hepatocellular carcinoma derived from 8 years human male juvenile. Analysis of the cell culture fluid from Hep3b revealed that 17of the major human plasma

proteins are synthesized and secreted by this cellline. Also, Hep3b produces the two major polypeptides of Hepatitis B virussurface antigen. This cell line was used to generate an animal model formetastatic hepatocellular carcinoma by injection into athymic mouse (Knowles *et al.,* 1980).

The Hep3b cell line was used to determine the cytotoxic effects of 6-xanthone compounds from the peel of fruits of *Garcinia mangostana* on hepatocellular carcinomas using the MTT proliferation assay (Ho *et al.,* 2002). A Similar study examined the effect of *Ginko biloba* extract on cell proliferation and cytotoxicity in human hepatocellular carcinoma cells using this cell line in addition to HepG2 cell line. In this study cell proliferation and cytotoxicity were determine by the (MTS) assay (Chao and Chu, 2004)

2.9.2 Cervical epithelial cell line (Hela)

The Hela cell line was derived from a cervical epithelial cancer from a 31 years old female and it has been reported to contain Human Papillomavirus18 (HPV-18). Hela cells have been used in many studies which aimed to investigate the anticancer role of artificial and natural products (Kaighn *et al*., 2007).

The antiproliferative effect of C*hlorophytum comosum* root extract was tested *in vitro* against Hela, HL-60, and U937 cells. The extract was found to have porliferation effect, and to induce apoptosis in these human cell lines (Matsushita *et al*., 2005). The cytotoxic effect of the anticancer drug Rhodamine and Herbal extracts of *Glacyrhizan radix*, *Rhei rhizome* and *Zingibers rhizome* on Hela cells was alsosuggested. Hela cells were used to indicate that the combination of anticancer drugs with some herbal extracts contributes to the enhancement ofclinical outcomes in cancer (Takara *et al.,* 2005).

In a cytotoxicological study of Iranian conifers on Hela cells, a colorimetric assay (MTT) was used and the findings showed that *Junperus sabina* extract possesan inhibitory effect against Hela cells by reducing the cell viability to less than 50%. Another similar study investigated the suppressant effects of *Vandellia cordifolia* on human cervical cancer Hela cell line. The result indicated that this plant extract suppressed Hela cell line proliferation by cell cycle progression inhibition at the G1 to S phase transition (Dehkordi *et al.,* 2004).

2.9.3 Prostate cell line (PC-3)

The Prostate cell line (PC-3) was initiated from a grade-IV bone metastasis prostate adenocarcinoma from a 62 years-old Caucasian male. The establishment, characterization, and tumorigenicity of (PC-3) cell line were reported. The cultured cells showed anchorage-independent growth in both monolayers and in soft agar suspension and can produce subcutaneous tumors in nude mice.

Electron microscopic studies revealed many features common to neoplastic cellsof epithelial origin including numerous microvilli, junctional complexes, abnormal nuclei and nucleoli,

abnormal mitochondria, annulate lamellae, and lipoidal bodies. In general, the functional and morphological characteristics of PC-3 are those of a poorly-differentiated adenocarcinoma .

The PC-3 cell line has been used *in vivo* and *in vitro* in many studies in the screening for new anticancer agents. PC-3 cells were used in a recent study to test the cytotoxic and antiproliferative effects of the crude extract of Thai medicinal plants for cancer treatment. A similar study evaluated the effects of plant hormones on PC-3 cell line *in vitro* reported that PC-3 cells exhibiteddifferential sensitivities to three hormones, and PC-3 proliferation wassignificantly inhibited. The human prostate carcinoma PC-3 was also used to determine the chemosensetivity of prostate cancer to genistein isoflavon (metabolites of soy) and -lapachone (plant product) *in vitro* using trypan-blue and MTS bioassay. The type of cell death, apoptosis or necrosis in response to treatment of PC-3 cells was determined using different assays such as annexin V-FITC and PI/TUNEL apoptosis assays (Dehkordi *et al.,* 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS AND ANALYTICAL PROCEDURE

3.1.1 Chemicals/Reagents

Solvents used were of General Purpose Grade and were distilled twice before use. Silica gel used for column chromatography was of mesh size 60-120 µm (Sigma Aldrich) while that for thin Layer chromatography was 60 GF_{254} aluminium precoated plate. Sephadex (Sigma Aldrich) was used for gel filteration chromatography.

3.1.2 Equipments

Melting Point (m.p.) Apparatus: The melting point of the isolated compounds\was obtained using Gallenkamp melting point apparatus.

Nuclear Magnetic Resonance (NMR) Spectrophotometer: The NMR experiment was carried out on a Bruker AVANCE (500 and 125 MHz for ^{1}H and ^{13}C) spectrometer, using the residual solvent peaks as internal standard.

Infrared spectrophotometer: The IR experiment was carried out using using a Perkin Elmer System 2000 FT-IR.

Polarimeter: The measurement of optical rotation was carried out using a Jasco P-2000 polarimeter, using aperture diameter - 1.8 mm, path length – 2.0 mm, wavelength of light 589nm.

Liquid Chromatography-Mass Spectrometry (LC-MS): The LCMS experiment was

carried out on an Agilent Technologies 6890 Network LC System in combination with a Hewlett Packard 5973 Mass Selective Detector.

Flash Column Chromatography apparatus: *Biotage® Flash* 400 fitted with 'SNAP' proprietary plastic column, containing a removable plastic insert that used to dry load the column.rsity of Surrey, Guildford, UK.

These equipments were available at the Department of Chemistry, University of Surrey, Guildford, UK.

3.1.3 Collection, identification and preparation of plant materials.

The plant material was collected from Turunku village, Igabi Local Government Area Kaduna state, Nigeria, in the month of July, 2013. It was identified at the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, by comparing with an existing specimen voucher number (1466). The leaves were manually separated from the branches, air-dried and made into powder using cleaned mortar and pestle.

3.2 METHODS

3.2.1 Extraction of plant material

The powdered plant material (2.5 Kg) was subjected to cold maceration using two different solvents: dichloromethane (DCM) and methanol with occasional shaking for three days. The extract was drained and filtered using Whatman (No1) filter paper, concentrated using rotary evaporator and was allowed to dry *in-vacuo*. This yielded a dark green residue (73.5 g) referred to as Dichloromethane Extract (DCME). The residue obtained after extraction with DCM was further extracted by the same method using absolute methanol. This yielded a dark brown residue (70.2 g) refered to as Methanol Extract (ME).

3.2.2 Preliminary Phytochemical Screening

The DCM and methanol extracts were subjected to preliminary phytochemical screening using standard methods as described by Silver *et al.,* 1998; Sofowora, 1993 and Trease and Evans, 2002.

3.2.3 Chromatographic separation and purification

Both DCM and methanol extracts were subjected to Flash Column Chromatography (FCC). Sufficient silica gel (Sigma-Aldrich) was added to the DCM extracts. The mixture was dried and grinded into a fine powder. The powdered mixture was loaded into a 50g SAP cartridge sample cartridge and fitted to the FCC instrument, along with a 150g sample-mass- cartridge packed with silica gel (Sigma-Aldrich). Gradient elution was used such that the mobile phase progressed from 100% n-hexane to a 1:1 mixture of DCM and ethylacetate. A total of 150 fractions (45mls each) were collected into 100ml beakers. Fractions which displayed similar retention factor (Rf) values with the same solvent mixture from TLC analysis and similar ^{1}H NMR spectrum were combined for either immediate NMR analysis or for further purification techniques. Twenty-one major (pooled) fractions were obtained. The same method was carried out on the methanol extract to investigate the polar fraction of the leaf of *Ficus sycomorus*. For the methanol extract fifteen major fractions were obtained. A combination of column chromatography over both silica gel and Sephadex® **(**a size exclusive cross-linked dextran gel) and preparative TLC (prep-TLC) were used for purification of the pooled fractions of both the DCM and methanol extract.

The FCC fractions 18-21 of the DCM extract which showed two spots was subjected to silica gel column chromatography eluted with n-hexane : ethylacetate (9:1). Thirty one collections (2 mls each) were made and collections 12-14 gave single homogenous spot on TLC chromatogram using the same solvent system. Its purities were also confirmed using their proton NMR spectrum. The compound was coded A1

The FCC fractions 33-38 of the DCM extract was subjected to repeated gel filtration using sephadex LH-20 eluted with n-hexane : ethylacetate (8:2). Collections 6-8 of the third running which gave single homogenous spot on TLC chromatogram using the same solvent system and had the same proton NMR spectra were combined together and coded A2.

The FCC fractions 41-45 of the DCM extract which showed two spots on TLC chromatogram was subjected to silica gel column chromatography eluted with n-hexane : ethylacetate (9:1). Twenty-three fractions (2 mls each) were collected. fractions 13 and 14 both yielded single homogenous spot on TLC chromatogram (Plate 4.5) using the same solvent system and had the same proton NMR chromatogram were combined together and coded A3.

The FCC fractions 41-45 of the DCM extract was subjected to gel filtration using sephadex LH-20 eluted with dichloromethane : methanol (50:50). Thirty one fractions (2 mls each) were collected. Collection 20-21 both gave single homogenous spot on TLC chromatogram using the same solvent system and had the same proton NMR spectra. They were combined together and coded A4.

The FCC fraction 48 of the DCM was subjected to silica gel column chromatography eluted with n-hexane : ethylacetate (9:1). Thirty five collections (2 mls each) were made. Collections 3 and 4 gave single homogenous spot on TLC chromatogram using the same solvent system. Their purity and similarities were also confirmed using their proton NMR spectrum. They were combined and coded A5.

The FCC fractions 88-93 of DCM extract was subjected gel filtration using sephadex LH-20 eluted with DCM : methanol (50:50). Twenty fractions (2 mls each) were collected. Collection 10 and 11 both gave single homogenous spot on TLC chromatogram using the same solvent system and had the same proton NMR spectra. They were combined together and coded A6.

The FCC fractions 142-149 of the DCM extract was subjected to silica gel column chromatography eluted with dichloromethane : ethylacetate (7:3). Twenty-eighteen fractions (2 mls each) were collected. Collection 13 gave single homogenous spot on TLC chromatogram using the same solvent system. The purity of the fraction was also confirmed from its proton NMR spectrum. The compound was coded A7.

The FCC fraction 48 of the DCM extract whose TLC chromatogram showed a single spot but its proton NMR spectrum showed that it had traces of impurity peaks. The fraction was dissolved in DCM, filtered, dried and subjected to proton NMR experiment again. The spectrum showed that the compound was pure. It was coded A8.

For the methanol extracts, fractions 1-66 were found to contain mainly fatty acids or compounds already obtained from the DCM extract. Fractions 67 and above were subjected to purification technics as follows:

Fractions 70-79 of the methanol extract which showed two prominent spots was subjected to silica gel column chromatography eluted with ethylacetate (100%). Thirty fractions (2 mls each) were collected. Collection 9 gave single homogenous spot on TLC chromatogram using the same solvent system. Its purity was further confirmed from its proton NMR spectrum. The compound was coded A9. Collections 21 and 22 also showed single spot on TLC chromatogram and clean proton NMR spectrum. This compound was coded A10.

Fraction 67-68 was subjected to silica gel column chromatography eluted with ethylacetate (100%). fifteen fractions (2 mls each) were collected. Collection 5 yielded single homogenous spot on TLC chromatogram using the same solvent system. Its purity was also confirmed from its proton NMR spectrum. The compound was coded A11.

Fractions 79-84 of the methanol extract which from its TLC chromatogram and proton NMR spectrum showed traces of fatty acids impurities was subjected to repeated silica gel column chromatography eluted with DCM : methanol (25:1). Fraction 17 and 18 (out of a total of twenty-two collections, 2 mls each) of the third column yielded a single homogenous spot on TLC chromatogram using the same solvent system. They were combined and coded A12. The purity was also confirmed from its proton NMR spectrum.

Fractions 86-87 of the methanol extract which showed 5 spots was subjected to repeated gel filtration using sephadex LH20 and eluted with methanol. After running the fifth sephadex column, collections 14 and 15 (out of the total of twenty-two collections, 2 mls each) were combined together and subjected to preparative TLC. This gave a compound which yielded a single spot on TLC chromatogram and whose purity was confirmed from its proton NMR spectrum. It was coded A13.

Fractions 95-101 of the methanol extract which showed two distinct spots on TLC chromatogram impurities was subjected silica gel column chromatography eluted with ethylacetate : methanol (8:2). A total of forty-one collections (2 mls each) were made. Fractions 17 and 19 yielded a single homogenous spot on TLC chromatogram using the same solvent system. They were combined and coded A14. The purity of this compound was further confirmed using its proton NMR spectrum.

Fractions 104-105 of the DCM extract which showed two prominent spots. The pooled fraction was subjected to silica gel column chromatography eluted with ethylacetate (100%). Twenty-one (2 mls each) were collected. Collection 4-5 gave single homogenous spot on TLC chromatogram using the same solvent system. Its purity was also confirmed from its proton NMR spectrum. The compound was coded A15.

3.2.4 Thin Layer Chromatography (TLC): Thin layer chromatography was carried out on silica gel precoated TLC plate (0.25mm thin layer).

Technique: One way ascending

Spotting and development: Spots were applied manually using capillary tube; plates were dried using air blower and developed at room temperature using a Shandon chromatotank.

Detection*:* Spots on TLC plates were visualised using ultra-violet (UV) imaging (at 254 nm and 366 nm) andspraying with *p*-anisaldehyde solution and 10% sulphuric acid, followed by heating at 110˚C for 5-10 min.

3.2.5 Column Chromatography

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

(a) Technique - Isocratic elution

(b) Column - Glass column packed with sintered disc at the bottom.

(c) Stationary phase - Silica gel, 60 – 120 mesh size

(d) Column packing - Wet slurry method.

(e) Sample loading - The sample was applied using dry load method (Cannell, 1998), the sample was dissolved in small amount of suitable organic solvent, it was then mixed with a small quantity of silica gel, dried, triturated and then loaded on top of the column.

(f) Solvent for elution - Various solvent systems were used depending on the TLC profile of the fraction to be purified

3.2.6 Gel Filtration Chromatography

Gel filtration was performed using Sephadex LH-20 (Sigma)

Packing method*:* The sephadex was suspended in DCM and was then poured to the column and allowed to settle tightly.

Sample application*:* The sample was dissolved in small volume of DCM and applied to the top of the column.

Solvent used: DCM : Methanol (1:1).

3.3 ANTIOXIDANT ACTIVITY STUDIES

3.3.1 Quantitative studies

Antioxidant studies of the DCM and methanol extracts of the leaves of *Ficus sycomorus* was carried out using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) model (Braca *et al.,* 2001). 0.004% w/v DPPH solution was prepared in 95% methanol. To 1ml of various concentrations of the extracts

(20, 40, 60, 80, 100 µg/ml), 2ml of DPPH (0.1 mM) was added. An equal amount of methanol and DPPH served as control. The mixture was shaken vigorously and was left to stand in dark for 30 mins. The absorbance of the resulting solution was measured using UV spectrophotomer at 520 nm. The experiment was performed in triplicate and the percentage scavenging activity of each extract on DPPH radical was calculated using the formular:

$$\text{Scavenging activity (\%)} = \left(\frac{Ab - Ae}{Ae}\right) \times 100$$

Where; Ab=absorbance of blank sample

Ae=absorbance of the extract

DPPH radical scavenging activities of the extract were expressed as IC_{50} values which were calculated from the graph of scavenging activity plotted against sample concentrations using Microsoft excel software (Viturro *et al*., 1999).

3.3.2 Qualitative analysis:

The crude extracts were spotted on a TLC plate and developed using suitable solvent system in the chromatographic tank. The developed chromatogram was sprayed with 0.15 w/v DPPH solution in methanol solution using an atomizer. The plate was observed for the presence of yellow colour on a pinkish/purple background on the TLC plate which is an indicator for the presence of antioxidant substance (Saha *et al.,* 2008).

3.4 ANTICANCER STUDIES

Human melanoma cancer cells MDA-MB-435, human breast cancer cells MDA-MB-231, human ovarian cancer cells OVCAR3 and human colon cancer cells HT-29 were purchased from the American Type Culture Collection (Manassas, VA). The respective cell lines were propagated at 37°C in 5% CO_2 in RPMI 1640 medium, supplemented with fetal bovine serum (10%), penicillin (100 units/ml), and streptomycin (100 µg/ml). Cells in log phase growth were harvested by trypsinization followed by two washings to remove all traces of enzyme. A total of 5,000 cells were seeded per well of a 96-well clear, flat-bottom plate (Microtest 96®, Falcon) and incubated overnight (37°C in 5% CO_2). Samples dissolved in DMSO were then diluted and added to the appropriate wells (concentrations: 50 µg/mL, 25 µg/mL, 5 µg/mL, 1 µg/mL, 0.2 µg/mL; total volume: 100µL; DMSO: 0.5%). The cells were incubated in the presence of test substance for 72

hours at 37°C and evaluated for viability with a commercial absorbance assay (CellTiter 96®AQ_{ueous}One Solution Cell Proliferation Assay, Promega Corp, Madison, WI) that measured viable cells. IC_{50} values are expressed in µg/mL relative to the solvent (DMSO) control.

CHAPTER FOUR

4.0 RESULTS

4.1 Result of preliminary phytochemical screening

The result of phytochemical screening revealed that the DCM extract contains alkaloids, terpenoids and tannins while the methanol extract contains alkaloids, saponins, terpenoids, flavonoids, tannins, cardiac glycosides and anthraquinones (Table 4.1).

Table 4.1: Results of preliminary phytochemical analysis of DCM and methanol extracts of *Ficus sycomorus* leaves

CONSTITUENTS	TEST	OBSERVATION	DCME	ME
Alkaloids	Mayer's reagent	cream colour observed	+	+
	Dragendroff reagent	Reddish-brown ppt observed	+	+
Saponins	Frothing test	persistent froth	-	+
oumarins	UV light test (365 nm)	No fluorescence observed	-	-
Steroids/terpenoids	Liebermann-Burchard	Brown-red ring	+	+
	Salkwoski test		+	+
Tanins	Gold-Beater's test	brownish-green colour	-	+
Anthraquinones	Hydrochloride test	pink colouration	-	-
Cardiac glycosides	Keller-Kiliani's test	green-blue colouration	+	+
Flavonoids	Aluminum chloride test	yellow colouration	-	+
	Shinoda test	yellow colouration observed	-	+

Keys: + = positive, - = negative

DCME = Dichloromethane extract

ME = methanol extract

4.2 Thin Layer Chromatography

The result of TLC analysis of the DCM and methanol extracts are presented in Table 4.2 and 4.3 and Plates 4.1 and 4.2,

Table 4.2: TLC Profile of DCM extract using DCM (100%) as solvent system

spot	Rf value	Colour in *p*-anisaldehyde

1	0.11	black
2	0.37	black
3	0.42	dark green
4	0.48	purple
5	0.54	purple
6	0.83	orange
7	0.95	purple



Plate 4.1: Thin layer chromatogram of DCM extract using DCM (100%) as solvent system

Table 4.3: TLC result Profile of methanol extract using DCM : ethylacetate (8 :2)

spot	R_f value	colour in *p*-anisaldehyde
1	0.23	black
2	0.39	dark green
3	0.47	purple
4	0.63	light purple
5	0.83	light purple
6	0.96	orange
7.	0.99	black

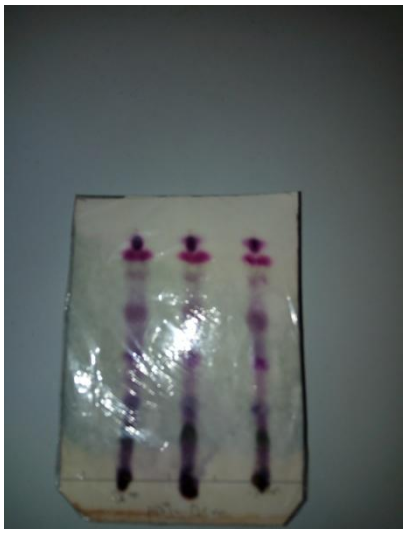


Plate 4.2: TLC chromatogram of methanol extract using DCM : ethylacetate (8 :2).

4.3 Isolation of A1

Compound A1 (15 mg) was isolated as a yellowish oil with an Rf value of 0.876 using n-hexane : ethylacetate (9:1) as solvent system (Plate 4.3).

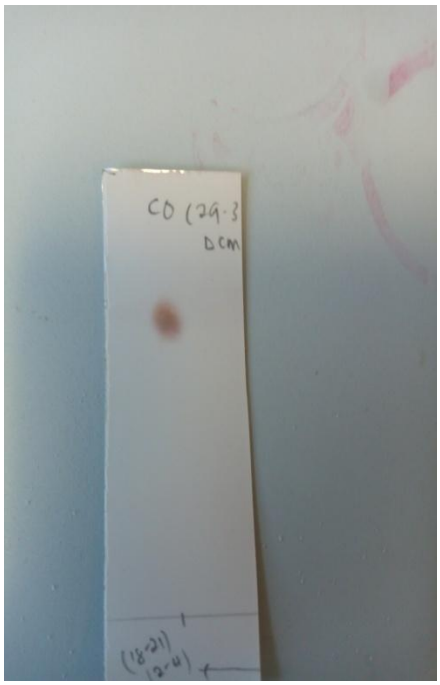


Plate 4.3: TLC chromatogram of A1 using n-hexane : ethylacetate 9:1 as solvent system

4.3.1 Physical tests carried out on A1

Solubility of A1

A1 was found to be soluble in chloroform.

4.3.2 SPECTRAL ANALYSIS OF COMPOUND A1

4.3.2.1 GCMS spectral analysis of A1

The GCMS spectra of A1 (Figure 4.1) showed molecular ion peak at 410, which corresponded to the molecular formula of $C_{30}H_{50}$.

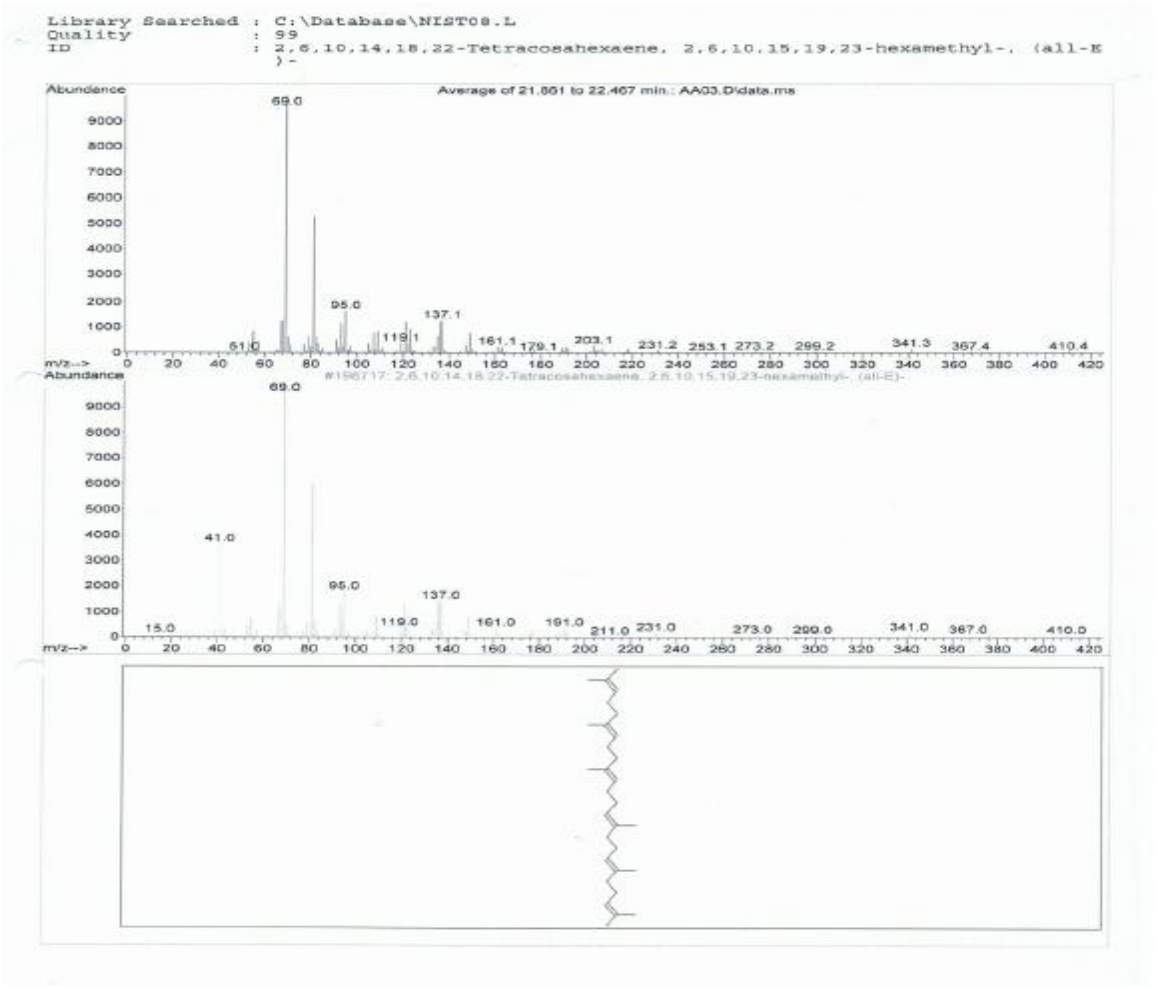


Figure 4.1: GCMS spectrum of A1

4.3.2.2 Proton Nuclear Magnetic Resonances (^{1}H NMR) spectral analysis of A1

The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of A1 (Figure 4.2) showed methyl groups resonances at δ_H 1.60 (s, 18H) and δ_H 1.68 (s, 6H), methylene groups resonances at δ_H 1.99- 2.03 (m, 20H), and internal olefinic signals at δ_H 5.06-5.15 (m, 6H).

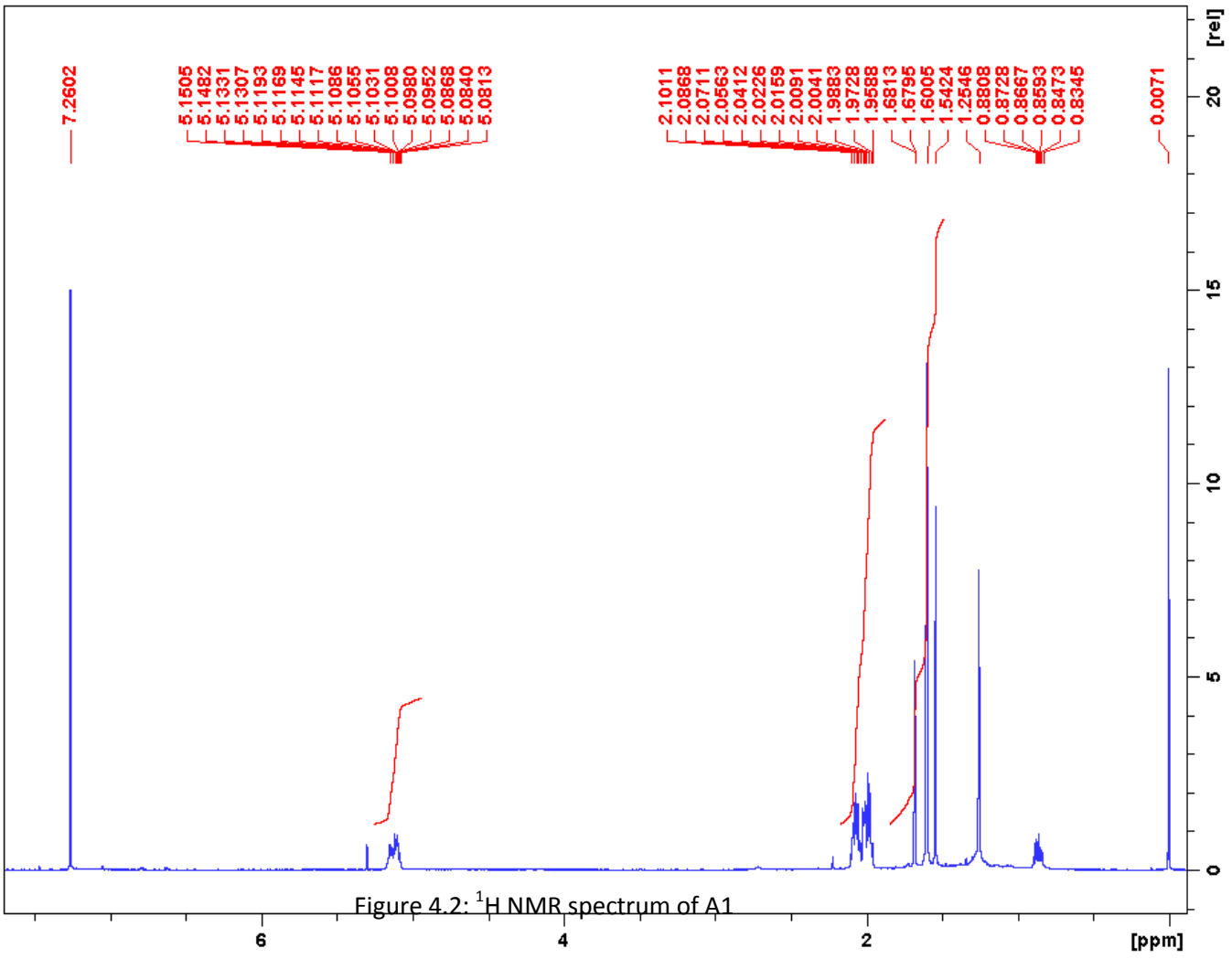


Figure 4.2: ^{1}H NMR spectrum of A1

4.3.2.3 ^{13}C Nuclear Magnetic resonance (^{13}C NMR) spectral analysis of A1

The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of A1 (Figure 4.3) showed signals at δc 25.70 (C-1/4), 131.2 (C-2/23), 124.2 (C-3/22), 39.8 (C-5/20), 134.8 (C-6/9), 124.4 (C-7/18), 26.6 (C-8/17), 135.0 (C-10/15), 124.3 (C-11/14), 28.3 (C-12/13), 17.6 (C-25/30), 16.0 (C-26/29), and 16.0 (C-27/28)

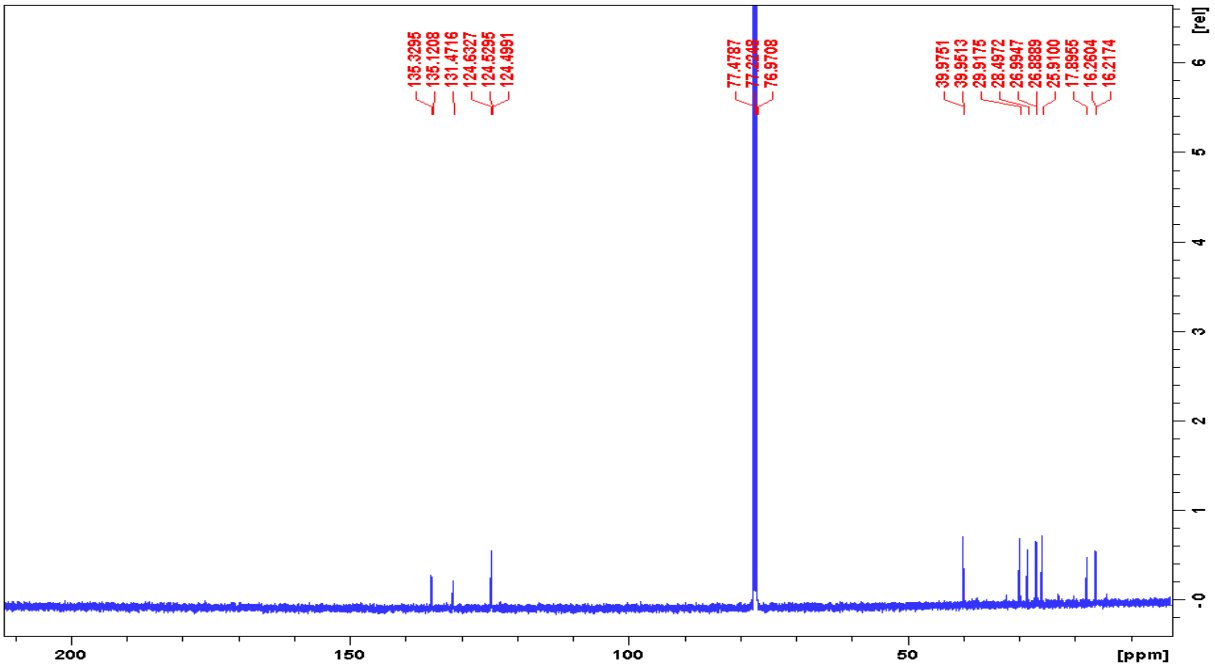


Figure 4.3: ^{13}C NMR spectrum of A1

4.3.2.4 Distortion lessEnhancement by Polarization Transfer (DEPT-135) spectral analysis of A1

The DEPT-135 spectrum of A1 (Figure 4.4) revealed eight methyl, ten methylene and six methine carbon resonances. Six quaternary carbons which disappeared in the spectrum were also noted.

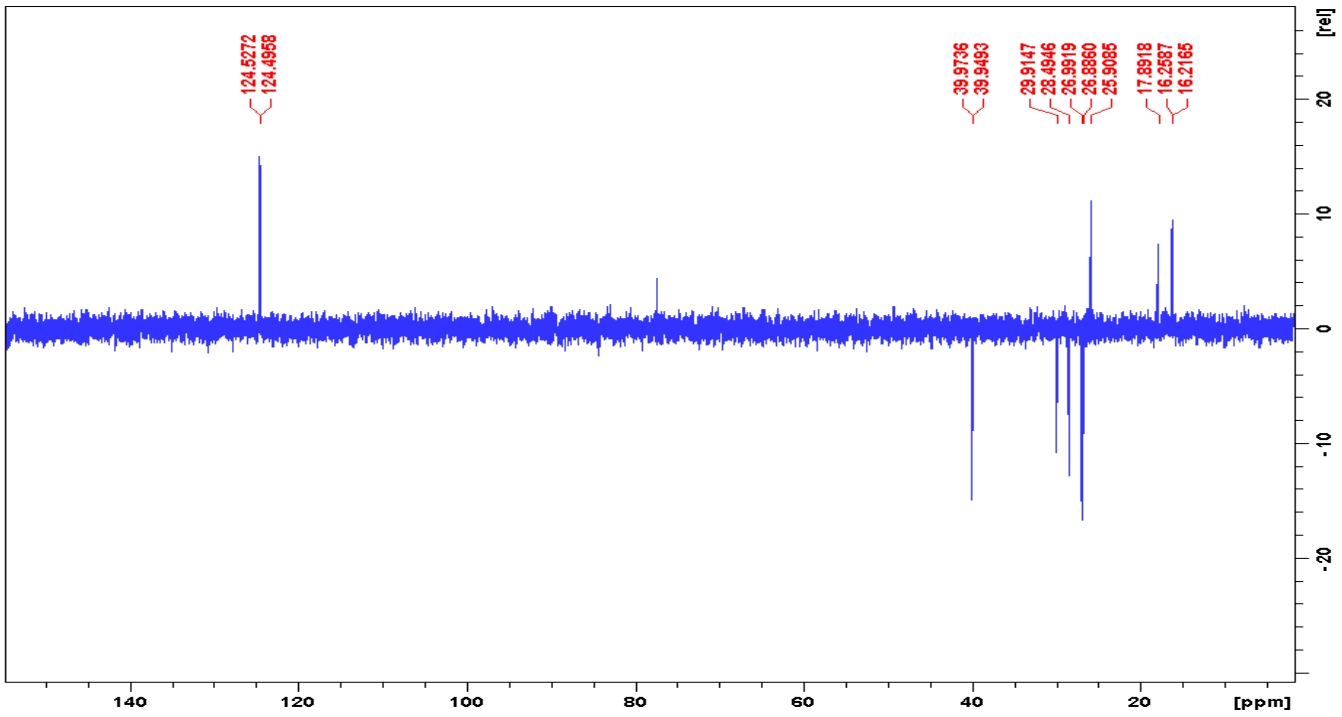


Figure 4.4 DEPT-135 spectrum of A1

4.3.2.5 Heteronuclear Single-Quantum Correlation (HSQC-DEPT) spectral analysis of A1

The HSQC-DEPT spectrum of A1 (Figure 4.5) was used to assigned each proton to its carbon atom.

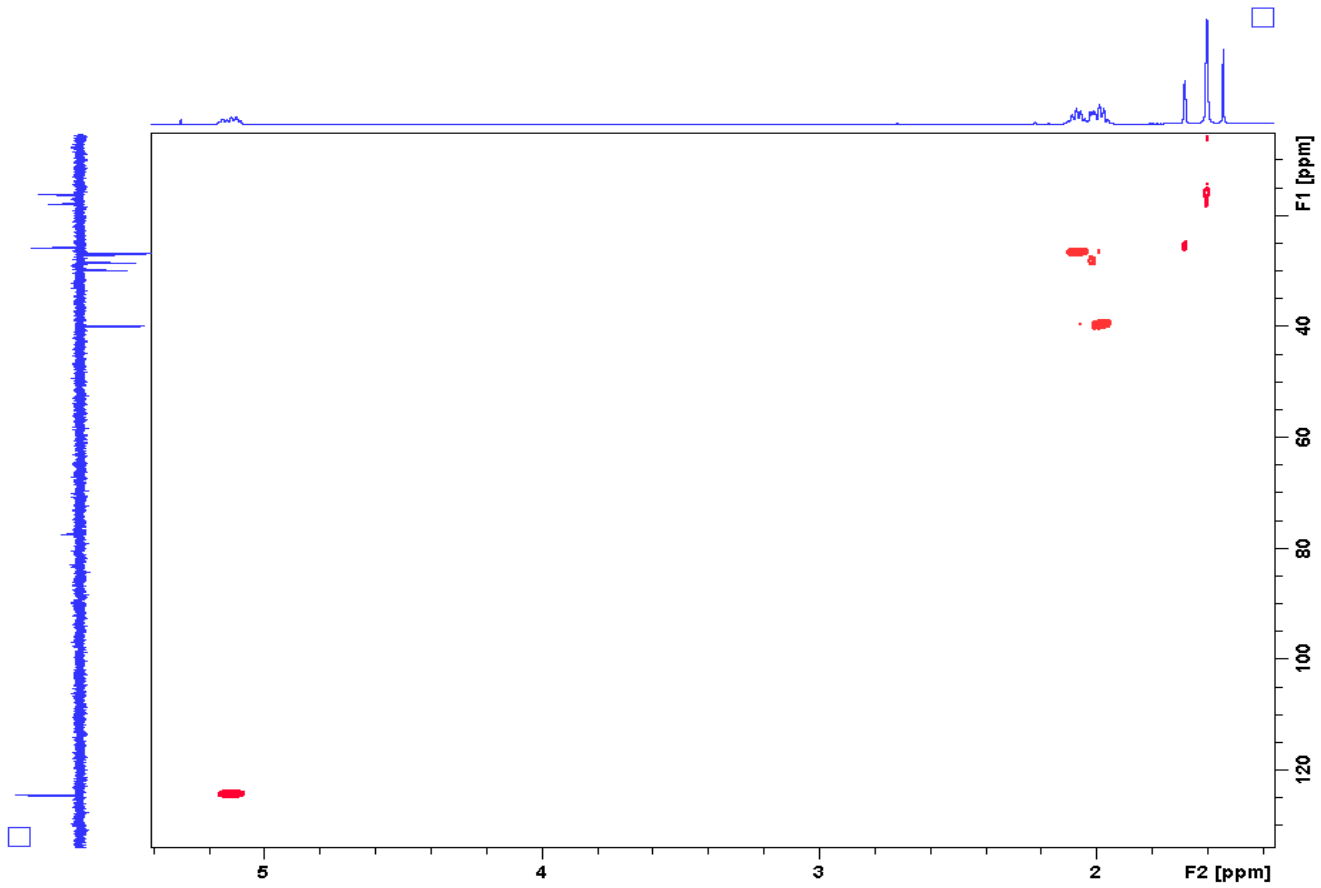


Figure 4.5: HSQC-DEPT spectrum of A1

4.3.2.6 Homonuclear COrrelation SpectroscopY (COSY) spectral analysis of A1

The COSY spectrum of A1 (Figure 4.6) showed cross peaks between proton resonance at δ_H 5.11 (H-11/14) and methylene proton signal at δ_H 2.01 (H-12/13). It also showed a long range correlation between methyl proton resonance at δ_H 1.68 (3H-1/24) as shown in Figure 4.6 below:

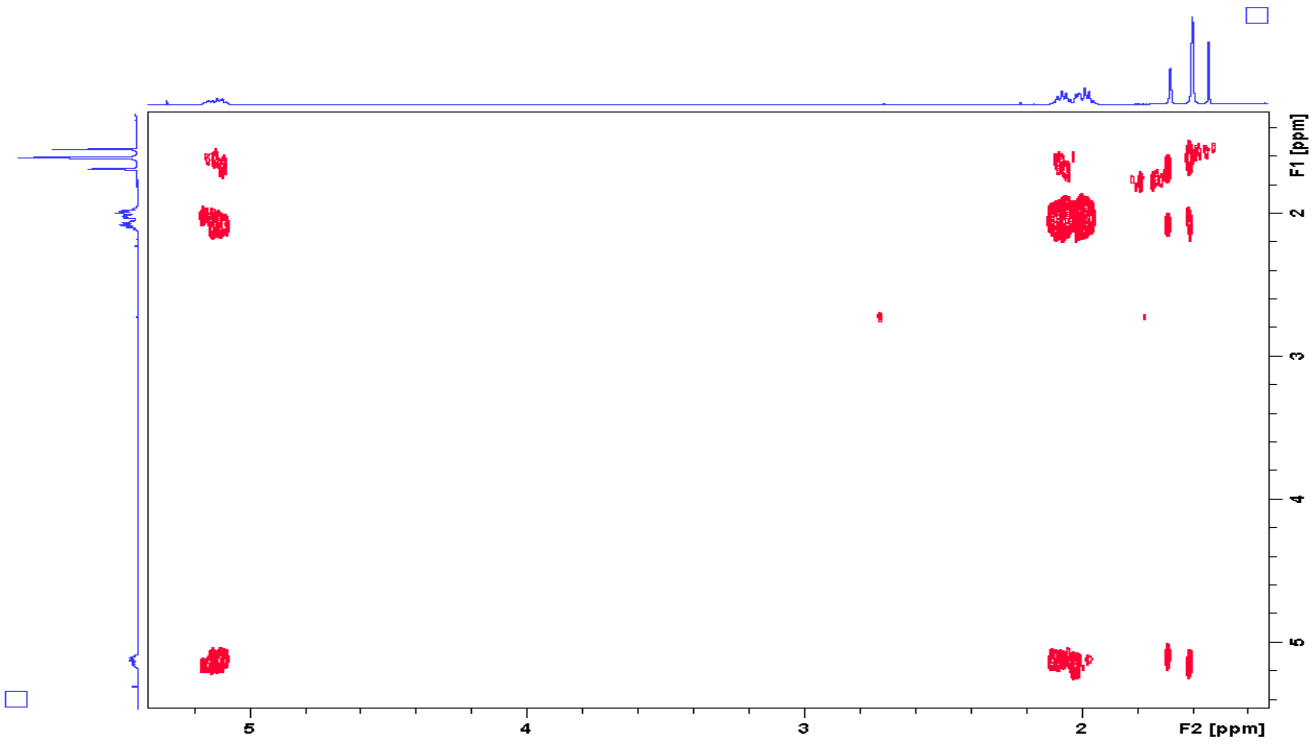


Figure 4.6 COSY spectrum of A1

4.3.2.7 Heteronuclear Multiple Bond Correlation (HMBC) spectral analysis of A1

The HMBC spectrum of A1 (Figure 4.7) showed correlation between two terminal methyl group protons signal at δ_H 1.60 (3H-26/29 and 3H-27/28) and carbon resonances at δ_c16.0 and 17.6 (C-25/30). These and other correlations observed were used to confirm the assignment of the protons and carbons positions in the proposed structure of A1.

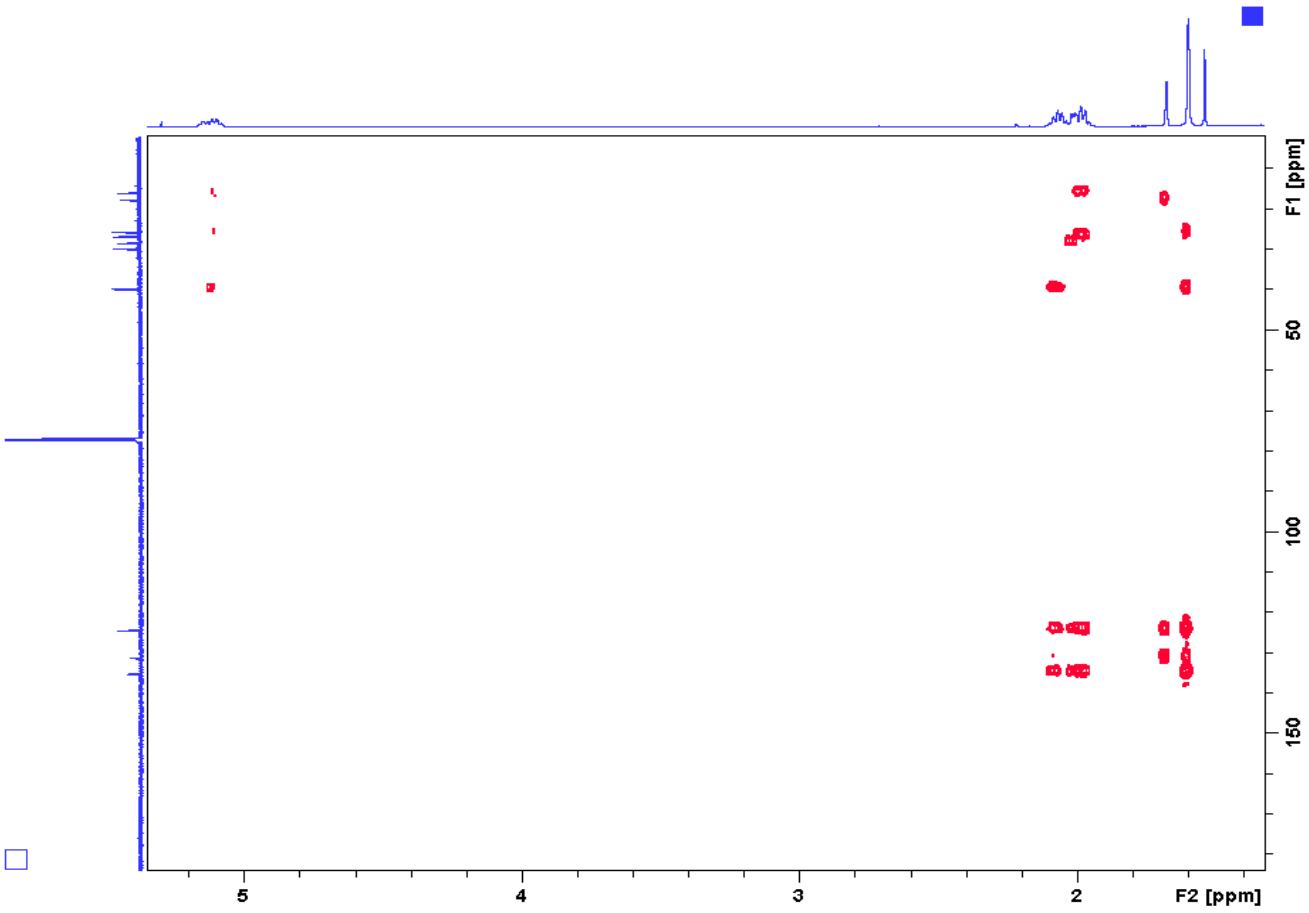


Figure 4.7: HMBC spectrum of A1

Table 4.4: NMR data of A1 compared with literature

No	**^{13}C NMR (400 MHz) $CDCl_3$**	**^{13}C NMR (500 MHz) $CDCl_3$**(Consolacion *et al.,* 2015)	**^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz)**	**^{1}H NMR Lit (400 MHz) $CDCl_3$** (Consolacion *et al.,* 2015)
1/24	25.7 CH_3	25.7	1.68 s*	-
2/23	131.2 C	131.2	-	

3/22	124.2 CH	124.4	5.11 m*	5.07-5.13*
4/21	26.8 CH_2	26.7	2.07 m*	2.08
5/20	39.8 CH_2	39.7	1.98 m*	1.94
6/19	134.8 C	134.9	-	
7/18	124.4 CH	124.3 \	5.11 m*	5.07-5.13
8/17	26.6 CH_2	26.6	2.07 m*	2.08
9/16	39.7 CH_2	40.0	1.98 m*	1.94
10/15	135.0 C	135.1	-	
11/14	124.3 CH	124.2	5.11 m*	5.07-1.13
12/13	28.3 CH_2	28.2	2.01 dd *J* (3.3, 6.8)	2.08
25/30	17.6 CH_3	17.6	1.60 s*	1.58
26/29	16.0 CH_3	16.0	1.60 s*	1.58
27/28	16.0 CH_3	16.0	1.60 s*	1.58

* Refers to overlapping resonance

4.4 Isolation of A2

Compound A2 (4.6 mg) was isolated as yellowish oil. It had an Rf = 0.47 using n-hexane : ethylacetate (8:2) as solvent system (Plate 4.4).



Plate 4.4: TLC Chromatogram of A2 using n-hexane : ethylacetate (8:2) as solvent system

4.4.1. Physical tests carried out on A2

Solubility of A2

A2 was found to be soluble in chloroform.

4.4.2 SPECTRAL ANALYSIS OF A2

4.4.2.1 ^{1}H NMR spectral analysis of A2

^{1}H NMR spectrum (500 MHz, $CDCl_3$) of A2 (Figure 4.8) showed methyl group proton resonances at δ_H 1.25 (s, 6H), δ_H 1.61 (s, 6H), and δ_H 1.740 (d, *J* = 1.25 Hz, 6H) overlapping methylene groups resonances at δ_H 1.97- 2.09 (m, 20H), and olefinic proton signals at δ_H 5.11-5.13 (m, 5H), carbinol and another upfield olefinic proton signal at δ_H 5.44 (ddd, *J* = 1.4 Hz, 7.2 Hz, H-7) and δ_H 4.09 (d, *J* = 5.3 Hz, 2H-26).

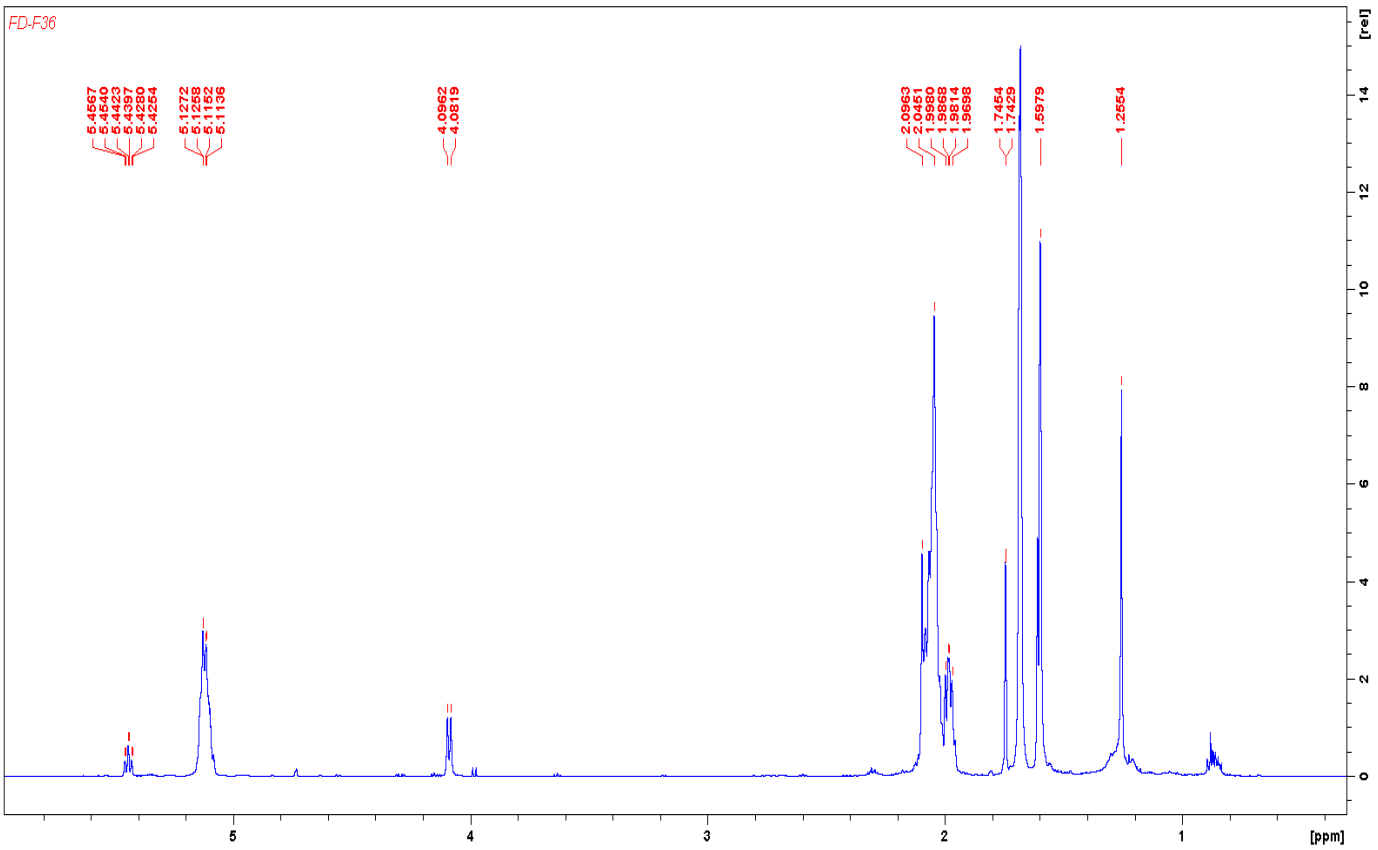


Figure 4.8: ^{1}H NMR spectrum of A2

4.4.2.2 ^{13}C NMR spectral analysis of A2

The ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of A2 (Figure 4.9) showed carbon resonances δc 25.9 (C-1/4), 131.4 (C-2/23), 124.3 (C-3/22), 26.7 (C-4), 39.9 (C-5/20), 135.2 (C-6), 124.5 (C-7), 26.9 (C-8/17), 39.91 (C-9), 135.1 (C-10/15), 124.4 (C-11/14), 27.3 (C-12/13), 17.6 (C-25/30), 59.2 (C-26), 16.1 (C-27/28) and 26.0 (C-29)

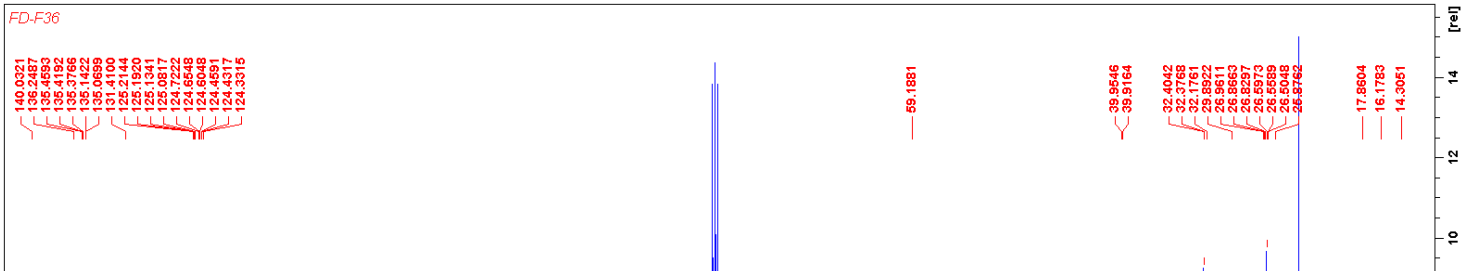


Figure 4.9: ^{13}C NMR spectrum of A2

4.4.2.3 DEPT-135 spectral analysis of A2

From the DEPT-135 spectrum of A2 (Fig 4.10) revealed seven methyl, eleven methylene and six methine carbon resonances. Six quaternary carbons which disappeared in the spectrum and other additional peaks were also noted.

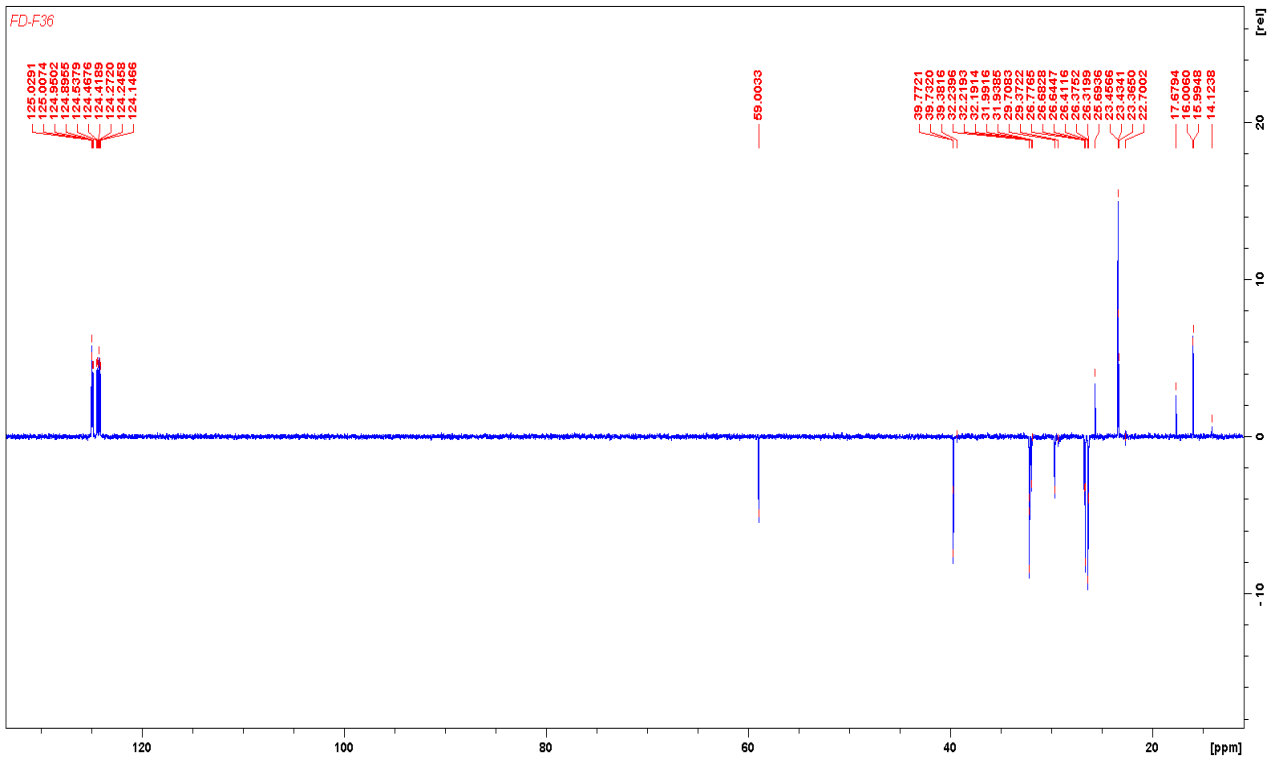


Figure 4.10: ^{13}C NMR spectrum of A3

4.4.2.4 HSQC-DEPT spectral analysis of A2

The HSQC-DEPT spectrum of A2 (Figure 4.11) was used to assigned each proton to its carbon atom.

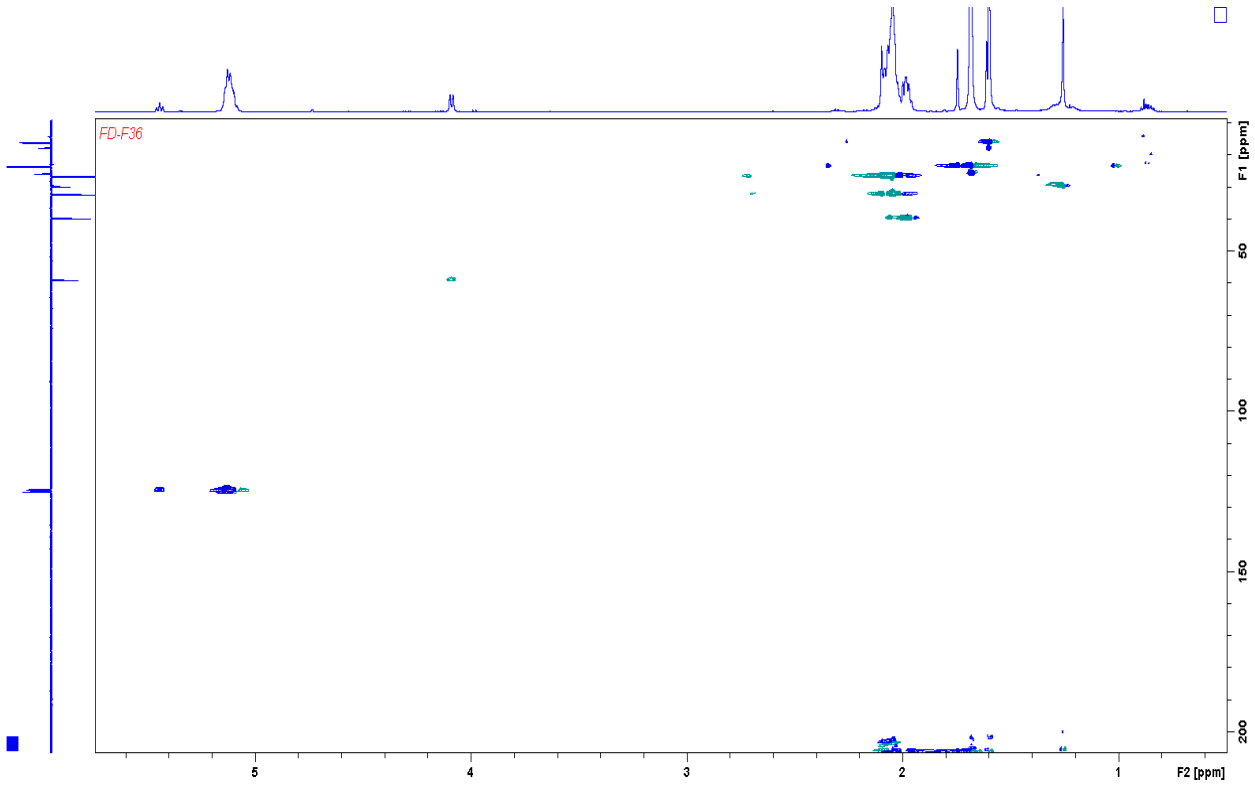


Figure 4.11: HSQC-DEPT spectrum of A2

4.4.2.5 COSY spectral analysis of A2

The COSY spectrum of A2 (Figure 4.12) showed important long range correlations between proton resonanceat δ_H 4.01 and the methine proton at δ_H 5.45. It also showed correlations between proton resonance at δ_H (H-4) and δ_H (H-5); δ_H (H-20) and δ_H (H-21) and between δ_H (H-13) and δ_H (H-14).

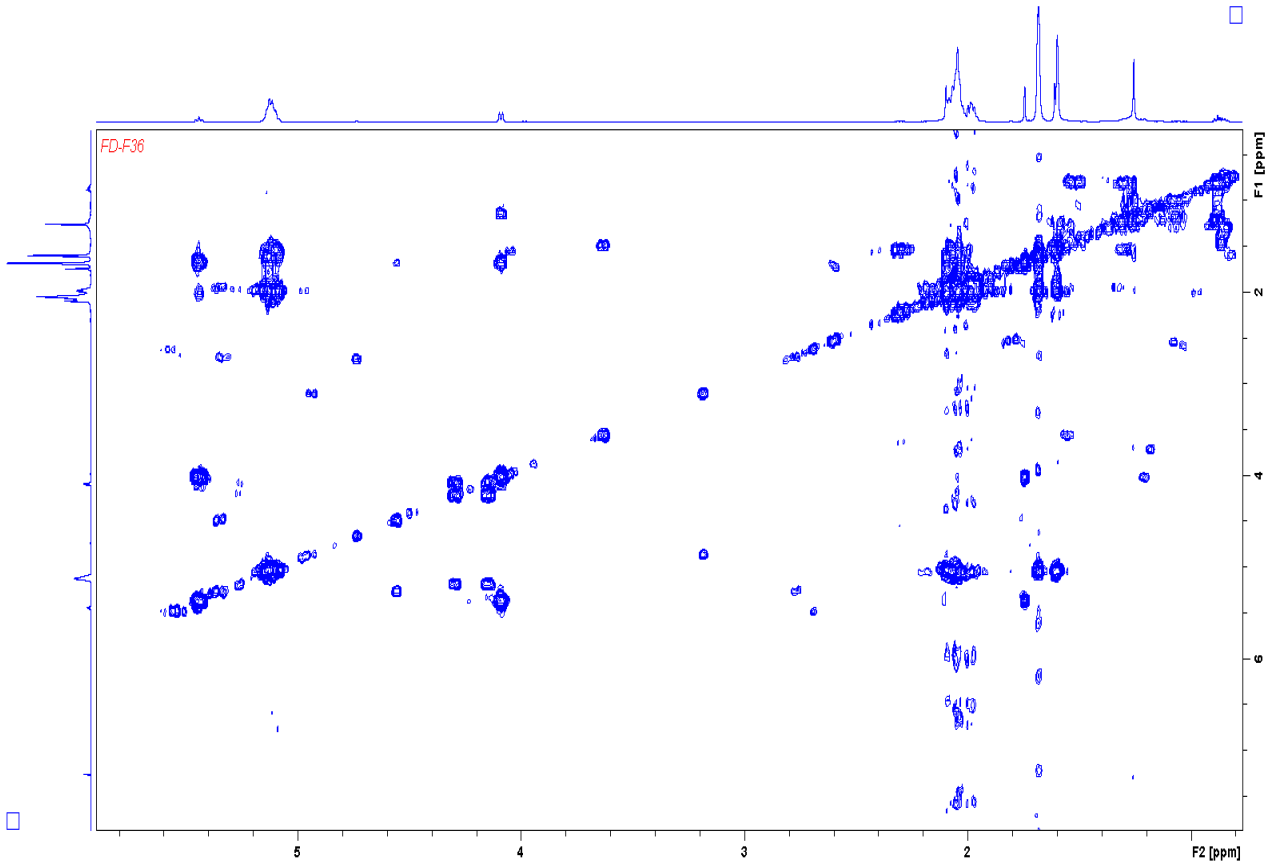


Figure 4.12: COSY spectrum of A2

4.4.2.6 HMBC spectral analysis of A2

The HMBC spectrum of A2 is shown in Figure 4.13 below it was used to confirm the connectivity network in the structure of the molecule

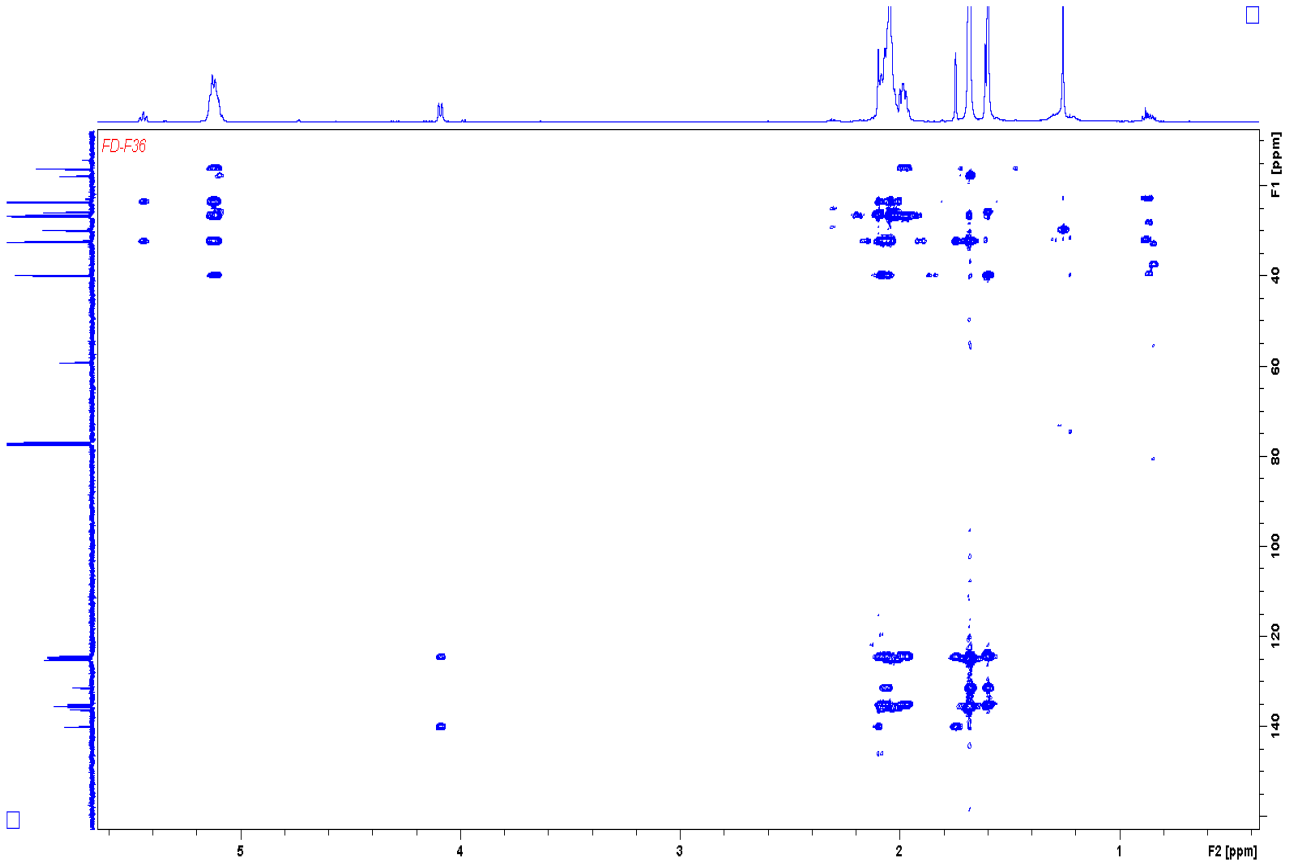


Figure 4.13: HMBC spectrum of A2

Table 4.5: NMR spectral data of A2

No	^{13}C NMR (400 MHz) $CDCl_3$		^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz)	HMBC (H→C)	COSY (H → H)
1/24	25.9	CH_3	1.68 s*		
2/23	131.4	C	-		
3/22	124.3	CH	5.12 m*		

4	26.7	CH_2	2.07 m		5
5/20	39.9	CH_2	2.00 m*		4
6/19	134.8	C	-		
7	135.2	CH	5.44 m	5,6,26	26
8/17	26.9	CH_2	2.07 m*		29
9	39.9	CH_2	2.00 m*		
10/15	135.1	C	-		
11/14	124.4	CH	5.12 m*		
12/13	27.3	CH_2	2.01 dd, (3.3, 6.8)		
25/30	17.6	CH_3	1.70 s		
26	59.2	CH_3	4.09 d, (5.3)	5,6,7	7
27/28	16.0	CH_3	1.68 s*		
29	26.0	CH_3	1.68*	5,7	18

*Refers to overlapped proton resonances

4.5 Isolation of A3

Compound A3 was isolated as white solid with Rf = 0.43 using DCM (100%) as solvent system (Plate 4.5).



Plate 4.5: TLC chromatogram of A3 using DCM (100%) as solvent system

4.5.1. Physical tests carried out on A3

Solubility of A3

A3 was found to be soluble in chloroform.

Melting point of A3

The compound was found to have a melting point range of 210 - 212^{0}C.

4.5.2 SPECTRAL ANALYSYSIS OF A3

4.5.2.1 LCMS spectrum of A3

The LCMS spectrum of A3 (Figure 4.14) showed molecular ion M^+ at *m/z* 426.

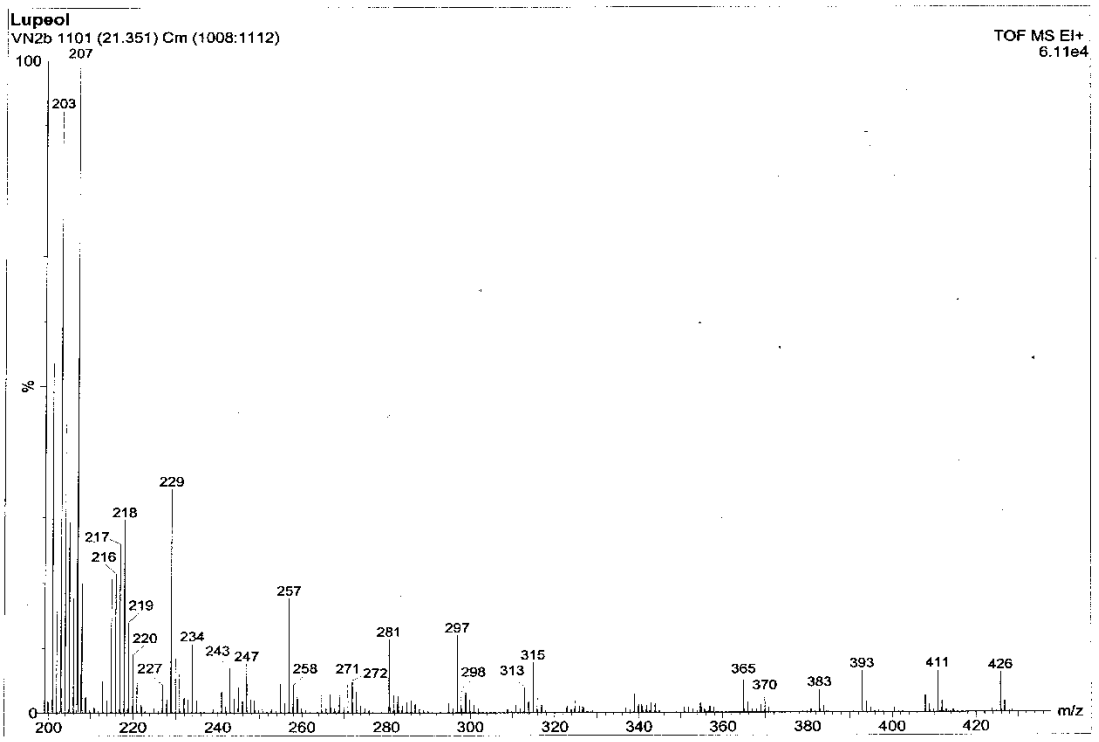
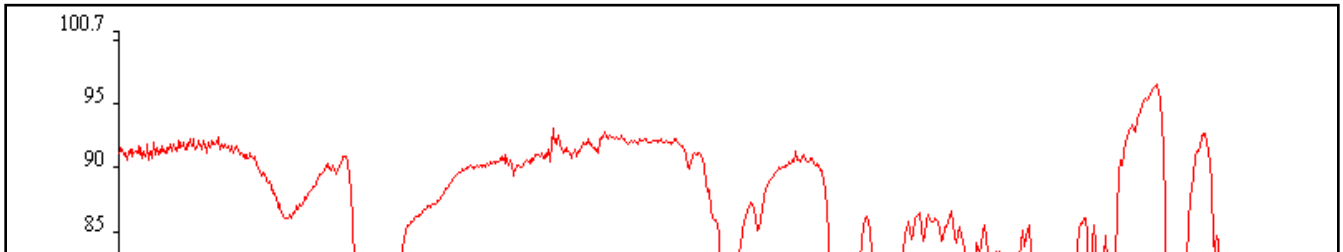


Figure 4.14: LCMS spectrum of A3

4.5.2.2 IR spectral analysis of A3

The IR spectrum of A3 (Figure 4.15) showed absorption band at 33855 cm^{1-} (O-H stretch) and 2922 cm^{1-} (CH_3 stretching and bending vibrations)



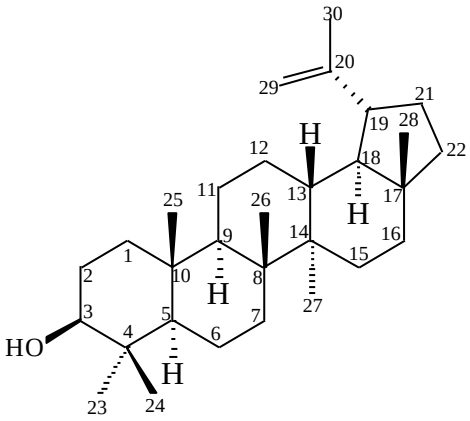
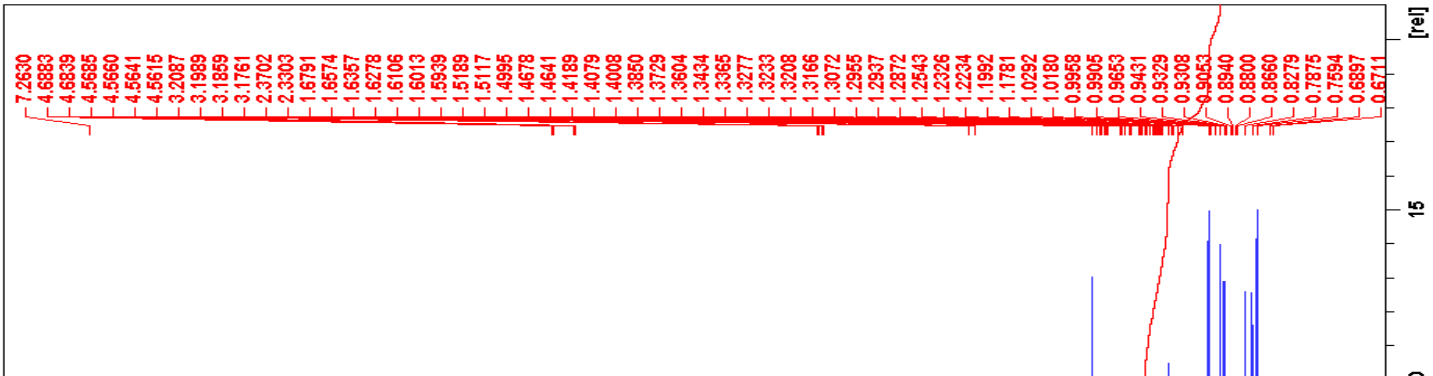


Figure 4.15: IR spectrum of A3

4.5.2.3 ^{1}H NMR spectral analysis of A3

The ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of A3 (Figure 4.16) showed proton resonances at δ_H 3.18 (dd, *J* = 4.85, 11.35 Hz H-3), 4.56 & 4.68 (*s*, H-29A and H-29B), 3.16 (dd, *J* = 4.76, 11.00 Hz, H-3), and methyl group proton resonances at δ_H 0.75, 0.78, 0.82, 0.93, 0.95, 1.02, 1.25 (each *s*,).



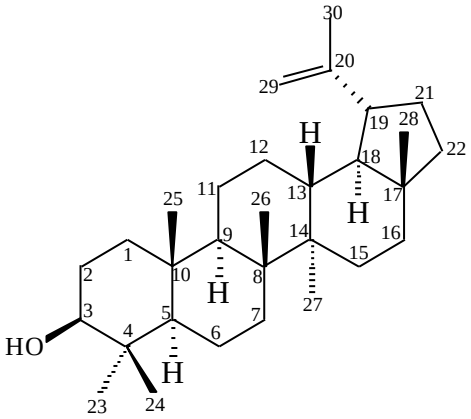


Figure 4.16:[1]HNMR spectrum of A3

4.5.2.4 [13]C NMR spectral analysis of A3

The[13]C NMR ($CDCl_3$, 100 MHz) spectrum (Figure 4.17) revealed thirty carbon signals. These include carbon resonances at δc 151.1 (C-20), 109.5 (C-29), 79.1 (C-3), 55.5 (C-5), 50.6 (C-9), 48.5 (C-18), 48.2 (C-19), 43.2 (C-17), 43.0 (C-14), 41.0 (C-8), 40.2 (C-22), 39.0 (C-13), 38.9 (C-4), 38.2 (C-1), 37.3 (C-10), 35.8 (C-16), 34.5 (C-7), 30.0 (C-21), 28.2 (C-23), 27.6 (C-15), 27.5 (C-12), 25.3 (C-2), 21.1 (C-11), 19.5 (C-30), 18.5 (C-6), 18.2 (C-28), 16.3 (C-25), 16.2 (C-26), 15.6 (C-24) and 14.7 (C-27).

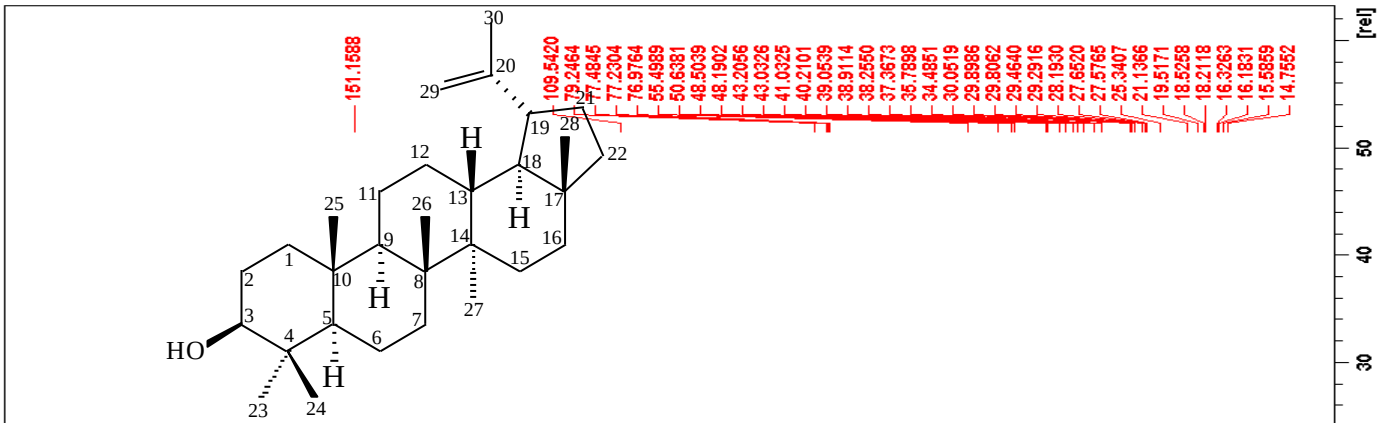


Figure 4.17: ^{13}C NMR spectrum of A3

4.5.2.5 DEPT-135 spectral analysis of A3

The DEPT-135 spectrum of A3 (Figure 4.18) showed eight methyl, eleven methylene and four methine carbon resonances. The remaining seven quaternary carbon resonances which did not appear in the spectrum were also noted.

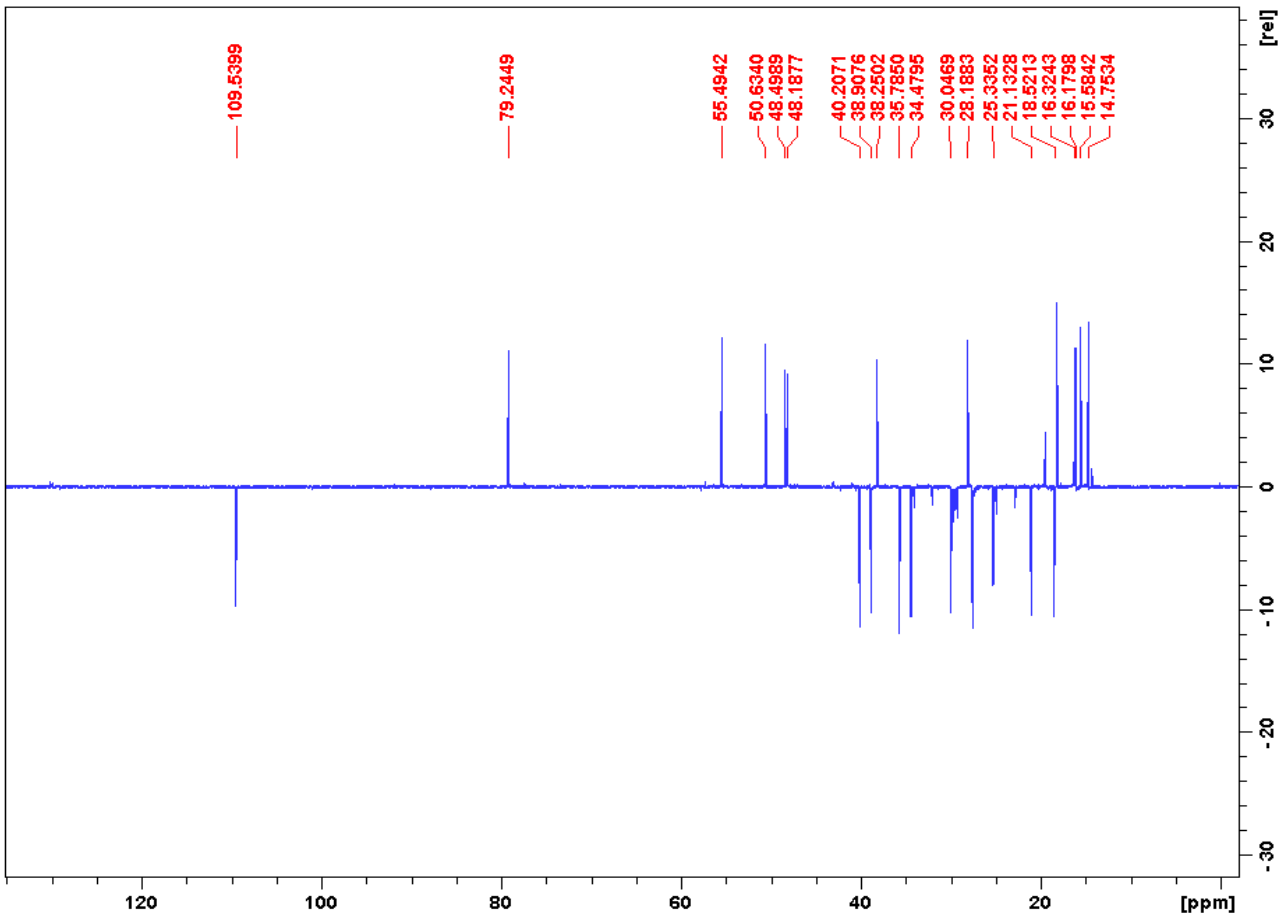


Figure 4.18: DEPT-135 spectrum of A3

4.5.2.6 HSQC-DEPT spectral analysis of A3

The HSQC-DEPT spectrum of A3 (Figure 4.19) was used to assigned protons to their respective carbons atoms.

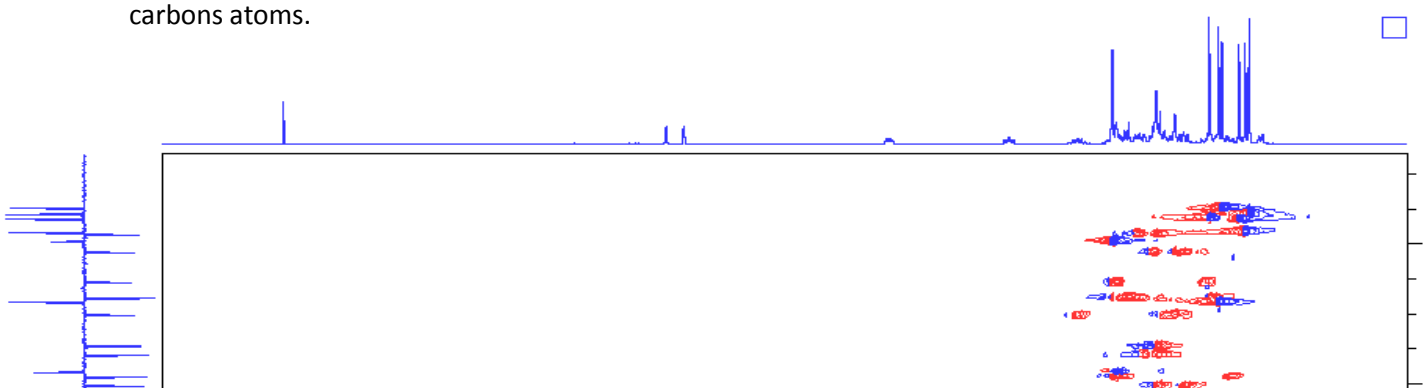


Figure 4.19: HSQC-DEPT spectrum of A3

4.5.2.7 COSY spectral analysis of A3

The COSY of A3 (Figure 4.20) showed cross peaks between methine proton signals at δ_H 0.69 and two methylene proton signals δ_H 1.51 and δ_H 1.39. Another important correlation observed was that between the methine proton signal at δ_H 1.27 and methylene proton signals at δ_H 1.41 and δ_H 1.21 etc

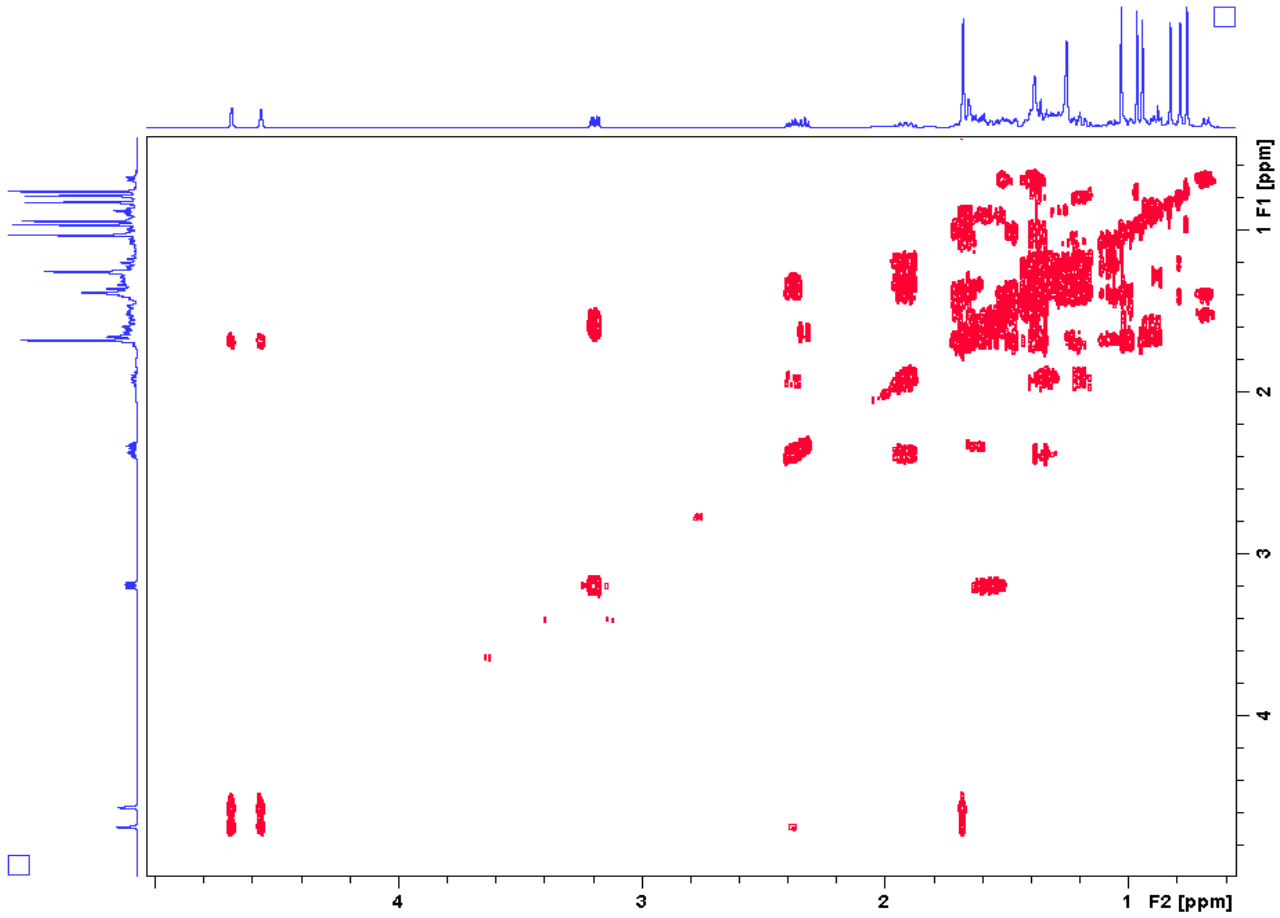


Figure 4.20: COSY spectrum of A3

4.5.2.8 HMBC spectral analysis of A3

The HMBC spectrum (Figure 4.21) showed correlations between proton resonance at δ_H 3.18 (H-3) and carbon δc 26.7 (C-2), 28.2 (C-23), and 15.6 (C-24). Other correlations observed were those between δ_H 4.56 (H-29) and δc 151.2 (C-20), 19.5 (C-30), and δc 48.2 (C-19); between δ_H

0.79 (3H-28) and δc 43.2 (C-17), 35.8 (C-16) and 40.2 (C-22

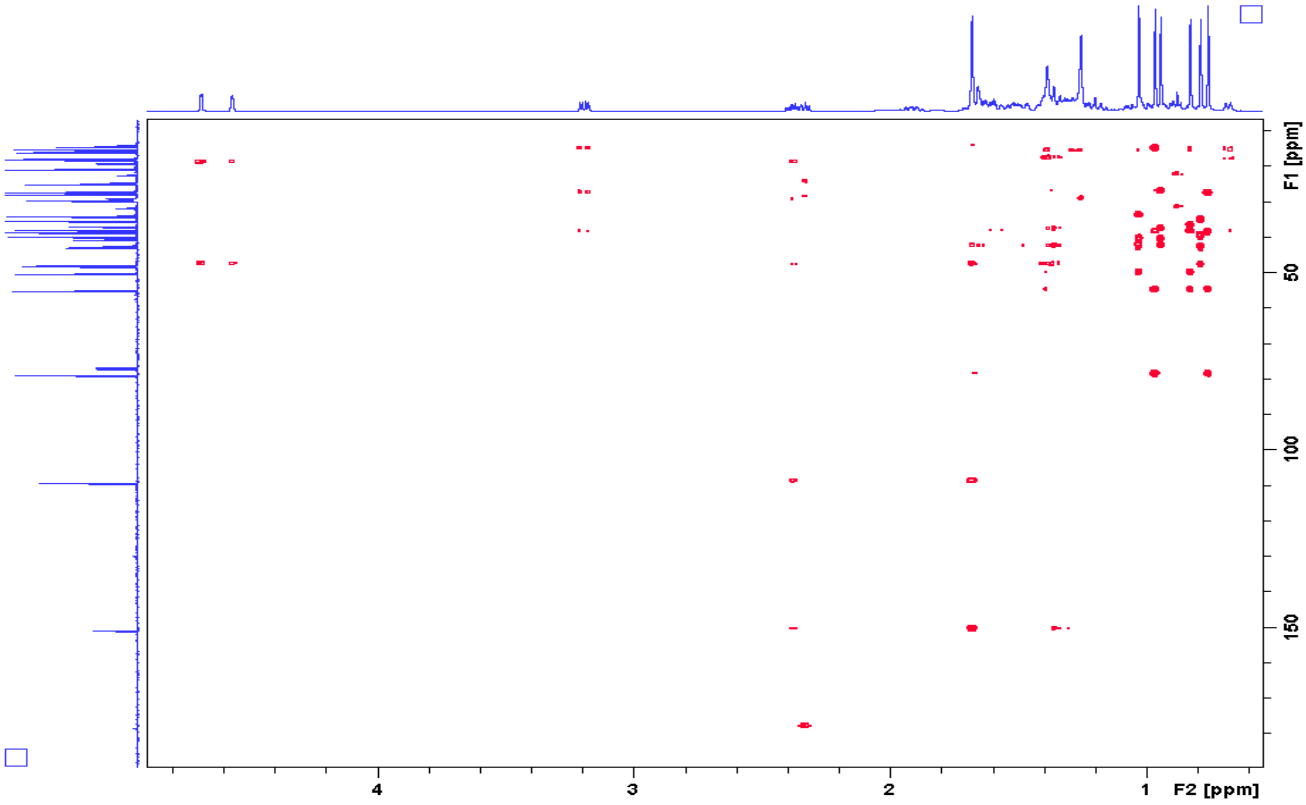


Figure 4.21: HMBC spectrum of A3

Table 4.6: NMR spectral data of A3 compared with literature

No.	**^{13}C NMR (500 MHz) $CDCl_3$**	**^{13}C NMR** (Mi-Young *et al.*, 2010)**(400 MHz) $CDCl_3$**	**^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz)**	**^{1}H NMR** (Mi-Young *et al.*, 2010) **(400 MHz) $CDCl_3$) (500 MHz) $CDCl_3$ (*J* in Hz)**	**HMBC (H → C)**	COSY **(H → H)**
1α	3.89 CH_2	38.7	1.65 *m*	1.62	5, 10	
1β			0.91 *m*	0.95	2,3,5, 10	
2 α	27.6 CH_2	27.9	1.60 *m*	1.70		
2β			1.56 *m*	1.50		
3	79.2 CH	79.0	3.18 *dd* J= 11.35, 4.85	3.16 *dd* J= 11.2, 5.2 Hz	2,23,24	

4	38.9	C	38.9		-		
5	55.5	CH	55.3	0.69 dd *J*= 11.35, 4.85	0.65		
6 α	18.5	CH_2	18.4	1.51 *m*	1.48	5,7	5
6β				1.39*	1.33	5,7	5
7 α	34.5	CH_2	34.3	1.38*	1.36		
7β				1.38*	1.36		
8	40.1	C	40.8		-		
9	50.6	CH	50.4	1.27 *m*	1.23	8,10,11,26	11 α, 11 β
10	37.4	C	37.2		-		
11 α	21.2	CH_2	21.0	1.41 *m*	1.33		9
11β				1.21 *m*	1.33		9
12 α	25.4	CH_2	25.2	1.06 *m*	1.06		
12β				1.67*	1.54		
13	38.3	CH	38.1	1.64*	1.59	128	18
14	43.1	C	42.8		-		
15 α	27.6	CH_2	27.5	1.00 *m*	1.01		
15β				1.67*	1.49		
16 α	35.8	CH_2	36.5	1.38*	1.36		
16β				1.47 d	1.44		
17	43.2	C	43.0		-		
18	48.5	CH	48.3	1.35*	1.35		13,19
19	48.2	CH	48.0	2.38	2.25		18, 21α
20	151.2	C	150.8		-		
21 α	29.9	CH_2	29.9	1.32*	1.23		
21β				1.92 *m*	1.84		
22 α	40.2	CH_2	40.0	1.19 *m*	1.18		3,9,5
22β				1.38*	1.36		
23	28.2	CH_3	28.0	0.97 *s*	0.94		
24	15.6	CH_3	14.4	0.77 *s*	0.73		
25	16.3	CH_3	16.2	0.83 *s*	0.80	1,5,9,10	
26	16.2	CH_3	16.0	1.02 *s*	1.00	8,9,14	
27	14.7	CH_3	14.6	0.98 *s*	0.92	14,15	
28	18.2	CH_3	18.0	0.79 *s*	0.76	22,17	
29A	109.5	CH_2	109.5	4.56 *br s*	4.54		
29B				4.68 *br s*	4.66		
30	19.5	CH_3	19.4	1.69 *br s*	1.65	20,29	

*Overlapped proton resonances

4.6 Isolation of A4

Compound A4 (6 mg) was isolated as a white powder, Rf = 0.52 using hexane : ethylacete (9:1) as solvent system (Plate 4.6).

.



Plate 4.6: TLC chromatogram of A4 using n-hexane : ethylacete (9:1) as solvent system

4.6.1. Physical tests carried out on A4

Solubility of A4

A4 was found to be soluble in chloroform.

Melting point of A4

The compound was found to have a melting point range of 216 - 217^{o}C.

4.6.2 SPECTRAL ANALYSIS OF A4

4.6.2.1 LCMS spectral analysis of A4

The mass spectrum of A4 (Figure 4.22) showed molecular ion peak $[M+H]^+$ at *m/z* 469 and M^+ at *m/z* 468.5

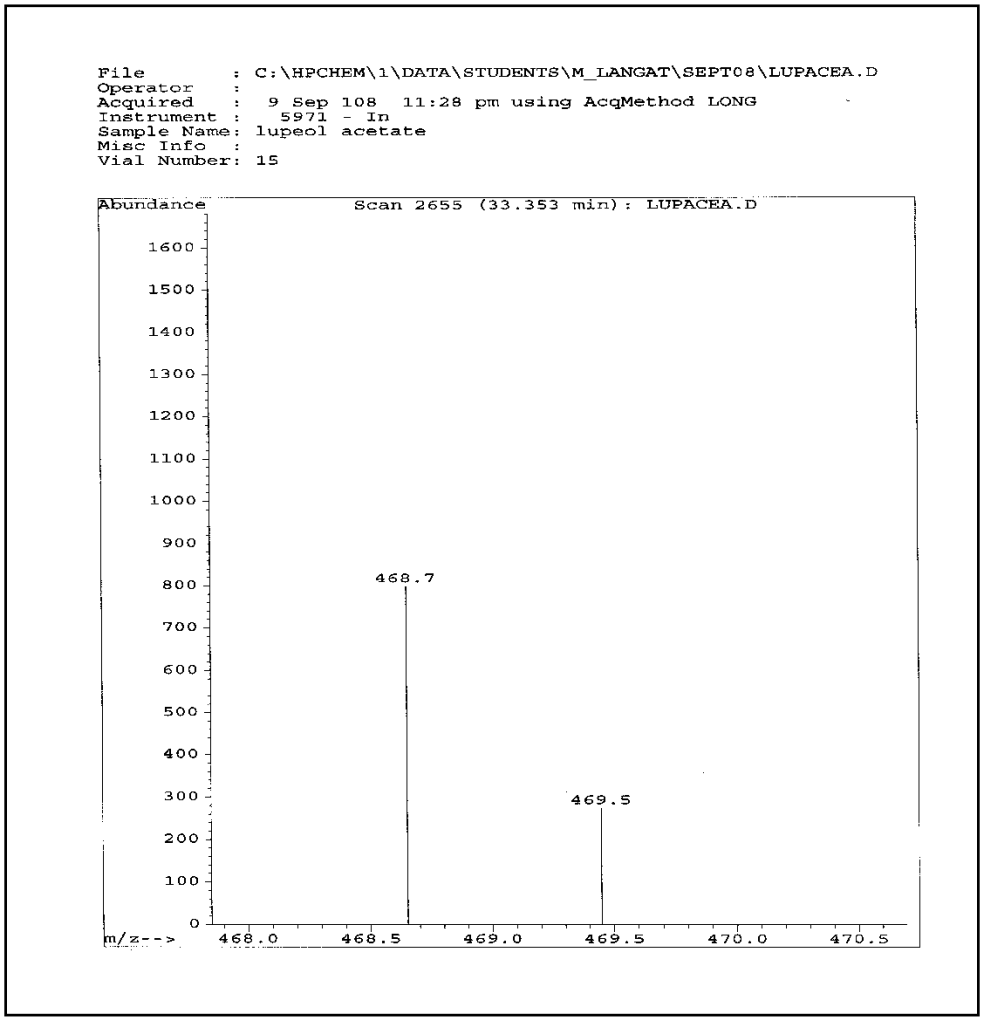


Figure 4.22: LCMS spectrum of A4

4.6.2.2 IR spectral analysis of A4

The IR spectrum of A4 (Figure 4.23) showed absorption peaks at1732.2 cm^{-1} (C=O stretching) 3070 cm^{-1} (exomethylene group), 2943 cm^{-1} (C-H stretching), 2416cm^{-1} (C=C), 1246cm^{-1} (C-O) and bending at 792cm^{-1}

.

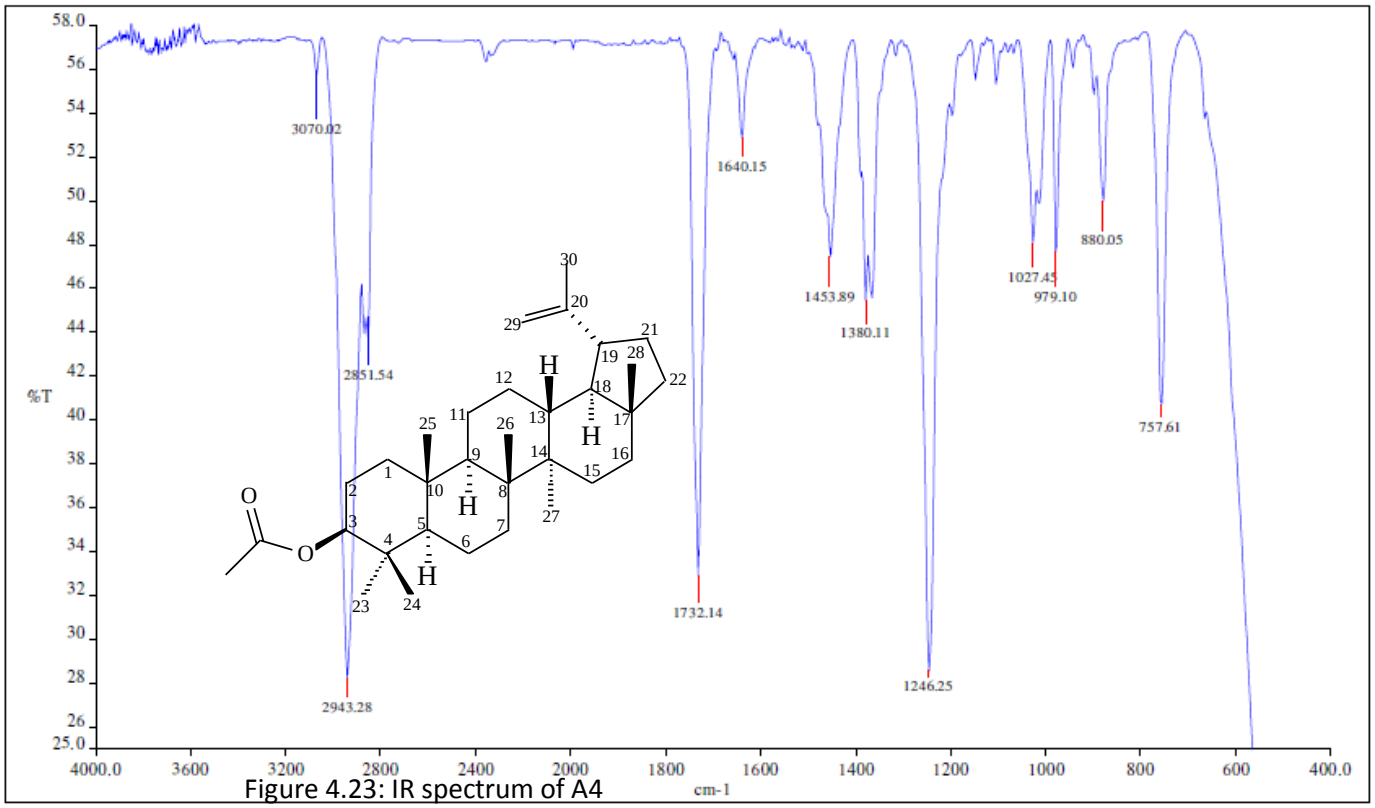
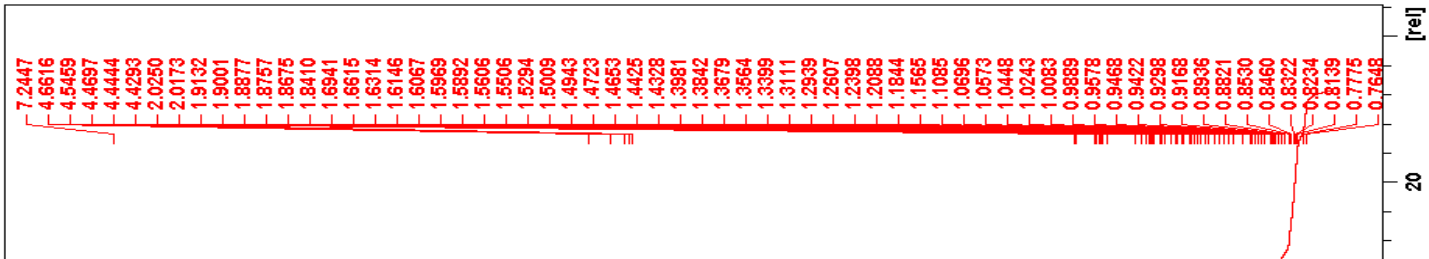


Figure 4.23: IR spectrum of A4

4.6.2.3 ^{1}HNMR spectral analysis of A4

The ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of A4 (Figure 4.24) showed proton resonances at δ_H 4.69 (1H, *s*, H-29b), 4.57 (1H, *s*, H-29a), 4.47 (1H, dd, *J* = 4.4, 12.8 Hz, H-3), 2.05 (3H, *s*, H-2/), 1.69 (3H, *s*, H-30), 1.03 (3H, *s*, H-25) 0.94 (3H, *s*, H-28), 0.85 (3H, *s*, H-23), 0.84 (3H, *s*, H-24), 0.83 (3H, *s*, H-26), and δ_H 0.79 (3H, *s*, H-27).



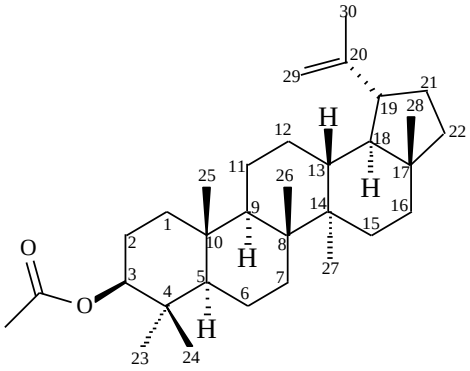


Figure 4.24:[1]HNMR spectrum of A4

4.6.2.4 [13]C NMR spectral analysis of A4

The [13]C NMR ($CDCl_3$, 100 MHz) spectrum of A4 (Figure 4.25) showed a total of thirty-two carbon resonances. These include signals at δc 171.3 (C-1'), 151.2 (C-20), 109.6 (C-29), 81.2 (C-3), 55.6 (C-5), 50.5 (C-9), 48.5 (C-18), 48.2 (C-19), 43.2 (C-17), 43.0 (C-14), 41.0 (C-8), 40.2 (C-22), 38.6 (C-1), 38.0 (C-4), 37.3 (C-10), 36.2 (C-13), 35.8 (C-16), 34.4 (C-7), 30.0 (C-21), 28.2 (C-2'), 27.6 (C-23), 25.3 (C-15), 24.0 (C-12), 21.7 (C-2), 21.1 (C-11), 19.5 (C-30), 18.4 (C-6), 18.2 (C-28), 16.7 (C-24), 16.4 (C-25), 16.2 (C-26) andδc14.7 (C-27).

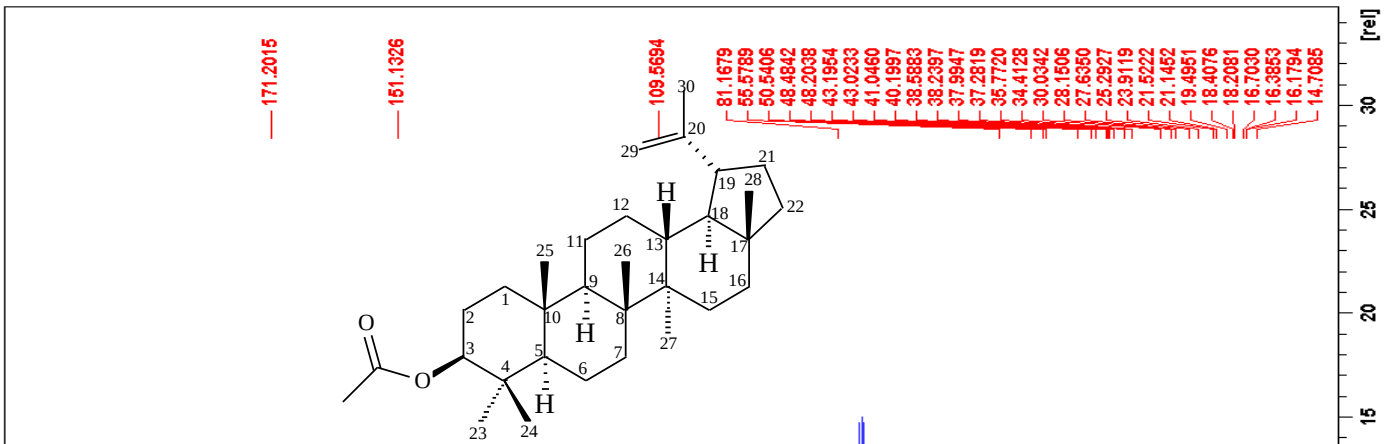


Figure 4.25: ^{13}C NMR spectrum of A4

4.6.2.5 DEPT-135 spectral analysis of A4

The DEPT-135 spectrum of A4 showed nine methyl, eleven methylene and four methine carbon resonances (Figure 4.26).

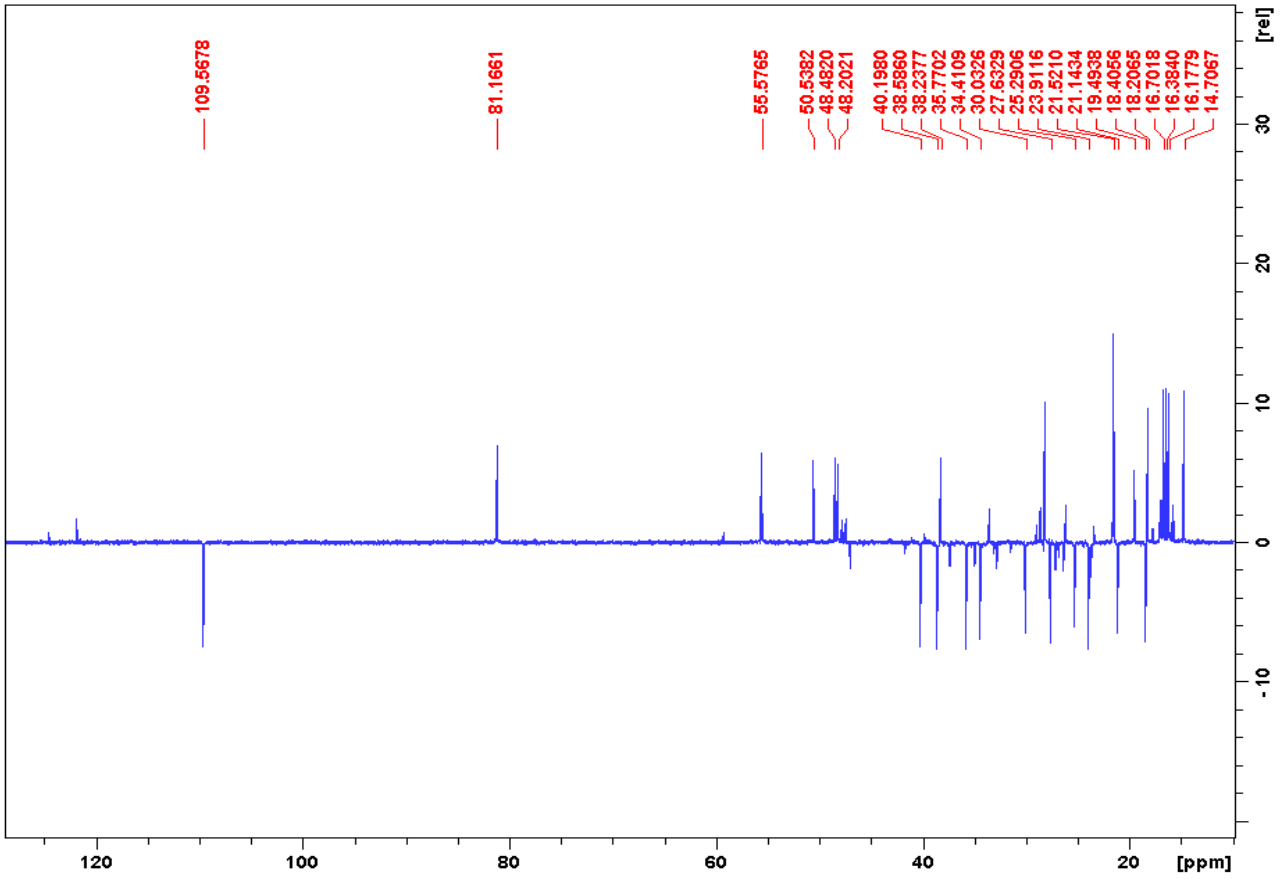


Figure 4.26: DEPT-135 NMR spectrum of A4

4.6.2.6 HSQC-DEPT spectal analysis of A4

The HSQC-DEPT spectrum of A4 (Figure 4.27) was used to assign protons to their respective carbons.

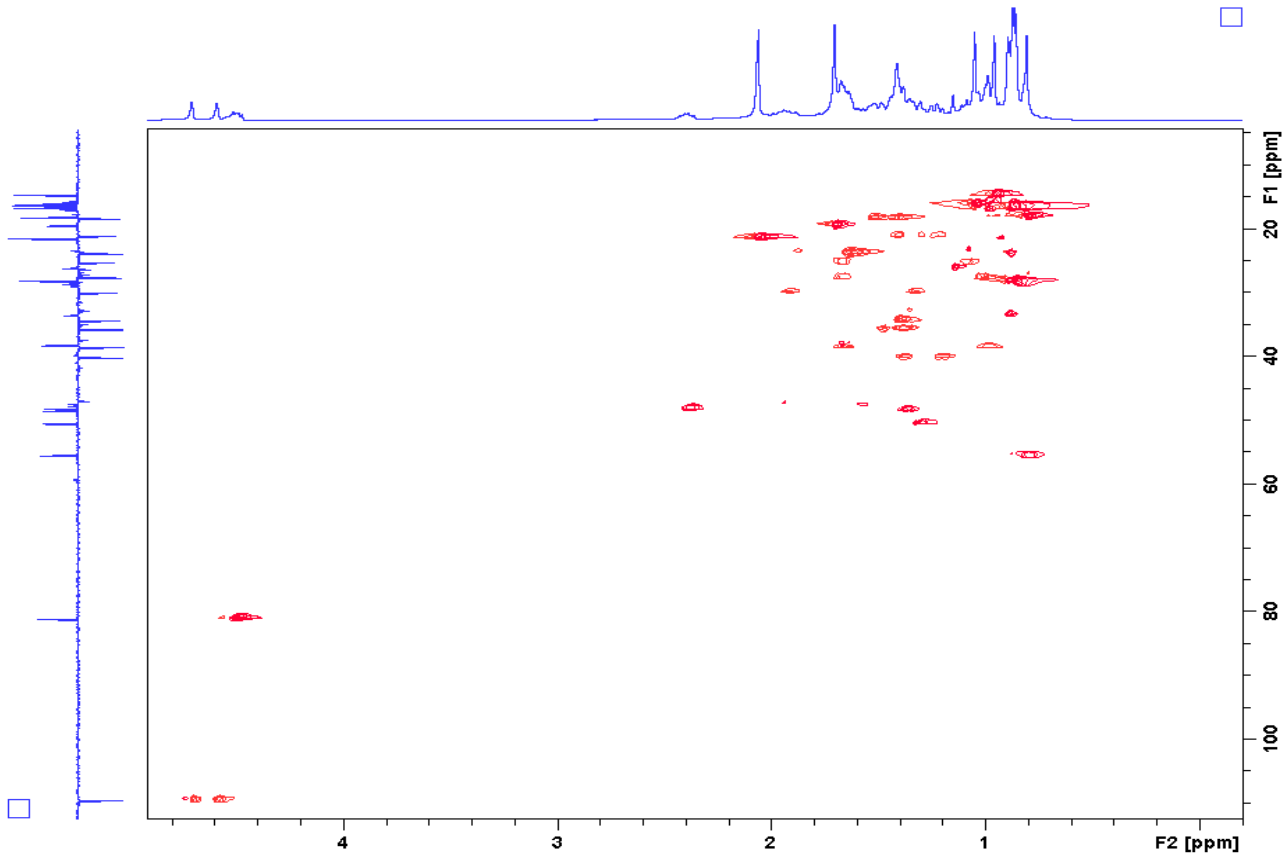


Figure 4.27: HSQC-DEPT spectrum of A4

4.6.2.7 COSY spectral analysis of A4

The COSY spectrum of A4 (Figure 4.28) was used to confirm the position of adjacent protons in the proposed structure of the compound.

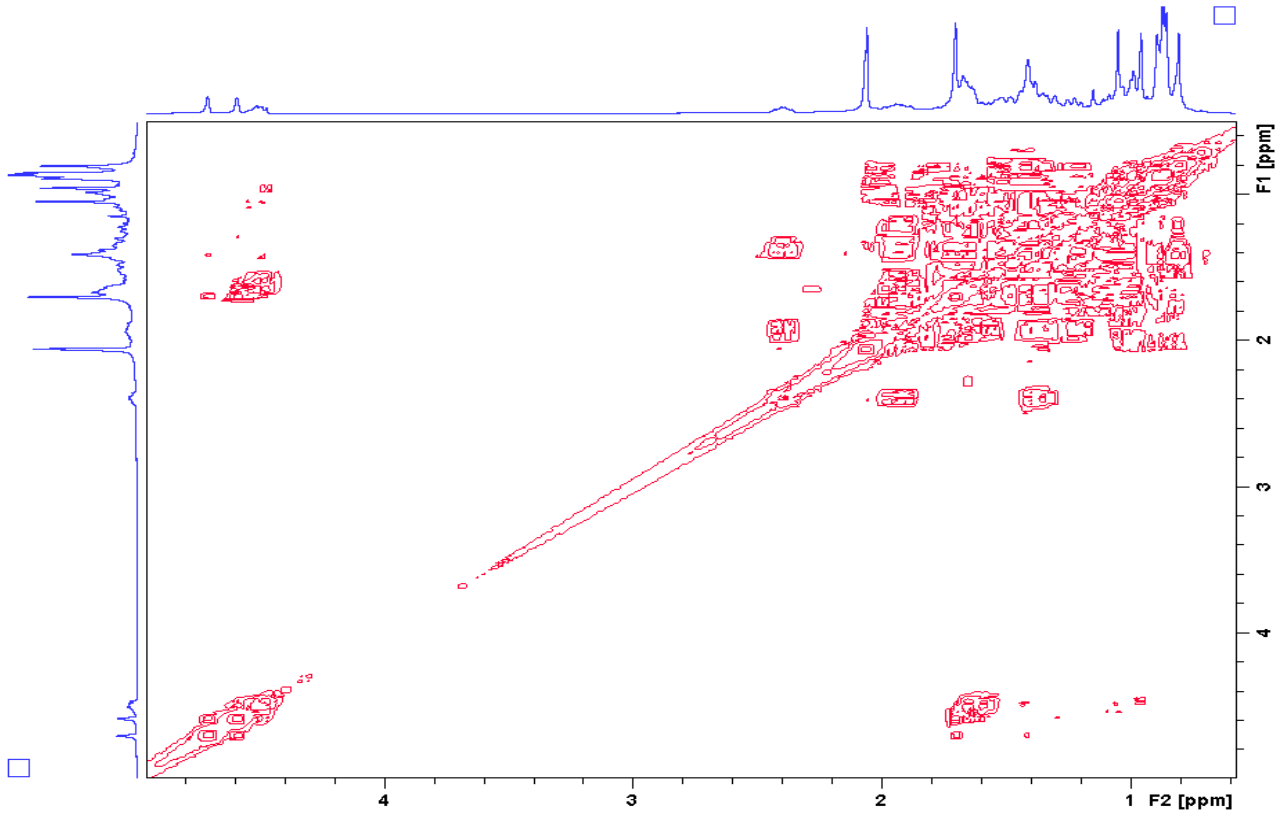


Figure 4.28: COSY spectrum of A4

4.6.2.8 HMBC spectral analysis of A4

The HMBC spectrum (Figure 4.29) is similar to that of A3. However, it showed an important correlation between the methine proton at δ_H 4.47 and the carbonyl carbon signal at δc 171.2. This and other correlations observed were also used to confirm the connectivity network in the proposed structure.

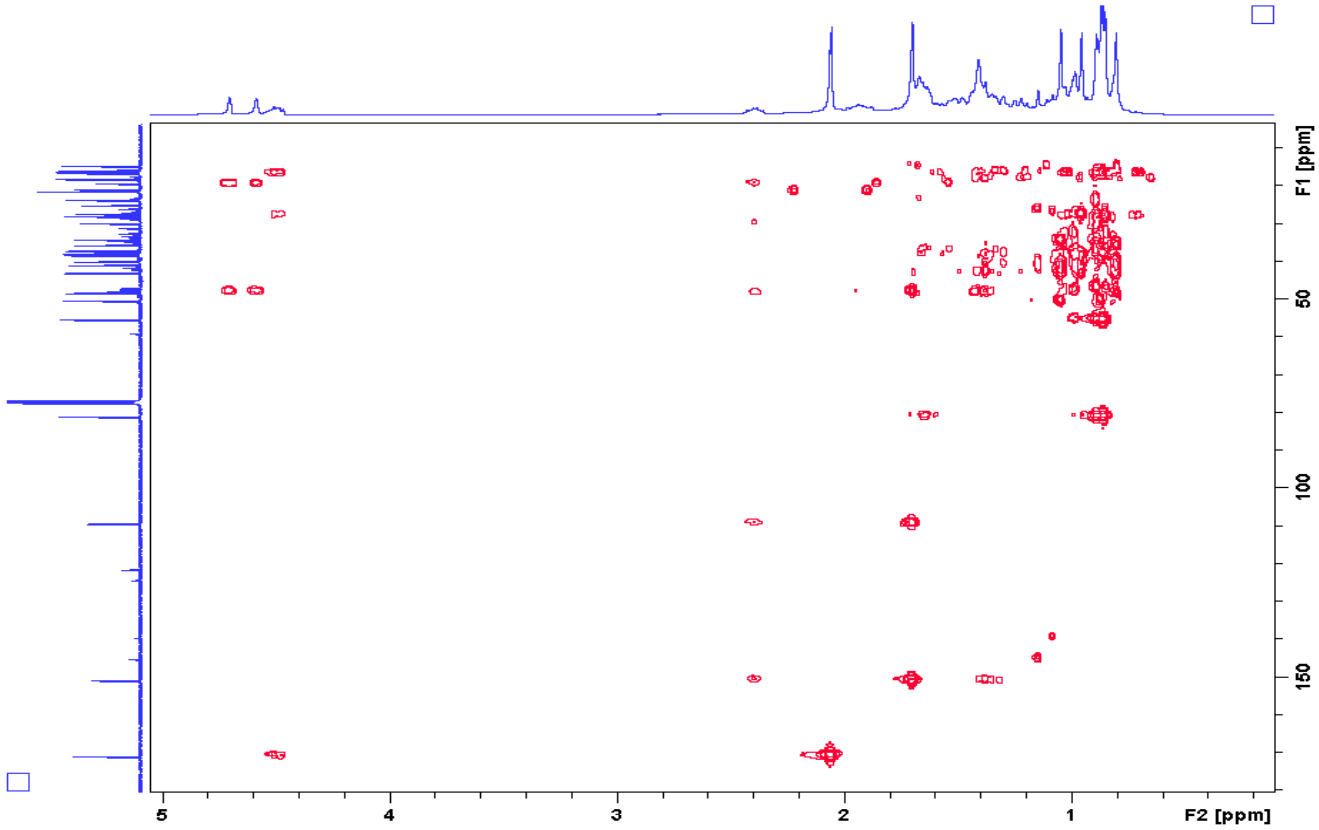


Figure 4.29: HMBC spectrum of A4

Table 4.7: NMR spectral data of A4 compared with Literature

No.	**^{13}C NMR (500 MHz) $CDCl_3$**	**^{13}C NMR (**Léa *et al.*, 2014) **(400 MHz) $CDCl_3$**	**^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz)**	**^{1}H NMR (**Léa *et al.*, 2014) **(400 MHz) $CDCl_3$) (*J* in Hz)**	HMBC **(H → C)**
1α	3.6 CH_2	38.50	1.69 *m*	1.62	3,10
1β			0.98 *m*	0.95	
2 α	23.9 CH_2	23.86	1.60* *m*	1.70	
2β			1.60* *m*	1.50	
3	81.2 CH	81.22	4.47 *dd*(11.0, 6.0Hz)	4.48 *dd* J= 11.0, 5.2 Hz	1,1',2,4,23,25
4	38.0 C	37.86		-	
5	55.5 CH	55.40	0.78 *m*	0.65	3,4,6,7,10,25
6 α	18.40 CH_2	18.39	1.50 *m*	1.48	

6β				1.40*	1.33	
7 α	34.5	CH_2	34.26	1.38*	1.36	
7β				1.38*	1.36	
8	41.1	C	40.96		-	
9	50.6	CH	50.49	1.30 *m*	1.23	5,8,10,11
10	37.9	C	37.23		-	
11 α	21.2	CH_2	21.00	1.40* *m*	1.33	
11β				1.22 *m*	1.33	
12 α	25.3	CH_2	25.01	1.07 *m*	1.06	
12β				1.68*	1.54	
13	38.3	CH	38.10	1.68*	1.59	
14	43.2	C	42.97		-	
15 α	27.7	CH_2	27.58	1.00 *m*	1.01	13,14,17
15β				1.67*	1.49	
16 α	35.8	CH_2	35.72	1.48*	1.36	
16β				1.48* *m*	1.44	
17	43.1	C	43.17		-	
18	48.2	CH	48.00	1.38*	1.35	7,16,19
19	48.5	CH	48.30	2.37	2.37	17,18,22
20	151.2	C	150.8		-	
21 α	30.1	CH_2	29.86	1.32* *m*	1.23	
21β				1.27 *m*	1.84	
22 α	40.2	CH_2	40.20	1.20 *m*	1.18	
22β				1.38*	1.36	
23	28.2	CH_3	28.09	0.84 *s*	0.94	3,4,5,24
24	16.7	CH_3	14.4	1.02 *s*	0.84 *s*	3,4,5,23
25	16.4	CH_3	16.33	0.85 *s*	0.85 *s*	1,5,9,10
26	16.2	CH_3	16.04	0.83 *s*	1.03 *s*	8,9,14
27	14.7	CH_3	14.50	0.94 *s*	0.94 *s*	8,13,14,15
28	18.2	CH_3	18.05	0.78 *s*	0.78 *s*	16,17,18,22
29A	109.6	CH_2	1.9.78	4.68 *br s*	4.54 *br s*	
29B				4.57 *br s*	4.66 *br s*	
30	19.5	CH_3	19.47	1.68 *br s*	1.69 *s*	19,20,29
$\underline{C}$=OCH_3	171.2	C	171.02	-		
C=O$\underline{C}H_3$	21.39	CH_3	2.42	2.04 *s*	2.03 *s*	

* = overlapping proton signals

4.7 Isolation of A5

Compound A5 (7.2 mg) was isolated as white powder, Rf = 0.75 using n-hexane : ethylacetate (8:2) as solvent system (Plate 4.7).



Plate 4.7: TLC chromatogram of A5 using n-hexane : ethylacetate 8:2 as solvent system

4.7.1. Physical tests carried out on A5

Solubility of A5

A5 was found to be soluble in chloroform.

Melting point of A5

The compound was found to have a melting point range of 243^0C - 245^0C.

4.7.2 SPECTRAL ANALYSIS OF A5

4.7.2.1 LCMS spectral analysis of A5

The LCMS of A5 (Figure 4.30) showed a $[M-H]^+$ ion peak at *m/z* 467.



Figure 4.30: LCMS spectrum of A5

4.7.2.2 IR spectral analysis of A5

The IR spectrum of A5 (Figure 4.31) showed absorptions bands at 2921 and 2851 cm^{-1} (C-H stretching), 1737 cm^{-1} (C=O streching) 1636 cm^{-1} (C=C stretch) and 1244 cm^{-1} (C-O stretching).

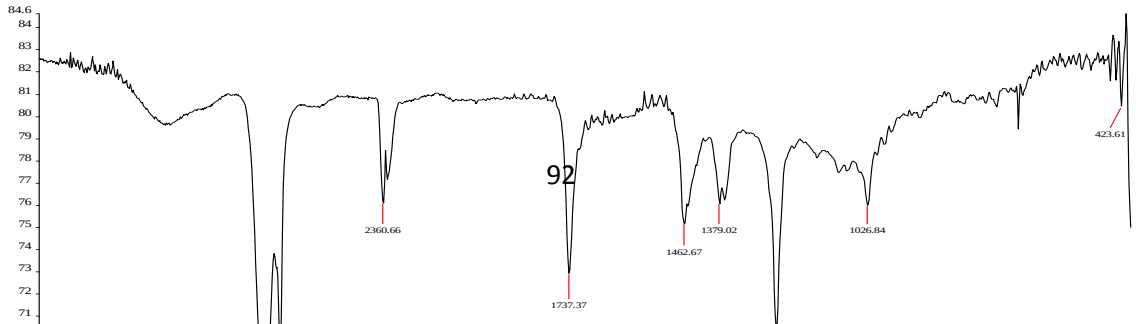


Figure 4.31: IR spectrum of A5

4.7.2.3 ^{1}H NMR spectral analysis of A5

The ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of A5 (Figure 4.32) showed proton signals at δ0.84 (s, 3H, –29), 0.89 (d, *J* = 7 Hz, H-30), 1.08 (d, *J* = 7.0 Hz, H-27), 1.27–1.30 (d, *J* = 7.0 Hz,), 1.48 (m, H-21), 1.52 (d, *J* = 11.5 Hz, H-18), 1.69 (s, 3H-2, 3H-7 and 3H-5), 1.83 (m, 2H-19), 1.86 (m, 2H-22), 1.90 (m, 2H-15), 2.05 (d, *J* = 11.5 Hz, H-1), 2.19 (s, H-9), 2.23–2.26 (d, *J* = 11.5 Hz, H-1 and H-16), 5.14 (s, 2H-11) and 5.55 (s, H-12).

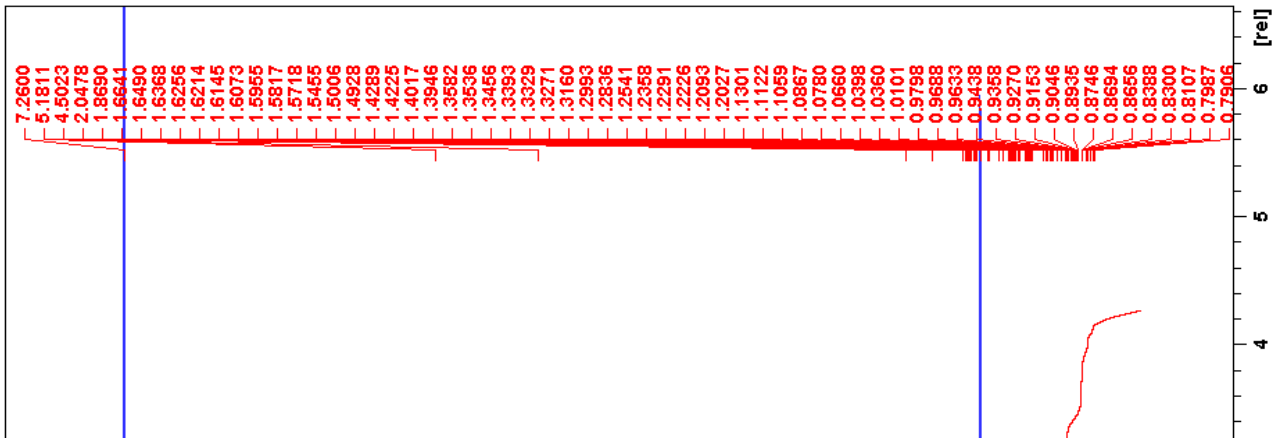


Figure.. 1H NMR spectrum of A..

Figure 4.32: ^{1}H NMR spectrum of A5

Figure 4.33: ^{1}H NMR spectrum of A5

4.7.2.4 ^{13}C NMR spectral analysis of A5

The ^{13}C NMR spectrum of A (Figure 4.33) showed a total of thirty-two carbon resonances at δc 39.6 (C-1), 27.9 (C-2), 80.8 (C-3), 39.6 (C-4), 55.2 (C-5), 18.2 (C-6), 33.7 (C-7), 38.4 (C-8), 47.4 (C-9), 35.6 (C-10), 23.6 (C-11), 121.6 (C-12), 145.2 (C-13), 42.1 (C-14), 28.7 (C-15), 27.9 (C-16), 32.9 (C-17), 59.0 (C-18), 40.0 (C-19), 41.5 (C-20), 31.2 (C-21), 42.0 (C-22), 29.7 (C-23), 15.7 (C-24), 15.7 (C-25), 16.8 (C-26), 23.6 (C-27), 28.7 (C-28), 17.5 (C-29), 21.4 (C-30), 170.8 and 21.3 (OAc).

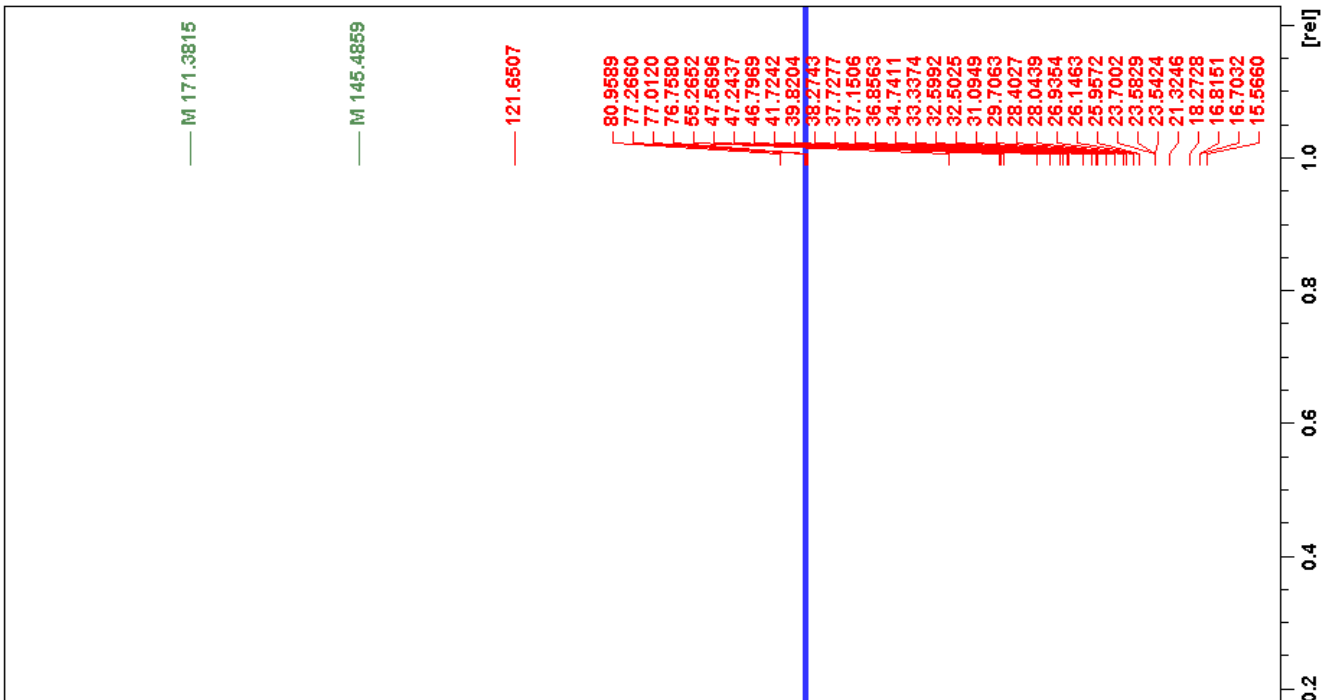


Fig 4.33: ^{13}C NMR spectrum of A5

Figure 4.33: ^{13}CNMR spectrum of A5

4.7.2.5 DEPT-135 spectralanalysis of A5

The DEPT-135 spectrum of A5 (Figure 4.34) revealed nine methyl, ten methylene and five methine carbon signals.

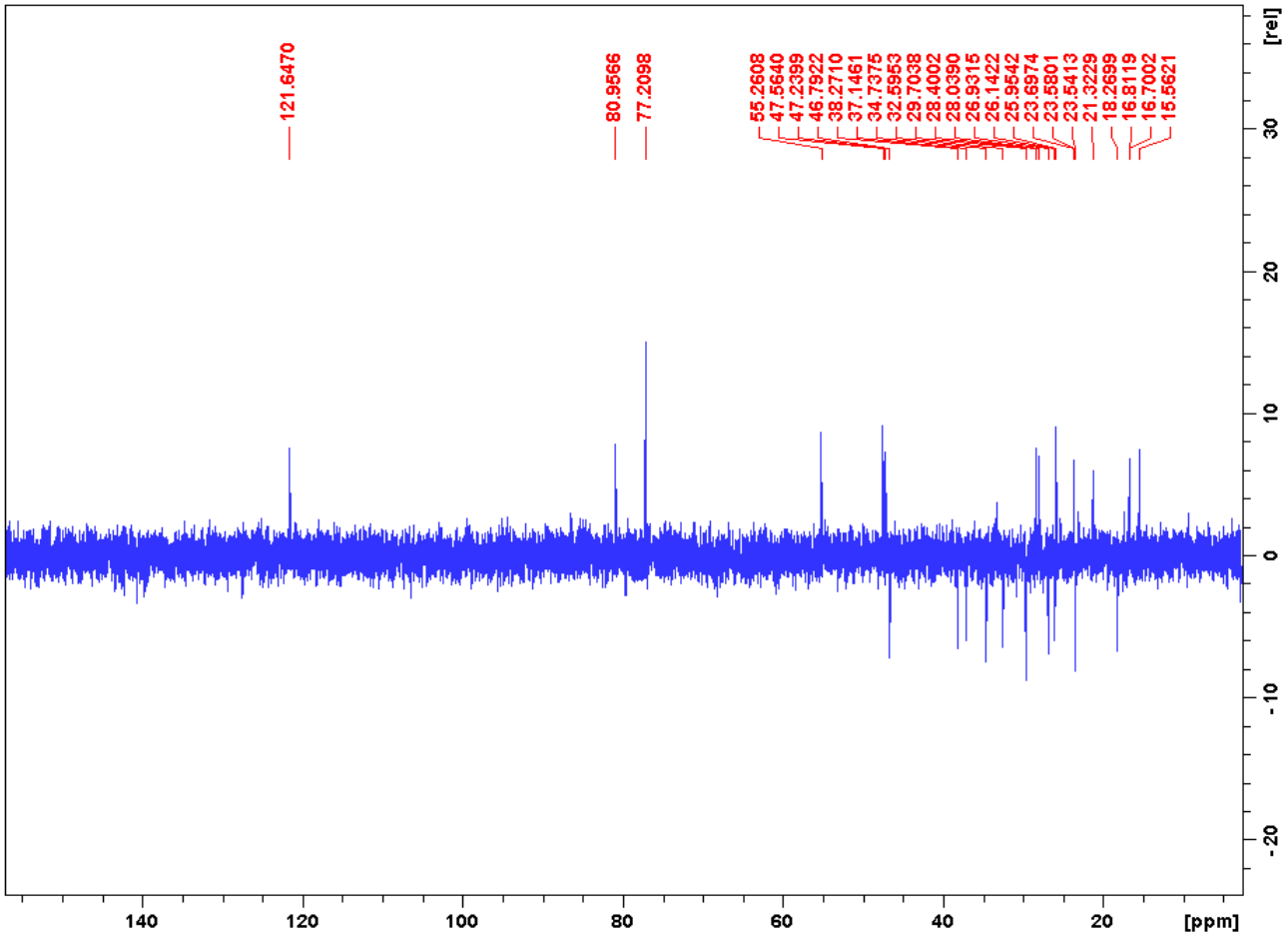


Figure 4.34: DEPT 135 spectrum of A5

4.7.2.6 HSQC-DEPT spectral analysis of A5

The HSQC-DEPT spectrum of A5 (Figure 4.35) was used to assign protons to their respective carbon atoms.

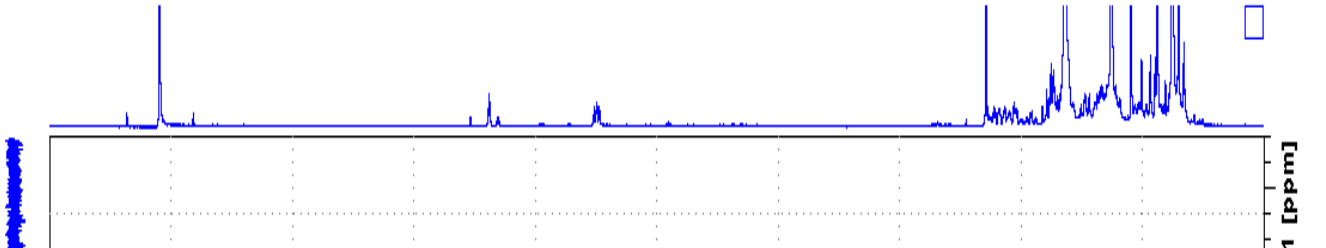


Figure 4.35: HSQC-DEPT spectrum of A5

4.7.2.7 HMBC spectral analysis of A5

The HMBC spectrum of A5 is shown in Figure 4.36 below:

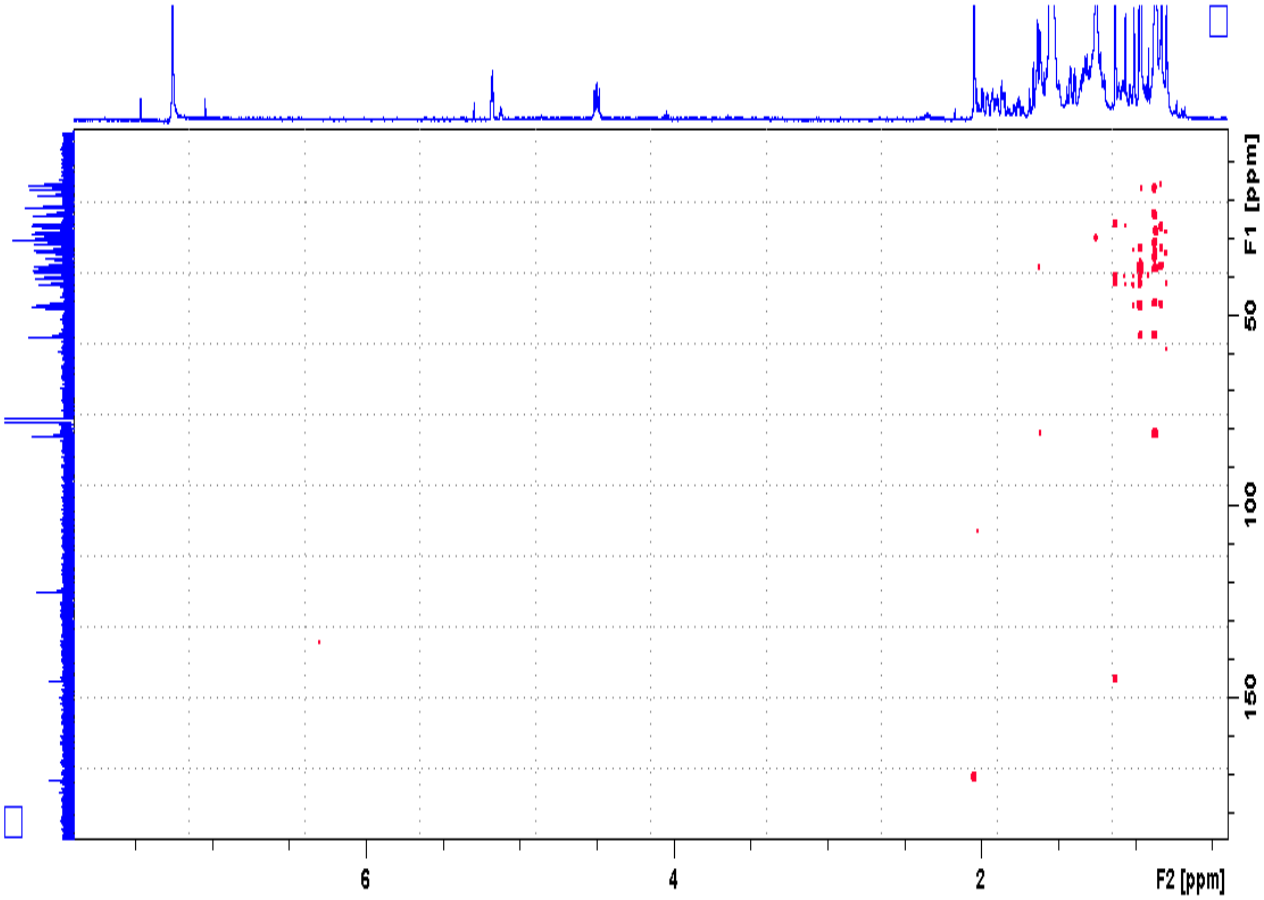


Figure 4.36: HMBC spectrum of A5

4.7.2.8 COSY spectral analysis of A5

The COSY spectrum of A5 is shown in Figure 4.37 below:

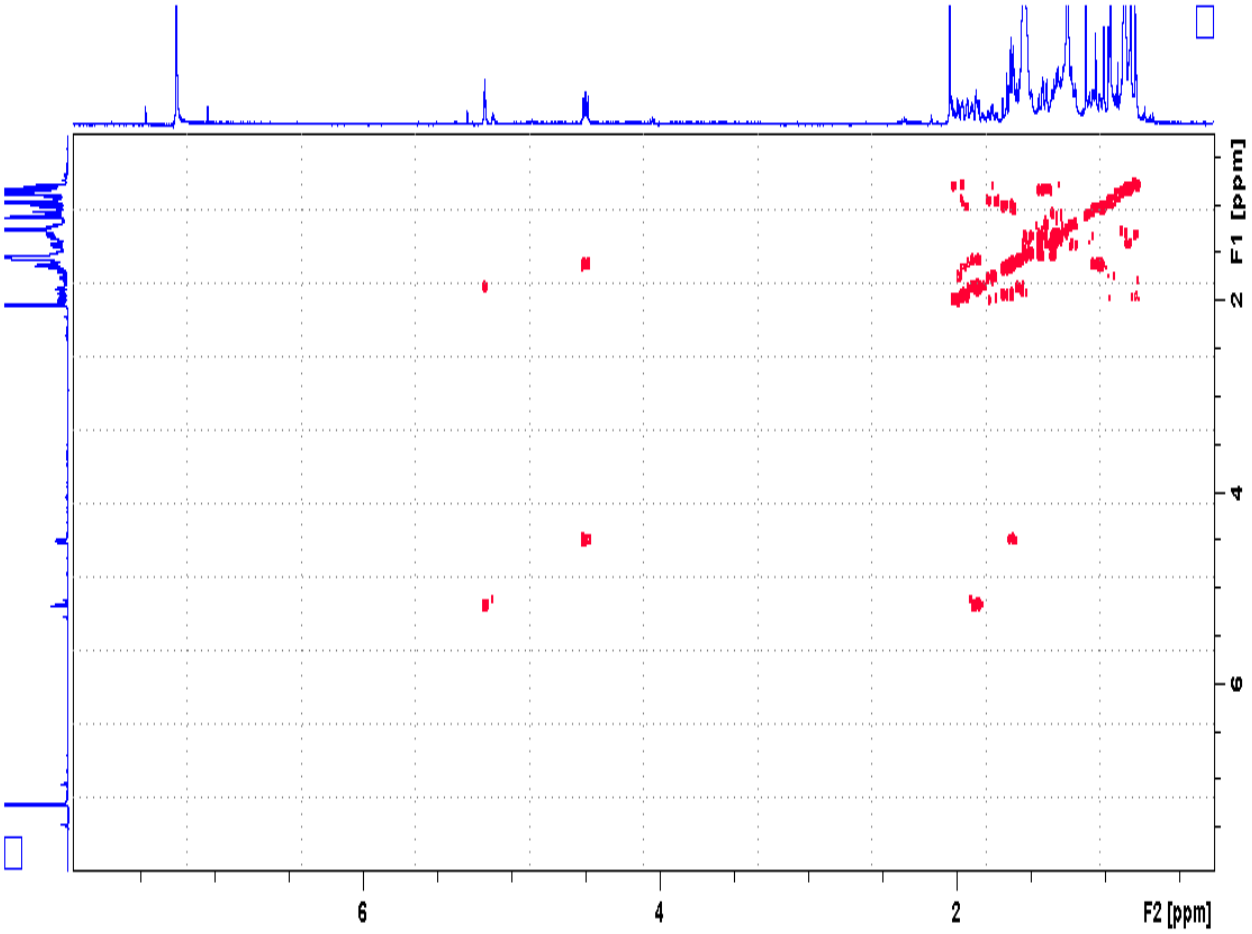


Figure 4.37: COSY spectrum of A5

4.7.8.9 NOESY spectral analysis of A5

The NOESY spectrum of A5 is shown in Figure 4.38 below. It was used to confirm the relative stereochemistry of the structure of the compound.

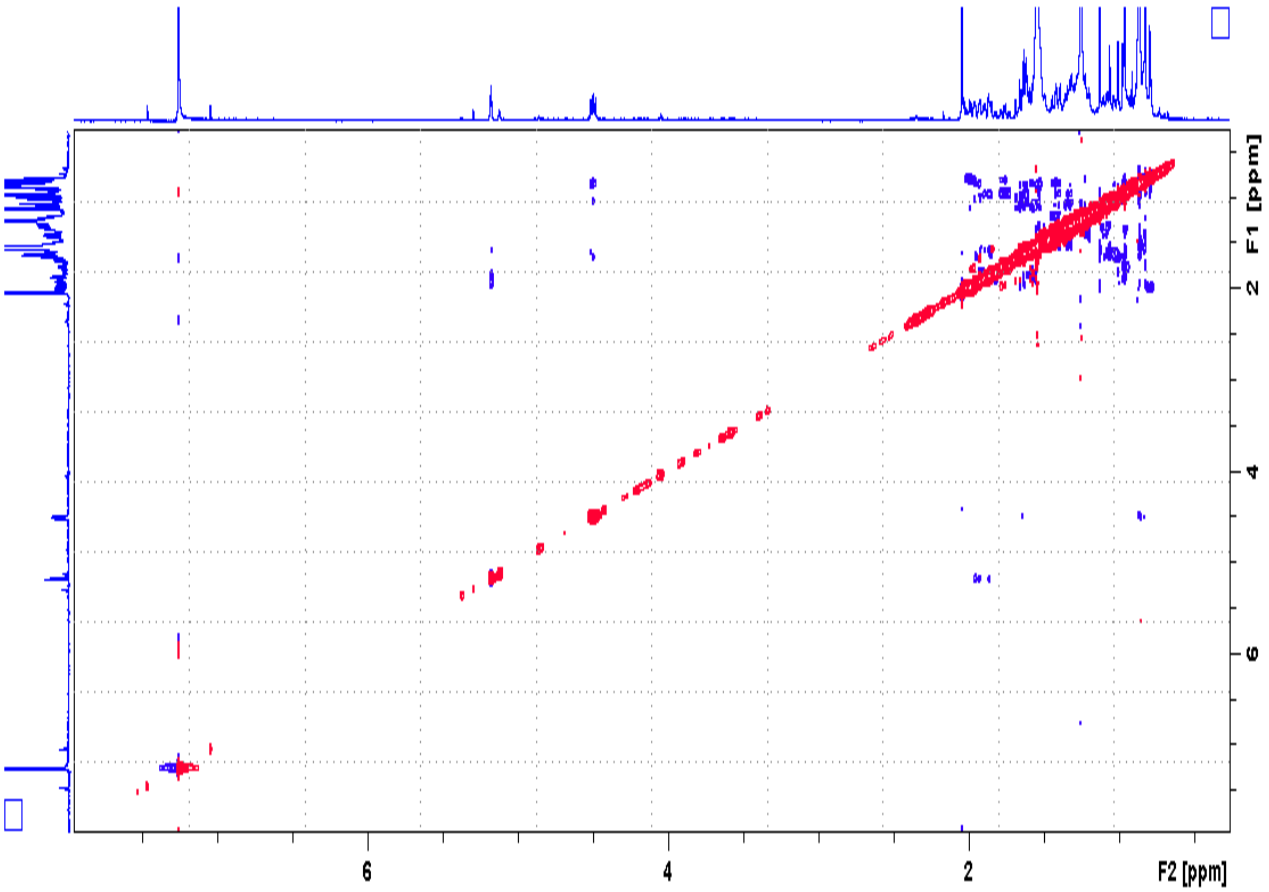


Figure 4.38 NOESY spectrum of A5

Table 4.8: NMR spectral data of compound A5 compared compared with literature

No.	^{13}C NMR (500 MHz)	^{13}C NMR (500 MHz) in $CDCl_3$ Lee *et al.*, (2014)	^{1}H NMR (500 MHz)	1H NMR (500 MHz) in $CDCl_3$Lee *et al.*, (2014)	HMBC (H → C)	COSY (H →H)	NOESY (H →H)
1α	38.5 CH_2	38.3	1.06 m	1.04	-	1β, 2	1β, 2, 3, 9
1β			1.63 m	1.64	3, 10	1α, 2	1α, 3, 11, 25

2	23.76 CH_2	23.5	1.63 m	1.88	3, 4, 10	1α, 1β, 3	1α, 3, 25
3	81.2 CH	81.0	4.5 dd *J* = 10.0, 5.5 Hz	4.50 *t J* = 8.0 Hz	-	2	1α, 1β, 2, 5, 9
4	37.9 C	39.8	-	-	-	-	-
5	55.5 CH	55.2	0.84 m	0.84 *m*	7, 10, 23, 24, 25	6α, 6β	3, 9, 6α, 6β, 7α
6α	18.5 CH_2	18.3	1.55 m	1.53 *m*	-	5, 6β, 7β	5, 6β, 7β, 27
6β			1.40 m	1.40 *m*	-	5, 6α, 7α, 7β	5, 6α
7α	32.8 CH_2	32.6	1.50 m	1.52 *m*	-	6β, 7β	5, 7β, 27
7β			1.34 m	1.33 *m*	-	6α, 6β, 7α	6α, 7α, 26
8	40.0 C	37.7	-	-	-	-	-
9	47.8 CH	47.6	1.58 m	1.58 *m*	-	11	1α, 3, 5, 27
10	37.1 C	36.8	-	-	-	-	-
11	23.8 CH_2	23.6	1.87 m	1.80 *m*	-	11, 12	1β, 12, 25
12	121.9 CH	121.6	5.18 t *J*=3.7 Hz	5.18 t J=3.5 Hz	9, 18	11	18, 26
13	145.5 C	145.2	-	-	-	-	-
14	41.9 C	41.7	-	-	-	-	-
15α	26.4 CH_2	26.1	1.78 m	1.76 *m*	-	15β, 16α, 16β	15β, 25, 28
15β			0.96 m	0.85 *m*	8, 14, 17	15α, 16α	15α, 16β, 18
16α	27.2 CH_2	26.9	2.0 m	1.98 *m*	-	15α, 15β, 16β	16β, 27, 28
16β			0.8 m	0.79 *m*	14, 17, 28	15α, 16β	15β, 16α, 18, 26
17	32.7 C	32.5	-	-	-	-	-
18	47.5 CH	47.2	1.96 m	1.93 d *J*=4.2 Hz	-	19α, 19β	12, 15β, 16β, 19β, 26, 28, 30
19α	47.0 CH_2	46.8	1.01 m	1.00 *m*	17, 18, 29	18, 19β	19β, 21α
19β			1.66 m	1.66 *m*	20, 29, 30	18, 19α	18, 19α, 21β, 26
20	31.3 C	31.1	-	-	-	-	-
21α	35.0 CH_2	34.7	1.34 m	1.32 *m*	-	21β, 22β	19α, 21β, 27
21β			1.10 m	1.08 *m*	-	21α, 22β	19β, 21α, 22β, 28
22α	37.4 CH_2	37.1	1.21 m	-	-	22β	22β
22β			1.41 m	-	-	21α, 21β, 22α	21β, 22α, 28
23	28.3 CH_3	28.0	0.87 s	0.88 s	3, 4, 5, 24	-	-
24	16.9 CH_3	15.6	0.86 s	0.86 s	3, 4, 5, 23	-	-
25	15.8 CH_3	15.7	0.98 s	0.96 s	1, 5, 9, 10	-	1β, 2, 15α, 28
26	17.0 CH_3	16.8	1.00 s	0.97 s	7, 8, 9, 14	-	7β, 16β, 18, 19β, 28
27	26.2 CH_3	26.0	1.13 s	1.13 s	8, 13, 14, 15	-	6α, 7α, 9, 16α
28	28.6 CH_3	28.4	0.83 s	0.83 s	16, 17, 18, 22	-	15α, 18, 21β, 22β, 25, 26
29	33.6 CH_3	33.4	0.87 s	0.87 s	19, 20, 21, 30	-	-
30	23.9 CH_3	23.7	0.87 s	0.87 s	19, 20, 21, 29	-	18
O-$\underline{C}$O-CH_3	171.4 C	171.1	-	-	-	-	-
O-CO-$\underline{C}H_3$	21.5 CH_3	21.4	2.05 s	2.05 s	O-$\underline{C}$O-CH_3	-	

4.8 Isolation of A6

Compound A6 (10.6 mg) was isolated as a white solid Rf = 0.54 using n-hexane : ethylacetate (9:1) as solvent system (Plate 4.8).

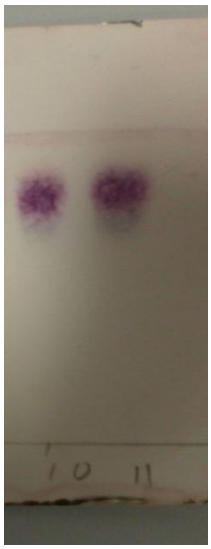


Plate 4.8: TLC chromatogram of A6 using n-hexane : ethylacetate (9:1) as solvent system

4.8.1. Physical tests carried out on A6

Solubility of A6

A6 was found to be soluble in chloroform.

Melting point of A6

The compound was found to have a melting point range of 145^0C - 147^0C.

4.8.2 SPECTRAL ANALYSIS OF A6

4.8.2.1 ^{1}H NMR spectral analysis of A6

The^{1}H-NMR (400 Mz, $CDCl_3$) spectrum of A6 (Figure 4.39) showed overlapping methyl and methylene proton signals at δ_H0.68 -2.28. Other signals appeared at δ_H 3.08 (m, H-3), 5.37 (t, *J* = 3.6 Hz, H-6) and 5.02 (m, H-9).

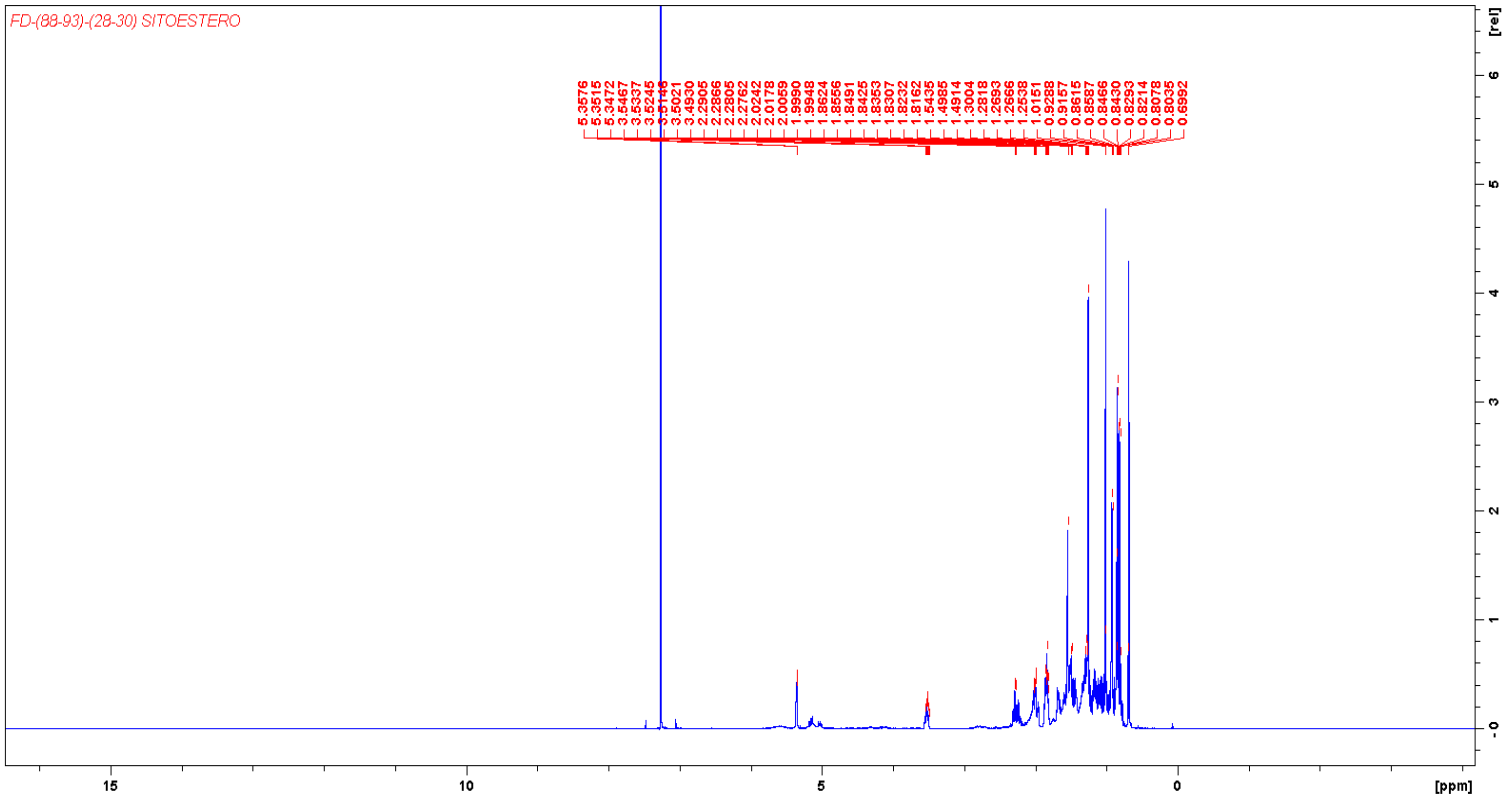


Figure 4.39: ^{1}H NMR spectrum of A6

4.8.2.2 ^{13}C NMR spectral analysis of A6

The ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of A6 (Figure 4.40) showed carbon resonances at δc 37.5 (C-1), 31.9 (C-2), 72.1 (C-3), 42.6 (C-4), 140.1 (C-5), 122.0 (C-6), 32.41 (C-7), 32.15 (C-8), 50.02 (C-9), 36.7 (C-10), 21.3 (C-11), 40.0 (C-12), 42.6 (C-13), 57.6 (C-l4), 26.3 (C-15), 28.5 (C-16), 56.3 (C-17), 19.2 (C-18), 12.1 (C-19), 36.4 (C-20), 19.0 (C-21), 26.3 (23) 46.1 (C-24), 29.3 (C-25), 19.6 (C-26), 19.8 (C-27), 23.3 (C-28), and 12.0 (C-29).

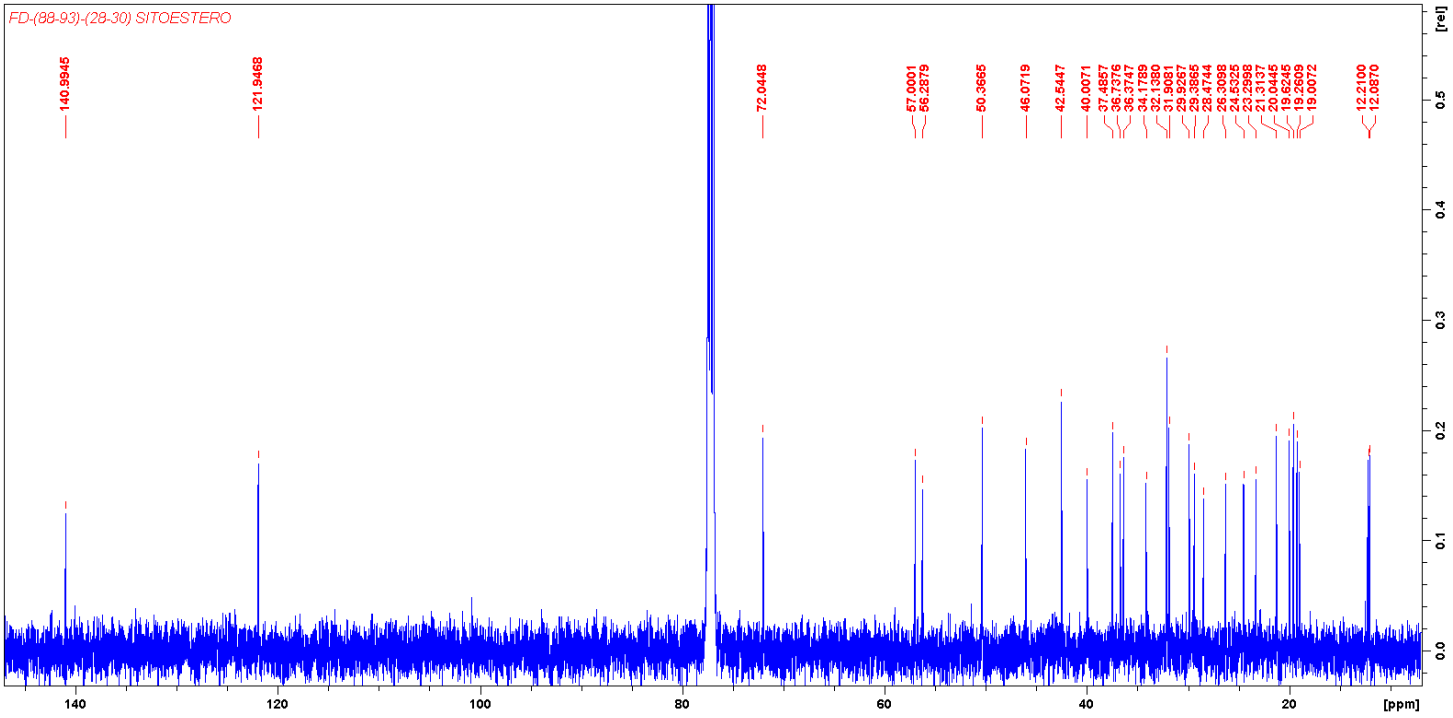


Figure 4.40: ^{13}C NMR spectrum of A6

4.8.2.3 DEPT-135 spectral analysis of A6

The DEPT-135 spectrum of A6 (Figure 4.41) showed six methyl, eleven methylene and nine methine carbon resonances. The three quaternary carbon signals which will not appear the spectrum were also noted.

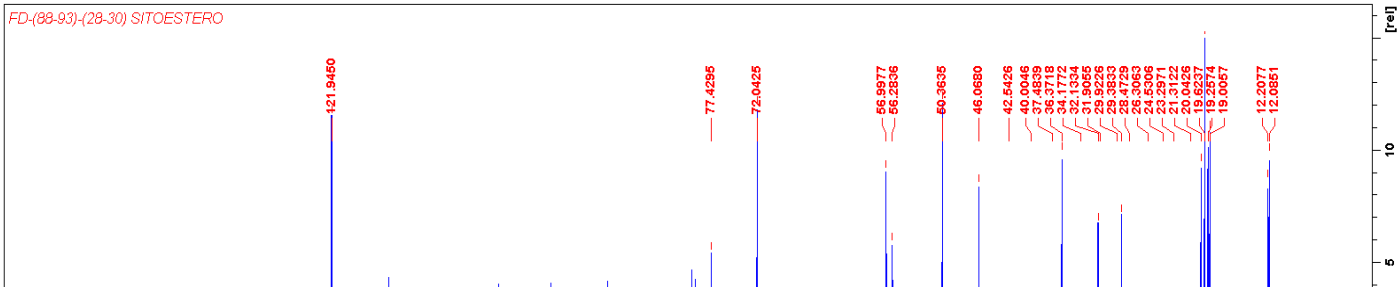


Figure 4.41: DEPT-135 spectrum of A6

4.8.2.4 HSQC-DEPT spectral analysis of A6

The HSQC-DEPT spectrum of A6 (Figure 4.42) was used to assign protons to their respective carbons.

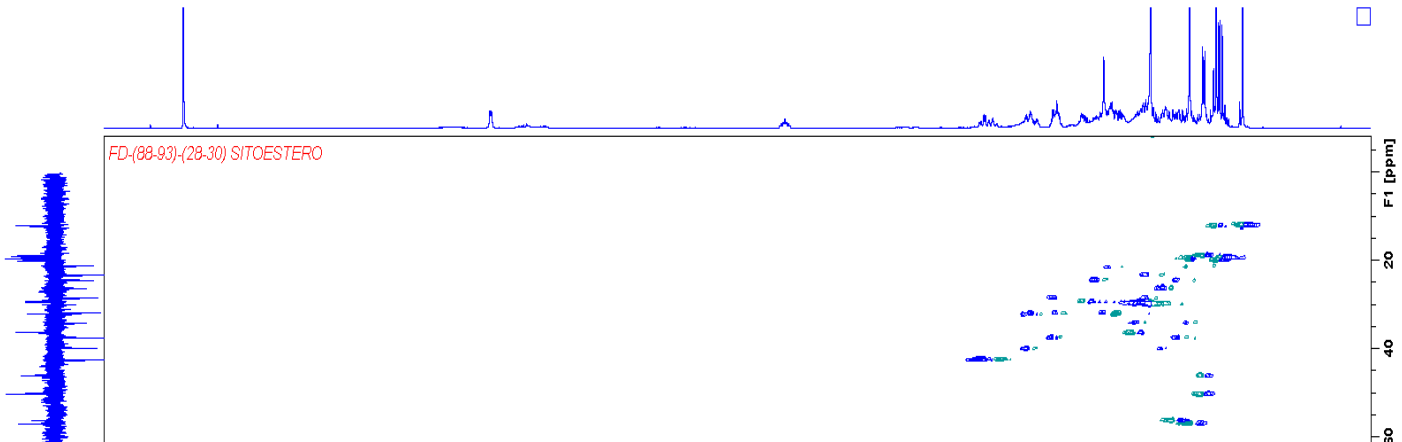


Figure 4.42: HSQC-DEPT spectrum of A6

4.8.2.5 COSY spectral analysis of A6

The COSY spectrum of A6 is presented (Figure 4.43) showed important correlations between proton resonance at δ_H 5.37 (H-6) and δ_H 2.03 (2H-7) and between proton signal at δ_H 1.61 (2H-15) and δ_H 1.41 (2H-16).

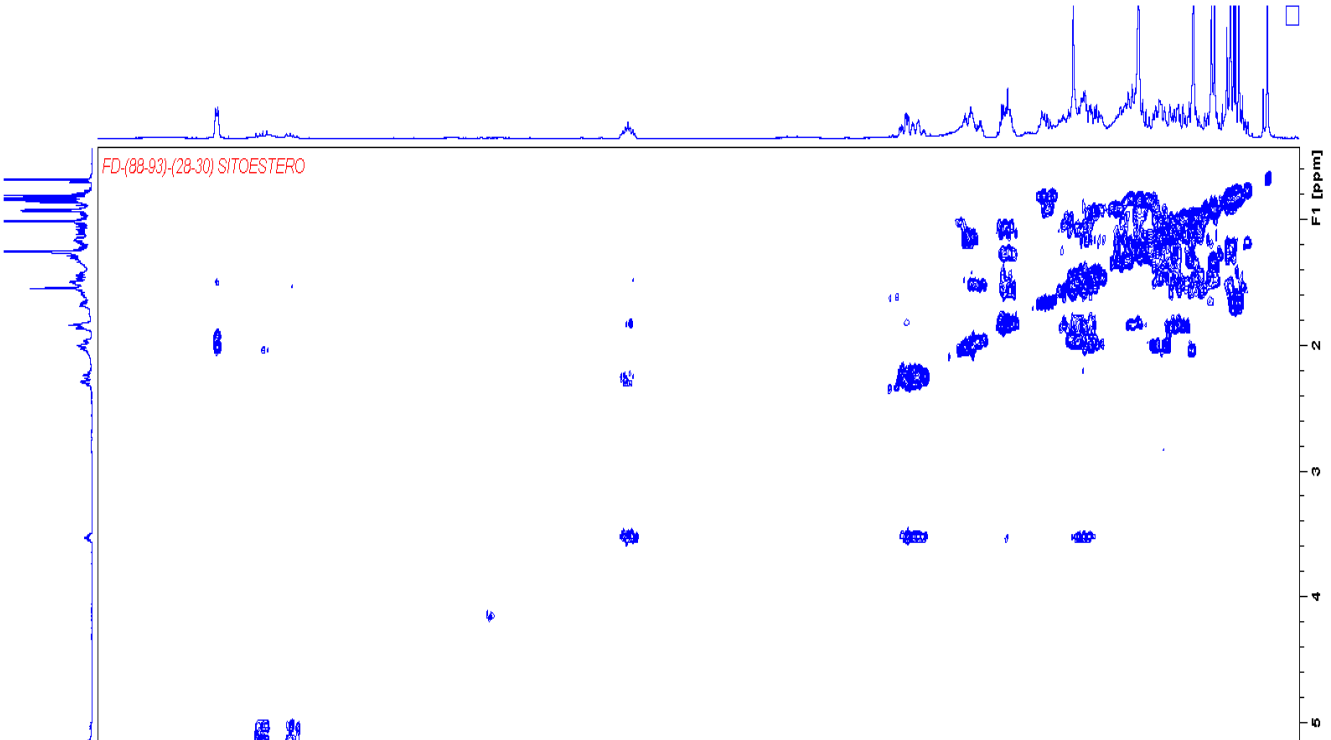


Figure 4.43: COSY spectrum of A6

4.8.2.6 HMBC spectral analysis of A6:

The HMBC spectrum of A6 (Figure 4.44) showed correlations between proton resonances at δ_H 5.37 (H-6) and carbon signals at δc 37.5 (H-3) and δc 31.9 (C-4). It also showed correlations between methyl proton signal at δ_H 0.91 (3H-21) and carbon resonance at δc 36.4 (C-20), 56.3 (C-17), and δc 34.2 (C-22). The methyl proton resonance at δ_H 0.84 (H-27) also showed cross peak with the carbon resonances at δc 29.3 (H-25) and δc 19.6 (H-26).

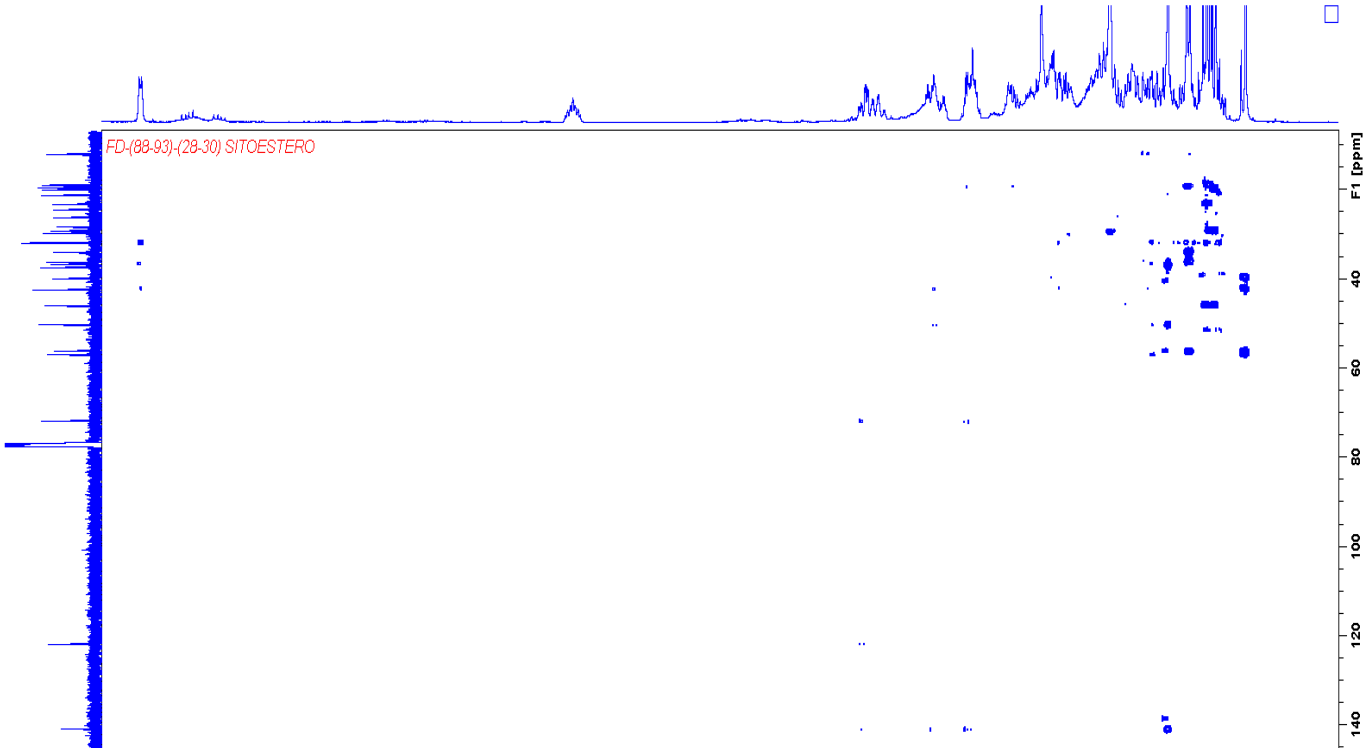


Figure 4.44: HMBC spectrum of A6

Table 4.9: NMR spectral data of A6 compared with literature

Carbon No.	**^{13}C NMR (500 MHz) $CDCl_3$**	**^{13}C NMR Lit (400 MHz) $CDCl_3$** (Manoharan *et al*., 2005)	**^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz)**	**^{1}H NMR Lit (500 MHz,$CDCl_3$)** (Manoharan *et al*., 2005)	**HMBC (H → C)**	**COSY (H → H)**
1α	37.5 CH_2	37.6	1.10 *m*		5	
1β			1.01 *m*		5	
2 α	31.9 CH_2	30.3	1.81 *m*			
2β			1.54 m			
3	72.1 CH	78.3	3.80 *m*	3.97	1, 2, 4α,4β	2,4
4α	42.6 CH_2	39.4	2.28* *dd* (4.69, 8.10)	2.38		
4 β			2.24* *m*	2.63	2 α	
5	140.1 C	141.0	-			
6	122.0 CH	122.0	5.37 t (3.6)	4,5,7,8		7α,
7 α	32.1* CH_2	32.2	2.03* *m*			6
7β			1.83 *m*			
8	32.15* CH	32.1	2.03* *m*			
9	50.2 CH	50.4	0.91* *m*		5,8,10,19	
10	36.7 C	37.0				
11β	21.3 CH_2	21.4	0.83* *m*			
12 α	40.0 CH_2	40.0	2.01* t (3.1, 12.6)			
12β			2.35 t (7.4, 15.3)			
13	42.6 C	42.6	-			
14	57.0 CH	57.0	0.98* d (6.4)	0.95	8,13,15	
15 α	26.3 CH_2	25.6	1.61* *m*	1.57	14	16α, 16β
15β			1.41* *m*	1.05		16α, 16β
16 α	28.5 CH_2	28.7	1.85 m	1.85		15α, 15β
16β			1.25*m	1.25		15α, 15β
17	56.3 CH	56.3	1.09 *m	1.0		
18	19.2 CH_3	19.3	0.68 s	0.67	5, 9, 10	
19	12.1* CH_3	12.0	1.01* s	0.94	19, 10,	
20	36.4 CH	36.5	1.3 m			
21	19.0 CH_3	19.1	0.91* *d* (6.4)	0.99	17, 20, 22	
22 A	34.2 CH_2	34.3	1.32 *m*	1.07		23A
22B			1.20*			
23 A	26.3 CH_2	26.4	1.6 *m*			22A,24
23 B			1.20* *m*			

24	46.1*	CH	46.1	0.91* *m*			23A
25	29.3	CH	29.5				27,26
26	19.6*	CH_3	19.5	1.01* *d* (2.6)	0.90		25
27	19.8	CH_3	20.1	0.84* *d* (1.86)	0.86	24,25,26	25
28A	23.3*	CH_2	23.4	0.83* *m*			
28B				0.84* m			
29	12.0*	CH_3	12.2	0.84* *dd* (2.4, 9.8)	0.87	24, 28	

*Overlapping signals

4.9 Isolation of A7

Compound A7 (5.2 mg) was isolated as a white amorphous solid, Rf = 0.67 using dichloromethane : ethylacetate (7:3) as solvent system (Plate 4.9).

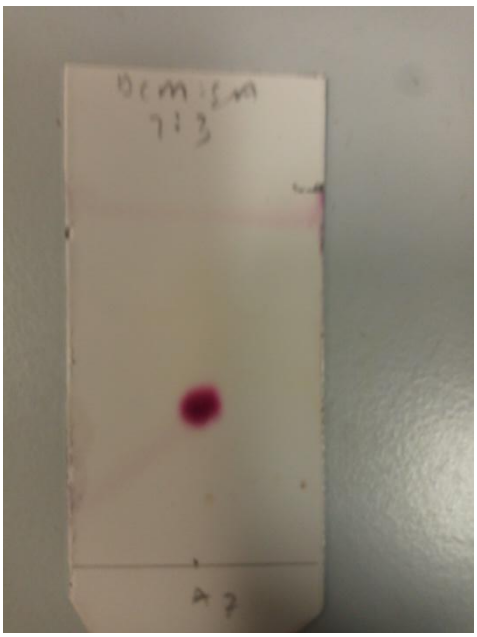


Plate 4.9: TLC chromatogram of A7 using dichloromethane : ethylacetate 7:3 as solvent system

Rf = 0.47

4.9.1. Physical tests carried out on A7

Solubility of A7

A7 was found to be soluble in chloroform and methanol.

Melting point of A7

The compound A7 was found to have a melting point range of 286^0C - 289^0C.

4.9.2 SPECTRAL ANALYSIS OF A7

4.9.2.1 IR spectral analysis of A7

The IR spectrum of A7 (Figure 4.45) showed an absorption band at 3412 cm^{1-} (-OH stretching), 2845 cm^{1-} and 2924 cm^{1-} (aliphatic CH_2 and CH_3 stretching), 1668 cm^{1-} (C=C stretching), and 1078 cm^{1-} (C-O-C),

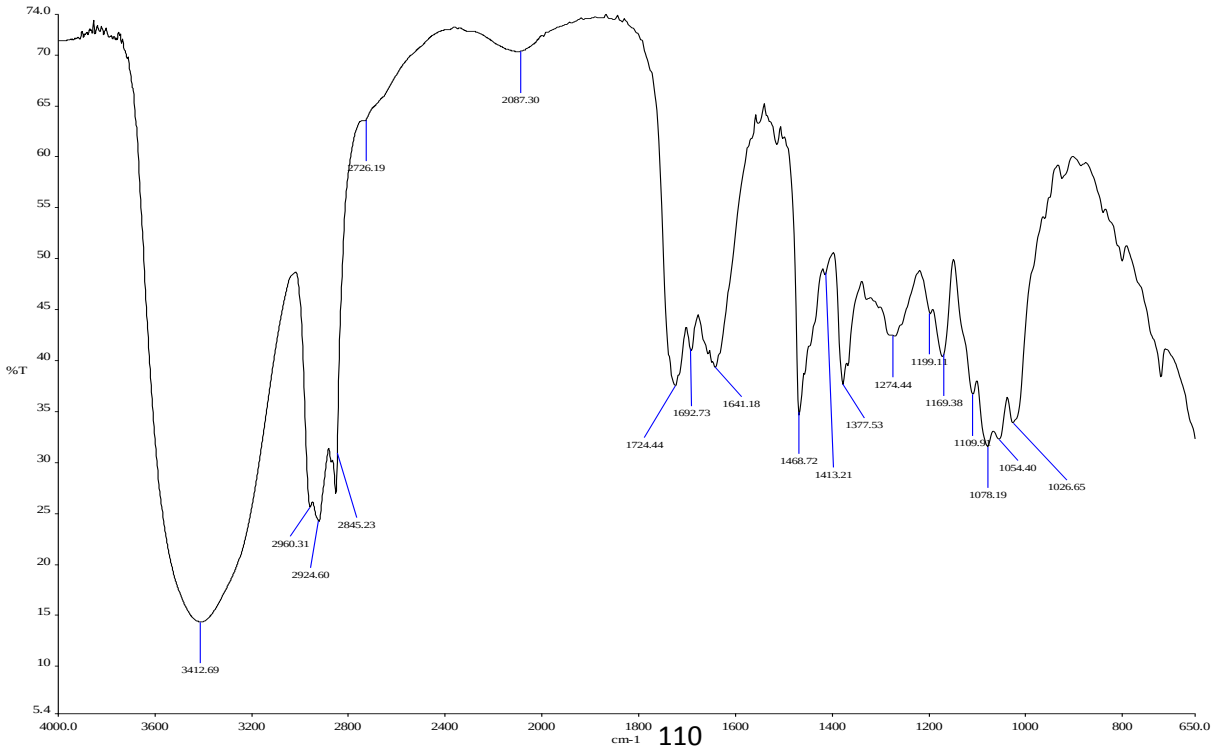


Figure 4.45: IR spectrum of A7

4.9.2.2 ^{1}H MNR spectral analysis of A7

The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of A7 (Figure 4.46) showed proton resonances at 4.37 (m, H-3), 1.01 (s, 3H-19), 0.91 (d, *J* = 6.4 Hz, H-21), 0.84(dd, *J* = 2.4 Hz; 9.8 Hz, H-29), 0.84 (d, 3H, *J* = 1.86,3H-27), 0.86 (d, *J* = 2.6, H-26), 0.68 (s,3H-18), 5.37 (s, H-1'), and 3.34 – 4.51 (protons of sugar moiety).

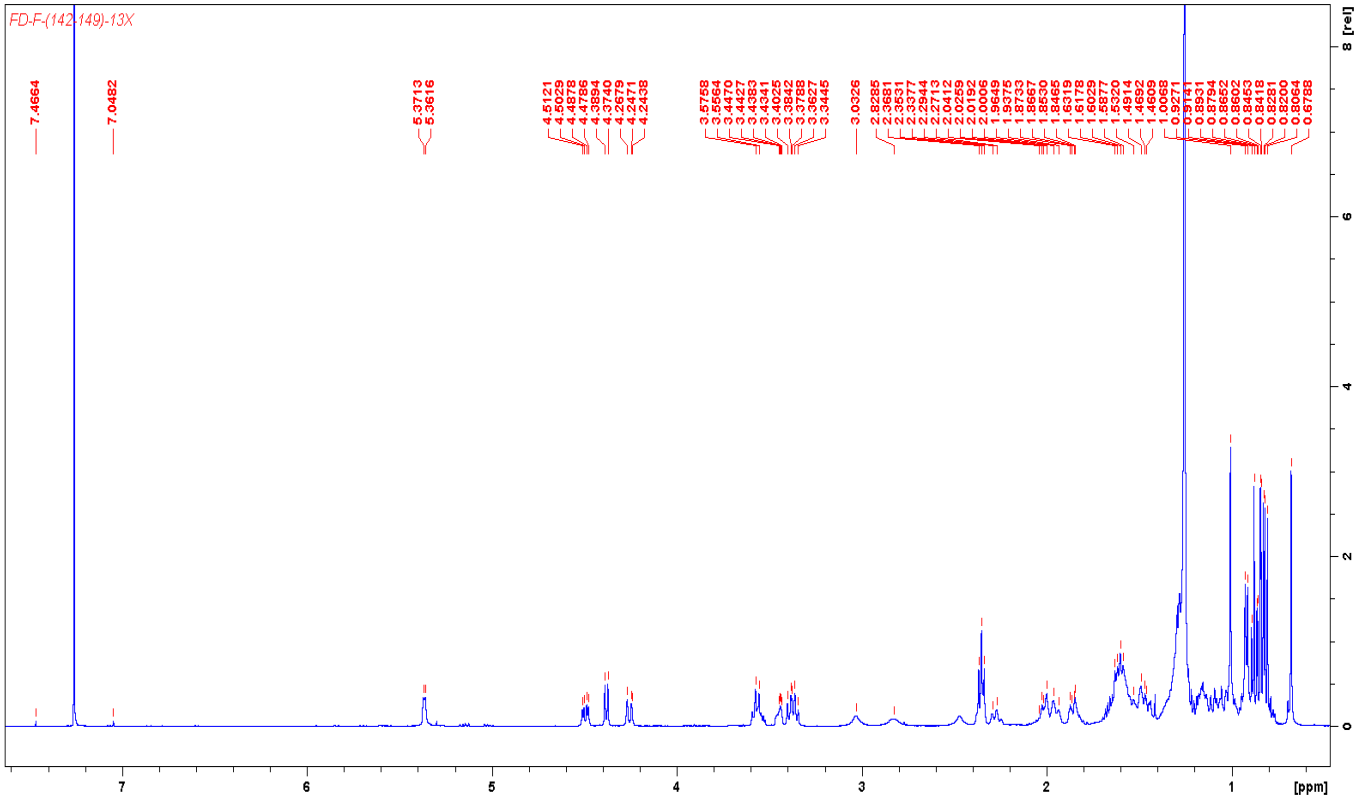


Figure 4.46: ^{1}H MNR spectrum of A7

4.9.2.3 ^{13}C MNR spectral analysis of A7

The 13C-NMR (400 Mz, $CDCl_3$) spectrum of A7 (Figure 4.47) showed a total of thirty five recognizable carbon resonances at δc 39.9 (C-1), 29.9 (C-2), 77.3 (C-3), 39.8 (C-4), 55.8 (C-5), 21.6 (C-6),39.2 (C-7), 29.7 (C-8), 48.7 (C-9), 29.4 (C-10), 21.6 (C-11), 27.2 (C-12), 50.8 (C-13), 30.2 (C-14), 62.1 (C-15), 77.3 (C-16), 124.3 (C-17), 118.26 (C-18), 130.2 (C-19), 151.87 (C-20), 146.47 (C-22), 29.2 (C-23), 14.1 (C-24), 62.12 (C-25), 76.7 (C-26), 63.75 (C-27), 111.14 (C-29) and δc 121.2(C-30)

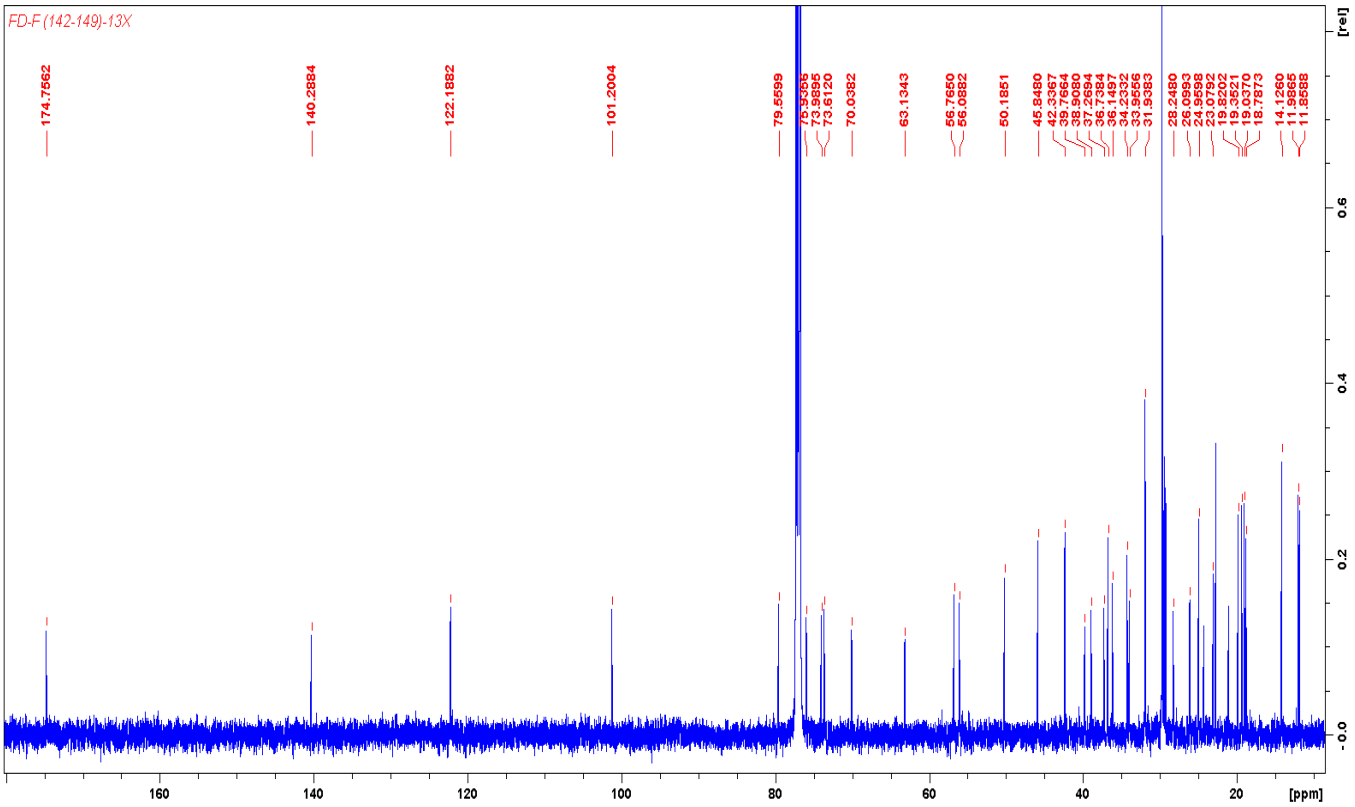


Figure 4.47: ^{13}C NMR spectrum of A7

4.9.2.4 DEPT-135 spectral analysis of A7

The DEPT-135 spectrum of A7 (Figure 4.48) showed six methyls, nine methylenes and eleven methine carbons. The quaternary carbon signals which will not appear the spectrum were also noted.

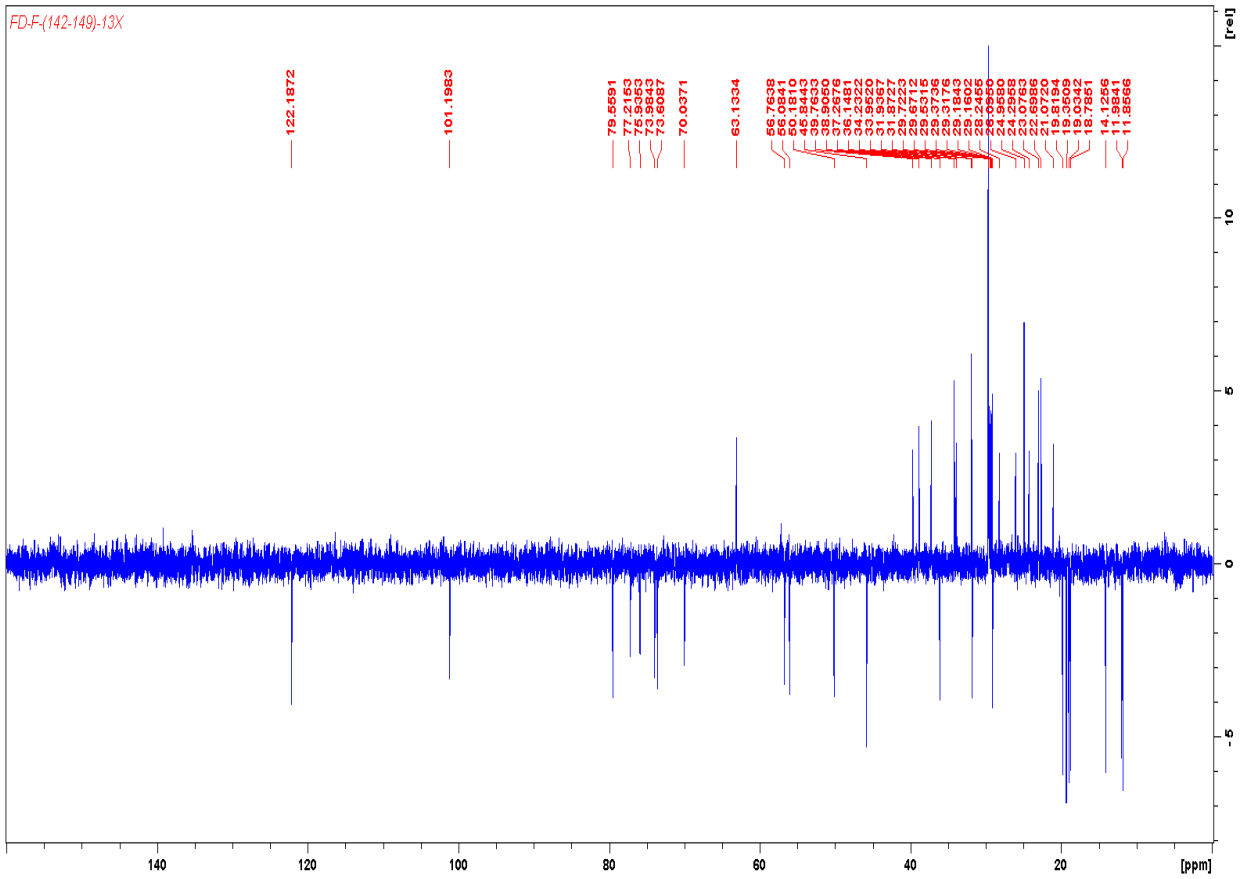


Figure 4.48: DEPT-135 spectrum of A7

4.9.2.5 HSQC-DEPT spectral analysis of A7

The HSQC-DEPT spectrum of A7 (Figure 4.49) was used to assign protons to their respective carbon atoms in the proposed structure.

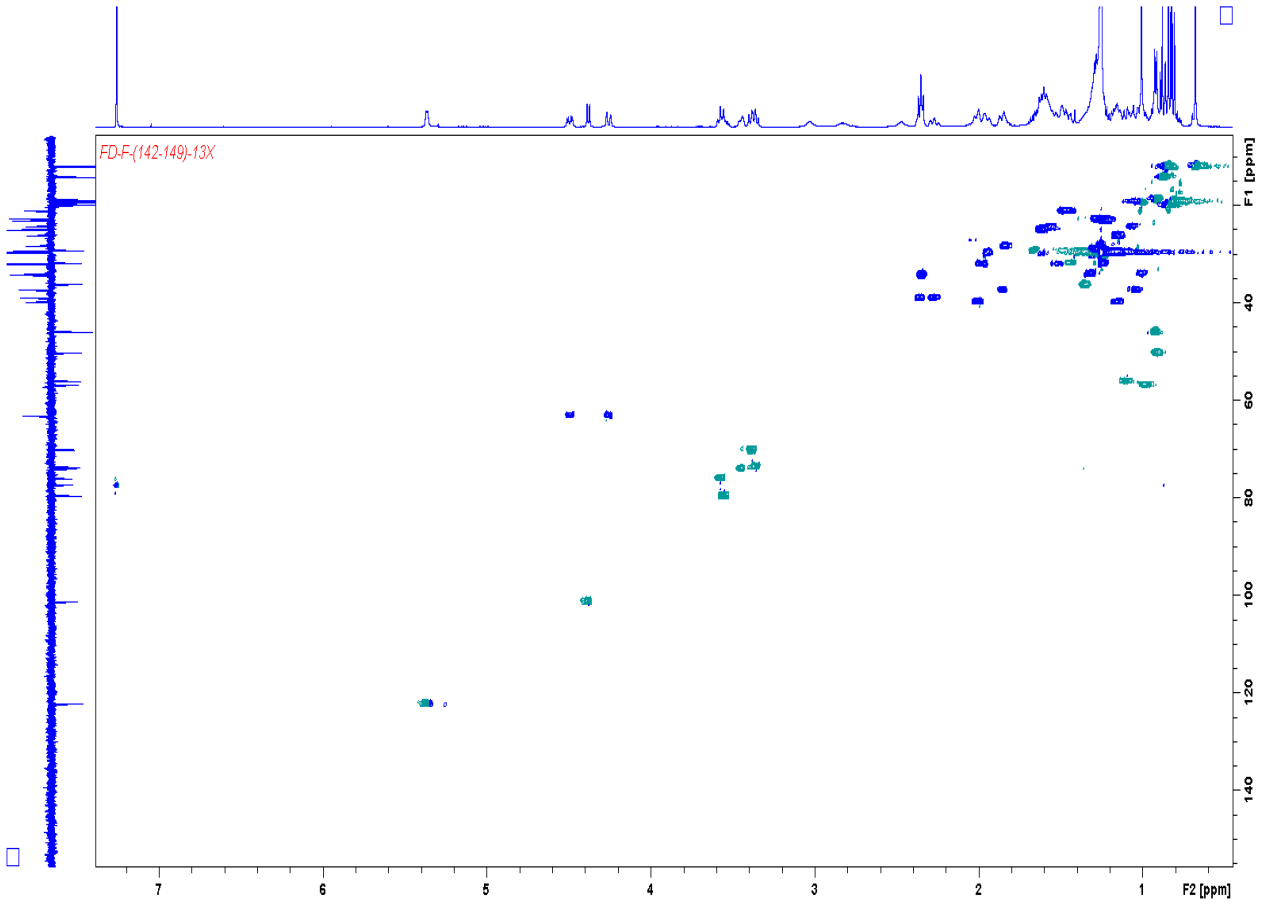


Figure 4.49: HSQC-DEPT spectrum of A7

4.9.2.6 COSY spectral analysis of A7

The COSY spectrum of A7 (Figure 4.50) is similar to that of A8. However, important correlation between sugar proton at δ_H 3.39 (H-5') and proton resonances at δ_H 3.38 (H-6A and 4.50 (6'B).

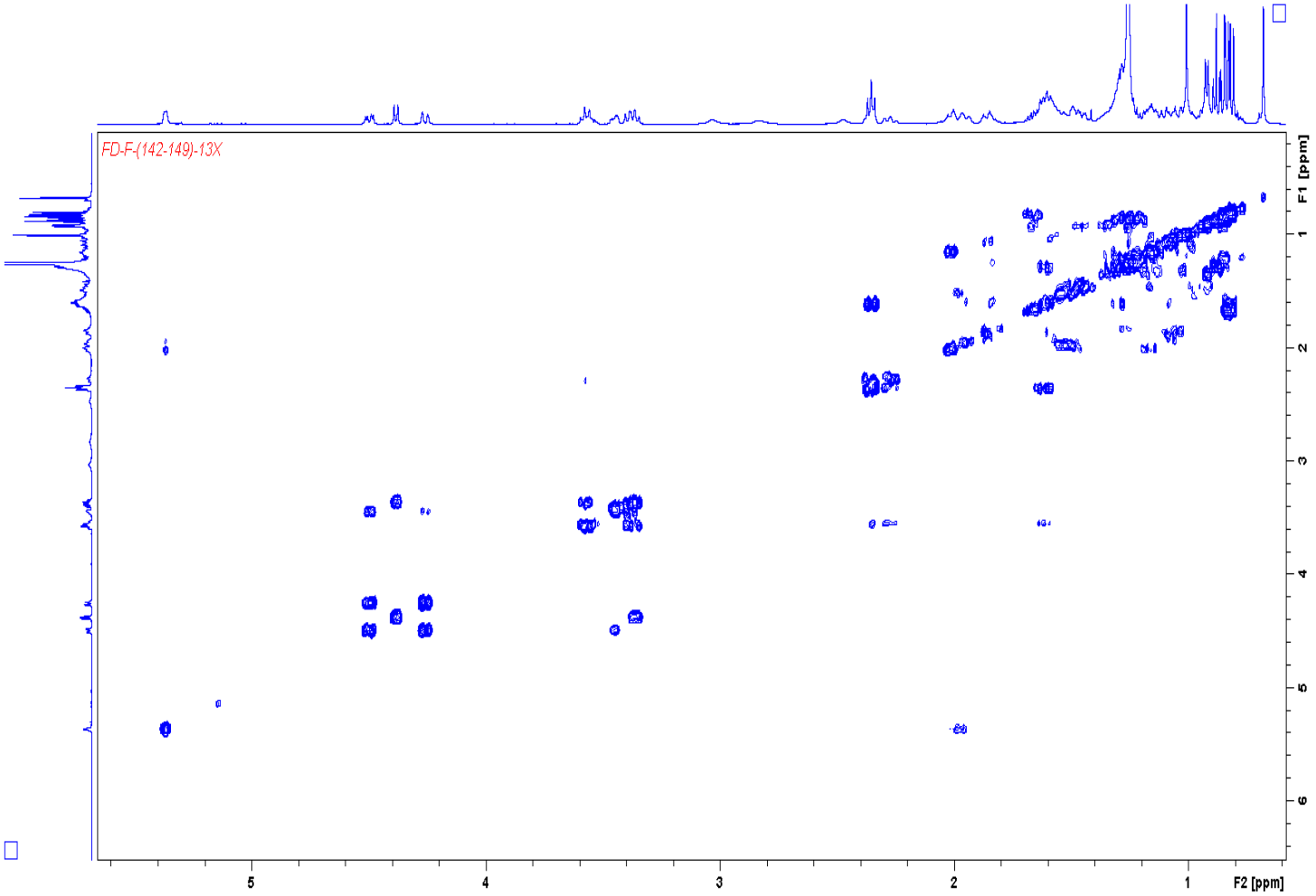


Figure 4.50: COSY spectrum of A7

4.9.2.7 HMBC spectral analysis of A7

The HMBC spectrum of A7 is presented in (Figure 4.51) Below: Important HMBC correlation was observed between the anomeric proton resonance at δ_H 5.37 and the carbon resonance at δc 77.5

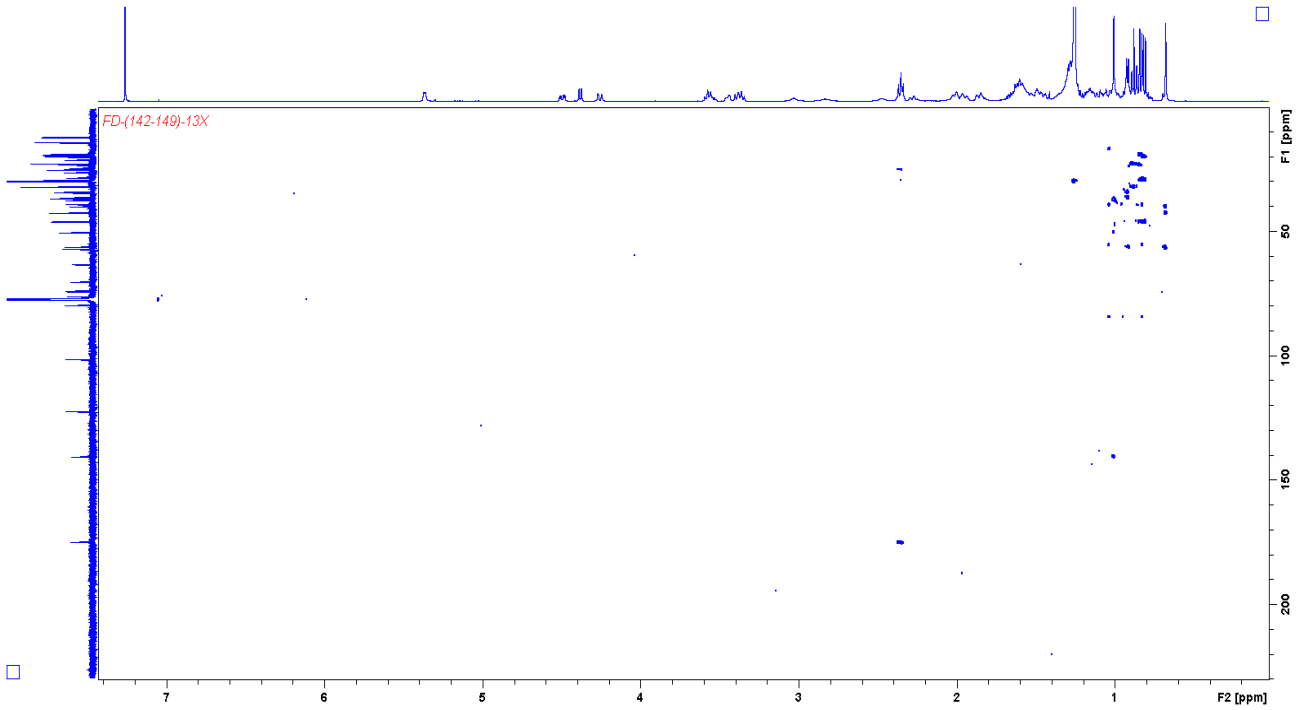


Figure 4.51: HMBC spectrum of A7

Table 4.10: NMR spectral data of A7 compared with literature

No.	**^{13}C NMR (500 MHz) $CDCl_3$**	**^{13}C NMR Lit (400 MHz) $CDCl_3$** (Hisashi *et al.*,1990)	**^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz)**	**^{1}H NMR Lit (500 MHz) $CDCl_3$** (Hisashi *et al.*,1990)	**HMBC (H ⟶ C)**	**COSY (H ⟶ H)**
1α	37.48 CH_2	37.6	1.85 *m*		5	

1β			1.01 *m*		5	
2 α	32.09 CH_2	30.3	1.96 *m*			
2β						
3	77.48 CH	78.3	4.37 *m*	4.49	1', 9, 10	
4α	39.7 CH_2	39.4	2.27 *dd* (4.69, 8.10)	2.38		
4 β			2.30 *m*	2.63	2 α	
5	140.3 C	141.0	-			
6	122.2 CH	122.0	5.37 t (3.6)			7α,
7 α	31.9 CH_2	32.2	1.96* *m*			6
7β			1.96* *m*			
8	32.15 CH	32.1	1.94* *m*			
9	50.2 CH	50.4	0.91* *m*			
10	37.2 C	37.0				
11β	19.9 CH_2	21.4	0.83* *m*			
12 α	39.98 CH_2	40.0	2.01 t (3.1, 12.6)			
12β			2.35 t (7.4, 15.3)			
13	42.4 C	42.6	-			
14	56.8 CH	57.0	0.98* d (6.4)	0.95		
15 α	25.5 CH_2	25.6	1.6* *m*	1.57	14	16α, 16β
15β			1.5* *m*	1.05		16α, 16β
16 α	28.3 CH_2	28.7	1.84 m	1.85		15α, 15β
16β			1.10*m	1.25		15α, 15β
17	56.2 CH	56.3	1.10 *m	1.0		
18	19.4 CH_3	19.3	0.68 s	0.67	5, 9, 10	
19	11.9* CH_3	12.0	1.01* s	0.94	13, 14, 17,	
20	36.7 CH	36.5	1.3 m			
21	19.1 CH_3	19.1	0.91* *d* (6.4)	0.99	17, 20, 22	
22 A	34.20 CH_2	34.3	1.32 *m*	1.07		
22B			1.20*			
23 A	26.1 CH_2	26.4	1.6 *m*			
23 B			1.20* *m*		28	
24	45.8* CH	46.1	0.91* *m*		28	
25	26.31 CH	29.5				
26	19.3* CH_3	19.5	0.86* *d* (2.6)	0.90		
27	19.8 CH_3	20.1	0.84 *d* (1.86)	0.86		
28A	23.1* CH_2	23.4	0.82* *m*			
28B			0.83* m			
29	11.9* CH_3	12.2	0.84* *dd* (2.4, 9.8)	0.87	24, 28	
1'	101.2 CH	102.6	4.37 *d* (6.6)	4.29	3'	
2'	75.8 CH	75.4	3.35 *m*			
3'	79.5 CH	78.7	3.87 *m*	3.87	1', 5'	
4'	70.1 CH	71.7	3.38 *d* (4.5)			5
5'	76.0 CH	78.5	3.39 *m*			4', 6'A, 6'B
6' A	63.1 CH_2	62.9	4.26 *dd* (1.7, 12.2)	3.40		5
6' B			4.50 *dd* (4.5, 11.0)	4.50		5

* = overlapping proton signals

4.10 Isolation of A8

Compound A8 (4.4 mg) was isolated as orange-red oil, Rf = 0.74 using dichloromethane : ether 19:1 as solvent system and $[\alpha]^{21}_{D}$ = -0.33(c = 0.063 CH_2Cl_2) (Plate 4.10)



Plate 4.10: TLC chromatogram of A8 using dichloromethane : ether 19:1 as solvent system

4.10.1. Physical test carried out on A8

Solubility of A8

A8 was found to be soluble in chloroform.

4.10.2 SPECTRAL ANALYSIS OF A8

4.10.2.1 IR spectral analysis of A8

The IR spectrum of A8 (Figure 4.52) showed an absorption band at 3448 (-OH stretching) 2920, 2865 (aliphatic CH_2 ans CH_3 stretching), 1641 (conjugated C=O stetching).

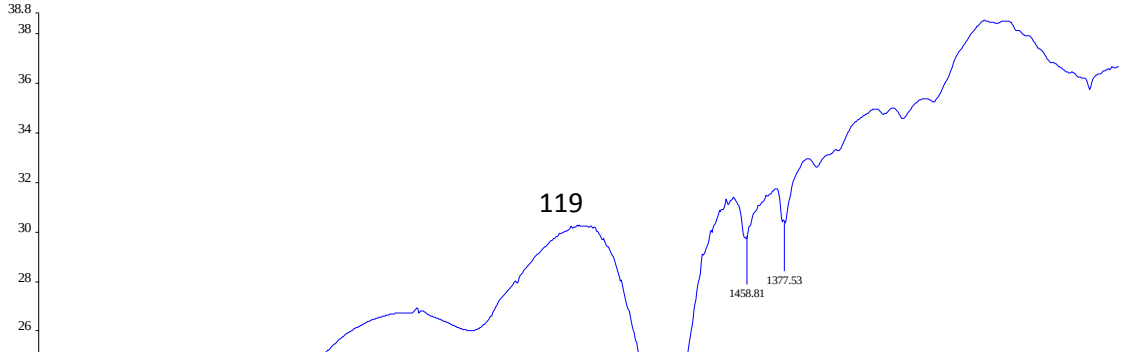


Figure 4.52: IR spectrum of A8

4.10.2.2 ^{1}H NMR spectral analysis of A8

The ^{1}HNMR(400 MHz, $CDCl_3$) spectrum of A8 (Figure 4.53) showed proton resonances at δ_H 2.03 (s, 3H-2a), 2.00 (s, 3-H3a), 1.96 (s, 3H-5a), 2.57 (m, 2H-7A), 1.42 (m, 2H-7b), 1.18 (s, 3H-9a), 1.45 (m, 2H-10), 1.47 (m, H-13), 1.38 (m, 2H-15), 1.31 (m, H-17), 1.49 (m, H-21), 0.89, (d, *J* = 1.5, 3H-21a) and 0.87 (d, *J* = 1.5 3H-22).

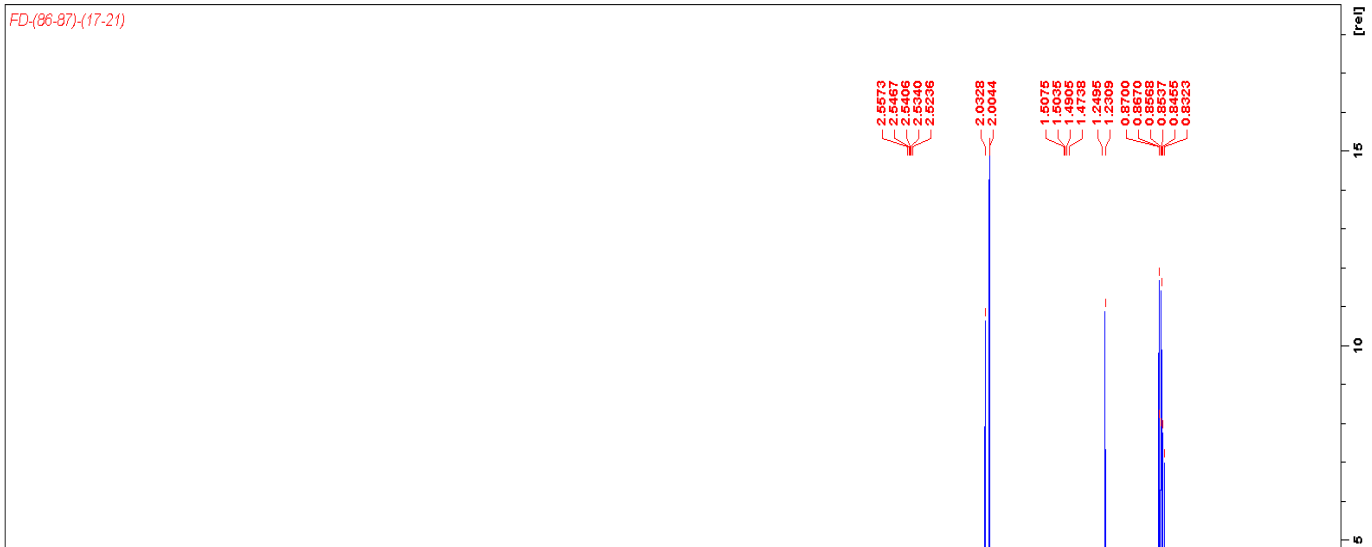


Figure 4.53:[1]H NMR spectrum of A8

4.10.2.3 ^{13}C NMR spectral analysis of A8

The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of A8 (Figure 4.54) showed a total of twenty nine carbon resonances at δc 187.4 (C-1), 187.9 (C-4), 140.8 (C-2), 140.7 (C-3), 140.4 (C-5), 12.2 (C-5a), 144.7 (C-6), 21.6 (C-7), 40.5 (C-8), 72.9 (C-9), 42.5 (C-10), 21.5 (C-11), 37.5 (C-12), 22.9 (C-13), 19.9 (C-13a), 37.7 (C-l4), 25.0 (C-15), 37.7 (C-16), 33.0 (C-17), 19.9 (C-17a), 37.8 (C-18), 24.7 (C-19), 39.6 (C-20), 28.2 (C-21), 21.6 (21a) and 22.8 (C-22).

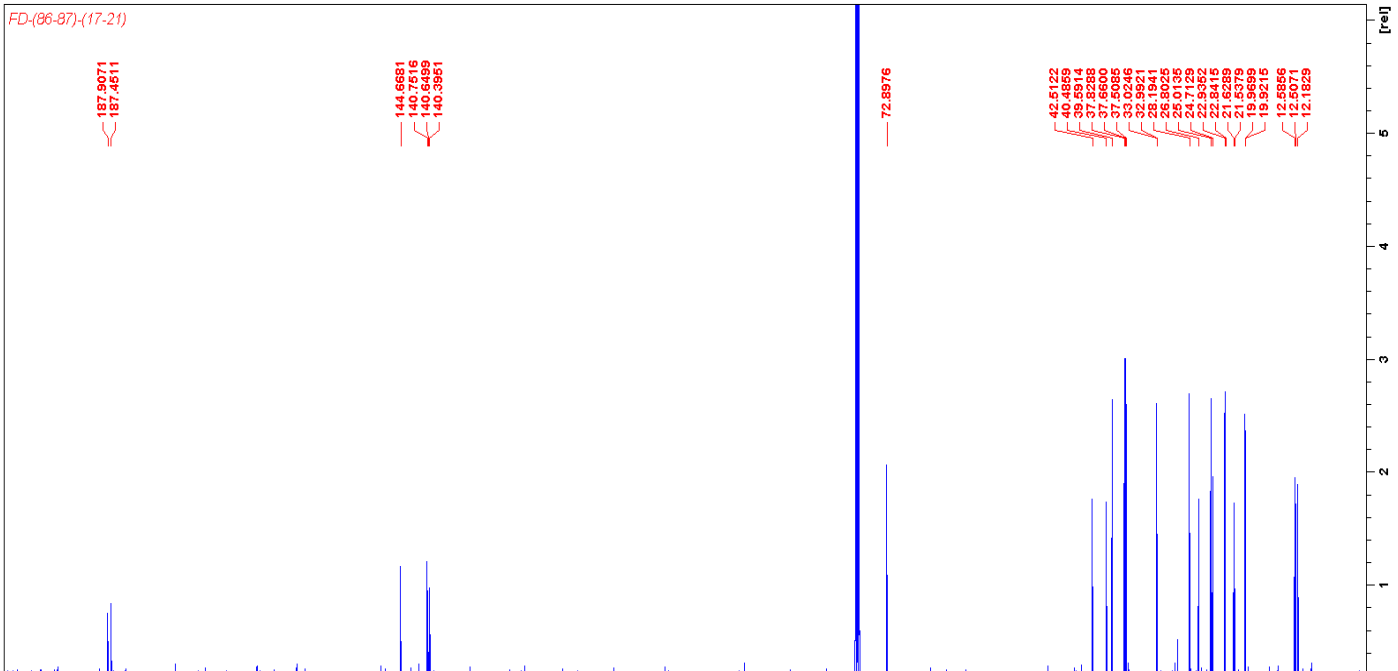


Figure 4.54: ^{13}C NMR spectrum of A8

4.10.2.4 DEPT 135 spectral analysis of A8

The DEPT-135 spectrum of A8 (Figure 4.55) showed eight methyl, eleven methylene and three methine carbon signals.

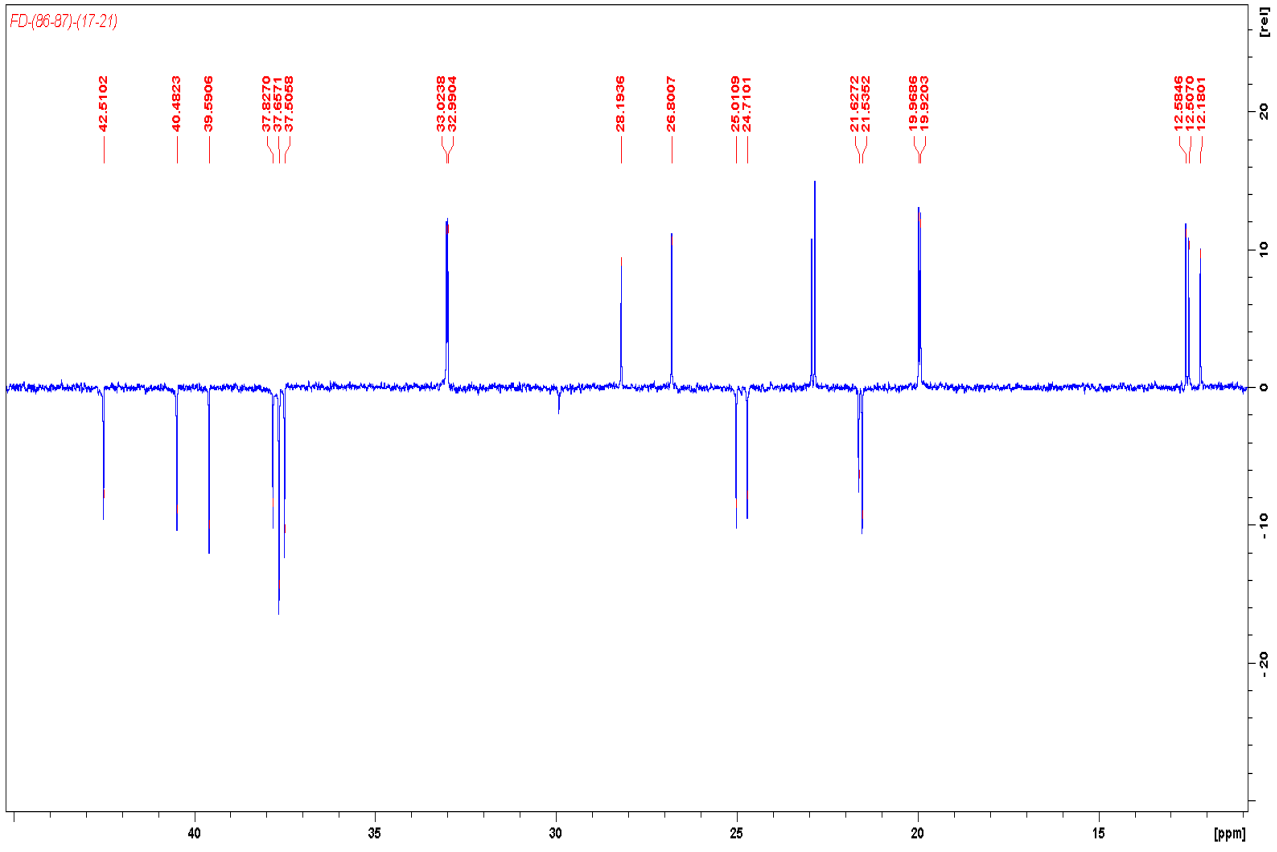


Figure 4.55: DEPT 135 spectrum of A8

4.10.2.5 HSQC-DEPT spectral analysis of A8

The HSQC-DEPT spectrum of A8, used to assigned protons to their respective carbon atoms is shown in Figure 4.56 below:

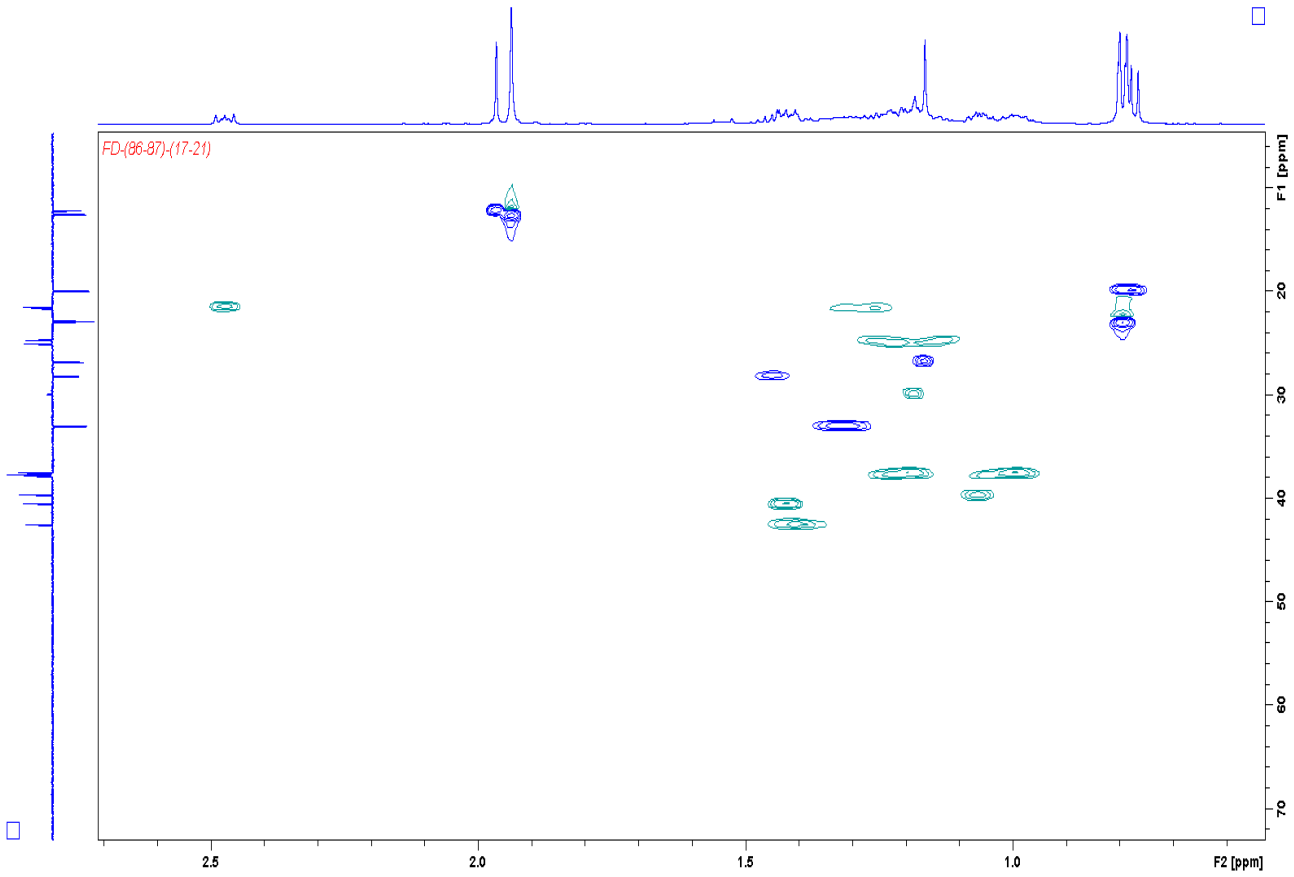


Figure 4.56: HSQC-DEPT spectrum of A8

4.10.2.6 COSY spectral analysis of A8

The COSY spectrum of A8 (Figure 4.57) showed cross peaks between proton resonance at δ_H1.38 (H-12) and proton resonances at δ_H 1.18 (2H-20), 0.89 (3H-21a) and 0.87 (3H-22). Other important correlations observed in the spectrum include those between δ_H 1.45 (2H-10) and δ_H 1.44 (2H-11) and between δ_H 1.14 (2H-11) and δ_H 1.38 (2H-12)

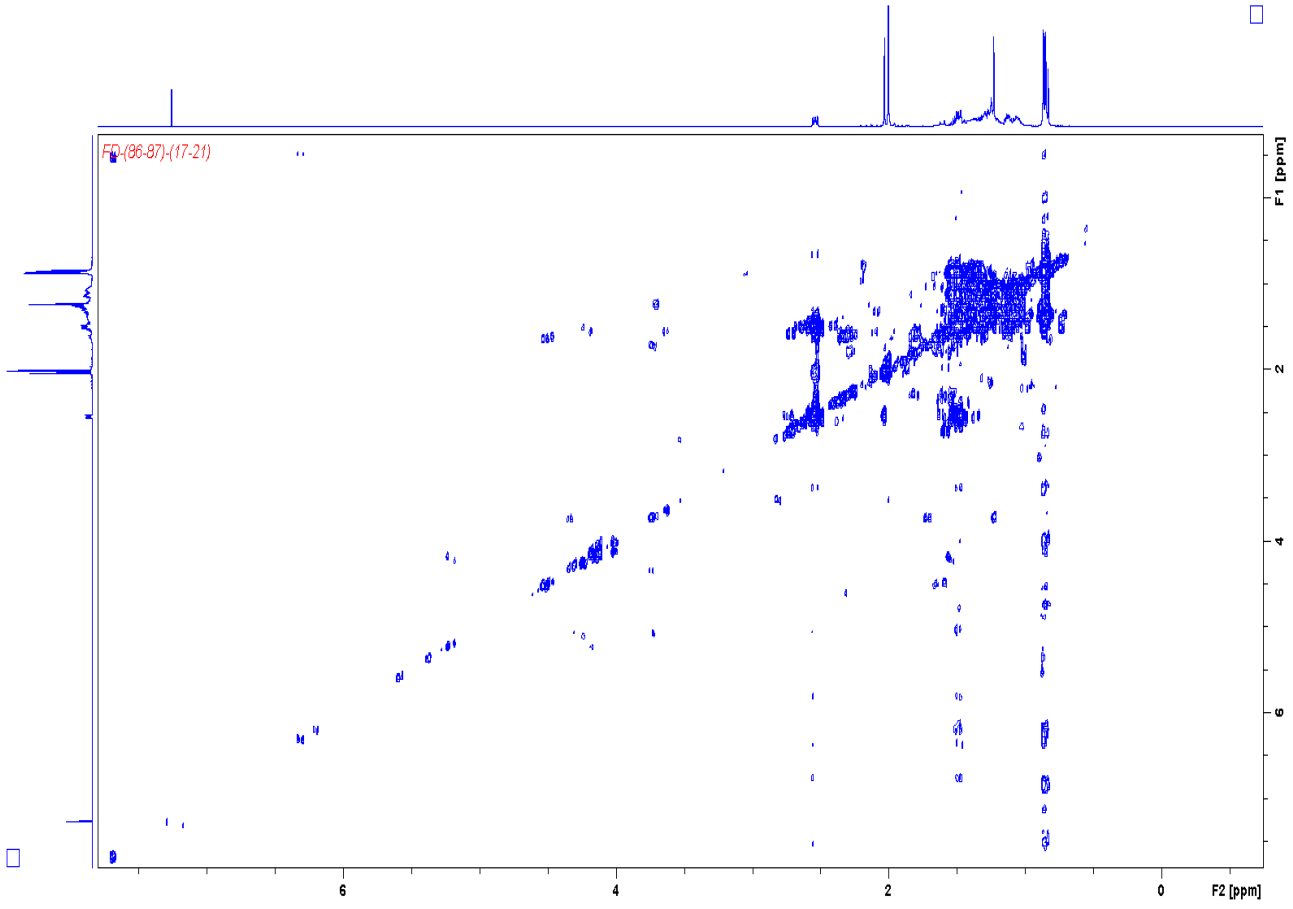


Figure 4.57: COSY spectrum of A8

4.10.2.7 HMBC spectral analysis of A8

The HMBC spectrum of A8 (Figure 4.58) showed correlations between the methyl group proton resonance at δ_H 2.03 (3H-2a) and the carbonyl carbon resonance at δc 186.9 (C-1). The two methyl group proton resonances at δ_H 2.00 (3-H-3a) and δ_H 1.96 (3-H-5a) also showed cross peaks with the carbonyl carbon resonance at δc 187.2. Correlation was also observed between proton signal at δ_H1.38 (H-12) and two methyl carbon resonances at δc at 21.6 (3H-21a) and 22.8 (C-22).

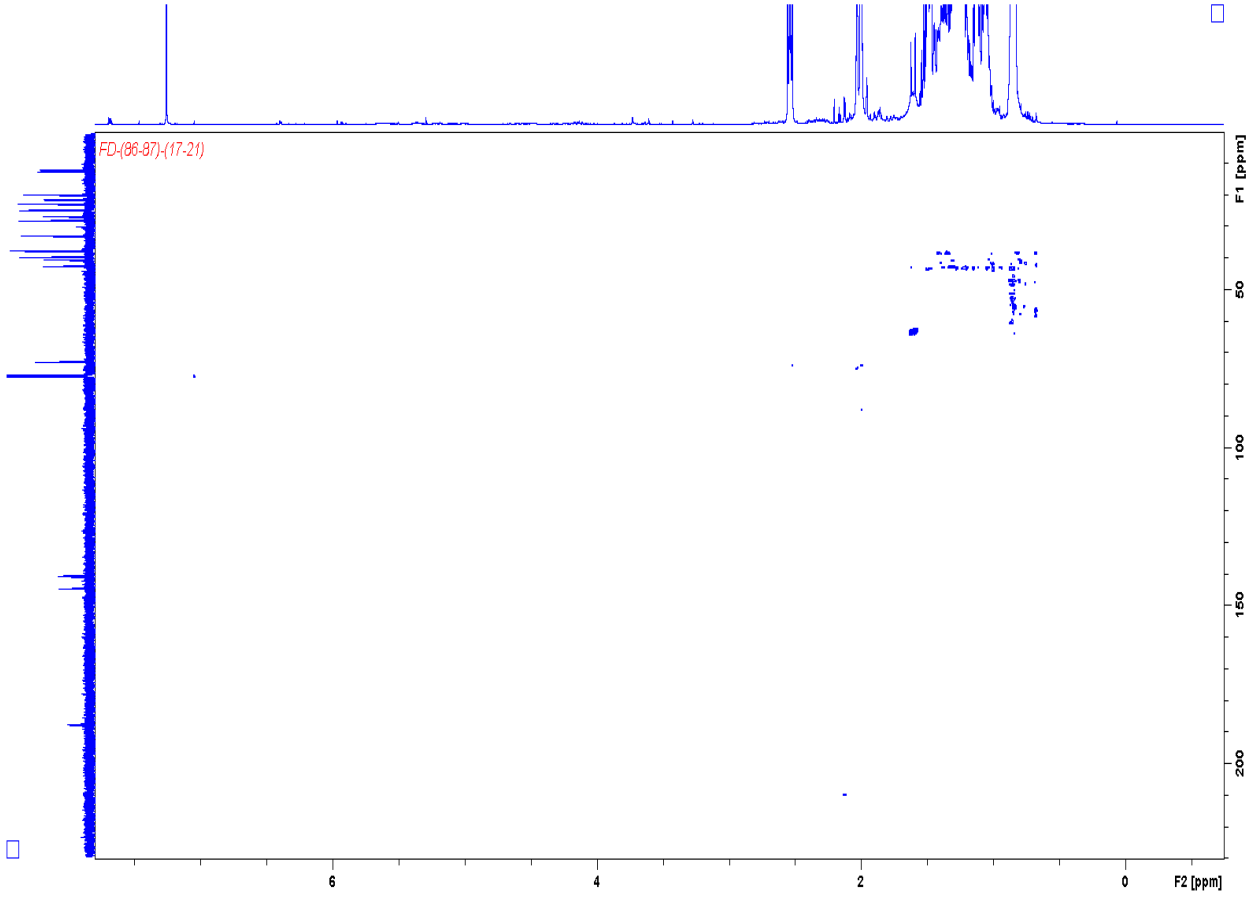


Figure 4.58: HMBC spectrum of A8

4.10.2.8 ROESYspectral analysis of A8

The ROESY spectrum of A8 was used to confirm the relative stereochemistry of the compound (Figure 4.59).

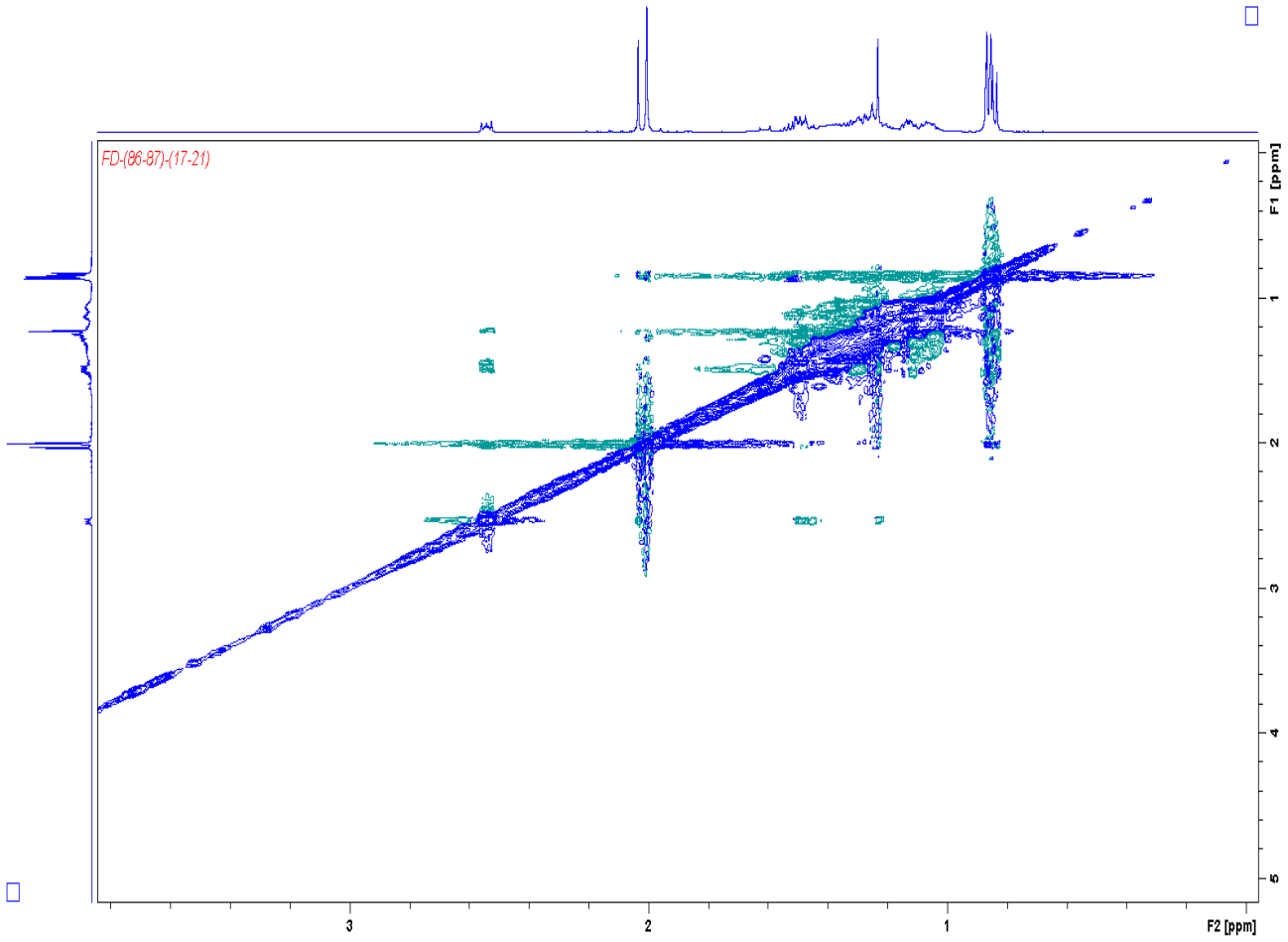


Figure 4.59: ROESY spectrum of A8

Table 4.11: NMR spectral data of A8 compared with literature

No.	^{13}C NMR (500 MHz) $CDCl_3$	^{13}C NMR (Williams *et al*., 2012) (400 MHz) $CDCl_3$	^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz)	^{1}H NMR (Williams *et al*., 2012) (500 MHz) $CDCl_3$ (*J* in Hz)	HMBC (H →C)	COSY (H →H)
1	187.4 C	186.9				
2	140.8 C	140.4				
2 a	12.6 CH_3	12.1	2.03 s	1.73	1,2,3,3a	
3	140.7 C	140.2				
3a	12.5 CH_3	12.0	2.00 s	1.72	2,3,4	
4	187.9 C	187.2				
5	140.4 C	140.02				
5a	12.2 CH_3	11.9	1.96 s	1.93	4,5,6,7	
6	144.7 C	144.6				
7	21.6 CH_2	21.7	2.57* m	2.51	1,5,6,9	8
8	40.5 C	40.7	1.42* m	1.40	6,7,9	7
9	72.9 C	72.1				
9a	26.8 CH_3	27.0	1.18 m	1.12	8,9,10	
10	42.5 CH_2	42.6	1.45* m	1.42	8,9,11	11
11	21.5 CH_2	21.9	1.44 m	1.43	9,10,15,13	10
12	37.5 CH_2	37.8	1.38 m	1.36		13
13	22.9 CH	33.0	1.47 m	1.46	12,13a,14	12
13a	19.9 CH_3	20.1	0.84	0.96	12,13,14	
14	37.7* CH_2	37.9	1.18* m	1.17		
15	25.0 CH_2	25.3	1.38* m	1.34		
16	37.7* CH_2	37.9	1.18* m	1.17		17,18
17	33.0 CH	33.2	1.48 m	1.47		16
17a	19.9 CH_3	20.1	0.99 d (9.5)	0.97	16,17,18	
18	37.8 CH_2	38.2	1.31 m	1.38		17,19
19	24.7 CH_2	25.1	1.33 m	1.35		18,20
20	39.6 CH_2	39.8	1.18 m	1.18		19,21
21	28.2 CH	28.4	1.49 m	1.52	21a,22	20,22
21a	21.6 CH_3	22.8	0.89 d (1.5)	0.91		
22	22.8 CH_3	22.9	0.87 d (1.5)	0.91	21,21a	21

4.11 Isolation of A9

Compound A9 (6.8 mg) was isolated as brown amorphous solid, Rf = 0.53 using

Dichloromethane : ethylacetae 8:2 as solvent system (Plate 4.11).



Plate 4.11: TLC chromatogram of A9 using ethylacetate (100%) as solvent system

4.11.1. Physical tests carried out on A9

Solubility of A9

A9 was found to be soluble in chloroform and methanol.

Melting point of A9

The compound was found to have a melting point range of 112-114^{0}C.

4.11.2 SPECTRAL ANALYSIS OF A9

4.11.2.1 LCMS spectral analysis of A9

The mass spectrum of A9 (Figure 4.60) showed molecular ion peak M^+ at *m/z* = 223.1.



Figure 4.60: LCMS spectrum of A9

4.11.2.2 IR spectral analysis of A9

The IR spectrum of A9 (Figure 4.61) showed absorption bands at 3400 cm^{-1} (O-H stretching), 1665 cm^{-1} (C=C stretching), 1679 (C=O) and 1126 cm^{-1} (C-O stretching).

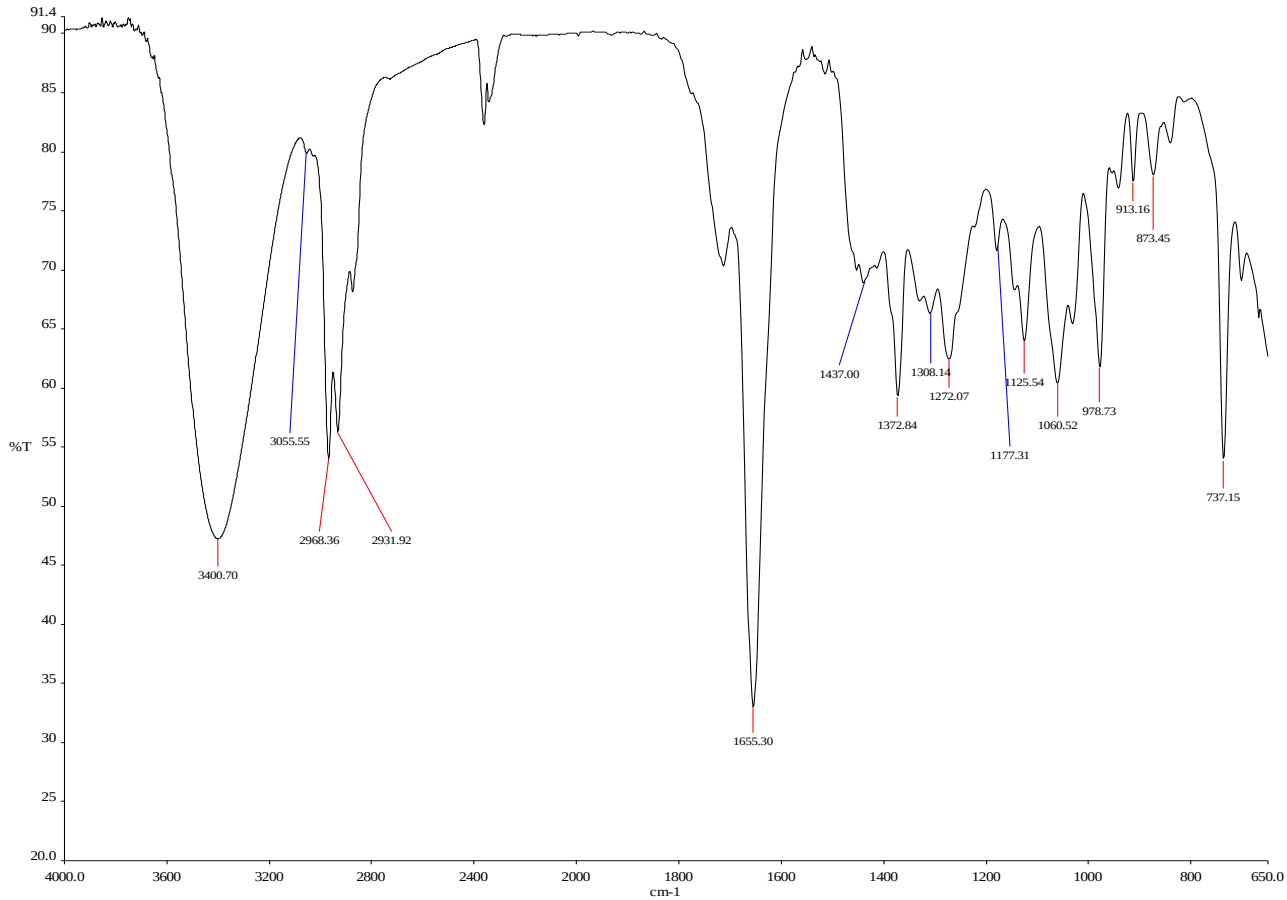


Figure 4.61: IR spectrum of A9

4.11.2.3 ^{1}H NMR spectral analysis of A9

^{1}H-NMR (500 MHz, CD_3OD) spectrum of A9 (Figure 4.62) showed proton signals at δ_H 2.19 (1H, d, *J*=17.1 Hz, H-2α), 2.48 (d, *J* = 17.1 Hz, H-2β), 5.89 (t, *J* = 1.33 Hz, H-4), 5.81 (overlap signal, H-7), 5.82 (overlap signal, H-8), 4.34 (dd, *J* = 4.5 Hz; *J* = 6.5 Hz, H-9), 1.27 (3H, d, *J* = 6.5 Hz, H-10), 1.06 (3H, s, 3H-11), 1.04 (s, 3H-12) and 1.93 (s, 3H-13).

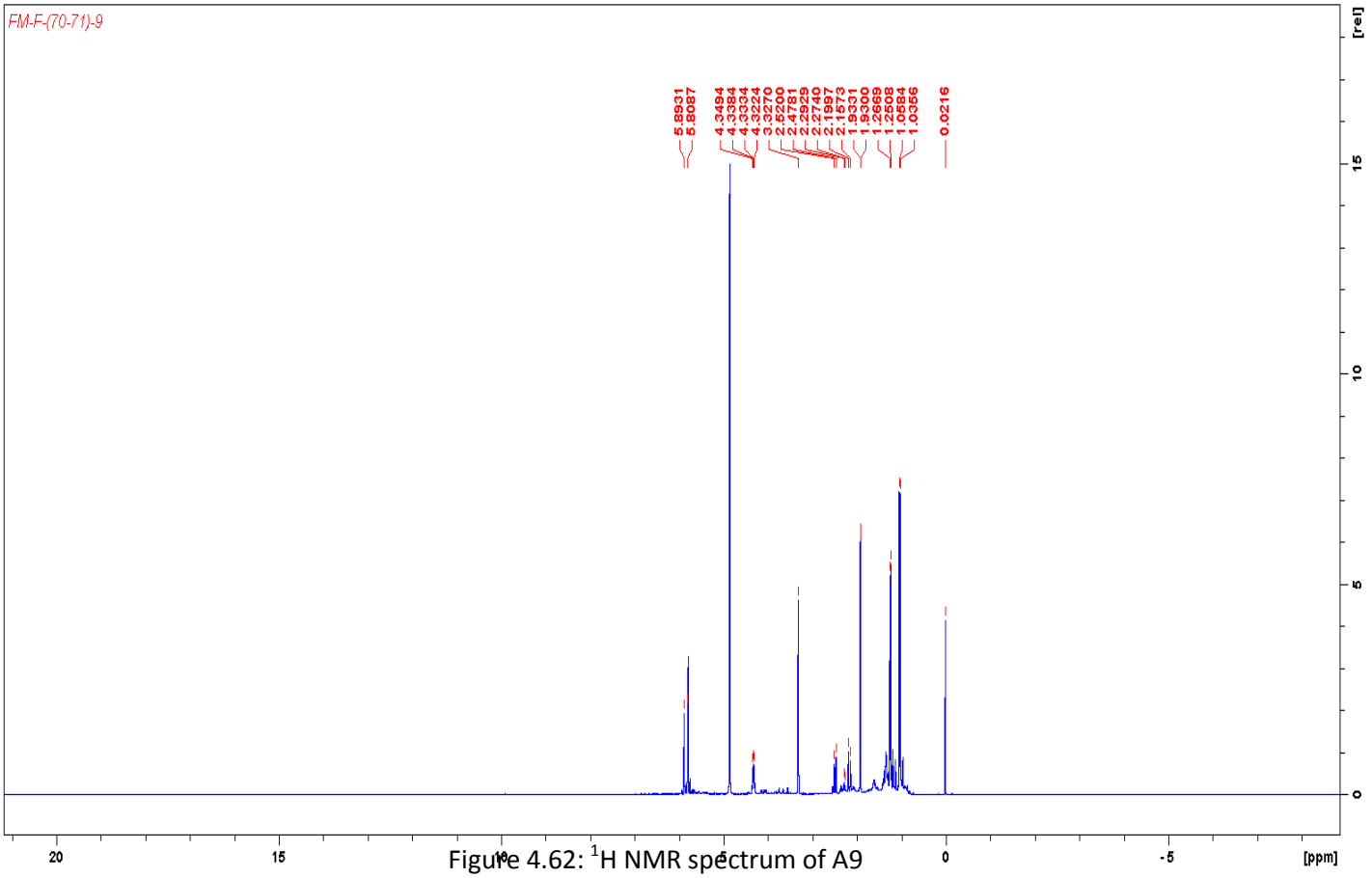


Figure 4.62: ^{1}H NMR spectrum of A9

4.11.2.4 ^{13}C NMR spectral analysis of A9

The ^{13}C-NMR (125 MHz, CD3OD) spectrum of A9 (Figure 4.63) showed a total of thirteen resonances at δc 42.6 (C-1), 50.9 (C-2), 201.4 (C-3), 127.6 (C-4), 167.3 (C-5), 80.1 (C-6), 130.7 (C-7), 137.3 (C-8), 68.8 (C-9), 24.0 (C-10), 23.6 (C-11), 24.6 (C-12) and 19.7 (C-13).

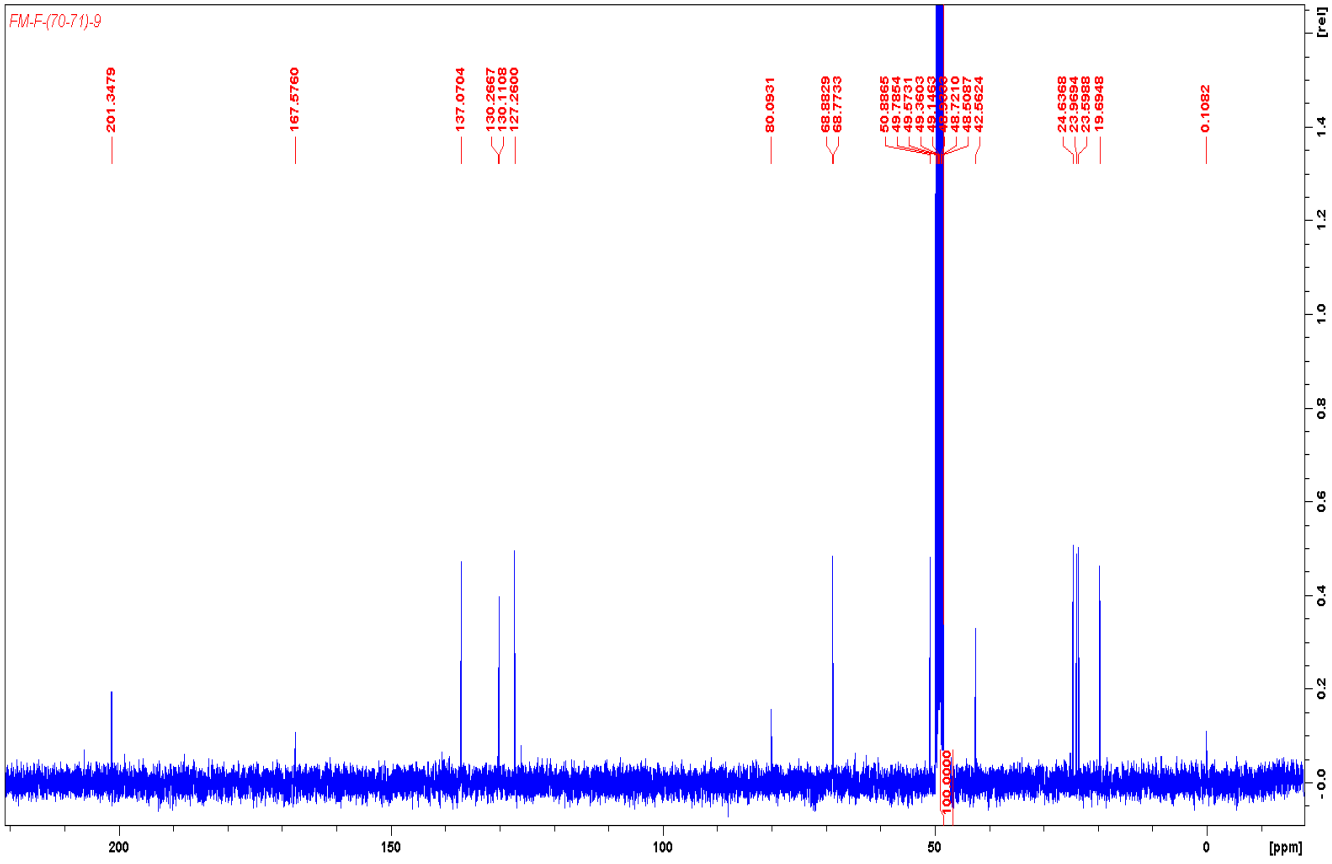


Figure 4.63:^{13}C NMR spectrum of A9

4.11.2.5 DEPT-135 spectral analysis of A9

The DEPT-135 spectrum of A9 (Figure 4.64) revealed four methyl, one methylene and four methine carbon signals. Four quaternary carbon signals which disappeared in the spectrum were also noted.

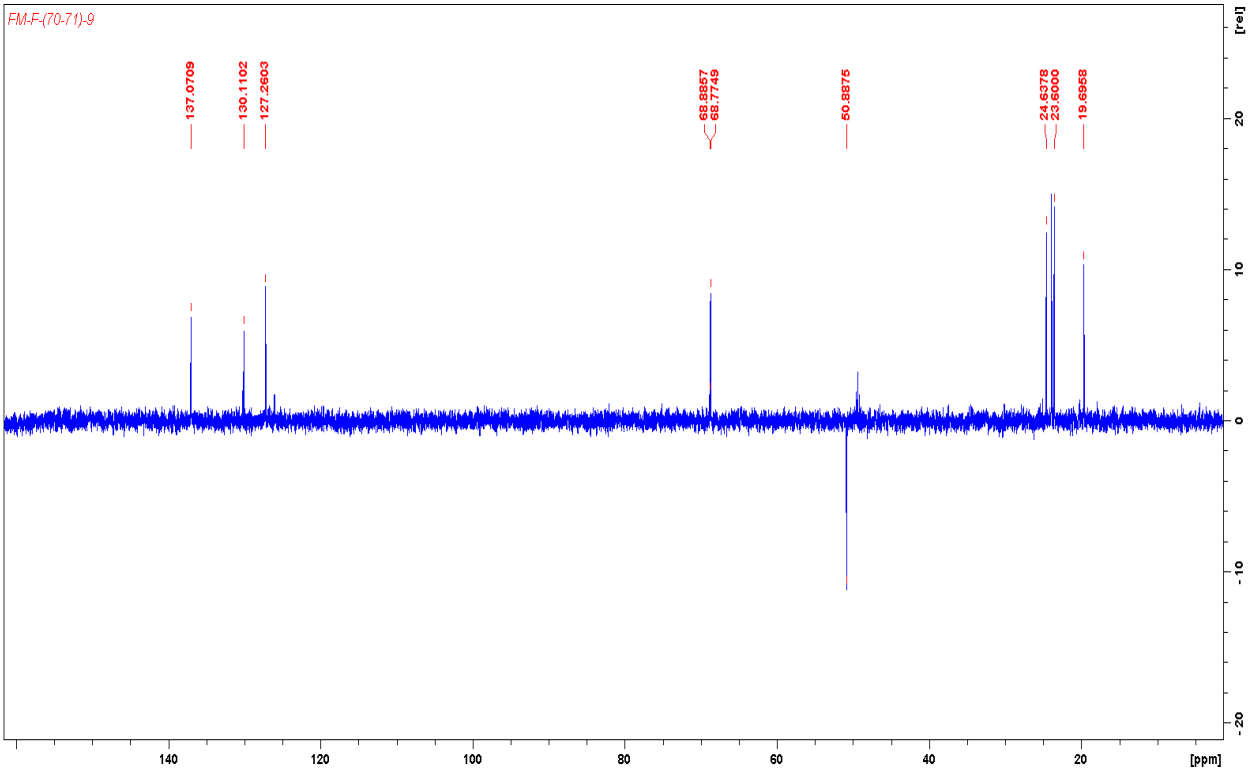


Figure 4.64: DEPT-135 spectrum of A9

4.11.2.6 HSQC-DEPT spectral analysis of A9

The HSQC-DEPT spectrum of A9 is shown in (Figure 4.65). It was used to assigned protons to their respective carbon atoms.

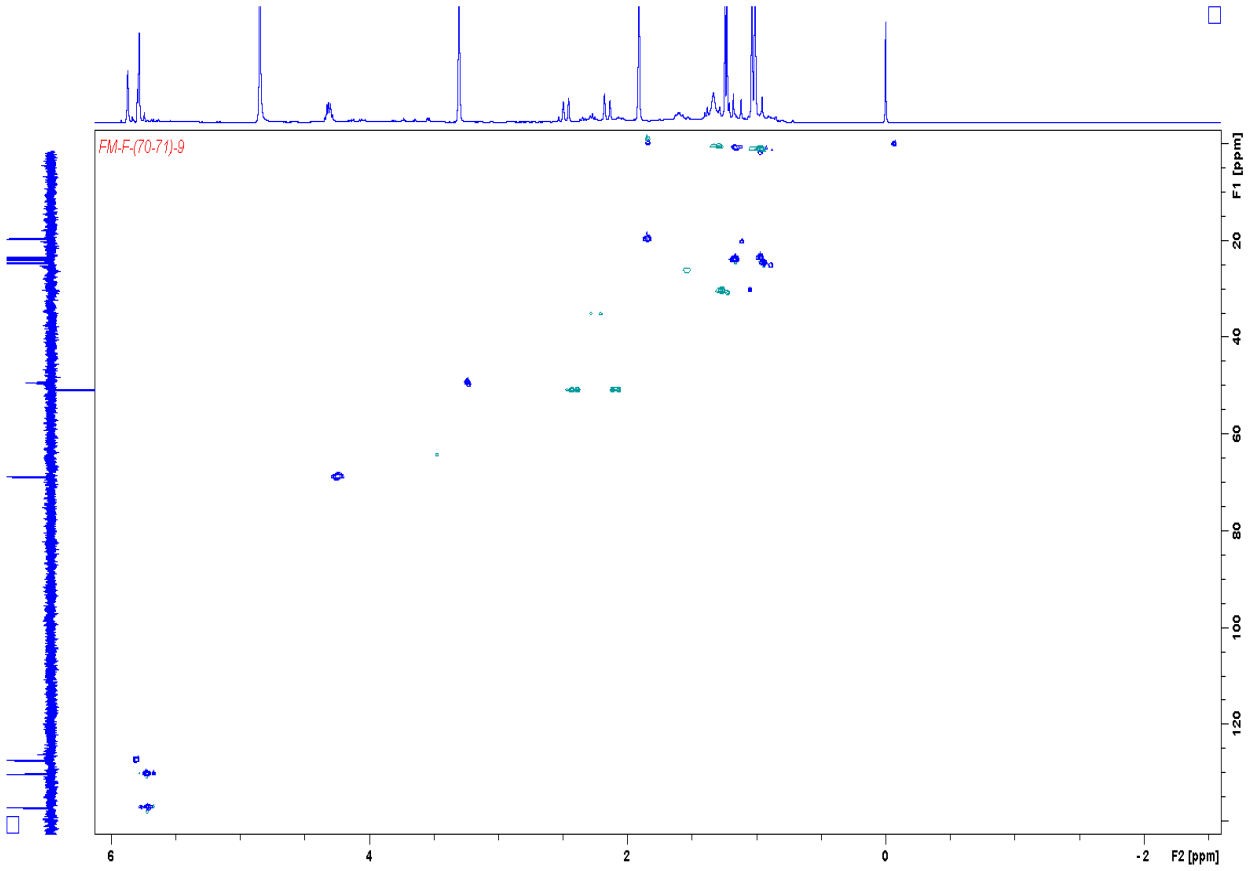


Figure 4.65: HSQC-DEPT spectrum of A9

4.11.2.7 COSY spectral analysis of A9

The COSY spectrum of A9 (Figure 4.66) showed correlation between the methyl proton resonance at δ_H 1.27 (3H-10) and the H-7 (δ_H 5.81), H-8 (δ_H 5.82) and H-9 (δ_H 4.34).

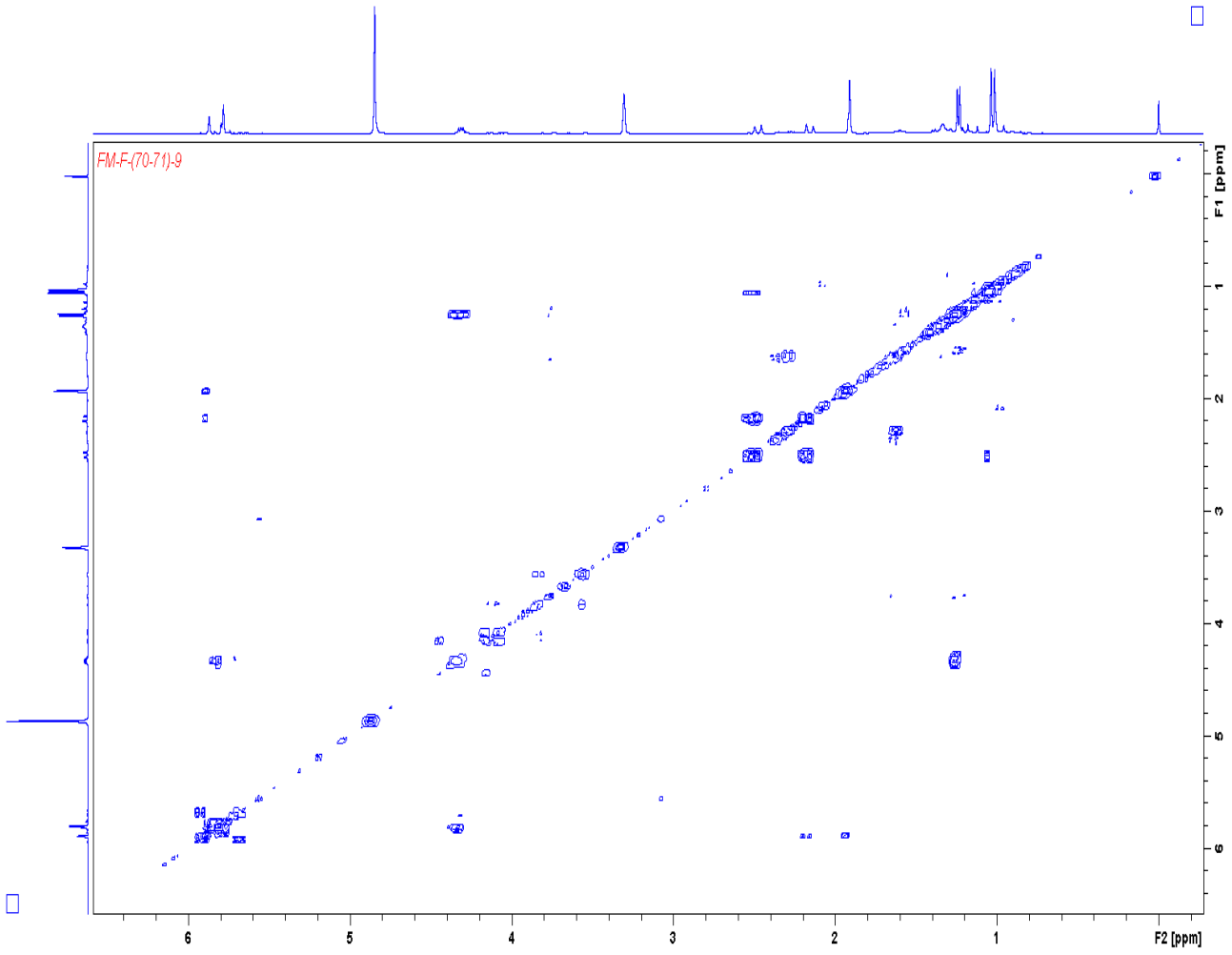


Figure 4.66: COSY spectrum of A9

4.11.2.8 HMBC spectral analysis of A9

The HMBC spectrum of A9 (Figure4.67) showed important correlations between the two germinal proton resonances at δ_H 2.19 (2H-2α) and δ_H 2.48 (2H-2β) and the carbon signals at δc127.6 C-4), δc 201.3 (C-3), δc 42.6 (C-1) and 80.1 (C-6). The methyl proton signal at δ_H 1.04 (3H-11) also showed correlations with carbon resonances at δc 50.1 (C-2) and δc 80.1 (C-6). These and other correlations observed were used to confirm the connectivity networks through the proposed structure.

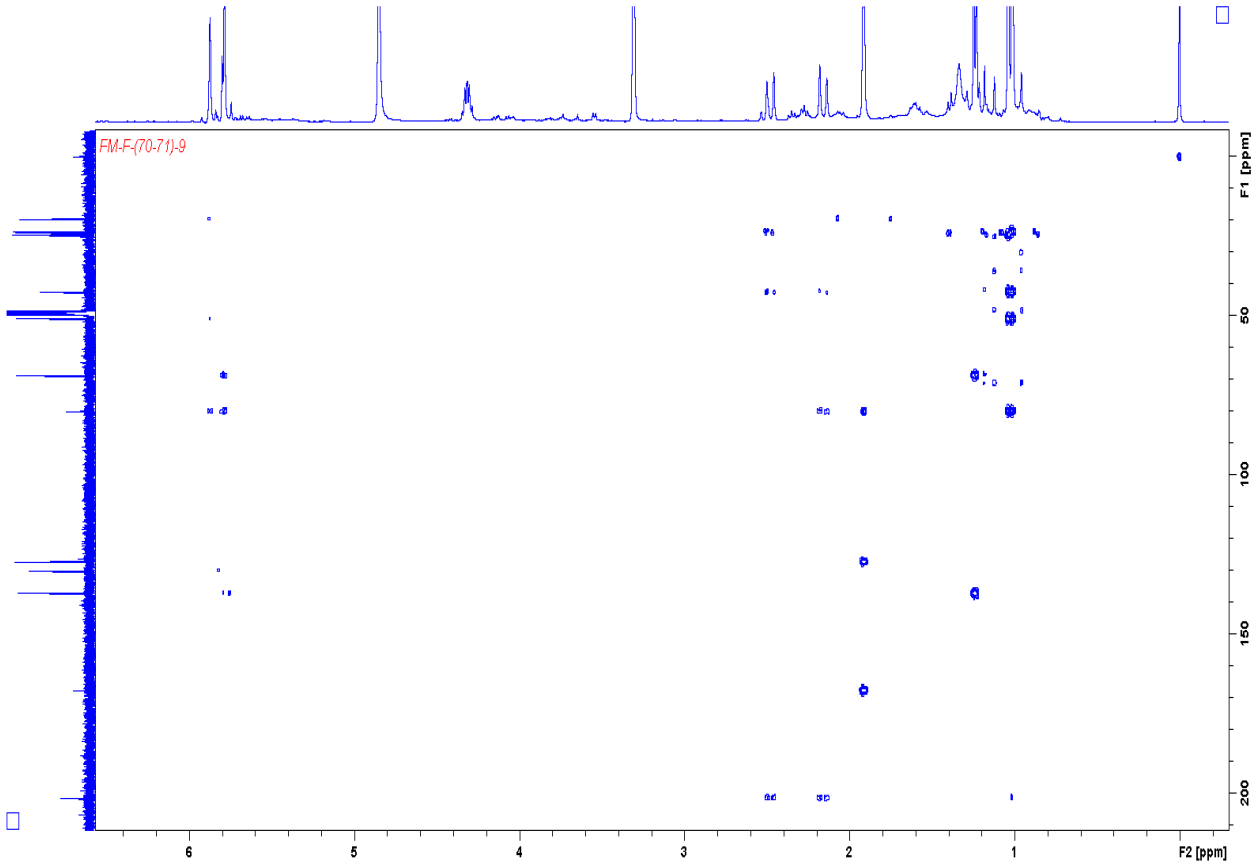


Figure 4.67: HMBC spectrum of A9

4.11.2.9 ROESY spectral analysis of A9

The ROESY spectrum of A9, shown in Figure 4.68, was used to confirm the relative stereochemistry of the structure of the compound.

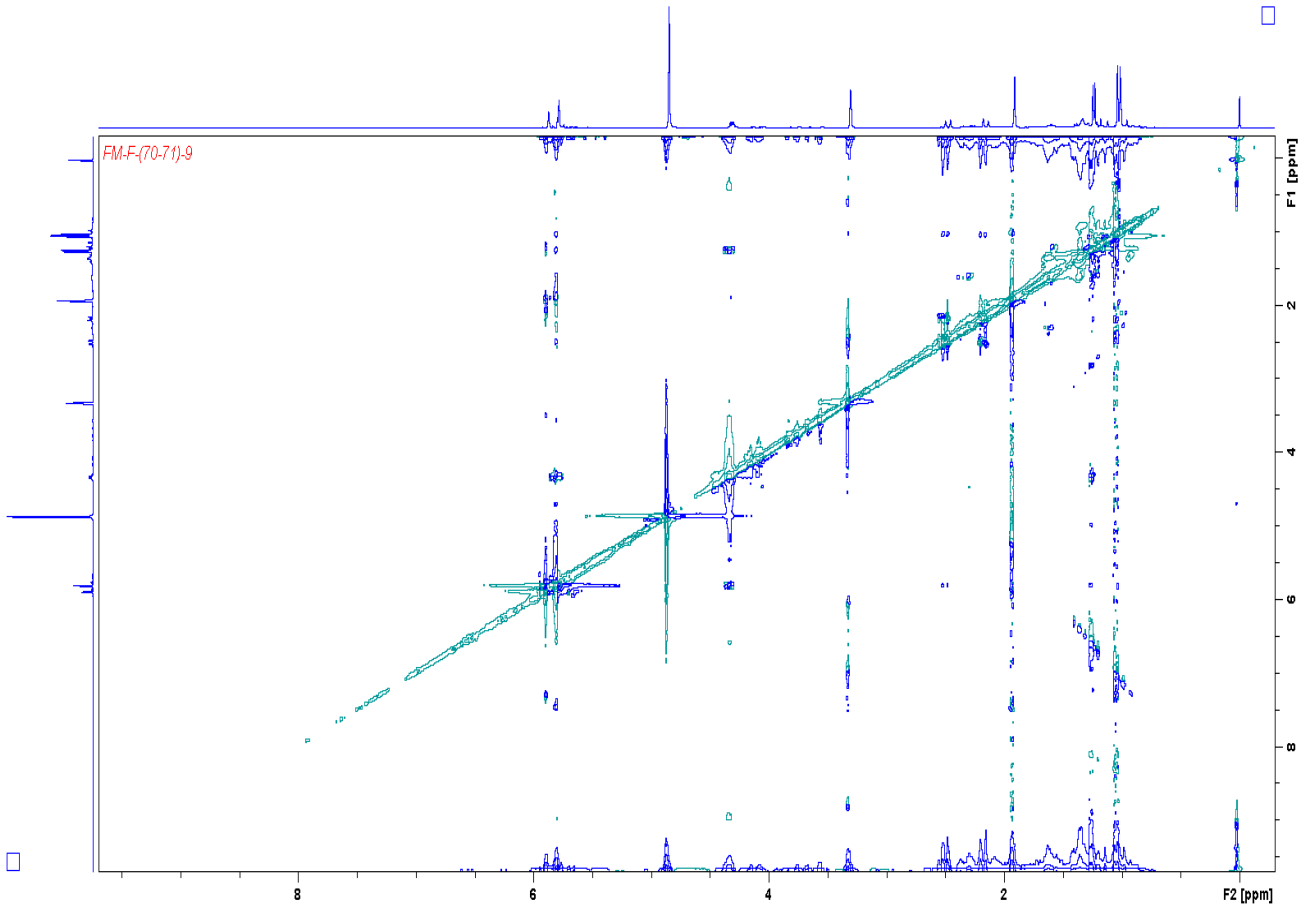


Figure 4.68: ROESYspectrum of A9

4.11.2.10 Electronic Circular Dicroism (ECD) spectral analysis of A9

The Electronic Circular Dicroism (ECD) spectrum of A9 (Figure 4.69) showed positive cotton effect at 252 nm.

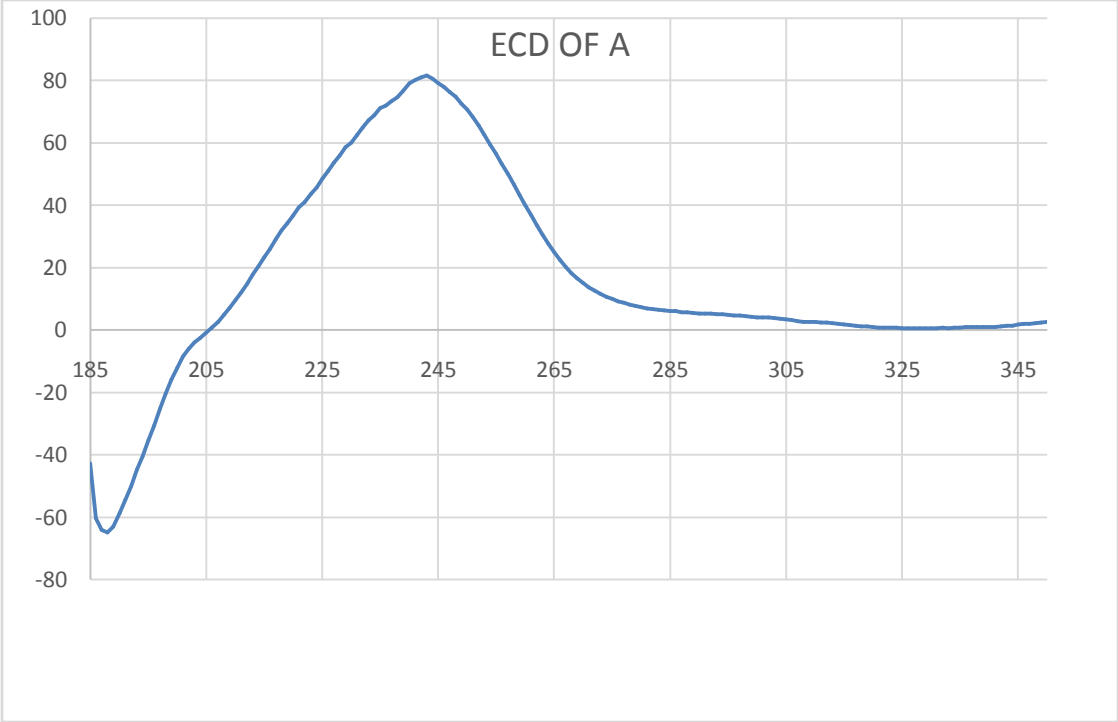


Figure 4.69: Electronic Circular Dicroism (ECD) spectrum of A9

Table 4.12: NMR spectral data of A9 compared with literature

NO.	**^{13}C NMR (500 MHz) $CDCl_3$**		**^{13}C NMR (500 MHz) $CDCl_3$ (Nguyen *et al.*, 2015)**	**^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz)**	**^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz)(Nguyen *et al.*, 2015)**	**HMBC (H→ C)**	**COSY (H→ H)**	**ROESY (H→ H)**
1	42.6	C	42.4	-	2.18			
2 α	50.9	CH_2	50.7	2.48 d (*J*=17.1)	2.53	1,6,9,11.	2β	4,7,11,12
2β				2.19 d (17.1)	2.18	3,6,9,10,11	2α,	11,12
3	201.4	C	201.3	-				
4	127.6	CH	127.1	5.89 s	5.90	2,3,5,6	-	2α,9,10, 11,12,13

5	167.3	C	167.5	-			-	
6	80.1	C	80.0	-			-	
7	130.7	CH	130.1	5.81 d (*2*.17)	5.82	6,7,8,9	8	2α,9,10, 11,12,13
8	137.3	CH	136.9	5.82 d (*2*.17)	5.81	6,7,9	9	2α,9,10,13
9	68.8	CH	68.7	4.34 m	4.34	4,7,8,10	8	4,7,8,10,13
10	24.0	CH_3	23.8	1.27 d (6.5)	1.26	8,9	9	4,7,8,9
11	23.6	CH_3	23.5	1.04 d (9.0)	1.06	1,2,3,6,11,12	2α,2β	2α7,13
12	24.6	CH_3	24.5	1.05 d (9.0)	1.04	1,2,6,11,		2α,2β,7
13	19.7	CH_3	19.6	1.93 d (1.23)	1.94		4	4,7,8,9,12
14								

4.12 Isolation of A10

Compound A10 (6.0 mg) was isolalated as brown amoprhouse solid, Rf = 0.67 using ethylacetate (100%) as solvent system (Plate 4.12).



Plate 4.12: TLC Chromatogram of A10 using ethylacetate (100%) as solvent system

4.12.1. Physical tests carried out on A10

Solubility of A10

A10 was found to be soluble in chloroform and methanol.

Melting point of A10

The compound was found to have a melting point range of 118^0C - 120^0C.

4.12.2 SPECTRAL ANALYSIS OF A10

4.12.2.1 LCMS spectral analysis of A10

The mass spectrum of A10 (Figure 4.70) showed molecular ion peak $[M + H]^+$ at *m/z* = 223.2

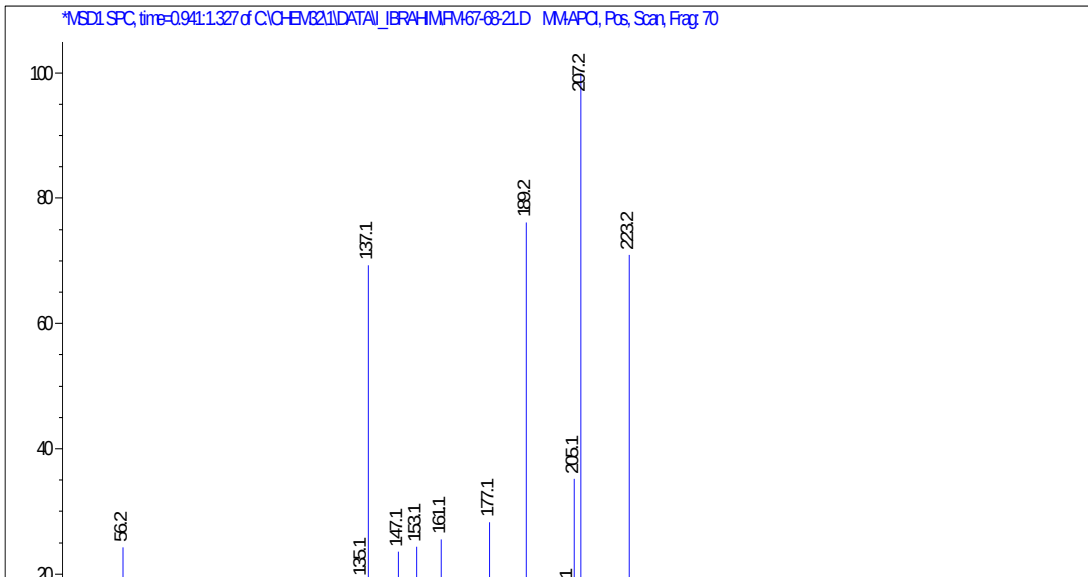


Figure 4.70: LCMS spectrum of A10

4.12.2.2 IR spectrum of A10

The IR spectrum of A10 (Figure 4.71) showed absorption bands at 3409 cm^{-1} (O-H stretching), 1653 cm^{-1} (C=O stretching), 1639 (C=C stretching) and 1122 $^{-1}$ (C-O stretches).

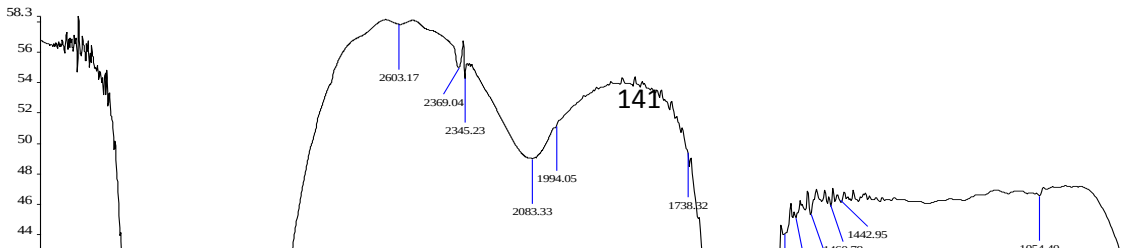


Figure 4.71: IR spectrum of A10

4.12.3.3 ^{1}H NMR spectral analysis of A10

The ^{1}H-NMR (500 MHz, CD_3OD) spectrum of A10 (Figure 4.72) showed proton resonances at δ_H 2.14 (d, *J* = 17.1 Hz, H-2α), 2.39 (d, *J* = 17.1 Hz, H-2β), 5.89 (t, *J* = 1.33 Hz, H-4), 6.18 (s, H-7), 5.60 (dd, *J* = 16.0, 8.3, H-8), 4.34 (m, H-9), 1.28 (d, *J* = 6.5 Hz, 3H-10), 0.99 (s, 3H-11), 1.04 (s, 3H-12), δ_H 4.21 and 4.11 (both d, 2H-13 A and B).

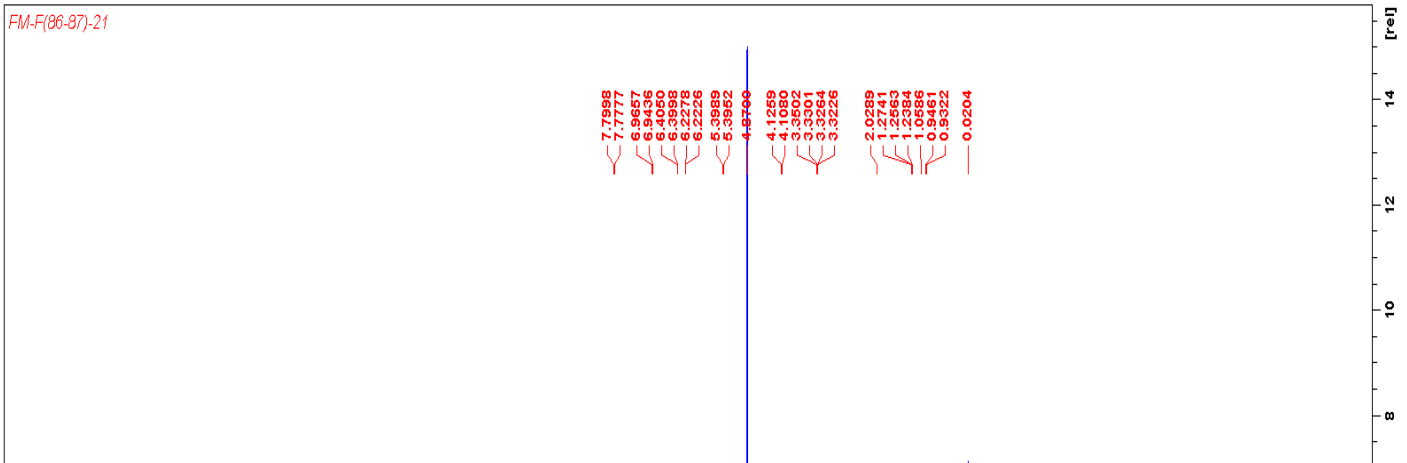


Figure 4.72: ^{1}H NMR spectrum of A10

4.12.3.4 ^{13}C NMR spectral analysis of A10

The ^{13}C NMR (125 MHz, CD_3OD) spectrum of A10 (Figure 4.73) showed a total of thirteen carbon resonances at δc 42.60 (C-1), 50.89 (C-2), 201.35 (C-3), 127.60 (C-4), 167.30 (C-5), 80.09 (C-6), 130.70 (C-7), 137.27 (C-8), 68.80 (C-9), 23.97 (C-10), 23.6 0(C-11), 24.61 (C-12) and δc 19.67(C-13).

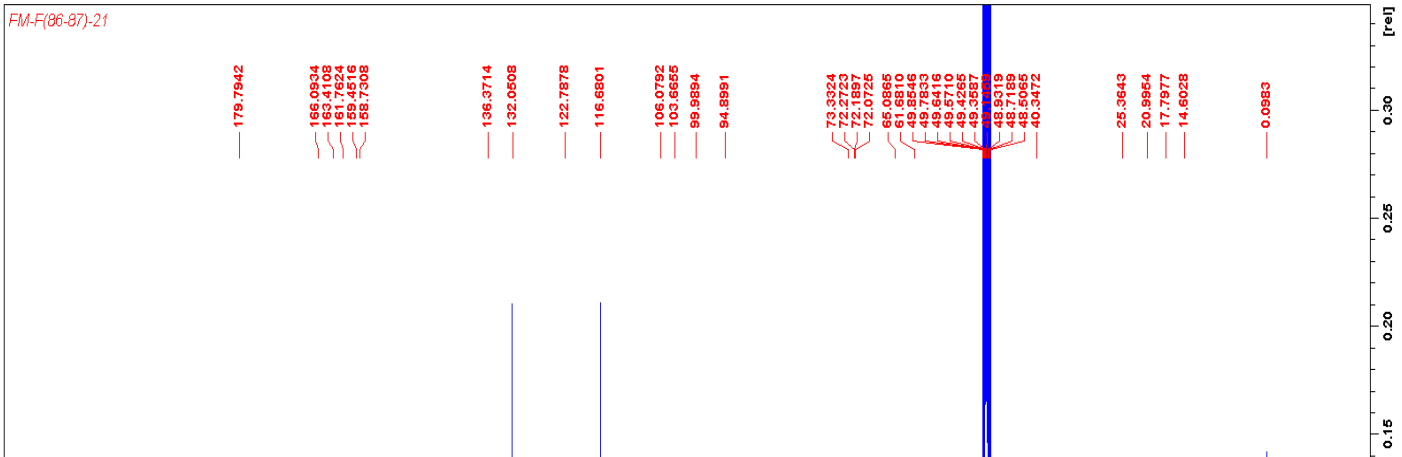


Figure 4.73: ^{13}C NMR spectrum of A10

4.12.3.5 DEPT-135 spectral analysis of A10

The DEPT-135 spectrum of A10 (Figure 4.74) revealed three methyl, two methylene and four methine carbon signals. Three quaternary carbon signals which disappeared in the spectrum were also noted.

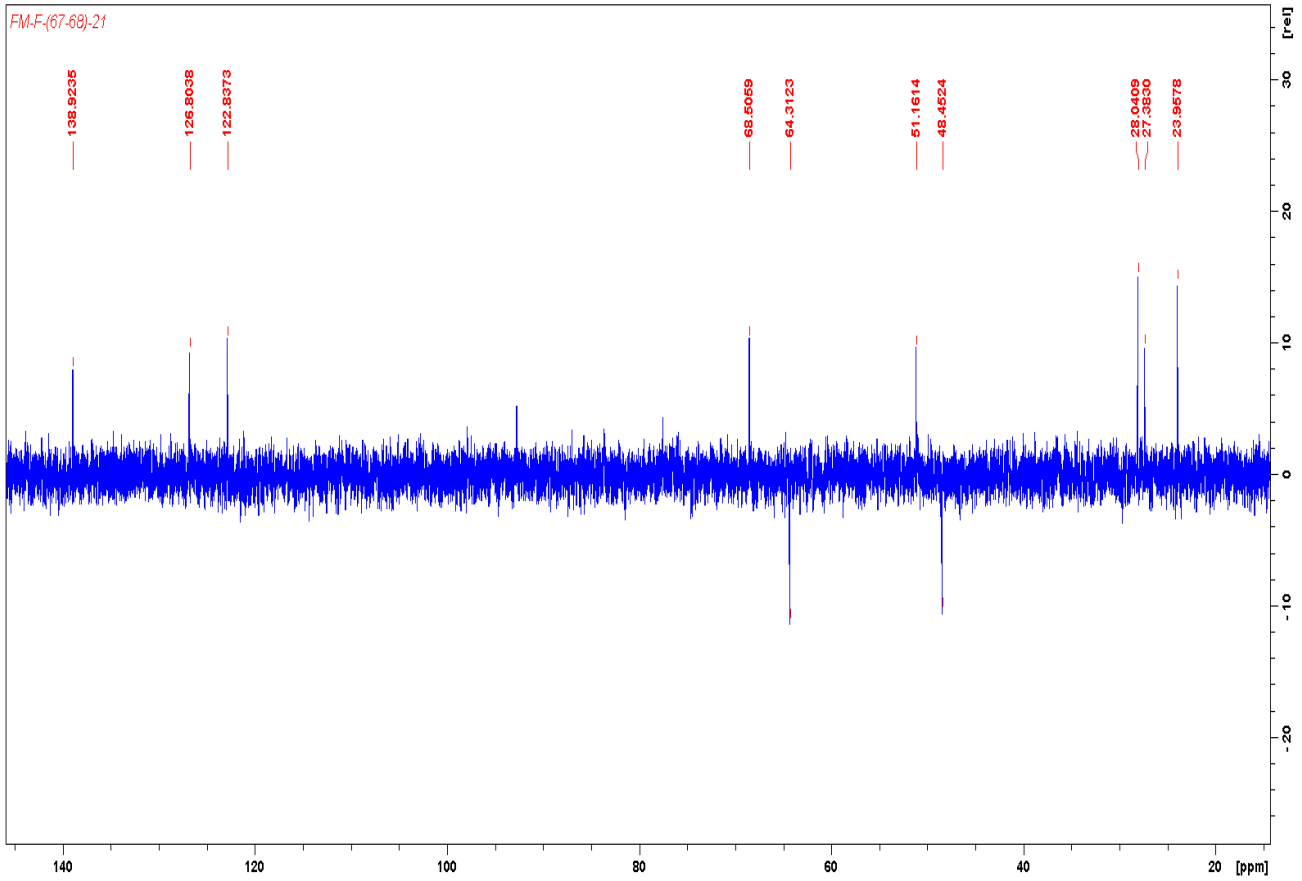


Figure 4.74: DEPT-135 spectrum of A10

4.12.3.6 HSQC-DEPT spectral analysis of A10

The HSQC-DEPT spectrum of A10 is shown in Figure 4.75. It was used to assigned protons to their respective carbon atoms.

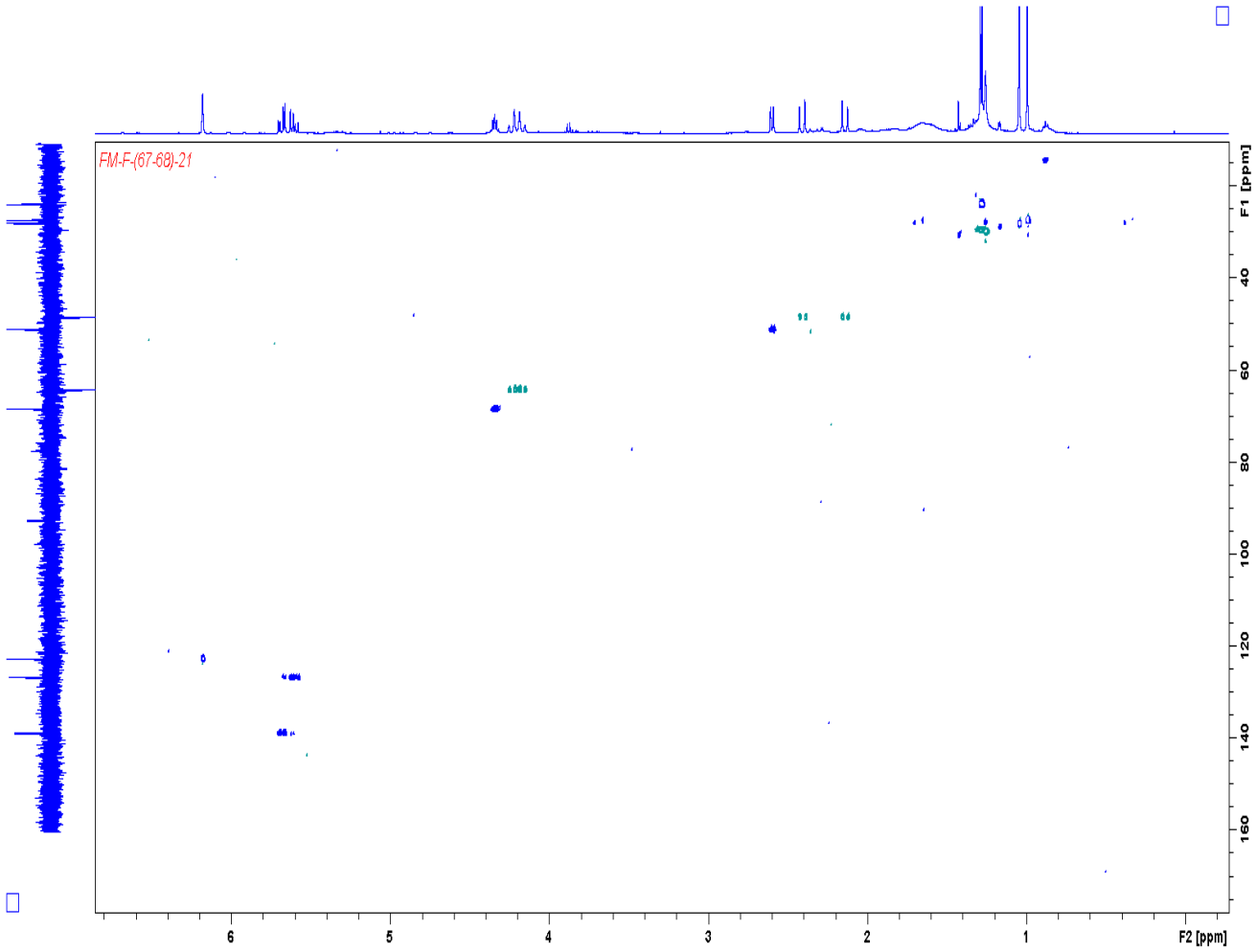


Figure 4.75: HSQC-DEPT spectrum of A10

4.12.3.7 COSY spectral analysis of A10

The correlations observed in the COSY spectrum of A10 (Figure 4.76) showed cross peaks between proton resonance at δ_H 1.28 (3H-10) and δ_H 4.34 (H-9); δ_H 4.34 (H-9) and δ_H 5.60 (H-8); δ_H 5.6 (H-8) and δ_H 5.69 (H-7) and between δ_H 5.69 (H-7) and δ_H 2.03 (H-6).

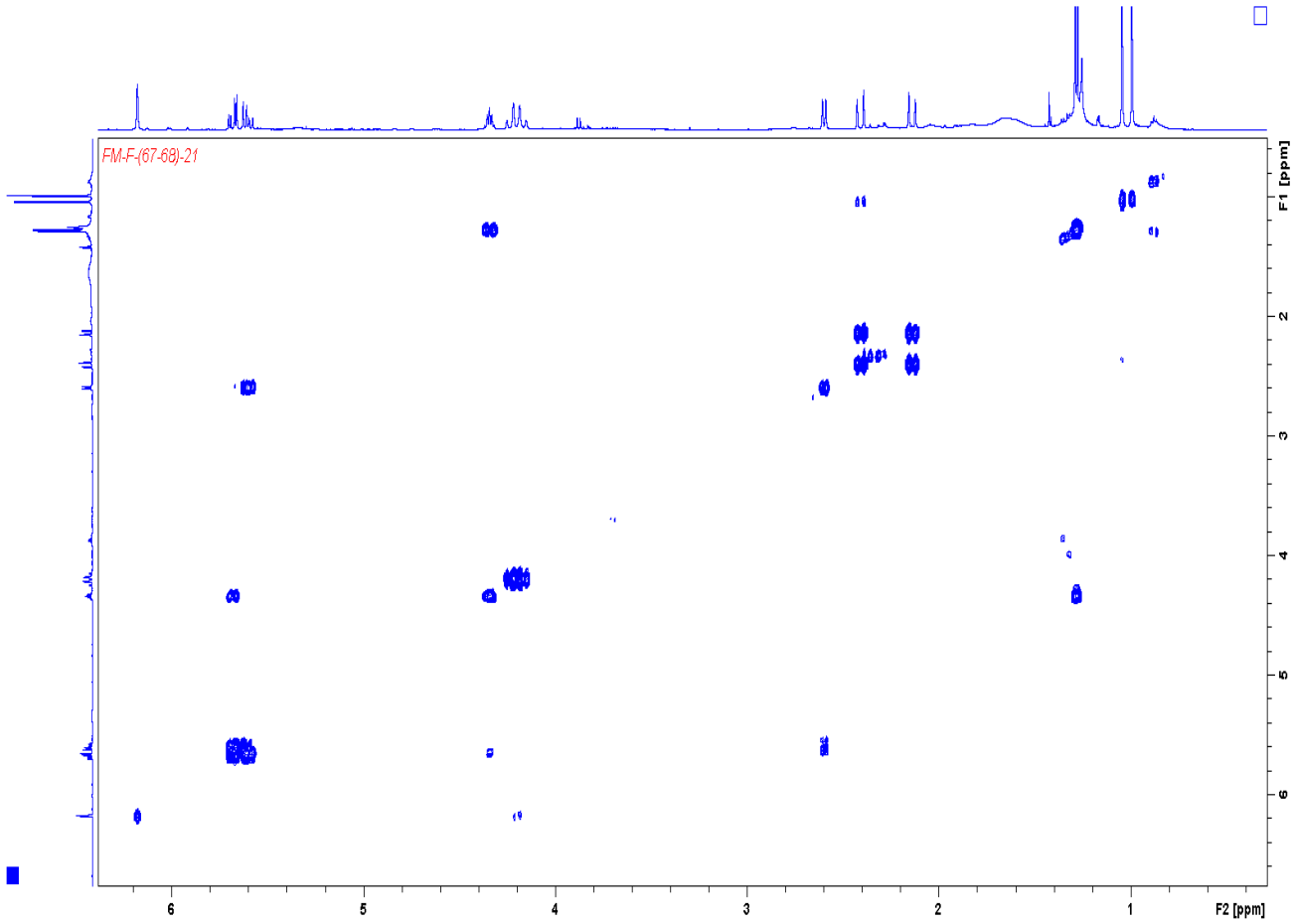


Figure 4.76: COSY spectrum of A10

4.12.3.8 HMBC spectral analysis of A10

The HMBC spectrum of A10 (Figure 4.77) showed that the two oxymethylene proton signal at δ_H 4.13 and 4.11 (H-13A and H-13B) showed correlation with carbon resonances at δc 122.7 (C-4) and δc 163.6 (C-5). The proton resonance at δ_H 6.18 (H-4) also showed cross peak with the carbinol carbon signal at δc 64.2. These and other correlations observed were used to confirm the assignment and positions of the proton and carbon atoms in the proposed structure of the compound.

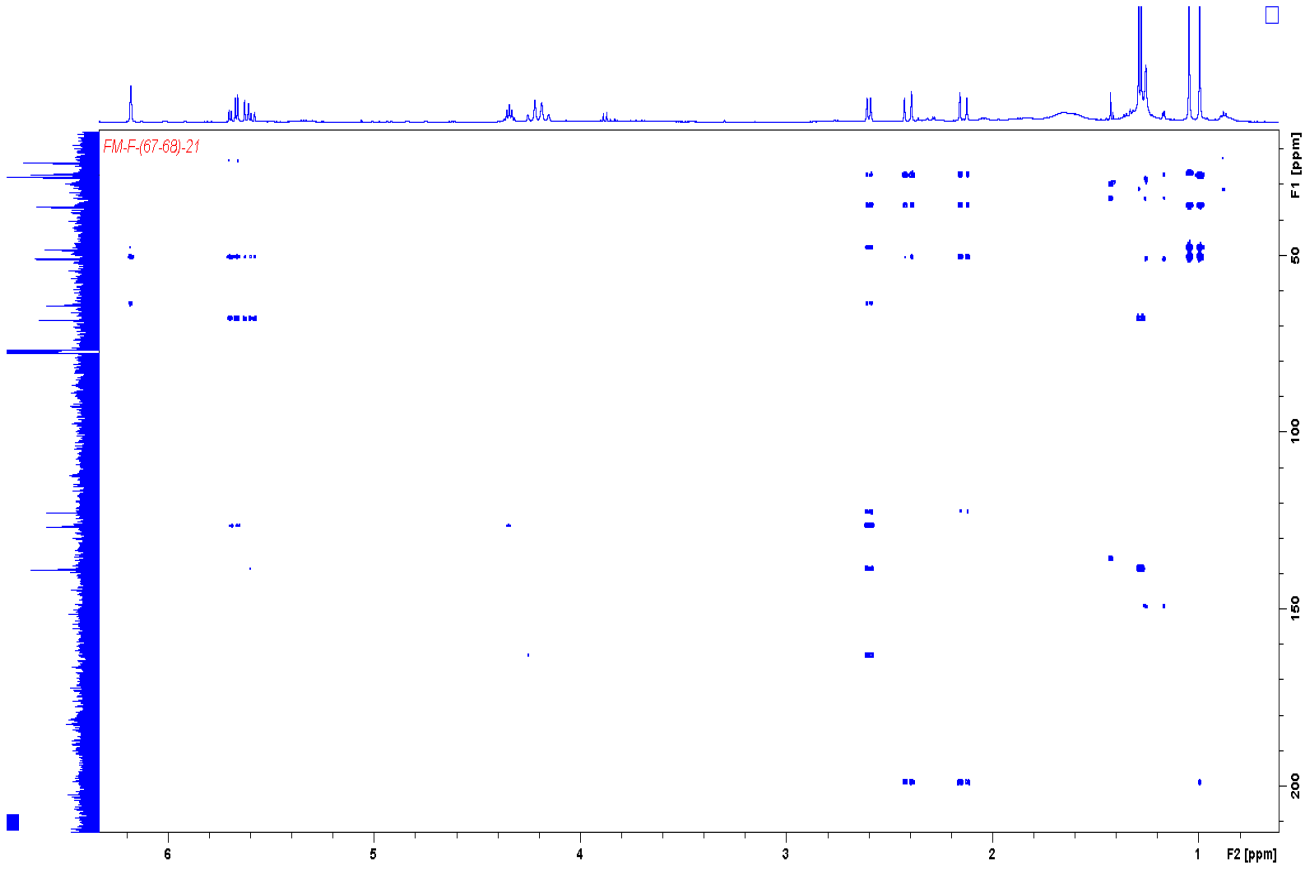


Figure 4.77: HMBC spectrum of A10

4.12.3.9 ROESY spectral analysis of A10

The ROESY spectrum of A10 shown in Figure 4.78 was used to confirm the relative stereochemistry of the proposed structure of the compound.

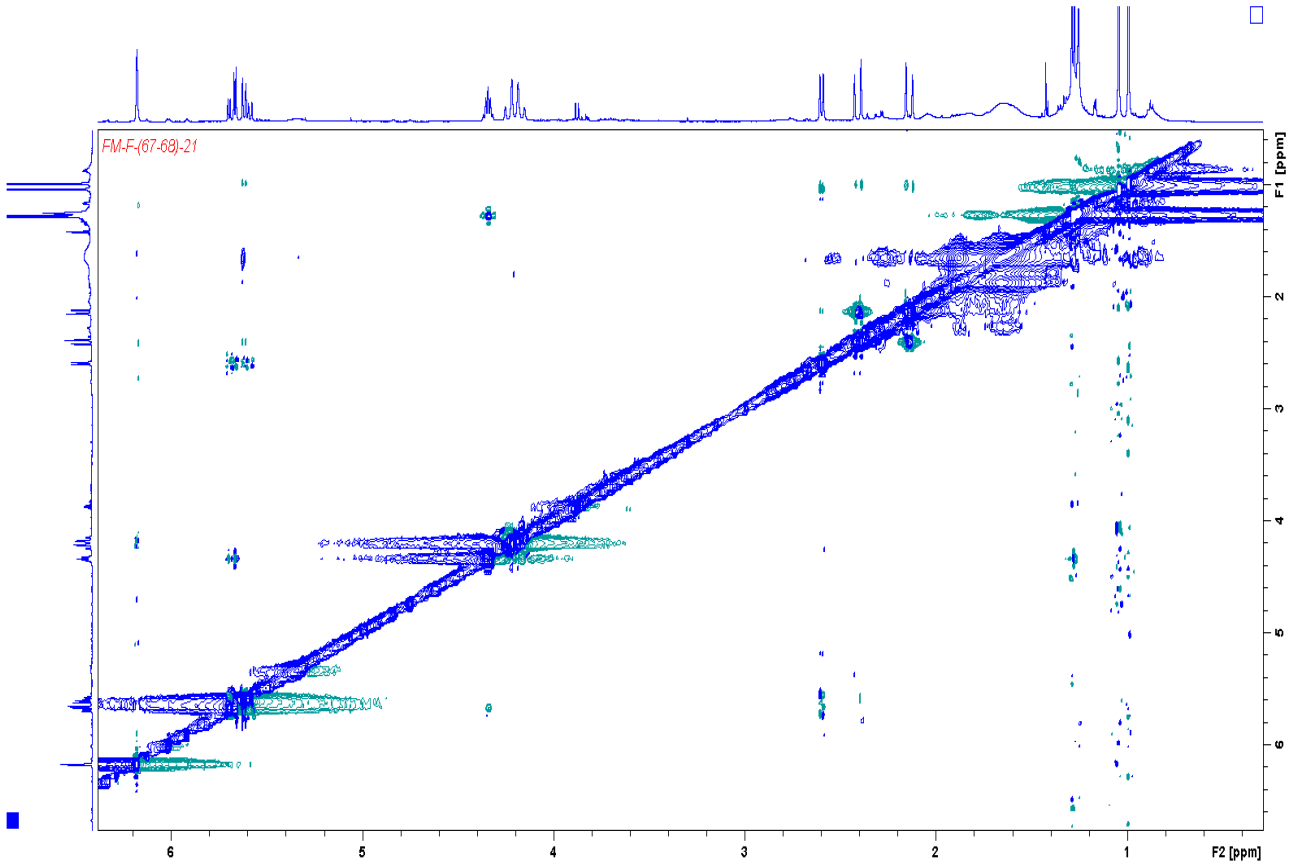


Figure 4.78: ROESY spectrum of A10

Table 4.13: NMR spectral data of A10.

NO.	**^{13}C NMR (500 MHz) $CDCl_3$**	**^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz)**	**HMBC (H → C)**	**COSY (H → H)**	**ROESY (H → H)**
1	36.4 C	-			

2α	48.3	CH_2	2.39 d (*J*=17.1)	1,3,6,12.	2β	4,7,12
2β			2.14 d (17.1)	1,4,6, 12,	2α	11,
3	199.4	C	-			
4	122.7	CH	6.18 s	2,4,5,6	13α	2α,9,10, 11,13
5	163.6	C	-		-	
6	51.1	CH	2.03 d (8.3)	1,2,5	7	
7	12.6	CH	5.69 dd (16.0, 8.3)	1,6,8	8,6	2α,9,10, 11,12,13
8	138.7	CH	5.60 dd (16.0, *2*.3)	6,7,9	7,9	2α,9,10,13
9	68.4	CH	4.34 m	8	8	4,7,8,10,13
10	23.9	CH_3	1.28 d (6.5)	7,9	-	4,7,8,9
11	27.3	CH_3	0.99 s	1,2,6	-	2α7,13
12	27.9	CH_3	1.04 d s	1,2,6,11	-	2α,2β,7
13A	64.20	CH_2	4.13 d (1.23)	4,5,6	4	4,7,8,9,12
13B			4.11 d (1.23)			

4.13 Isolation of A11

Compound A11 (12 mg) was isolated as white crystal with Rf 0.41 using DCM : ethylacetate (7:3) as solvent system (Plates 4.13) $[\alpha]^{21}_{D}$ = +550 (c = 0.04, CH_2Cl_2).

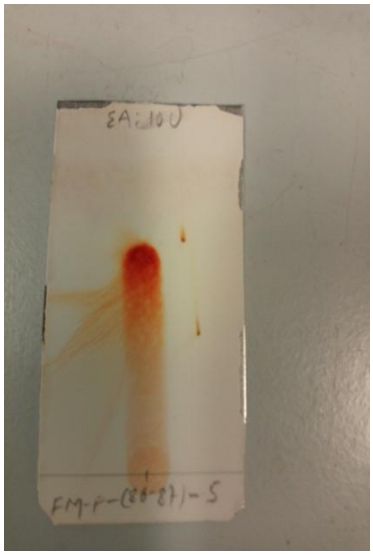


Plate 4.13: TLC chromatogram of A11 using DCM : ethylacetate (7:3) as solvent system

4.13.1. Physical tests carried out on A11

Solubility of A11

A11 was found to be soluble in methanol.

Melting point of A11

The compound was found to have a melting point range of 237 - 238^{0}C.

4.13.2 SPECTRAL ANALYSIS OF A11

4.13.2.1 Mass spectral analysis of A11

The mass spectrum of A11 (Figure 4.79) showed molecular ion peak at $[M+H]^+$ at *m/z* = 290.



Figure 4.79: LCMS spectrum of A11

4.13.2.2 IR spectral analysis of A11

The IR spectrum of A11 (Figure 4.80) showed absorption bands at 3420 cm^{-1} (O-H stretching), 2920 cm^{-1} (aliphatic CH_3 stretching) and 1637 cm^{-1} (aromatic C=C stretching).

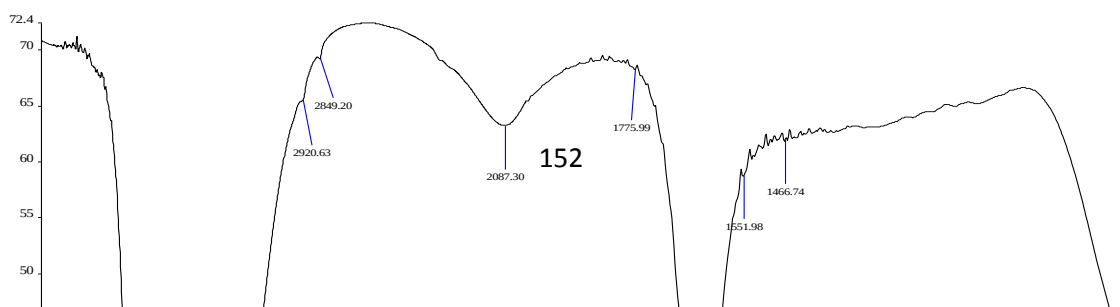


Figure 4.80: IR spectrum of A11

4.13.2.3 ^{1}H NMR spectral analysis of A11

The ^{1}H NMR (500 MHz, CD_3OD) spectrum of A11 (Figure 4.81) showed proton signals at δ_H 4.84 (m, H-2), 4.20 (bs, H-3), 2.83 (dd, *J* = Hz, H-4 α & β), 5.96 (d, *J* = H- 6), 5.94 (d, H-8), 7.0 (d, *J* = H-2'), 6.7 (d, *J* = *Hz,* H-5') and 6.8 (dd, *J* = Hz, H-6').

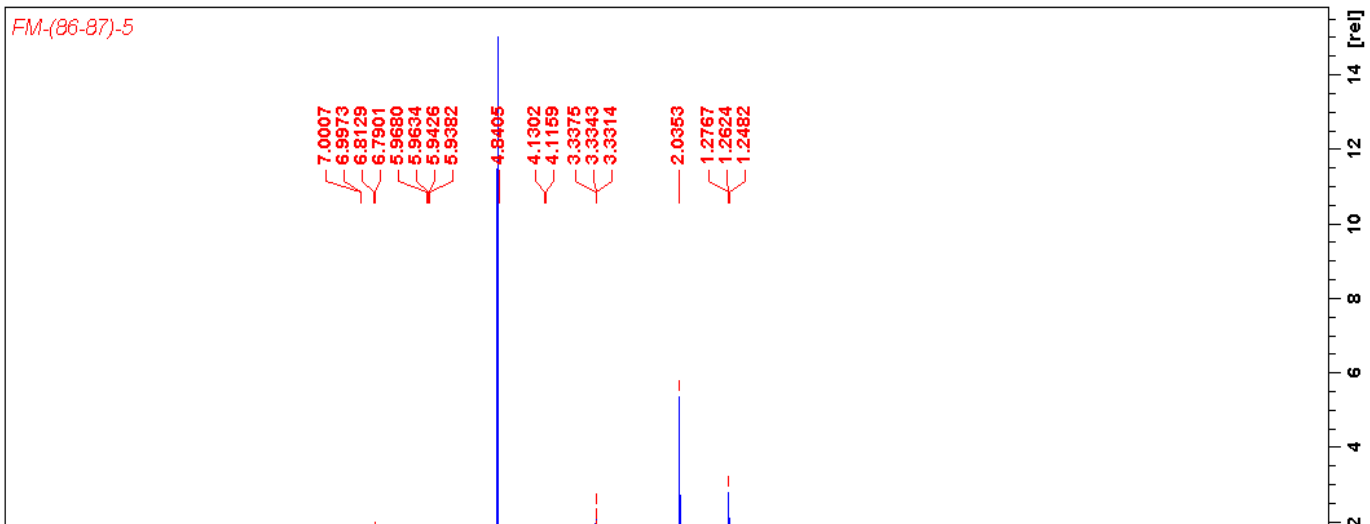


Figure 4.81:[1]H NMR spectrum of A11

4.13.2.4 [13]C NMR spectral analysis of A11

The [13]C NMR (125 MHz, CD_3OD) spectrum of A11 (Figure 4.82) showed fifteen carbon signals at δc 80.01 (C-2), 67.6 (C-3), 29.4 (C-4), 157.8 (C-5), 96.5 (C-6), 158.2 (C-7), 96.03 (C-8), 157.5 (C-9), 100.2 (C-10), 132.4 (C-1'), 115.3 (C-2'), 145.9 (C-3'), 146.1 (C-4'), 116.0 (C-5') and 119.5 (C-6').

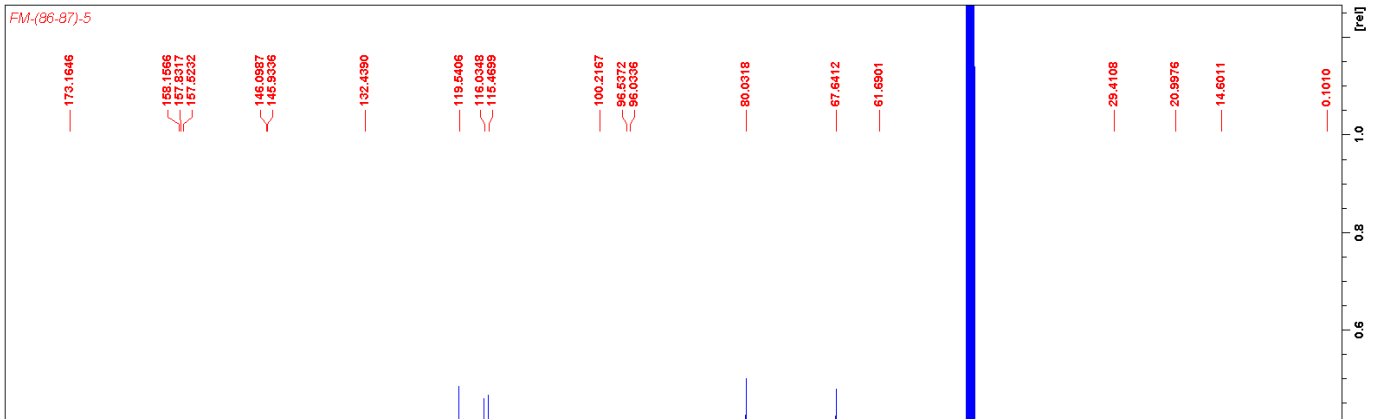


Figure 4.82: ^{1}H NMR spectrum of A11

4.13.2.5 DEPT-135 spectral analysis of A11

The DEPT-135 spectrum of A11 **(**Figure 4.83) showed one methylene, seven methine and the seven quaternary carbons resonances which will not appear in the spectrum were noted.

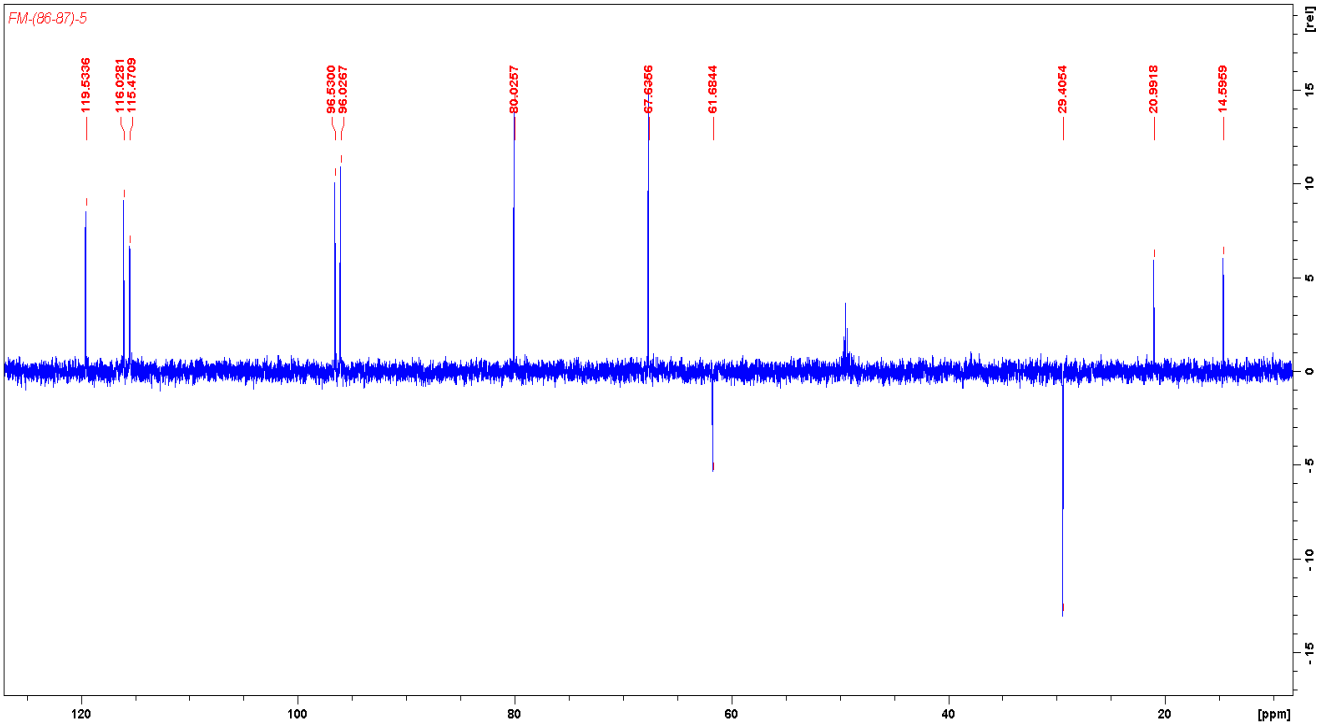


Figure 4.83: DEPT-135 spectrum of A11

4.13.2.6 HSQC-DEPT spectral analysis of A11

The HSQC-DEPT spectrum of A11 is shown in Figure 4.84 It was used to assigned protons to their respective carbon atoms.

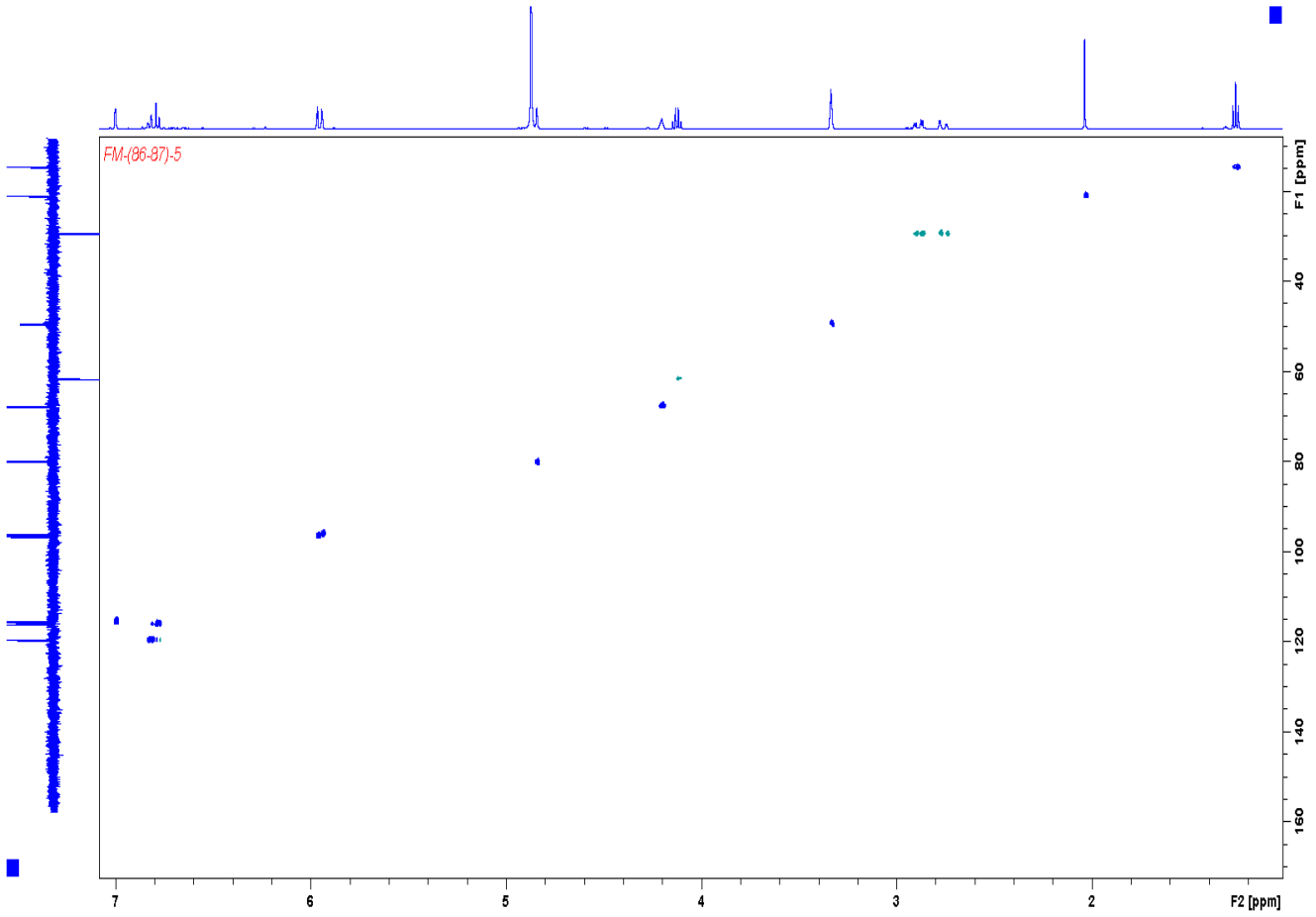


Figure 4.84: HSQC-DEPT spectrum of A11

4.13.2.7 COSY spectral analysis of A11

The COSY spectrum of A11 (Figure 4.85) showed cross peaks between δ_H 5.96 (H-6) and δ_H 5.94 (H-8); δ_H 6.70 (H-5') and δ_H 6.80 (H-6'); δ_H 6.80 (H-6') and δ_H 7.01 (H-2); 4.19 (H-2) and δ_H 4.12 (H-3) and δ_H 2.83 (2H-4α and δ_H 4.12 (H-3).

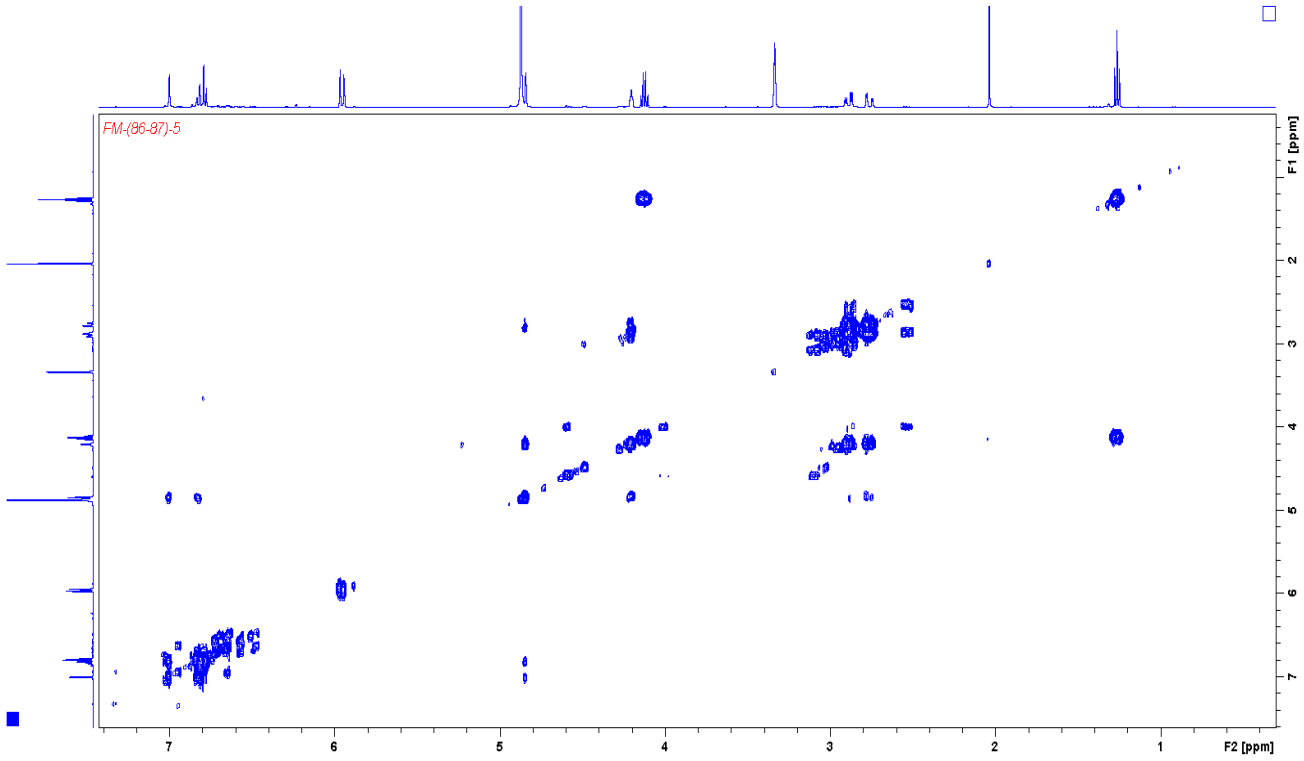


Fig 4.85: COSY spectrum of A11

4.13.2.8 HMBC spectral analysis of A11

The HMBC spectrum of A11 (Figure 4.86) showed important correlation between proton resonance at δ_H 4.84 (H-2) and carbon resonances at δc 67.6 (C-3), 29.4 (C-4), 132.4 (C-1'), 115.3 (C-2') and 119.48 (C-6'). These and other correlations observed in the spectrum were used to confirm the connectivity network in the proposed structure of the molecule.

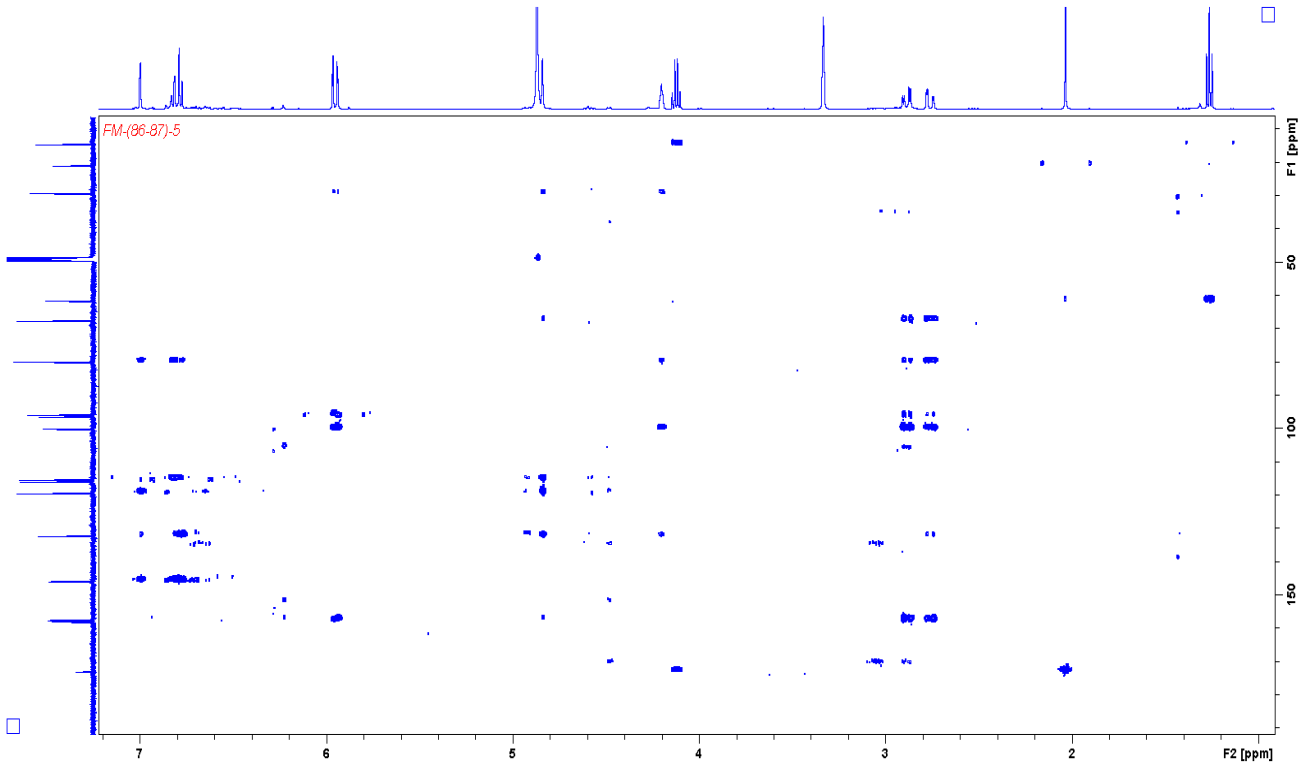


Figure 4.86: HMBC spectrum of A11

Table 4.14: NMR spectral data of A11 compared with literature

NO.	**^{13}C NMR (500 MHz) $CDCl_3$**	**^{13}C NMR (500 MHz) $CDCl_3$** (Abdullahi *et al.*. 2016)	**^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz)**	**^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz)** (Abdullahi *et al.*, 2016)	**HMBC (H →C)**	**COSY (H →H)**	**ROESY (H →H)**
2	80.09 CH	79.9	4.84 bs	8.34	3,4,9,1', 2' 6'	3	2'
3	67.61 CH	67.5	4.12 t (6.8)	4.19	2,4,1'	2,4β	4α
4α	29.43 CH_2	2.83	2.76 dd 1H (2.7,16.2)	2.86	2,3,5,9,10		
4β		2.76	2.89 dd 1H (4.7, 9.7)	2.73		3	
5	157.80 C	157.4					
6	96.53 CH	96.4	5.96 d 1H (2.3)	5.93	5,7,8,10		

7	158.19 C	157.9					
8	96.03 CH	95.9	5.94 d 1H (2.3)	5.96	6,7,9,10		
9	157.48 C	157.7					
10	100.22 C	100.1					
1’	132.38 C	132.3					
2’	115.34 CH	115.3	7.01 d 1H (1.7)	6.99	2,6,1’,4’	6’	2
3’	145.91 C	145.9					
4’	146.10 C	145.8					
5’	116.03 CH	115.9	6.70 d 1H (8.1)	6.77	1’3’4	6’	
6’	119.48 CH	119.4	6.80 dd 1H (1.6,8.1)	6.81	2,1’,4’,5’	5’,2’	

4.14 Isolation of A12

Compound A11 (5.8 mg) was isolated as reddish brown solid, Rf = 0.49 using ethylacetate : methanol 25:1 as solvent system (Plate 4.12).

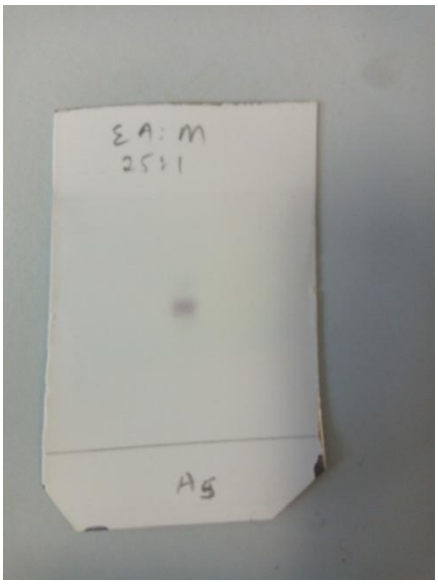


Plate 4.14: TLC chromatogram of A12 using ethylacetate : methanol 25:1 as solvent system

Rf = 0.49

4.14.1. Physical tests carried out on A12

Solubility of A14

A12 was found to be soluble in methanol.

Melting point of A12

The compound was found to have a melting point range of 277^{0}C - 279^{0}C.

4.14.2 Spectral analysis of A12

4.14.2.1 ^{1}H NMR spectral analysis of A12

The ^{1}H NMR (CD_3OD, 400 MHz) of A12 (Figure 4.87) revealed proton resonances at δ 6.20 (dd, *J* = 2.08, 8.1 Hz, H-6); 6.39 (d, *J* = 2.08 Hz, H-8); 6.93 (2H, d, *J* = 8.1 Hz, H3' and H-6') and 8.03 (d, *J* = 8.04 Hz, H2' and 5').

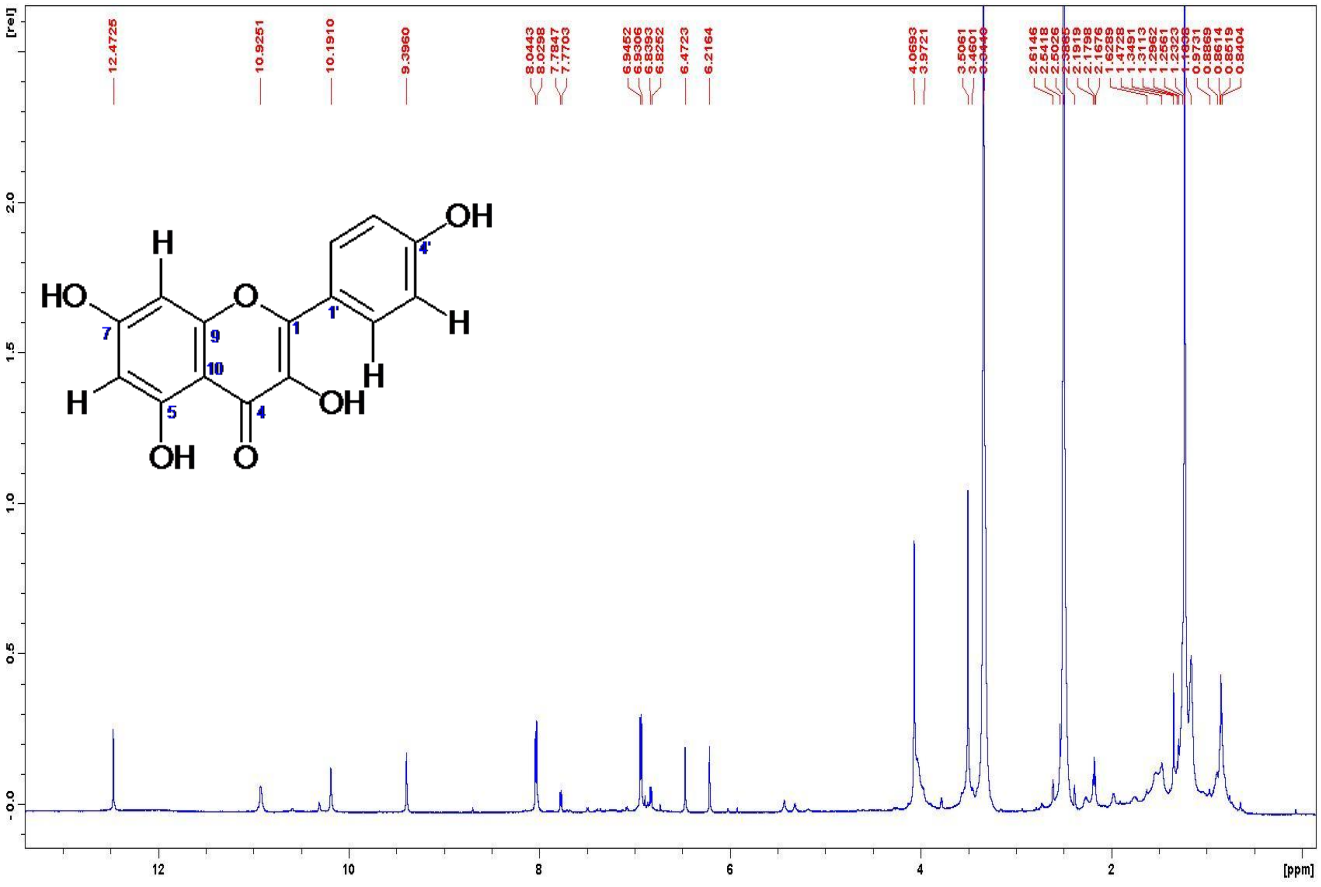


Figure 4.87: ^{1}H NMR Spectrum of A12

4.14.2.2 Attaches Proton Test (APT) spectrum of A12

The APT spectrum of A12 (Figure 4.88) showed carbon resonances at δ179.0 (C-4), 168.8 (C-7), 161.5 (C-5), 160.7 (C-4'), 157.1 (C-2), 157.5 (C-9), 134.0 (C-3), 131.5 (C-2'/5'), 129.9 (C-6'), 121.4 (C-1'), 151.5 (C-3'/6'), and 101.6 (C-10), 100.3 (C-6) and 94.0 (C-8). The spectrum also showed six methine and fifteen quaternary carbon signals.

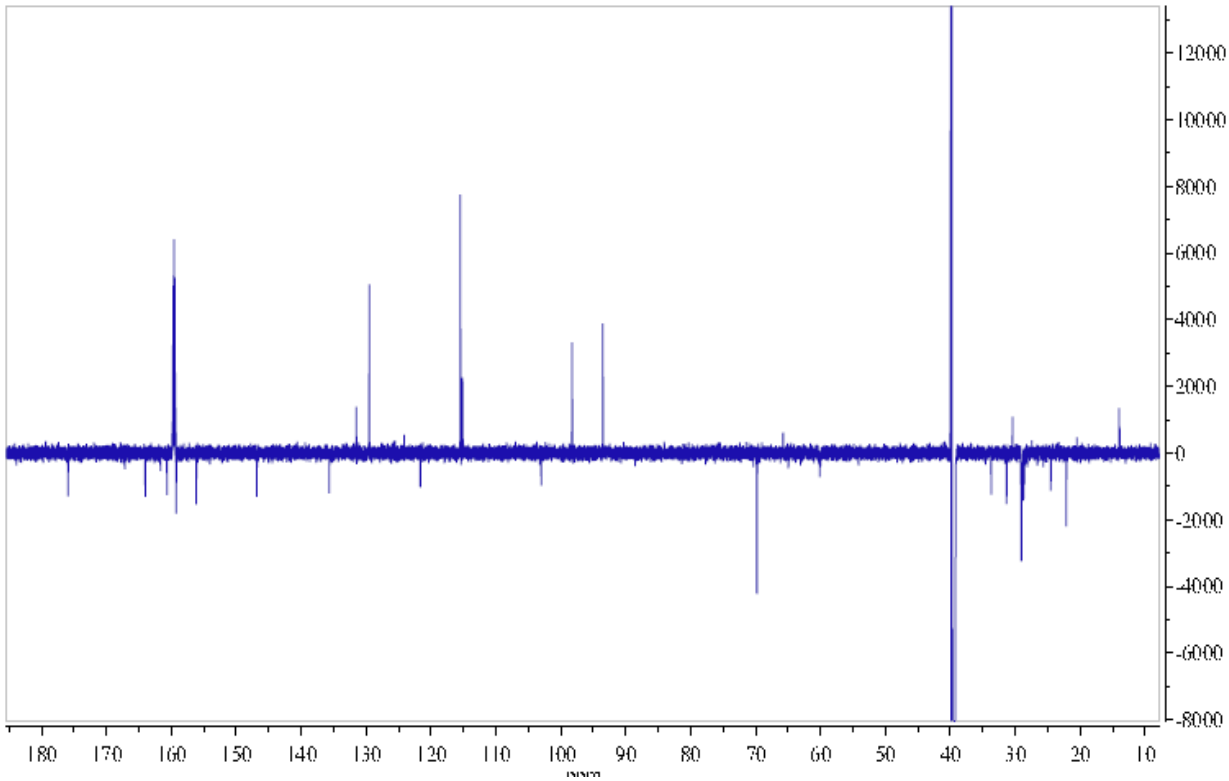


Figure 4.88: ^{13}C NMR/DEPT Spectrum of A12

4.14.2.3 HSQC spectral analysis of A12

The HSQC spectrum of A12 shown in Figure 4.89 was used to assign protons to their respective carbon atoms.

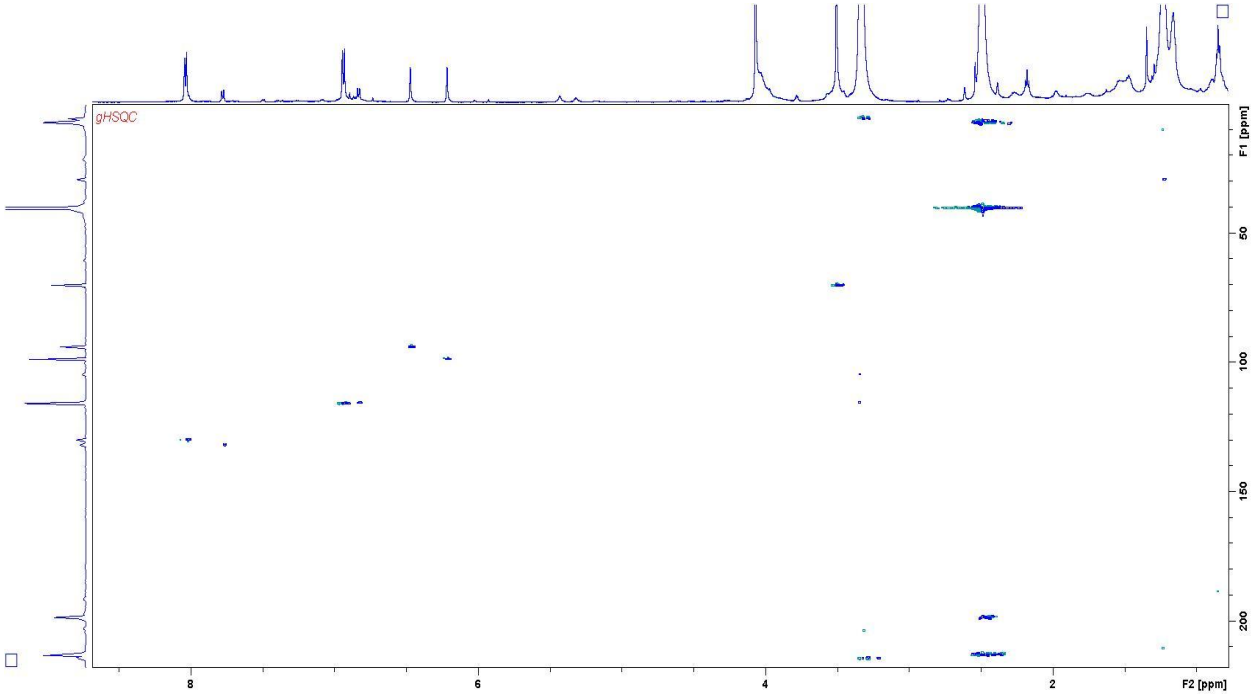


Figure 4.89: HSQC Spectrum of A12

4.14.2.4 HMBC spectral analysis of A12

The HMBC spectrum of A12 (Figure 4.90) was used to confirm connectivity network in the proposed structure of the molecule.

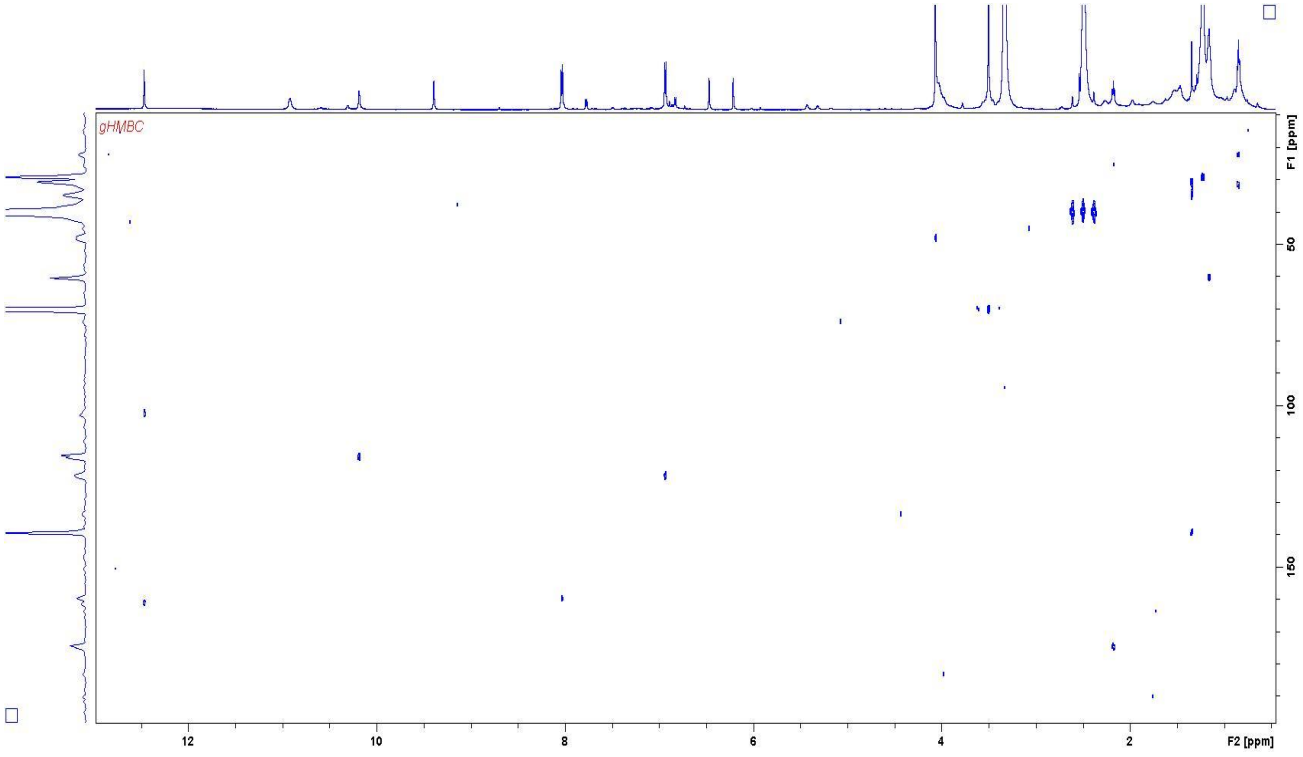


Figure 4.90: HMBC spectrum of A12

Table 4.15: NMR spectral data of A12 compared with literature

NO.	**^{13}C NMR (500 MHz)** CD3OD	**^{13}C NMR (500 MHz)** CD3OD **(**Gangwal *et al.,* 2010)	**^{1}H NMR (500 MHz)** CD3OD **(*J* in Hz)**	**^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz)** **(**Gangwal *et al.,* 2010)
2	157.3 C	157.2		
3	134.0 C	135.5		
4	179.0 C	177.6		
5	161.5 C	160.8		
6	100.3 CH	99.6	6.20 dd (2.08, 8.1)	6.21
7	168.8 C	162.7		
8	94.0 CH	94.8	6.39 d (2.08)	6.32
9	157.5 C	156.0		
10	103.6 C	105.7		
1’	121.6 C	120.6		
2’/5’	131.5 CH	130.9	6.93 d, (8.1)	6.86
3’/6’	115.1 CH	115.2	8.03 d, (8.1)	7.97
4’	160.7 C	160.2		

4.15 Isolation of A13

Compound A13 (6.4 mg) was isolated as yellow powder, Rf = 0.51 using ethylacetate : methanol 9:1 as solvent (Plate 4.15) and $[\alpha]^{21}_{D}$ = + 0.33 (c = 0.063, CH_2Cl_2).



Plate 4.15: TLC chromatogram of A13 using ethylacetate : methanol 9:1 as solvent system

4.15.1. Physical tests carried out on A13

Solubility of A13

A13 was found to be soluble in methanol.

Melting point of A13

The compound was found to have a melting point range of 182-184^{0}C.

4.15.2 SPECTRAL ANALYSIS OF A13

4.15.4.1 LCMS spectral analysis of A13

The LCMS spectrum of compound A13 (Figure 4.91) showed molecular ion peak $[M+H]^+$ at *m/z* 432.1



Figure 4.91: LCMS spectrum of A13

4.14.4.2 IR spectral analysis of A13

The IR spectrum of A13 (Figure 4.92) showed absorption band at 3381 cm^{-1} (O-H stretching), 1650 cm^{-1} (C=O stretching) and 1492 cm^{-1} (aromatic C=C stretching).

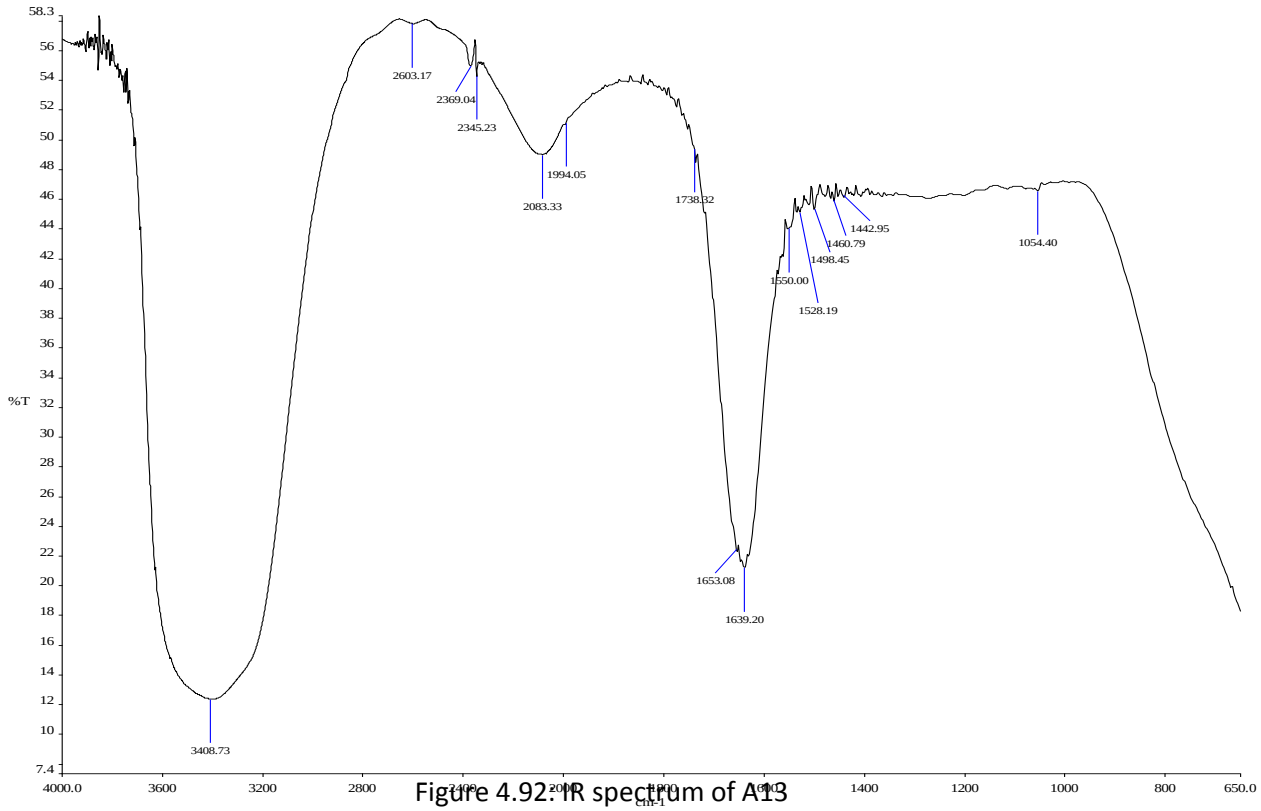


Figure 4.92: IR spectrum of A13

4.15.4.3 ^{1}H NMR spectral analysis of A13

The ^{1}H NMR of A13 (500 MHz, CD_3OD) of A13 (Figure 4.93) revealed signals at δ_H 6.22 (d, *J* = 2.08 Hz, H-6), 6.41 (d, *J* = 2.08H z, H-8), 7.71 (d, *J* = 8.84Hz, H-2'and H-5'), 6.90 (d, *J* = 8.84 H-3' and H-6'), 0.85 (d, H-6''), 5.39 (d, *J* = 1.5 Hz, H-1''), and 3.40-3.90 (sugar protons).

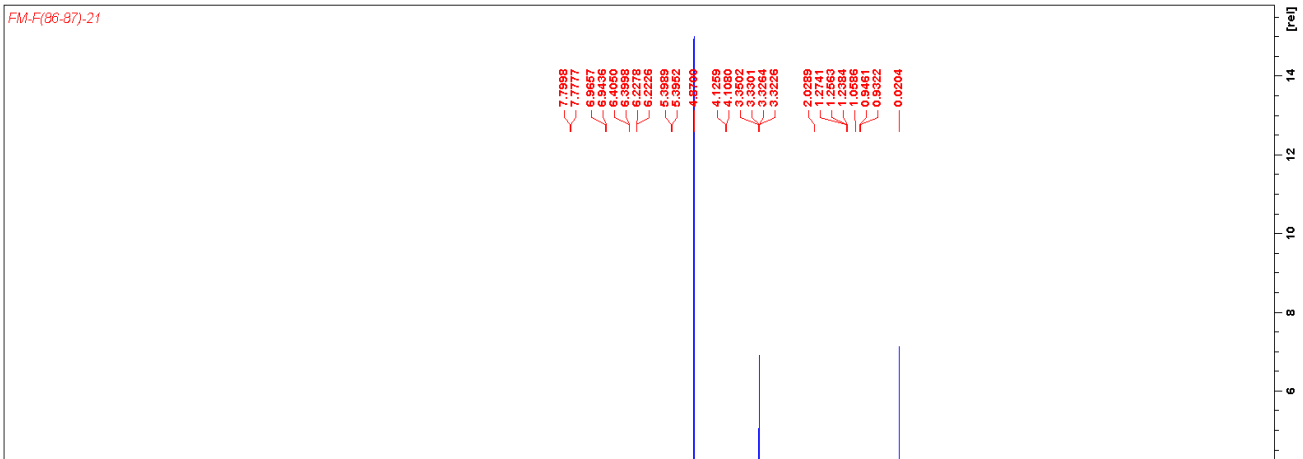


Figure 4.93: ^{1}H NMR spectrum of A13

4.15.4.4 ^{13}C NMR spectral analysis of A13

The ^{13}C NMR(100 MHz, CD_3OD) spectrum of A13 (Figure 4.94) showed proton signals at δc 94.7 (C-8), 99.0 (C-6), 104.1 (C-10), 115.6 (C-3', C-5'), 122.4 (C-1'), 131.0 (C-2',C-6'), 134.9 (C-3), 157.8 (C- 9),161.8 (C-2), 164 (C-5), 161.8 (C-7), 160.5 (C-4') & 178.2(C-4) Sugar moiety appeared at δ:102.3 (C-1''), 74.5 (C- 2''),77.51 (C-3''),70.31 (C-4''), 78.1 (C-5'') and 17.8 (C-6'').

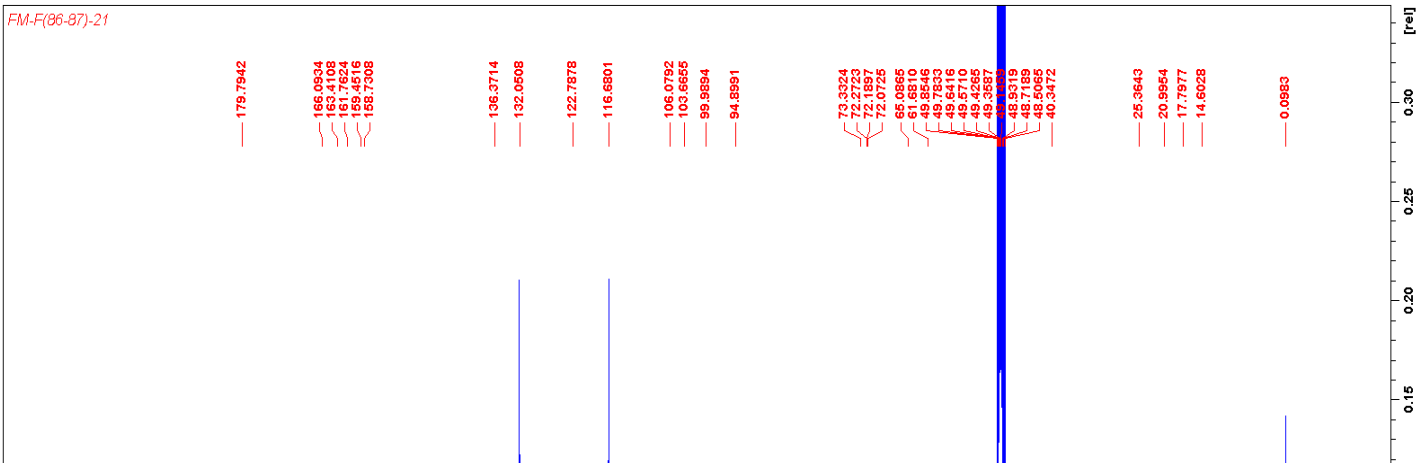


Figure 4.94: ^{13}C NMR spectrum of A13

4.15.4.5 DEPT-135 spectral analysis of A13

The DEPT-135 spectrum of A13 (Figure 4.95) revealed one methyl and eleven methine carbon resonances. The nine quaternary carbons resonances which disappeared in the spectrum were also noted.

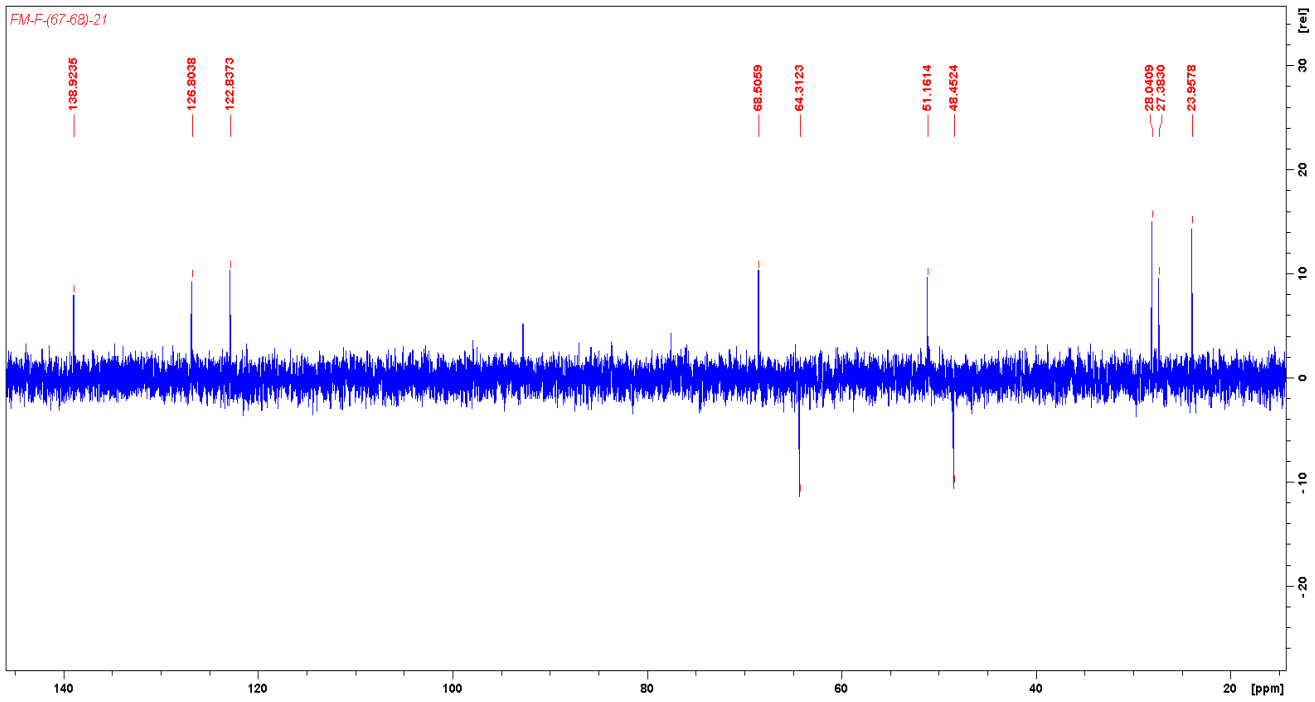


Figure 4.95: DEPT-135 spectrum of A13

4.15.4.6 HSQC-DEPT spectral analysis of A13

The HSQC-DEPT Spectrum of A13 shown in Figure 4.96 was used to assign protons to their respective carbon atoms.

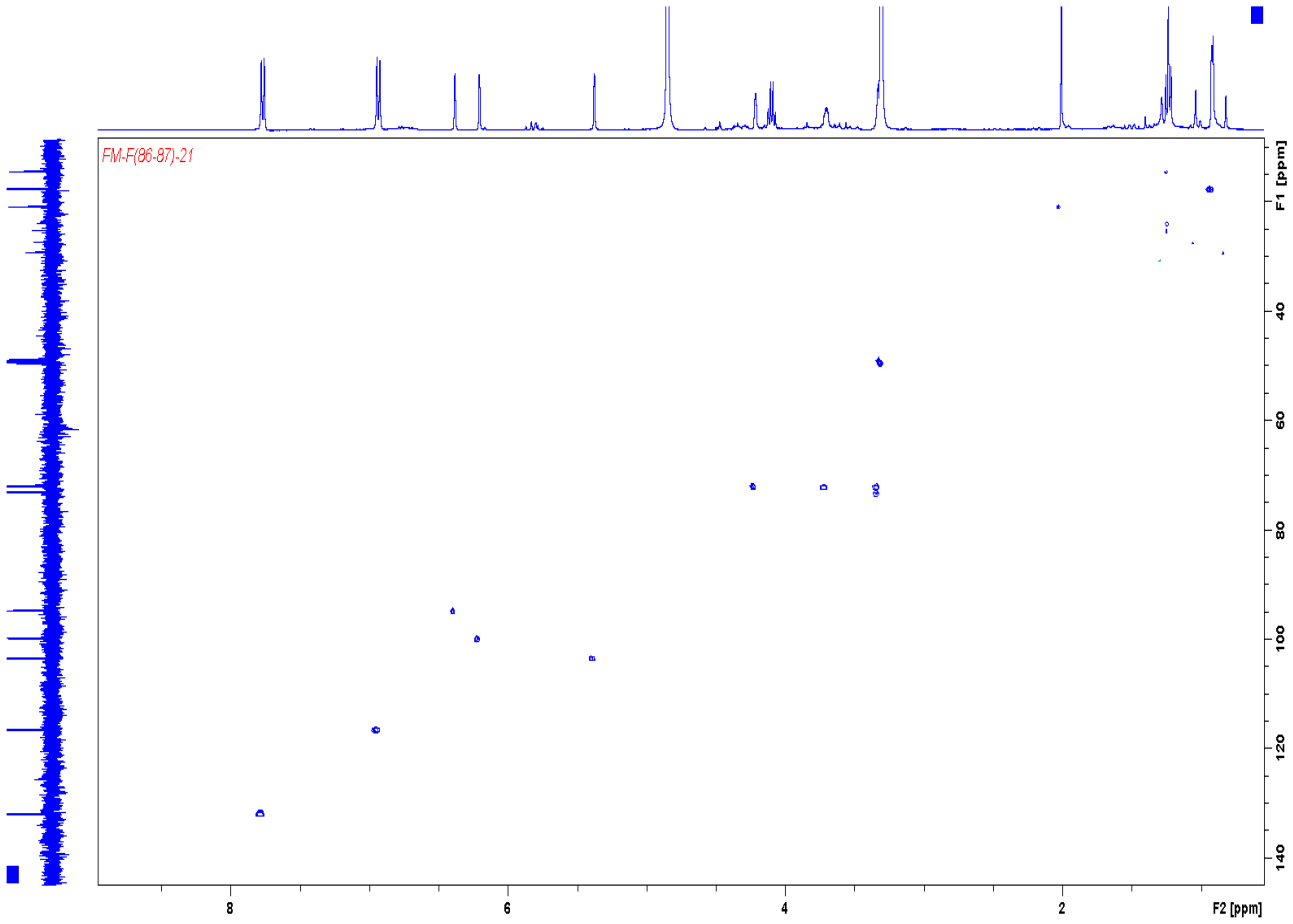


Figure 4.96: HSQC-DEPT spectrum of A13

4.15.4.7 COSY spectral analysis of A13

The H-H COSY spectrum of A13 (Figure 4.97) showed correlation between the proton resonance at δ_H 7.71 (H-2'/5'), and proton resonances at δ_H 6.90 (H-3'/6'). Correlation was also observed between proton resonance at δ_H 6.22 (H-6) and δ_H 6.41 (H-8).

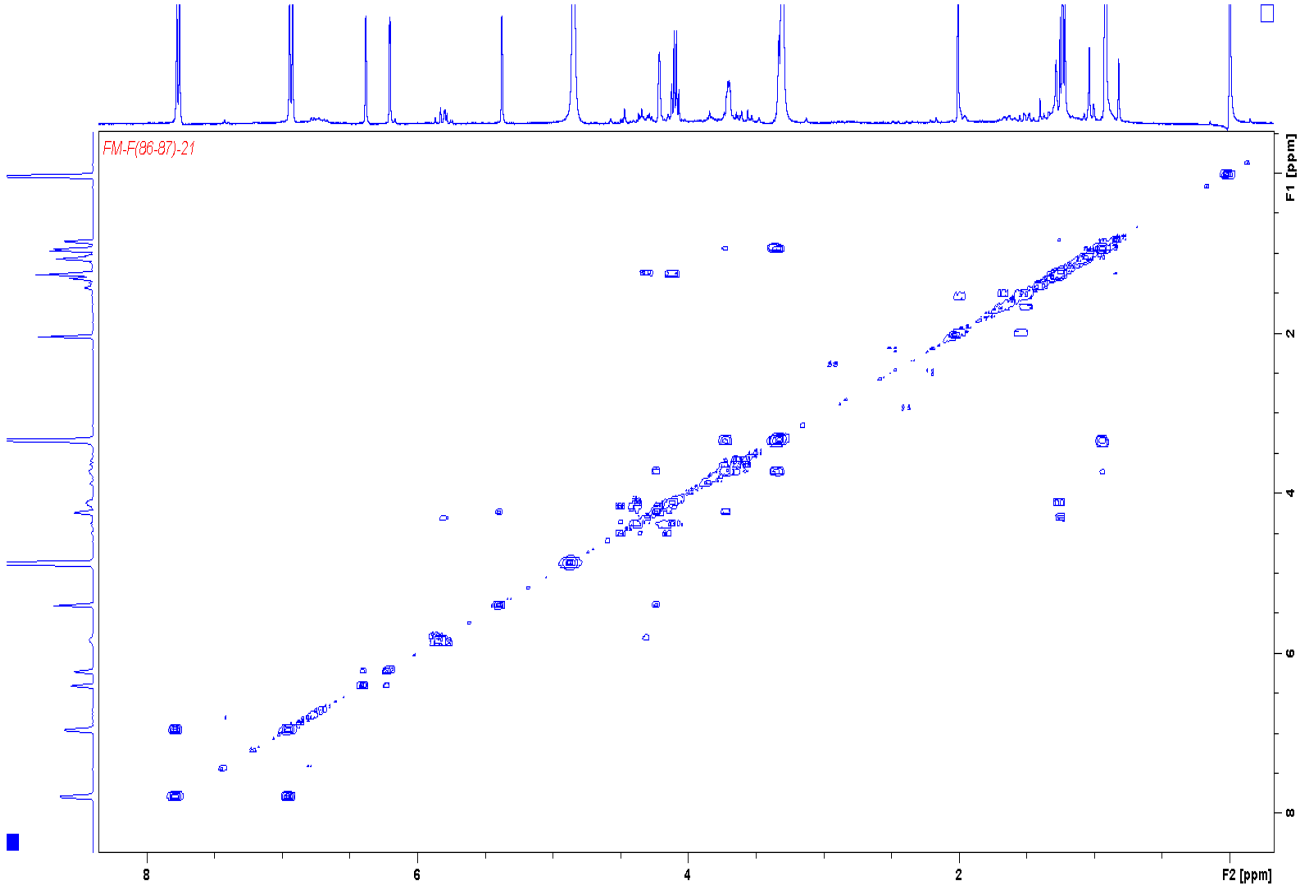


Figure 4.97: COSY spectrum of A13

4.15.4.8 HMBC spectral analysis of A13

The HMBC spectrum of A13 (Figure 4.98) revealed that the proton signals at δ_H 5.39 (H'') showed cross peaks with a carbon at δc 136.1(C-3).

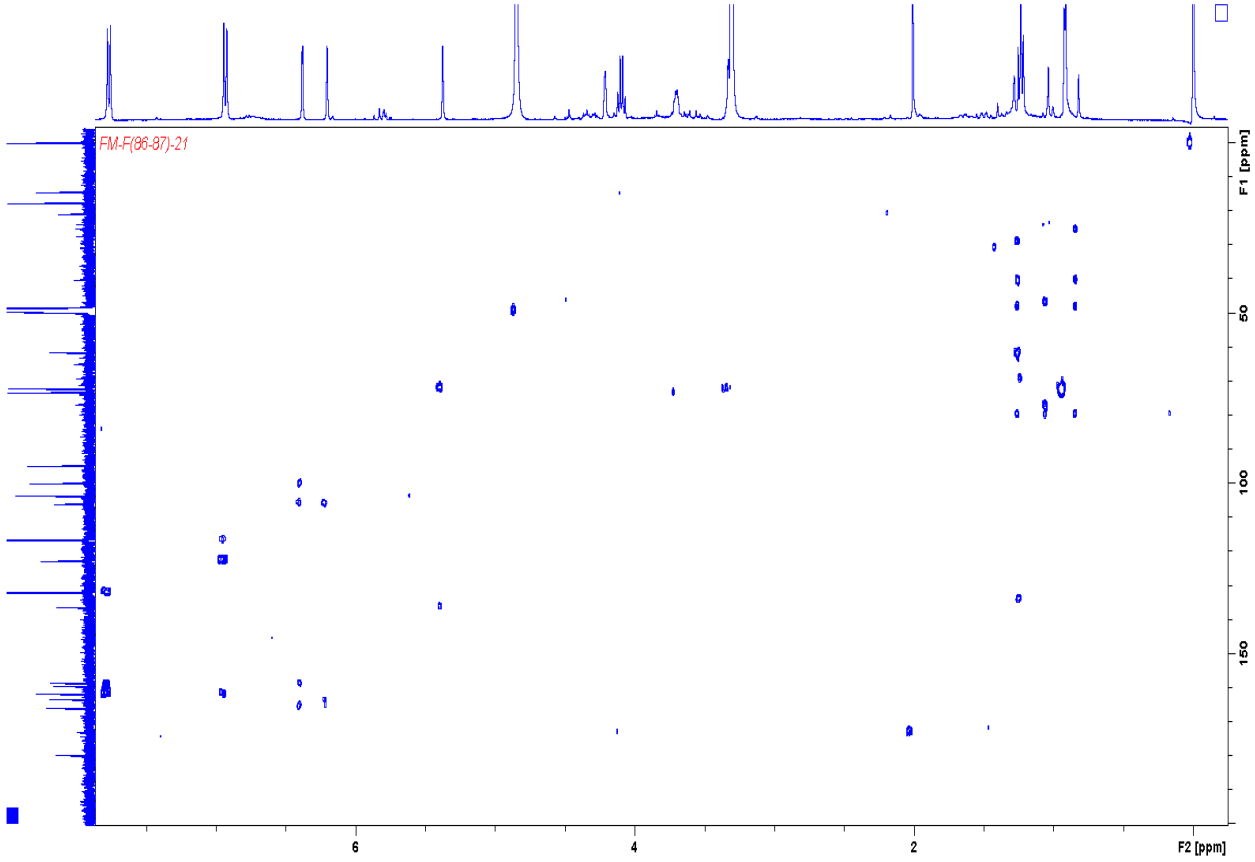


Figure 4.98: HMBC spectrum of A13

4.15.4.9 ROESY spectral analysis of A13

The ROESY spectrum of A13 (Figure 4.99) was used to confirm the relative stereochemistry of the proposed structure of the molecule.

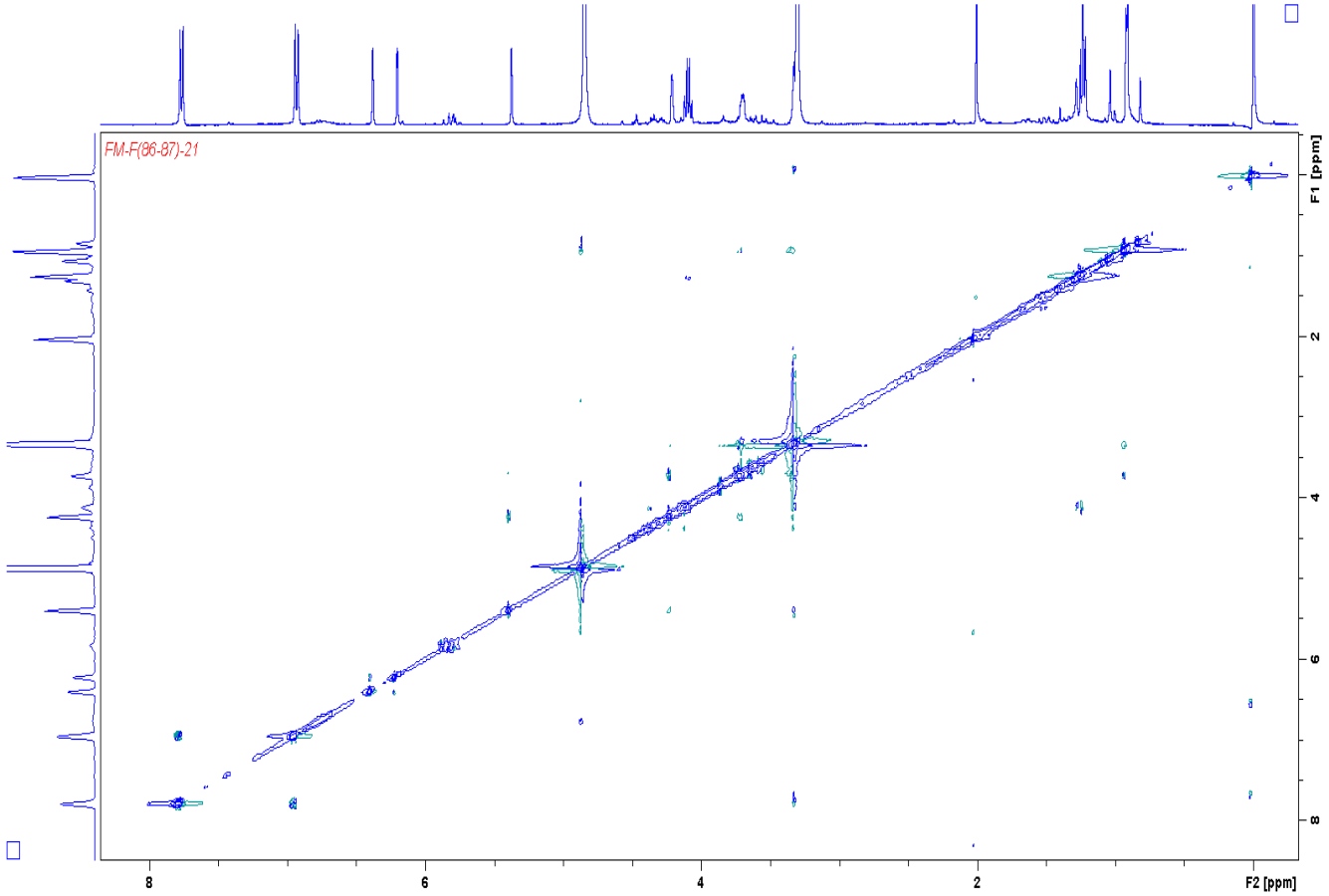


Figure 4.99: ROESY spectrum of A13

Table 4.16: NMR spectral data of A13 compared with literature

NO.	**^{13}C NMR (500 MHz)** $CDCl_3$	**^{13}C NMR (500 MHz)** $CDCl_3$ **(Venktata and Prakash, 2011)**	**^{1}H NMR (500 MHz)** $CDCl_3$ **(*J* in Hz)**	**^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz) (Venktata and Prakash, 2011)**	**HMBC (H → C)**	**COSY (H → H)**	**ROESY (H → H)**
2	159.0 C	158.2					
3	136.4 C	136.1					

4	179.8	C	179.0					
5	161.8	C	162.3					
6	99.9	CH	99.7	6.22 dd (2.08)	6.27	7,10,	7	
7	163.4	C	162.8				6	
8	94.9	CH	94.7	6.41 d (2.08)	6.72	6,7,9,10		
9	158.7	C	157.2					
10	106.1	C	107.1					
1’	122.7	CH	121.4				2”	3’,6’
2’/5’	132.1	CH	131.3	7.71 d 2H(8.84)	7.80	2,5,(2’/5’),	3’/6’	2’,5,5”
3’/6’	116.7	CH	116.6	6.90 d 2H (8.84)	6.98	5,1’,(3’/6’)	2’/5’	
4’	163.1	C	161.6					
1”	103.67	CH	104.4	5.39 d 1H (1.5)	5.42	2”,3”,5”	2”	2”,5
2”	72.19	CH	72.2	4.19 dd 1H (1.7.,3.3)	4.22	1”,3”	1’ 1”,3”	1”,3”
3”	72.27	CH	72.5	3.71 dd 1H (3.9,5.7)	3.37*		2”,4”	2”
4”	73.3	CH	73.7	3,35 m	3.36*	2”,3”,5”	5”,3”	
5”	72.07	CH	71.4	3.33 m	3.38*		6”	2’,5’,6”
6”	17.79	CH_3	18.4	0.98 s	0.93	4”,5”	5”	1”,5”

4.16 Isolation of A14

Compound A14 (4.9 mg) was isolated as pink powder, Rf = 0.73 using ethylacetate : methanol 8:2 as solvent system (Plate 4.16) and $[\alpha]^{21}_{D} = +55^{0}$ (c = 0.04, CH_2Cl_2).



Plate 4.16: TLC chromatogram of A14 using ethylacetate : methanol 8:2 as solvent system

4.16.1. Physical tests carried out on A14

Solubility of A14

A14 was found to be soluble in methanol.

Melting point of A14

The compound was found to have a melting point range of 175^0C - 177^0C.

4.16.2 SPECTRAL ANALYSIS OF A14

4.16.2.1 LCMS spectral analysis of A14

The mass spectrum of A14 (Figure 4.100) showed molecular ion M^+ peak at *m/z* = 464

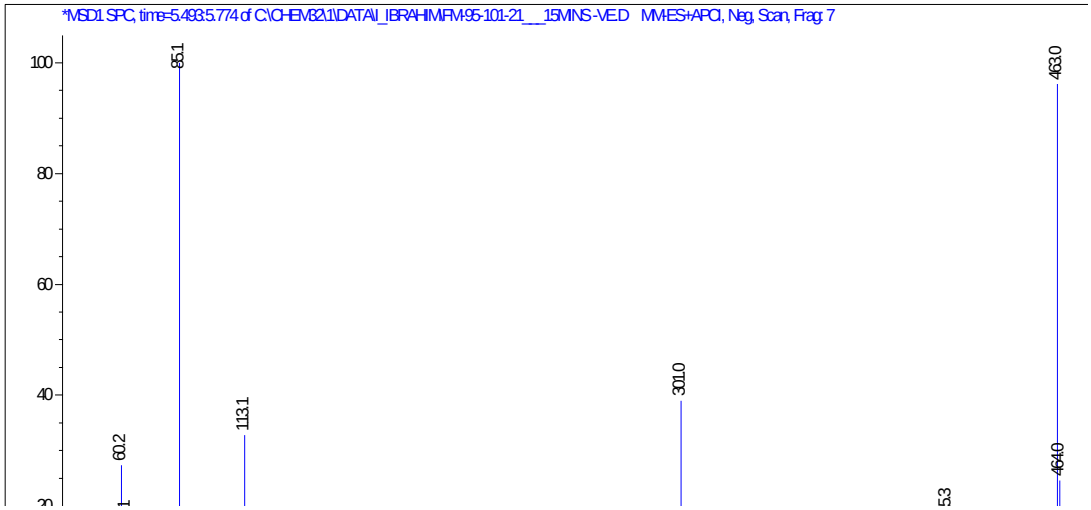


Figure 4.100: LCMS spectrum of A14

4.16.2.2 IR spectrum of A14

The IR spectrum of A14 (Figure 4.101) showed absorption peak at 3388 cm^{-1} (O-H stretching), 1687 cm^{-1} (C=O stretching) and 1499 cm^{-1} (aromatic C=C stretching)

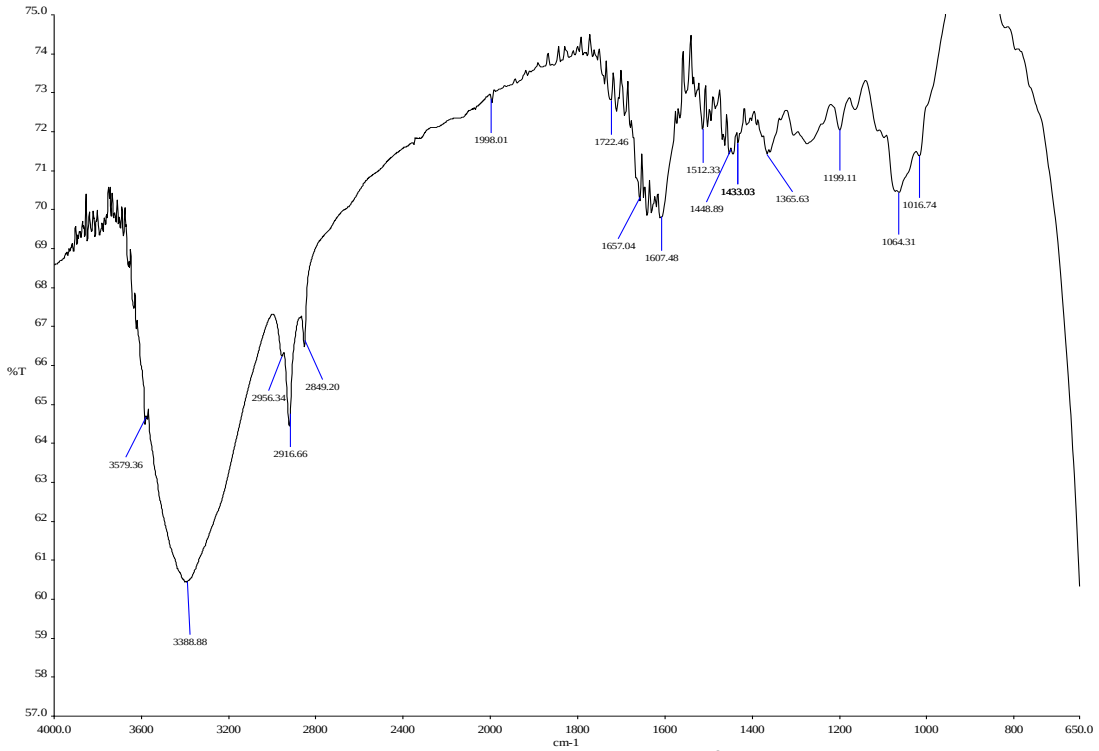


Figure 4.101: IR spectrum of A14

4.16.2.3 ^{1}H NMR spectral analysis of A14

The ^{1}H-NMR (CD_3OD, 400 MHz) of A14 (Figure 4.102) revealed signals at δ_H 7.68 (d, *J* = 2.02 Hz, H-2′), 7.55 (dd, *J* = 2.14, 6.4 Hz, H-6′), 6.83 (d, *J* = 8.4 Hz, H-5′), 6.26 (d, *J* = 1.76 Hz, H-8), 6.09 (d, *J* = 1.89 Hz, H-6), 5.13 (d, *J* = 7.68 Hz, H-1′′), 3.75 (dd, *J* = 2.34, 12.0 Hz, H-6′′) and 3.20-3.90 (sugar protons).

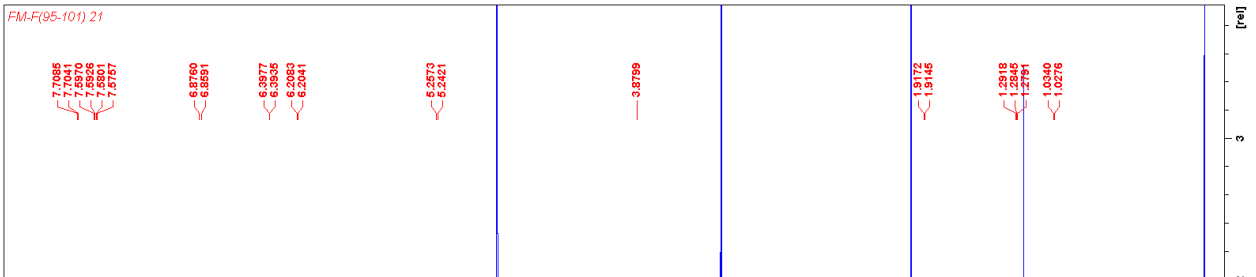


Figure 4.102: ^{1}H NMR spectrum of A14

4.16.2.4 ^{13}C NMR spectral analysis of A14

The ^{13}C NMR(125 CD_3OD) spectrum of A14 (Figure 4.103) showed signals 21 carbon signals which include signals at δc 179.4 (C-4), 168.8 (C-7), 161.5 (C-5), 157.2 (C-2), 148.0 (C-4′), 144.8 (C-3′), 134.0 (C-3), 121.8 (C-6′), 121.6 (C-1′), 116.1 (C-2′), 114.7 (C-5′), 104.4 (C-1′′), 100.3 (C-6), 94.0 (C-8), 103.3 (C-10), 78.3 (C-5′′), 78.1 (C-2′′), 75.7 (C-3′′), 71.2 (C-4′′), 62.6 (C-6′′) etc.

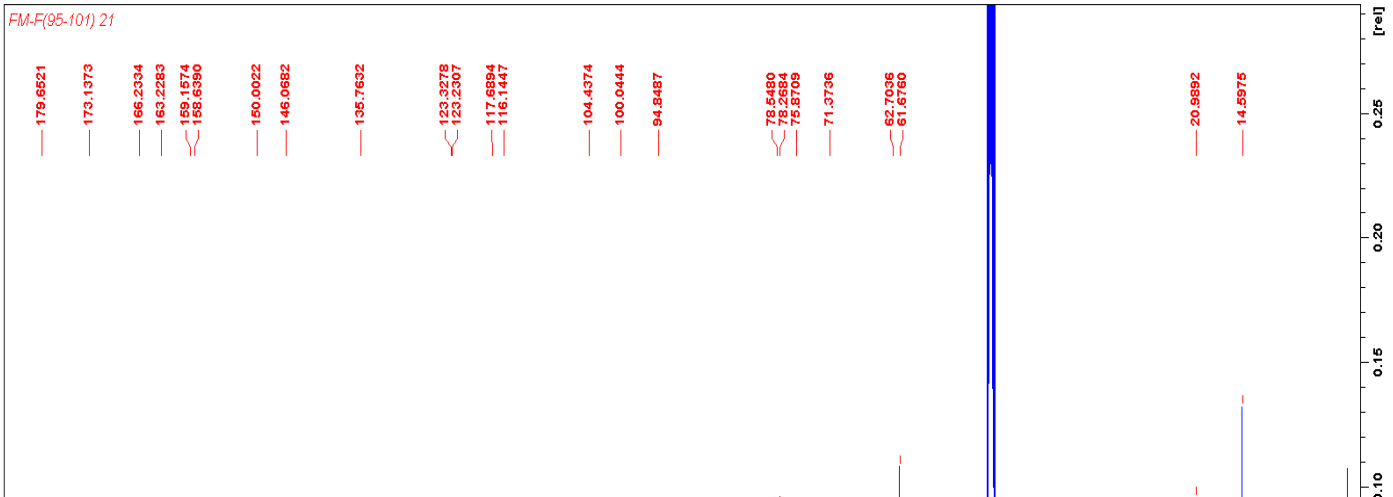


Figure 4.103: ^{13}C NMR spectrum of A14

4.16.2.5 DEPT-135 spectral analysis of A14

The DEPT experiment of compound A14 (Figure 4.104) revealed ninemethine and one methylene carbon resonances. Quaternary carbons that will not appear on the DEPT-135 spectrum were also noted.

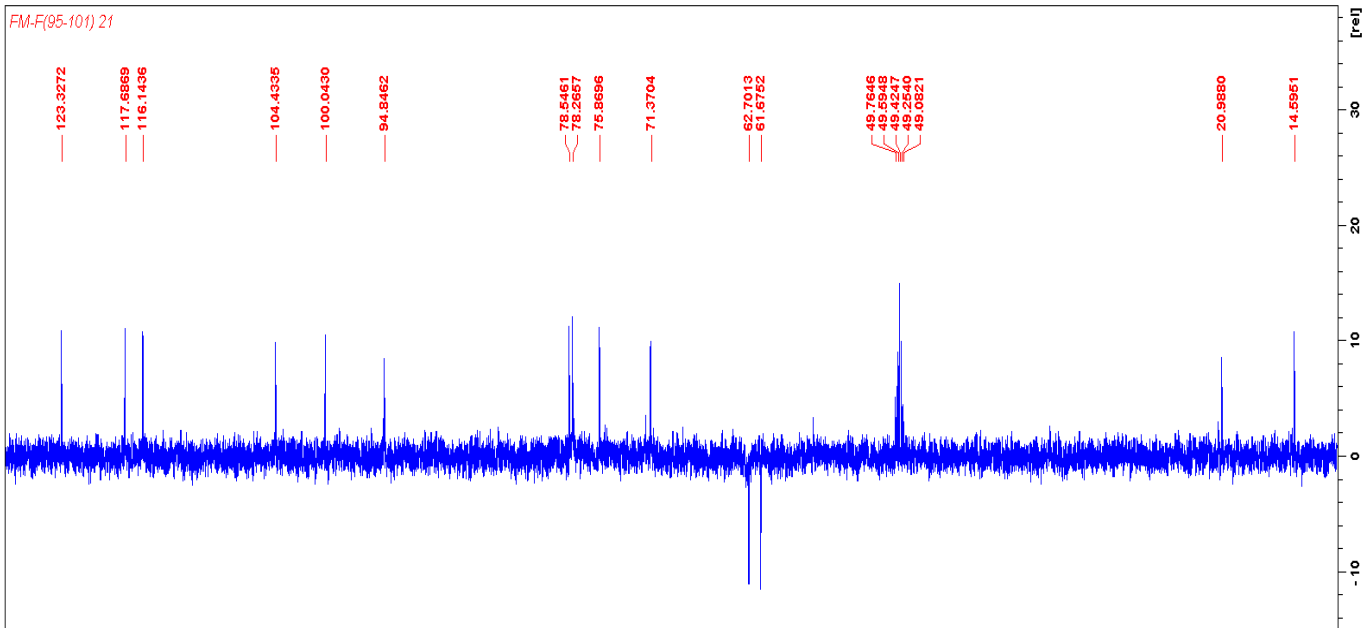


Figure 4.104: DEPT-135 spectrum of A14

4.16.2.6 HSQC-DEPT spectral analysis of A14

The HSQC-DEPT spectrum of A14 shown in Figure 4.105 was used to assign protons to their respective carbon atoms.

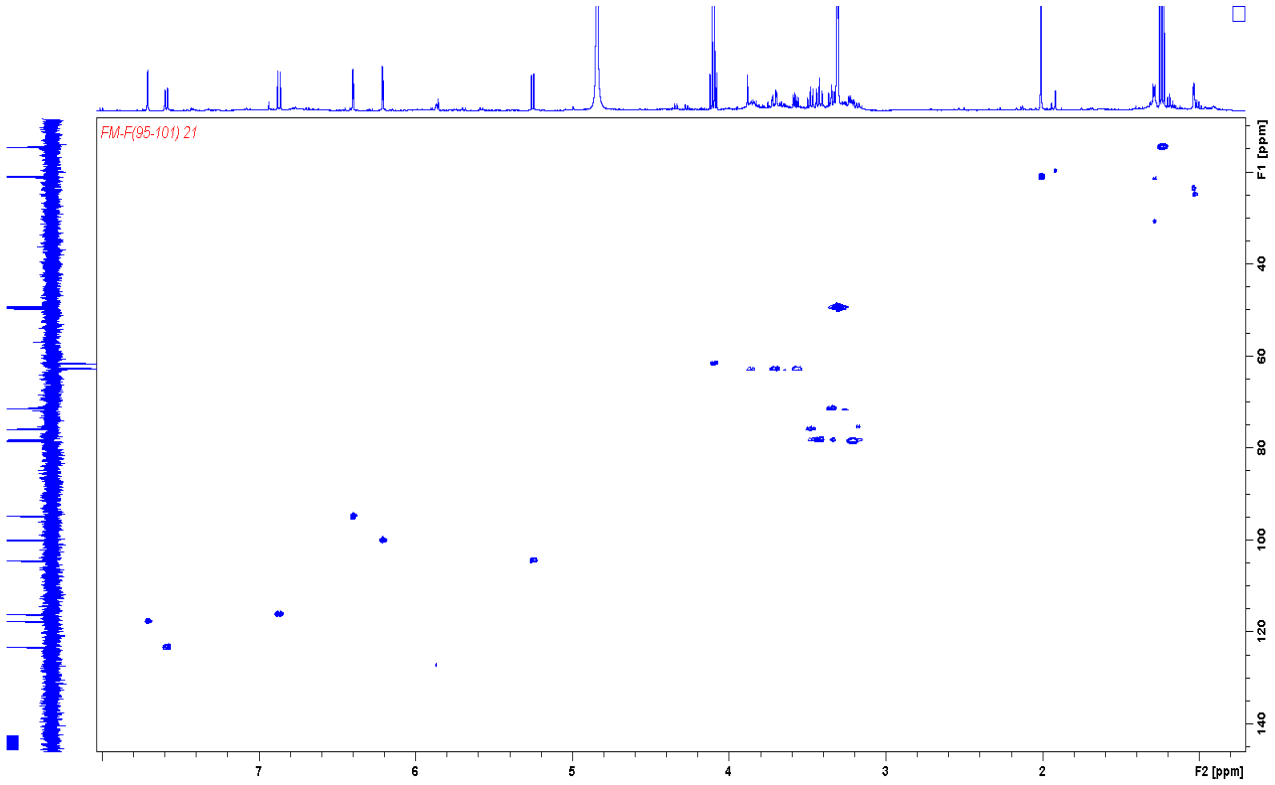


Figure 4.105: HSQC-DEPT spectrum of A14

4.16.2.7 COSY spectral analysis of A14

The COSY spectrum of A14 (Figure 4.106) showed cross peaks between proton resonance at δ_H 7.71 (H-2'/5') and δ_H 6.90 (H-3'/6') and between δ_H 6.22 (H-6) and δ_H 6.41 (H-8). The correlations among the adjacent proton resonances of the sugar moiety were also observed.

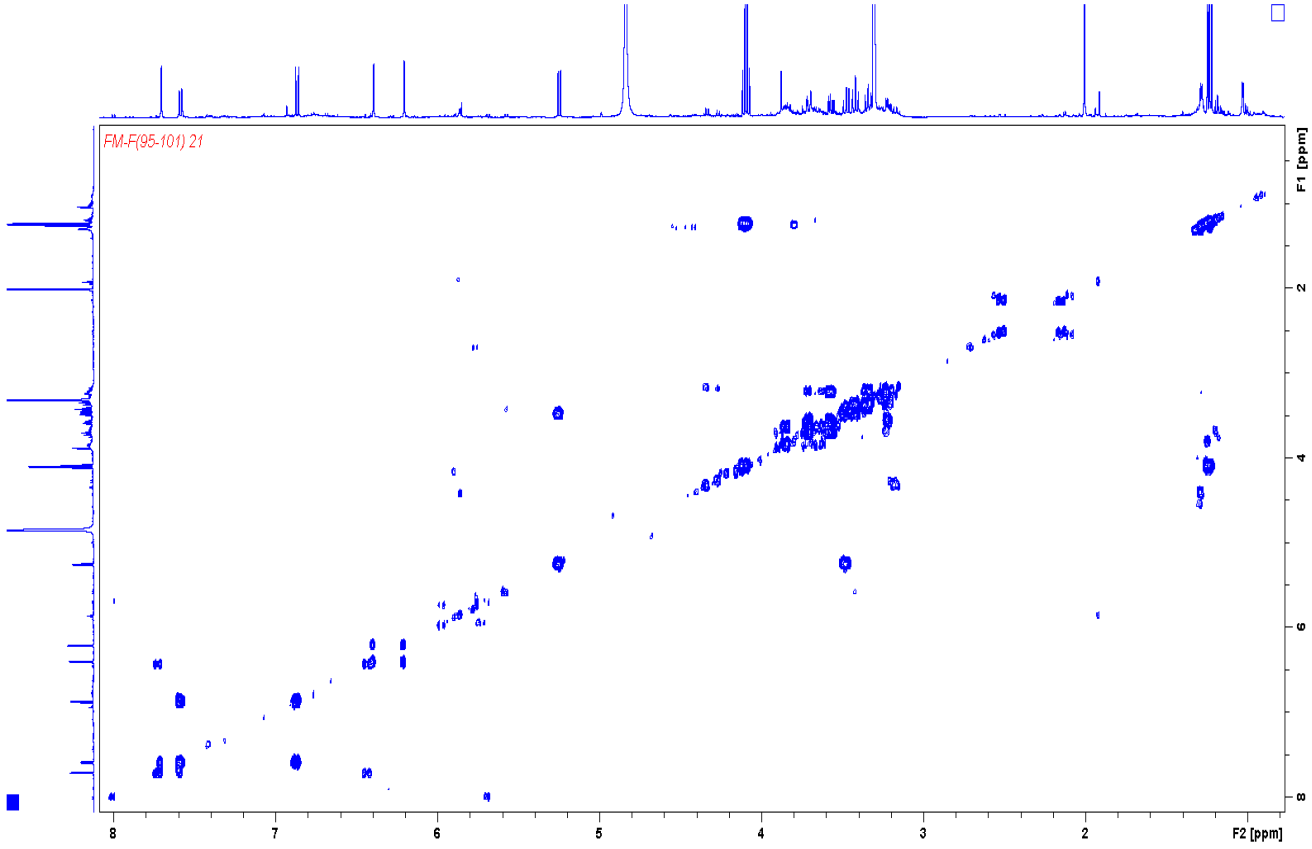


Figure 4.106: COSY spectrum of A14

4.16.2.8 HMBC spectral analysis of A14

The HMBC spectrum of A14 (Figure 4. 107) revealed that the proton signals at δ_H 7.71 (H-2'/5') showed cross peaks with carbon resonances at δc 159.0, (C-2), 161.8 (C-5), 116.7 (C-3'/6'). Proton signals at δ_H 6.90 (H-3'/6') showed cross peaks with a carbon resonance at δc 161.8 (C-3), 122.7 (C-c1') and 132.1 (C-2'/5') while anomeric proton signals at δ_H 5.19 (H-1'')showed cross peaks with a carbon at δc 136.4 (C-3).

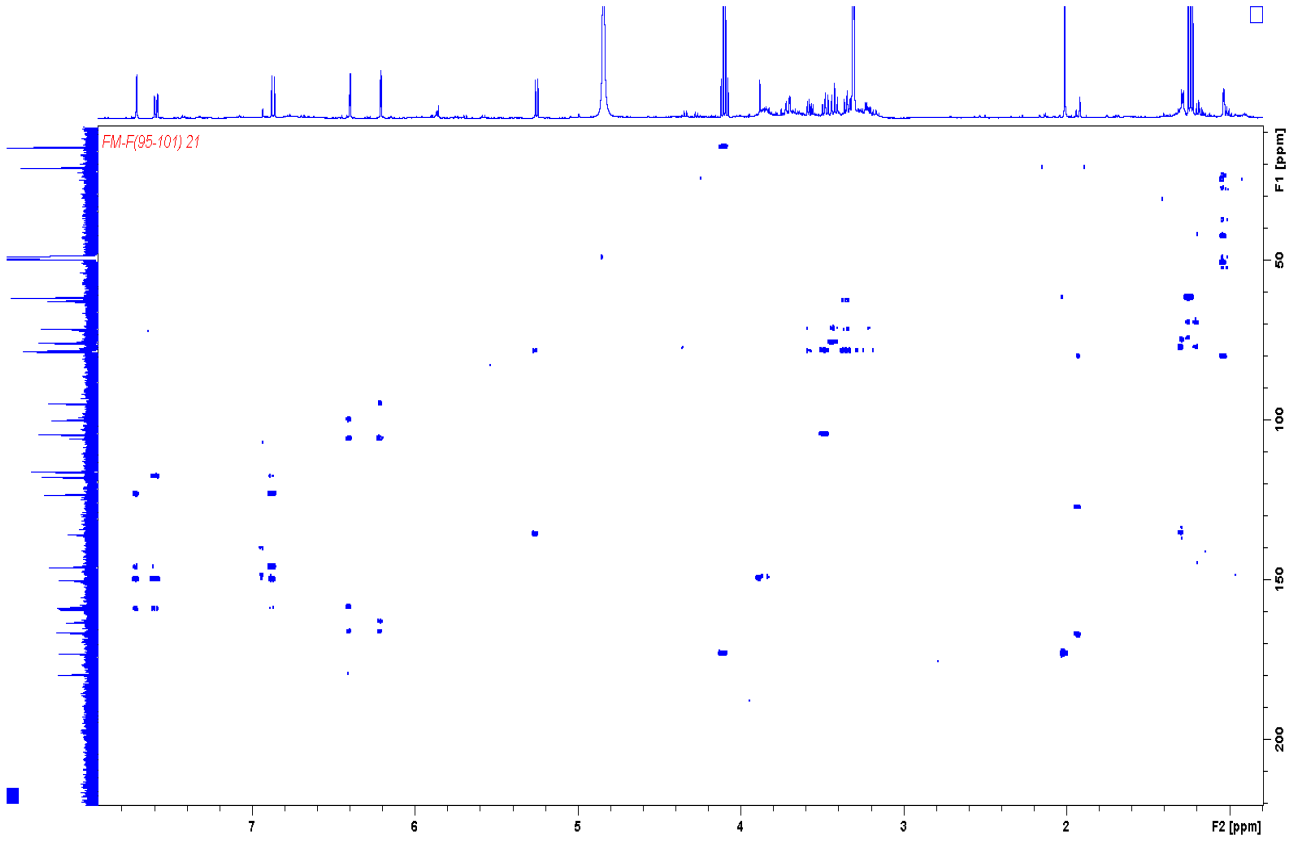


Figure 4.107: HMBC spectrum of A14

Table 4.17: NMR spectral data of A14 compared with literature

NO.	**^{13}C NMR (500 MHz) $CDCl_3$**	**^{13}C NMR (500 MHz) $CDCl_3$ (Hadi *et al.*, 2017)**	**^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz)**	**^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz) (Hadi *et al.*, 2017)**	**HMBC (H → C)**	**COSY (H → H)**	**ROESY (H → H)**
1							
2	159.17 C	158.44					
3	136.4 C	135.64					
4	179.76 C	179.48					
5	163.23 C	162.99					
6	100.04 C	99.89	6.20 d 1H (2.1)	6.19	5,7,8,10	-	
7	166.23 C	165.97					
8	94.85 CH	94.73	6.39 d 1H (2.1)	6.38	6,7,9,10		6

9	158.7 CH	158.44					
10	105.8 C	106.68					
1’	123.04 C	123.08					
2’	116.14 CH	117.59	7.58 dd 1H (2.2, 8.5)	7.71	1’,3’,6’		3’
3’	146.04 CH	145.87	6.86 d 1H (8.5)	6.91	2’,4’,5,		2’
4’	150.00 C	149.33					
5’	117.61 C	116.01				6’	
6’	123.4 CH	123.20	7.70 d 1H (8.5)	7.59	1’,2’,4’,5’	5’	8
1”	104.44 CH	104.39	5.2 d 1H (7.7)	5.23	3,3’	2”	2”,4”,3”
2”	75.87 CH	75.37	3.50	3.48	1”,3”	1”,3”	1”
3”	78.54 CH	78.11	3.24	3.35	4”	2”,4”	1”
4”	71.37 CH	71.22	3.37	3.43	3”,5”,6”	3”,5”	1”
5”	78.27 CH	78.35	3.50	3.24	1”,3”,4”	6” α, 6” β	
6”a	61.67 CH_2	62.58	4.12 dd 1H (1.8, 12.0)	3.37	5”	5”	
6”b			3.57 d 1H (4.5, 11.6)	3.56		5”	

4.17 Isolation of A15

Compound A15 (11.3 mg) was isolated as dark green amorphous solid, Rf = 0.59 using dichloromethane : ethylacetate 20:1 as solvent system (Plate 4.15).

.



Plate 4.17: TLC Chromatogram of A15 using dichloromethane : ethylacetate 20:1 as solvent system

Rf = 0.59

4.15.1. Physical tests carried out on A15

Solubility of A15

A15 was found to be soluble in methanol.

Melting point of A15

The compound was found to have a melting point range of 163 - 165^{0}C.

4.17.2 SPECTRAL ANALYSIS OF A15

4.17.2.1^{1}H NMR spectral analysis of A15

The 1H NMR (500 MHz, $CDCL_3$) spectrum of A15 (Figure 4.108) showed proton resonances at δ_H 3.21 ***(s ,*** H-2^1), 7.98 (t, *J*=17.8 Hz,H-3^1), 6.11 (dd, *J*=1.4, 17.8 Hz, Hz, H-3^{2}E), 6.23 (dd, *J* = 1.4 Hz, 11.6 Hz, H-3^{2}Z), 9.32 (s, 3H-5), 3.30 ***(s,*** 3H-7^1), 8.48 (s, H-20), 3.61 ***(s,*** 3H-12^1), 1.73 ***(s,*** 3H-18^1), 1.53 ***(****Sept., J =* 6.6 Hz, 3H-P5/3H-P15'), 0.77 (m, 3H-18/3H-19) and 1.5 0 (d, *J* = 4.2 Hz).

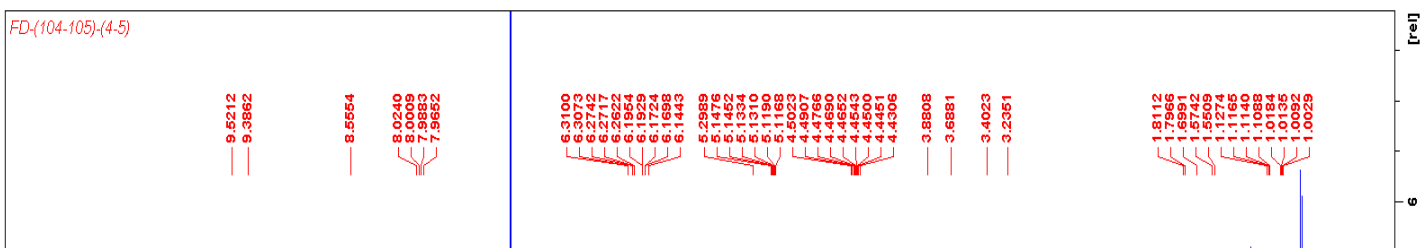


Figure 4.108: ^{1}H NMR spectrum of A15

4.17.2.2 ^{13}C NMR spectral analysis of A15

The ^{13}C NMR spectrum of A15 displayed fifty five carbon resonances as shown in Figure 4.109.

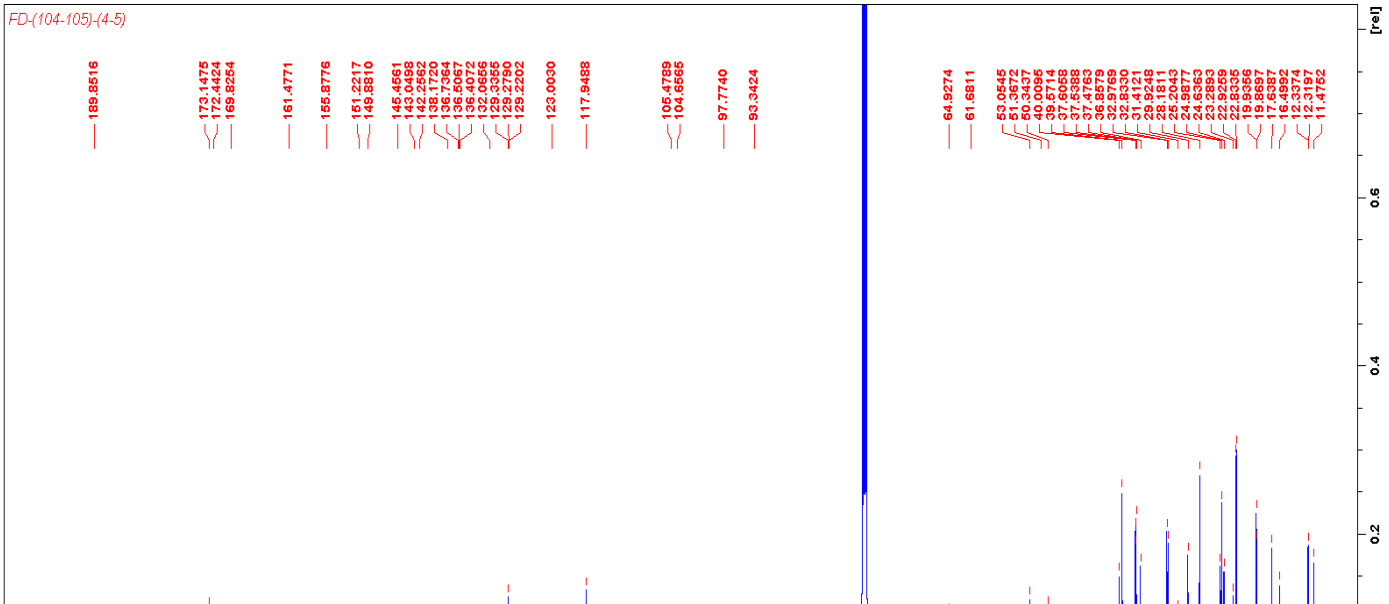


Figure 4.109: ^{13}C NMR spectrum of A15

4.17.2.3 DEPT-135 spectral analysis of A15

The DEPT-135 spectrum of A15 (Figure 4.110) showed eleven methyl, thirteen methylene and ten methine carbon resonances. Twenty-one quaternary carbon signals which will not show in the spectrum were also noted.

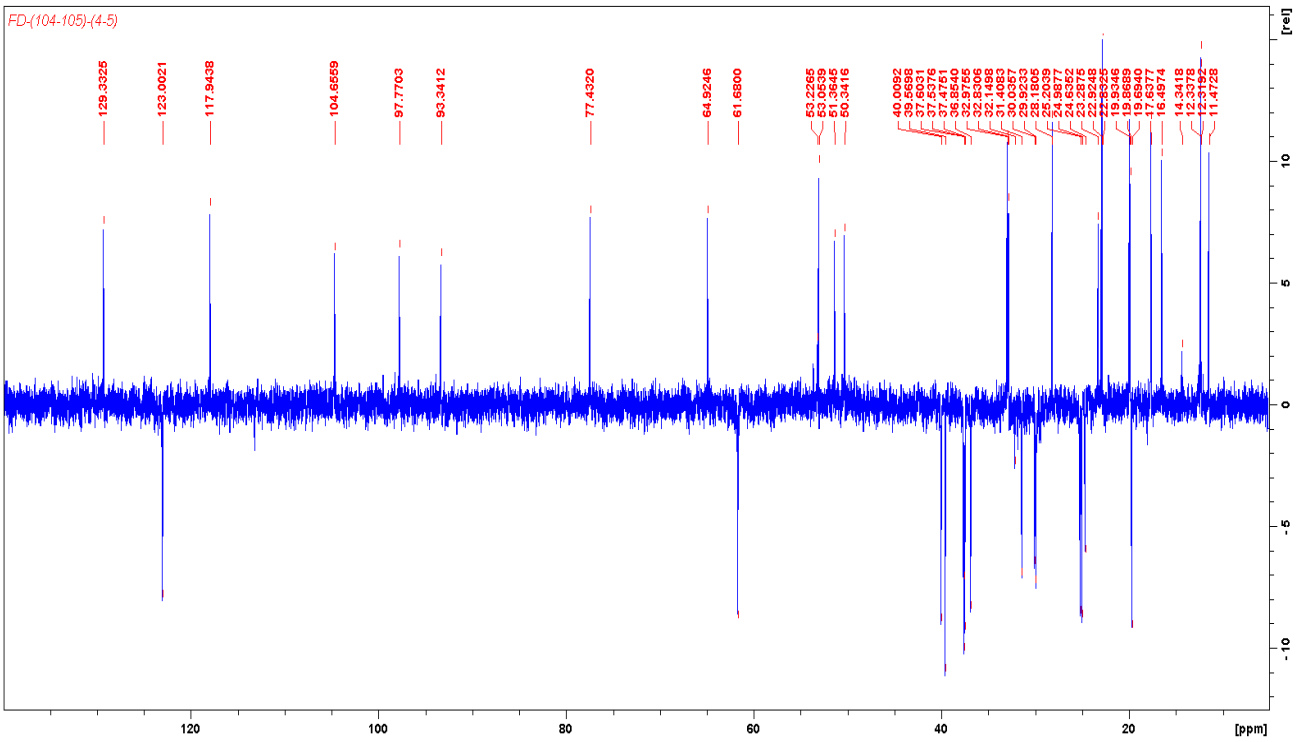


Figure 4.110: DEPT-135 spectrum of A15

4.17.2.4 HSQC-DEPT spectral analysis of A15

The HSQC-DEPT spectrum of A15 (Figure 4.111) was used to assign protons to their respective carbon atoms.

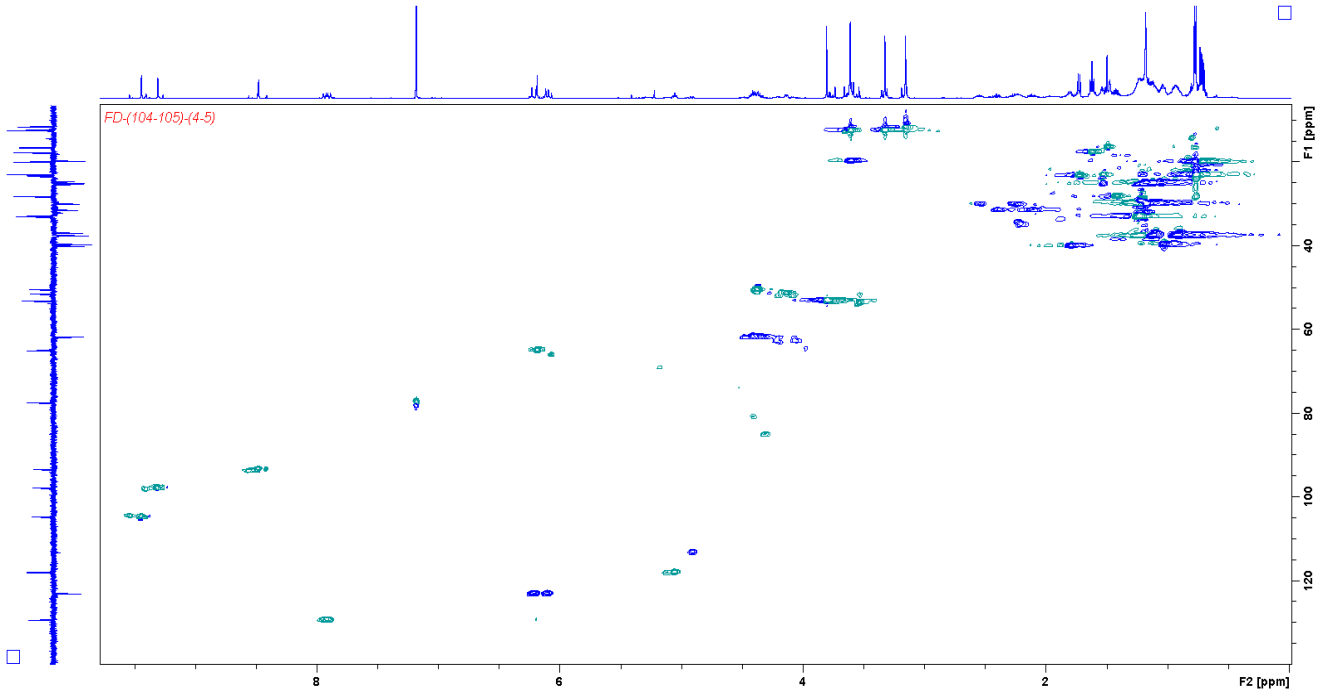


Figure 4.111: HSQC-DEPT spectrum of A15

4.17.2.5 COSY spectral analysis of A15

The COSY spectrum of A15 is shown in Figure 4.112

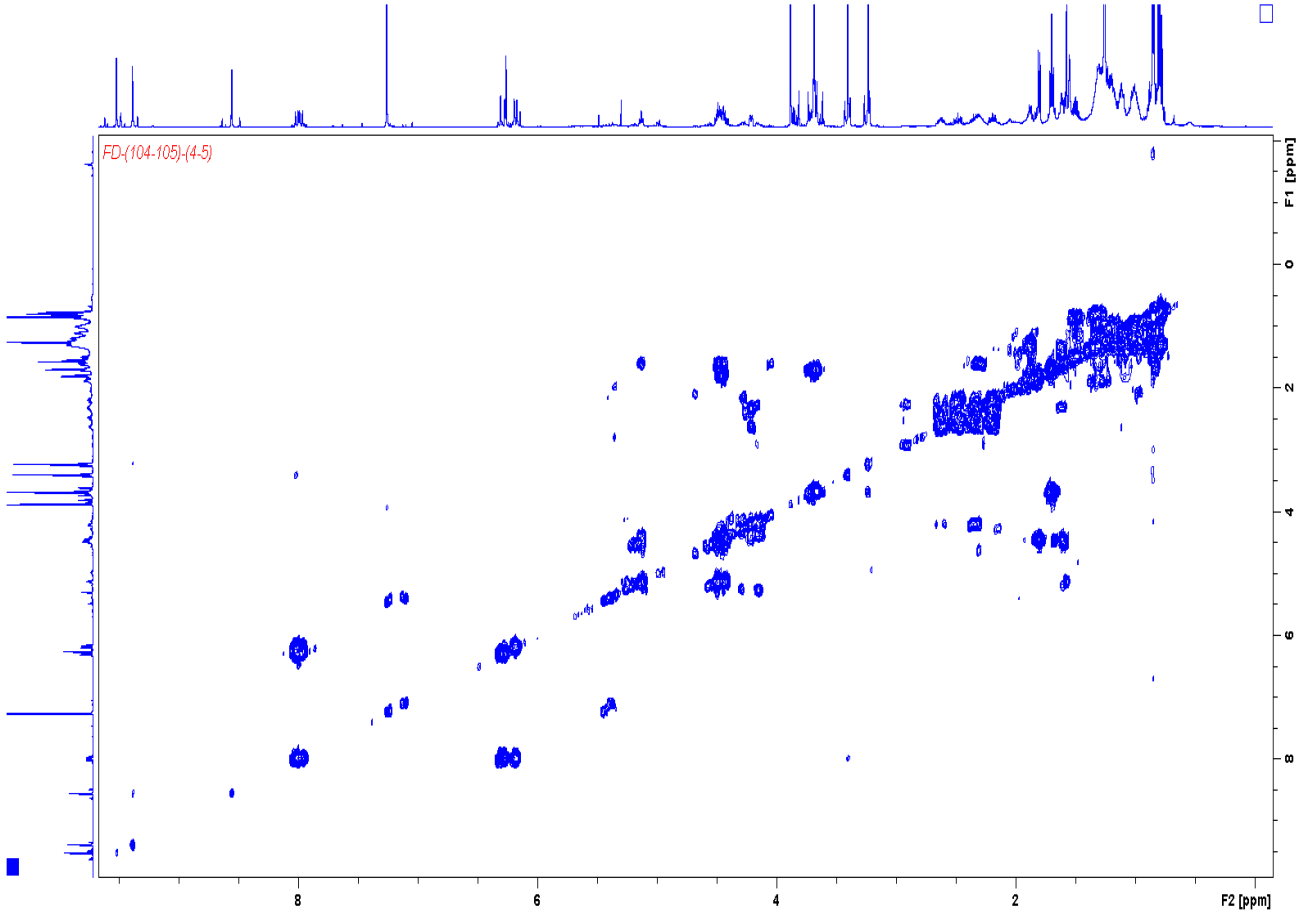


Figure 4.112: COSY spectrum of A15

4.17.2.6 HMBC spectral analysis of A15

The HMBC spectrum of A15 shown in Figure 4.113 was used to confirm the connectivity network in the proposed structure of the compound

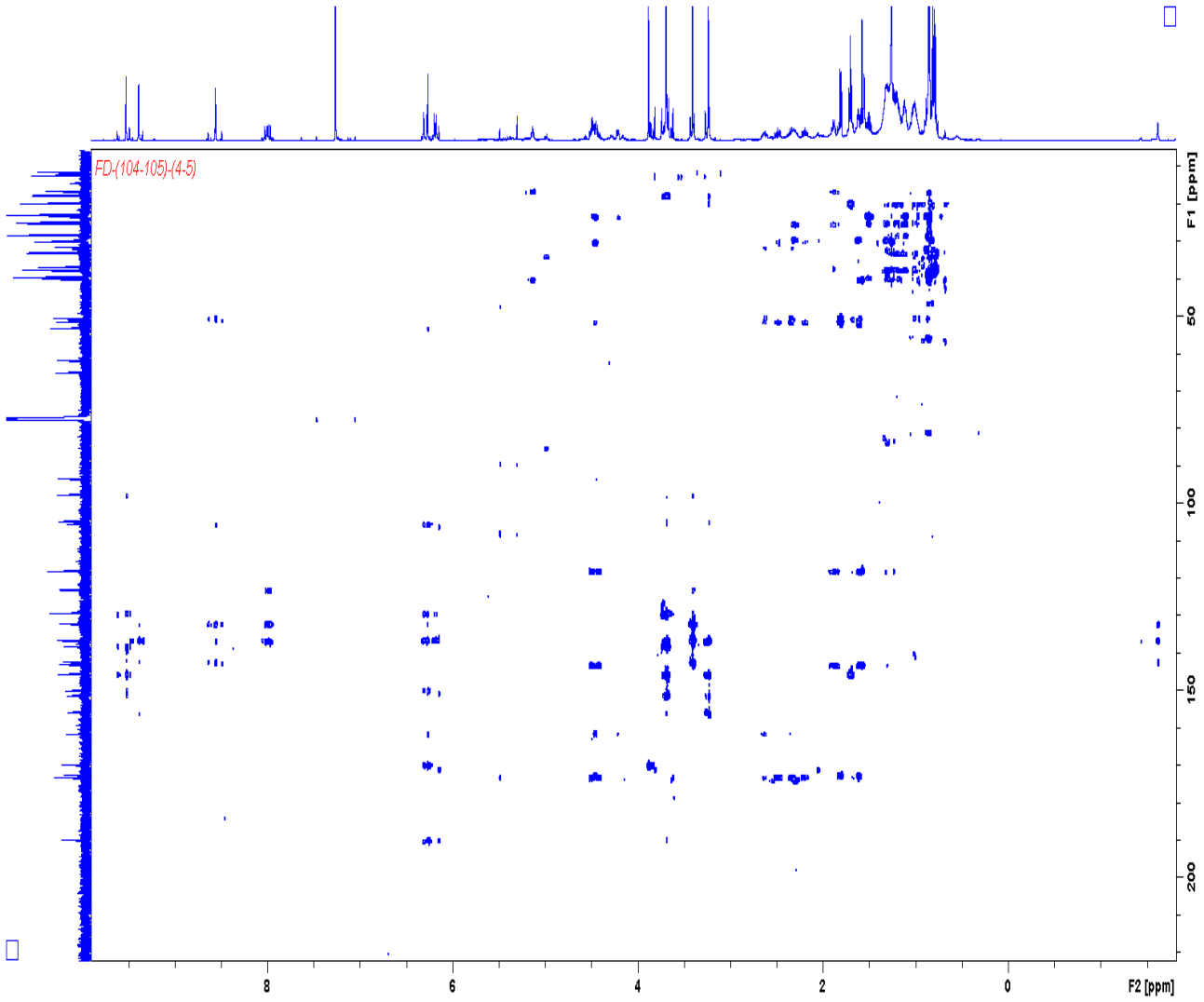


Figure 4.113: HMBC spectrum of A15

Table 4.18: NMR spectral data of A15 compared with literature

No.	^{13}C NMR (500 MHz) in $CDCl_3$	^{13}C NMR (100 MHz) (Akihiko, 1996)	^{1}H NMR (400 MHz) $CDCl_3$(*J* in Hz)	^{1}H NMR (100 MHz) (Akihiko, 1996)	HMBC (H→C)	COSY (H→H)
1	143.1 C	142.8	-		-	-
2	132.1 C	131.8	-		-	-
2^1	12.3 CH_3	12.1	3.20 s	3.39 dd	1, 2, 3	-
3	136.7 C	136.6	-		-	-
3^1	129.0 CH	129.1	7.98 dd, (6.3, 17.8)	7.98 dd	2, 3, 3^2, 4	3^2E, 3^2Z
3^2E	123.0 CH_2	122.9	6.23 dd, (1.4, 17.8)	6.28 dd	3, 3^1	3^1, 3^2Z
3^2Z			6.11 dd, (1.4, 11.6)	6.17 dd	3, 3^1	3^1, 3^2E
4	136.5 C	136.3	-		-	-
5	97.8 CH	98.0	9.30 s	9.36 s	3, 4, 7	-
6	155.9 C	155.4	-		-	-
7	136.4 C	136.2	-		-	-
7^1	11.5 CH_3	11.3	3.3 s	3.21 s	6, 7, 8	-
8	145.5 C	145.8	-		-	-
8^1A	19.7 CH_2	19.5	3.70 q, (7.8)	3.66 q	7, 8, 8^2, 9	8^1A, 8^2
8^1B			3.70 q, (7.8)	3.66 q	7, 8, 8^2, 9	8^1A, 8^2

8^2	17.6 CH_3	17.4	1.62 t, (7.8)	1.68 t	8, 8^1	8^1A, 8^1B
9	151.2 C	151.1	-		-	-
10	104.57 CH	104.3	9.52 s	9.50 s		-
11	138.2 C	137.8	-		-	-
12	129.4 C	129.4	-		-	-
12^1	12.3 CH_3	12.3	3.73 s	3.88 s	11, 12, 13	-
13	129.3 C	127.0	-		-	-
13^1	189.8 C	192.0	-		-	-
13^2	64.9 C	89.0	9.19 s	6.26 s	13,13^1,13^2,13^3,14, 15	`
13^3	172.5 C	172.8	-		-	-
13^4	55.3 OCH_3	53.4	3.68 s	3.68 s	13^3	-
14	150.0 C	149.8	-		-	-
15	105.5 C	107.7	-		-	-
16	161.5 C	162.5	-		-	-
17	51.4 CH	51.8	4.15 ddd, (3.3, 9.0, 11.8)	4.21 ddd	17^1, 17^2, 18^1, 19	17^1A, 17^1B
17^1A	29.9 CH_2	31.1	2.39 m		16, 17, 17^2, 17^3, 18	17, 17^1B, 17^2A, 17^2B
17^1B			2.11 sep, (4.3)		17, 17^2, 17^3, 18	17, 17^1A, 17^2A, 17^2B
17^2α	31.4 CH_2	31.6	2.40 m		17, 17^1, 17^3	17^1A, 17^1B, 17^2B

17^2β			2.26sep, (4.3)		17, 17^1, 17^3	17^1A, 17^1B, 17^2A
17^3	173.1 C	173.6	-		-	-
18	50.3 CH	50.3	4.32 m	4.46 dq	16, 17, 17^1, 18^1, 19	18^1
18^1	23.3 CH_3	22.7	1.73 d, (7.3)	1.80 d	17, 18, 19	18
19	172.4 C	172.2	-		-	-
20	93.3 CH	93.6	8.56 s	8.55	1, 2, 18	-
P1A	61. CH_2		4.35 m		P2, P3, 17^3	P1B, P2, P20
P1B			4.35 m		P2, P3, 17^3	P1A, P2, P20
P2	118.0 CH		5.23dt, (1.1, 7.1)		P4, P20	P1A, P1B, P20
P3	143.1 C		-		-	-
P4A	40.0 CH_2		1.82 m		P2, P3,P20	P4B, P5A, P5B
P4B			1.82 m		P2, P3,P20	P4A, P5B, P5B
P5A	25.2 CH_2		1.20 m		P3, P4, P6	P4A, P4B, P5B, P6A, P6B
P5B			1.20 m		P3, P4, P6	P4A, P4B, P5A, P6A, P6B
P6A	36.9 CH_2		1.23* m		P4, P5, P7, P8, P19	P5A, P5B, P6A, P7
P6B			1.02* m		P5, P7, P8, P19	P5A, P5B, P6A, P7
P7	33.0 CH		1.22* m		P6, P8	P6A, P6B, P8A, P8B, P19
P8A	37.6 CH_2		1.20* m		P6, P7, P9, P10, P19	P7, P8B, P9A, P9B

P8B			1.02* m		P6, P7, P9, P10, P19	P7, P8A, P9A, P9B
P9A	24.6 CH_2		1.04 m		P8, P10	P8A, P8B, P9B, P10A, P10B
P9B			1.04 m		P8, P10	P8A, P8B, P9A, P10A, P10B
P10A	37.5 CH_2		1.20* m		P8, P9, P11, P12, P18	P9A, P9B, P10B, P11
P10B			1.02* m		P8, P9, P11, P12, P18	P9A, P9B, P10A, P11
P11	32.8 CH		1.22* m		P9, P10, P12, P13, P18	P10A, P10B, P12A, P12B, P18
P12A	37.5 CH_2		1.20* m		P10, P11, P13, P18	P11, P12A, P13A, P13B
P12β			1.02* m		P10, P11, P13, P14, P18	P11, P12A, P13A, P13B
P13α	25.0 CH_2		1.20* m		P11, P12, P14	P12A, P12B, P13B, P14A, P14B
P13β			1.20* m		P11, P12, P14	P12A, P12B, P13A, P14A, P14A
P14A	39.6 CH_2		1.03 m		P12, P13, P16, P17	P13A, P13B, P14A, P15
P14B			1.03 m		P12, P13, P16, P17	P13A, P13B, P14A, P15
P15	28.2 CH		1.42 sep, (6.6)		P13, P14, P16, P17	P14A, P14B, P16, P17
P16	22.8 CH_3		0.82 d, (6.6)		P14, P15, P17	P15, P17

P17	22.9	CH_3		0.82 d, (6.6)		P14, P15, P16	P15, P16
P18	19.9	CH_3		0.82 d, (6.6)		P10, P11, P12	P11
P19	19.9	CH_3		0.81 d, (6.6)		P6, P7, P8	P7
P20	16.5	CH_3		1.57 d,(4.2)		P2, P3, P4	P1A, P1B, P2

4.18 RESULTS OF ANTIOXIDANT ACTIVITY STUDIES

4.18.1 Quantitative analysis

The results of quantitative *invitro* DPPH radical scavenging activity of the DCM and methanol extracts of the leaf of *Ficus sycomorus* showed an increase in antioxidant activity with increase in concentration of extract. The linear graph in (Figure 4.114 and 4.115) showed good association between the antioxidant activity and concentration of the plant extracts as the correlation coefficient tends toward one. The IC_{50} ^{DPPH} (the concentration of antioxidant which reduces the free radical DPPH by 50%) parameter was determined using Microsoft excel 2007, on linear graph section of the percentage of inhibition according to the concentration of the extract, a trend line equation y = ax + b was generated. IC_{50}^{DPPH} was calculated by transforming the equation above and the expression x at which y-value is 50% was accepted as unknown.

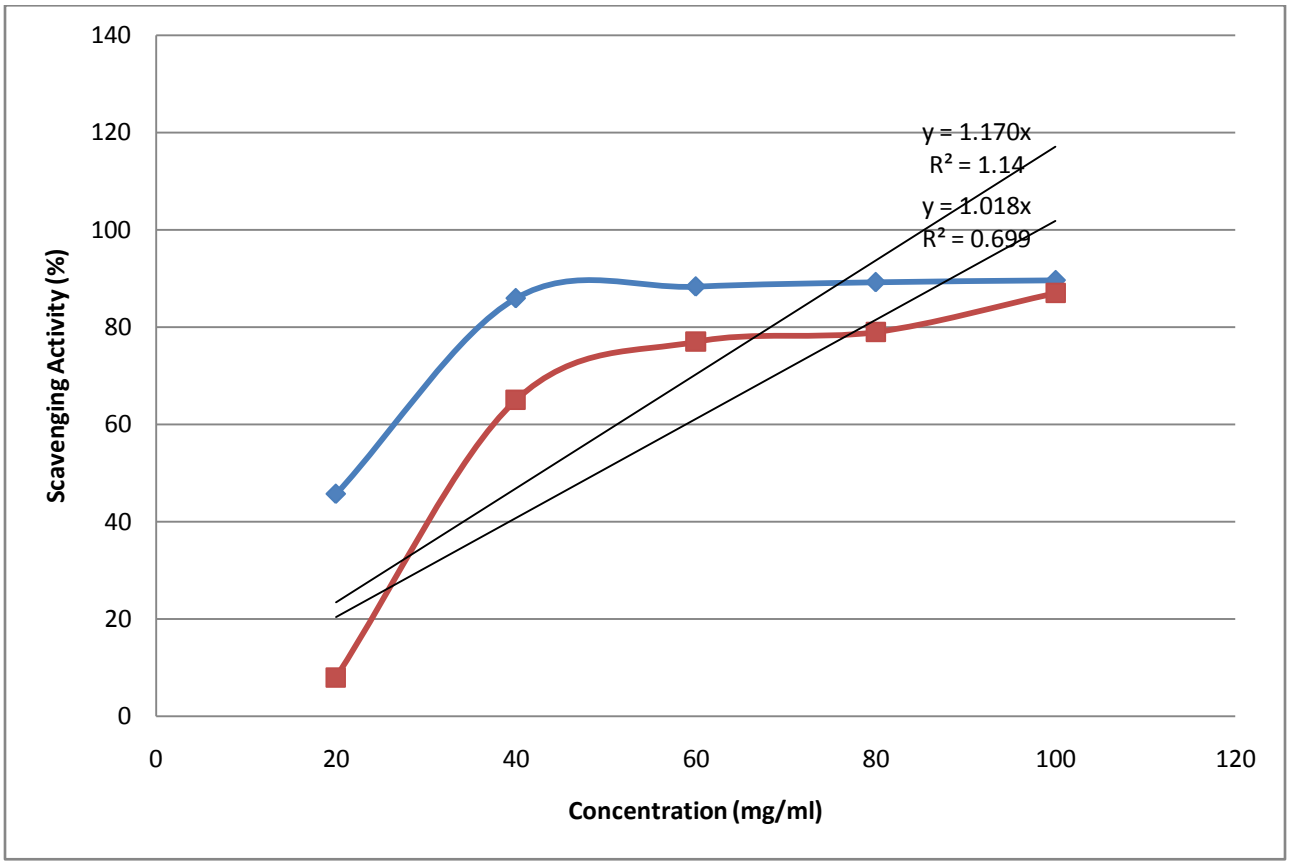


Figure 4.114: Graph of % inhibition (scavenging activity) against concentration (µg/ml) of the DCM and methanol leaf extracts of *Ficus sycomorus*

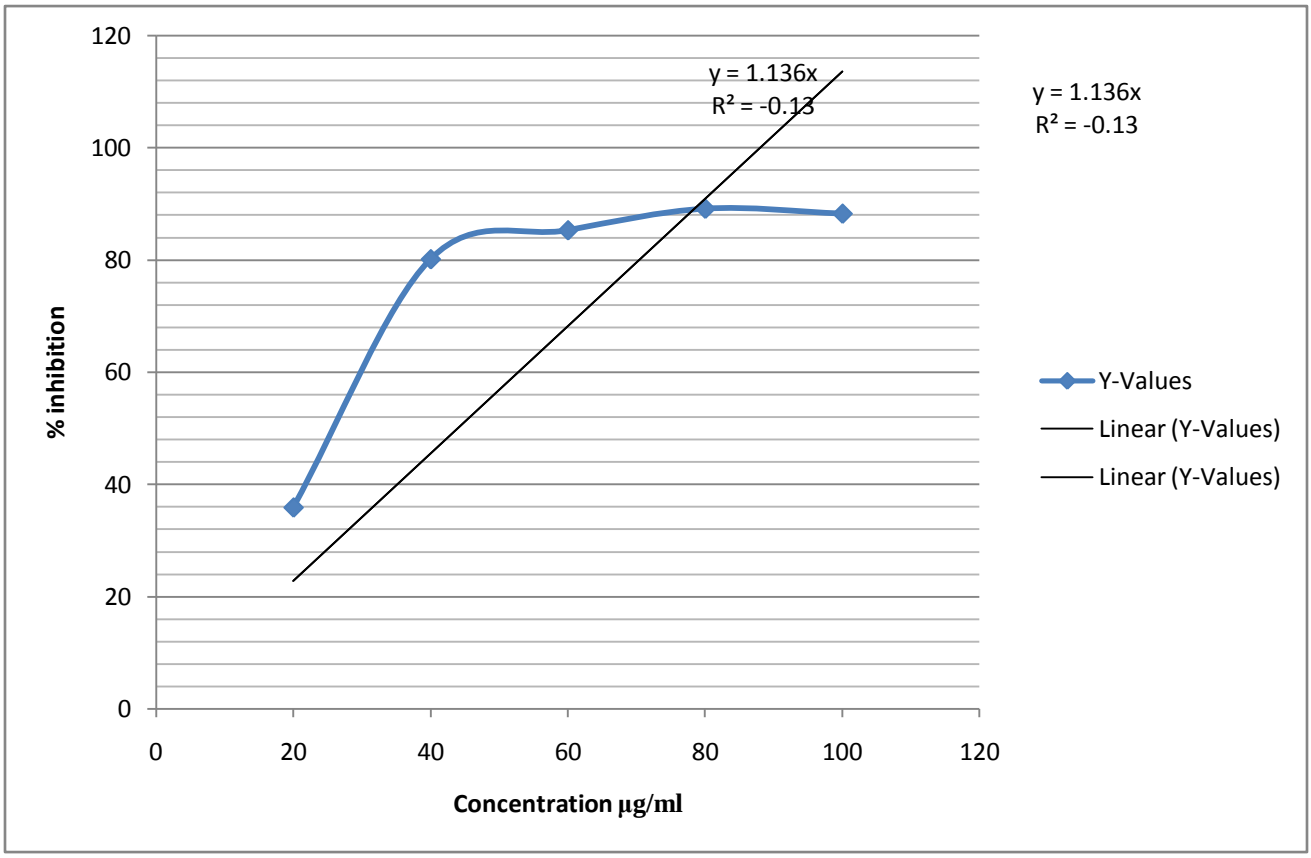


Fig 4.115: Graph of % inhibition (scavenging activity) against concentration µg/ml of Vitamin C

4.18.2 Qualitative Analysis: The qualitative antioxidant screening of the DCM andmethanol extractsusing different systems shows that the plant posses antioxidant activity due to the appearance of a yellow spot on a purple background as shown in plate 4.18 and 4.19.



Plate 4.18: Qualitative DPPH radical antioxidant studies of DCM extract using n-hexane : ethylacetate (8:2) as solvent system



Plate 4.19: Qualitative DPPH radical antioxidant studies of methanol extract using DCM : methanol (9:1) as solvent system

Table 4.19: IC_{50} values of DCM extract, methanolextract and Vitamin C

SAMPLE	IC_{50} (µg/ml)
DCM extract	42.83
Methanol extract	34.00
Vitamin C	25

4.19 Results of Anti-cancer studies

Results of anticancer studies of DCM and mehanol extracts, as well as the compounds A11, A12 A13 and A14 from the leave of *Ficus sycomorus* are summarized in Table 4.20

Table 4.20: Results of anticancer studies of the DCM extract, methanol extract and compounds A11, A12 A13 and A14

	% survival							
	MDA-MB-435[a]		HT-29[b]		MDA-MB-231[c]		OVCAR3[d]	
Extract	20 µg/mL	2 µg/mL	20 µg/mL	2 µg/mL	20 µg/mL	2 µg/mL	20 µg/mL	2 µg/mL
ME	100	100	95	98	74	81	68	88
DCME	100	100	100	100	68	87	85	95
A11	95	100	93	100	81	79	80	84
A12	83	100	92	92	88	86	88	100
A13	95	100	91	88	74	84	76	100
A14	85	94	85	89	67	76	73	77

[a] melanoma. [b] colorectal adenocarcinoma. [c] breast adenocarcinoma. [d] ovary adenocarcinoma

ME = Methanol extract

DCME = Dichloromethane extract

CHAPTER FIVE

5.0 DISCUSION

5.1 Phytochemical Screening

The results of phytochemical analysis revealed that the DCM extract of *Ficus sycomorus* leaves contains terpenoids, alkaloids andcardiac glycosides while the methanol extract containsalkaloids, flavonoids, tannins, saponins, cardiac glycosides, anthraquionones and terpenoids but coumarins and anthraquinones were found to be absent. This result is comparable to that reported by Sandabe *et al.*, 2002 which showed that the ethanolic extract of *Ficus sycomorus* contains cardiac glycosides, saponins,tannins but alkaloid was absent in hexane fraction of the plant. Hassan *et al*., 2007 also reported that the plant contains flavonoids, cardiac glycosides, saponins and tannins but devoid of coumarins. Flavonoids are most commonly known for their antioxidant activity; they are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants (Spencer *et al.,* 2008). Flavonoids have been reported to have anti-viral, anti-inflammatory, antitumor and antioxidant activities while tannins have been reported to be active against ulcerated and inflammed tissue as well as possessing antioxidant activity (Subhuti, 2003). Alkaloids are known to have antimalarial, analgesic and even antitumor activities. Cardiac glycosides are known to improve cardiac activities (Syke, 2011).

5.2 Spectral analysis

5.2.1 Spectral analysis of A1

Compound A1 identified as squalene, an acyclic triterpenoid.

The GC-MS spectrum of compound A1 (Figure 4.1) showed a molecular ion peak M^+ at *m/z* 410 which corresponded to molecular formula $C_{30}H_{50.}$ The ^1H NMR spectrum (Figure 4.2) showed six olefinic proton resonances between δ_H 5.08-5.15 which were assigned to H-3, 7, 11, 14, 18, and 22; ten methylene proton resonances between δ_H 1.96 and δ_H 2.10 and eight methyl groups between δ_H 1.25 and 1.68. The ^{13}C NMR spectrum (Figure 4.3) displayed fifteen carbon resonances representing thirty carbons indicating that the molecule was symmetrical. These include eight methyl carbons (between δc 16.2 and 17.9), ten methylene carbons (between δc 25.9 and 40.0), and six vinyl methine carbons (between δc 124.5 and 135.3). The ^1H and ^{13}C NMR spectral features of this compound were comparable with those reported for squalene by Consolacion *et al.,* 2015 (Table 4.4). This was further confirmed by a search for molecular ion peak of the compound and its fragmentation pattern in the NIST standard reference library of the GC-MS instrument and was found to (99%) match that of squalene. Hence compound A1 was confirmed to be squalene.

Based on our literature search this is the first report of squalene from this plant. However, the compound was previously isolated from *F. pseudopalma, F. linearifolia, F. triangularis,F. odorata*, and *F. nervosa* (Langusei, 2017). This compound was also reported to be isolated from stem bark and leaves of *Ficus sarmentos* (Xue-gui *et al*., 2011), and *F. Minahassae* (Ragasa *et al.,* 2016).

Squalene, a precursor to the synthesis of terpenoids and phytosterols, has been reported to possess cardioprotective effects which are related to inhibition of lipid accumulation and/or its antioxidant properties (Farvin *et al.,* 2006). It was reported that the compound significantly suppresses azoxymethane-induced colonic aberrant crypt foci (ACF) formation and crypt multiplicity which could lead to possible chemopreventive activity against colon carcinogenesis (Rao *et al.,* 1998).

Squalene is a structurally unique triterpene compound that is one of the main components (about 13%) of skin surface lipids (Passi *et al*., 2002). It is transported in serum generally in association with very low density lipoproteins and is distributed ubiquitously in human tissues, with the greatest concentration in the skin. Squalene is not very susceptible to peroxidation and appears to function in the skin as a quencher of singlet oxygen radicals, protecting human skin surfaces from lipid peroxidation due to exposure to UV light and other sources of oxidative damage (Kelly, 1999). Experimental studies have shown that squalene can effectively inhibit chemically induced skin, colon, and lung tumorigenesis in rodents (Gershbein and Singh, 1969).

5.2.2 Spectral analysis of A2

Compound A2 was identified as squalenol.

Most of the NMR spectral data of A2 matched with those of compound A1 (squalene) except that A2 is not symmetrical and was found to be more polar than A1, hence more than fifteen carbon resonances were observed in the ^{13}C NMR spectrum. Similarly, an additional carbon resonance was observed at δ_c 59.0 shown by the HSQC-DEPT spectrum (Figure 4.11) to be correlated to the proton resonance at δ_H 4.09. The down field shift observed for C-6 (δ_c 140.1) and H-7 (δ_H 5.45) as well as the HMBC correlation between that carbon resonance and the carbinol protons suggested that the additional $-CH_2OH$ groups may be attached at position C-6. However considering the similarities of chemical environment in the structure of squalene, this group could be attached at other similar positions such as C-10, C-15 and C-19. This explained the additional (overlapping) carbon resonances observed in the ^{13}C NMR spectrum δ_c 124.4 - 125.2, 135.1 - 136.2 and 25.9 - 26. The C-H and H-H connectivities were established from the HMBC spectrum (Figure 4.12) and COSY spectrum (Figure 4.13) respectively. A long COSY correlation was observed between the carbinol proton δ_H 4.01 (2H-26) and the

methine proton at δ_H 5.45 (H-7). Using these spectral data, compound A2 was identified as squalenol. To the best of our literature search this is the first report of this compound.

5.2.3 Spectral analysis of A3

Compound A3 was was identified as lupeol.

The LCMS spectrum (Figure 4.14) showed a molecular ion peak at *m/z* 426 which corresponded to a molecular formular $C_{30}H_{50}O$. Six degrees of unsaturation was calculated, which accounted for one C=C double bond and five rings. The FTIR spectrum of A3 (Figure 4.15) showed characteristic absorption frequencies at 3437 and 1265 cm^{-1} typical of the O-H stretching and C-O bond vibrations, respectively. The absorption bands observed at 897 cm^{-1}, 1635 cm^{-1}, 2928cm^{-1}, 1593 cm^{-1} and 1463 cm^{-1} were due to an unsaturated out of plane C-H vibration, C=C vibrations, stretching and bending vibrations due to methyl groups, and methylene group respectively.

The ^{1}H NMR spectrum of A3 (Figure 4.16) showed the presence of seven methyl proton resonances at δ_H 0.97, 0.77, 0.83, 1.02, 0.98, 0.79, and 1.69 ppm (each integrating for 3H). From the HSQC-DEPT spectrum (Figure 4.18) and comparism with literature values, these corresponded to carbon resonances at δc 28.2 (C- 23), 15.6 (C-24), 16.3 (C-25), 16.2 (C-26), 14.7 (C-27), 18.2 (C-28) and 19.5 ppm (C-30) respectively. One proton sextet at δ_H 2.38 ascribable to H-19β is characteristic of lupeol. The H-3 proton resonance appeared at δ 3.18 while a pair of broad singlets at δ 4.56 and 4.68 were characteristic of the terminal (exocyclic) methylene group protons of lupane type triterpenoids and were ascribed to H-29a and H-29b, respectively. The ^{13}C NMR spectrum of A3 (Figure 4.17) showed the typical 30 carbons signals of a pentacyclic lupane-type triterpene. When used in conjunction with the DEPT spectrum (Figure 4.18), it was

found that the compound contained a total of six quaternary carbons, which provided further evidence for the presence of a pentacyclic triterpenoid. The structure was confirmed to be lupeol due to the appearance of terminal methylene carbon resonances for C-20 and C-29 at δc 151.2 and δc 109.5 respectively and the C-3 oxymethine carbon resonance at δc 79.2. The HMBC sprctrum of A3 (Figure 4.21) showed correlation between the methyl proton resonances at δ_H 0.97 (3H-23) and δ_H 0.77 (3H-24) with the carbon resonance at δc 79.2 (C-3). Two methyl proton resonances at δ_H 0.83 (H-25) and 1.02 (H-26) both showed correlations with the two carbon resonance at δc 50.6 (C-9) and δc 48.2 (C-19). The positions of the adjacent protons in the structure were established using the COSY spectrum (Figure 4.20). Based on the spectral data and comparison with data reported in the literature (Mi-Young *et al.*, 2010) compound A3 was identified as 2β-hydroxylup-20(29)-ene, commonly known as lupeol (clerodol, fagarsterol lupenol or fagarsterol). Based on our intensive literature search, this is the first report on the isolation of lupeol from *F. sycomorus*. It was, however, reported to be isolated from *Ficus hispida* (Chaudhary *et al*., 2014), *Ficus nervosa* (Consolacion *et al.*, 2013), *Ficus pseudopalma (*Librado and Anna, 2014) and *Ficus mysorensis* (Hatem, 2015). Lupeol has been reported by many researchers to possess anti inflammatory (Geetha and Varalakshmi, 2001), antioxidant (Librado and Anna, 2014 and Michel *et al.,* 2016) and anti-diabetic activity (Vijai *et al.,* 2014) among others.

5.2.4 Spectral analysis of A4

Compound A4 was identified as lupeol acetate.

The LCMS spectrum of A4 (Figure 4.22) showed a molecular ion peak at *m/z* 469 indicating a molecular formular of $C_{32}H_{52}O_2$. This required seven degrees of unsaturation, indicating one C=C double bond, one C=O and five rings, indicating the presence of a pentacyclic triterpenoid. The IR spectrum of A5 (Figure 4.33) displayed an absorbance at 1724 cm^{-1} indicating the presence of

(>C=O) stretching of an aliphatic ester (Mizanur Rahman *et al*., 2009) and absorption bands at 1640 and 880 cm^{-1} were ascribed to terminal double bond stretch.

The ^{1}H NMR spectrum of A4 (400 MHz, $CDCl_3$) (Figure 4.24) is similar to that of A3 (lupeol). However, in addition to resonances found for lupeol, the H-3 peak had shifted downfield to δ_H 4.47 and an extra acetate methyl group proton resonance was seen at δ_H 2.04. The ^{13}C NMR spectrum showed resonances due to acetate carbonyl goroup at δc171.2 and an extra methyl group resonance at δc 21.39. A HMBC correlation between this carbon signal and the protons signal at δ_H 1.60 (H-2α and β) showed that the acetoxy group is attached at position 3.

Based on these spectral data and comparism with those reported in the literature (Léa *et al.*, 2014) (Table 4.7), A4 was confirmed to be 3β-acetoxylup-20(29)-ene also known as lupeol acetate.

Based on our intensive literature search, lupeol acetate has never been isolated before from *F. sycomorus*. It was however reported to be isolated from *Ficus hispida* (Wang and Coviello, 1975), *Ficus odorata* (Tsai *et al.,* 2012), *Ficus hispida* (Chaudhary *et al.,* 2014) and *Ficus cordata* (Herve *et al.,* 2008). Lupeol acetate was reported to have antinociceptive and anti-inflammatory activities (Yuh Fung *et al*., 2012). Varghese *et al*., (2009) also reported that the compound possesses antibacterial and anti-fungal activities.

5.2.5 Spectral analysis of A5

Compound **A5** was identified as β-amyrin acetate.

The LCMS spectrum of A5 (Figure 4.30) showed a $[M-H]^+$ ion peak at *m/z* 467.3903 which indicated a molecular formula of $C_{32}H_{52}O_2$ for the compound. A double bond equivalence of seven was calculated, accounted for by the presence of one C=C double bond, one C=O bond and five rings. The IR spectrum (Figure 4.31) showed absorptions bands at 2921 and 2851 cm^{-1} for C-H stretches, 1737 $cm^{-1}$for the carbonyl stretch of an acetate group, 1636 cm^{-1} for a double bond stretch and 1244 cm^{-1} for a C-O stretch.

The ^{1}H NMR spectrum (Figure 4.32) showed H-3 proton resonance at δ_H 4.50 (dd, *J*=10.0, 5.9 Hz), which corresponded to the carbon resonance at δ_c 81.2 in the HSQC spectrum (Figure 4.35). The observed coupling constants suggested that the configuration of H-3 was α with the acetate group in the β-position. Acetate methyl group proton resonances appeared at δ_H 2.01. The triplet observed at δ_H 5.18 was assigned to the vinyl methine proton at C-12.

The ^{13}CNMR spectra of A5 displayed thirty-two carbon resonances which according to the DEPT-135 spectrum (Figure 4. 34) included nine methyl, ten methylene, six methine, and seven fully substituted carbons resonances. This indicated that compound A5 was an acetylated pentacyclic triterpenoid derivative**.** The HMBC spectrum of A5 (Figure 4.36) showed correlation between the methine proton resonance at δ_H 0.84 with the two methyl group carbon resonances at δc 28.3 (C-23) and δ_H 16.9 (C-24). Similarly, the olefinic proton resonance, H-12 (δ_H 5.18, t, *J*=3.7) showed correlations with the C-9 (δ_c 47.8) and C-18 (δ_c 47.5) carbon resonances, and it showed further coupling with the H-11 (δ_H 1.87) resonance in the COSY spectrum (Fig 4.37). The HMBC correlation between the proton resonance at δ_H 1.13 (3H-27) and δ_c145.5 (C-13) confirmed this placement for the alkene group.

In the NOESY spectrum (Figure 4.38), the H-3 resonance at δ_H 4.50 showed correlations with the H-5 (δ_H0.84) and H-9 (δ_H1.58) resonances. The H-9 resonance showed also correlations with the H-5 (δ_H 0.84) and 3H-27 (δ_H 1.13) resonances. All these protons (H-5, H-9, 3H-27) are in the α-orientation according to the biosynthesis, while the H-18 (δ_H 1.96), 3H-25 (δ_H 0.98) and 3H-26 (δ_H 1.00) resonances, which showed correlations with each other in the NOESY spectrum, are in the β-orientation (Oliveira *et al*., 2010). The NMR data compared well with literature values (Lee *et al.*, 2014). Previous research showed that β-amyrin acetate posses anti-inflammatory activity (Okoye *et al*., 2014), sedative, anxiolytic and anticonvulsant properties (Aragao *et al*., 2009).

5.2.6 Spectral analysis of A6

Compound A6 was identified as sitosterol.

The ^{1}H NMR spectrum (Figure 4.39) showed one olefinic proton signal appearing as doublet at δ_H 5.37 which was assigned to H-6. The up field signals at δ_H 1.01(m), 0.84 (d, *J* = 1.86 Hz), 0.83 (m) and 0.91 (d, *J* = 6.4 Hz) were assigned to the methyl groups at positions 26, 27, 29 and 21

respectively. H-3 proton resonance as multiplet at δ_H 3.80 While the methyl proton resonances at δ_H 0.68 (s), and 1.01 (s) corresponded to the angular methyl protons H-18 and -19 proton respectively. The spectrum also showed a multiplet at δ_H 3.51 which was ascribed to an oxymethine proton (H-3). The ^{13}C NMR spectrum of A6 (Figure 4.40) revealed twenty-nine recognizable carbon signals which, based on the DEPT-135 spectrum (Figure 4.41), included six methyl, eleven methylene, nine methine and three quaternary carbons. The spectrum showed two alkene carbon signals at δc 140.1 and 122.0, which were assigned to C-5 and C-6 respectively of the sitosterol nucleus (Manu and Kalia, 2013). C-3 oxymethine carbon resonance appeared at δc 72.1 while resonances at δc 19.6 and 19.8 were assigned to the two terminal methyl groups at position 26 and 27 respectively. Three up field resonances observed at δc 12.1, and δc 19.2 were ascribed to the angular methyl carbons linked at C-18 and C-19 respectively. Other signals observed in the ^{13}C NMR spectrum are at δc 36.4, 34.2, 26.3, 46.1.8, 26.3, and 23.3 assigned to the carbon numbers 20, 22, 23, 24, 25 and 28, respectively. These are part of the side chain linked at position 17 (δc 56.3) of the cyclopentyl ring. The ^{1}H NMR and ^{13}C NMR spectral data of A6 were in well agreement with the reported spectral data in the literature, A6 was therefore confirmed to be 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17- dodecahydro-1H-cyclopenta[a]phenanthren-3-ol also called beta sitosterol. This is a very common plant constituent. It was previously isolated from the leaves *Ficus sycomorus* (Abu-Mustapha *et al.,* 1964). Sitosterol was reported to posses anti-cancer (Bin Sayeed and Ameen, 2015, Novotny *et al.*, 2017) anti-oxidant and anti diabetic activities (Gupta *et al*., 2011).

5.2.7 Spectral analysis of A7

Compound A7 was identified as sitosterol-3-*O-β*-D-glucopyranoside.

The IR spectrum of A7 (Figure 4.45) showed an absorption band at 3412.69 cm^{-1} indicating the presence of a hydroxyl group (-OH). The absorption band at 1624 cm^{-1} indicated the presence of (C=C) stretching. The ^{1}H NMR of A6 (Figure 4.46) is similar to that of sitosterol (compound A6) described above. But additional chemical shifts observed in the region δ_H 3.34-3.58 together with anomeric proton signal at δ_H 4.37 (d, *J* = 7.5Hz) indicated the presence of a sugar moiety. Similarly, the^{13}C NMR spectrum of A7 (Figure 4.47) showed additional six well resolved oxygenated carbon resonances of the sugar moiety at δc 101.4 (C-1‘), 73.8 (C-2’), 76.2 (C-3’), 70.3 (C-4’), 73.9 (C-5’), and 63.1 (C-6’). The carbinol carbon of the CH_2OH group observed at δc 63.1 suggested the sugar moiety to be glucose and the large coupling constant the anomeric proton (6.6 Hz) suggested that the glucose has a β-configuration (Zhao *et al*., 1989; Jares *et al*., 1990). The HMBC correlation between the anomeric proton, H-1’ (δ_H 4.37, *d*, *J* = 7.5 Hz), and C-3 (δc 79.78) confirmed the attachment of the sugar moiety to C-3. From the above data and comparison with what was reported literature (Hisashi *et al*., 1990) compound A7 was identified as sitosterol-3-*O-β*-D-glucopyranoside. This compound was previously isolated from the leaf of the same plant by Mortada *et al*., (2010).

5.2.8 Spectral analysis of A8

Compound A8 was identified as α-tocopherylquinone.

The IR spectrum of A8 (Figure 4.53) Showed bands at 3450cm^{-1}, corresponding to a hydroxyl group and at 1641 cm^{-1}, due to an α,ß-unsaturated ketone.

The ^{1}H NMR ($CDCl_3$, 500 MHz) spectrum of A8 (Figure 4.54) showed characteristic features of tetra-alkyl benzoquinones (Mahmood *et al*., 1984). The three methyl singlets at δ_H 2.00 (3H-2a), 2.00 (3H-5a) and 2.03 (3H-3a) coupled with the absence of any quinonoid proton signal suggested that the quinone ring was fully substituted. Other methyl proton signals observed at δ_H 0.96, 0.97, and 0.91 were assigned to 3H-13a, 3H-17a and H-21a/H-22 respectively. The ^{13}C-NMR and DEPT-135 spectra of A8 (Figure 4.55 and Figure 4.56) showed a total of twenty-one carbon signals. These included eight methyl, eleven methylene, three methine and nine quaternary carbons. The resonance at δc 72.9 was ascribed to the quaternary carbon bearing hydroxyl group at C-9. Two carbonyl carbon signals appearing at δc 187.4 and δc 187.9 were assigned to the two carconyl carbons at C-1 and C-4 respectively of the benzoquinone ring. Three methyl carbon resonances attached to the ring appeared at δc 12.6 (C-2a), 12.5 (C-3a) and 12.2 (C-5a). The carbon resonance due to methyl group carbon attached to the quaternary carbinol carbon (C-9a) appeared at δc 26.8. Signals appearing at δc 19.9, 21.6, and 22.8 were assigned to the methyl groups at C-13a/17a, C-21a and C-22 respectively. The assignment of these chemical shifts and the connectivities through the molecule were confirmed using COSY (Figure 4.57) and HMBC (Figure 4.58) spectra.

The HMBC correlation observed between the methyl group protons at δ_H 2.00 (3H-5a) and the to the methylene carbon at δc 21.6 (C-7) indicating that the methylene group to be neighbored to the benzoquinone moiety. The Hydroxyl bearing quaternary carbon C-9 showed correlations with 2H-7, 2H-8, 2H-10 and to a methyl group, suggesting its position to be between C-8 and C-10. Similarly, the HMBC spectrum showed long range correlations between the two methyl groups at C-21a and C-22. Both protons of these groups correlated with the methine carbon at δc 28.2 (C-21). This indicated that two methyl groups were bond to this same carbon atom. These spectral which were similar to those reported in the literature (Williams *et al*., 2012) confirmed compound A8 was to be α-tochopherylquinone.

5.2.9 Spectral analysis of A9

Compound A9 identified as (6*R*,9*R*)-9,13-dihydroxy-4,7-megastimadiene-3-one (also called vomifoliol or blumenol A).

The LCMS of A9 (Figure 4.60) spectrum of A9 showed a molecular ion peak at *m/z* 223.1, corresponding to the molecular formula $C_{13}H_{20}O_{3.}$ This gave four degree of unsaturation accounted for by the presence one C=O group and two C=C double bonds and one ring. The IR spectrum (Figure 4.61) showed an absorption band at cm^{-1} 3400, 1,655 and 1125 cm^{-1} for O-H, C=O and C=C stretch respectively.

The ^{1}H-NMR spectrum of A9 (Figure 4.62) showed a proton singlet at δ_H 5.89 which was assigned to an alkene proton at C-4. Additionally, the spectrum showed an AB system of non-equivalent proton resonances at δ_H 5.81 and 5.82 (H-7 and H-8), both having the characteristic coupling constant (*J* = 16 Hz). The two germinal methylene proton signal at δ_H 2.19 and δ_H 2.48 (*J*= 17.1 Hz) were ascribed to the two non-equivalent protons C-2. The resonance at δ_H 1.26 (d, *J*= 6.5) was assigned to the 3H-10 terminal methyl group protons while the two methyl proton resonances (both singlets and integrating for three protons) at δ_H 1.06 and 1.04 were assigned to 3H-11 and 3H-12 respectively.

The ^{13}C-NMR and DEPT spectra (Figure 4.63 and 4.64) of A9 showed a total of thirteen carbon signals. These include three methyl, two methylenes, four methines, three quaternary carbons and one carbonyl carbon. A downfield carbon signal observed at δc 201.3 was assigned to the carbonyl group of C-3 this carbonyl group showed an α-β unsaturated system with C-4 (δ_C 127.6) and C-5 (δ_C 167.5). Two oxygenated carbon signals appeared at δ_C 80.1 and 68.8 which were assigned to C-6 and C-9 respectively. The two geminal methyl groups at δ_H 1.04 (3H-11) and δ_H 1.05 (3H-12) showed cross peak in the HMBC spectrum (Figure 4.67) with the C-2 methylene

carbon (δc 50.9) and the oxygenated fully substituted C-6 (δc 80.1). The corresponding geminal H-2 resonances (δ_H 2.48 and δ_H 2.19 *J* = 17.1 Hz) showed correlation with the C-3 carbonyl resonance (δc 201.3), alkene carbon resonance at δc 127.6 (C-4), quaternary carbon at δc 42.6 (C-1) and the oxygenated carbon C-6 (δc 80.1). The C-6 resonance correlates with the alkene proton resonace at δ_H 5.89 (H-4) and H-7 (δ_H 5.81,*J* = 2.17 Hz) and H-8 (δ_H 5.82 *J* = 2.17 Hz) of the *trans* alkene. The COSY spectrum (Figure 4.66) showed coupling between the 3H-10 methyl proton resonance at δ_H 1.27 and the H-7 (δ_H 5.81), H-8 (δ_H 5.82) and H-9 (δ_H 4.34). The placement of hydroxyl group at C-9 was confirmed by the downfield chemical shift observed for H-9. The Elecronic Circular Dichroism (ECD) spectrum of A9 (Figure 4.69) showed a positive Cotton effect at 252 nm. This, according to the ECD helicity rule (Oritani and Yamashita,1972), showed that the chiral center in the molecule at position 6 has an *S* configuration. Almeida *et al*., 2010, showed that there are two diastereomers; (6S, 9*R*) and (6*R*, 9*R*) for natural blumenols. Similarly comparison between the CD and NMR spectral data of A9 with those in the literature (Yumiko and Ito, 2005) revealed that the compound to have either (6S,9S) or (6S,9R) configuration.

Finally, since positive optical rotation value was obtained, compound A9 was concluded to have (6*S*, 9*R*) configuration. Hence compound A9 was identified to be **(6*S*,9*R*)-6,9-dihydroxy-4,7-megastimadiene-3-one**. The stereochemistry of this compound was further confirmed using its ROESY spectrum (Figure 4.68). From the spectrum it was observer that H-2α showed NOESY correlations to H-7 and H_3-12while H-2β gives showed correlation with H_3-11. This showed that C-11-methyl substituent is axial, while the 3H-12 methyl group is equatorial. 12-H_3 showed a ROESY correlation with both 7-H and 8-H, indicating that the exocyclic double bond is extended perpendicular to the ring. 9-H gives major correlations to 7-H, 8-H and 10-H_3, but weak correlations were also observed to H-4 and 13-H_3. That would place 9-H closer to 2-Hβ and 13-H_3 in the most stable confirmation, and the observed strong correlation from 10-H_3 to 8-H suggests that C-9 has an axial configuration. To the best of our literature search this is the first report of this compound from *Ficus sycomorus*. However, the compound has recently been reported to be isolated from *F. thonningii* and *F. pumila* (Fongang *et al.,* 2017).

5.2.10 Spectral analysis of A10

Compound A10 was identified as (6R,9R)-6,9-dihydroxy-4,7-megastimadiene-3-one.

The LCMS spectrum of A10 (Figure 4.70) showed a molecular ion peak at *m/z* 223.1, corresponding to the formula $C_{13}H_{20}O_3$. This showed that the compound has four degrees of unsaturation which are accounted for by the presence one carbonyl group, two C=C double bonds and a single ring. The IR spectrum of A10 (Figure 4.71) showed a broad absorption band at 3408, 1,724 and 1,641 cm^{-1} which were attributed to hydroxyl (O-H) group, carbonyl group (C=O) and C=C stretch respectively.

The compound A10 was found to have similar structural skeleton with compound A9 because they have similar ^1H NMR and ^{13}C NMR spectral data. However, compound A10 differed from A8 in that the methyl group at C-13 had been oxidized to an alcohol. This is shown by a pair of non-first order coupled doublets at δ_H 4.21 d (*J* = 1.23) which based on the HSQC-DEPT spectrum (Figure 4.75) was found to correspond to an oxymethylene carbon resonance at δ_c 64.2. The position of the CH_2OH group was confirmed by the HMBC correlation between these oxymethylene protons and the two alkene carbons at δ_c 122.7 (C-4) and δ_c 163.6 (C-5) as well as the correlation observed between the methine proton signal at δ_H 6.18 (H-4) and the carbinol carbon. Similarly, the tertiary carbon at position 6 of compound A9 was replaced by a methine carbon (δc 51.1). This further confirmed by the correlation observed in the COSY spectrum (Figure 4.76) between the H-6 methine proton (δ_H 2.03) and H-7 methine proton (δ_H 5.69). The stereochemistry of this compound was also found to be the same as A9. This is possible since they are from the same plant, thus are biologically related. From the spectral data above and comparism with the data reported in the literature for this compound, A10 was identified as **(6*S*,9*R*)-9,13-dihydroxy-4,7-megastimadiene-3-one**.

To the best of our literature search this is the first report of isolation and full characterization of this compound from natural source. However, Toshiyuki *et al.*, (2001) reported that they obtained this compound by enzymatic hydrolysis of its glycoside (called apocynoside) using β-glucosidase. The ^1H and ^{13}C of the resulting compound is much similar to that of compound A10.

5.2.11 Spectral analysis of A11

Compound A11 was identified as (+)-catechin.

The LCMS spectrum of A11 (Figure 4.79) showed molecular ion peak at m/z 291, correspondind to molecular formular $C_{15}H_{13}O_6$. The compound thus, required nine degree of unsaturation which was accounted for by the presence of six C=C double bond and three rings. The IR of A11 (Figure 4.80) showed broad bands at 3420.63 cm^{-1} and 1637.22 cm^{-1} due to phenolic and alcoholic O-H stretches and C=C bond stretching in aromatic rings respectively.

The ^{1}H NMR spectrum (Figure 4.81), showed five aromatic proton signals at δ_H 5.96 ppm (H- 6), 5.94 (H-8), 7.0 ppm (H-2'), 6.70 (H-5') and 6.81 (H-6'). The proton at H-2' (δ_H 7.01, *J* = 1.7 Hz) appeared as doublet because it was splitted by H-6' (δ_H 6.80 Hz) in a *meta* position. H-5' was split by H-6' in an *ortho* position yielding a doublet at δ_H 6.7 (d, *J* = 8.1 Hz) and H-6' was split by H-2' and H-5' given a doublet of doublet at δ_H 6.8 (*dd*, 1.7, 8.2 Hz). This is typical of ABX system of ring B of flavan nucleus. The two *meta*-coupled proton signal at δ_H 5.94 and 5.96 (d, *J* = 2.3 Hz, both) which are *meta*-coupled to each other were assigned to position 6 and 8 respectively of ring A. The basic structure of this compound was therefore deduced as 3, 3', 4', 5, 7-pentahydroxyflavan. The protons at H-4, were split by H-3, giving a doublet of doublet at δ_H 2.76 (H-4α) and 2.89 (H-4β). The coupling constant (6.8 Hz) between H-2 (δ_H 4.84) and H-3 (δ_H 4.12) indicated that these protons were trans to each (Pizzolatti *et al*., 2002). These signals are attributed to ring C.

The ^{13}C-NMR spectrum of A11 (Figure 4.82) showed 15 signals indicating 15 carbons atoms which (based on DEPT 135 spectrum shown in Figure 4.83) include seven of quaternary carbons at which appeared at δc 157.8 (C-5), 158.2 (C-7), 157.3 (C-9), 100.2 (C-10), 132.4 (C-1'), 149.5 (C-3') and 146.1(C-4'); seven of tertiary carbons at δc 80.1 (C-2), 67.2 (C-3), 96.5 (C-6), 93.03 9C-8), 115.3 (C-2'), 116.03 (C-5') and 119.48 (C-6') and one of secondary carbon at δ_C 29.4 (C-4).

The ^{1}H-^{1}H COSY correlations showed coupling between H-3 and H-2, and also between H-3 and H-4. The connectivities through the proposed structure were further confirmed by correlations observed in the HMBC spectrun (Figure 4.86 and Table 4.14). The value of the specific rotation ($+55^0$) and the large *J* 2,3 coupling constant confirmed the configuration of A11 to be *trans* at C-2 and C-3. Based on these data and their comparison with the ^{1}H and ^{13}C NMR data reported in the literature (Oh *et al*., 2016; Abdullahi *et al.,* 2016) compound A11 was confirmed to be (+)-catechin. This compound has been reported to showed antioxidant activity (Gadow *et al.,* 1997), anti-ulcer and anti-inflammatory activity (Lans, 2007) as well as good antilisterial activity at IC50 greater than 200 µg/mg and antimicrobial activity at MIC value greater than 500µg/ml (Nyila *et al*., 2012).

5.2.12 Spectral analysis of A12

Compound A12 was identified as kaempferol.

The ^{1}H NMR (CD_3OD 500 MHz) spectrum of A12 (Figure 4.87) showed the presence of four aromatic proton resonances representing six aromatic protons of a flavanol structure. The characteristic AA'BB' pattern of a a *para*–disubstituted ring B showed by a pair of *auto*-coupled doublets each integrating to two protons at δ_H 7.7 (H-2'/H-6') and δ_H 6.92 (H-3'/H-5'). A pair of *meta*-coupled proton resonances at were observed at δ_H 6.40 (1H *d*, *J* = 2.1 Hz) and 6.20 (1H, *d*, *J* = 2.1 Hz) and were ascribed to H-8 and H-6 of ring A respectively. These assignments were confirmed using the HMBC spectrum (Figure 4.90).

The ^{13}C-NMR/DEPT-135 spectra (Figure 4.88) showed a total of fifteen carbon signals. These included six methine, four oxygenated methines, nine quaternary carbons, one methyl, and one methine carbon signals. The spectrum also showed Ring C showed an α,β unsaturated ring system with resonances at δc 157.2, 134.0 and 179.0 which were ascribed to C-2, C-3 and C-4 of ring C respectively. This spectral data were in agreement with that of kaempferol reported in the literature (Markham *et al.,* 1978; Singh and Singh*,* 2015). To the best of our knowledge this is the first time this compound is isolated from the leaf of this plant. However, it was previously isolated from the stem bark of *Ficus indica* (Eun *et al*., 2003).

Kaempferol has been reported to posses anti-cancer activity by inducing apoptosis and cell cycle arrest in various cancer cell lines, including colon cancer cell (Nguyen *et al.,* 2003, Choi and Ahn, 2008, Li *et al*., 2009). It was also reported to have antioxidant and anti-inflammatory activities (García-Lafuente *et al.,* 2009).

5.2.13 Spectral analysis of A13

Compound A13 was identified as kaempferol-3-*O*-α-L-rhamnoside.

The LCMS spectrum of A3 (Figure 4.91) showed a $[M-H]^+$ peak at 432.1, which corresponded to the molecular formula of $C_{21}H_{20}O_{10}$and thus required 12 degrees of unsaturation. These were accounted for by the presence of the seven C=C double bonds, one C=O group, three rings for the flavanoid structure and one hexose sugar ring. The IR spectrum (Figure 4.92) showed a broad absorption band at 3408 cm^{-1} due to O-H stretching in alcohols and phenols. Other bands were observed at 1738 cm^{-1} (due to C=O stretching of the ketone), and 1653 cm^{-1} (due to C=C bond stretching of aromatic systems).

The ^1H NMR and ^{13}C NMR (CD_3OD 500 MHz) spectrum of A13 (Figure 4.93 and 4.94) showed that the compound had similar structural skeleton as that of A12. Moreover, the ^{13}C-NMR and DEPT-135 spectra of A13 showed a total of twenty-one carbon signals which included six methine, four oxygenated methines, nine quaternary carbons, one methyl, and one methine carbon signals. Anomeric carbon signal was observed at δc 103.7 which showed a correlation in the HSQC-DEPT correlation with the anomeric proton signal observed at δ_H 5.39. This indicated the presence of a sugar moiety which suggested that the compound A13 was a flavonoid glycoside. The HMBC spectrum (Figure 4.98) was used to determine where the sugar unit was attached to the flavonoid moiety. A correlation seen between the anomeric proton H-1" (δ_H 5.39) and the carbon signal at δc 136.4 (C-3) confirming that the sugar was attached at C- 3. The sugar was determined to be rhamnose by the presence of the typical rhamnose C-6 methyl carbon signal at δc 17.8 (C-6") which corresponded to methyl proton signal at δ_H 0.98 (3H, *s*) in the HSQC-DEPTspectrum (Figure 4.96). The coupling constant value for the anomeric proton, (*J*=1.5 Hz) indicated that it had an axial-equatorial relationship between H-5" and H-2" suggesting an α-L-rhamnoside (Julinton *et al*., 2015). The coupling of the rhamnose protons was confirmed with the ^1H-^1H-COSY spectrum (Figure 4.97). The spectral data reported here for compound A13 is in agreement with the NMR data of kaempferol-3-*O*-α-rhamnoside reported by Venktata and Prakash, (2011) as shown in Table 4.16. Therefore, compound A13 was identified as kaempferol-3-*O*-α-L-rhamnoside also called afzelin. From our literature search, this is the first report of this compound from *Ficus sycomorus*. However, this compound was recently isolated from the leaves of *Ficus septic* (Anonymous, 2015) and *Ficus pumila* (Pham *et al*., 2017). Kaempferol-3-*O*-

β-D-glucopyranoside was reported to have anti-cancer activity against prostate cancer cells (Kai-Chang *et al*., 2015); inhibit the growth of breast cancer cells by stimulating apoptosis (Diantini *et al*., 2015). It has been also shown to scavenge superoxide anion radicals in RAW264.7 cells (Mao *et al*., 2011). Afzelin was also reported to have antibacterial, DNA-protective, antioxidant, anti-inflammatory, anti-complement activity, inhibitor of angiotensin converting enzyme (ACE) (Hansen *et al*., 1996; Min *et al*., 2003; Shin *et al*., 2013; Lee *et al*., 2014).

5.2.14 Spectral analysis of A14

Compound A14 was identified as quarcetin-3-*O*-β-*D*-glucopyranoside

The LCMS spectrum of A14 (Figure 4.100) showed a molecular ion peak at *m/z* 464.3 corresponding to a molecular formula of $C_{21}H_{20}O_{12}$, requiring twelve degrees of unsaturation, indicating the presence of seven C=C double bonds, one C=O group and four rings. The IR spectrum (Figure 4.101) showed absorption bands at 3388, 1722 and 1657 cm^{-1} due to O-H stretching, C=O stretching and C=C bond stretching on an aromatic ring. The ^{1}H NMR sprctrum (Figure 4.102) showed aromatic proton signals at δ_H 6.20 (1H, d, *J*=2.0 Hz) and δ_H 6.39 (1H, d, *J*=2.1 Hz) consistent with the *meta*-coupled protons, H-6 and H-8, of ring A respectively. Signals at δ_H 7.71 (1H, d, *J*=2.2 Hz,), δ_H 7.59 (1H, *dd*, *J*=8.5, 2.3 Hz), and δ_H6.87 (1H, d, *J*=8.5 Hz) were assigned to the protons H-2', H-6' and H-5' respectively of ring B (Ebada *et al*., 2008). A doublet at δ_H 5.25 (1H, *J* =7.6 Hz) was assigned to the anomeric proton (H-1") of a hexose sugar (Yekta *et al*., 2008, Li and Kuo, 1998). The remaining proton resonances due to the sugar moiety were observed between δ_H 3.42 and 3.71. The ^{13}C NMR spectrum (Figure 4.103) showed a total of twenty-one carbon signals. This, according to the DEPT-135 spectrum (Figure 4.105), included 10 quaternary carbons at δc159.2, 135.8, 179.7, 163.2, 168.2, 158.7, 105.6, 123.0, 146.0 and 150.0 assigned to C-2, C-3, C-4, C-5, C-7, C-9, C-10, C1', C-3' and C-4' respectively; ten methine carbons at δc100.4, 94.9, 116.1, 117.6, 123.4, 104.4, 175.9, 78.5, 71.4 and 78.2 assigned to C-6, C-8, C-2',

C-5’ and C-6’, 1”, 2”, 3”, 4” and 5” respectively as well as one methylene carbon at δc 62.7. The anomeric carbon signal was seen to appear at δc 104.4 which corresponded to the anomeric proton at δ_H 5.25. This showed that compound A13 is a flavanoid glycoside. The sugar unit was identified as glucose due to the typical glucose –CH_2OH group observed at δc 62.7 (C-6”). The coupling constant of the anomeric proton (*J* =7.6 Hz) defined the stereochemistry of glycosidic linkage as β (Guo *et al*., 2012). The HMBC correlation observed between the anomeric proton and the carbon signal at 135.8 (C-3) confirmed that the glucose moiety was attached at C-3. Based on these spectral studies, it was confirmed that the isolated compound was quercetin-3-*O*-β-D-glucoside. This compound was previously isolated from the leaves of the same plant by Mohamed *et al.,* (2010). It was also isolated from *Ficus pandurata* (Ramadan *et al*., 2009), and *Ficus auriculata* (Ahlam *et al*., 2011). Quercetin-3-*O*-β-D-glucoside has been reported to have anti-cancer (Yamazaki *et al*., 2014), anti-aging (Yang *et al*., 2014), and anti-oxidant properties (Messer *et al*., 2015).

5.2.15 Spectral analysis of A15

Compound A15was identified as phaophytin A.

The ^{1}H NMR spectrum of A15 (Figure 4.108) displayed two olefinic proton signals appearing as singlets at δ_H 9.30 and δ_H 9.52 which were ascribed to H-5 and H-10 respectively. A vinyl group was found to be present at C-3 as indicated by the H-3^1 double doublet at δ_H 7.95 (*J* = 15.19 Hz, 11.39 Hz) while the H-3^2 (E) and the H-3^2 (Z) appeared as double doublets at δ_H 6.25 (*J* = 15.19 Hz, 2.28 Hz) and 6.15 (*J* = 11.39 Hz, 2.28 Hz) respectively. The spectrum also showed four methyl

group proton resonances, each appearing as sharp signals, at δH: 3.20, 3.30, 3.61 and 3.81 assigned to 3H-2^1, 3H-7^1, 3H-12^1 and 3H-13^4 (methyl ester) respectively. Two other methyl proton resonances observed at δ_H, 1.73 (d, *J*= 7.3 Hz) and 1.62 (d, *J* = 7.8 Hz) were ascribed to 3H-18^1 and 3H-8^2 methyl protons respectively. The signal for H-17 (δ 4.15, 1H, d, *J* = 3.3 Hz), indicated that H-17 and H-18 were trans-oriented. The 2H-8^1 proton resonance appeared as a quartet at δ_H 3.70 (*J*= 7.8 Hz) which together with 3H-8^2 constituted the ethyl group at C-8.

The ^{13}C NMR spectrum (Figure 4.109) showed a total of fifty-five carbon atoms. These based on the DEPT-135 spectrum (Figure 4.110) included eleven methyl, thirteen methylene and ten methine carbon resonances. Three carbonyl carbon resonances appeared at δc 189.8, 173.1 and 172.5 which were assigned to carbonyl carbons at C-13^1, C-13^3 and C-17^1 respectively. 2^1/12^1, 7^1, 8^2 and 18^1 methyl carbon resonances appeared as singlets at δc 12.3, 11.5, 17.6 and 23.3 respectively. Carbon resonances at 129.0 and 130.0 were due to terminal alkene carbons at C-3^1 and C-3^2 respectively. In addition, a phytyl moiety was confirmed by extensive 2D NMR (COSY and HMBC) spectroscopic data analyses, and the position of this unit was confirmed by the HMBC correlation observed between the methylene proton at δ_H 4.35 and the carbonyl carbon at δc 173.1 (C-17^3). These spectral data were in agreement with those reported for the same compound by Nakatani *et al.* 1981. The configuration of C-13^2 was assigned to be S, based on the up-field shift of H-17 (δ_H: 4.15), which was in agreement with that reported by the same researcher.

5.3 Antioxidant activity studies

Free radicals are easily formed when a covalent molecular bond is broken and one electron remains with each newly formed atom. To prevent free radical damage, the body has a defense system of antioxidant. When 100 µg/ml of the extract was spotted on a TLC Plate, the result obtained when the chromatogram was sprayed with DPPH solution wasa yellowishcolour development on pinkish back ground of the TLC plate. This was an indicator for the presence of antioxidant substance.

The results of the quantitative free radical scavenging activity of DCM and methanol extract of *Ficus sycomorous* leaf evaluated using DPPH method is presented in Figure 4.114 and 4.115. It is evident from the result, that both extractsshowed increase in the percentage scavenging activity with increase in concentration. This is due to decrease in UV absorbance observed with increase in concentration of the extract.

This decrease in absorbancewas a result of the radical being scavenged by the plant extracts (as indicated by a colour change from purple to yellow) through donation of hydrogen to form a stable DPPH molecule (Brand-Williams *et al.,* 1995).

The values of the IC_{50} showed that methanol leaf extract showed higher antioxidant activity (with $IC_{50}$of 32.30) than the DCM extract (with IC_{50} of 42.83). This is due to bioactive compounds such as polyphenols including tannins and flavonoids which were found to be present in the

polar extracts. Polyphenols are one of the major plant compounds with antioxidant activity. The antioxidant activity of phenolic compounds was reported to be mainly due to their redox properties (Galato *et al.,* 2001), which play an important role in scavenging and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Organic extracts may be more beneficial than isolated constituents, because other compounds present in the extracts can change the chemical or biological properties of bioactive individual component (Borcher *et al.,* 2004).Comparing these values with that of vitamin C (used as standard drug), it was evident that vitamin C with IC_{50} Value of 2.5 µg/ml had a higher antioxidant activity than both the extracts.

However, the result shows that the plant has a weaker antioxidant activity when compared to *Ficus carica* (Common fig) known to have a high antioxidant activity with IC_{50} value of 0.22 ± 0.17 mg/ml (Safaa *et al*., 2010). The result of the analysis carried out by Alphonsine *et al*., (2012) also indicated that the extracts of the fruits of*Ficus sycomorus* produced the highest antiradical activity with IC_{50} of 9.60 $\pm$ 0.02 µg/ml as against 31.83 ± 0.05 µg/ml for *Ficus sur*.

5.4 Anticancer studies

The result of the anticancer studies (Table 4.19) showed that the cancer cells inhibition property of the leaves of *Ficus sycomorus* is a selective dose-dependent cytotoxic action. The concentrations (µg/ml) used in the study were 20 and 2µg/ml. The methanol extract showed no activity against MDA-MB-435(100% survival) at both concentrations significantly inhibited the growth of breast adenocarcinoma and ovary adenocarcinoma cells at both concentrations while DCM extract and compound A11 had no effect on HT-29 cancer cells at both concentrations. This effect of the methanol extract is probably due to the presence of the flavonoids present in the leaves. The result showed that the extract can be a significant source of promising anticancer compounds particularly against Human Melanoma and Ovary adenocarcinoma cells. 5% and 2% inhibition was found against colorectal adenocarcinoma cell line for the methanol at concentrations of 20 and 2 µg/ml respectively. However, the crude extracts and the compounds tested were found to have activity against MDA-MB-231 and OVCAR-3 at both concentrations with the exception of compounds A12 and A13 which showed no activity against OVCAR3 cancer cells both at concentration of 2 and 20 µg/ml. Compound A14 showed significant inhibition against all the cancer cells with highest activity (survival of 68%) on MDA-MB-231 cells at a concentration of 20 µg/ml. The extracts and the fractions from the leaves of *Ficus sycomorus* could be said to inhibit the growth of the cell lines by inducing apoptosis through any or combination of various mechanisms like mitochondrial transmembrane depolarization, increase cytochrome C release, caspase-3 and caspase-7 activation or increased poly ADP-ribose polymerase degredation (Badaboina *et al.,* 2013). Similarly the isolated compounds A1, A2 and A3 were found to have high effects on HT-29, MDA-MB-231 and OVCAR3 cell lines at both concentrations when compared with the crude extract and the fractions, although no cell inhibition was recorded for all the compounds for MDA-MB-435 cell lines. Preliminary phytochemical study indicated the presence of flavonoid, saponins and tannins in methanol

extract. Flavonoids such as quercetin, kaemferol and their glycosides have been shown to possess anticancer activities. Furthermore, flavonoids have a chemopreventive role in cancer therapy through their effect on signal transduction in cell proliferation and angiogenesis (Fotsis *et al.,* 1997). The cytotoxicity and anticancer activity of methanol extract is probably due to the presence of these flavonoids.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Based on the findings in this work, it can be concluded that the plant *Ficus sycomorus* contains important phytochemical constituents such as flavonoids, saponins and alkaloids with significant anti-oxidant and anti-cancer activity against some cancer cell lines. Similarly fifteen compounds namely: squalene, squalenol, lupeol, lupeolacetate, beta amarinacetate, sitosterol, sitosterol-3-O-β-D-glucopyranoside, α-tocopherylquinone, bluminol A and its derivative, (+)-catechin,

kaempferol, afzeline, quercetin, isoquercetin and pheophytin were isolated from the leaf of the plant. To the best of our search this is the first report on the isolation of squalene, squalenol, α-tocopherylquinone, bluminol A and its derivative, (+)-catechin, kaempferol, and afzeline from the leaf of this plant.

6.2 Recommendations

i. Further studies should be carried out to isolate alkaloids, and saponins present in the leaves and screen them for anticancer activities.

ii Further anticancer studies should be carried out on the plant using other cancer cells

iii the leaves should also be subjected to other biological activities such as anti-inflammatory, antibacterial, anticonvulsant and anxiolitic activities.

iv. There is also need to carry out acute toxicity studies on the plant so as to rule out the possibility of any deleterious effect that may arise as a result of prolong use of the plant.

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APPENDIX

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PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY STUDIES OF THE ETHANOL LEAF EXTRACT OF *FICUS SYCOMORUS* L. (FAMILY: MORACEAE)

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ABSTRACT

Ficus sycomorus (Sycamore fig.) Family Moraceae is a plant used in African traditional medicine to treat mental illness, dysentery, cough, diarrhea, tuberculosis and cancer. The crude ethanol extract, n-Hexane and ethyl acetate fractions of the leaves of the plant were subjected to preliminary phytochemical screening using standard procedure as well as qualitative and quantitative antioxidant activity studies using 1, 1- diphenyl-2-picryl hydrazyl (DPPH) assay. The results of phytochemical screening showed that the crude ethanol extract contains alkaloids, flavonoids, saponins, tannins, terpenoids and anthraquinones, the n- hexane fraction contains, terpenoids, alkaloids and anthraquinones while the ethylacetate fraction contains alkaloids, saponins, tannins, terpenoids, flavonoids, anthraquinones and cardiac glycosides coumarins were found to be absent in the leaves of the plant. The results of the antioxidant activity studies revealed that *Ficus sycomorus* leaf extract has IC_{50} of 46.83µg/ml, 58.46.07µg/ml and 42.00ug/ml for crude ethanol extract, n-hexane and ethylacetate fractions respectively. Vitamin C which is the standard drug used was found to have IC_{50} of 25.00ug/ml. The plant exhibited antioxidant potential and could be useful in diseases involving oxidative stress such as age related cancers, stroke, Rheumatoid, arthritis and heart diseases.

Keywords: Antioxidant; *Ficus sycomorus*; Moraceae

INTRODUCTION

Ficus is the Latin form of fig, derived from the Persian 'fica'. In Greek 'syka' means fig. The name of the species comes from the Greek 'sykamorea' (sycamore), used in the Gospel according to St. Luke; it was the tree that Jesus cursed because it was barren (Hyde *et al.*, 2013). *Ficus sycomorus* is a large, semi-deciduous spreading savannah tree, up to 21 (max. 46) m; it is occasionally buttressed. Its leaves are broadly ovate or elliptic, the sub base is cordate, apex is rounded or obtuse and is scabrous above; petiole is 1-5 cm long, with five to seven pairs of yellow lateral veins; lowest pair originates at the leaf base. The plant is widely distributed in tropical Africa stretching from Senegal to South Africa, Nigeria, Niger, Mali, South Africa, Guinea, Kenya, Tanzania, Somalia, Ethiopia and Ivory Coast. In Nigeria the plant is mostly found in semi-arid regions (Williams *et al.*, 1980).

The plant is referred to by number of local names such as Sycamore fig (English), Baure (Hausa, Northern Nigeria), Tarmu (Kanuri), and Kamda (Babur/Bura), among others (Hyde *et al.*, 2013).

Ficus sycomorus is used traditionally in the treatment of snake bites, jaundice, chest pains, dysentery, cool, coughs and throat

infections (Sofowora, 1993). In northern Nigeria, the stem bark of *Ficus sycomorus* is used traditionally to treat fungal infections, jaundice and dysentery (Berg and Corner, 2005). The Hausa and Fulani tribes of northern Nigeria use the stem-bark of *F. sycomorus* to treat diabetes mellitus, fungal infection, jaundice and dysentery (Hassan *et al.*, 2007; Aduom *et al.*, 2012). The parts of *F. sycomorus* used traditionally for the treatment of tumors and diseases associated or characterized by inflammation include the fruits in different stages of ripening, fresh or dry, tree bark, leaves, twigs and young shoots, and also latex from the bark, fruit and young branches (Lansky *et al.*, 2008).

MATERIALS AND METHODS

Collection, and Identification, of plant materials

The leaves of *Ficus sycomorus* were collected in April, 2013, at the fields of Ahmadu Bello University Zaria, Nigeria. The plant was identified by Mallam U.S. Gallah of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria, (voucher specimen number 1466). The plant material was air dried under shade and size reduced manually using clean mortar and pestle.

Preparation of the extract

2.5 Kg of the plant material was subjected to cold maceration using 75% ethanol for 2d hours. The extract was filtered using Whatman filter paper (No. 1) and the filtrate evaporated under reduced pressure and dried to afford 18.20 %(w/w) of a brownish residue referred to as crude ethanol extract. The crude ethanol extract was further fractionated successively using n-hexane, chloroform, ethylacetate and n-butanol. The crude ethanol extract, n-hexane fraction and ethylacetate fraction were used for this research.

Phytochemical screening

Preliminary qualitative phytochemical tests were carried out on Crude Ethanol Extract, n- hexane and ethylacetate fractions employing standard procedures (Sofowora, 1982).

Antioxidant studies

Qualitative antioxidant studies

The crude extract and the various fractions were spotted on a TLC plate and developed using hexane-ethylacetate mixture at the ratio of 8:2 as a solvent system in a chromatographic tank. The developed chromatogram was sprayed with 0.15%w/v DPPH in methanol solution using an atomizer. The plate was observed for the presence of yellow colour on a pinkish/purple background on the TLC plate which is an indication for the presence of antioxidant principles (Saha *et al.*, 2008).

Quantitative antioxidant studies

The free radical scavenging activity of the leaves of *Ficus sycomorus* was determined using DPPH (Braca *et al.*, 2001). 0.004% w/v DPPH solution was prepared in 95% methanol. To 1ml of various concentrations of the extract (20, 40, 60, 80, and 100µg/ml) 2ml of DPPH solution was added. An equal amount of methanol and DPPH served as control. The mixture was shaken vigorously and was left to stand in dark for 30mins. The absorbances of the resulting solutions were measured using UV spectrophotometer at 520nm. The experiment was performed in triplicate and the percentage scavenging activity of each extract on DPPH radical was calculated as follows.

$$\text{Scavenging activity (\%)} = \left(\frac{Ab - Ae}{Ae}\right) \times 100$$

Where; Ab = absorbance of blank sample
Ae = absorbance of the solution containing the extract

The IC_{50} (concentrations of the extracts that inhibit 50% of the free radicals) were calculated from the graph of scavenging activity plotted against sample concentrations using Microsoft excel software (Viturro *et al*., 1999).

RESULTS

Phytochemical screening

The result of Phytochemical screening of the Crude ethanol extract, n-hexane and Ethylacetate fractions of the leaves of *F. sycomorus* revealed that the crude ethanol extract contains alkaloids, saponins, terpenoids, flavonoids, tannins, cardiac glycosides and anthraquinones; the N-hexane fraction contains alkaloids, terpenoids and cardiac glycosides while ethylacetate fraction contains Alkaloids,Saponins. Tannins, Flavonoids, Anthraquinones and Cardiac glycosides (Table I).

Table I: Results of phytochemical analysis of leaves of *F. sycomorus*

Phytochemical constituent	CEE	NHF	EAF
Alkaloids	+	+	+
Saponins	+	-	+
Coumarins	-	-	-
Terpenoids	+	+	-
Tannins	+	-	+
Anthraquinones	+	-	+
Cardiac glycosides	+	-	+
Flavonoids	+	-	+

Keys: + = Present; - = Absent; CEE = Crude ethanol Extract; EAF = Ethylacetae fraction; NHF = n- hexane Fraction

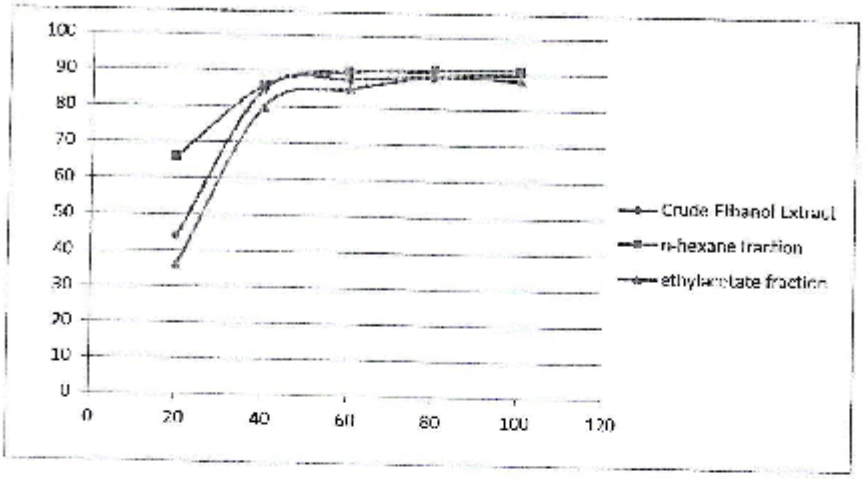


Figure. 1: A graph % inhibition against concentration of crude ethanolic extract and n-hexane fraction the of leaf of *Ficus sycomorous*

Table II: IC_{50} values of th ecrude ethanol extract, n- hexane fraction and ethylacetate fraction of leaf extract *ficus sycomorous*

SAMPLE	IC50 (µg/ml)
Crude Ethanolic extract	44.83
Hexane Fraction	58.46
Ethyl acetate fraction	42.00
Vitamin C	25.00

On addition of 2mL of DPPH solution to the various concentrations of the prepared crude ethanol, n-hexane and ethylacetate fractions of the leaf extract of *Ficus sycomorus*, the purple colour of DPPH was altered and as concentration decreases, the purple colour of DPPH gradually reappears although in the least concentration (20µg/mL), which increases with the intensity of the DPPH. The colour observed when DPPH was added to the plant extract was yellowish grey, although yellow colour is the expected colour, but due to complex nature of our plant extract, it contains a diverse class of constituent unlike the vitamin C. The yellowish grey is intermediate between the original colour of the extract and the expected yellow colour of the resultant solution, the appearance of yellow colour in the colourless solution of vitamin C on addition of DPPH signifies the scavenging of free radicals of DPPH hence antioxidant property. As mentioned above, these plants contain flavonoids saponins, alkaliods and terpenoids. The antioxidative effect is mainly due to phenolic components, such as flavonoids and phenolic diterpenes (Shahidi *et al.*, 1992). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994).

From this observation, it can be inferred that the crude ethanol and n-hexane fractions of leaf of *Ficus sycomorus* have some degree of antioxidant property.

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