ISOLATION AND CHARACTERIZATION OF THE BIOACTIVE COMPOUNDS IN THE STEM BARK OF *ALBIZIA CORIARIA*

BY

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DECLARATION

I hereby declare that this dissertation entitled "Isolation and characterization of the bioactive compounds in the stem bark of *Albizia coriaria*" is my original work and has not been submitted for any degree to any University before.

Signature Date

…….. ……

Ganza Barbara

APPROVAL

This dissertation has been submitted for examination with the approval of the following supervisors:

1. **Assoc. Prof. Byamukama Robert**

Signature Date

…… ……

2. **Prof. Kiremire T. Bernard**

Signature Date

……. …….

DEDICATION

Dedicated to Leah Kirabo Nandera

My loving daughter and best friend.

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ABSTRACT

In this study, *Albizia coriaria* was investigated for the bioactive compounds present in its stem bark. The plant was selected on the basis of its widespread use in traditional herbal medicine.

The stem bark was collected from a plant of *Albizia coriaria* in Mukono District in June 2012. Extraction of the plant material was done with ethyl acetate, methanol and water and the bioactivity of each extract was tested against *E. coli* and *P. aeruginosa.* Separation and purification of the compounds in the most active (ethyl acetate) extract was done using a combination of column chromatography and thin layer chromatography. The compounds were identified by 1D and 2D -^{1}H and ^{13}C NMR techniques as well as Mass spectrometry (MS).

The results of the bioactivity tests carried out in this study indicate that *A. coriaria* has potential anti microbial activity (Table 4.1.1). Six compounds, namely: Lupeol (**1**), Lupenone (**2**), Betulinic acid (**3**), Acacic acid lactone (**4**), (+) – Catechin (**5**) and Benzyl alcohol (**6**) were identified and characterized from the most bioactive (ethyl acetate) extract (Fig. 4.2.1g, 4.2.2g, 4.2.3f, 4.2.4f, 4.2.5g and 4.2.6d). Five of the characterized compounds have a wide range of biological activity reported in literature. This justifies the use of this plant in traditional medicine and indicates a promising potential for the development of medicinal agents from *Albizia coriaria* stem bark.

CHAPTER ONE

INTRODUCTION

1.1. Background

Research on plants with medicinal properties and identification of the chemical components responsible for their activities have justified the ancient traditional healing wisdom and have proven the enduring healing potential of many plant medicines (Babu et al., 2009). Wild plants have always been a major source of primary health care and other necessities of daily life for local communities throughout the world, an indication that medicinal plants can provide the best alternative source to obtain a variety of drugs. Drugs of natural origin now play an ever more important role in medical and healthcare services because metabolites produced by plants constitute a major source of bioactive substances which can be used as an alternative for cheap and effective herbal drugs against common infections (Sati and Joshi, 2011).

Despite the extensive use of antibiotics and vaccination programmes, infectious diseases continue to be a leading cause of morbidity and mortality worldwide (WHO, 2002). Widespread antibiotic resistance, the emergence of new pathogens in addition to the resurgence of old ones, and the lack of effective new therapeutics exacerbate the problems. According to the World Health Report of infectious diseases of 2002, overcoming antibiotic resistance is one of the major issues of the World Health Organization (WHO) for the present millennium (WHO, 2002). This scenario has been complicated by the emergence of HIV/AIDS, which renders the victims immuno-compromised and open to opportunistic infections such as chronic cough, diarrhea,

candidiasis, tuberculosis, typhoid among others. At the same time, most of the African population lives below the poverty line and cannot afford the expensive conventional medicines. These challenges call for renewed strategies on treatment, especially in the development of new antimicrobial drugs.

Plants are possible sources of antimicrobial agents (Sati and Joshi, 2011). The discovery of modern drugs such as quinine, vincristine, digoxin and digitoxin, emetine, artemisinin, etc., from medicinal plants signifies the huge potential that still exists for the production of many more novel pharmaceuticals (Geyid et al., 2005). For this reason, the ethno pharmacology of medicinal plants has attracted increasing attention in new drugs research and development (Jazari et al., 2011). According to Tagboto and Townson (2001), it is estimated that two-thirds of the world population rely on traditional remedies due to the limited availability, the high prices of most pharmaceutical products and the various side effects that they cause. This further justifies the search for alternative products from plants used in folklore medicine.

Uganda is well known for its rich ethno botanical wealth, particularly regarding medicinal plants (Tabuti et al., 2003) which are traditionally used in the treatment of ailments and could be a good source for discovery of new, safe and biodegradable drugs (Basha et al., 2010). As wide spread as infectious diseases are in Uganda, the number of medicinal plant species prescribed traditionally against infectious diseases runs into the hundreds (Namukobe et al., 2011). Many of these plants have a prolonged and uneventful use that may serve as an indirect testimony to their efficacy. Unfortunately, by the year 2000, only 5-15 % of the higher plants had been

systematically investigated for the presence of bioactive compounds worldwide (Newman et al., 2000), and there is no report of the current status.

Albizia coriaria **(**Welw. ex Oliver**)** is one of the many plants used in folklore medicine to treat a variety of ailments. In Uganda, *Albizia coriaria* is known locally as Musita (Lusoga), Mugavu (Luganda) and Musiisa (Rukiga). The bark of *Albizia coriaria* is harvested locally for the treatment of syphilis, skin diseases, jaundice, eye diseases, cough, sore throats, and for use as a general tonic (Namukobe et al., 2011). Its bark has also been reported to have anti-plasmodial activity (Kigondu et al., 2009). In ethno veterinary, the bark has been reported to be useful in the treatment of anaplamosis, liver flukes and diarrhea (Tabuti and Mugula, 2007).

Albizia coriaria belongs to the genus *Albizia*, which consists of about 150 species of mostly fast-growing subtropical and tropical trees and shrubs in the subfamily Mimosoideae of the legume family Fabaceae, with the greatest diversity in Africa and South America (Abdel-Kader et al., 2001).

Albizia coriaria is a deciduous slow growing tree 6-36 m tall, the tree crown spreading and flat, with an often twisted trunk. Young branchlets of *Albizia coriaria* usually appear hairy; the leaves are bipinnate, oblong to elliptic 13-33 mm long, 5-17 mm wide and rounded (Fig. 1.1.1). The bark of *Albizia coriaria* is gray-black, rough and raggedly scaling (Fig 1.1.2). Its fruit is a pod 10-21 cm long, 2-5 cm wide, brown or purplish-brown in colour with a tapered or acute apex. The specific epithet '*coriaria*' describes the leathery texture of its upper leaf surfaces. Direct

seeding is used in the propagation of *Albizia coriaria*; however, seedlings and wildings may also be used (Abdel-Kader et al., 2001).

Figure 1.1.1: *Albizia coriaria* plant

Figure 1.1.2: *Albizia coriaria* stem bark

1.2. Statement of the problem

Albizia coriaria stem bark is traditionally used in the treatment of several bacteria caused ailments such as cough, diarrhea and syphilis (Namukobe et al., 2011). However, the compounds responsible for the physiological actions of this traditional remedy were not known. This has not only hindered the standardization and development of this herb, but also made its recognition, acceptance and utilization remain locally restricted. Therefore, this study was done to isolate, characterize and identify the bioactive compounds in the stem bark of *A. coriaria* responsible for some of its medicinal properties.

1.3. Objectives

1.3.1. General objective

To characterize the constituent compounds of *Albizia coriaria* which are responsible for its medicinal properties.

1.3.2. Specific objectives

The General objective was achieved through the following specific objectives:

i. Extraction and isolation of the phytochemical constituents of *Albizia coriaria* stem bark.

ii. Testing for antimicrobial activity of the crude extracts against *Pseudomonas aeruginosa* and *Escherichia coli*.

iii. Structure elucidation of the compounds isolated from the most active extract against the selected organisms.

1.4. Significance of the study

This study has yielded important lead molecular structures for possible antimicrobial drug development especially in the production of synthetically improved therapeutic agents. The identified compounds may as well be used as markers for the standardization of herbal formulations from *Albizia coriaria*. Furthermore, the antimicrobial activity of the crude extract was tested against selected organisms. The bioactivity results of *Albizia coriaria* provided preliminary scientific justification for the traditional medicinal uses of this ethno remedy, an important step towards its acceptance and development as alternative therapeutic agent.

1.5. Justification of the study

Albizia coriaria can provide an alternative source for antimicrobial drugs. However, it can only be developed and standardized if its bioactivity is known and its bioactive ingredients are identified and characterized. Therefore there was need to investigate *Albizia coriaria* in order to understand better its chemical composition, properties, safety and efficacy.

CHAPTER TWO

LITERATURE REVIEW

2.1. *Albizia coriaria*

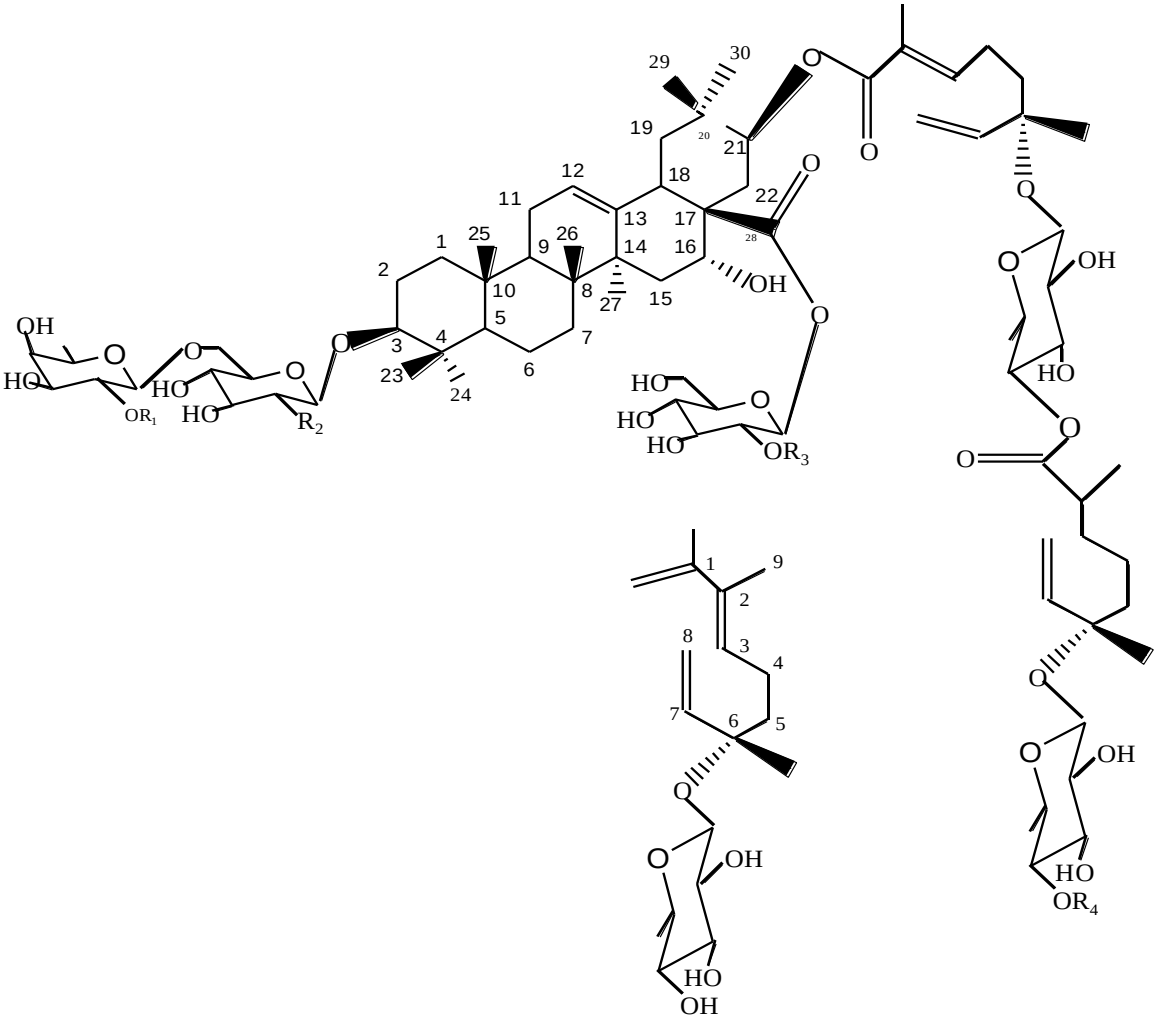
Albizia coriaria is one of about 150 species that belong to the genus *Albizia*. This genus is pan tropical, occurring in Asia, Africa, Madagascar, southern North America and Australia.

Triterpenoid saponins are commonly described in the genus *Albizia* (Gupta et al., 2005). Acacic acid glycosides have been reported in *Albizia coriaria* root bark as well as in the related species, such as in *Albizia adianthifolia*, *Albizia chinensis*, *Albizia grandibracteata*, *Albizia gummifera*, *Albizia julibrissin*, and *Albizia procera* (Note et al., 2010). Preliminary phytochemical analysis of *Albizia coriaria* bark revealed the presence of alkaloids, steroids, triterpenoids, coumarins, tannins and anthracenoside glycosides (Wanyama et al., 2011).

Phytochemical studies carried out on several *Albizia* species led to the isolation of several triterpene glycosides; The root bark of *Albizia anthelmintica* (Carpani et al., 1989), the seeds of *Albizia lucida* (Orsini et al., 1991), the stem of *Albizia subdimidiata* afforded a number of *N*-acetyl-d-glucosamine derivatives of oleanolic and echinocystic acids (Abdel-Kader et al., 2001). Three acacic acid lactone glycosides (Albizia saponins) were obtained from the bark of *Albizia lebbeck* (Pal et al., 1995). Proceraosides obtained from the seeds of *Albizia procera* were acacic acid glycosides bearing a common oligosaccharide moiety at C-28 and acylated at C-21 with a monoterpenic carboxylic acid linked to a monoterpene quinovoside (Yoshikawa et al., 1998).

Similar antitumor acacic acid glycosides julibrosides with variable monoterpene quinovosyl units at C-21 and oligosaccharide moities at C-3 (trisaccharide substituted by *N*-acetamido unit or tetrasaccharide), were reported from *Albizia julibrissin* (Zheng et al., 2006).

Three antitumor acacic acid glycosides acylated by an *o*-aminobenzoyl unit were isolated from leaves of *Albizia grandibracteata* (Krief et al., 2005). Cytotoxicity guided fractionation of the extract obtained from the roots of Madagascan plant *Albizia gummifera*, led to the isolation of three cytotoxic acylated acacic acid glycosides gummiferaosides (Cao et al., 2007). Two other reports described the isolation of further cytotoxic acylated acacic acid glycosides albizosides from *Albizia chinensis* (Liu et al., 2009) and coriariosides from *Albizia coriaria* (Note et al., 2009). The coriariosides isolated from the roots of *Albizia coriaria* (Fig 2.1.1) were characterized as: 3-O-[β-D-xylopyranosyl-(1-2)-β-D-fucopyranosy-l-(1-6)-2-(acetamido)-2-deoxy-β-D-glucopyranosyl]-21-O-{(2E,6S)-6-O-{4-O-[(2E,6S)-2,6-dimethyl-6-O-(β-D-quinovopyranosyl) octa-2,7-dienoyl}acacicacid-28-O-β-D-xylopyranosyl-(1-4)-α-rhamnopyranosyl-(1-2)-β-D-glucopyranosyl ester (**I**), 3-O-{β-D-fucopyranosyl-(1-6)-[β-D-glucopyranosyl-(1-2)-β-D-dlucopyranosyl}-21-O-{(2E,6S)-6-O-{4-O-[(2E,6S)-2,6-dimethyl-6-O-(β-D-quinovopyranosyl) octa-2,7-dienoyl]-β-D-quinovopyranosyl-2,6-dimethylocta-2,7-dienoyl}acacic acid-28-O-α-L-rhamnopyranosyl-(1-2)-β-D-glucopyranosyl ester (**II**) and 3-O-[β-D-fucopyranosyl-(1-6)-β-D-glucopyranosyl]-21-O-{(2E,6S)-6-O-{4-O-[(2E,6S)-2,6-dimethyl-6-O-(β-D-quinovopyranosyl)octa-2,7-dienoyl)-β-D-quinovopyranosyl]octa-2,7-dienoyl}acacic acid-28-O-β-D-glucopyranosyl ester (**III**) (Note et al., 2009).



S

	R_1	R_2	R_3	R_4
I	Xyl	NHAc	Xyl(1→ 4) Rha	S
II	H	O-Glc	Rha	S
III	H	OH	H	H

Figure 2.1.1: Acacic acid type saponins from the roots of *Albizia coriaria.*

The plants in the genus *Albizia* are commonly used in traditional medicine in different parts of the world (Table 2.1.1).

Table 2.1.1: Ethnomedicinal uses of some Albizia species.

Species name	**Ethnomedicinal uses**	**Reference**
Albizia coriaria	The bark infusion is drunk for the treatment of diarrhea, cough and snake bite; the root or bark infusion is bathed for the treatment of amoebiasis while the decoction is drunk for the treatment of syphilis.	Tabuti et al., 2003; Namukobe et al., 2011
Albizia anthelmintica	The stem bark is used in Sudanese folk medicine for treatment of tape worm infection, stomach trouble, amoebic dysentery and less frequently for malaria.	Koko et al., 2000.
Albizia gummifera	The root decoction is taken to treat malaria, stomach pains, scabies, other skin diseases and psychiatric problems.	Kokwaro, 1993.

Albizia julibrissin	The stem bark has been recorded in Chinese Pharmacopoeia as a sedative agent and an anti-inflammatory drug for treating injuries due to falls, and removing carbuncles, skin ulcers, and wounds.	Pharmacopoedia Committee of China, 2005.
Albizia zygia	The bark decoction is taken to treat uterine fibroids, bark infusion is taken to treat diarrhea while sap is applied to the eye to treat cataract.	Tabuti et al., 2003
Albizia malacophylla	The root decoction is applied to incisions to treat migraine.	Tabuti et al., 2003
Albizia ferruginea	The bark infusion is efficient in the treatment of malaria, stomach pain, dysentery and skin infections.	Kareru et al., 2007.

2.2. Plants as sources of medicine

Several investigations and modern methods have confirmed folkloric accounts of the use of higher plant preparations for the treatment of infections. It is known that the antimicrobial activity of natural products isolated from higher plants is comparatively low. However the natural products commonly have reasonable potency, are comparatively easy to synthesize and their synthetic analogues can possess enhanced therapeutic potential (Aburjai et al., 2001).

2.2.1. Plant metabolites

Plant metabolites include compounds that aid in the growth and development of plants; however some of them are not required for the plant to survive. Each plant family, genus, and species produces a characteristic mix of these chemicals, and they can sometimes be used as taxonomic parameters in classifying plants. Humans use some of these compounds as medicines, flavoring agents, or recreational drugs such as the alkaloids nicotine and cocaine, and the terpene cannabinol. Plant metabolites largely fall into three major classes of compounds: alkaloids, terpenoids, and phenolics. Thousands of metabolites have been isolated from plants, and many of them have powerful physiological effects in humans and are used as medicines. In Traditional Chinese Medicine (TCM) for example, many medicinal herbs have been used for hundreds of years to treat respiratory complaints such as bronchial inflammation, pneumonia, expectoration and cough, and have shown less or no side effects as compared to synthetic drugs (Shang et al., 2010).

2.3. A review of some analytical methods used in natural products research

The qualitative and quantitative studies of bioactive compounds from plant materials mostly rely on the selection of proper methods. In this section, some of the commonly used methods in natural products research are discussed.

2.3.1. Extraction

Extraction is the first step of any medicinal plant study and plays a significant and crucial role on the final outcome of the study. Extraction methods are sometimes referred to as "sample preparation techniques". It is true that the development of modern chromatographic and spectrometric techniques make bioactive compound analysis easier than before but the success still depends on the extraction methods, input parameters and the exact nature of plant parts. The most common factors affecting extraction processes are the matrix properties of the plant part, the solvents used, temperature and extraction time. It is only possible to conduct further separation, identification, and characterization of bioactive compounds if the extraction process has been appropriately done. Bioactive compounds from plant materials can be extracted by various classical extraction techniques. Most of these techniques are based on the extracting power of different solvents used and the application of heat and/or mixing. The commonly used classical techniques are: soxhlet extraction, maceration and hydro distillation to obtain a crude extract which is then concentrated using a rotary evaporator (Azmir et al., 2013).

2.3.2. Column chromatography

In column chromatography, the stationary phase (a solid adsorbent) is placed in a vertical glass column and the mobile phase (a liquid) is added to the top and flows down through the column (by either gravity or external pressure). Column chromatography is generally used as a purification technique to isolate desired compounds from a mixture (Kenkel, 2003).

The crude extract to be purified by column chromatography is applied at the top of the column. The liquid solvent (the eluent) is passed through the column by gravity or by the application of air pressure. Equilibrium is established between the solute adsorbed on the adsorbent and the eluting solvent flowing down through the column. Because the different components in the mixture have different interactions with the stationary and mobile phases, they will be carried along with the mobile phase to varying degrees and a separation will be achieved. The individual components, or elutants, are collected as the solvent drips from the bottom of the column (Harvey, 2000).

Silica gel (SiO_2) and alumina (Al_2O_3) are the two adsorbents commonly used for column chromatography. These adsorbents are sold in different mesh sizes, indicated by a number on the bottle label. The polarity of the solvent which is passed through the column affects the relative rates at which compounds move through the column (Harvey, 2000). Polar solvents can compete more effectively with the polar molecules of a mixture for the polar sites on the adsorbent surface and will also solvate the polar constituents better. Consequently, a highly polar solvent will move even highly polar molecules rapidly through the column. If a solvent is too polar, movement becomes too rapid, and little or no separation of the components of a mixture will

result. If a solvent is not polar enough, no compounds will elute from the column. Proper choice of an eluting solvent is thus crucial for the successful application of column chromatography as a separation technique. Often a series of increasingly polar solvent systems are used to elute a column. A non-polar solvent is first used to elute the less-polar compounds. Once the less-polar compound is off the column, a more-polar solvent is added to the column to elute the more-polar compounds (Kenkel, 2003).

2.3.3. Thin layer chromatography

Thin layer chromatography (TLC) is often used to analyze the fractions obtained from column chromatography to determine if the fraction contains more than one component and if fractions can be combined without affecting their purity (Kenkel, 2003). The separation by TLC depends on the relative affinity of compounds towards stationary and mobile phase. The compounds under the influence of mobile phase (driven by capillary action) travel over the surface of the stationary phase. During this movement, the compounds with higher affinity to the stationary phase travel slowly while those with less affinity to the stationary phase travel faster. Thus separation of components in the mixture is achieved. Once separation occurs, the individual components are visualized as spots on the plate after staining with iodine vapour (Harvey, 2000).

2.3.4. High performance liquid chromatography

High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is

forced through under high pressures of up to 400 atmospheres. That makes it move faster. It also allows the use of a smaller particle size for the column packing material which gives it a greater surface area for interactions between the stationary phase and the molecules flowing through it. This allows a much better separation of the components of the mixture (Harvey, 2000). Preparative high-performance liquid chromatography (HPLC) has become a favorite method of natural product isolation and purification. The various modes available (e.g., normal-phase, reversed-phase, size exclusion, and ion-exchange) to date can be used to purify most classes of natural products. Although preparative HPLC is very similar to analytical HPLC, instead of injecting a small amount of sample to maximize the resolution, the amount of feed is very high in order to maximize the purification productivity and minimize the amount of solvent used (Mbaveng et al., 2008).

2.3.5. Mass spectrometry (MS)

Mass spectrometry allows the determination of the molecular mass and the molecular formula of a compound, as well as certain structural features. A small sample of the compound is vaporized and then ionized as a result of an electron being removed from each molecule, producing a molecular ion (a radical cation). Many of the molecular ions break apart into cations, radicals, neutral molecules, and other radical cations (McMurry, 2000). The bonds most likely to break are the weakest ones and those that result in the formation of the most stable products. These fragments of the molecules are detected individually on the basis of their mass-to-charge ratios (Bruice, 2000). The details of exactly how these positively charged fragments are separated and detected differ according to the specific design of the mass analyzer portion of the instrument. In

any case, the information acquired and displayed by the data system (the so-called mass spectrum) allows the analyst to reconstruct the original molecule and thereby identify it. Besides the significant applicability to molecular compound identification, mass spectrometry also finds application in elemental analysis, such as to determine what isotopes of an element might be present in a sample (Kenkel, 2003).

2.3.6. Nuclear magnetic resonance (NMR) spectroscopy

Nuclear magnetic resonance spectroscopy depends on the absorption of energy when the nucleus of an atom is excited from its lowest energy spin state to the next higher one. Many elements are difficult to study by NMR, and some cannot be studied at all. Fortunately though, the two elements that are the most common in organic molecules (carbon and hydrogen) have isotopes (^{1}H and ^{13}C) capable of giving NMR spectra that are rich in structural information. A proton nuclear magnetic resonance (^{1}H NMR) spectrum tells us about the environments of the various hydrogen atoms in a molecule; a carbon-13 nuclear magnetic resonance (^{13}C NMR) spectrum does the same for the carbon atoms (Carey, 2000). Together, ^{1}H and ^{13}C NMR is used in determining a substance's molecular structure. It is often used in conjunction with other spectrometric techniques such as FTIR and mass spectrometry.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Plant collection, authentication and extraction

3.1.1. Plant collection and authentication

The fresh stem bark of *Albizia coriaria* was collected in June 2012, from Mukono district, Uganda after identification and authentication by a taxonomist from the department of Botany, Makerere University. The stem bark sample was packed in polythene bags and transported to Makerere University.

3.1.2. Preparation of the extracts

The bark was chopped into small pieces and air dried at room temperature for 21 days. The dry bark was ground into fine powder using an electric grinder. The powdered plant material (1 Kg) was sequentially extracted three times with 5 litres of ethyl acetate, methanol and water at room temperature for 48 hours. The extracts were filtered through cotton wool and Whatman No. 1 filter paper and concentrated with a rotary evaporator at 40 ^{0}C to dryness. The dried extracts were transferred to sample bottles which were placed in a dessicator containing anhydrous sodium sulphate to remove any traces of water that could have been present. The dry extracts were later kept in tightly stoppered bottles in a refrigerator for further analysis.

3.2. Testing for selected antimicrobial activity

The activity of the plant extracts was tested against two American Type Culture Collection (ATCC) bacterial strains: *Pseudomonas aeruginosa* and *Escherichia coli* (ATCC 25922) both of which were obtained from the Department of Pharmacology (Makerere University Medical School, Kampala). The bacterial strains were selected on the basis of the diseases against which *Albizia coriaria* is used. The bark of *Albizia coriaria* is used locally for the treatment of cough (commonly caused by *P.aeruginosa*) and diarrhea (caused by *E.coli*). Testing of the plant extracts for antibacterial activity was done by the modified agar disc diffusion method (Baris et al., 2006). The most active extract was selected for fractionation and further analysis.

3.2.1. Modified Agar disc diffusion assay for bacterial screening

The test micro organism was aseptically inoculated (approx. 1.0×10^8 colony forming units/ml) on sterile Mueller Hinton agar by surface spreading to make a uniform microbial inoculum. Using sterile glass cork borers (6 mm in diameter), two wells were carefully made on the agar plate without distorting the media; both wells contain the test extract. A separate agar plate was used to test the control drug; ciprofloxacin (0.5 mg/ml) against the bacteria. The second plate containing dimethyl sulfoxide (DMSO) was used as a negative control. Fifty microlitres (50 µl) of the extract and the controls were carefully dispensed into the respective wells and the plates left on the bench for 60 minutes to allow the system stabilize as the inoculated microorganisms get acclimatized to the new environment. The culture plates were then incubated at 37 ^{0}C for 24 hours. Using a metric ruler, the diameter of the zone of inhibition (the diameter of the area of no

growth of the micro organism around the disc) was measured for the ethyl acetate, methanol and aqueous extracts (Baris et al., 2006).

3.2.2. Determination of Minimum Inhibitory Concentration (MIC) using Broth dilution method

MIC was performed on organisms that exhibited highest sensitivity, to the ethyl acetate extract, of diameter above 12 mm upon screening. Five hundred microlitres of the test extract was serially diluted from 2-fold to 4-fold dilution in sterile Mueller Hinton broth. Five hundred microlitres (500 µl) of the test organism was aseptically inoculated in each of the four tubes containing the extract in order of increasing dilution (500, 250, 125 and 62.5 mg/ml). Thereafter, the tubes were incubated at 37 ^{0}C for 24 hours (Baris et al., 2006). After incubation, the tube next to the one showing no microorganism turbidity was considered as containing the MIC of the extract in question.

3.2.3. Determination of Minimum Bactericidal Concentration (MBC)

Following the MIC determination using the Broth dilution method, after the recommended period of incubation, four millilitres (4 ml) of solution from each of the four tubes containing the ethyl acetate extract in order of increasing dilution (500, 250, 125 and 62.5 mg/ml) was inoculated on Mueller Hinton agar and incubated at 37 ^{0}C for 24 hours. The highest dilution which showed growth of the microorganism was considered as the Minimum Bactericidal

Concentration (MBC). The purpose of this test was to determine the lowest concentration at which the ethyl acetate extract would kill *E. coli* and *P. aeruginosa* (Baris et al., 2006).

3.3. Isolation and purification of active compounds from the bioactive fractions

3.3.1. Column chromatography

The most active crude extract (Ethyl acetate extract) was subjected to silica gel (70-230 mesh) column chromatography. Briefly, silica gel (500 g) was mixed with n-hexane to form a homogenous suspension/slurry and stirred using a glass-stirring rod to remove bubbles. The silica gel slurry was then poured into a glass column. The sample to load on the column was prepared by dissolving 15 g of the ethyl acetate extract in 100 ml of methanol. To the solution, 30 g of silica was added and mixed by stirring with a glass rod. The mixture was allowed to dry at room temperature. The dried silica extract mixture was layered on the column layer bed. The column was first eluted with n-hexane as the mobile phase with the polarity increasing by 5 % increments of ethyl acetate. After getting to 100 % ethyl acetate, the polarity was further increased by 5 % increments of methanol. For each eluent system, two litre volumes were used and 250 ml fractions collected in 250 ml glass beakers. The collected fractions were concentrated to dryness using a rotary evaporator at 40 ^{0}C. The fractions were further fractionated using sephadex LH-20 by column chromatography with dichloromethane and methanol (1:1) as the eluent system to obtain the pure compounds (Harvey, 2000).

3.3.2. Thin layer chromatography

The concentrated fractions collected from the column chromatography were subjected to thin layer chromatography (TLC). Briefly, a spot of each fraction was carefully applied onto a thin layer chromatographic plate (coated with silica) and left to dry. After about five minutes, the plate was dipped in a suitable solvent which allowed the compounds in the spot to move upwards by capillary attraction. The plate was then removed from the solvent and left to dry. The positions of different compounds were observed by fluorescence under UV-light followed by staining with iodine vapour. Fractions with similar TLC profiles were combined (Mbaveng et al., 2008).

3.4. Structure determination of the bioactive compounds

The structures of the bioactive compounds were determined using spectroscopic methods i.e. Mass spectrometry (MS) and Nuclear magnetic resonance (NMR) spectroscopy. For MS, 0.5 mg of each pure compound was put in a labeled sample vial and diluted with an appropriate solvent of HPLC grade up to a concentration of one micro molar (1 μM). The solution in each case was filtered in order to remove any insoluble material which would otherwise block the sample introduction line. Two ml of each sample solution was introduced into the mass spectrometer for analysis. For NMR, both one dimensional (1D) and two dimensional (2D) proton (^{1}H) and carbon (^{13}C) NMR experiments such as COSY, NOESY, HMBC and HSQC were employed. For each sample, 10 mg of the pure compound was dissolved in 1 ml of a suitable deuteriated solvent and then filtered through a pasteur pipette equipped with a glass wool plug that discharged into a

5 mm NMR tube (Aldrich Z412848) which was labeled clearly with a concentric label. The purpose of the filtration was to remove any undissolved sample, particulates and dust from the solution, which could affect the resolution and the line shape of the NMR spectra. The 1D and 2D spectra were recorded on Bruker AV-600 instruments (McMurry, 2000). All the spectra were analysed and the results compared with published information in literature in order to elucidate the structures of the isolated compounds.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. In vitro activity of the crude extracts against selected bacteria

The ethyl acetate extract yielded 83.06 g of a brick red powder upon concentration. The methanol extract yielded 127 g of a dark coloured powder while the aqueous extract yielded 4.26 g of a brown powder. Three grams (3 g) of each extract was subjected to bioactivity tests. When the activity of the plant extracts ware tested against two bacterial strains namely: *Pseudomonas aeruginosa* and *Escherichia coli*, the ethyl acetate extract's zone of inhibition diameter was 18 mm and 17 mm for *Escherichia coli* and *Pseudomonas aeruginosa* respectively (Table 4.1.1); while the methanol extract exhibited a zone of inhibition diameter of 8 mm against *Pseudomonas aeruginosa* and had no inhibition of growth on *Escherichia coli*. The aqueous extract had no inhibition of growth of any of the bacterial strains tested (Table 4.1.1).

Table 4.1.1: Antibacterial activity of plant extracts against *Pseudomonas aeruginosa* and *Escherichia coli* (Fig. 4.1.1).

	Zone of inhibition diameter (mm)					
	Extract			**Controls**		
	Ethylacetate (1000 mg/ml)	Methanol (1000 mg/ml)	Aqueous (1000 mg/ml)	Positive (Cipro)	Negative (DMSO)	Blank
Micro-organism						
E. coli	18[a]	0[c]	0[e]	48	0	0
P. aeruginosa	17[b]	8[d]	0[f]	49	0	0

Legend: Antibacterial activity of the plant extract (1000 mg/ml) each in ethylacetate, methanol and water were tested against *P.aeruginosa* and *E.coli* bacteria. The diameters corresponding

to zone of inhibition were measured for each extract (in mm). The letters a-f denote the bacteria culture plate on which the tests were done. Cipro = Ciproflaxin, DMSO = Dimethylsulfoxide.

Both bacterial strains (*P.aeruginosa* and *E.coli*) were found to be susceptible to the ethyl acetate extract as the zone of inhibition diameters (17 mm and 18 mm for *P.aeruginosa* and *E.coli* respectively, Table 4.1.1) were within the range for standard antibiotics such as ampicillin (inhibition diameter 16-22 mm), doxycycline (inhibition diameter 18-24 mm) and tetracycline (inhibition diameter 18-25 mm), as reported by the Clinical and Laboratory Standards Institute – CLSI (2007). The methanol extract showed no antibacterial activity towards *E. coli* while the zone of inhibition diameter of 8 mm (Table 4.1.1) indicated that *P. aeruginosa* was resistant to the extract (Fig. 4.1.1). The aqueous extract did not show any antibacterial activity towards both strains (Fig 4.1.1). The bioactivity results were considered evidence of the presence of more compounds in the ethyl acetate extract compared to the methanol and aqueous extracts, the probable reason being that the compounds in *A. coriaria* stem bark were more soluble in ethyl acetate as compared to methanol and water.

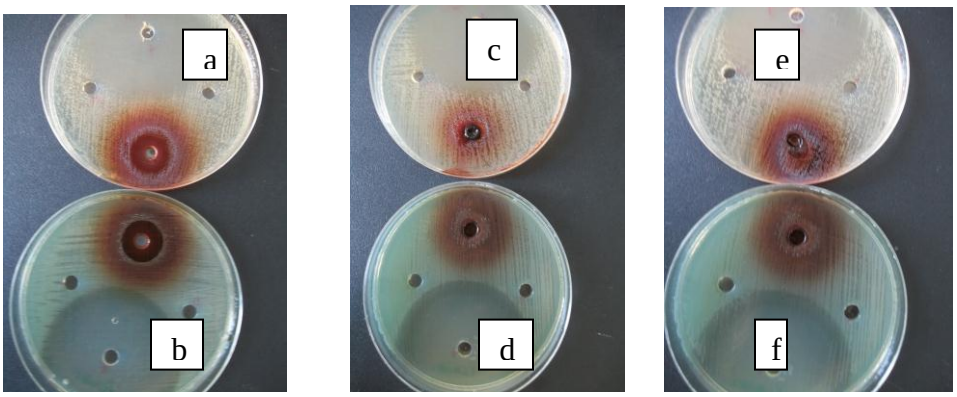


Figure 4.1.1: Bioactivity tests. The upper culture plates (a, c and e) are of *E. coli* and the lower ones (b, d and f) are for *P. aeruginosa*.

Since the ethyl acetate extract showed the largest zone of inhibition, it was considered for further tests. Its Minimum Inhibitory Concentration (MIC) against each of the bacterial strains was investigated as well as its Minimum Bactericidal Concentration (MBC). It showed a minimum inhibory concentration (MIC) of 125 mg/ml on *E. coli* and 250 mg/ml on *P. aeruginosa* while its Minimum Bactericidal Concentration (MBC) was 125 mg/ml on *E. coli* and was not detected on *P. aeruginosa* (Table 4.1.2).

Table 4.1.2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the ethyl acetate extract (see fig 4.1.2 and 4.1.3 for culture plates)

	MIC in mg/ml	**MBC in mg/ml**
	Ethyl acetate extract	
Microorganism		
E. coli (ATCC25922)	125	125
P. aeruginosa	250	Not detected

The ethyl acetate extract showed a minimum inhibitory concentration (MIC) of 125 mg/ml on *E. coli* and 250 mg/ml on *P. aeruginosa*. This showed that the ethyl acetate extract was more effective against *E. coli* as compared to *P. aeruginosa*. The Minimum Bactericidal Concentration (MBC) of 125 mg/ml on *E. coli* and none on *P. aeruginosa* also showed that the extract has a stronger activity towards *E. coli* than *P. aeruginosa*. However, the high MIC values call for a higher dose for effective treatment. This result supports the high dosage (3-4 cupfuls per day) used by the traditional healers for an adult.

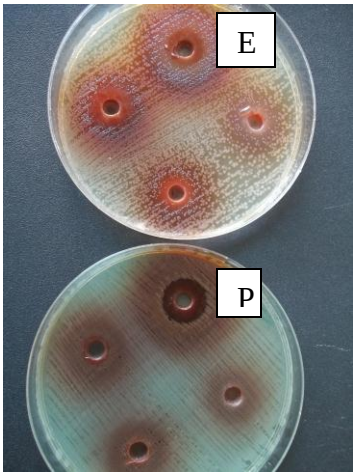


Figure 4.1.2: Minimum Inhibitory Concentration (MIC) tests. **E** represents *E. coli*; moving anticlockwise from E are decreasing concentrations of the extract (500, 250, 125 and 62.5 mg/ml). **P** represents *P.aeruginosa*; moving anticlockwise from P are decreasing concentrations of the extract (500, 250, 125 and 62.5 mg/ml respectively).

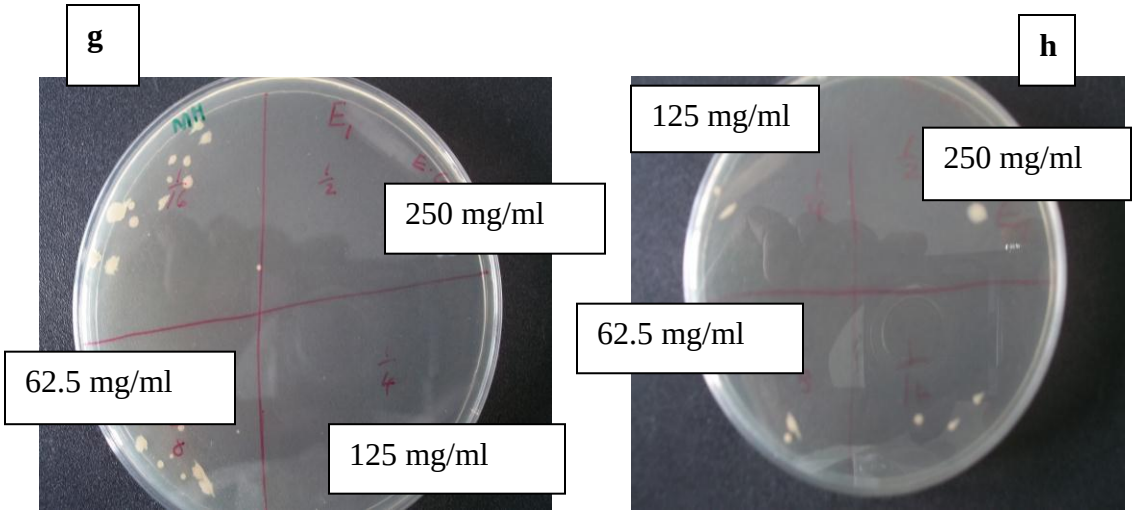


Figure 4.1.3: Minimum Bactericidal Concentration (MBC) tests. Plate g represents *E.coli*: MBC = 125 mg/ml; the highest dilution which showed growth of the microorganism. Plate h represents *P. aeruginosa*: MBC was not detected as visible growth occurred at each of the concentrations used.

4.2. Characterization of the compounds in the most active (ethyl acetate) extract

Six compounds were isolated from the ethyl acetate extract of the stem bark of *Albizia coriaria* by a combination of chromatographic techniques. The structures of these isolated compounds were identified by NMR spectroscopy and Mass spectrometry as Lupeol (**1**), Lupenone (**2**), Betulinic acid (**3**), Acacic acid lactone (**4**), (+) - Catechin (**5**) and Benzyl alcohol (**6**). This is the first time these compounds are being reported in this plant.

4.2.1. Compound 1 (Lupeol)

Compound **1** was obtained as a white powder, soluble in methylene chloride, having been eluted with methylene chloride: methanol 1:1 from the sephadex column. The mass spectrum of compound **1** (Fig 4.2.1a) exhibited a molecular ion $[M]^+$ peak at *m/z* 426 observed from EI-MS; its molecular formula was assigned to be $C_{30}H_{50}O$. Fragments at *m/z* 189 and 218, characteristic of pentacyclic triterpenes (Pereira et al., 1996) were also present. The other prominent fragment ion peak was at *m*/*z* 411 $[M-CH_3]^+$, which is characteristic of a pentacyclic triterpene with an isopropenyl group (Pereira et al., 1996).

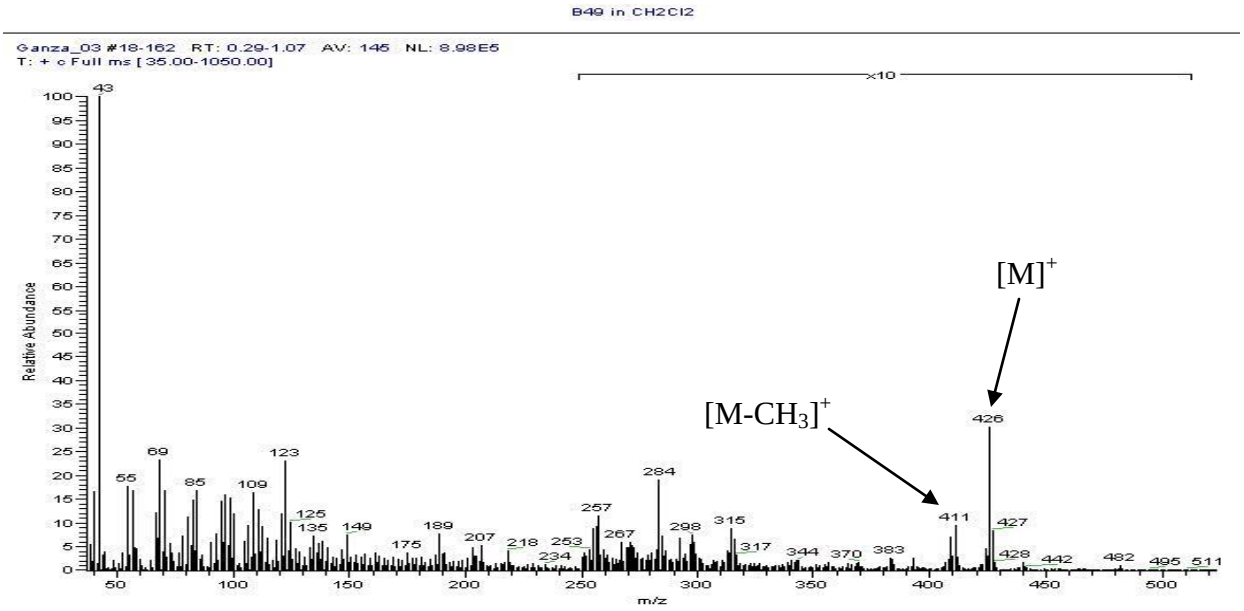


Figure 4.2.1a: Mass spectrum of compound **1**

The ^{1}H NMR spectrum of **1** (Fig 4.2.1b) showed resonances assignable to olefinic methylene protons at 4.68 ppm and 4.55 ppm, a carbinyl proton at 3.15 ppm, a vinyl methyl singlet at 1.68 ppm, and six methyl singlets at 0.94 ppm, 0.74 ppm, 0.83 ppm, 1.03 ppm, 0.95 ppm and 0.79 ppm. The vinyl methyl at 1.68 ppm was found to be coupled to one of the two methylene protons (H-29b, 4.55 ppm) in the COSY experiment, thus indicating the presence of an isopropenyl group as well as a lupane skeleton (Shai et al., 2008). A methine signal at 2.40 ppm (ddd, *J*=11.1, 11.1, 5.8 Hz) was attributed to the proton at C-19 position.

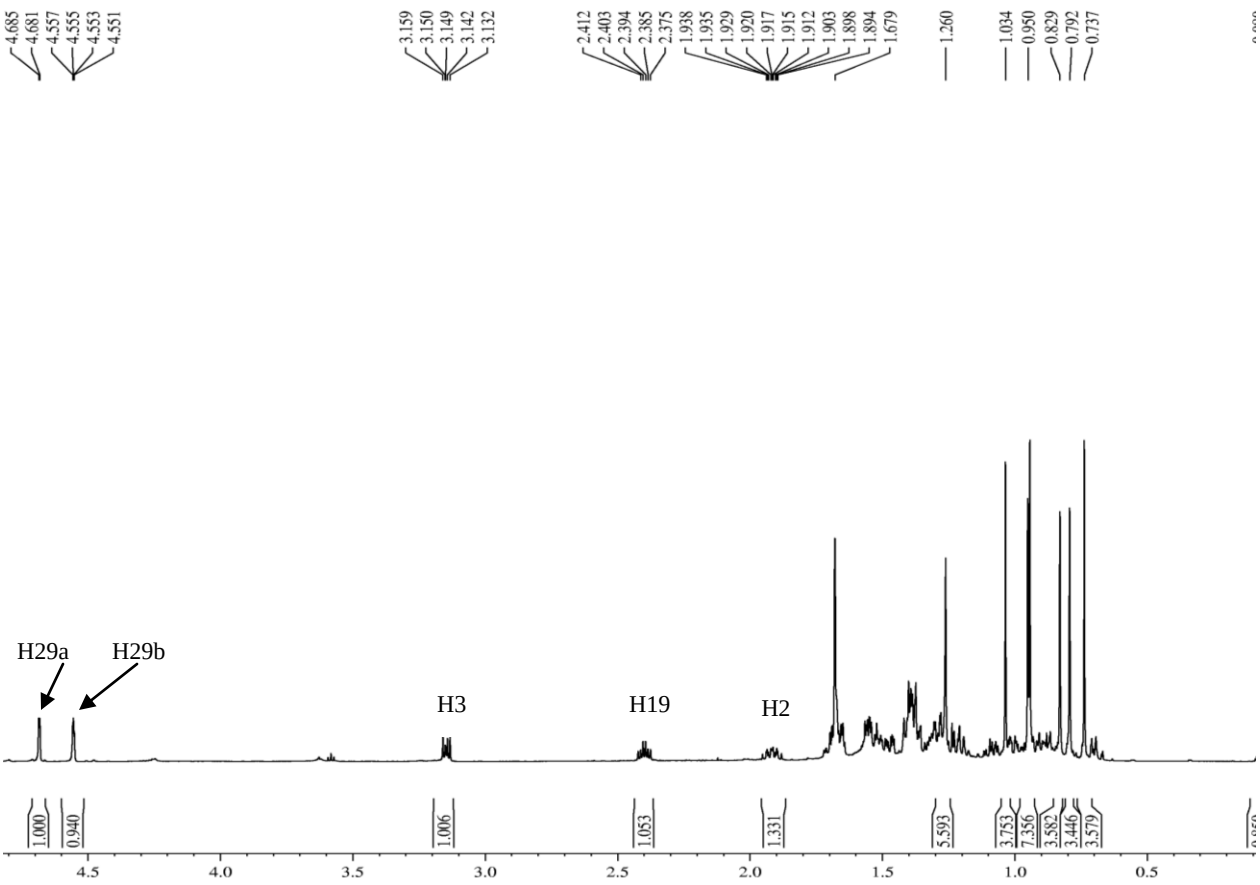


Figure 4.2.1b: ^{1}H NMR spectrum of compound **1**

The ^{13}C NMR data (Fig 4.2.1c) of compound **1** were in agreement with the MS data, as it revealed 30 carbon signals, which were shown by the HSQC experiment to be seven methyls, ten methylene, six methine carbons, five quaternary carbons and two olefinic carbons. The ^{13}C NMR data was also in complete agreement with the existence of an isopropenyl group, in particular, the characteristic vinylic carbon atom resonances at 151.2 ppm and 109.3 ppm, corresponding to carbon atoms 20 and 29 respectively. This supported the olefinic methylene protons seen as singlets at 4.68 ppm and 4.55 ppm in the ^{1}H NMR spectrum of compound **1**. The signal at 78.7 ppm was characteristic for the oxymethine carbon at position 3.

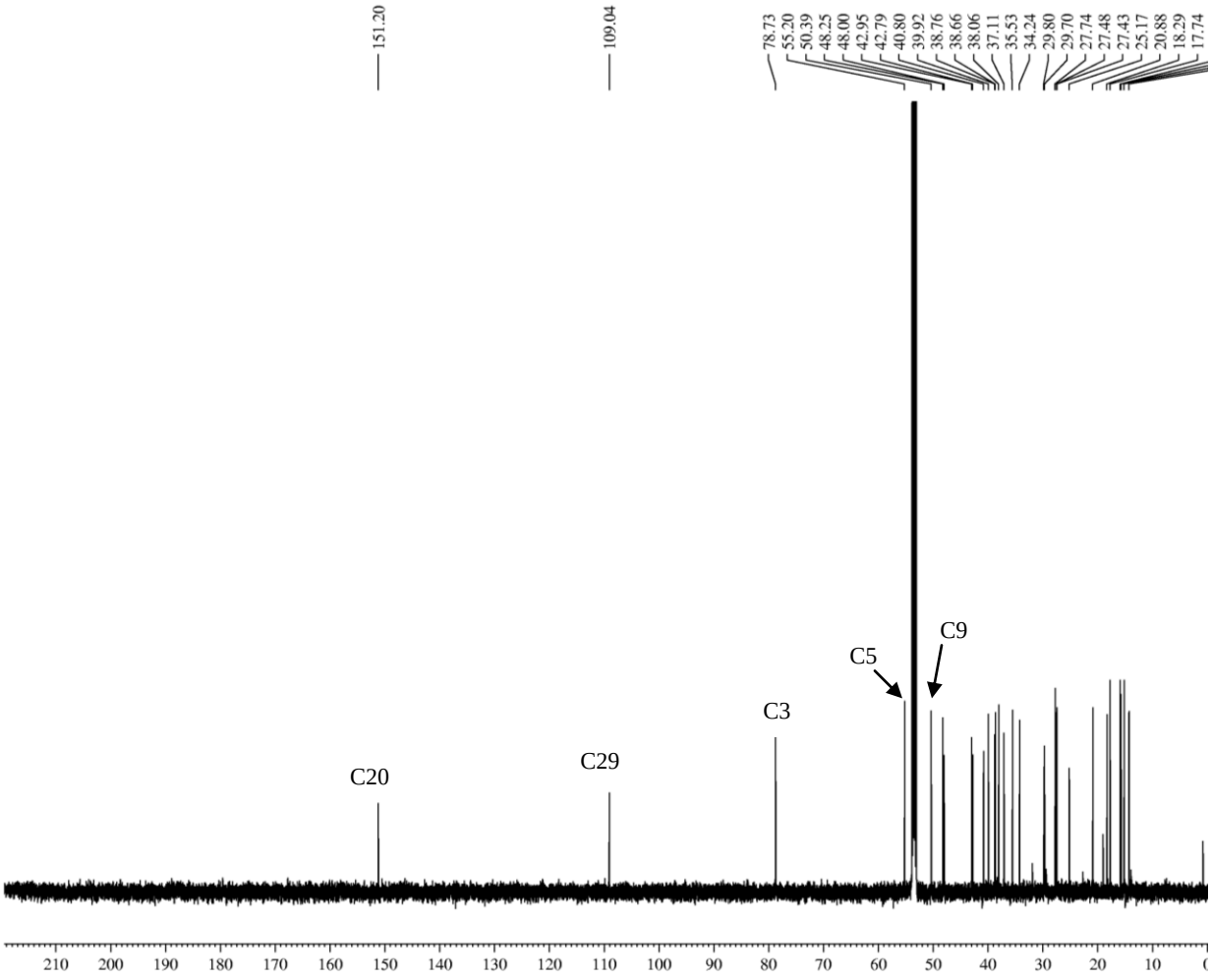


Figure 4.2.1c: ^{13}C NMR spectrum of compound **1**

With the help of the HSQC experiment (Fig 4.2.1d), a correlation was observed between the olefinic methylene protons: H-29a (4.68 ppm), H-29b (4.55 ppm) and C-29 (109.3 ppm). From the same spectrum, all the ^{1}H resonances were assigned to their corresponding ^{13}C resonances (Table 4.2.1).

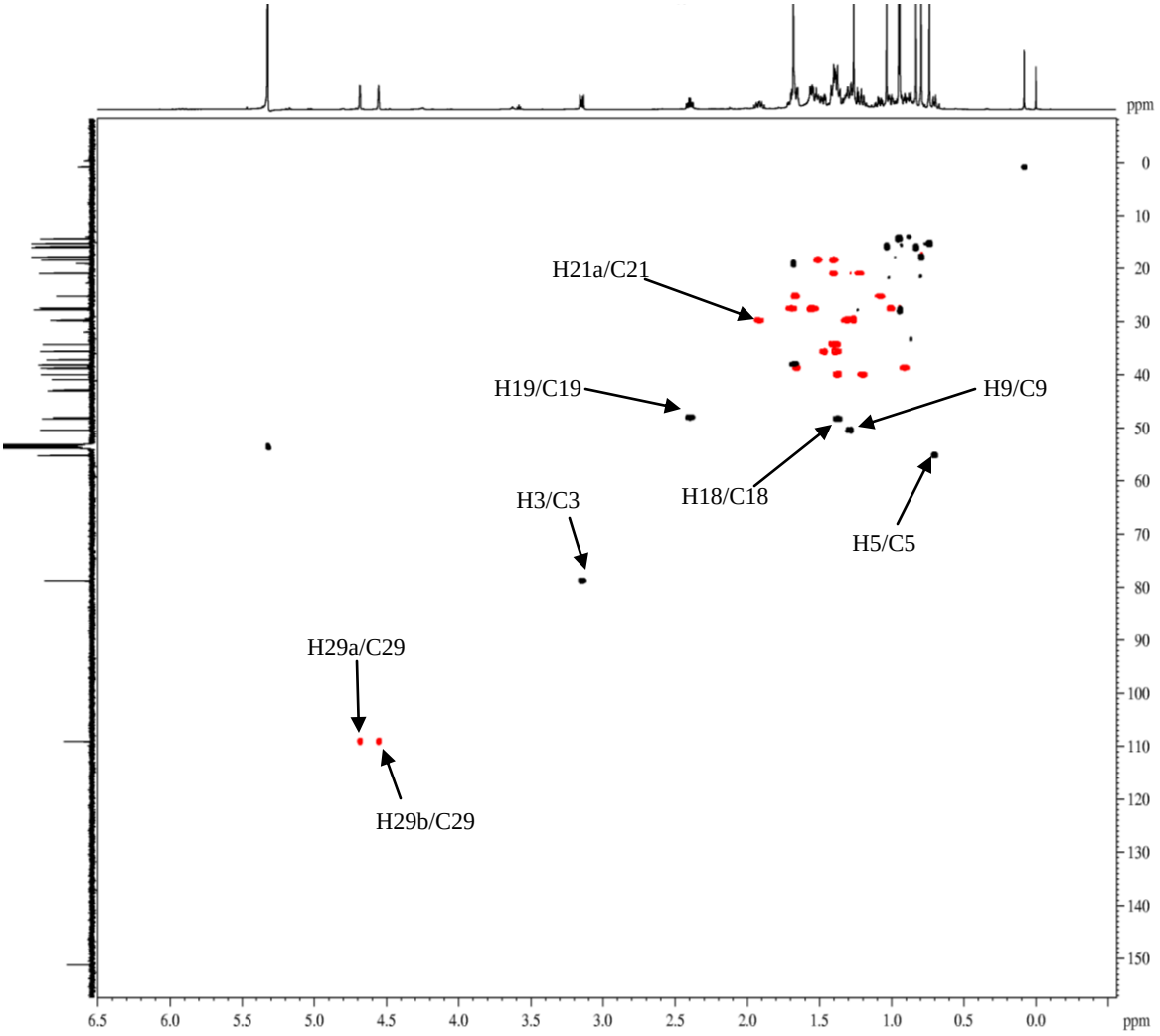


Figure 4.2.1d: ^{1}H-^{13}C HSQC spectrum of compound **1**

The HMBC spectrum of compound **1** (Fig 4.2.4e) showed correlations between the carbinyl proton, H-3 (3.15 ppm) and the carbon atoms C-24 (15.4 ppm) and C-23 (28.0 ppm) as well as between the olefinic methylene protons: H-29a (4.68 ppm), H-29b (4.55 ppm) and the carbon atoms C-30 (19.2 ppm) and C-19 (48.3 ppm). Several other correlations were observed from the HMBC spectrum of compound **1** that were helpful in the assignment of quaternary carbon atoms (Table 4.2.1).

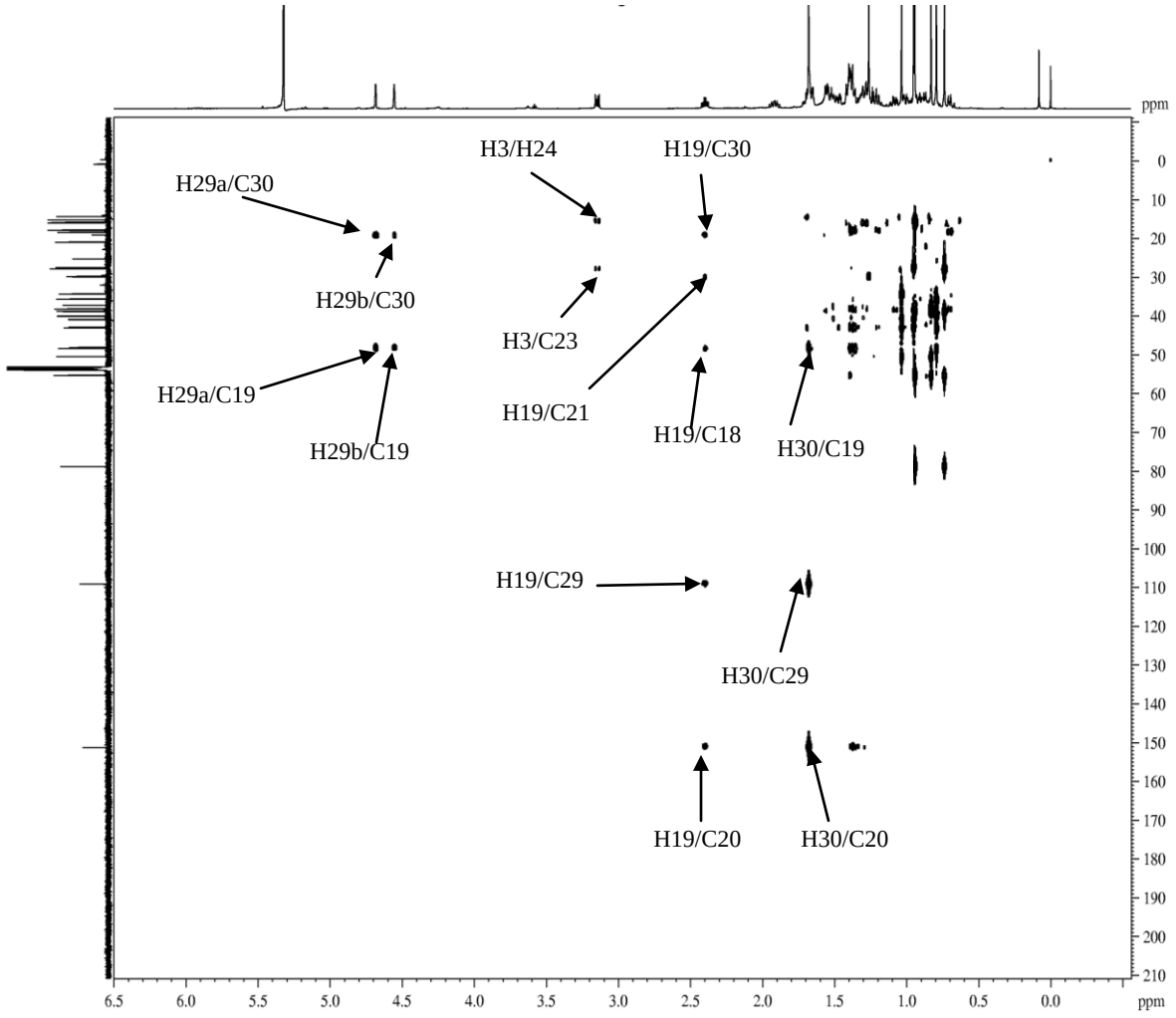


Figure 4.2.1e: ^{1}H-^{13}C HMBC spectrum of compound **1**

Several cross peaks were observed from the COSY spectrum of compound **1** (Fig 4.2.1f) including those between H-29a (4.68 ppm) and H-30 (1.68 ppm), H-3 (3.15 ppm) and H-2 (1.55 ppm), H-19 (2.40 ppm) and H-18 (1.37 ppm) as well as that between H-21a (1.92 ppm) and H-22a (1.37 ppm). The spectral evidence given above, as well as the ^{1}H and ^{13}C NMR chemical shifts (Table 4.2.1) of compound **1** were found to be in close agreement with the ^{1}H and ^{13}C

NMR chemical shifts reported for Lupeol isolated from *Holarrhena floribunda* (Fotie et al., 2006). Hence compound **1** was assigned as Lupeol (Fig 4.2.1g).

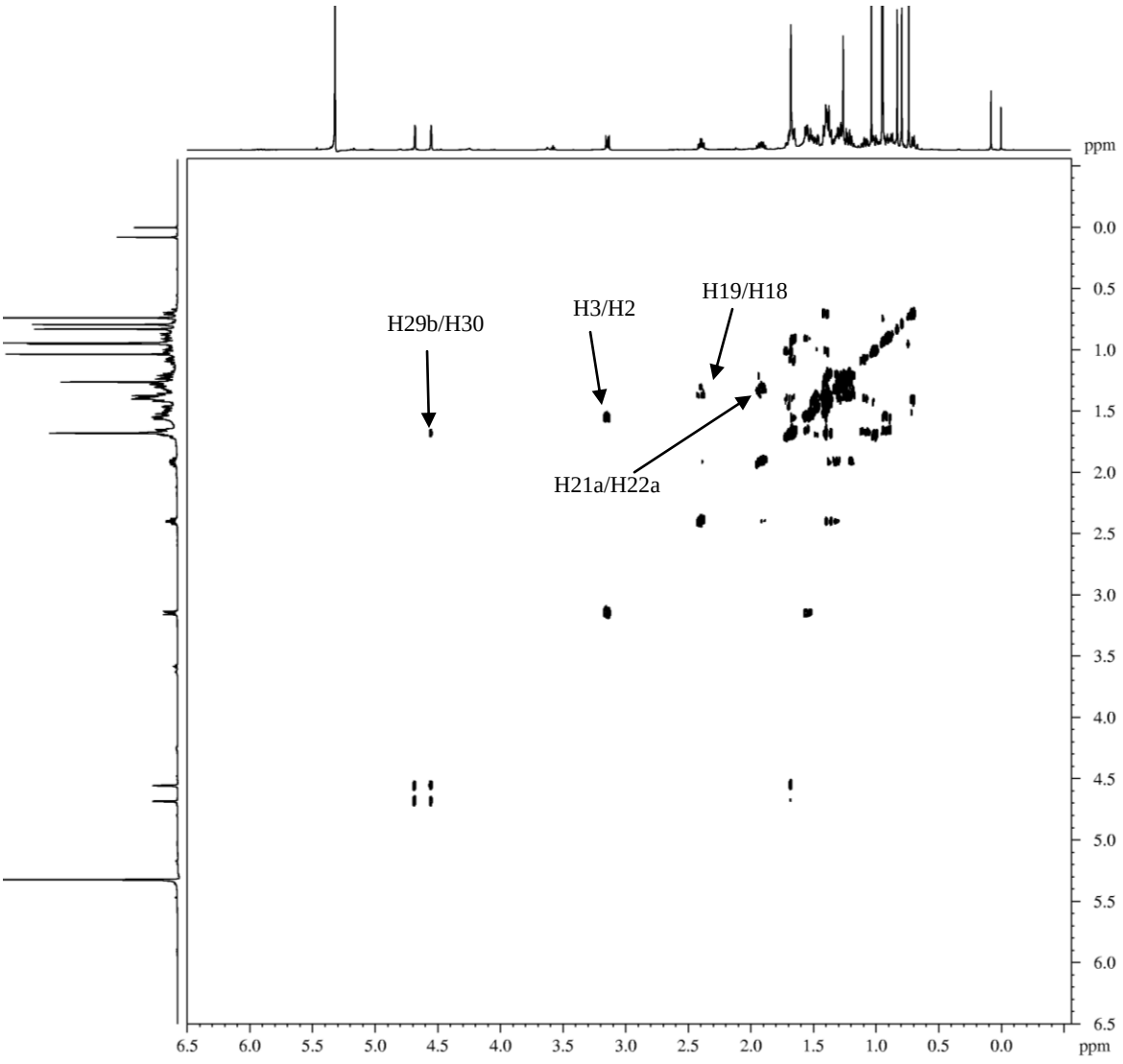


Figure 4.2.1f: ^{1}H-^{1}H COSY spectrum of compound **1**

The spatial arrangement of the olefinic methylene protons (H-29a and H-29b) was assigned basing on their coupling constants. H-29a (4.68 ppm) was observed as a doublet (J = 2.5 Hz) in the ^{1}H spectrum of compound **1**, hence suggesting its coupling with H-30. This supports its position that is trans to C-30 (Fig. 4.2.1g). H-29b (4.55 ppm) was observed as a triple doublet (J

= 2.7 Hz, 2.6 Hz, 1.3 Hz). This suggested its coupling with H-19 (J = 2.7 Hz), H-30 (J = 2.6 Hz) and H-18 (J = 1.3 Hz). H-29b was therefore assigned the position that is cis to C-30 (Fig. 4.2.1g).

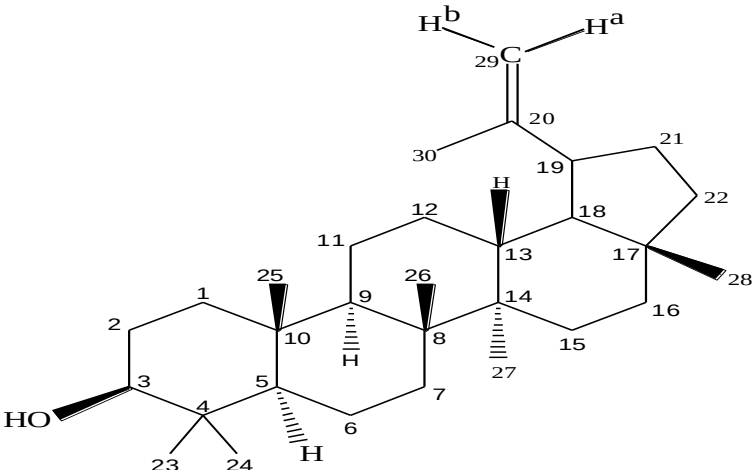


Figure 4.2.1g: The structure of compound **1** (Lupeol).

According to Fotie et al. (2006), Lupeol inhibits the chloroquine sensitive and chloroquine-resistant malaria strains. Another published report by Siddique and Saleem (2011) indicates that Lupeol exhibits a wide spectrum of pharmacological activities against various disease conditions such as inflammation, arthritis, diabetes, cardiovascular ailments, renal disorder, hepatic toxicity, microbial infections and cancer. The same report indicates that Lupeol acts as an effective antibacterial agent when tested against both gram positive and gram negative bacteria such as *Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus, Staphylococcus lutea, Salmonella paratyphi, Salmonella typhi, Shigella boydi, Shigella dysentriae, Vibrio mimicus, E.coli, Klebsiella pneumoniae* and *P.aeruginosa*. The reported pharmacological

activities of Lupeol support the traditional use of *Albizia coriaria* for the treatment of cough, diarrhoea and typhoid. Furthermore, according Siddique and Saleem (2011), Lupeol inhibited the growth of a variety of pathogenic fungal species such as *Sporothrix schenckii*, *Microsporum canis, Aspergillus fumigates* and *Candida albicans*; which also supports the traditional use of *Albizia coriaria* in the treatment of candidiasis and skin diseases.

Table 4.2.1: ^{1}H (600 MHz) and ^{13}C (600 MHz) NMR spectral data together with the HMBC correlations of compound **1** in CD_2Cl_2.

#	**^{1}H** (ppm, Hz)	**^{13}C** (ppm)	**HMBC**
1a 1b	1.65 (m) 0.91 (m)	38.7 CH_2	
2	1.55(m)	27.7 CH_2	
3	3.15 (dd, 10.6, 5.91)	78.7 CH	C-24, C-23
4		38.8 C	
5	0.70 (m)	55.2 CH	
6a 6b	1.51 (m) 1.40 (m)	18.3 CH_2	
7	1.34 (m)	34.2 CH_2	
8		40.8 C	
9	1.26 (dd, 12.9, 2.7)	50.4 CH	
10		37.1 C	
11a	1.40 (m)	20.9 CH_2	

11b	1.20(m)		
12a 12b	1.67 (m) 1.09 (dd, 13.3, 4.8)	25.2 CH_2	
13	1.67 (m)	38.7 CH	
14		43.0 C	
15a 15b	1.69 (m) 1.01 (ddd, 13.8, 4.2, 2.5)	27.7 CH_2	
16	1.47 (ddd, 12.9, 4.5, 2.7)	35.5 CH_2	C-15, C-17
17		43.2 C	
18	1.37 (m)	48.4 CH	
19	2.40 (ddd, 11.1, 11.1, 5.8)	48.3 CH	C-30, C-21, C-18, C-29, C-20
20		151.2 C	
21a 21b	1.92 (dddd, 13.5, 10.5, 10.5, 8.6) 1.31 (m)	29.8 CH_2	
22a 22b	1.37 (m) 1.20 (m)	40.2 CH_2	
23	0.94	28.0 CH_3	
24	0.74	15.4 CH_3	C-5
25	0.83	16.1 CH_3	C-1, C-5, C-9, C-10
26	1.03	16.0 CH_3	C-7, C-9, C-14
27	0.95	14.5 CH_3	C-8, C-13, C-14, C-15
28	0.79	18.0 CH_3	C-16

29a 29b	4.68 (d, 2.5) 4.55 (ddd, 2.7,2.6, 1.3)	109.3 CH_2	C-30, C-19 C-30, C-19
30	1.68 (m)	19.2 CH_3	C-19, C-29, C-20

4.2.2. Compound 2 (Lupenone)

Compound **2** was obtained as brown oil, soluble in methylene chloride, having been eluted with methylene chloride: hexane 1:1 from the silica column. The mass spectrum of compound **2** exhibited a molecular ion peak $[M]^+$ at *m/z* 424, corresponding to a molecular formula $C_{30}H_{48}O$. Fragments at *m/z* 203 and 218, characteristic of pentacyclic triterpenes (Pereira et al., 1996) were also present in the mass spectrum of compound **2** (Fig 4.2.2a). The mass spectrometry data also suggested the presence of a carbonyl group (fragment at *m/z* 205) and the accurate mass of this fragment indicated that it was attached to rings A or B of the pentacyclic ring (Pereira et al., 1996). The fragment at 409 corresponding to $[M\text{-}CH_3]^+$ was also observed.

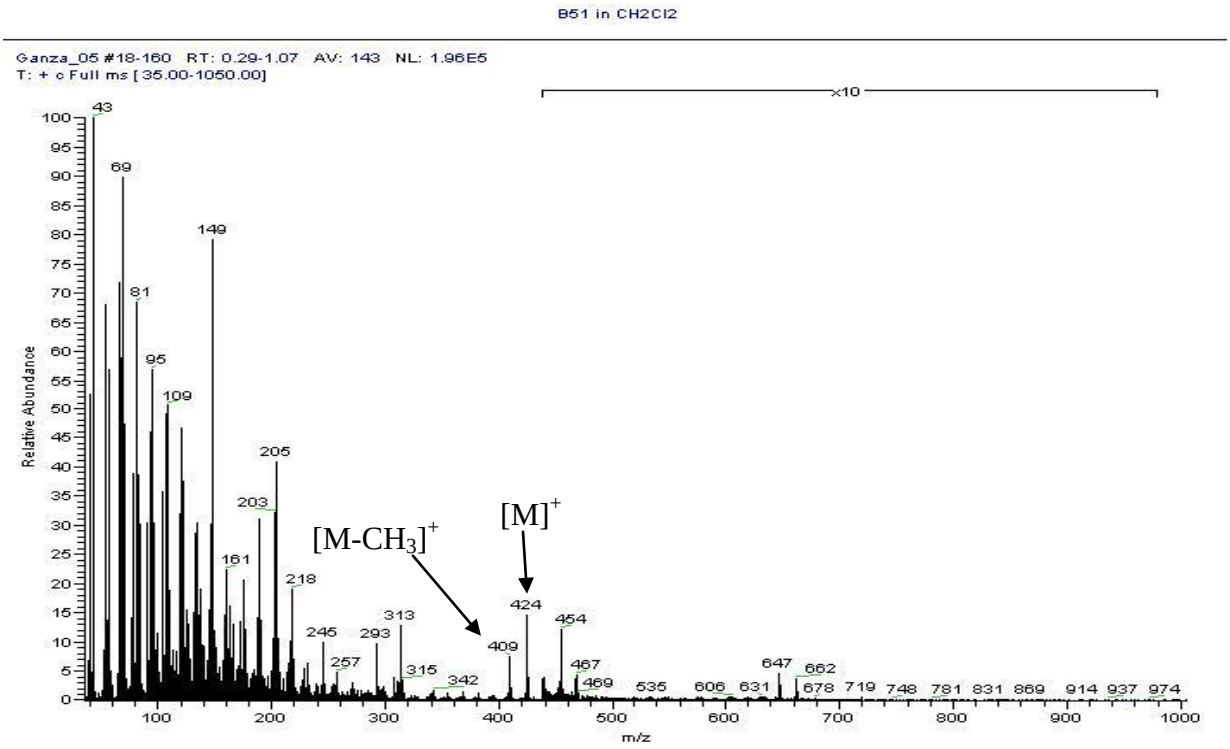


Figure 4.2.2a: Mass spectrum of compound **2**

The ^{1}H NMR data of compound **2** (Fig 4.2.2b) were found to be closely similar to the ^{1}H NMR data of compound **1**. Just like in compound **1**, the ^{1}H NMR spectrum of compound **2** showed signals for six tertiary methyl groups, which were observed as singlets at 0.79 ppm, 0.97 ppm, 1.04 ppm, 0.84 ppm, 0.74 ppm and 0.92 ppm. The same spectrum also showed resonances for olefinic methylene protons at 4.74 ppm and 4.60 ppm and a vinyl methyl singlet at 1.68 ppm which was shown to be coupled to one of two vinylic protons (H-29a, 4.74 ppm), thus indicating the presence of an isopropenyl group as well as a lupane skeleton. The ^{1}H NMR spectrum of compound **2** however did not show the carbinyl proton signal at 3.15 ppm as was the case for compound **1**.

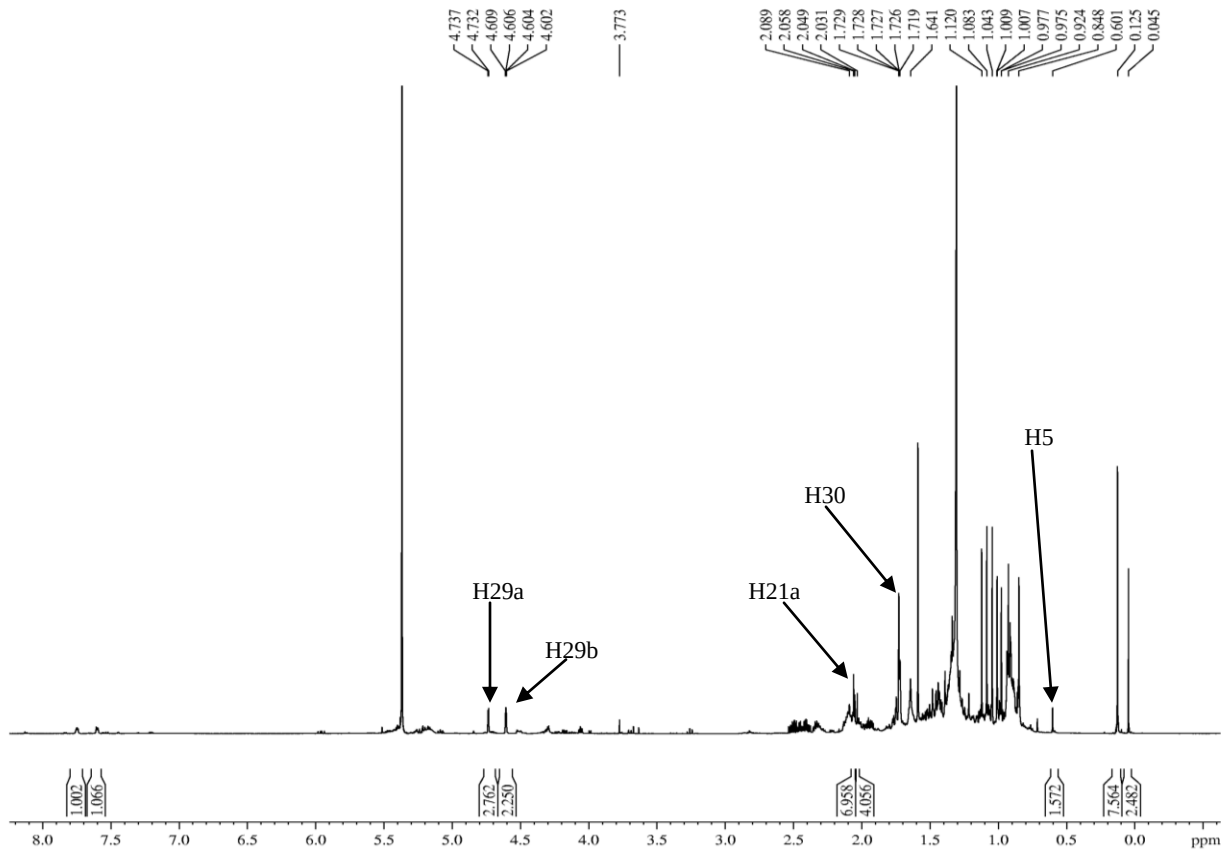


Figure 4.2.2b: ^{1}H NMR spectrum of compound **2**

The ^{13}C NMR data of compound **2** were in agreement with the MS data, as it revealed the presence of 30 carbon atoms. Similar to compound **1**, the ^{13}C NMR data of compound **2** was also in agreement with the existence of an isopropenyl group, evidenced by the characteristic vinylic carbon atom resonances at 151.6 ppm and 109.6 ppm, corresponding to carbon atoms 20 and 29 respectively. The existence of an isopropenyl group was also supported by the olefinic methylene protons seen as singlets at 4.74 ppm and 4.60 ppm in the ^{1}H NMR spectrum of compound **2**. The carbon resonance at 218.0 ppm, in the ^{13}C NMR spectrum (Fig 4.2.2c) was assigned to a carbonyl carbon and a resonance at 1.55 ppm, in the ^{1}H NMR spectrum, to two alpha protons of

the carbonyl group. The existence of the carbonyl group was further supported by the fragment observed at m/z 205 in the mass spectrum of compound **2**. The assignment of this carbonyl group to position 3 in ring A was possible from the results of a COSY experiment clearly showing the coupling between the two hydrogen atoms at position 2 and those at position 1.

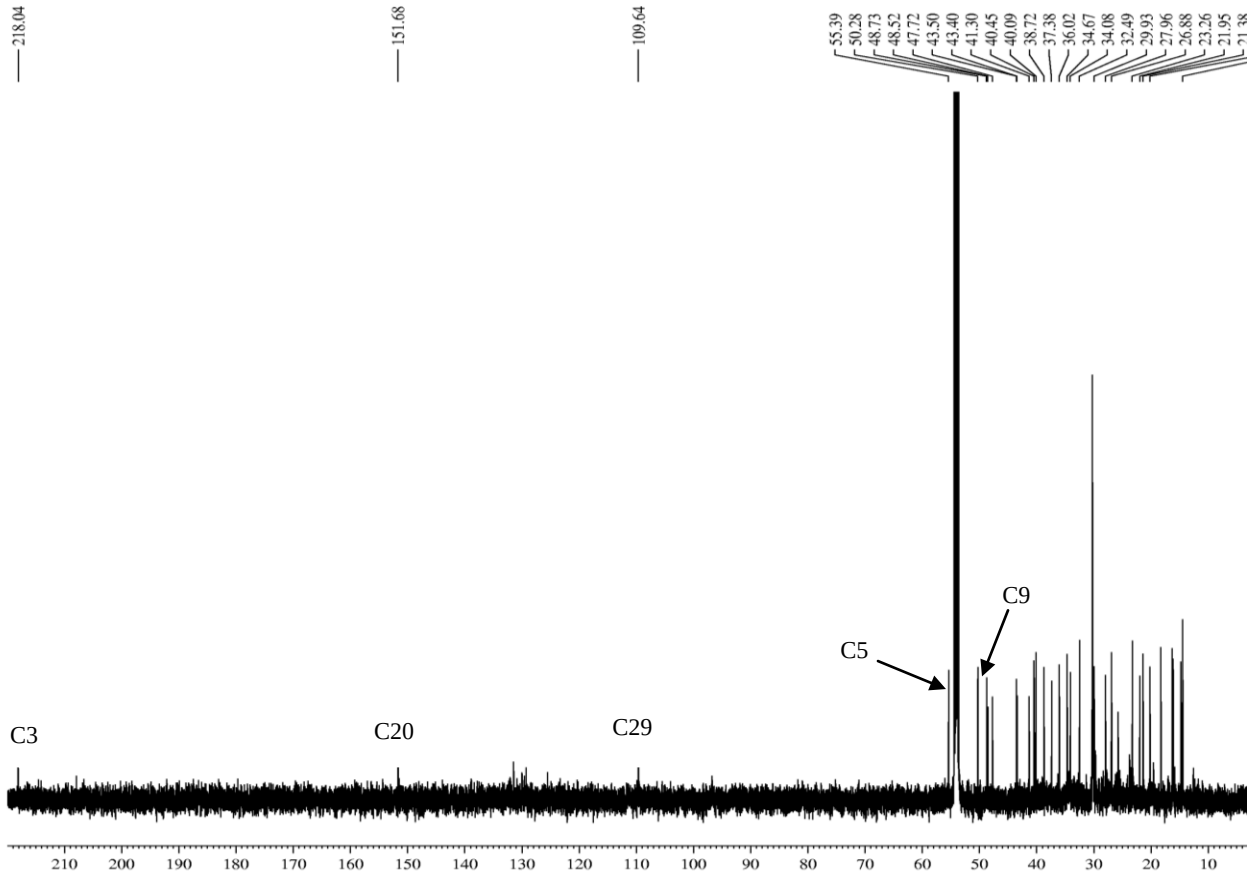


Figure 4.2.2c: ^{13}C NMR spectrum of compound **2**

From the HSQC spectrum of compound **2** (Fig 4.2.2d), ^{1}H resonances were assigned to their corresponding ^{13}C resonances (Table 4.2.2). The HSQC spectrum of compound **2** was found to be very similar to that of compound **1**. It showed a correlation between the olefinic methylene protons: H-29a (4.74 ppm), H-29b (4.60 ppm) and the carbon atom C-29 (109.6 ppm) as was the

case in compound **1**. However, instead of six quaternary carbons in compound **1**, the HSQC spectrum of compound **2** showed seven quaternary carbon atoms.

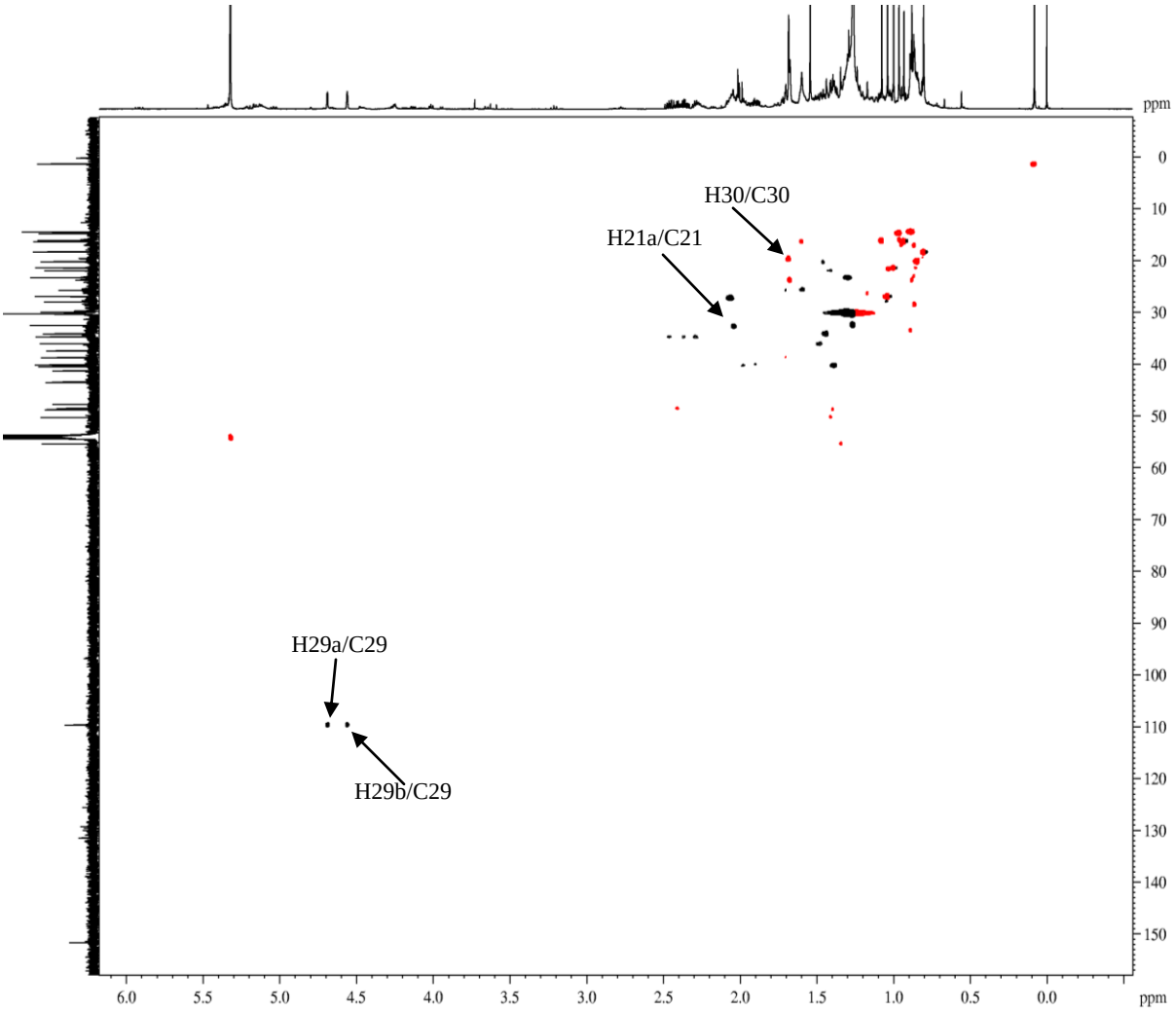


Figure 4.2.2d: ^{1}H-^{13}C HSQC spectrum of compound **2**

The HMBC spectrum of compound **2** (Fig 4.2.2e) showed a correlation between H-29a (4.74 ppm) and the carbon atoms C-30 (21.9 ppm) and C-19 (at 48.5 ppm) as well as between H-19 (2.40 ppm) and the carbon atoms C-30 (21.9 ppm), C-29 (109.6 ppm) and C-20 (151.6 ppm).

Several other correlations in the HMBC spectrum of compound **2** made the assignment of the quaternary carbon atoms possible (Table 4.2.2).

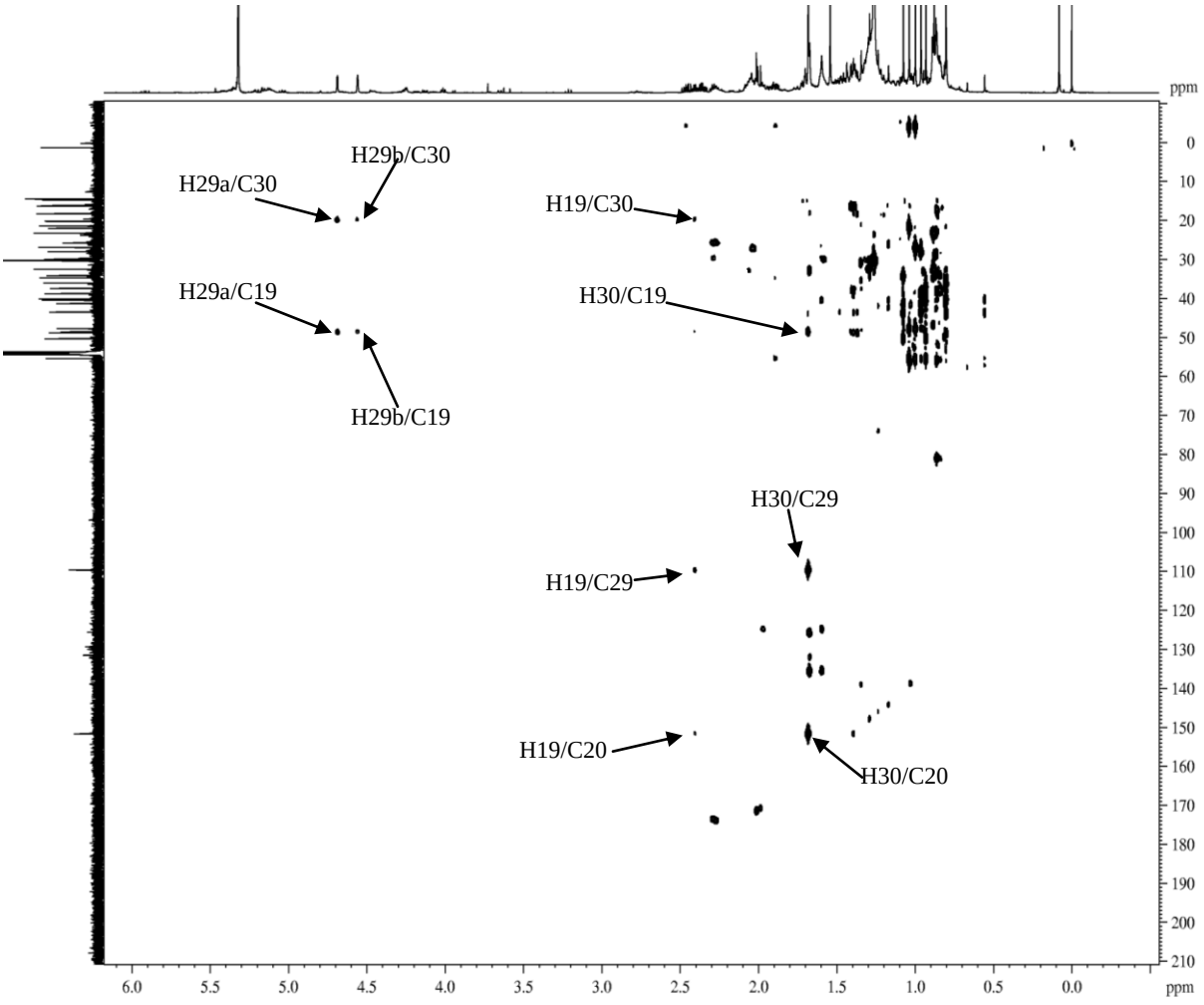


Figure 4.2.2e: ^{1}H-^{13}C HMBC spectrum of compound **2**

From the COSY spectrum of compound **2** (Fig 4.2.2f), some of the cross peaks that were observed include that between H-29b (4.60 ppm) and H-30 (1.68 ppm), H-18 (1.35 ppm) and H-

19 (2.40 ppm), H-21a (2.03 ppm) and H-22a (1.37 ppm) as well as that between H-7 (1.34 ppm) and H-26 (1.04 ppm), among others.

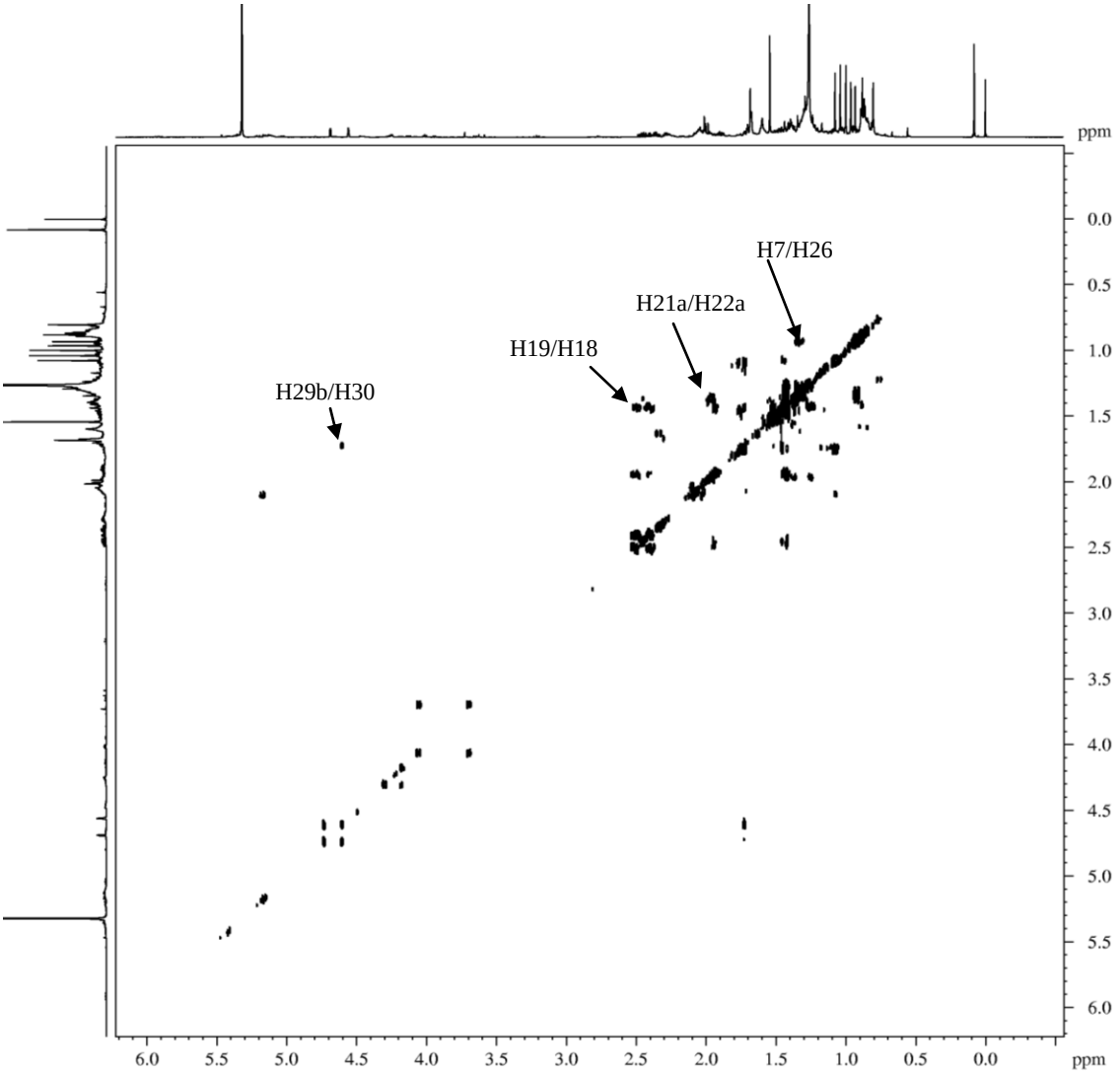


Figure 4.2.2f: ^{1}H-^{1}H COSY spectrum of compound **2**

The ^{1}H and ^{13}C NMR chemical shifts (Table 4.2.2) of compound **2** were found to be in close agreement with the ^{1}H and ^{13}C NMR chemical shifts and structure reported for Lupenone isolated from *Glochidion sphaerogynum* (Puapairoj et al., 2005). Hence compound **2** was assigned as Lupenone (Fig 4.2.2g).

Similar to compound **1**, the spatial arrangement of the olefinic methylene protons (H-29a and H-29b) in compound **2** was assigned basing on their coupling constants. H-29a (4.74 ppm) was observed as a doublet (J = 2.5 Hz) in the ^{1}H spectrum of compound **2**, hence suggesting its coupling with H-30. Therefore, it was assigned the trans position to C-30. H-29b (4.60 ppm) was observed as a triple doublet (J = 2.7 Hz, 2.6 Hz, 1.3 Hz), suggesting its coupling with H-19 (J = 2.7 Hz), H-30 (J = 2.6 Hz) and H-18 (J = 1.3 Hz). Therefore, H-29b was assigned the position that is cis to C-30 (Fig. 4.2.2g).

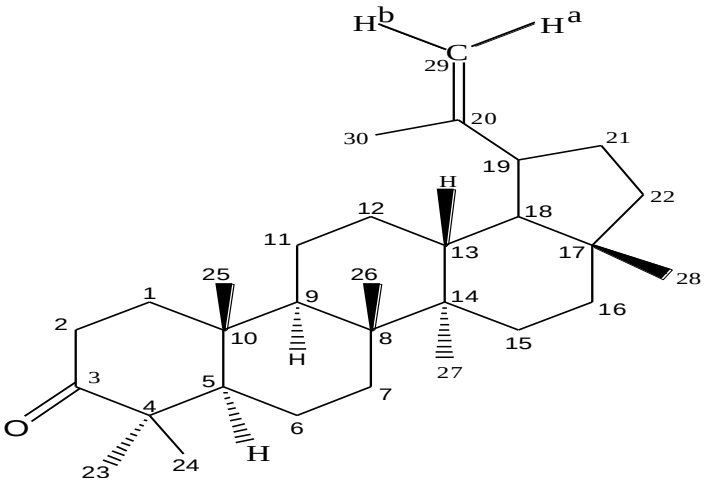


Figure 4.2.2g: The structure of compound **2** (Lupenone)

Lupenone is reported to have antimicrobial potential as it exhibited antifungal activity against *C.albicans*, *Trichophyton mentagrophytes* and *Aspergillus niger* (Po-wei et al., 2012). It is also reported to have exhibited low antibacterial activity against *staphylococcus aureus*, *E.coli*, *P.aeruginosa* and partial inhibition of *bacillus subtilis* (Po-wei et al., 2012).

Table 4.2.2: ^{1}H (600 MHz) and ^{13}C (600 MHz) NMR spectral data together with the HMBC correlations of compound **2** in CD_2Cl_2.

#	**^{1}H** (ppm, Hz)	**^{13}C** (ppm)	**HMBC**
1a 1b	1.6 (m) 0.92 (m)	38.7 CH_2	C-2 C-2
2	1.55 (m)	27.9 CH_2	C-1
3		218.0 C	
4		40.0 C	
5	0.60 (m)	55.4 CH	
6a 6b	1.51(m) 1.40 (m)	20.18 CH_2	
7	1.34 (m)	34.9 CH_2	
8		41.3 C	
9	1.22 (dd, 12.9, 2.7)	50.2 CH	
10		37.4 C	
11a 11b	1.40 (m) 1.12 (m)	21.4 CH_2	
12a 12b	1.64 (m) 1.08 (dd, 13.3, 4.8)	26.9 CH_2	
13	1.64 (m)	37.4 CH	
14		43.4 C	
15a	1.71(m)	29.9 CH_2	

15b	1.01(ddd, 13.8, 4.2, 2.5)		
16	1.47(ddd, 12.9, 4.5, 2.7)	36.0 CH_2	C-15, C-17
17		43.5 C	
18	1.35 (m)	48.7 CH	
19	2.40 (ddd, 11.1, 11.1, 5.8)	48.5 CH	
20		151.6 C	
21a 21b	2.03 (dddd, 13.5, 10.5, 10.5, 8.6) 1.31 (m)	32.5 CH_2	
22a 22b	1.37 (m) 1.20 (m)	40.4 CH_2	
23	0.92	23.2 CH_3	
24	0.74	20.1 CH_3	C-5
25	0.84	14.4 CH_3	C-1, C-5, C-9
26	1.04	16.1 CH_3	C-7, C-9, C-14
27	0.97	14.8 CH_3	C-8, C-13, C-14, C-15
28	0.79	18.0 CH_3	C-16
29a 29b	4.74 (d, 2.5) 4.60 (ddd, 2.7,2.6, 1.3)	109.6 CH_2	
30	1.68 (m)	21.9 CH_3	

4.2.3. Compound 3 (Betulinic acid)

Compound **3** was obtained as a white amorphous powder, soluble in methanol, having been eluted with methylene chloride: methanol 9:1 from the silica gel column. The NMR spectral data of compound **3** were very similar to those of compound **1**. The ^{1}H NMR spectrum of compound **3** (Fig 4.2.3a), just like that of compound **1**, showed resonances for olefinic methylene protons at 4.70 ppm and 4.59 ppm, a carbinyl proton at 3.13 ppm, a vinyl methyl singlet at 1.69 ppm, which was found to be coupled to one of the two methylene protons (H-29, 4.59 ppm), thus indicating the presence of an isopropenyl group as well as a lupane skeleton. However, in addition to the vinyl methyl singlet at 1.69 ppm, the ^{1}H NMR spectrum of compound **3** showed five methyl singlets at 0.94 ppm, 0.75 ppm, 0.89 ppm, 0.97 ppm and 0.96 ppm, and not six methyl singlets as was the case for compound **1**. The spectrum also showed a methine signal at 2.40 ppm (ddd, *J*=11.1, 11.1, 5.8 Hz) which was attributed to the proton at C-19 position, as was the case in compound **1**.

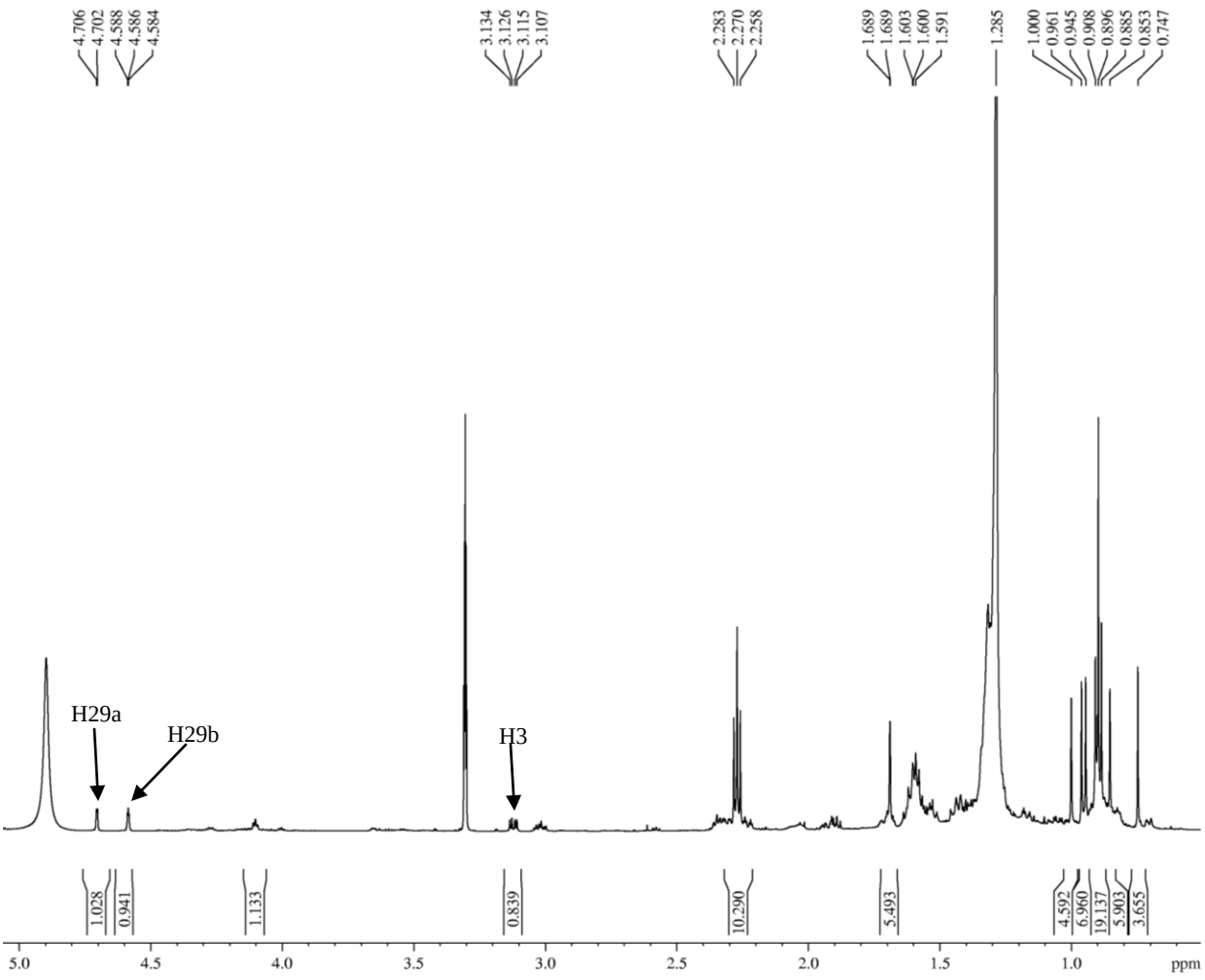


Figure 4.2.3a: ^{1}H NMR spectrum of compound **3**

The ^{13}C NMR spectrum (Fig 4.2.3b) of compound **3** revealed 30 carbon signals, which were shown by the HSQC experiment to be six methyl, ten methylene, six methine, five quaternary carbons, one carboxylic acid, and two olefinic carbons. This showed that one of the methyl groups in compound **1** had been replaced by a carboxylic acid in compound **3**. Similar to compound **1**, the ^{13}C NMR data of compound **3** was also in complete agreement with the existence of an isopropenyl group due to the characteristic vinylic carbon atom resonances at

150.5 ppm and 108.8 ppm, corresponding to carbon atoms 20 and 29 respectively. This supported the olefinic methylene protons seen as singlets at 4.70 ppm and 4.59 ppm in the ^{1}H NMR spectrum of compound **3**. The signal at 78.2 ppm was characteristic for the oxymethine carbon at position 3. The down field part of the ^{13}C NMR spectrum of compound **3** showed a signal at 176.3 ppm, which was attributed to the carboxylic carbon atom.

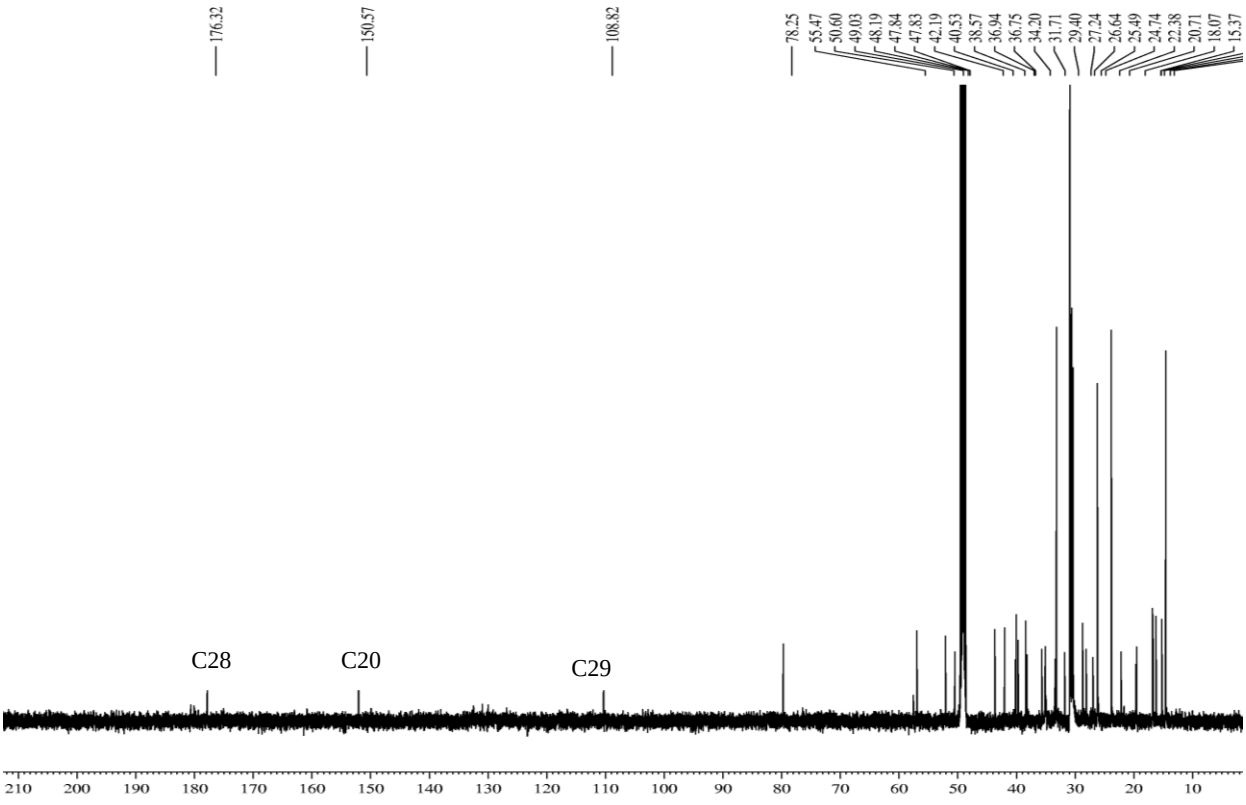


Figure 4.2.3b: ^{13}C NMR spectrum of compound **3**

From the HSQC spectrum of compound **3** (Fig 4.2.3c), ^{1}H resonances were assigned to their corresponding ^{13}C resonances (Table 4.2.3). Similar to compounds **1** and **2**, the HSQC spectrum

of compound **3** showed a correlation between the olefinic methylene protons: H-29a (4.70 ppm), H-29b (4.59 ppm) and the carbon atom C-29 (108.8 ppm). The same spectrum showed seven quaternary carbon atoms as was the case with compound **2**, but a difference in the number of methyl groups was observed. The HSQC spectrum of compound **3** showed six methyl groups and not seven as was the case for compound **2**.

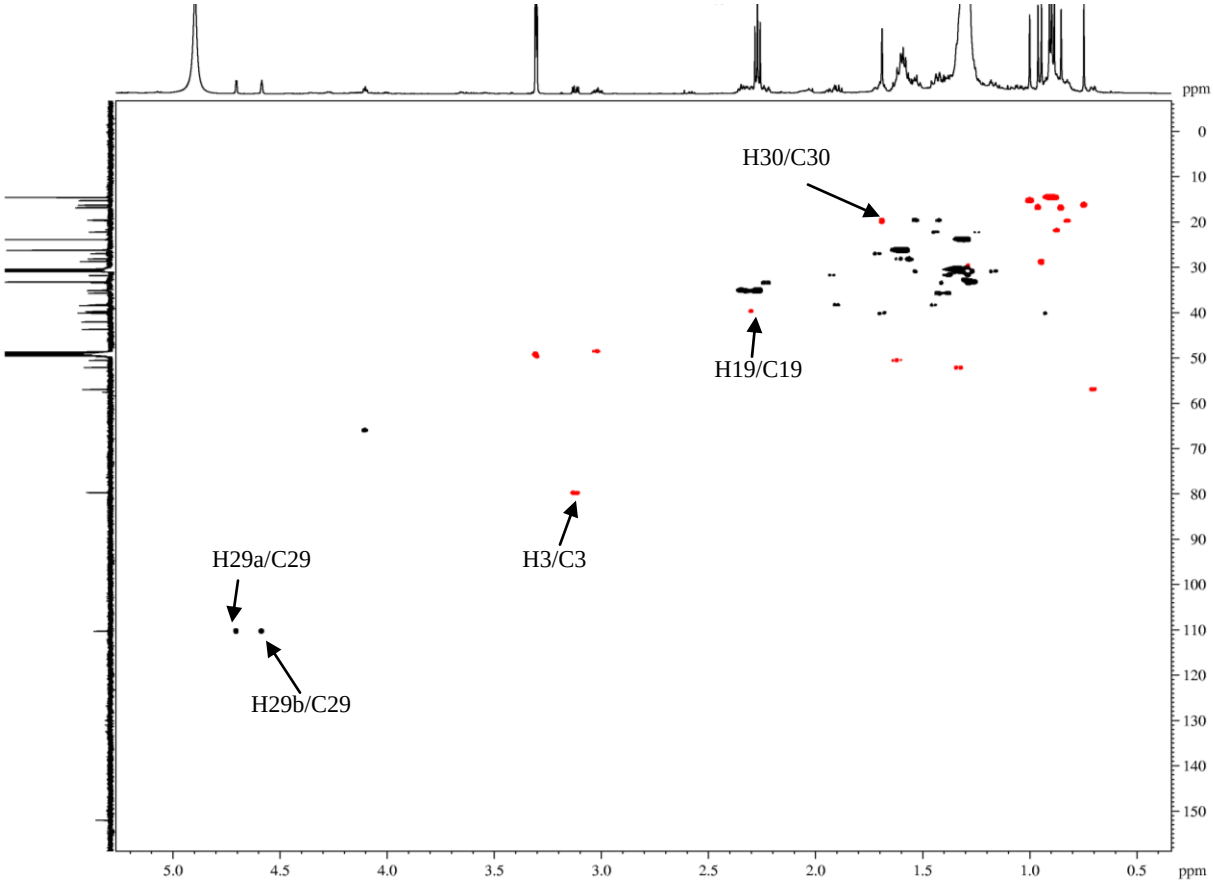


Figure 4.2.3c: ^{1}H-^{13}C HSQC spectrum of compound **3**

The HMBC spectrum of compound **3** (Fig 4.2.3d) showed correlations between H-29a (4.70 ppm) and the carbon atoms C-30 (19.5 ppm) and C-19 (49.0 ppm). Correlations between H-30

(1.69 ppm) and the carbon atoms C-19 (49.0 ppm), C-29 (108.8 ppm) and C-20 (150.5 ppm) were also observed. Many other correlations in the HMBC spectrum of compound **3** made the assignment of the quaternary carbon atoms possible (Table 4.2.3).

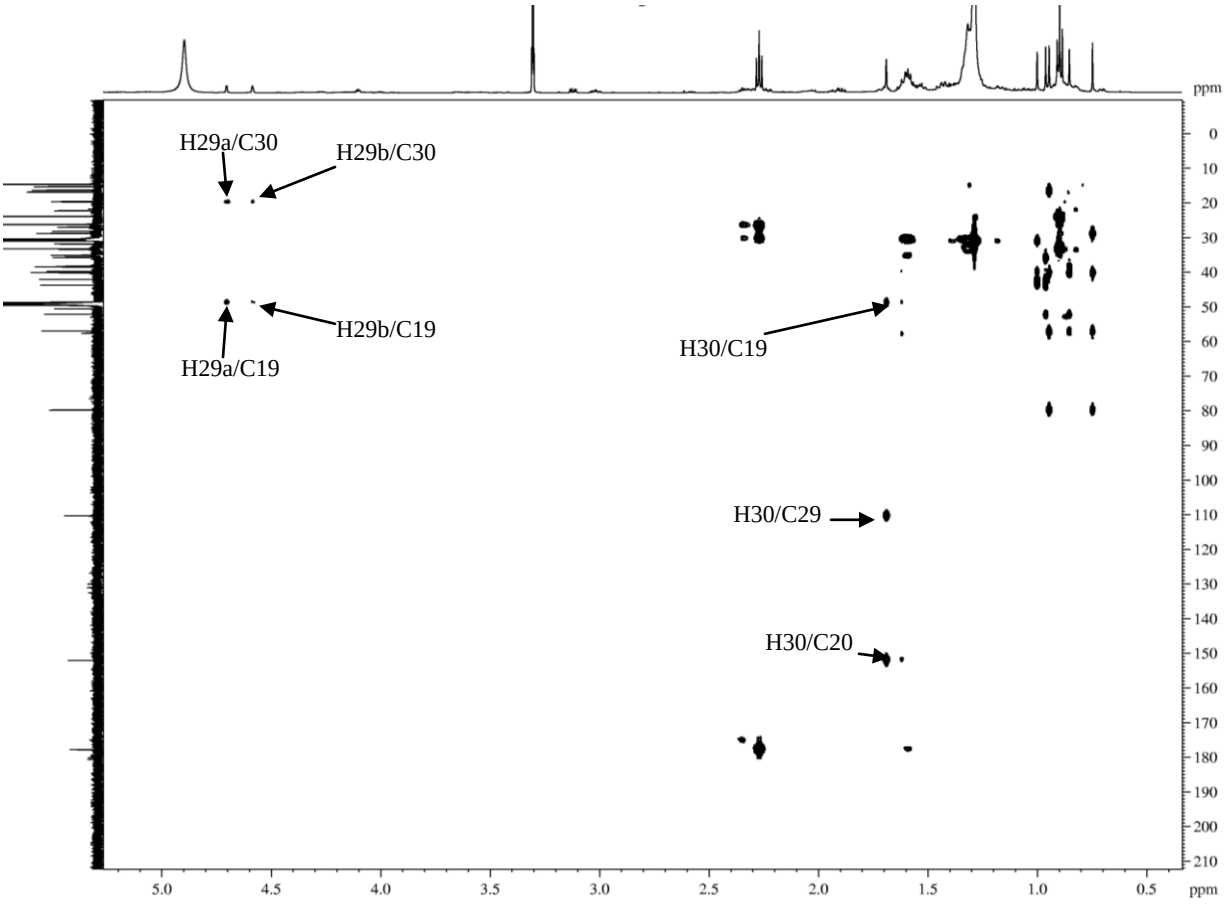


Figure 4.2.3d: ^{1}H-^{13}C HMBC spectrum of compound **3**

From the COSY spectrum of compound **3** (Fig 4.2.3e), a number of cross peaks were observed including that between H-29a (4.70 ppm) and H-30 (1.69 ppm), H-2a (1.60 ppm) and H-3 (3.13 ppm), H-18 (1.34 ppm) and H-19 (2.40 ppm), H-7 (1.42 ppm) and H-26 (0.96 ppm) as well as H-21a (2.03 ppm) and H-22 (1.70 ppm) among others.

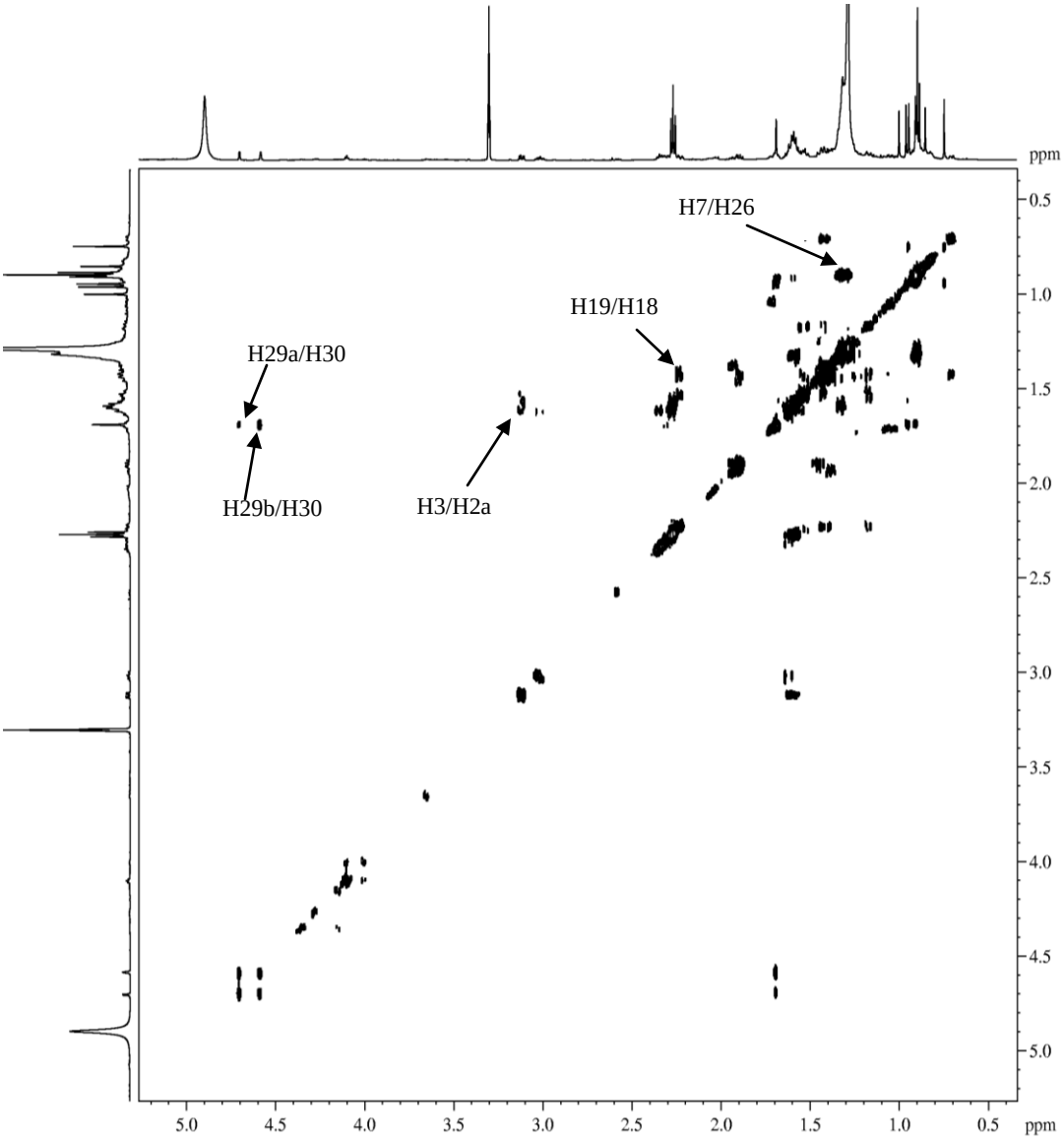


Figure 4.2.3e: ^{1}H-^{1}H COSY spectrum of compound **3**

The ^{1}H and ^{13}C NMR chemical shifts (Table 4.2.3) of compound **3** were found to be in close agreement with the ^{1}H and ^{13}C NMR chemical shifts and structure reported for Betulinic acid (Sholichin et al., 1980). Compound **3** was therefore assigned as Betulinic acid (Fig 4.2.3f).

Similar to compounds **1** and **2**, the spatial arrangement of the olefinic methylene protons (H-29a and H-29b) in compound **3** was assigned basing on their coupling constants. H-29a (4.70 ppm) was observed as a doublet (J = 2.5 Hz) in the ^{1}H spectrum of compound **3**, hence suggesting its coupling with H-30. It was therefore assigned the position that is trans to C-30 (Fig. 4.2.3f). H-29b (4.59 ppm) was observed as a triple doublet (J = 2.7 Hz, 2.6 Hz, 1.3 Hz), suggesting its coupling with H-19 (J = 2.7 Hz), H-30 (J = 2.6 Hz) and H-18 (J = 1.3 Hz). Therefore, H-29b was assigned the position that is cis to C-30 (Fig. 4.2.3f).

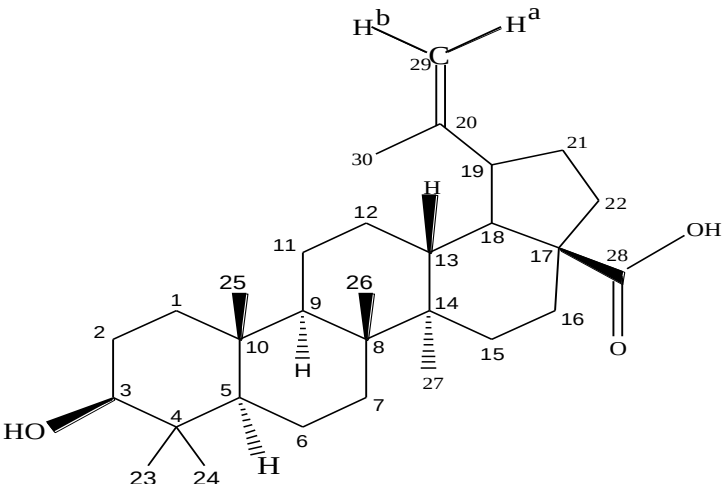


Figure 4.2.3f: The structure of compound **3** (Betulinic acid).

Betulinic acid is reported to have antiviral, antiplasmodial, antibacterial (against *E.coli* and *staphylococcus aureus*), anthelmintic as well as antidepressant effects (Carmona et al., 2010; Hess et al., 1995; Machado et al., 2013; Shai et al., 2008). This supports the use of *Albizia*

coriaria for the treatment of diarrhoea, skin infections, candida albicans and respiratory diseases in African folk medicine.

Table 4.2.3: ^{1}H (600 MHz) and ^{13}C (600 MHz) NMR spectral data together with the HMBC correlations of compound **3** in MeOH.

#	**^{1}H** (ppm, Hz)	**^{13}C** (ppm)	**HMBC**
1a 1b	1.67 (m) 0.92 (m)	38.5 CH_2	
2a 2b	1.60 (m) 1.56 (m)	27.2 CH_2	
3	3.13 (dd, 11.4, 4.7)	78.2 CH	
4		40.5 C	
5	0.70(m)	55.5 CH	
6a 6b	1.53 (m) 1.41 (m)	18.0 CH_2	
7	1.42 (m)	34.2 CH_2	
8		42.2 C	
9	1.62 (m)	50.6 CH	
10		36.9 C	
11	1.44 (dd, 8.9, 2.8)	20.7 CH_2	
12a 12b	1.67 (m) 1.09 (dd, 13.3, 4.8)	25.5 CH_2	

13	1.67 (m)	38.5 CH	
14		42.1 C	
15a 15b	1.97 (dd, 14.1, 5.0) 1.90 (dd, 14.0, 12.2)	31.7 CH_2	
16	2.27 (ddd, 12.9, 4.5, 2.7)	34.2 CH_2	C-15, C-17
17		50.6 C	
18	1.34 (dd, 11.9, 6.4)	47.8 CH	
19	2.40 (ddd, 11.1, 11.1, 5.8)	49.0 CH	
20		150.5 C	
21a 21b	2.03 (dddd, 13.5, 10.5, 10.5, 8.6) 1.31 (m)	29.4 CH_2	
22	1.70 (m)	36.7 CH_2	
23	0.94	28.2 CH_3	
24	0.89	15.4 CH_3	
25	0.75	16.1 CH_3	C-1, C-5, C-9,
26	0.96	16.7 CH_3	
27	0.97	14.8 CH_3	C-8, C-13, C-14, C-15
28		176.3 C	
29a 29b	4.70 (d, 2.5) 4.59 (ddd, 2.7,2.6, 1.3)	108.8 CH_2	C-19, C-29 C-19, C-29
30	1.69	19.5 CH_3	C-19, C-20, C-30

4.2.4. Compound 4 (Acacic acid lactone)

Compound **4** was obtained as a white amorphous powder, soluble in methanol. It was eluted with methylene chloride: methanol 98:2 from the silica column. The ^{1}H NMR spectrum of compound **4** showed signals assignable to seven methyl singlets at 0.99 ppm, 1.01 ppm, 1.25 ppm, 0.93 ppm, 0.95 ppm, 0.79 ppm and 0.98 ppm, three oxygenated methine groups at 3.15 ppm, 3.90 ppm and 4.26 ppm, an olefinic group at 5.42 ppm as well as a carbonyl group at 183.2 ppm. The same spectrum also showed signals corresponding to eight methylene groups (from 1.00 ppm to 2.33 ppm) and two methine groups (0.78 ppm and 1.52 ppm). The low field signals at 7.60 ppm and 7.75 ppm suggested their attachment to a highly electronegative atom. This supported the presence of two hydroxyl groups.

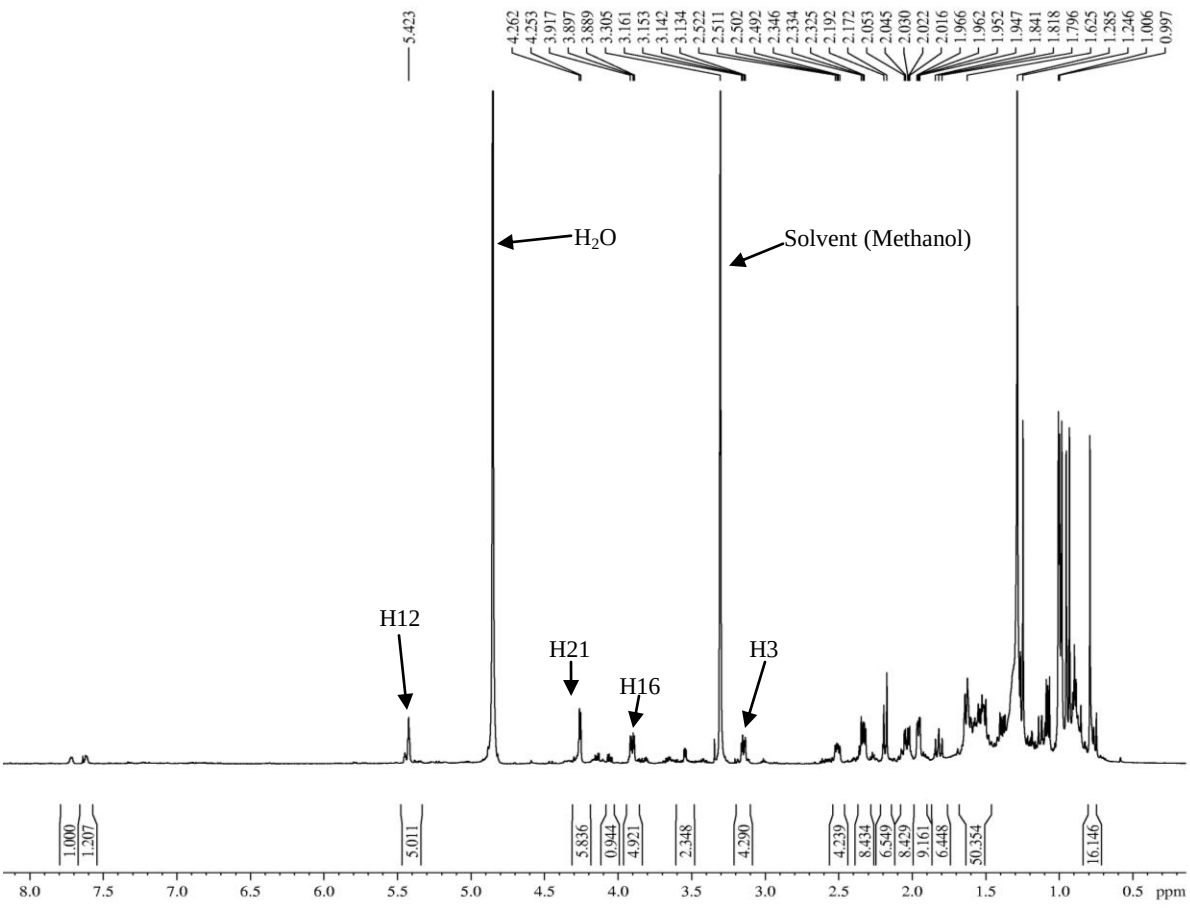


Figure 4.2.4a: ^{1}H NMR spectrum of compound **4**

The ^{13}C NMR spectrum (Fig 4.2.4b) of compound **4** revealed 30 carbon signals, which were shown by the HSQC experiment to be seven methyl, eight methylene, six methine, six quaternary carbons, one carbonyl and two olefinic carbons. Similar to compound **3**, the ^{13}C NMR data of compound **4** was also in complete agreement with the existence of a carbonyl group due to the characteristic carbon atom resonance at 183.2 ppm. The signal at 79.7 ppm was characteristic for the oxymethine carbon at position 3. This supported the proton seen as a double doublet at 3.15 ppm. The two olefinic carbon resonances were observed at 126.1 ppm and 140.8 ppm.

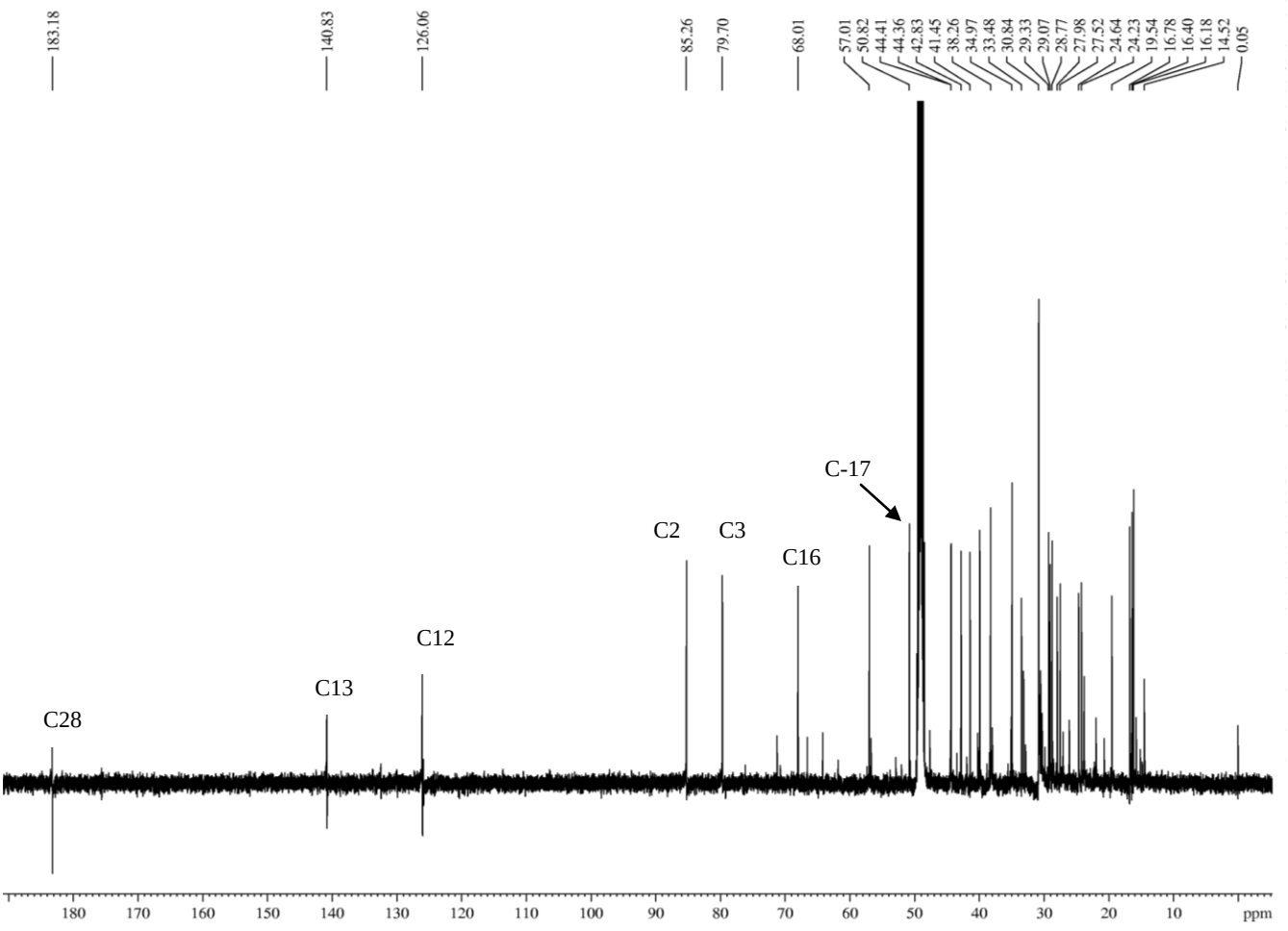


Figure 4.2.4b: ^{13}C NMR spectrum of compound **4**

Correlation of the carbon resonances with the resonances of their directly attached protons (Table 4.2.4) was made possible by the HSQC experiment (Fig 4.2.4c). The proton at 5.42 ppm was found to be attached to one of the olefinic carbons, C-12 (126.1 ppm) while the proton at 3.15 ppm was found to be attached to one of the three oxygenated methine groups, C-3 (79.7 ppm).

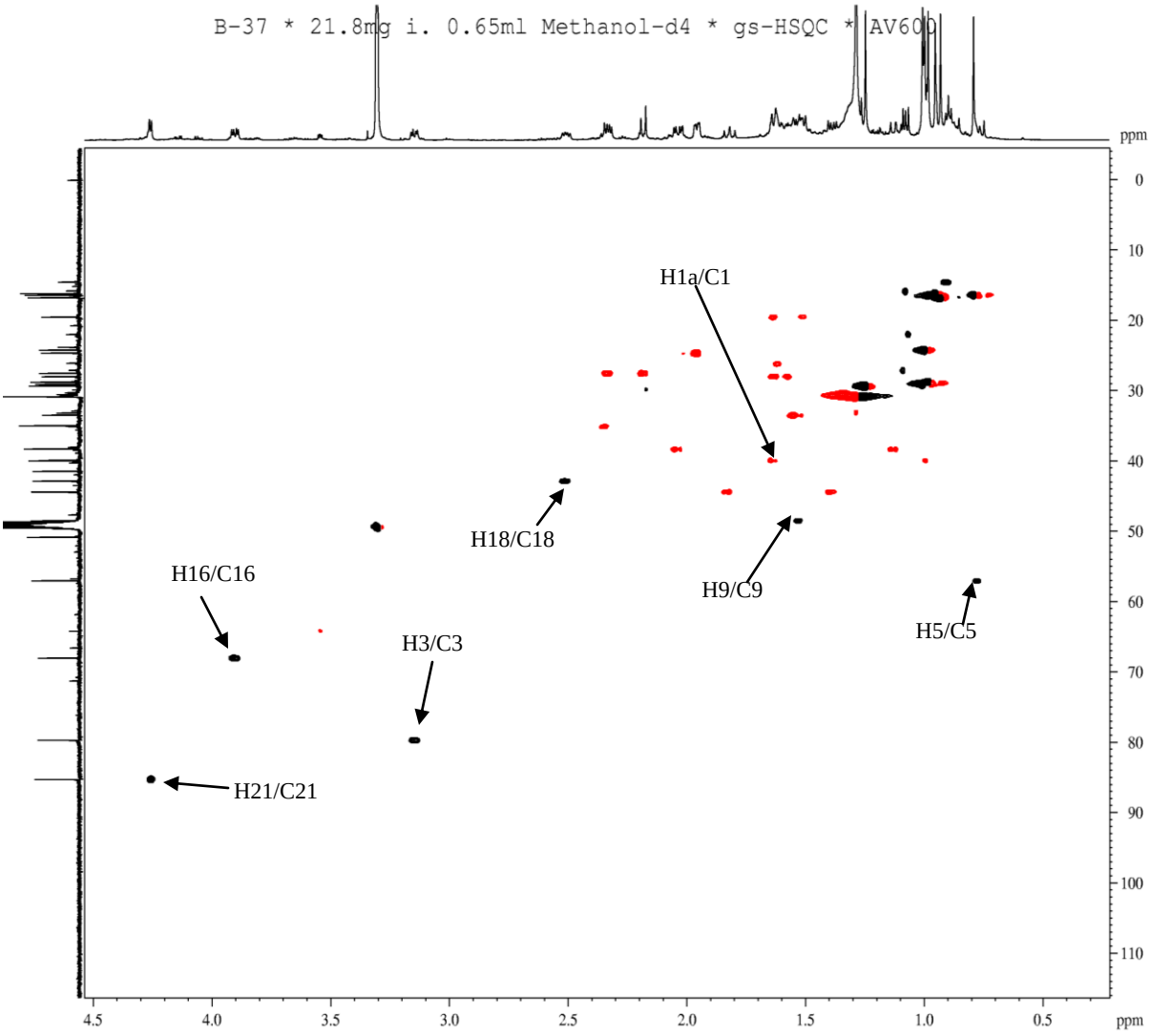


Figure 4.2.4c: ^{1}H – ^{13}C HSQC spectrum of compound **4**

From the HMBC spectrum of compound **4** (Fig 4.2.4d), assignment of all carbon atoms was made possible (Table 4.2.4). The carbonyl carbon, C-28 (183.2 ppm) was assigned basing on its HMBC correlation with C-16 (68.0 ppm), C-18 (42.8 ppm), C-21 (85.3 ppm) and C-22 (27.5 ppm) while the olefinic carbon, C-13 (140.8 ppm) was assigned basing on its HMBC correlation with C-11 (24.7 ppm), C-18 (42.8 ppm), C-19 (44.4 ppm) and C-27 (29.3 ppm).

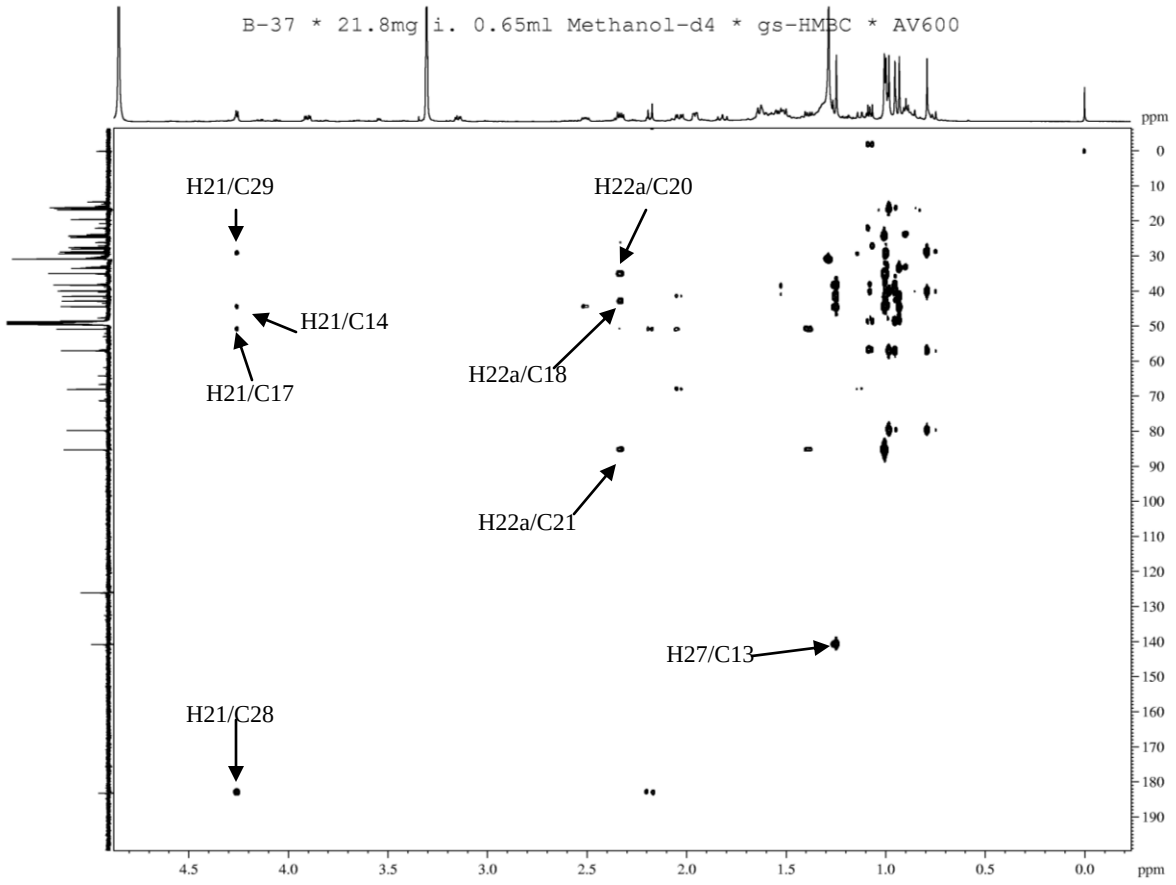


Figure 4.2.4d: $^{1}H - ^{13}C$ HMBC spectrum of compound **4**

From the COSY spectrum of compound **4** (Fig 4.2.4e), cross peaks between H-3 (3.15 ppm) and H-2a (1.63 ppm), H-6a (1.63 ppm) and H-5 (0.78 ppm), H-12 (5.42 ppm) and H-11 (1.96 ppm), H-16a (3.90 ppm) and H-15a (2.04 ppm), H-19a (1.82 ppm) and H-18 (2.51 ppm), H-21 (4.26 ppm) and H-22a (2.33 ppm) were observed.

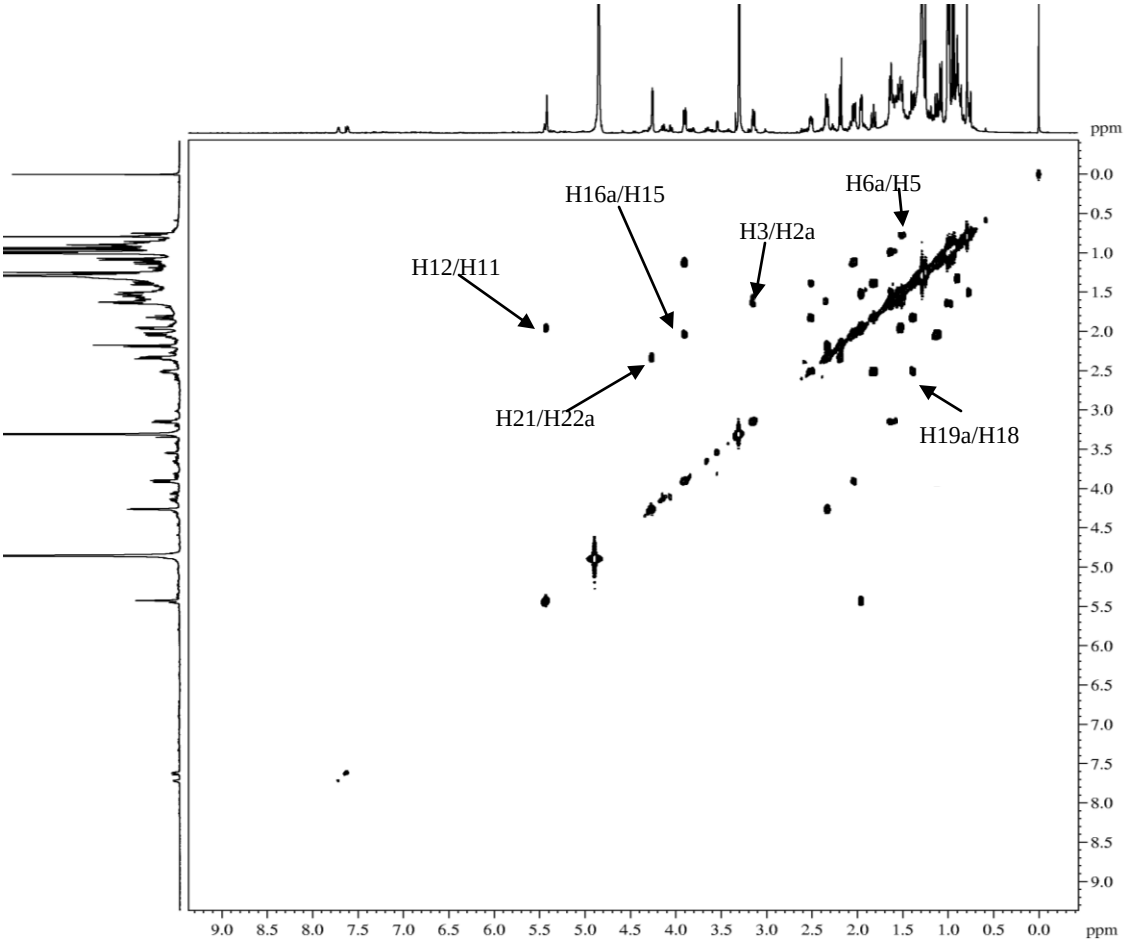


Figure 4.2.4e: $^{1}H - {}^{1}H$ COSY spectrum of compound **4**

The ^{1}H and ^{13}C NMR chemical shifts (Table 4.2.4) of compound **4** were found to be consistent with the ^{1}H and ^{13}C NMR chemical shifts and structure reported for Acacic acid lactone (3β,16α,21β)-3,16-Dihydroxy-21,28-epoxyolean-12-en-28-one) isolated from *Albizia versicolor* (Rukunga and Waterman, 2001). Hence compound **4** was assigned as Acacic acid lactone (Fig 4.2.4f).

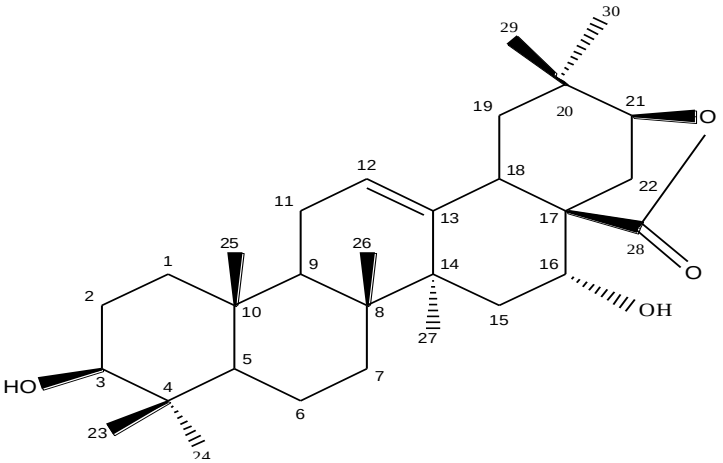


Figure 4.2.4f: The structure of compound **4** (Acacic acid lactone).

Table 4.2.4: ^{1}H (600 MHz) and ^{13}C (600 MHz) NMR spectral data together with the HMBC correlations of compound **4** in MeOH.

#	**^{1}H** (ppm, Hz)	**^{13}C** (ppm)	**HMBC**
1a 1b	1.63 (m) 1.00 (m)	40.0 CH_2	
2a 2b	1.63 (m) 1.57 (m)	28.0 CH_2	
3	3.15 (dd, 11.4, 4.7)	79.7 CH	C-1, C-24
4		39.9 C	
5	0.78 (m)	57.0 CH	C-4, C-7, C-6, C-24
6a 6b	1.63 (m) 1.51 (m)	19.5 CH_2	
7	1.55 (m)	33.5 CH_2	

8		41.5 C	
9	1.52 (m)	48.5 CH	
10		38.3 C	
11	1.96 (dd, 8.9, 2.8)	24.7 CH_2	C-13, C-12, C-9, C-8
12	5.42 (d, 3.2)	126.1 CH	C-9, C-14, C-11
13		140.8 C	
14		44.4 C	
15a 15b	2.04 (dd, 14.1, 5.0), 1.12 (dd, 14.0, 12.2)	38.3 CH_2	
16	3.90 (dd, 11.8, 4.7)	68.0 CH	C-28, C-17, C-22
17		50.8 C	
18	2.51 (dd, 11.9, 6.4)	42.8 CH	C-28, C-13,C-12,C-17,C-14,C-22
19a 19b	1.82 (t, 13.4) 1.39 (dd, 14.3, 6.6)	44.4 CH_2	C-13, C-18, C-20, C-29, C-30, C-21, C-17.
20		35.0 C	
21	4.26 (d, 5.5)	85.3 CH	C-28, C-17, C-14, C-29
22a 22b	2.33 (m) 2.18 (d, 12.2)	27.5 CH_2	C-28, C-17, C-18, C-20, C-21 C-28, C-18
23	0.98	28.8 CH_3	C-3, C-5, C-4, C-24
24	0.79	16.4 CH_3	C-3, C-5, C-4, C-23
25	0.95	16.2 CH_3	C-5, C-9, C-1
26	0.93	16.8 CH_3	C-9, C-19, C-8, C-7

27	1.25	29.3 CH_3	C-13, C-14, C-8, C-15
28		183.2 C	
29	1.01	29.1 CH_3	C-21, C-14, C-20,
30	0.99	24.2 CH_3	C-21, C-14, C-20, C-29

4.2.5 Compound 5 ((+) – Catechin)

Compound **5** was obtained as a dark brown powder, soluble in acetone. It was eluted from the silica gel column with ethyl acetate: hexane 85: 15. It was characterized by NMR, MS and comparisons of spectral analytical data to literature values. The mass spectrum of compound **5** (Fig 4.2.5a) showed a molecular ion peak $[M]^+$ at *m/z* 290, corresponding to a molecular formula $C_{15}H_{14}O_6$.

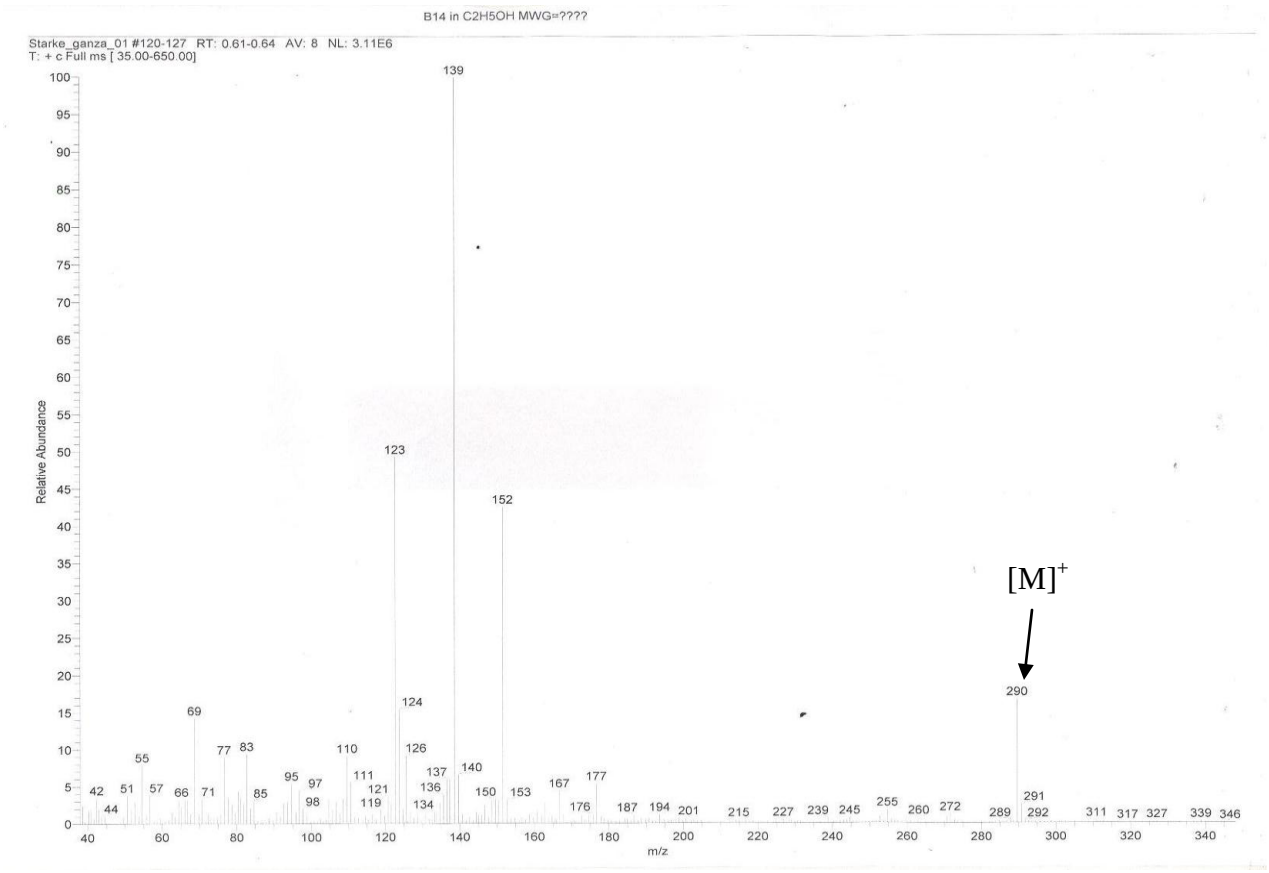


Figure 4.2.5a: Mass spectrum of compound **5**

The ^{1}H NMR spectrum of **5** (Fig 4.2.5b) showed signals due to seven methine and one methylene groups between 2.97 ppm and 6.90 ppm. The downfield part of the spectrum showed signals at 8.22 ppm, 8.04 ppm, 7.91 ppm and 7.87 ppm which all had no direct attachment to carbon atoms in the HSQC experiment. This was attributed to the protons of the four hydroxyl groups attached at the aromatic carbon atoms 3', 4', 5 and 7. The chemical shift of the hydroxyl proton attached to the non aromatic carbon 3 was observed at 3.95 ppm.

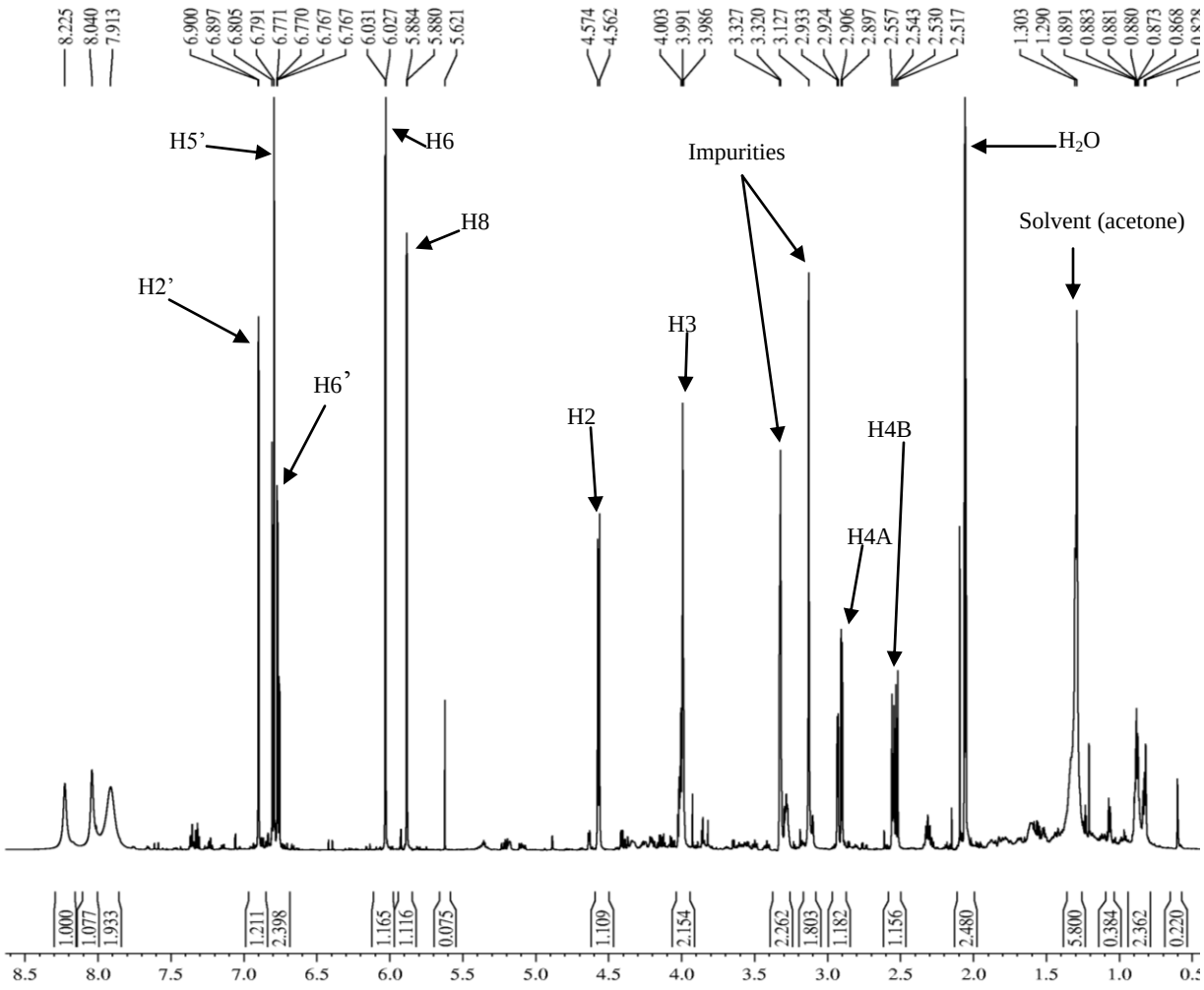


Figure 4.2.5b: ^{1}H NMR spectrum of compound **5**

The ^{13}C NMR spectrum of compound **5** (Fig 4.2.5c) was consistent with the MS data as it revealed the presence of 15 carbon atoms, which were shown by the HSQC experiment to be one methylene (28.8 ppm), seven methine (82.7 ppm, 68.3 ppm, 96.1 ppm, 95.4 ppm, 115.2 ppm, 115.7 ppm and 120.1 ppm) and seven quaternary (100.6 ppm, 157.2 ppm, 157.7 ppm, 156.9 ppm, 132.2 ppm, 145.6 ppm and 145.7 ppm) carbons atoms. The downfield signals at 158.4 ppm, 157.2 ppm, 156.9 ppm, 145.6 ppm and 145.7 ppm were all in agreement with the presence of five aromatic carbon atoms bonded to an oxygen atom each. This was found to correspond to

C-7, C-5, C-8a, C-3’ and C-4’ respectively. The chemical shifts at 132.2 ppm, 120.1 ppm, 115.7 ppm, 115.2 ppm, 100.6 ppm, 96.1 ppm and 95.4 ppm were attributed to the presence of the aromatic C-1’, C-6’, C-5’, C-2’, C-4a, C-6 and C-8 carbon atoms respectively (Table 4.2.5).

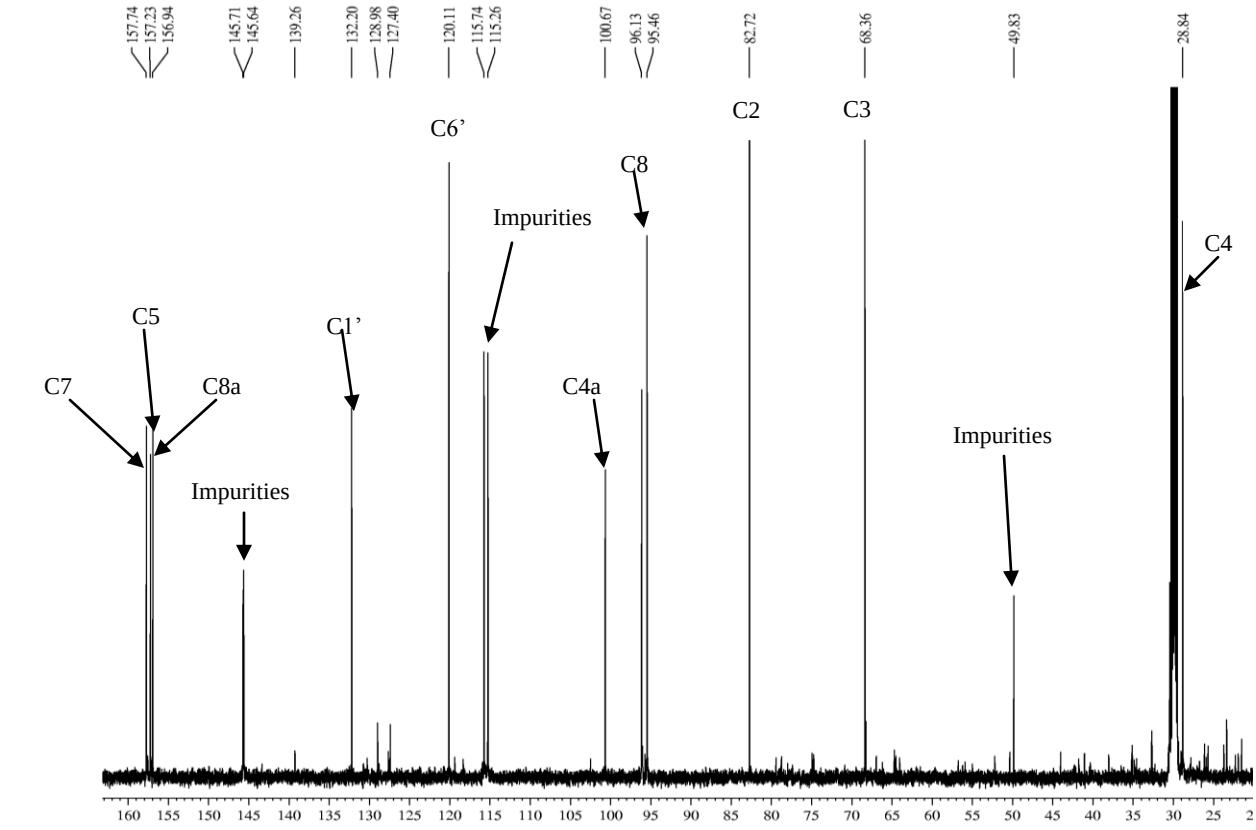


Figure 4.2.5c: ^{13}C NMR spectrum of compound **5**

Correlation of the carbon resonances with the resonances of their directly attached protons (Table 4.2.5) was made possible by the HSQC spectrum of compound **5** (Fig 4.2.5d).

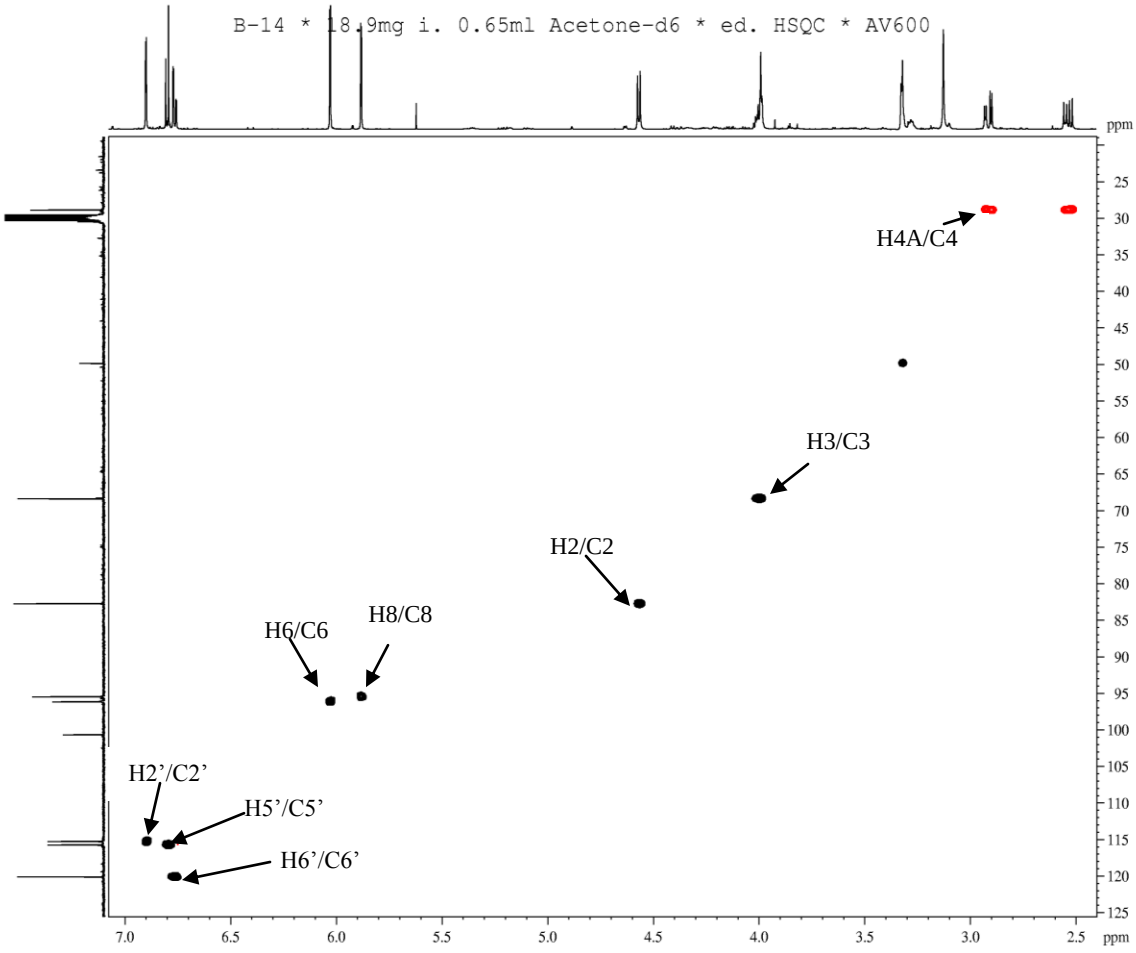


Figure 4.2.5d: ^{1}H-^{13}C HSQC spectrum of compound **5**

The HMBC spectrum of compound **5** (Fig 4.2.5e) made the assignment of all the carbon atoms possible (Table 4.2.5). The connectivity of rings A and C was confirmed by the HMBC correlations between C-8a (156.9 ppm) and C-2 (82.7 ppm), C-8 (95.4 ppm) as well as C-6 (96.1 ppm). This was further supported by the correlations between C-4a (100.6 ppm) and C-4 (28.8 ppm), C-8 (95.4 ppm) as well as C-6 (96.1 ppm).

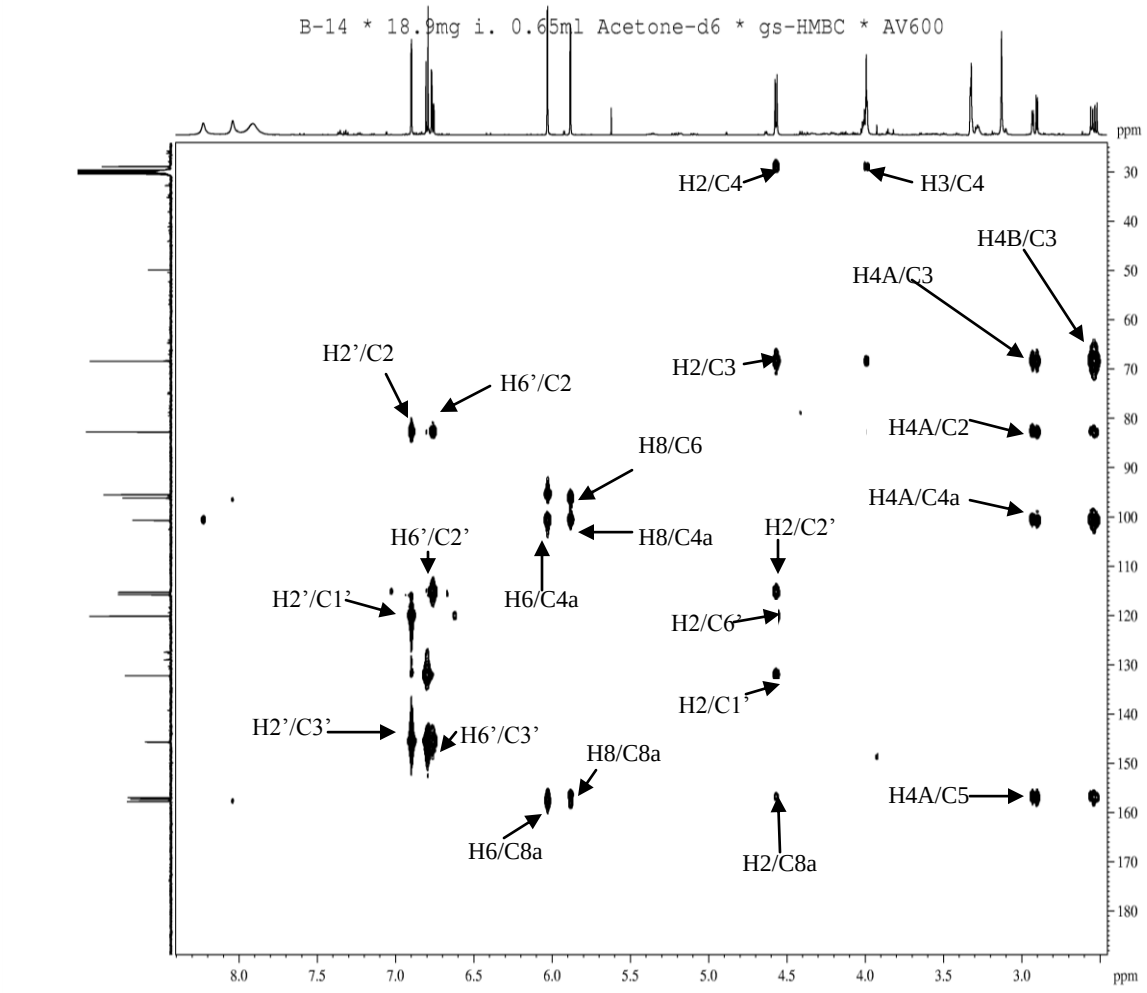


Figure 4.2.5e: ^{1}H-^{13}C HMBC spectrum of compound **5**

The COSY spectrum (Fig 4.2.5f) of compound **5** displayed cross peaks between H-2 (4.56 ppm) and H-3 (4.00 ppm), H-3 (4.00 ppm) and H-4A (2.93 ppm) as well as between H-5' (6.80 ppm) and H-6' (6.76 ppm) among others.

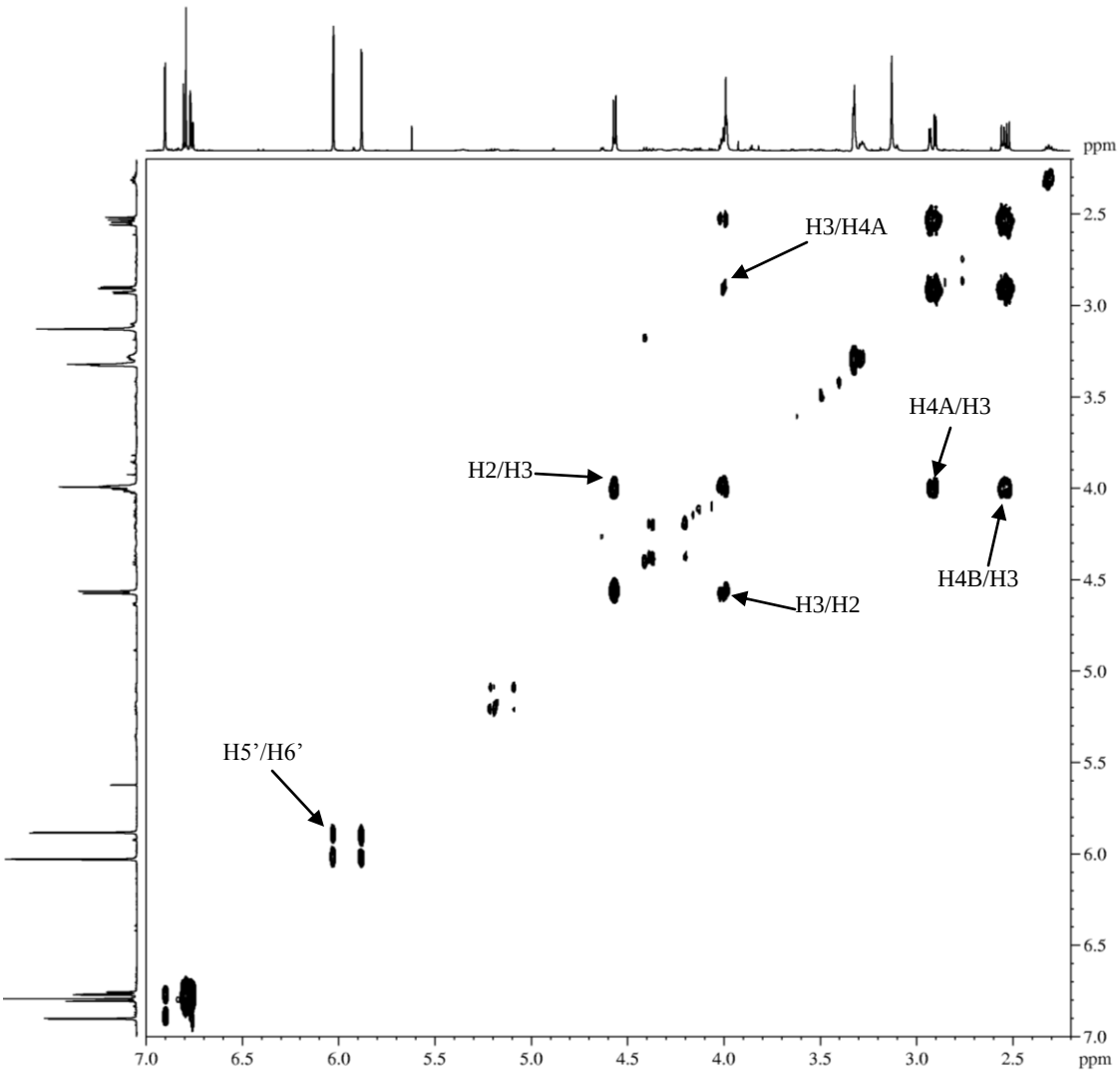


Figure 4.2.5f: ^{1}H-^{1}H COSY spectrum of compound **5**

The ^{1}H and ^{13}C NMR chemical shifts (Table 4.2.5) of compound **5** were found to be consistent with the ^{1}H and ^{13}C NMR chemical shifts and structure reported for (+) – Catechin isolated from green tea (Davis et al., 1996). Hence compound **5** was assigned as (+)-Catechin (Fig 4.2.5g).

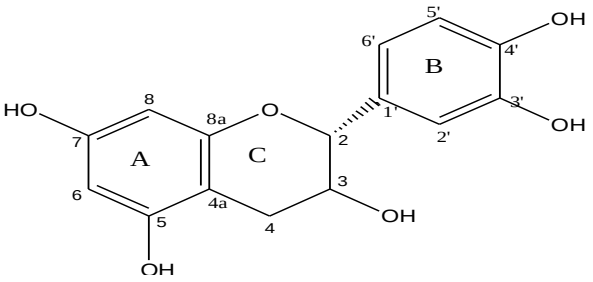


Figure 4.2.5g: The structure of compound ((+) – Catechin).

(+) – Catechin belongs to the flavonoids, a class of metabolites with a wide range of pharmacological properties. It is a polyphenolic compound naturally abundant in green tea, grape seeds and other plants and is reported to exhibit physiological effects, including antibacterial, antifungal, antiviral, antioxidative and antitumor activities (Tamura and Ochiai, 2012). This supports the traditional use of *Albizia coriaria* to treat diseases of bacterial origin and also as a skin tonic.

Table 4.2.5: ^{1}H (600 MHz) and ^{13}C (600 MHz) NMR spectral data together with the HMBC correlations of compound **5** in Acetone.

#	**^{1}H** (ppm, Hz)	**^{13}C** (ppm)	**HMBC**
2	4.56 (d, 7.7)	82.7 CH	C-8a, C-1', C-6', C-2', C-3, C-4
3	4.00 (m)	68.3CH	C-4
4A 4B	2.93(dd,16.1, 5.6) 2.53(dd,16.0,8.5)	28.8 CH_2	C-5, C-4a, C-2, C-3
4a		100.6 C	
5		157.2 C	

6	6.03 (d, 2.3)	96.1 CH	C-4a, C-8a
7		157.7 C	
8	5.88 (d, 2.3)	95.4 CH	C-8a, C-4a, C-6
8a		156.9C	
1’		132.2 C	
2’	6.90 (d, 2.0)	115.2 CH	C-3’, C-6’, C-2, C-1’
3’		145.6 C	
4’		145.7 C	
5’	6.80 (d, 8.0)	115.7 CH	C-3’, C-1’
6’	6.76 (dd, 8.1, 2.0)	120.1 CH	C-3’, C-2’, C-2

4.2.6. Compound 6 (Benzyl alcohol)

Compound **6** was obtained as a brown powder, soluble in methanol. It was eluted with ethyl acetate: methanol 9:1 from the column packed with silica gel. The ^{1}H NMR spectrum of compound **6** (Fig 4.2.6a) showed signals due to five aromatic protons (7.33 ppm) as well as two non aromatic protons (4.58 ppm) with an oxygen neighbor.

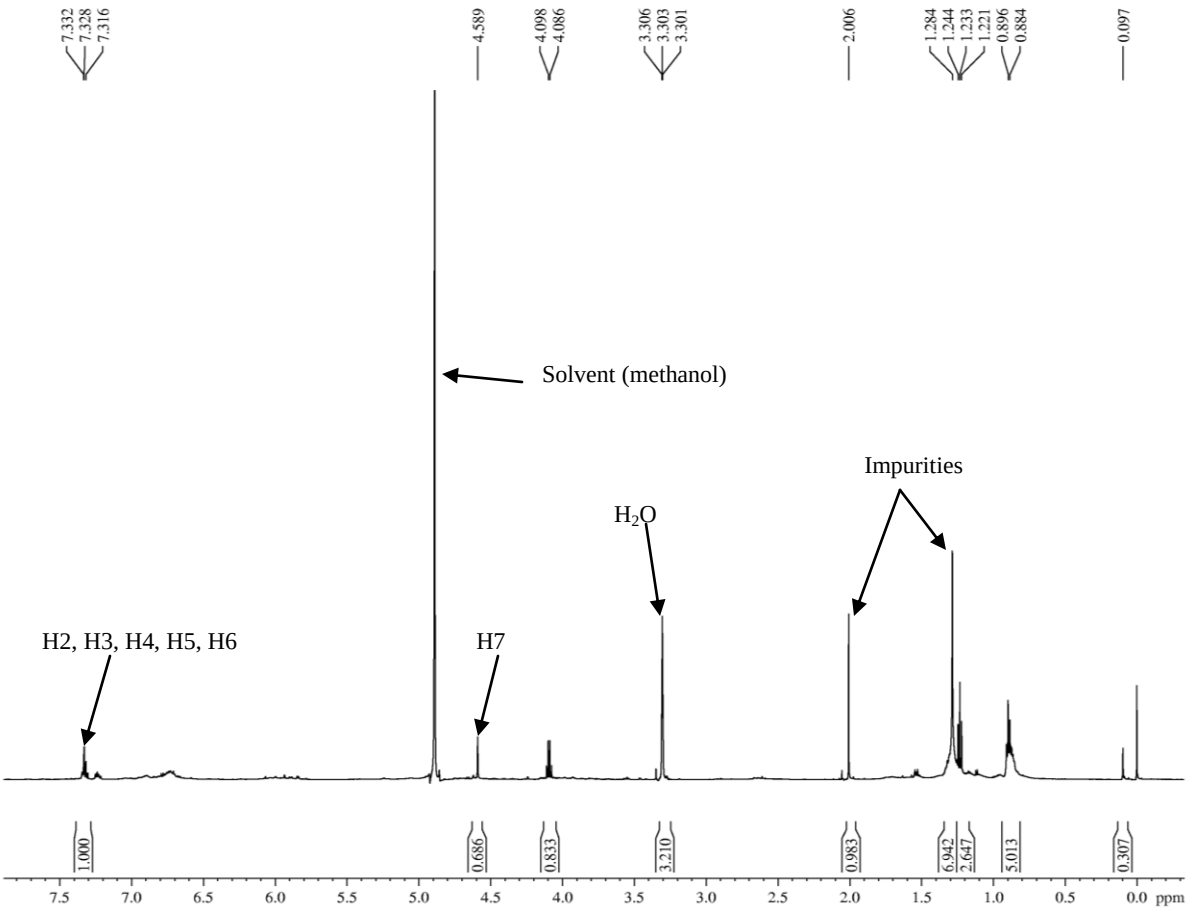


Figure 4.2.6a: ^{1}H NMR spectrum of compound **6**

The ^{13}C NMR data of compound **6** (Fig 4.2.6b) revealed the presence of seven carbon atoms. The downfield part of the spectrum showed six signals between 128.0 and 142.7 ppm, which was typical of aromatic carbons. Two carbon atoms showed a signal at 128.0 ppm, while other two showed a signal at 129.4 ppm. The chemical shift at 65.3 ppm was indicative of the presence of a C-O bond.

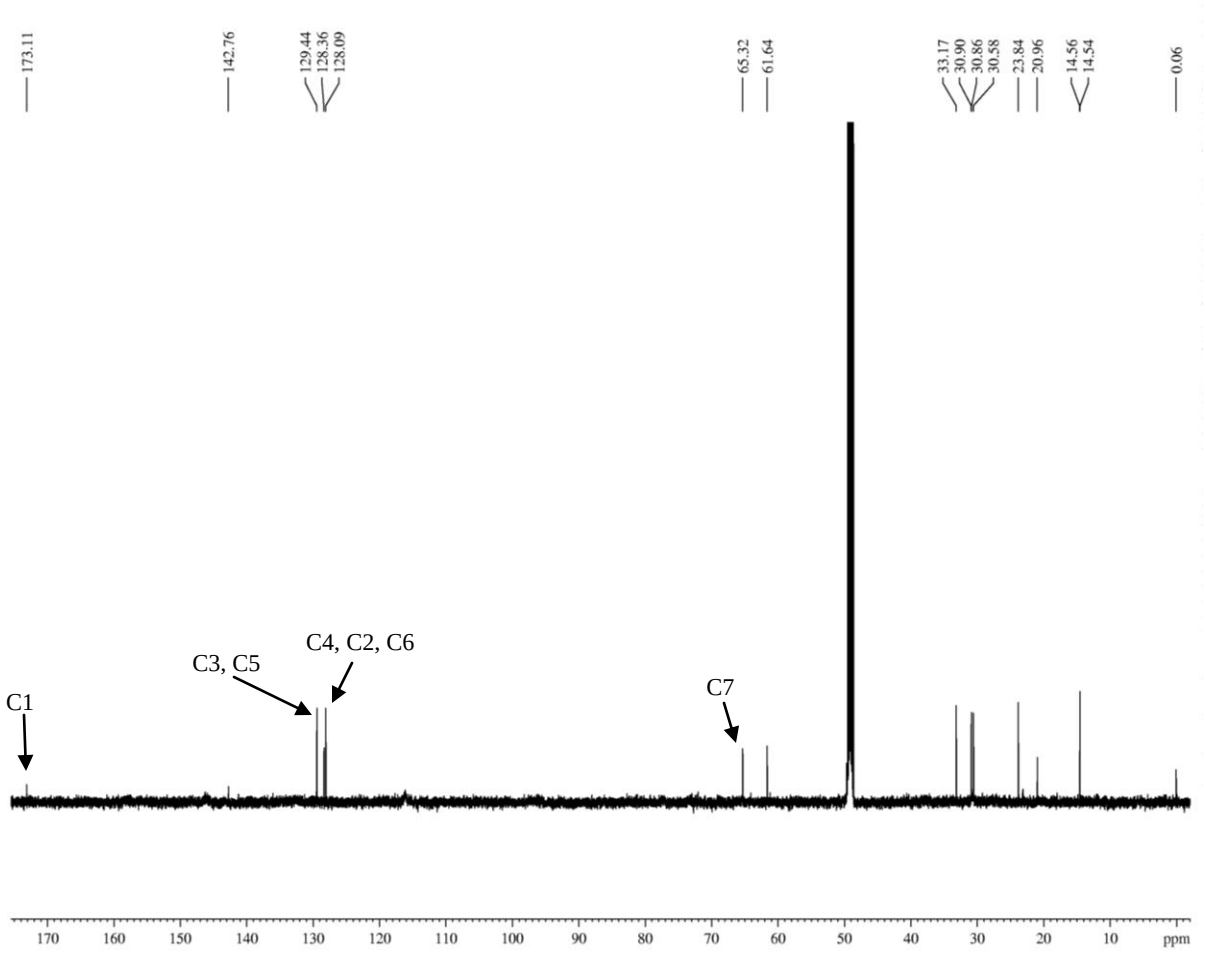


Figure 4.2.6b: ^{13}C NMR spectrum of compound **6**

Assignment of the protonated carbon atoms (Table 4.2.6) was done with the help of the HSQC spectrum (Fig 4.2.6c).

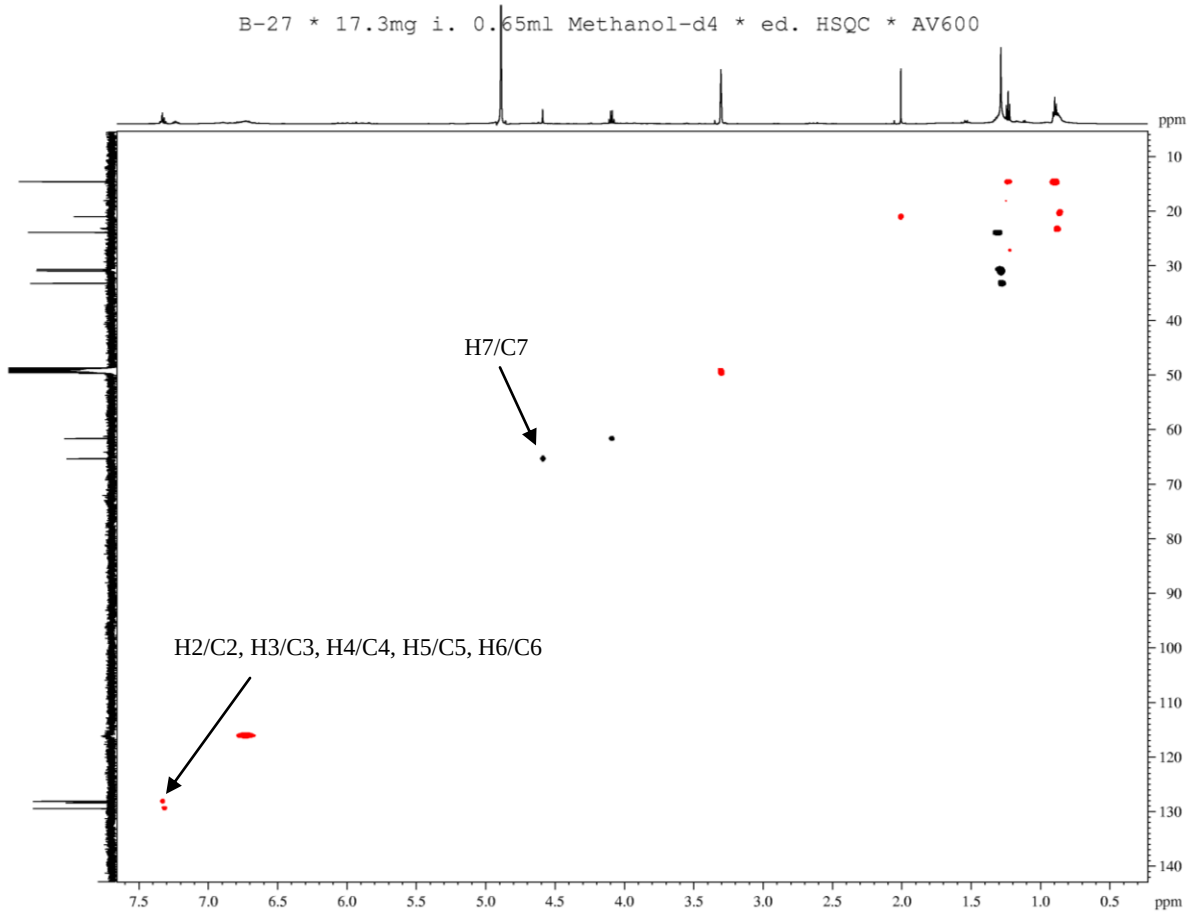


Figure 4.2.6c: ^{1}H-^{13}C HSQC spectrum of compound **6**

From the ^{1}H and ^{13}C NMR data (Table 4.2.6) as well as the HSQC correlations, it was concluded that compound **6** was Benzyl alcohol (Fig 4.2.6d).

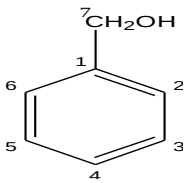


Figure 4.2.6d: The structure of compound **6** (Benzyl alcohol)

Table 4.2.6: ^{1}H (600 MHz) and ^{13}C (600 MHz) NMR spectral data together with the HMBC correlations of compound **6** in MeOH.

#	**^{1}H** (ppm)	**^{13}C** (ppm)	**HMBC**
1		142.7 C	
2	7.33	128.0 CH	C-3, C-4, C-6
3	7.33	129.4 CH	
4	7.33	128.3 CH	
5	7.33	129.4 CH	
6	7.33	128.0 CH	
7	4.58	65.3 CH_2	C-6, C-2, C-1

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

In this study, six compounds have been isolated and characterized from the stem bark of *Albizia coriaria* as: Lupeol (**1**), Lupenone (**2**), Betulinic acid (**3**), Acacic acid lactone (**4**), (+) - Catechin (**5**) and Benzyl alcohol (**6**). This therefore is the first time these compounds are reported in this plant. The compounds that have been characterized in this study exhibited a wide range of bioactivity on bacterial growth. This, coupled with the findings from the bioassay studies on the crude extract, justify the use of *Albizia coriaria* in traditional medicine. Furthermore, standardization of herbal formulations from *Albizia coriaria* bark can be possible by using the characterized compounds as markers in the improved traditional medicine.

5.2. Recommendation

Phytochemical studies on the leaves of *Albizia coriaria* are recommended in order to establish if they contain the same compounds as those in the bark; this will solve the problem of harvesting the bark for the treatment of diseases. With the exception of Lupeol which is a non toxic agent and does not cause any systemic toxicity in animals at doses ranging from 30 to 2000 mg/kg (Siddique and Saleem, 2011), as well as (+)-Catechin which is known to be abundant in many edible fruits (Savova et al., 2004), toxicity studies on the other characterized compounds are recommended, so as to establish their safety to human beings.

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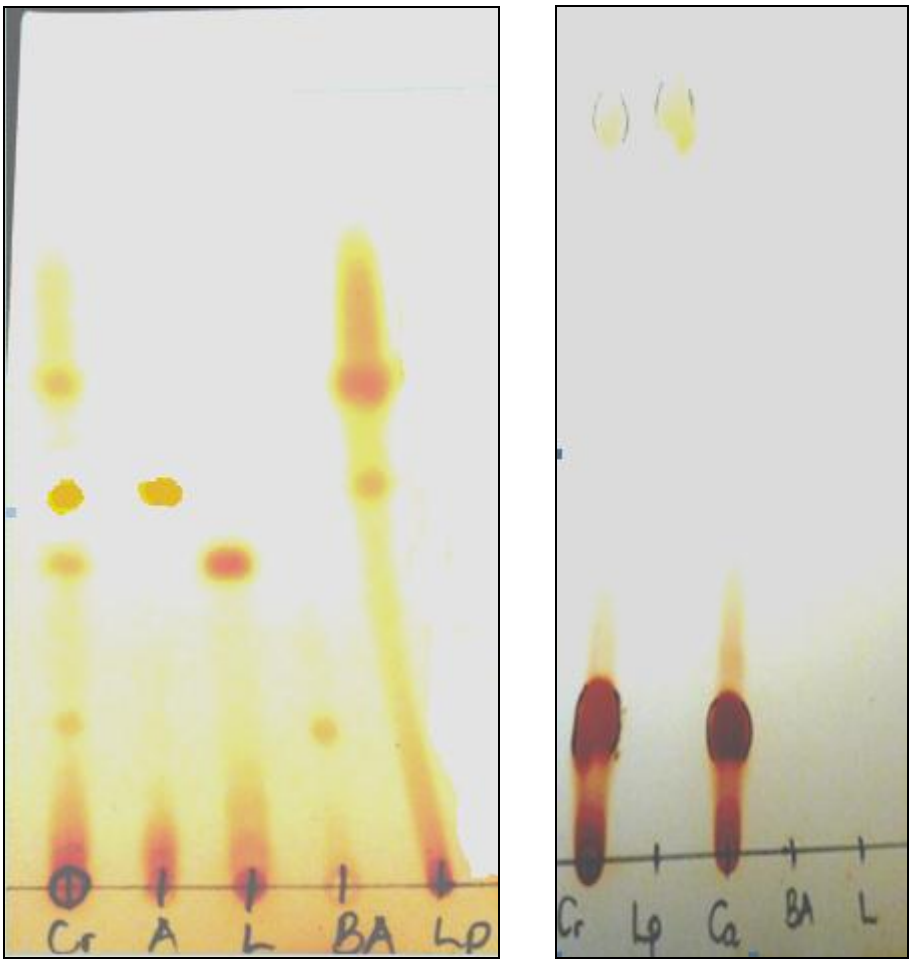
APPENDIX



Appendix 5.1: TLC profile of the crude extract (Cr) and the isolated compounds: acacic acid lactone (A), lupeol (L), betulinic acid (BA), lupenone (Lp) and (+)-catechin (Ca); mobile phase: Left-Hexane : ethyl acetate 8:2, Right-Methylenechloride : methanol 9:1.