ISOLATION AND CHARACTERISATION OF BIOACTIVE PRINCIPLES FROM *Raphia africana* (OTEDOH)

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DECLARATION PAGE

I declare that the work in this thesis entitled "Isolation and Characterisation of Bioactive Principles from *Raphia africana* (Otedoh)" has been carried out by me in the Department of Chemistry, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and in the list of references provided. No part of this dissertation has been previously presented for another degree or diploma at this or any other institution.

Anyam, John Vershima	_____	_____
Name of Student	Signature	Date

CERTIFICATION PAGE

This thesis entitled "ISOLATION AND CHARACTERISATION OF BIOACTIVE PRINCIPLES FROM *Raphia africana* (OTEDOH)" by John Vershima ANYAM, meets the regulations governing the award of the degree of Doctor of Philosophy in Organic Chemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABBREVIATIONS

Table of Abbreviations

HMBC	Heteronuclear multiple bond correlation Spectroscopy
HPLC	High performance liquid chromatography
HSQC	Heteronuclear single quantum correlation Spectroscopy
MAE	Microwave assisted extraction
MBC	Minimum bactericidal concentration
MFC	Minimum fungicidal concentration
MIC	Minimum inhibitory concentration
NMR	Nuclear magnetic resonance
TLC	Thin layer chromatography
VLC	Vacuum liquid chromatography
ACDLABS	Advanced chemistry development, Inc.
COSY	Correlation spectroscopy
DMAPP	Dimethylallyl diphosphate
FTIR	Fourier transform infrared (spectroscopy)
GC	Gas chromatography
IPP	Isopentenyl pyrophosphate
IR	Infrared (spectroscopy)
KGaA	Kommanditgesellschaft auf aktien
PUFA	Polyunsaturated fatty acid
TMS	Tetramethylsilane
UV	Ultra violet

ABSTRACT

Raphia africana (Otedoh) is a member of the palm family Arecaceae. The plant was collected from banks of the creeks Ukoko and Uertsor in Buruku Local Government Area of Benue State. The fruit mesocarp was extracted and subjected to phytochemical and antimicrobial screenings. The ethyl acetate extracts, obtained via different extraction protocols (soxhlet extraction, microwave assisted extraction and maceration) were subjected to *Artemia salina* lethality assay. Extracts (ethyl acetate and methanol) were subjected to Gas Chromatography Mass Spectrometry (GCMS) and High-Performance Liquid Chromatography (HPLC) as part of pre-isolation analysis. Phytochemical screening showed the presence of steroids, triterpenes, alkaloids, anthroquinones, cardiac glycosides and flavonoids. Antimicrobial screening of the extracts showed the ethyl acetate extract to have the broadest and highest activity against the test microbes, having lower minimum inhibitory concentration (0.625 mg/mL) and a wider zone of inhibition diameter (27 mm) on *Klebsiella pneumoniae.* The ethyl acetate extract also showed good minimum bactericidal/fungicidal concentration values on *Staphylococcus aureus* (2.5 mg/mL), *Klebsiella pneumoniae* (1.25 mg/mL) and *Candida albicans* (2.5 mg/mL). The hexane extract was the least active against the test microbes with chloroform and methanol extracts showing intermediate activity. Extracts obtained by soxhlet extraction and microwave assisted extraction showed toxicity at a concentration of 269.2 mg/mL, while the extract obtained through maceration showed the lowest toxicity (467.7 mg/mL). The GCMS profiles showed the extracts to consist of lipids and diterpene alcohols while the selectively targeted HPLC showed traces of sterols (campesterol, cholestanol, cholesterol, ß-sitosterol, stigmasterol and tocopherol) and flavonoids (isovitexin,

kaempferol and orientin). Crude extracts were extensively purified using vacuum liquid chromatography (VLC) and gel filtration (GF). Fractions from VLC and GF sessions were characterised using Nuclear Magnetic Resonance Spectroscopy (NMR) and found to be mainly lipids. Extensive chromatography allowed the isolation of saturated fatty acids (margaric and myristic acids), a monounsaturated fatty acid methyl ester (methyl oleate), triglycerides, a sterol methyl ether, tentatively identified diosgenin-type steroidal saponins, tentatively identified monogalactosyl diacylglycerides, monoacyl glycerides and diacylglycerides. This is the first time these substances have been identified in the mesocarp of this plant. The isolated compounds were tested against microbes and they showed higher but similar activity compared to the crude extracts. Ja 83 (a monogalactosyldiacylglyceride) showed the largest zone of inhibition against *Staphylococcus aureus* (30 mm), against *Klebsiella pneumonia*e (31 mm) and 32 mm against *Shigella dysenteriae* while showing the least zone against *Microsporum sp.* Isolated compounds were inactive towards *Aspergillus fumigatus* and *Aspergillus niger*. Relatively mild behaviour of the crude extracts against *Artemia salina*, and the lipidic nature of the phytoconstituents reaffirm its use as a food source and it is recommended that this, now increasingly neglected tropical fruit tree, be actively cultivated for human use provided the toxicity studies allow them for such purpose.

CHAPTER ONE

1.0 INTRODUCTION

Thousands of plants have already been investigated for metabolites/phytochemicals by scientists (Dias *et al.,* 2012). The number of novel compounds isolated from plants has steadily fallen, from its peak in the heyday of isolation (1960s and 1970s). Many new applications are being discovered every day for previously isolated compounds (Dias *et al.,* 2012).

Many neglected tropical diseases have no viable cure yet (Lawal *et al.,* 2010). For instance, the best drugs for treatment of trypanosomiasis kill about 5% of patients (Pépin *et al.,* 1995; Kayser *et al.,* 2003). All anti-malarials are hepatotoxic (Alkadi, 2007; German *et al.*, 2007) while human immunodeficiency virus induced Acquired Immune Deficiency Syndrome (HIV/AIDS) is still ravaging millions world-wide. Worse yet, a new crop of epidemics from Zika, Chikungunya, Yellow fever, Ebola haemorrhagic fever, to Lassa fever are ravaging the third world; disturbingly, these epidermics have no viable cure (Musso and Gubler, 2016; Phillips and Neyland, 2016).

Species of *Raphia* like *R. farinifera*, *R. hookeri*, *R. regalis* and *R. sudanica* are used for treating mouth ulcers, stomach problems and for treating heart disease (heart pain). A search on internet databases, like Elsevier, National Center for Biotechnology Information (NCBI) yielded no result on *R. Africana*; offline resources like Kashim Ibrahim Library (KIL) Ahmadu Bello University Library showed that no scientific study investigating *Raphia africana's* ethnomedicinal applications had been reported prompting further interest in the plant.

1.1.1 Justification of the Study

There is lack of scientific information on *Raphia africana.* This study seeks to investigate the traditional claims on *Raphia africana* (Otodeh) *Arecaceae* with a view to contributing to the knowledge base in the fight against tropical diseases like mouth ulcers.

1.1.2 Aim

The aim of this research is to authenticate or otherwise, the claims by traditional medicine practioners on the fruit mesocarp of *Raphia africana* by isolating and characterising some of the compounds from it.

1.1.3 Objectives of the Research:

The aim will be achieved through the following objectives:

i. Extraction of the powdered plant material using different solvents based on the eluotropic series i.e. non-polar pet-ether (60-80 ^{O}C), chloroform, ethyl acetate and methanol (polar),

ii. phytochemical screening of the crude solvent extracts,

iii. antibacterial and antifungal screening of the various extracts,

iv. lethality assay of the crude extracts using Brine shrimp (*Artemia salina*),

v. separations and purifications of the crude extracts using several steps of chromatographic techniques,

vi. spectroscopic analyses (NMR, IR, GCMS) of the isolated compounds.

CHAPTER TWO

2.0 LITERATURE REVIEW

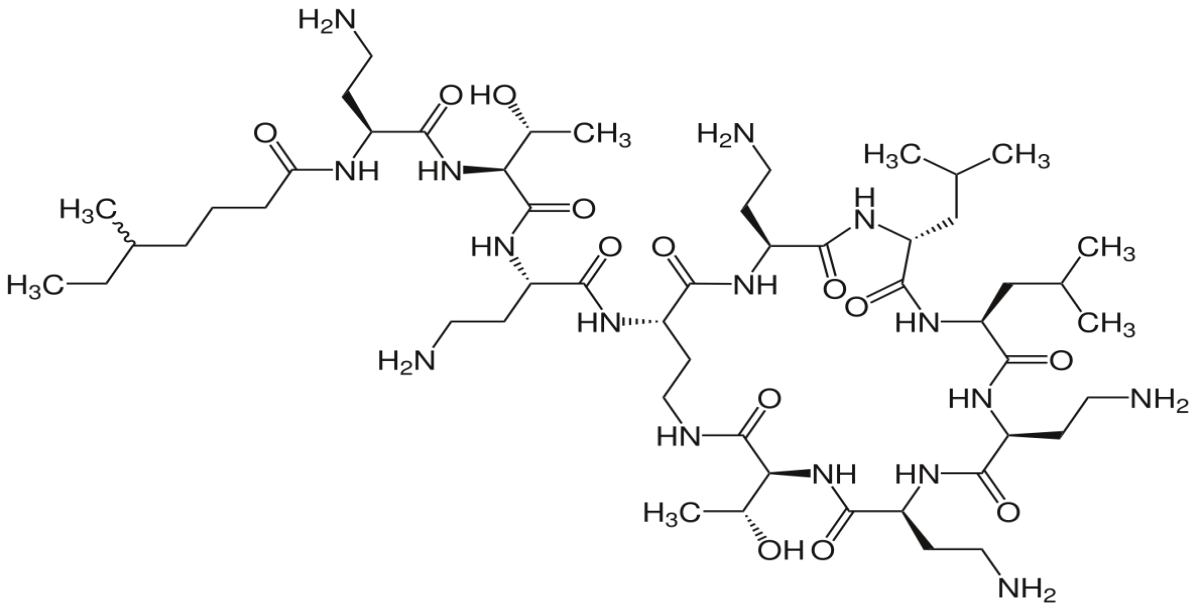
1.1 Man and Medicinal Plants

Plants occupy a very important place in human history (Pitchai *et al.,* 2010). Plants have been important sources of material for shelter, food, transport, clothing, utensils, weapons, tools and medicines (Husain *et al.,* 2008). A medicinal plant is a plant used for treating or managing an ailment (Mesfin *et al.,* 2009). The use of plants as medicines is thought to be as old as human history (Springob and Kutchan, 2009). The *Ötzi* man, a mummy found in the Ötzal valley on the Alps around the Austro-Italian border in the year 1991, had medicinal herbs (*Papaver somniferum* seeds in his stomach) and mushrooms on him; carbon dating has placed the time of his death to be around 5, 300 years ago (Jacomet, 2009; Jeraj *et al.,* 2009).

There has been a recourse to medicinal plants around the world in the past few decades because they are low cost, considered safer, are part of a religion or belief system or tradition and pathogens have become resistant to even antibiotics of last resort. Up to 700000 people die every year due to antibiotics resistance and, by some estimates, this figure could easily rise to 10 million a year by 2050 if nothing dramatic is done (Li *et al.,* 2006; Hoffman *et al.,* 2015). The cost of such a huge number (10 million) is estimated to be around 66 trillion dollars. For over twenty years, there has not been a major breakthrough in antibiotics development. We have become accustomed to simply replacing one antibiotic with another; we do not have another anymore. Public health

matters cannot be left to market forces alone; big pharmaceutical companies are running away from financial implications arising from numerous expensive and time consuming drug trials that yield no results (Goozner and Moore, 2004; Butler *et al.,* 2014). The observation by Pollan (2013) that: "As a civilisation, we've just spent the better part of a century doing our unwitting best to wreck the human-associated microbiota with a multifronted war on bacteria", is a warning knell of the situation at hand and a dire harbinger of the future (Pollan, 2013; Shen *et al*., 2013; O'Toole and Jeffery, 2015; Van Olden *et al*., 2015; Nasaruddin *et al*., 2016). Most diseases of today from obesity to cancer to Alzheimer's are now ascribed to an impoverished microbiome induced by antibiotics. It is now left for scientists to delve into resources at hand in order to safeguard our future.

Colistin (**1**) for a long time has been used as antibiotic of last resort; it has unfortunately become resistant to certain superbugs (Falgenhauer *et al.,* 2016).



Colistin (1)

A strain of *Escherichia coli*, for instance, was found to be resistant to Colistin (**1**); an antibiotic of last resort, prompting fears of an emerging antibiotic apocalypse (Ninh *et al.,* 2015).

In many parts of the world where no written records of ancient times were kept, oral traditions preserved knowledge of medicinal plant use (Luo *et al.,* 2016). These oral traditions are not without their problems. One big issue is the association of some of these traditional methods with tenuous magic or superstition that in turn throws in misleading ideas about proper utility of plants (Broendegaard, 1985).

Scientific investigations of claims by traditional medicine practitioners play an important role in teasing apart fact from fable (Meetei *et al.,* 2012). Another issue is that some plants are very harmful when not utilised appropriately and so authorities throughout history have tried to destroy or misdirect knowledge of their deleterious applications. Two good examples of such plants is *Strophanthus kombé* reportedly destroyed by the British colonial authorities in their colonies on account of its use as a potent arrow poison and *Cannabis sativa*, banned by many governments today, for its misuse as a potent psychoactive drug (Hokkanen, 2012; Osseo-Asare, 2008). Scientific studies have unearthed new applications or better ways of using medicinal plants. Extracts from *Strophantus kombé* are now utilised as heart medication while the much vilified *C. sativa* is now employed in controlled applications in the management of pain associated with glaucoma and cancer; quinine and derivatives from the cinchona plant have been relegated to the side-lines in the treatment of malaria leaving the stage to artemisinins from *Artemisia annua* (Farag and Kayser, 2015; Hokkanen, 2012; Ilesanmi *et al.,* 2011).

The principles behind the therapeutic activities of medicinal plants centre on secondary metabolites, which are by products of plant metabolism. Other medicinal principles include fatty acids and triglycerides (Figure 2.1) which play important roles in heralding immune systems to potential infection and other immuno-regulatory activities (Ben-Menachem *et al.*, 2003).

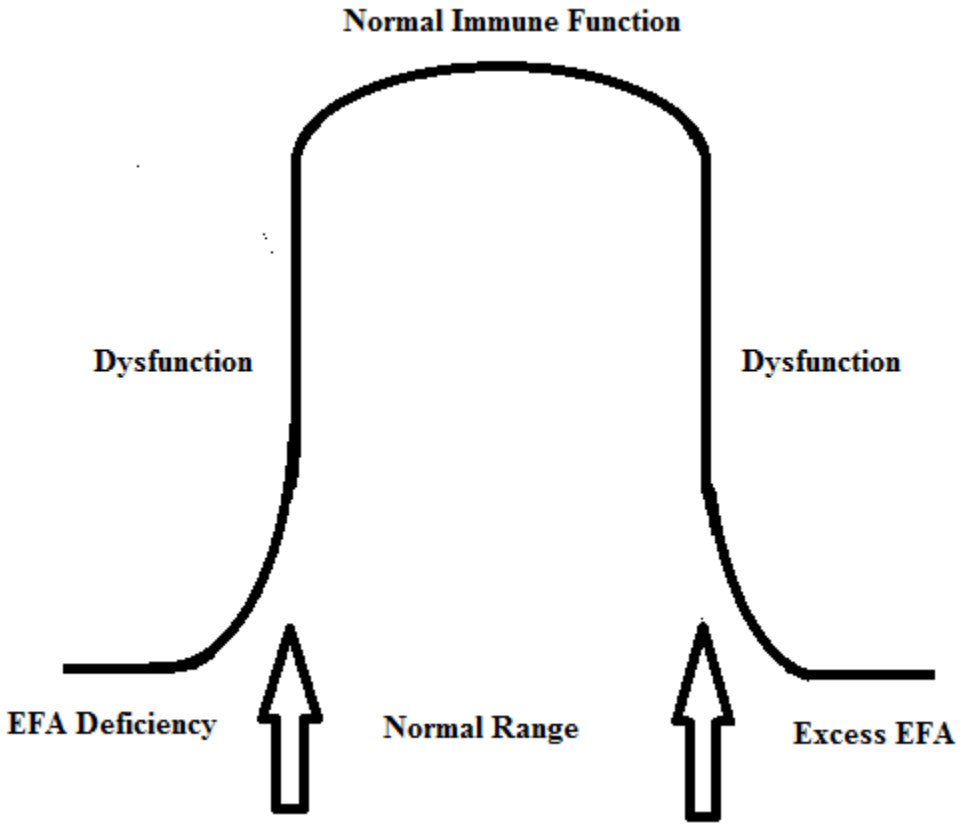


Figure 2.1 Triglycerides, fatty acids and immunity**;** Immune responses as a function of dietary essential fatty acid (EFA)(Gunstone and Seth, 1994; Gunstone, 2011)

Among the secondary metabolites are an important class; the alkaloids.

2.1 Plant Secondary Metabolites

2.1.1 Alkaloids

Alkaloids are nitrogenous bases. Alkaloids are secondary metabolites that mostly contain basic nitrogen atoms (Wells, 2011). The term alkaloids has been used to classify synthetic compounds of similar structure. Alkaloids are purified from crude extracts of plants and other animals by acid base extraction. Alkaloids have been employed variously in many medicinal applications (**Table 2.1**) (Cushnie *et al.,* 2014).

While the boundary between alkaloids and many other nitrogen-containing compounds is not clearly defined, amines, antibiotics, proteins, nucleic acids and peptides are not strictly regarded as alkaloids. When a compound contains nitrogen in the exocyclic position, it is usually classified as an amine rather than an alkaloid (Prescott, 1903; Helm, 2000; Schardl *et al.,* 2007) have argued alkaloids for a special case of amines.

More recently, alkaloids have been classified based on the nature of their carbon skeleton or biochemical precursor (Table **1.2).** Nicotine (**3**) and alkaloids which contains both pyridine and pyrrolidine fragments deriving from nicotinamide and ornithine precursors respectively could be assigned to both classes, it is one of the border line cases that require compromise (Zenk and Juenger, 2007).

2.1.2 Terpenes and Terpenoids

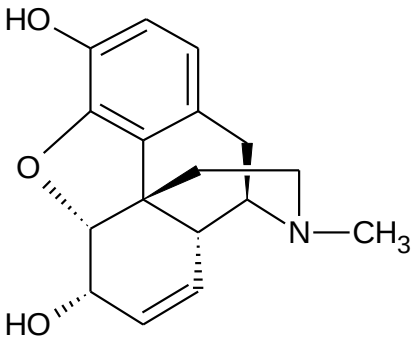
Terpenes, which number over 20, 000 currently, can be used more broadly to include terpenoids (Boutanaev *et al.,* 2015). Terpenoids are terpenes, which contain oxygen atoms, in which case may exist as aldehydes, ketones and alcohols (Andre *et al.,* 2016).

Table 2.1: Alkaloids in Medicinal Applications

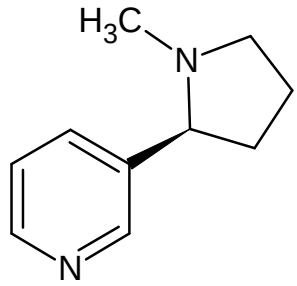
Alkaloids	Pharmacological Activity	Ethnomedicine	Reference
Quinine	Antimalarial, Antiseptic	South American first peoples as an Antimalarial	(Wells, 2011)
Vincamine	Vasodilatory		(Hesse, 2002)
Morphine	Analgesic	Opium by Greeks/Chinese	(Hesse, 2002)
Cocaine	Stimulant (Narcotic)	South American first peoples in Entheogenic rituals	(Morton, 1968)
Ephedrine	Antiasthma	Ephedra by Greeks	(Song *et al.*, 2010)
Tubocurarine	Muscle-relaxant	South American first peoples for Poisoning arrows	(Shinwari, 2010)

Table 2.2: **Alkaloid Classification**

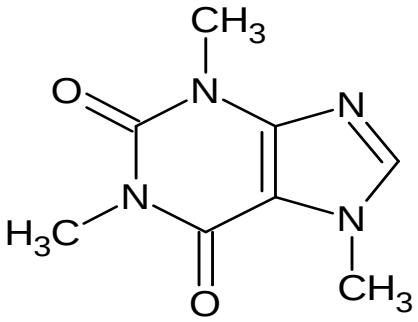
Group	Characteristic	Example	Reference
True alkaloids	- Nitrogen in heterocycle - Origin: amino acids	Morphine (**2**) Nicotine (**3)**	(Cushnie *et al.*, 2014; Saxton, 1971)
Proto-alkaloids	- Nitrogen containing - Origin: amino acids	Mescaline (**5**)	(Cushnie *et al.*, 2014)
Polyamine alkaloids	Derived from spermine, spermidine and putrescine		(Cushnie *et al.*, 2014)
Peptide and cyclopeptide alkaloids			(Hesse, 2002)
Pseudo-alkaloids	- are sterol like - are terpene like - origin: not from amino acid - some are purine like	Caffeine (**4**) Theophylline (**6**)	(Pan *et al.*, 2003)



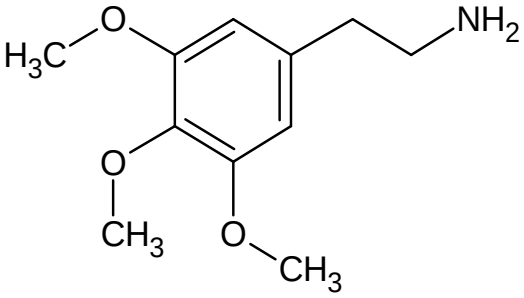
Morphine (**2**)



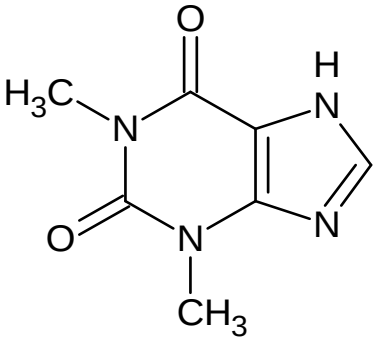
Nicotine (**3**)



Caffeine (**4**)

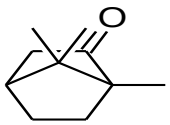


Mescaline (**5**)

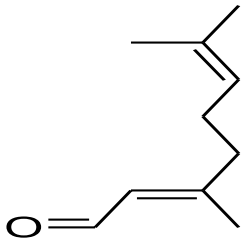


Theophylline (**6**)

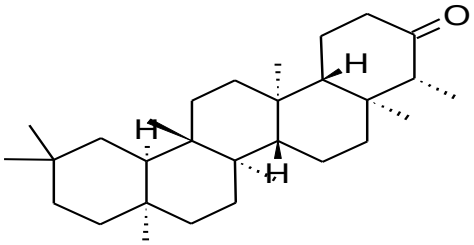
Terpenoids are the largest group of natural products. They make up 60 % of known natural products. Terpenoids have been used in traditional herbal remedies, their aromatic properties contribute to the scent of eucalyptus, the flavours of cinnamon, cloves, ginger and the yellow colour in carrots (Thimmappa *et al.*, 2014).



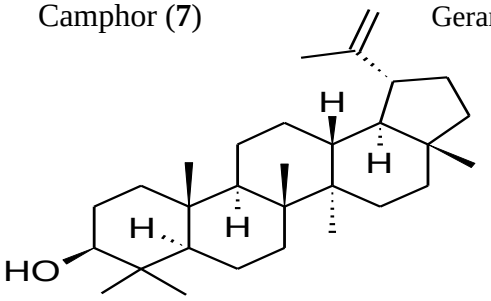
Camphor (**7**)



Geranial (**8**)



Friedelan-3-one (**9**)



Lupeol (**10**)

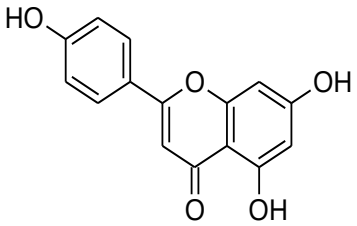
Geranial (**8**) together with its isomer neral are found in many plants; together they are referred to as citral. They are important terpenoids used variously for the synthesis of vitamin A, for aromatherapy, as antimicrobial agents, allelochemicals and insecticides (Robacker and Hendry, 1977; Kuwahara and Suzuki, 1983; Onawunmi, 1989; Chaieb *et al.*, 2007).

Table 2.3: Terpene and Terpenoids in Traditional Herbal Applications

Terpene / Terpenoids Isolated	**Plant Source**	**Utility of Plant**	**References**
Citral (**8**)	*Eucalyptus citriodora*	Aromatherapy, pesticide	(Onawunmi, 1989)
Camphor (**7**)	*Salvia divinorium*	Aromatherapy, insecticide	(Hanson, 2010)
Cannabinoids	*Cannabis sativa*	Pain relief agents	(Andre *et al.*, 2016)
Friedelan-3-one (**9**)	*Pterocarpus santalinoides*	Anti-inflammatory	(Bessong *et al*., 2006; Igoli *et al.*, 2008; Mann *et al.*, 2011; Odeh *et al*., 2016)
Lupeol (**10**)	*Malachanta alnifolia*	Anti-inflammatory	(Geetha and Varalakshmi, 2001; Saleem *et al*., 2004; Wal *et al*., 2011a; Mann *et al.*, 2011; Anyam, 2011; Wal *et al*., 2015)

2.1.3 Flavonoids

Flavonoids like terpenoids are an important class of fungal and plant secondary metabolites (Cook and Samman, 1996). They like terpenoids are widely distributed in nature (Havsteen, 1983). They are made up of a fifteen carbon skeleton consisting of two phenyl rings and a heterocyclic ring named rings A, B, and C (heterocyclic) also referred to as C6-C3-C6 (Cazarolli *et al.,* 2008). They are further classified as neoflavonoids, isoflavonoids and bioflavonoids (Cushnie and Lamb, 2005).

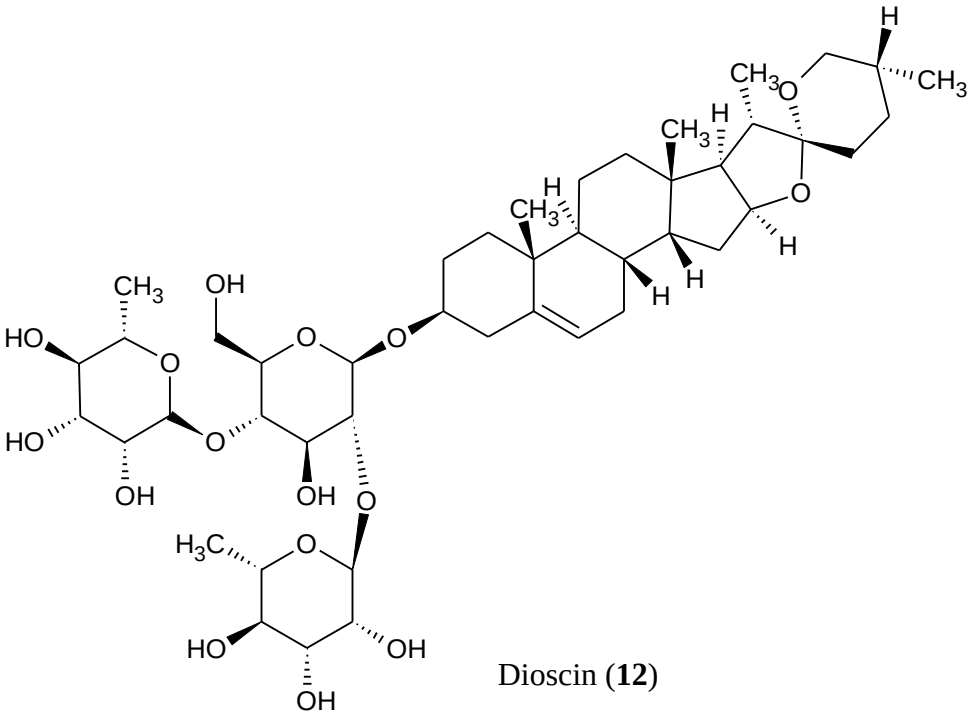


Apigenin (**11**)

Many *in vitro* studies have shown flavonoids to be antioxidant (Fongang *et al.,* 2015), anti-allergic (Tan *et al.,* 2011), antibacterial (Djouossi *et al.,* 2015), antifungal (Cushnie and Lamb, 2005), antiviral (Friedman, 2007), anticancer (Lee, 1999), anti-inflammatory and anti-diarrhoeal (Cruz-Vega *et al.,* 2009). Apigenin (**11**) occurs widely in nature and while its direct effect on the human system is still being studied, it has been used to dye wool and numerous *in vitro* studies have affirmed its potential as a natural drug (Shukla and Gupta, 2010).

2.1.4 Saponins

Saponins are amphipathic glycosides noted for their soap-like behaviour (Hostettmann and Marston, 1995; Augustin *et al*., 2011). They consist of one or more hydrophilic glycoside moieties (most commonly D' glucose and D' galactose) linked to a lipophilic triterpenes. Saponins are used in several controlled chemical and biomedical applications as reagents (Majinda, 2012). Plants rich in saponins have been employed as piscicides (killing fish) for millennia (Xu *et al.,* 1996). Steroidal sapogenins, like dioscin (**12**), have been used as starting points for the semisynthesis of steroid hormones (Hardman and Sofowora, 1972).



Dioscin (**12**)

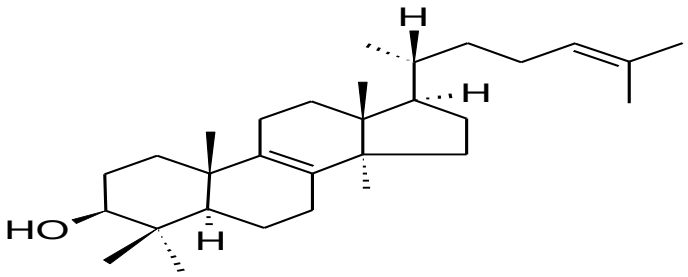
2.1.5 Steroids

Steroids are important biological molecules that play an important role in cell signalling and cell fluidity (Bode *et al.,* 2003). The steroids, testosterone and estradiol are important sex hormones. Sterols are derived from cholestane; they are forms of steroids with C-3 hydroxyl group. Sterols may also vary remarkably in terms of ring structure, as is the case with the secosteroid vitamin D3 (that is found in animals and fungi) (Todd *et al.,* 2015). Sterols arise biosynthetically from lanosterol and cycloartenol (plants) which are in turn derived from the triterpene squalene (Pascal *et al.,* 1993; Piironen *et al.,* 2000; Bode *et al.,* 2003; Schaller, 2010).

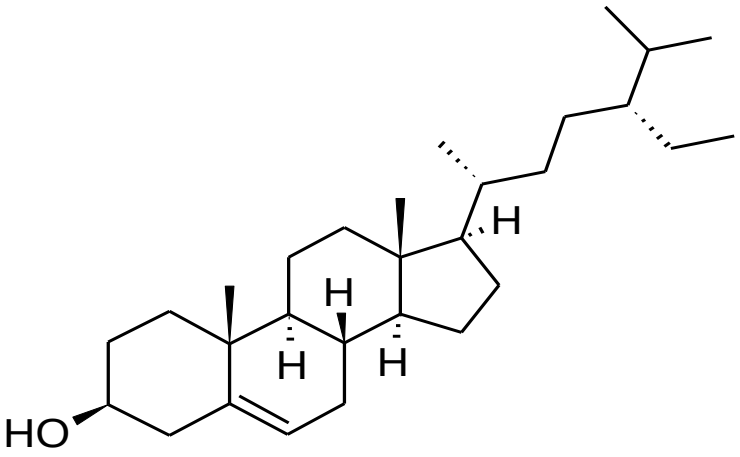
Steroids are reportedly associated with disorders like malignancies like prostate cancer where they have also been known to promote tumour cell aggression (Piironen *et al.,* 2000). Steroids have been used as statins to reduce elevated cholesterol levels by targeting the mevalonate pathway (Gunstone and Herslöf, 2012).

The mevalonate biosynthetic pathway starts with Acetyl-CoA and terminates in dimethylallyl pyrophosphate (DMAPP) and isopentenyl (IPP) (Flores-Sanchez *et al.,* 2002).

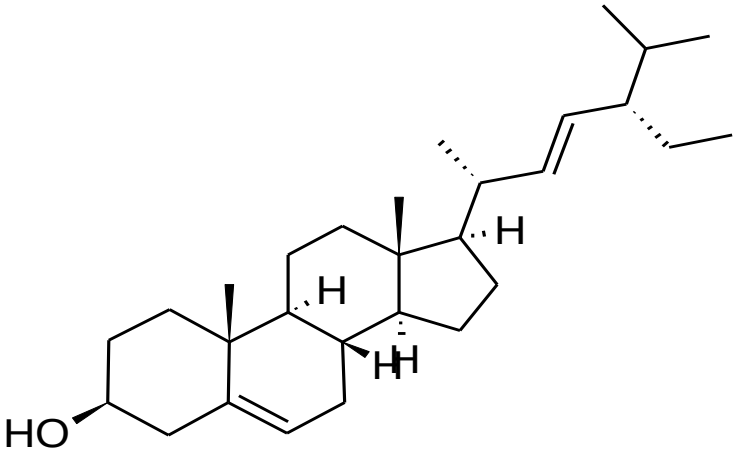
DMAPP and IPP are generators of isoprene units (Shanker *et al.,* 2003). The isoprene units are combined into squalene and rearranged into rings to make lanosterol (**13**) (Schaller, 2010).



Lanosterol (**13**)



Sitosterol (**14)**



Stigmasterol **(15)**

β-sitosterol (**14**) and stigmasterol (**15**), frequently occurring phytosterols, have been studied for their effect on blood cholesterol and benign prostatic hyperplasia (Ikeda and Sugano, 1983; Becker *et al*., 1993; Wilt *et al.*, 1999; Saeidnia *et al*., 2014). Plant, fungal and microbial metabolites are therefore important in the battle against pathogens.

2.2 ARECACEAE

The *Arecaceae* (Palm Trees) are a family of perennial climbers, shrubs, acaules, and trees also called Palmae. They are monocots in the order *Arecales* consisting of 81 genera, distributed around tropical regions of the world, are very closely related.They are closely related clans and subdivided into about 2, 200 species (Asmussen *et al.*, 2006; Martins *et al*., 2014).

The Palmae are very important trees used as food products e.g. coconut products, oils, dates, palm syrup, ivory nuts, carnauba wax and construction materials: (Rattan cane, raffia, and palm wood). Human uses for this family are legion (Yu *et al.*, 2008; Gruca *et al*., 2014). Very popular members of this family are *Cocos nucifera* (Coconut palm) and *Elaeis guineesis* (Oil palm) (Plate 1.). One important genus in the large family is Raphia.

2.2.1 The Genus *Raphia*

The *Raphia* palms are tropical trees that belong to the family *Arecaceae* and consists of about 20 species. Members are found throughout tropical Africa (West Africa to Madagascar) and in South America (Brazil) (Douglas, 1958; Edem *et al*., 1984; Rainey *et al.*, 2009). Scientific reports and investigations have shown that *Raphia* palms are

indigenous to West Africa, specifically along the swampy areas of the tropical forests (Keay *et al.*,1960; Moore, 1975; Otedoh, 1977; Otedoh, 1981; Ndon, 2003).

Contrary to the posit by Obahiagbon *et al*. (2007) and Obahiagbon (2009)**,** *Raphia* palms are found throughout Nigeria; in the drier northern regions, though, they thrive only along banks of water bodies (Russel, 1965)**.** The *Raphia* is among the eleven indigenous genera of the palm trees found in Nigeria. The *Raphia* palm is hapazanthic; that is, after a period of vegetative growth, it produces flowers and fruits only once and dies subsequently (Obahiagbon, 2009). Russel (1965) describes the leaves as being the largest in the plant kingdom. They require high rainfall per annum, high temperature, high relative humidity and long hours of sunshine for proper growth and development (Otedoh, 1977; Ndon, 2003). Fibre from the plants is utilised in many crafts. Most importantly they are tapped to produce a wine unrivalled in taste among all the palms (Russel, 1965; Edem *et al.,* 1984; Ibegbulem *et al.,* 2013). Members of the *Raphia* genus include:

1. *R. africana* Otedoh
2. *R. australis* Oberm. *and* Strey
3. *R. farinifera* (Gaertn.) Hyl.
4. *R. gentiliana* De Wild.
5. *R. hookeri* G. Mann *and* H. Wendl.
6. *R. laurentii* De Wild.
7. *R. longiflora* G. Mann *and* H. Wendl.
8. *R. mambillensis* Otedoh
9. *R. mannii* Becc.
10. *R. matombe* De Wild.
11. *R. monbuttorum* Drude
12. *R. palma-pinus* (Gaertn.) Hutch.
13. *R. regalis* Becc.
14. *R. rostrata* Burret
15. *R. ruwenzorica* Otedoh
16. *R. sese* De Wild.
17. *R. sudanica* A. Chev.
18. *R. taedigera* (Mart.) Mart.
19. *R. textilis* Welw.
20. *R. vinifera* P. Beauv.



Plate 1. Palms: *Beccariophoenix madagascariensis* (Jum. and H. Perrier)[1], *Elaeis guineensis* (Jacq.)[2], *Cocos nucifera* (L)[3] and *Raphia taedigera* (Mart.)[4].

2.2.2 Traditional Usage of Raphia

Sap obtained from the *Raphia* palms has been reported to play active roles in human physiology and health; it is used to ameliorate symptoms of malaria, measles, and jaundice. (Obahiagbon, 2009) reported that the sap aids the flow of milk in nursing mothers. Together with other species of *Raphia* like *R. farinifera*, *R. hookeri*, *R. regalis*, *R. sudanica*; *Raphia africana* is consumed: the cooked fleshy part of fruit, the mesocarp (which is very bitter) is eaten by a small subset of the Nigerian population (the Tiv of Central Nigeria) and in parts of South-Western Cameroon (Russel, 1965). The cooked mesocarp is used for treating mouth ulcers, stomachache and for treating symptoms of heart disease like heart ache. Uncooked fleshy part of the fruit is taken with palmwine in parts of South-Western Cameroon. The fruits are used as fish poison in Nigeria and Cameroon. The sap of the *Raphia* palm is made into a delicious palm wine consumed by millions in Africa (Obahiagbon, 2009). The larva of *Oryctes owariensis* (Plate 2.) which feeds on dead trunks of *Raphia* palms is relished as a delicacy in parts of Tivland (Central Nigeria and South-Western Cameroon) and Igboland (Eastern Nigeria) (Onyeike *et al.*, 2005). The leaves are used for roofing, rope making, designing costumes for masquerades, making baskets, mats, brooms, etc (Foss, 1978a). The principal petiole is used for construction of chairs, beds, ladders, hut rafters, toys, fishing tools, and drying platforms (Yapna *et al.*, 2013). It is true that before the advent of industrialisation, products from *Raphia* palms were used in almost every aspect of social life; in many rural areas in West Africa this remains true even up till date (Douglas, 1958; Russel, 1965; Foss, 1978; Nicklin, 1980; Faparusi, 1981; Edem *et al.*, 1984). As swamps are

being destroyed for rice farming, oil exploration and construction projects; the conservation status of many *Raphia* species is unknown (Oboutor *et al*., 2007; Mitsch *et al.,* 2008; Sandy and Bacon, 2008; Rainey *et al*., 2009; Muafor *et al*., 2014). Also as many other commercial products replace aspects of *R. africana's* once ubiquitous utility, the plant has sadly become ignored and largely disappeared in many areas. *Raphia* plants are critically endangered in many areas (Udofia, 2009).

2.2.3 Phytochemistry of Raphia

The *Raphia* palm sap is reported to be composed of sugars, proteins, titrable organic acids, alcohol, vitamins (ascorbic acid, thiamine, riboflavin, etc), mineral elements and water (Obahiagbon *et al.,* 2007).

Phytochemicals like alkaloids, saponins, flavonoids and phenols have been identified in the seeds and pulp of *Raphia hookeri* (Okwu and Nnamdi, 2008). Some vitamins (Vitamins A, E, thiamine, riboflavin and niacin) as well as some toxic elements (Pb, Hg, Cd and As) have been identified in the seeds and pulp of the *Raphia hookeri* fruit (Okwu *and* Nnamdi, 2008). Obahiagbon (2009) reported the presence of the following elements in the sap of *Raphia hookeri* palm namely Ca, Cl, Cu, Fe, K, N, Na, Mg, Mn, P, and Zn. Opute (1978) reported the fatty acid constituents of some *Raphia* species (Table 2.1.). *Raphia hookeri* is the most studied member of the genus (Edem *et al.,* 1984; Imogie *et al*., 2007; Ogbuagu, 2008; Umoren and Ebenso, 2008; Afolayan *et al.,* 2014); *Raphia africana* has been largely neglected or probably misidentified by researchers as another member of the *Raphia* genus.

A study of the seed and mesocarp oils of some Raphia species showed them to have higher levels of unsaturated fatty acids than other palms (Table 2.1 and Table 2.2) (Opute, 1978).

Yapna *et al.* (2013) have isolated eight compounds: [diosgenin (**16**), ellargic acid (**17**), diosgenin 3-O-α-Lrhamnopyranosyl(1→2)-β-D-glucopyranoside (**18**), catechin (**19**), epiafzelechin (**20**), β-sitosterol (**21**), aurantiamide acetate (**22**), and crotonic acid (**23**)] from seed coats of *Raphia farinifera* (Structure 6).

2.2.4 Raphia africana *(Description and Geographical Spread)*

Raphia africana (Arecaceae) is commonly found in the rain forests of Nigeria and Cameroon and along riverbanks and streams in the drier regions of West Africa (Majekodunmi, 2016). The plant is smaller than other members of its genera (16-25 metres), it grows up to 5 - 10 metres tall and has large compound pinnate leaves (13 - 17 metres long), slightly smaller than other *Raphia* members reported to have the longest leaves in the plant kingdom. The leaves of some *Raphia* plants grow up to 25 m (82.38 feet) long and 3 m (9.84 feet) wide (Burkill, 1985). The plants are either monocarpic, flowering once and then dying after the seeds are mature; or hapaxanthic, with individual stems dying after fruiting but the root system remaining alive and sending up new stems (Otedoh, 1977; Otedoh, 1981). The plant is now being cultivated for its many uses (Huxley and Griffiths, 1992).

Table 2.4: Fatty acid composition of some Raphia fruit coat and seed

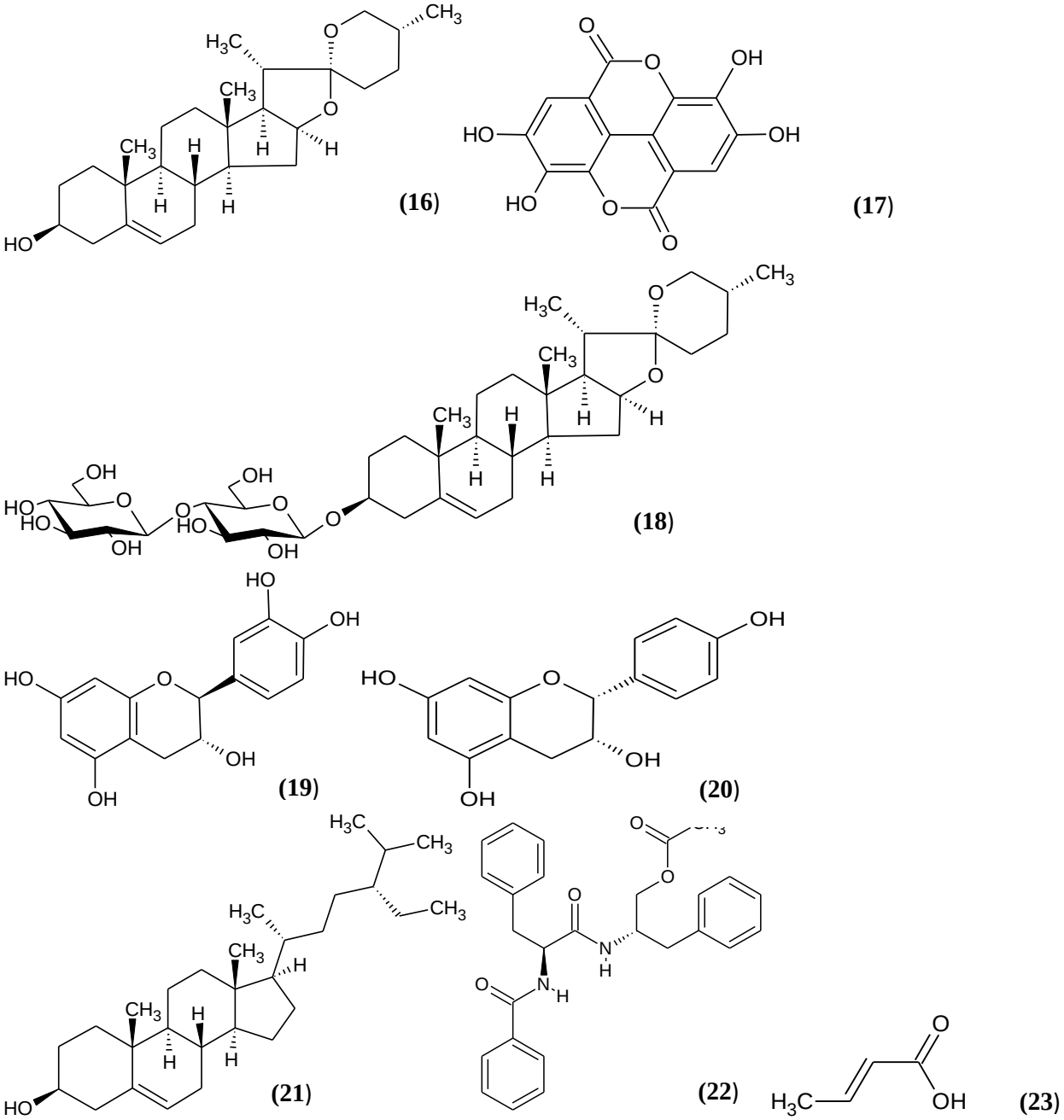
Raphia Species	Fatty acid %																	
	Capryloyl (8:0)		Capriyl (10:0)		Lauryl (12:0)		Myristoyl (14:0)		Palmitoyl (16:0)		Stearoyl (18:0)		Oleoyl (18:1)		Linoleoyl (18:2)		Linolenoyl (18:3)	
	Seed	**Fruit coat**	**Seed**	**Fruit coat**	**Seed**	**Fruit coat**	**Seed**	**Fruit coat**	**Seed**	**Fruit coat**	**Seed**	**Fruit coat**	**Seed**	**Fruit coat**	**Seed**	**Fruit coat**	**Seed**	**Fruit coat**
Raphia sudanica	trace	✕	trace	-	0.8	-	1.7	-	23.0	29.7	1.8	1.6	31.1	39.7	32.5	12.6	4.2	1.8
Raphia hookeri	trace	✕	trace	-	1.0	-	1.2	0.5	27.0	32.3	3.9	5.2	35.8	13.2	28.8	47.3	1.6	1.1
Raphia farinifera	trace	✕	trace	✕	0.7	✕	1.1	✕	28.3	✕	3.7	✕	22.9	✕	38.6	✕	2.5	✕
Raphia vinifera	3.1	✕	trace	-	trace	trace	1.9	trace	38.7	35.2	4.5	2.6	35.6	32.8	14.7	29.8	trace	trace
Raphia regalis	7.3	✕	trace	-	4.0	trace	6.3	trace	25.6	17.0	5.3	33.2	19.3	48.8	30.5	trace	trace	trace

Key: ✕= no data provided for field; - = not detected; Source: (Opute, 1978).

Table 2.5: Fatty acid distribution in Raphia mesocarp oils

Raphia sp.	Per cent fatty acid methyl esters									
	Lauryl	Myristoyl		Palmitoyl			Stearoyl	Oleoyl	Linoleoyl	Linolenoyl
	12:0	14:0	15:0	16:0	16:l	17:0	18:0	18:l	18:2	18:3
R. hookeri	t	0.5	t	32.3	0.6	t	5.2	13.2	47.3	1.1
R.sudanica	t	t	t	29.7	0.6	t	1.6	39.7	26.6	1.8
R. regalis	t	t	1.1	17	t	t	33.2	48.8	t	t
R. vinifera	t	t	t	35.2	t	t	2.6	32.8	29.8	t

Key: t=trace; Source: (Opute,1978).



Structure 6: Compounds Isolated from *R. farinifera* (Yapna *et al*., 2013)

2.2.5 Vernacular names of *Raphia africana*

There is much confusion among botanists about the proper nomenclature for this group of interesting palms. Among the peoples of Nigeria, these vernacular names are used for different species at times interchangeably (Otedoh, 1981). The Yoruba call 'them' *eriko*; the Bini *oha*; *ekian* among the Urhobo; Itsekiri, *ebeje*; the Igbo call *Raphia, ude*; the Ibibio *'iya or aya'* and Ijaw, '*bou'* (Russel, 1965).

For *Raphia hookeri*, the vernacular names are, *ori* (Igede), *ichol* (Tiv), *nduvui* (Mende); *adobe*, (Twi, Fanti, Ga, Ashanti); *doka* (Nzima); and *ala* (Ewe); *oguro*, (Yoruba); ngwo, (Igbo); *ukot idien*, (Ibibio); and *koro*, (Ijaw).

Raphia sudanica which is more common in northern Nigeria, is called *tukurwa* (Hausa); paje (Fulani); *eba* (Nupe); and *ichol* (Tiv) (Russel, 1965). These vernacular names do not however satisfy all the classification demands by the over 20 *Raphia* species and are used interchangeably at times (Hockings *et al.*, 2015).

2.3 Vacuum Liquid Chromatography

Vacuum liquid chromatography (VLC) is a suction aided chromatography technique. It has emerged as a very important tool for the rapid fractionation of extracts prior to high performance liquid chromatography (HPLC) and isolation (Sticher, 2008). VLC employs different stationary phases, such as alumina, silica gel, reversed phase silica gel, nitrile, diol and polyamide gels. While VLC has been used in conjunction



Plate 2. *Raphia africana* (Otedoh) palms, Ukoko Creek, Mbaade, Benue State, Nigeria

with other purification steps like gel filtration as reported by Tor-Anyiin *et al*. (2015), it has been in many cases the only separation step (Dikko *et al.,* 2016).

Most columns use a sintered glass funnel (porosity 3), packed under vacuum to a height of 5 cm onto which pre-adsorbed sample on silica gel is added. The columns are eluted initially with hexane [or any least polar member (s) of a solvent system] while gradient increments of the ethyl acetate [or more polar member (s) of a solvent system] component are added gradually in 1% gradient ratios or 5% gradient increments (Anyam *et al.,* 2015).

2.3.1 Gel filtration or Gel permeation Chromatography

Gel filtration chromatography is a technique that separates molecules according to differences in size as they pass through a gel medium regulated filtration column (Rouessac and Rouessac, 2007). Thus it is called size exclusion chromatography or molecular sieve chromatography. It becomes gel permeation chromatography when an organic solvent is used as the mobile phase. It is well suited to situations where sensitivity to pH may be a problem. It can also separate sample components by partition between the stationary and mobile phase (which may be dependent on the solvent used) (Moore, 1964; Trathnigg, 1995).

Sephadex LH-20 is a gel filtration medium commonly utilised in the purification of natural products. It is unique in being hydrophilic and lipophilic and hence swells in water and many organic solvents. It has been used in the isolation of steroids, flavonoids, terpenoids, lipids and low molecular weight peptides.

The compounds are usually separated by a form of liquid/liquid partitioning or

absorption chromatography. It is made from crosslinked hydroxypropylated dextran beads that have a polysaccharide network. It has been utilised both as an initial purification step and final purification step (Da Silva and Parente, 2010; Tor-Anyiin *et al.,* 2015).

2.4 Hyphenated Techniques in Compound Isolation

2.4.1 High Performance Liquid Chromatography (HPLC)

HPLC is a chromatographic technique that employs a pressurised mobile phase to enhance column efficiency (Karger, 1997). Like every other chromatographic method, it exploits differences in analyte-mobile phase and analyte-stationary phase affinities which leads to different analyte flow rates (retention times). Pressures ranging from (50-350 bar) are used. Column dimensions are usually within 2.1 to 4.5 mm (diameter) and 30-250 mm (length) (Middlesworth and Cannell, 1998). The combination of smaller sorbent particles (2-50 micrometres) and high pressure give HPLC very high resolving power, hence a popular chromatographic technique (Bucar *et al*., 2013).

Time of emergence of a specific analyte from a column is called its retention time. Retention times observed under specific conditions like column type, mobile phase kind, pressure, temperature, and flow rates are considered important identifying characteristics of analytes. A suitable column method is usually arrived at on a trial and error basis (Sundström *et al.,* 2013).

Wide varieties of detectors are used on HPLC machines. Multiple detectors are sometimes used on one machine. Common detectors are Ultra Violet (UV)-visible

absorbance detector, refractive index detector and a mass spectrometer (Gerber *et al.*, 2004).

2.4.2 Gas Chromatography – Mass Spectrometry (GC-MS)

GC-MS is an analytical method that, as the name implies, employs both gas chromatography and mass spectrometry for separation and identification of analytes. It is an excellent technique for known and unknown samples (Sarker and Nahar, 2012).

The gas chromatograph utilises a mostly 30 m long capillary column with stationary phases selected to promote separation of analytes. Here again as is the case with other chromatographic techniques, differences in the analytes-stationary phase affinity aids separation. The mass chromatograph captures, ionises, accelerates, deflects, and detects molecules eluted from the column. Components are identified by characteristic retention times and fragmentation patterns of mass to charge (M/Z) ratios. Libraries containing hundreds of thousands of previously identified compounds are consulted by the machine to aid identification of analytes (Halket *et al.*, 2005).

2.5 Spectroscopic Techniques

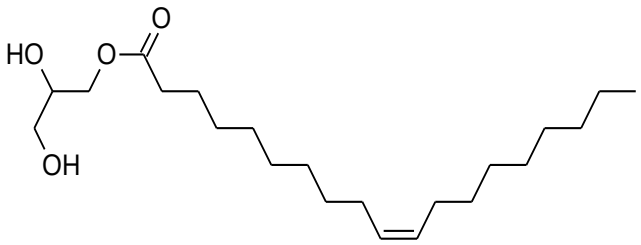
2.5.1 Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear Magnetic Resonance Spectroscopy exploits the magnetic properties of certain atomic nuclei that possess a nuclear spin to provide data on the electronic structure of

a molecule and its functional groups. NMR has come to be a highly tractable and predictable tool for small molecules (Zangger, 2015). It has replaced many time consuming wet methods of identification and is now used to confirm the identity of organic molecules (Guillén and Ruiz, 2003b). NMR spectroscopy operates on the principle of nuclear magnetic resonance; that isotopes of certain nuclei when placed in a magnetic field absorb radio frequency radiation typical of the spin of the nuclei (Yang *et al*., 2015; Sommer, 2015). That spin (1/2) align with and against external magnetic fields leading to a spin state separation. That radiation with energy commensurate with this spin state separation can cause a spin flip (excitation from lower to high energy state). Energy separation between spin states correspond to different magnetic moments that are due to specific electronic environments of nuclei of interest. These electronic environments are due to local magnetic fields generated primarily by local moving electrons (protons) and para contributions (for heavier nuclei) in response to the applied field (Keeler, 2002). Signals arising from different chemical environments in response to the applied fields are reported relative to tetramethylsilane (TMS) ppm. The "ppm" values are arrived at by dividing the local field value (Hz) with the spectrometer (applied) frequency (MHz) and resulting values multiplied by a million to give values also called chemical shifts (Winschel *et al*., 2015).

Chemical shifts are characteristics of a particular molecule and provide detailed information about the structure. NMR has emerged as a valuable and currently unrivalled tool in the analysis of small molecule (Parker *et al*., 2014). Structured determination of glycerides used to be carried out mostly by wet methods, which were non-specific because of problems like chain migration and man-hour loss (Nieva-echevarría *et al*., 2015). Currently, valuable information can be acquired on a

triglyceride detailing its degree of unsaturation, iodine value, stereochemistry, molecular mass and many others by just a few minutes of NMR generated data (Crowther, 2008; Nieva-Echevarría *et al.*, 2016). For example, the NMR of monoglyceride (**16**) can be assigned thus:



1-monoglyceride (16)

One would expect nine signals, one for CH_3, methylenes, H-3 acyl, allylic, H-2, bisallylic, olefinic protons, and three for the glyceryl protons (Figure 12). Undisturbed methyls have shifts around 1 ppm, undisturbed methylenes around 1.24 while on the glyceryl moiety withdraws electron density at the Sn-1 and Sn-2 part (Figure 2.1).

2.5.2 Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR) of Lipids

The use of Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR) to study lipids was pioneered 55 years ago in the seminal work by Johnson and Shoolery (1962). They arrived at accurate values for the degree of unsaturation of fatty acids and their triglycerides. They were also able to determine the average molecular weight of triglycerides using area measurements of signals assignable to olefinic protons in fatty acid chains, protons of the glycerol backbone and protons at the methyl chain ends. Iodine value was also obtained (Johnson and Shoolery, 1962);

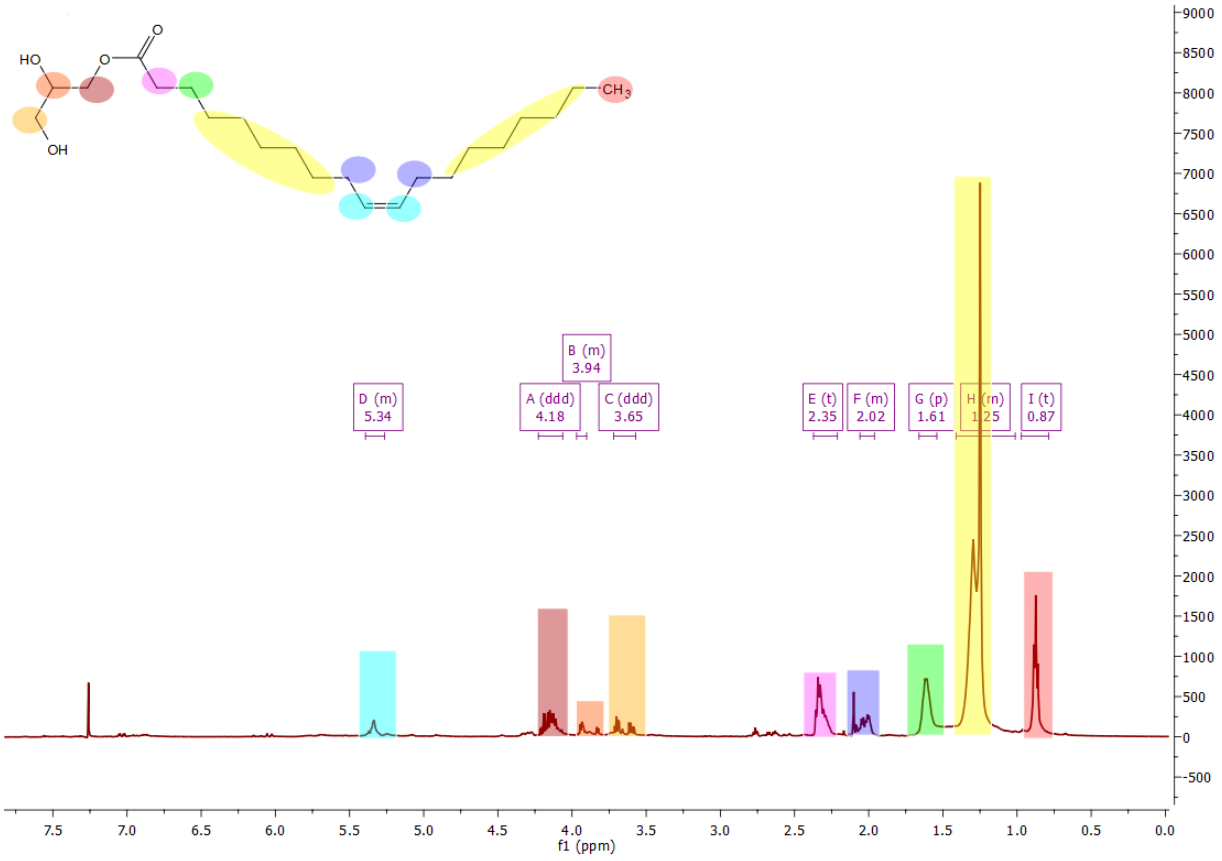


Figure 2.2 [1]HNMR of 1-monoglyceride of nonadecenoic acid [signals have been assigned courtesy of Nieva-echevarría *et al.* (2015)].

these values, which were very similar to those, obtained on the same sample by the lengthy and laborious method of Wij's.

Several studies have validated [1]H-NMR techniques in the study of fats and oils as these NMR techniques show very high agreements with those obtained by other methods (Guillén and Ruiz, 2001; Guillén and Ruiz, 2003a; Sopelana *et al.*, 2013; Ibargoitia *et al.*, 2014; Nieva-echevarría *et al.*, 2014; Nieva-echevarría *et al.*, 2015). High resolution NMR techniques have been employed in qualitative and quantitative aspects in assessing acylglycerol contents (Siciliano *et al.*, 2013). One major advantage of NMR over other spectroscopic methods is its accuracy, as it does not

involve transformation of matrices; only facile sample preparation/handling and short analysis time (Nieva-Echevarría *et al.,* 2015).

A comprehensive survey of journals showed that very few have used NMR in the analysis of fats and oils as most analysis rely on GC or HPLC, wet chemical methods or electrochemical methods (Sedman *et al*., 2010). NMR is excellent for the analysis of fats and oil because it provides equivalent information without the stress of cumbersome wet chemical methods (Nieva-Echevarría *et al*., 2015).

Naturally, occurring triglycerides present in a wide variety of isomeric combinations that can be hard to resolve with proton NMR alone. ^{1}H-NMR can however provide valuable information on the average chain length and average degree of unsaturation for triglycerides (Crowther, 2008). These are critical in defining physical properties of triglycerides as well as their health implications (Prasain *et al*., 2015).

2.5.3 Infrared Spectroscopy of Lipids

One major advantage of Infrared Spectroscopy (IR spectroscopy) over other spectroscopic techniques in the analysis of lipids is its ability to identify the presence of trans-fatty acids (Crowther, 2008).

The principal utility of fourier transform infrared spectroscopy (FT-IR) in the study of fats and oils is to characterise rather than to determine the exact fatty acid composition (Guillén *et al.,* 2003). The most popular measured characteristic from FT-IR spectra of fats and oils has been the iodine value (IV) which can be determined from the *cis* double bond peak at 3006 cm^{1} (Guillén and Cabo, 1997). Other important

attributes of FT-IR of fats and oils is also the amount of free fatty acids in oil samples where a peak at 1744 cm^1 denotes the carbonyl of triglycerile esters while that at 1711 cm^1 implies the carbonyl of free fatty acids (Ismail *et al*., 1993).

2.6 Microwave Assisted Extraction (MAE)

Microwave Assisted Extraction (MAE) uses electromagnetic radiation from the microwave region (2450 MHz) in a more selective and rapid (within 30 minutes) fashion (Chan *et al*., 2011). MAE has reportedly better recovery than traditional extraction processes like soxhlet extraction, maceration and supercritical fluid extraction. It has been championed as a green method because it enables significant reduction in solvent usage and energy.

Chan *et al.* (2014) have developed a microwave assisted extraction model based on total amount of microwave energy absorbed per unit volume during an extraction that is useful for scaling up processes, estimating suitable extraction times and determination of equilibrium-absorbed energy density. This predictive model can be used to determine the range of extraction conditions prior to the optimisation study thereby making it possible to utilise MAE profiles for different microwave machines setups by adjusting the applied microwave power to the required absorbed power density value.

MAE has been carried out using domestic microwave ovens and while being less efficient to commercial extractors, customized microwave systems are essential to improve the performance of commercial varieties (Young, 1995; Pednekar *et al.*,

2011; Gao *et al.,* 2012; Das *et al.,* 2013). In addition, the high cost of commercial microwave extractors has made use of modified domestic ovens attractive. It is important to note that MAE is influenced strongly by solvent nature, extraction time, temperature, microwave power, sample characteristic, solvent feed ratio and effect of stirring (Ganzler *et al.,*1990).

The method has been used for the extraction and isolation of several natural products (Wang *et al.,* 2010; Zou *et al.,* 2012; Chan *et al.,* 2011; Crawford *et al.,* 2011; Chan *et al.,* 2014; Shimbe *et al.,* 2016).

It is considered a very promising technique in natural product isolation (Xiao *et al.,* 2008).

MAE of natural products started in the 1980s and has become the most popular and cost effective extraction method today (Chan *et al.,* 2011; Routray and Orsat, 2012).

2.7 Lipids and Lipid Biosynthesis

Christie (2012) has defined lipids as fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds. Triglycerides are made up of three fatty acids attached to a glycerol backbone. They are non-polar liquid molecules. Polyunsaturated fatty acids (PUFA) like α-linolenic acid (ω-3) and linoleic acid (ω-6) are termed essential dietary nutrients since humans cannot biosynthetically produce them. In fact, a diet low in PUFA is deemed a potential health hazard (Ailhaud *et al.,* 2006).

2.7.1 Fatty Acids

Fatty acids are defined as the simplest and most fundamental lipids found in nature. They exist in nature as the deep protonated, carboxylate ion or esterified to glycero lipids, glycero phospholipids, wax esters, cholesterol esters, and saccharolipids (Murphy, 2015).

2.8 Bacteria

Bacteria are prokaryotic microorganisms (few micrometres in length) (Komba *et al.,* 2013; Igietseme *et al.,* 2014; Falgenhauer *et al.,* 2016). Bacteria come in all shapes and sizes. Some are rod-like, some spiral-like or oval shaped or spherical. Bacteria are known to exist in all manner of habits; even nuclear waste is exempt. Bacteria are able to maintain symbiotic relationships with animals and plants. They form a biomass that exceeds that of animals and plants put together (Bhuvaneswari *et al.,* 2014).

They have been variously applied to bioremediation efforts, mining, fermentation processes, antibiotics manufacture, and sewage treatment and so on (Rubin *et al.,*

2012; Rubin *et al.,* 2015). Bacteria are also responsible for numerous ailments of humankind like pneumonia, skin infections, sexually transmitted diseases, gastritis, upper respiratory tract infection, eye infections, gastritis, food poisoning, urinary tract infections and bacteria meningitis among a host of others diseases (Figure 2.2.). Antibiotic resistance has become a problem; stimulating fears of an impending antibiotic apocalypse (Capone *et al.*, 2013; Falgenhauer *et al.,* 2016; Liu *et al.,* 2016; Malhotra-Kumar *et al.,* 2016; Zhi *et al.,* 2016). Some very commonly isolated clinical isolated human pathogenic bacteria are *Staphylococcus aureus*, *Escherichia coli*,

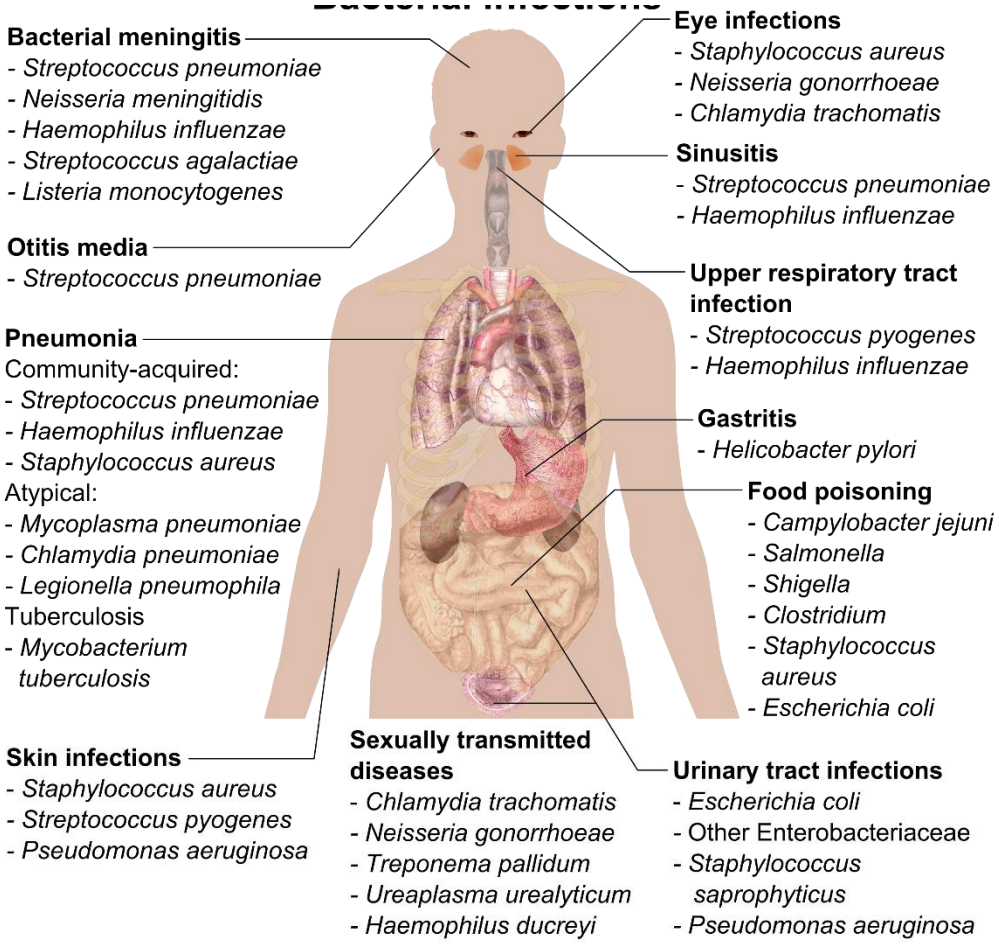


Figure 2.3: Overview of bacterial infections

Klebsiella pneumoniae, *Shigella dysenteriae*, *Salmonella typhi* and *Pseudomonas aeruginosa*. *E.coli* is a very frequently opportunistic human pathogen (Rubin *et al.*, 2015). It is reported to cause about 90% of urinary tract infections, many diarrhoeal infections, meningitis and sepsis (newborns) (Adetutu *et al.*, 2012).

2.8.1 *Shigela dysenteriae*

Shigela dysenteriae is a rod shaped non-spore forming, non-motile, gram negative and facultative anaerobic bacterium (Chakraborty *et al.*, 2001). It is responsible for the

most severe form of dysentery. It is principally spread by unsanitary conditions. Dysentery due to *S. dysenteriae* causes malnutrition, central nervous system problems, anaemia and renal failure. Many *in vitro* studies have been carried out on this pathogen using elements of Nigeria ethnobotany but no *in vivo* human studies have been trialled yet.

2.8.2 *Salmonella typhae*

Salmonella typhae is a bacterium that grows in the blood and gastro intestinal tracks. Symptoms include high fever, weakness, headaches, mental confusion and vomiting. It is spread principally through poor hygiene and poor sanitation.

Bacterial infections are usually treated with antibiotics; today the main thrust of natural products chemistry is finding agents against the rising tide of antibiotic resistance. Another major worry in antibiotic resistance is that caused by fungi (San-Blas and Calderone, 2008).

2.9 Fungi

Pathogenic fungi are responsible for fungal infections (Nandagopal *et al.,* 2011). Immunocompromised patients are especially susceptible to fungal infections. Fungi cause very stubborn infectious diseases like ringworm and athlete's foot.

Species of the Candida constitute the most common opportunistic human pathogens causing vaginal (yeast infections), oropharyngeal (oral thrush). Candidemia or invasive candidiasis occurs when the blood becomes infected. Some frequent fungal pathogens are *Candida albicans*, *Aspergillus fumigatus*, *Aspergillus niger*, *Microsporum sp* and *Trichophyton rubrum* (San-Blas and Calderone, 2008).

Antifungal resistance has become a problem especially in patients with compromised systems. Antifungal agents like Amphotericin-B have side effects on the liver and kidneys (Melkusova and Bujdakova, 2004).

Anti-fungal agents with side-effects are now being combined with plant derived natural products to ameliorate harmful effects of these drugs (Ogungbe *et al.*, 2012).

2.10 Brine Shrimp Lethality Assay

Brine shrimp lethality bioassay is used widely by scientists as a cytotoxicity test of bioactive compounds (Wu, 2014). This assay which utilises brine shrimp (Artemia salina) was introduced by Michael *et al*. (1956) and modified by Van Walbeek (1971), Vanhaecke *et al*. (1981) and Wu (2014). The bioassay has been used in the evaluation of toxicity of natural products which may indicate the effectiveness of a drug. It is regarded a useful tool in preliminary toxicity screening prior to experiments on higher animals (Wu, 2014).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

The solvents (hexane, chloroform, ethyl acetate and methanol) were obtained from Sigma-Aldrich® through their vendors in Nigeria and were of analytical grade. The solvents were redistilled to remove possible traces of impurities and dried using anhydrous $MgSO_4$ (administered until observation of snow-globe effect) then finally run through a column of silica gel to further dry. Solvents were maintained in sealed glass bottles before use. Silica gel 60 F_{254} for Thin Layer Chromatography Plates and Silica gel G were purchased from Merck® KGaA, Germany; column type silica gels were re-activated at 120 ºC for two h before use. Sephadex LH-20 was also used in the separation procedures. Used sephadex LH 20 was cleaned between column runs using commercial bleaches containing sodium hypochlorite and re-equilibrated using increasing amounts of methanol in water until only methanol.

3.2 Equipment

An Agilent GC-MS supplied with a HP-5MS 5 % phenyl siloxane column (number 19091S-433) was used for part of pre-isolation analysis at the Department of Chemical Engineering, University of Ilorin. HPLC analysis was performed using a Perkin Elmer

HPLC with UV-Visible spectra focus detector, P4000 pump, and injector-autosampler AS4000 belonging to the Lighthouse Petroleum Engineering Company limited, Warri Delta State, Nigeria.

A Thermo Fisher Scientific FT-IR Spectrometer (Nicolet IS10) in Multi User Scientific Research Laboratory (MUSRL) ABU, Zaria was used to acquire the FT-IR data for the isolated compounds.

Agilent (Varian) 500MHz for ^{1}H 125 MHz for ^{13}C NMR spectrometers were of the Royal Melbourne Institute of Technology, Melbourne, Austrailia and Multinuclear NMR spectrometer (Varian INOVA 500 MHz NMR system) from The University of Alabama, in Huntsville, USA were used to acquire NMR spectra of the isolated compounds.

3.3 Sample Collection

Ripe fruits were collected from grooves of Raphia along the tributaries (Streams: Ukoko, Uatsor and Kungwa) of river Dura in Mbaade, Buruku Local Government Area of Benue State, Nigeria (Longitude 7.141949 N, Latitude 9.209512 E) in December 2011. Fruits and fronds from the trees were taken to the Department of Forest Production and Products, University of Agriculture, Makurdi, Benue State, Nigeria, for proper identification. It was given the voucher number FHI28200.

The fruits were kept in plastic sacks for two days to facilitate dehulling. The fruits were dehulled and the thin yellow mesocarp scraped with a sharp knife and dried under airy

and shady conditions for three weeks then pulverised in a wooden mortar into a coarse powdered material. The pulverised plant material was placed in plastic containers, properly labelled until further work.

3.4 Extraction Protocol

3.4.1 Extraction 1: Cold Maceration

The plant material (1.0 Kg) was extracted sequentially by cold maceration using petroleum spirit (60-80 °C) (2.5 L) chloroform (2.0 L), ethyl acetate (2.0 L) and methanol (1.5 L) according to methods described by Bucar *et al.* (2013) and Dinan (1998). Extracts (petroleum spirit, chloroform, ethyl acetate and methanol) were concentrated *in vacuo* at 40 – 60 ^{o}C and labelled RCA, RCB, RCC and RCD respectively.

3.4.2 Extraction 2: Microwave Assisted Extraction (MAE)

The plant material (1.0 Kg) was introduced into winchester bottles (2.5 L) and extracted sequentially using an LG *iwave* conventional microwave oven with model number MS2022DB. The oven was set at the defrost function (70 W) and the sample irradiated at three-minute intervals for a total of 30 minutes according to methods (Chan *et al.*, 2011, 2014). Extracts obtained [petroleum spirit (60-80 ^{o}C), chloroform, ethyl acetate and methanol]; labelled RMA, RMB, RMC and RMD respectively, were concentrated *in vacuo* at 40 - 50 ^{o}C and dried in a fume hood until constant weight/volume.

3.4.3 Extraction 3: Soxhlet Extraction.

The plant material (1.0 Kg) was extracted sequentially by soxhlet extraction using Petroleum spirit (60-80 ^{o}C) (2.5 L) chloroform (2.0 L), ethyl acetate (2.0 L) and methanol (1.5 L) according to methods described (Dinan, 1998; Middlesworth and Cannell, 1998). The petroleum spirit, chloroform, ethyl acetate and methanol extracts were concentrated *in vacuo* at 40 – 60 ^{o}C and labelled RA, RB, RC and RD respectively. Extracts were dried in a fume cupboard until constant weight. Portions of these were taken for GCMS, biological assays and phytochemical screening as part of pre-isolation analyses; an attempt at dereplication (Brkljača and Urban, 2011, 2014; Dias *et al.*, 2012; Eyres *et al.*, 2008).

3.5 Sample Analysis

3.5.1 Pre-isolation Analyses

The extracts were subjected to phytochemical screening, high performance liquid chromatography, and gas chromatography mass spectrometry to screen for potential compounds in the plant.

3.5.1.1 Analysis of methanol extracts for flavonoids (HPLC)

The sample was prepared thus:

Dried and pulverised mesocarp (1.0 g) after extraction with chloroform was refluxed with methanol (50 mL, 3 x) and then with 50 % methanol (50 mL, 1 x) for 2 hours. Extracts were combined and concentrated. The residue was refluxed with 10 % HCl (5 mL). The hydrolysate was extracted with ethyl acetate (50 mL, 6 x). The ethyl acetate extract was evaporated to dryness and reconstituted with methanol (10 mL) (Hostettmann *et al.*, 1998).

The sample was separated on a 250 mm x 4.5 mm i.d 5-µm particle, RP – C_{18} Vydae column maintained at 40 ^{o}C.

Kaempferol, vitexin, and orientin used were isolated from known concentrations of bay leaves (*Laurus nobilis*). Quercetin and isoorientin were purchased from Sigma-Aldrich. Calibration curves were constructed for each flavonoid in the range of sample quantity 0.02 – 0.5 µg.

A mobile phase consisting of solvent A (0.05 % trifluoroacetic acid) and solvent B (0.03 % trifluoroacetic acid in 83 % acetonitrile v/v) with the following gradient 0 – 15 minutes, 15 % B in A: 5 -10 minutes, 15 – 70 % B in A; 10 – 15 minutes, 70 % B in A. The flow rate was set at 1 mL/minute and the injection volume 10 µL. The UV detection was at 342 nm.

3.5.1.2 HPLC analysis of Ethyl acetate extract for Phytosterols

Standard solutions (cholesterol, cholestanol, campesterol, stigmasterol, β-sitosterol and tocopherol) were prepared in dichloromethane for phytosterols. Linearity of dependence

of response on concentration was verified by regression analysis. Identification was based on comparison of retention times and spectral data with standards. Quantification was performed by establishing calibration curves for each compound determined using the standards.

The pulverised sample (3 g) was extracted three times with ethyl acetate (30 mL) for 15 minutes at 400 ^{o}C. The extract was filtered into a pre-cleaned beaker. The acidic compounds were extracted (3 times) with 5% potassium hydroxide (10 mL). The basic compounds were extracted with 5% HCl (10 mL). Extracts were concentrated to 30 mL, before centrifuging at 6000 rpm for 10 minutes. After removal of suspended particles, solvent was removed by evaporation and residue dissolved in methylene chloride for chromatographic analysis (Hostettmann *et al.,* 1998).

HPLC Analysis

Each sample (2 µL) was injected into the HPLC. The detection wavelength was set at 220 nm. The flow rate was set at 1 mL/min with methanol/water/tetrahydrofuran (94: 5:1, v/v/v), pH 5 (adjusted with acetic acid) as mobile phase.

3.5.1.3 Gas Chromatography Mass Spectroscopy (GC-MS) Profile of Ethyl Acetate Extract

The extracts (2 mg) were comminuted and introduced into chloroform (10 mL) then filtered into clean test tubes. The mixtures (1 µL) were injected into an Agilent GC-MS (Column #1 Agilent 19091S-433 HP-5MS 5 % Phenyl Silox). The injection port

temperature was 325 ^{o}C; the column dimensions: 30 m x 250 µm x 0.25 µm). The flow rate was 79.5 mL/min with pressure of 11.604 psi and run time of 45.75 min.

The identification of components was done based on comparison of retention indices and mass spectra with those of the NIST11 library (Sarker and Nahar, 2012)**.**

3.5.2 Phytochemical Screening

Phytochemical screening was carried out on all the extracts according to methods reported by Odebiyi (1978) and Dinan (1998). These procedures were carried out with very small modifications followed thus:-

3.5.2.1 Fehling's test for free reducing sugars:

A mixture (1:1) of Fehling's solutions A (aqueous solution of copper (II) sulfate) and B (aqueous potassium sodium tartrate in sodium hydroxide) (5 mL) was added to the extract (2 mL) which was dissolved in water and the mixture boiled on a water bath for 5 minutes (Horner and Klufers, 2016).

3.5.2.2 Test for anthraquinone glycosides.

Benzene (10 mL) was added to the extract (0.2 g) and shaken for 5 minutes and the ammoniacal lower phase observed for colour change on addition of 10 % ammonia (5 mL) solution.

For anthraquinone glycosides: the extract (0.2 g) was boiled with dilute sulphuric acid (10 mL), filtered while hot and the filtrate shaken with benzene (5 mL). The benzene layer was separated and to it, 10 % ammonia solution (3 mL) was added and the mixture shaken (Sofowora, 1993; Harris, 2003a).

3.5.2.3 Test for saponin glycosides

Frothing test: the extract (0.2 g) was shaken with water (5 mL) in a test tube for 30 seconds (Sofowora, 1993; Harris, 2003b).

3.5.2.4 Tests for steroids and terpenoids

a) Salkowski's test: the extract (0.5 g) was dissolved in chloroform. Concentrated sulphuric acid (2 mL) was carefully added down the side of the test tube to form a lower layer. Brown interfacial rings were observed in reaction to all extracts (Harris, 2003b).

b) Liebermann - Burchard's test: the extract (0.5 g) was dissolved in chloroform (2 mL) and filtered into a clean, dry test tube. Acetic anhydride (2 mL) was added to the filtrate and shaken. Few drops of concentrated sulphuric acid were added carefully down the side of the tube to form a lower layer (Odebiyi, 1978; Dinan, 1998).

3.5.2.5 Test for tannins

a. Ferric chloride test: the extract (0.2 g) was stirred with water (5 mL) and filtered. To the filtrate (2 mL), in a test tube, two drops of 0.1 % ferric chloride solution were added. b. Lead subacetate test: To the aqueous extract (1 mL), three drops of lead subacetate solution was added and observed for colour change (Odebiyi, 1978; Dinan, 1998).

3.5.2.6 Test for alkaloids

General test: An extract (0.2 g) was stirred with 1% hydrochloric acid (5 mL) on a water bath and to the filtrate (1 mL) was added, 2-3 drops of the following reagents were added in that order: Wagner reagent (solution of iodine in potassium iodide),

Mayer's reagent (potassium mercuric iodide solution), Dragendroff's reagent (potassium bismuth iodide solution) (Odebiyi, 1978; Dinan, 1998).

3.5.2.7 Test for cardiac glycosides

The extract (1.0 g) was dissolved in water. Lead subacetate solution (1 mL) was added and filtered. On the filtrate, the following test was carried out:-

Keller-killiani test (for deoxysugars): To the filtrate was added chloroform (2 mL), the chloroform layer (lower) was separated and evaporated to dryness on a water bath. The residue was dissolved in 3.5 % ferric chloride (3 mL) solution in glacial acetic acid. The mixture was allowed to stand for a minute before it was transferred to a test tube.

Concentrated sulphuric acid (1.5 mL) was added down the side of the test tube to form a lower layer on standing; colour changes were observed (Odebiyi, 1978; Dinan, 1998).

3.5.2.8 Test for flavonoids

Shinoda's test: the extract (0.2 g) was diluted with ethanol. Some pieces of magnesium filing were added followed by dropwise addition of concentrated hydrochloric acid (5 drops). Colour transformations were observed (Odebiyi, 1978; Dinan, 1998).

Sodium hydroxide test: the extract (0.2 g) was dissolved in water (2 mL) and filtered. 10 % aqueous sodium hydroxide (2 mL) solution was added (Odebiyi, 1978; Dinan, 1998).

Ferric chloride test: the extract (0.1 g) was boiled with water and filtered. To the filtrate (2 mL), 10 % ferric chloride solution (2 drops) were added (Odebiyi, 1978; Dinan, 1998).

3.6 Biological Studies

3.6.1 Brine Shrimp Lethality Test

The brine shrimp lethality test was carried out according to the method of Meyer *et al.* (1982) with slight modification. Extracts (45 mg) were dissolved in diethyl ether and allowed to evaporate to dryness and then volumes were made up to (3 mL) to give stock solutions (1500 µg/L). Concentrations of 150 µg/L, 450 µg/L, 750 µg/L, 1050 µg/L and 1350 µg/L were obtained by serial dilution. The experiments were performed in triplicate.

Hatched nauplii were transferred from the hatchery chamber using a micropipette into test tubes and control test-tubes. Test-tubes containing the nauplii were allowed to stand for 24 h and surviving nauplii counted. The number of deaths at each concentration and control were recorded.

LC_{50} at 24 hours was determined at 95% confidence interval using linear regression analysis as described previously (Throne *et al.*, 1995).

3.6.2 Antimicrobial Screening

Test organisms

The following microbes (clinical isolates): *Staphylococcus aureus; Escherichia coli; Salmonella typhi; Shigella dysenterea; Pseudomonas aeruginosa; Klebsiella pneumoniae, Trichophytom rubrum, Microsporum sp., Aspergillus fumigatus* and *Aspergillus niger* were used for the antimicrobial assay. They were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. All microorganisms were checked for purity and maintained agar slants.

3.6.2.1 Cultivation and standardisation of test organisms

Test organisms were taken from agar slants and sub-cultured into bijou bottles containing sterile nutrient broth for bacteria and Sabourand dextrose broth (20 mL) for fungi. They were incubated for 24 hours at 37 ^{o}C. The broth culture was standardised using sterile normal saline to obtain a density of 10^6 cfu/mL for bacteria. A sporulated test fungal spore was harvested with 0.05 % Tween80 in sterile normal saline and standardised to 10^6 spores/mL (Apak and Olila, 2006).

3.6.2.2 Antimicrobial Sensitivity Test

Antibacterial screening was carried out using agar diffusion method as described by Apak and Olila (2006) with slight modifications. Mueller Hinton and Sabourand dextrose agar were the media used for the growth of bacteria and fungi respectively. All media were prepared according to the manufacturer's instructions, sterilised at 121 ºC for 15 minutes, and poured into sterile petri dishes. Plates were allowed to cool, solidify and were stored by inversion. Diffusion method was used for screening extracts. Sterilised Mueller Hinton agar was seeded with 0.1 mL of standard inoculum of test bacteria and Sabourand dextrose was seeded with 0.1 mL of standard inoculum of test fungi. Inocula were then evenly spread over the surface of the media using a sterile swab. A sterile standard cork borer (6 mm) was used to cut a well at the centre of inoculated media. A solution of extract (0.1 mL) of 5 mg/mL concentration was introduced into wells in prepared media. Incubation for bacteria was at 37 ºC for 24 h and at 27 ºC for one week for fungi. Plates were then observed for zone of inhibition of growth and recorded in millimetres (Apak and Olila, 2006).

3.6.2.3 Determination of minimum inhibitory concentration (MIC)

MIC was determined using broth dilution method as described by Vollekova *et al.* (2001) and modified by Usman *et al.* (2009). Mueller Hinton and Sabourand dextrose broth were prepared according to manufacturer's instructions. The broth (10 mL) was dispensed into

test tubes, separated and sterilised at 121 ^{o}C in an autoclave for 15 minutes and allowed to cool. McFarland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Normal saline was prepared and was used to make a turbid suspension of microbes. Normal saline (10 mL) was dispensed into five test tubes, test microbes were inoculated and incubated for 6 h at 37 ^{o}C. Dilution of microorganisms in normal saline was continuously done until the turbidity (1.5 x 10^{6} cfu/mL) matched that of the McFarland scale by visual comparison. Two-fold serial dilution of extract in sterile broth was done to obtain concentrations of 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL and 0.3125 mg/mL. Having obtained different concentrations of extracts in the broth, a standard inoculum (0.1 mL) of the microbes was inoculated in to the different concentrations. Incubation for bacteria was at 37 ^{o}C for 24 h and at 27 ^{o}C for one week for fungi. Test tubes were then observed for turbidity. The lowest concentration of extract in the broth, which showed no turbidity, was recorded as MIC.

3.6.2.4 Determination of minimum bactericidal/fungicidal concentration (MBC/Minimum Fungicidal Concentration (MFC))

MBC/MFC was carried out to determine whether test microbes were killed or only their growth was inhibited at what least possible concentration. Mueller Hinton and Sabourand dextrose agar were prepared by manufacturer instruction, sterilised at 121 ^{o}C for 15 minutes, and poured into sterile petri dishes. Plates were allowed to cool and solidify. Contents of the MIC in serial dilutions were then sub-cultured on to prepared plates and then incubated at 37 ^{o}C for 24 h for bacteria and at 30 ^{o}C for one week for fungi, after which plates were observed for colony growth. MBC/MFC was determined from plates

with lowest concentration of extracts free from colony growth. Results were recorded after 24 h (Vollekova *et al.*, 2001; Usman *et al.*, 2009).

3.7 Chromatographic Purification of compounds

3.7.1 Vacuum Liquid Chromatography (VLC)

Based on observations from the biological studies and pre-isolation analysis, extracts obtained via the soxhlet extraction (deemed less controversial than the conventional microwave assisted technique) were subjected to VLC using pet-ether-ethyl acetate and chloroform-methanol as solvent systems.

Extracts were pre-adsorbed on celite and the solvents removed *in vacuo* until a fine flowing powder was obtained. A sintered glass funnel of 7 cm height by 3 cm diameter with a porosity rating of 3 was used for the VLC experiment. It was loaded uniformly with silica gel G, thin layer chromatography grade, under vacuum. The procedure was as that previously described (Bowden, 1986; Hostettmann *et al.*, 1998; Kakar *et al.*, 2017).

To check for evenness in the column packing, petroleum ether 60 - 80 (40 mL) was added to the column and ran through under suction.

The pre-adsorbed extract was evenly laid atop the silica gel allowing some headspace for the solvent and covered with a filter paper (Whatman number 1) cut to size to prevent erosion of the extract bed by solvent.

Very gentle gradients with gradual increases (1 %) in the polar members of the solvent binaries were utilised in the separation. Fractions (93) obtained from the Pet-ether extract were combined based on thin layer chromatography (TLC) similarity, reduced to 20 fractions.

The chloroform extract derived fractions were very similar to the hexane extract and therefore based on TLC were discontinued.

The ethyl acetate extract (30 mL) furnished 70 fractions. Similar fractions JL 19-25, JL 28-JL 35 were combined and separated again using VLC to give purer fractions.

Based on preliminary observations from the ^{1}H NMR of collected fractions and the rather very oily nature of extracts, ethyl acetate extract (100 mL) was saponified using KOH (2.5 N) as reported previously (Da Mota *et al.*, 2014; Inchingolo *et al.*, 2014; Gu *et al.*, 2016). The non-saponifiables separated by VLC to furnish three fractions JSEA 2, JSEA 30 and JSEA46. The saponification went thus: methanol potassium hydroxide (2.5 N) was added to the oily ethyl acetate extract. The reaction vessel (1 litre round bottomed flask) was and connected to a reflux condenser and the mixture heated at 75 ºC for one hour until complete saponification. Highly saturated sodium chloride solution was added extracted severally (3 times) with diethyl ether. The combined organic phase was washed using water and concentrated under vacuum to yeild an extract for further purification.

Methanol extract (3.5 g) was fractionated using repeated VLC to yield 20 fractions.

3.7.2 Thin Layer Chromatography (TLC)

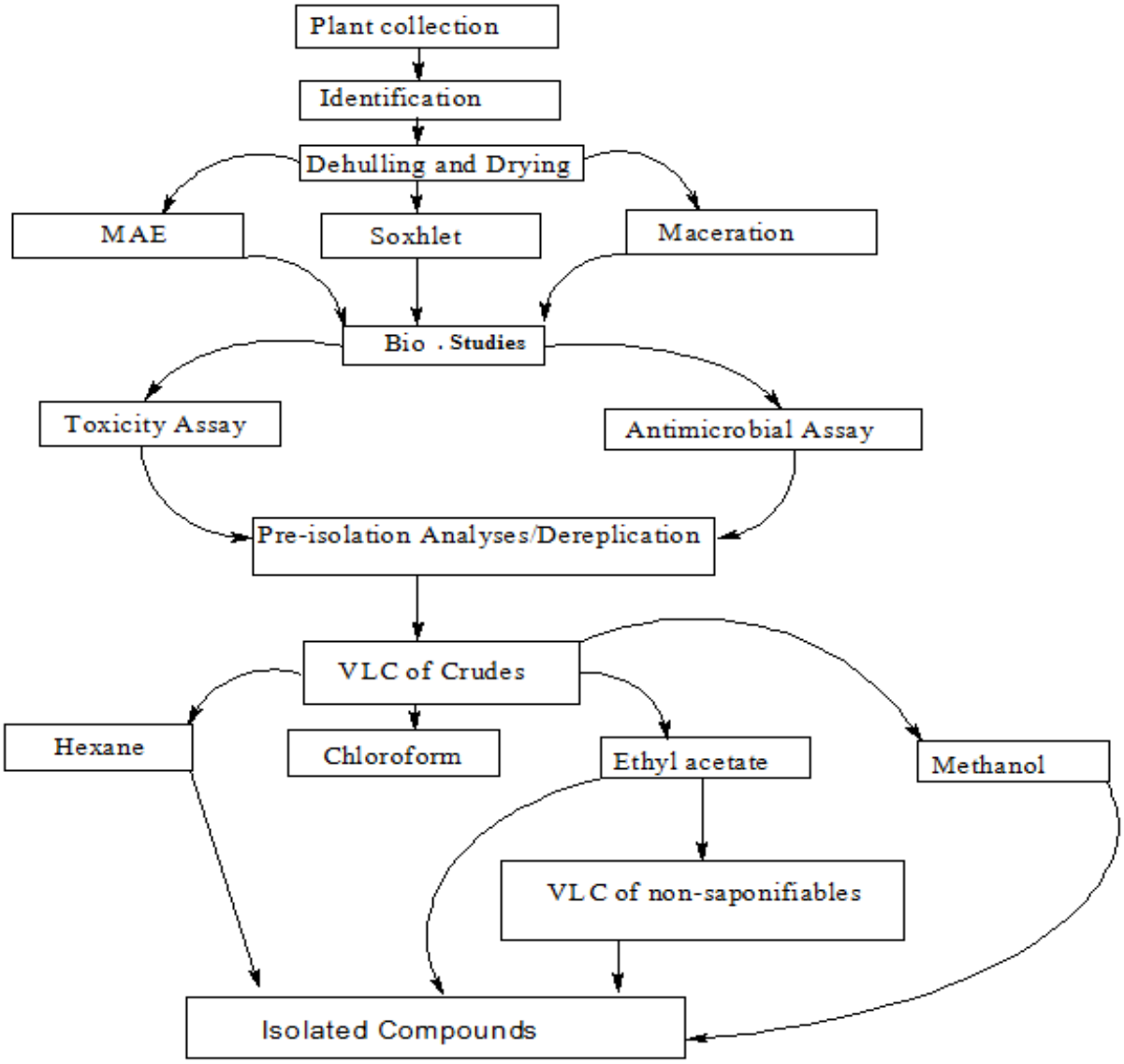
TLC using aluminium backed Pre-coated F_{254} silica gel TLC plates was carried out carried out on fractions obtained from the column. The procedures for TLC were as described by Marston *et al.* (1986); Harris (2003); Sarker and Nahar (2012). In all cases the TLC tank was an improvised setup made up of a 250 mL beaker and a matching glass trough for cover. Plates were cut into 5 cm by 5 cm sizes and marked lightly in pencil (HB) to demarcate a baseline (1 cm from the bottom of TLC plate) and to mark likely points for spoting. Fine bore capillary tubes were used during spotting. A piece of filter paper was placed in the tank to aid saturation of solvent vapour prior to a run. Solvents were prepared afresh after lengthy delays (20 minutes) between runs. The following solvent systems hexane: ethyl acetate (9:1; 8:2; 7:3 or 1:1) and chloroform: Methanol (9:1) were found to produce high degrees of resolution throughout the chromatographic stage and hence duely employed. Liebermann-Burchards reagent was used extensively during the exercise and was prepared thus: acetic anhydride (10 mL) in a beaker placed in an ice bath was mixed with concentrated sulphuric acid (10 mL) and the mixture cautiously added to a tap cooled conical flask of ethanol (100 mL). Fractions of insufficient purity were further purified by gel filtration using sephadex LH-20. Samples (50 fractions) with sufficient TLC purity were sent for spectroscopic analyses (Kakar *et al.*, 2017).

3.8 Spectroscopic Analyses of Isolated Compounds

3.8.1 Nuclear Magnetic Resonance Spectroscopy

Samples were dissolved in deuterated chloroform and depending on the difficulty in spectral interpretation; other NMR experiments were carried out in addition to proton (^{1}H). Carbon (^{13}C), heteronuclear single quantum correlation (HSQC), correlation spectroscopy (COSY) and heteronuclear multiple bond correlation (HMBC) experiment were performed on most of the samples. In most of the cases NMR spectra were processed and analyzed using Mestrenova 6®, Mestrenova 9® and Advanced Chemistry Development (ACDLABS) Chemsketch® software.

3.9 Isolation profile:



Scheme 1: A summary of Methods (Isolation Protocol)

CHAPTER FOUR

4.0 RESULTS

4.1 Extraction

The petroleum ether extracts were the most quantitative [332 mL (113.4 g)] followed by chloroform [247 mL (85.1 g)], ethyl acetate [190 mL (64.2 g)] and methanol in that order (Table 4.1). All extracts had pleasant aromas; those of petroleum ether and chloroform were the most pleasant with the methanol being little pungent. The least polar extracts were pale yellow (petroleum ether) to amber (chloroform) in colour (Table 4.1). The more polar extracts (ethyl acetate and methanol) were darker; methanol (dark brown) being the darkest.

Table 4.1: Yields from Extractions of the Mesocarp of *R. africana*

Extract	Volume	Colour	Precipitation	Weight	Percentage
Petroleum ether	332 mL	Pale yellow	Dense, white, waxy	113.4 g	11.3 %
Chloroform	247 mL	Amber	Light, white, waxy	85.1 g	8.5 %
Ethyl acetate	190 mL	Brown	Clear	64.2 g	6.4 %
Methanol	110 mL	Dark Brown	Clear	38.1 g	3.8 %

Percentage yield was calculated thus:

$$\left[\% = \frac{weight}{1000} \times 100\right]$$

4.2 Phytochemical Screening of the Mesocarp of Extracts of *R. africana*

Phytochemical screening of extracts of the mesocarp of *Raphia africana* are summarized in Table 4.2. Results indicate the presence of the following secondary metabolites: (Hexane extract) - steroids and triterpenes; (Chloroform extract)- saponins, steroid/triterpenes, alkaloids and cardiac glycosides;(Ethyl acetate extract)-free anthraquinones, steroids/triterpenes, alkaloids, cardiac glycosides, anthraquinone glycosides and flavonoids; (Methanol extract)- steroids/triterpenes, alkaloids, cardiac glycosides and flavonoids. The following observations were made for the various phytochemical tests:

4.2.1 Fehling's test for free reducing sugars

Colours observed were hexane, chloroform, ethyl acetate extract (blue); and methanol extract (green) (Table 4.2).

4.2.2 Test for anthraquinone glycosides

The extracts: hexane (colourless), chloroform (sky blue precipitate), methanol (light brown solution) were not positive to the free anthraquinone test except for the ethyl acetate extract (prussian blue solution). The extracts: hexane (colourless), chloroform (cloudy), ethyl acetate extract (blue solution) and methanol (white solution) were not positive to the anthraquinone glycosides test (Table 4.2).

4.2.3 Test for saponin glycosides

All extracts, except the chloroform (little frothing), showed no persistent froth even on warming (Table 4.2).

4.2.4 Tests for steroids and terpenoids

The extracts: hexane, ethyl acetate, chloroform and methanol showed colour reactions, reddish, brownish, brownish and reddish, respectively; indicative of the presence of sterols and terpenes (Table 4.2).

4.2.5 Test for tannins

a. Ferric chloride: Various colour reactions were observed for the extracts; yellow for the methanol and colourless for the hexane, ethyl acetate and chloroform (Table 4.2).

b. Lead subacetate: again, various colour reactions were observed for the extracts; cloudy for the methanol and colourless for the hexane and chloroform; white for ethyl acetate (Table 4.2).

4.2.6 Test for alkaloids

Colour change and precipitation were observed for; precipitation of all the extracts with the above reagents, with the exception of the hexane extract, indicated presence of alkaloids, that is: for Wagner's reagent, a light-brown (methanol and chloroform extract) to brownish precipitate (ethyl acetate extract) was a positive while a yellow solution was observed for the hexane extract. For Mayer's reagent, a creamy white (ethyl acetate extract), orange (chloroform extract), light brown precipitate (methanol extract) and

yellow solution (hexane extract); Dragendroff's reagent, an orange precipitate (ethyl acetate extract), orange solution (chloroform extract), orange precipitate (methanol extract) and yellow solution (hexane extract) (Table 4.2).

4.2.7 Test for cardiac glycosides

A yellow precipitate (positive reaction) was observed for the methanol extract, a creamy solution (positive reaction) for the ethyl acetate extract, a pale solution (negative reaction) for the chloroform extract and a yellow solution (negative reaction) for hexane extract (Table 4.2).

4.2.8 Test for flavonoids

Shinoda's test: Clear solutions (methanol, ethyl acetate, chloroform extracts) were observed while the hexane extract was observed to have a colourless reaction to test reagents.

Sodium chloride test: Colour changes heretofore were observed on basification and subsequent acidification, thus: a pale yellow solution turning colourless on the addition of dilute hydrochloric acid (positive reaction) was observed for the methanol extract, a trace yellow reversible on addition of dilute hydrochloric acid (positive reaction) for the ethyl acetate extract, a colourless solution (negative reaction) for the chloroform extract and a cloudy solution (negative reaction) for hexane extract (Table 4.2).

Ferric chloride test: A green or blue colour indicating presence of phenolic nucleus was observed for. Yellow solutions (negative) were observed in all extracts except the ethyl acetate, which was a trace yellow solution (Table 4.2).

4.3 Antimicrobial Assay of the Mesocarp of Extracts of *R. africana*

Result for antimicrobial sensitivity tests and diameter zone of inhibition for hexane, chloroform, ethyl acetate and methanol extracts of the mesocarp of *Raphia africana* pathogens are presented in Tables (4.3). All extracts exhibited similar sensitivities against test pathogens. The ethyl acetate extract however elicited the widest zones of inhibiton against *S. aureus* (23 mm), *K. pneumoniae* (27 mm), *S. typhi* (24 mm) and *C. albicans* (23 mm). The hexane extract on the other hand produced the weakest inhibition against *E. coli* (20 mm), *K. pneumoniae* (24 mm) and *C. albicans* (20 mm). The chloroform extract was slightly more active against test microbes.

All extracts showed antifungal activity against *C. albicans*. The pathogens *S. dysenteriae*, *P. aeruginosa*, *A. fumigatus*, *A. niger* and *T. rubrum* were however resistant to all the extracts.

4.4 Minimum Inhibitory Concentration for the Mesocarp extracts of *R. africana*

Minimum inhibitory concentration for hexane, chloroform, ethyl acetate and methanol are presented in Table 4.4. The MIC results showed the ethyl acetate extract to have the highest MIC value (0.625 mg/mL) against *K. pneumoniae*. Other MIC values for ethyl acetate were identical to those of hexane, chloroform and methanol extracts.

Table 4.2: Phytochemical Screening of Mesocarp Extracts of *Raphia africana*

Constituents	**Test**	**Observation**				**Inference**			
		CH_3OH	EtOAc	Chloroform	Hexane	Me	Ea	Cf	Hx
Carbohydrates	a. Fehling test	Green solution	Blue solution	Blue solution	Blue solution	-	-	-	-
Anthraquinones	a. Free anthraquinone	Light-brown solution	Prussian blue solution	Sky blue ppt. on boiling	Colourless	_	+	_	_
	b. Anthraquinone glycosides	White lower	Blue lower	Cloudy soln.	Colourless	_	+	_	_
Saponins	a. Frothing test	No frothing	No Frothing	small frothing	No frothing	_	_	+	_
Steroids and triterpenes	Lieberman Burchard's test	Brownish red ring	Brown ring	Brown ring	Brown ring	+	+	+	+
	a. Salkowski test	Red brown ring	Brown ring	Brown ring interface	Reddish brown	+	+	+	+
Tannins	a. Lead subacetate	Cloudy ppt	White ppt	Colourless	Colourless	_	_	_	_
	b. $FeCl_3$ test	Yellow solution	Clear solution	Clear solution	Clear solution	_	_	_	_
Alkaloids	a. Mayer's test	Light-brown	Creamy white ppt	Orange ppt	Yellow soln	+	+	+	_
	b. Wagner's test	Light-brown ppt.	Brown ppt	Brown ppt	Yellow solution	+	+	+	_
	c. Dragendoff's test	Orange ppt	Orange ppt	Light-brown ppt. Orange soln	Orange sol	+	+	+	_
Cardiac glycosides	a. Kella-killiani test	Yellow ppt	Creamy solution	Pale green solution	Yellow solution	+	+	+	_
Flavonoids	a. Sodium hydroxide	Pale yellow	Trace yellow	Colourless	Cloudy	+	+	_	_
	b. $FeCl_3$ test	Yellow soln	Trace yellow	Yellow soln	Yellow sol	_	_	_	_
	c. Shinoda's Test	-ve observation	-----	Clear solution	Colourless	_	_	_	_

Key : +ve = Positive; -ve = negative; soln = Solution; ppt = precipitate; Me=Methanol; Ea = Ethyl acetate; Cf =Chloroform; Hx=Hexane

4.5 Minimum bactericidal concentration and minimum fungicidal concentration for the Mesocarp extracts of *R. africana*

Results of the minimum bactericidal concentration and minimum fungicidal concentration for hexane, chloroform, ethyl acetate and methanol extracts of the mesocarp of *Raphia africana* pathogens are presented in Table 4.5. The best minimum bactericidal value (1.25 mg/mL) was exhibited by ethyl acetate against *K. pneumonieae*. Other extracts (chloroform hexane and methanol) showed similar MBC/MFC values. The methanol extract however had a higher MBC value (2.5 mg/mL) against *S. typhi* than the chloroform (5 mg/mL) and hexane (5 mg/mL) extracts.

4.6 Brine Shrimp Lethality Assay

The Lethality (LC_{50}) values were determined from graphs of probit values versus Log_{10} of concentration values (Appendix XXVIII-A to XXVIII-C). All extracts displayed mild toxicities against the brine shrimp larva (Table 4.6). Ethyl acetate extracts obtained via soxhlet and microwave assisted extraction showed the highest cytotoxicity (269.2 mg/L) while the ethyl acetate extract obtained via cold maceration showed the lowest activity (467.7 mg/L). Details and tables on the LC_{50} calculations are found in the appendices (Appendix XXVIII-A to XXVIII-C).

Table 4.3: Antimicrobial Sensitivity and Diameter of Zone of Inhibition (mm) for Mesocarp Extracts of *Raphia africana*

Pathogen	Hexane	Chloroform	Ethyl acetate	Methanol	Sparfloxacin®	Fluconazole®	Fulcin®
S. aureus	S(22)	S(21)	S(23)	S(20)	S(37)	R	R
E. coli	S(20)	S(21)	S(22)	S(22)	S(35)	R	R
K. pneumoniae	S(24)	S(25)	S(27)	S(25)	S(40)	R	R
S. dysenteriae	R	R	R	R	S(42)	R	R
S. typhi	S(21)	S(20)	S(24)	S(23)	S(37)	R	R
P. aeruginosa	R	R	R	R	S(34)	R	R
C. albicans	S(20)	S(21)	S(23)	S(21)	R	S(35)	R
A. fumigatus	R	R	R	R	R	R	S(32)
A. niger	R	R	R	R	R	R	S(30)
Microsporum sp	S(20)	S(21)	S(21)	S(20)	R	R	S(37)
T. rubrum	R	R	R	R	R	R	S(34)

Key: S = Sensitivity; R = Resistance; (n) = Diameter of Zone of Inhibition in millimetres

Table 4.4: MIC for Extracts of *Raphia africana*

Micro-organisms	**Concentration (mg/mL)**																			
	Hexane extract					Chloroform extract					ethyl acetate extract					Methanol extract				
	5	***2.5***	***1.25***	***0.625***	***0.3125***	***5***	***2.5***	***1.25***	***0.625***	***0.3125***	***5***	***2.5***	***1.25***	***0.625***	***0.3125***	***5***	***2.5***	***1.25***	***0.625***	***0.3125***
S. aureus	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++
E. coli	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++
K. pnuemoniae	_	_	µ	+	++	_	_	µ	+	++	_	_	_	µ	+	_	_	µ	+	++
S. dysenteriae	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я
S. typhi	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++
P. aeruginosa	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я
C. albicans	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++
A. fumigatus	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я
A. niger	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я
Microsporum sp	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++
T. rubrum	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я

KEY: *µ* = MIC, - = No turbidity (no growth), + = Turbid (light growth), ++ = moderate turbidity, +++ = high turbidity; *Я* = pathogen is resistant to extract at this concentration

Table 4.5. MBC and MFC of the Mesocarp Extracts of *Raphia africana*

Micro organisms	**Concentration (mg/mL)**																			
	Hexane extract					**Chloroform extract**					**ethyl acetate extract**					**Methanol extract**				
	5.0000	***2.5000***	***1.2500***	***0.6200***	***0.3125***	***5.0000***	***2.5000***	***1.2500***	***0.6250***	***0.3125***	***5.0000***	***2.5000***	***1.2500***	***0.6250***	***0.3125***	***5.0000***	***2.5000***	***1.2500***	***0.6250***	***0.3125***
S. aureus	***µ***	+	++	+++	+++ +	***µ***	+	++	+++	+++ +	_	***µ***	+	++	+++	***µ***	+	++	+++	++++
E. coli	***µ***	+	++	+++	+++ +	***µ***	+	++	+++	+++ +	***µ***	+	++	+++	++++	***µ***	+	++	+++	++++
K. pnuemoniae	_	***µ***	+	++	+++	_	***µ***	+	++	+++	_	_	***µ***	+	++	_	***µ***	+	++	+++
S. dysenteriae	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я
S. typhi	***µ***	+	++	+++	+++ +	***µ***	+	++	+++	+++ +	_	***µ***	+	++	+++	_	***µ***	+	++	+++
P. aeruginosa	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я
C. albicans	***µ***	+	++	+++	+++ +	***µ***	+	++	+++	+++ +	_	***µ***	+	++	+++	***µ***	+	++	+++	++++
A. fumigatus	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я
A. niger	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я
Microsporum sp	***µ***	+	++	+++	+++ +	***µ***	+	++	+++	+++ +	***µ***	+	++	+++	++++	***µ***	+	++	+++	++++
T. rubrum	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я	я

KEY: *µ* = MBC/MFC, - = No Colony growth, + = Scanty colonies growth, ++ = moderate colonies, +++ = heavy colony growth; я = pathogen is resistant to extract at the concentration

4.7 Thin Layer Chromatography of Crude Extracts

Thin layer chromatography (TLC) of crude extracts (hexane, chloroform, ethyl acetate and methanol) showed the extracts to be very similar [R_f values (0.875/diethyl ether in pet ether 19:1)]. Other spots were hard to decipher (centring roughly on 0.6) based on R_f values as spot lineation was obscured by the rather oily nature of the extracts.

Ethyl acetate and methanol extracts exhibited similar tendencies in the more polar solvent systems (hexane/ethyl acetate, 9:1; 7:3). Here better resolution was attained for spots; occurring around 0.3, 0.4 and 0.5. This behaviour had not been observed in the hexane and chloroform extracts as they had shown a tendency to migrate with the solvent front on application of a more polar solvent system.

4.8 HPLC analysis of methanol and ethyl acetate extracts of *Raphia africana*

Results of HPLC analsis of methanol and ethyl acetate extracts are presented in Table 4.7 and 4.8 respectively. The protocol observed prior to HPLC analysis did not involve a defatting step. The ethyl acetate extract as referred to here is more or less a combination of hexane, chloroform and ethyl acetate extract. Identification of compounds was based on comparison of retention times and spectral data with those of the standards. Quantification of the sterols and flavonoids was done by establishing linear calibration curves for each compound determined using different concentrations (linearised) of the standards.

Table 4.6 Percentage Mortality and Lethal Concentration (LC¬50) of Extracts obtained via Different Extraction Protocols

Crude Extract	Conc (µg/mL)	Mortality %			LC_{50} (µg/mL)		
Ethyl acetate		MAE	SOX	MAC	MAE	SOX	MAC
	1350	100	90	80			
	1050	90	90	70			
	750	60	70	40	269.2	269.2	467.7
	450	60	60	30			
	150	50	40	30			
	0	10	10	10			

Each % mortality value is a mean value of three replicates; MAE=Microwave Assisted Extraction; SOX = Soxhlet Extraction; MAC = Maceration

β-sitosterol was the most prominent sterol (1.32 mg/Kg) in the ethyl acetate followed by stigmasterol (1.22 mg/Kg), tocopherol (0.82 mg/Kg), cholestanol (0.65 mg/Kg), cholesterol (0.36 mg/Kg) and campesterol (0.34 mg/Kg).

Flavonoid content (Table 4.7) in the methanol extract ranged as follows: Isovitexin (0.06 mg/g) and kaempferol (0.06 mg/g) were found to be the most abundant flavonoid principles in *Raphia africana* followed by orientin (0.02 mg/g); isoorientin, vitexin and kaemferol were found in trace quantities (Structure 3 and 4).

4.9 GC-MS Analysis of Ethyl acetate extract of *Raphia africana*

GC-MS profiles of ethyl acetate and methanol extracts are presented in Tables 4.9 and 4.10 respectively. Structures of some of the identified compounds are presented in Structure 1, 2 and 3. Components of the extracts were identified using the National Institute for Standards and Technology (NIST 11) database. Database comparisons of Kovats retention indices and mass spectra of the constituents with those of known compounds led to assignments of Reverse Match Factors (normalised dot products obtained by scaling and comparing experimental mass spectra with library mass spectra) using the Masshunter programme. Match factors were assigned between the values of 999 (perfect match), to 900 or greater (excellent match), to 800 – 900 (good match), to 700 – 800 (fair match); reverse match factors (RMF) less than six hundred were deemed poor matches.

Hexadecanoic acid (38.83 %) and E-9-octadecanoic acid were not only the most abundant principles in the ethyl acetate extract but also had excellent match factors. Other ethyl acetate components were octadecanoic acid (3.79 %), oleic acid (2.68 %), E- 15-heptadecenal (2.44 %), cis hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ester (8.20 %). The 2-monoglyceride of linolenic acid (12.55 %) was the most abundant principle of the methanol extract followed by n-hexadecanoic acid (12.09 %); here however the overarching abundance of one constituent over other principles was much smaller. Other components detected in significant quantity were identified in the methanol extract were phytol (8.27 %), 17-pentatriacontene (3.02 %) linoleic acid ethyl ester (3.56 %), hexadecanoic acid methyl ester, E-15-heptadenal.

In total, 60-80 % of the constituents were identified in the methanol extract. It was observed that while the less polar solvent, ethyl acetate, selected for saturated lipids, the more polar methanol had polyunsaturated lipids and alcohols as major principles.

4.10 Sample (VLC fractions) coding for Antimicrobial and NMR Studies

Samples from the chromatography sessions were re-coded for convenience before being presented for antimicrobial and NMR studies (Table 4.11). The sequence of fraction divergence from the crude extracts is presented in Table 4.12. Since the fruits belong to an edible plant; fractions were tasted and smelt with results ranging from “soapy” to “smoky vegetable oil taste” (Table 4.11).

Table 4.7: Result of HPLC analysis for methanol extract of *Raphia africana*

S/N	Flavonoid	Concentration (mg/g)
1	Isoorientin	Trace
2	Orientin	0.02
3	Vitexin	Trace
4	Isovitexin	0.06
5	Quercetin	Trace
6	Kaempferol	0.06

Table 4.8. Result of HPLC analysis for ethyl acetate extract of *Raphia africana*

S/N	Steroid	Concentration (mg/kg)
1	Cholesterol	0.36
2	Cholestanol	0.65
3	Campesterol	0.34
4	Stigmasterol	1.22
5	β-sitosterol	1.32
6	Tocopherol	0.82

Table 4.9**: GC-MS Results for Ethyl acetate extract of *Raphia africana***

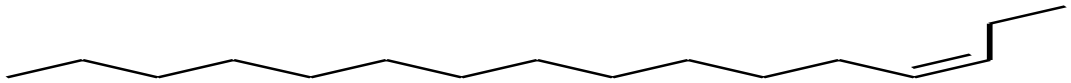
S/N	RT (minutes)	Area (%)	MS-Library Hit	Reverse Match Factor	Match value
1	35.227	1.94	1-Hexadecanol	932	Excellent
2	36.935		trans-13-Octadecenoic acid	754	Fair
3	37.247		Azelaic acid	920	Excellent
4	38.818	2.44	E-15-Heptadecenal	916	Excellent
5	39.524	1.43	Phthalic acid, butyl tetradecyl ester	887	Good
6	39.925	1.95	Hexadecanoic acid, methyl ester	906	Excellent
7	40.269	38.83	n-Hexadecanoic acid	914	Excellent
8	40.369	2.23	1-Eicosene	917	Excellent
9	41.288	12.93	9-Octadecenoic acid, (E)-	936	Excellent
10	41.413	3.79	Octadecanoic acid	872	Good
11	41.576	2.68	Oleic Acid	812	Good
12	42.321	1.82	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	816	Good
13	43.090		17-Pentatriacontene	807	Good
14	43.966		1,3-Dipalmitin trimethylsilyl ether	703	Fair
15	44.084		Octadecanoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis-	729	Fair
16	44.372	8.20	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	913	Excellent
17	44.885	21.74	Di-n-octyl phthalate	870	Good

Key: RT = Retention time; MS = Mass Spectra

Table 4.10: GC-MS Results for Methanol extract *Raphia africana*

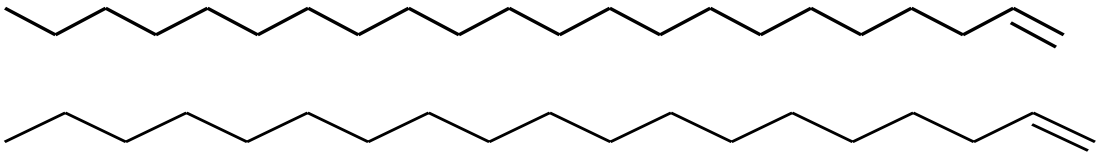
S/N	RT (minutes)	Area (%)	MS-Library Hit	RMF	MATCH VALUE
1	35.227	1.9	1-Hexadecanol	927	Excellent
2	38.811	2.95	E-15-Heptadecenal	902	Excellent
3	39.243	1.61	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	918	Excellent
4	39.456	3.69	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	942	Excellent
5	39.593	2.97	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	942	Excellent
6	39.925	1.84	Hexadecanoic acid, methyl ester	896	Good
7	40.187	12.09	n-Hexadecanoic acid	919	Excellent
8	40.369	6.17	Hexadecanoic acid, ethyl ester	845	Good
9	40.988	1.4	8,11-Octadecadienoic acid, methyl ester	851	Good
10	41.238	2.68	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	819	Good
11	41.282	6.85	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	840	Good
12	41.395	3.56	Linoleic acid ethyl ester	839	Good
13	41.445	3.02	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	846	Good
14	41.576	3.03	17-Pentatriacontene	739	Fair

Key: RT = Retention time; MS = Mass Spectra; RMF = Reverse Match Factor

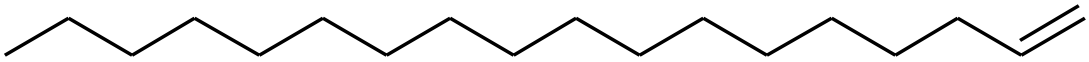


(i)

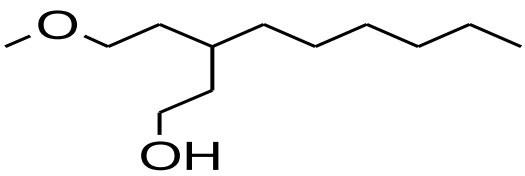
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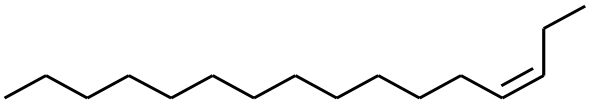


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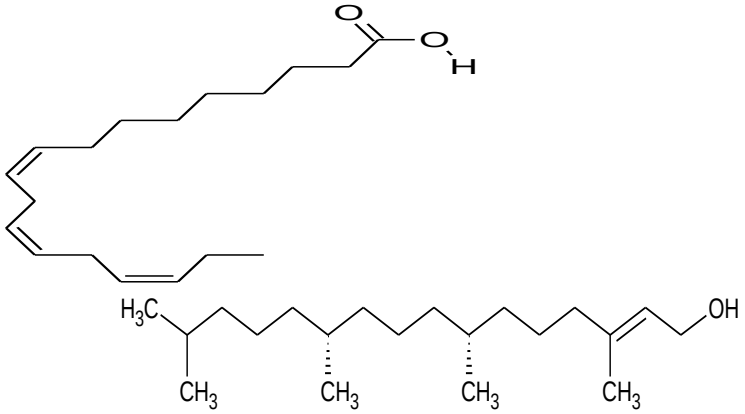


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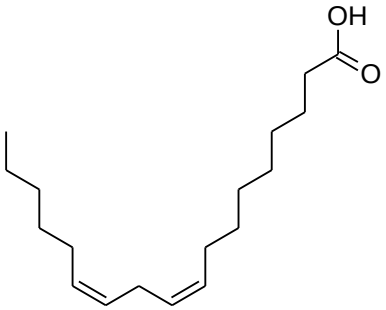


(v) **(vi)**

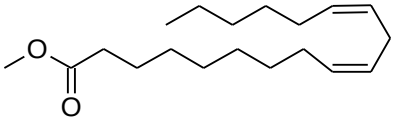


(vii) **(viii)**

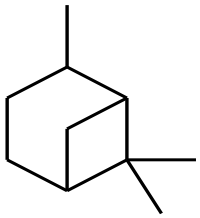
Structure 1: Structures of compounds Identified Using GC-MS: **(i).** (3Z)-3-Hexadecene **(ii).** 1-Docosene **(iii).** 1-Nonadecene **(iv).** 1-Octadecene Octadec-1-ene **(v).** 3-(2-Methoxyethyl)-1-nonanol **(vi).** 3-Hexadecene, (Z) - C16H32 3Z)-3-Hexadecene (**vii)**. 9, 12, 15-Octadecatrienoic acid, (Z,Z,Z) (**viii**) Phytol



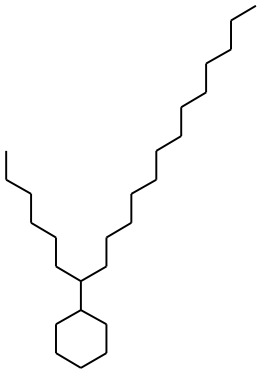
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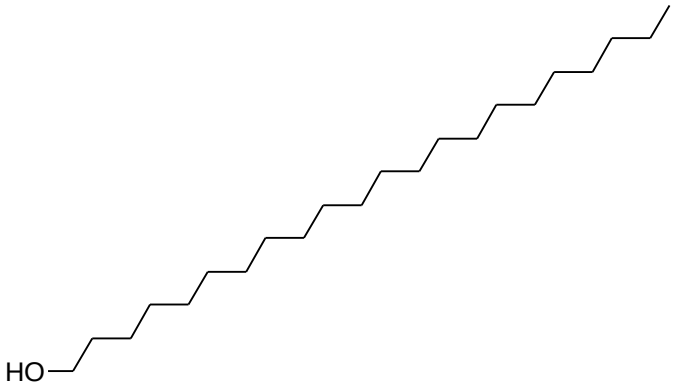
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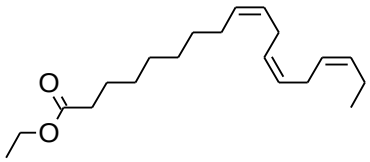
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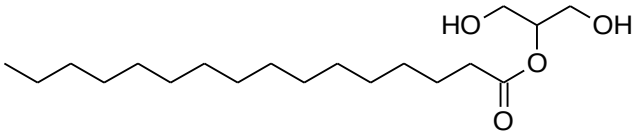
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(v)

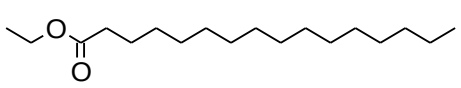


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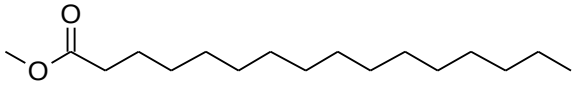


(vii)

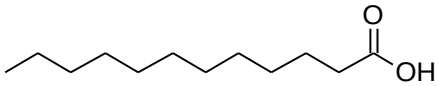
Structure 2: Compounds Identified Using GC-MS: **(i)**. 9, 12-Octadecadienoic acid (Z,Z) **(ii)**. 9, 12-Octadecadienoic acid (Z,Z)-,methyl ester **(iii)** Bicyclo [3.1.1] heptane, 2, 6, 6-trimethyl-, [1R-(1.alpha.,2.beta.,5.alpha.)]- **(iv)**. Cyclohexane, (1-hexyltetradecyl)- **(v)**. Docosanol **(vi)**. Ethyl 9, 12, 15-octadecatrienoate **(vii)**. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester)



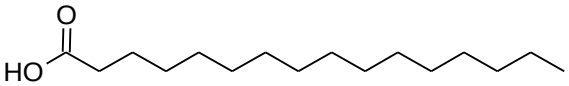
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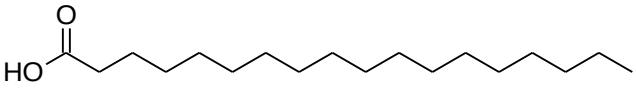
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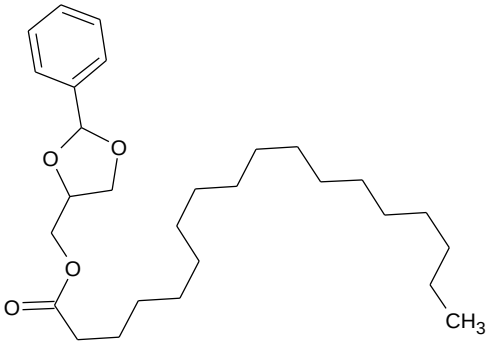


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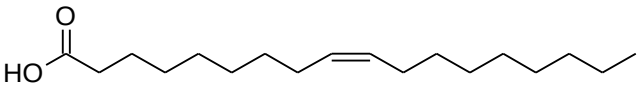
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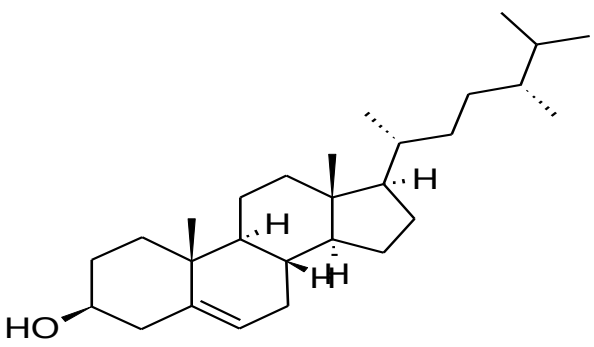
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(vi)

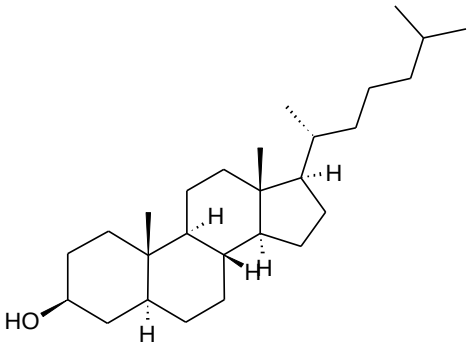


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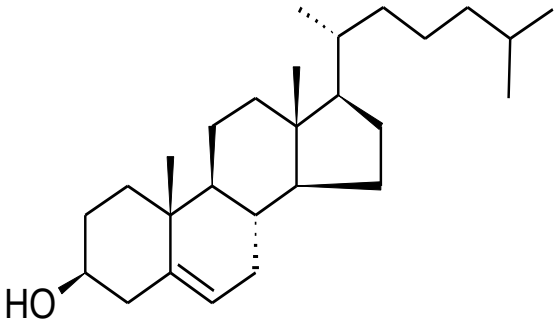
Structure 3: Structures of compounds Identified Using GC-MS: (i). Hexadecanoic acid, ethyl ester **(ii)**. Hexadecanoic acid, methyl ester **(iii**). Lauric acid (**iv**). n-Hexadecanoic acid **(v**). Octadecanoic acid **(vi).** Octadecanoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis- **(vii)**. Oleic acid



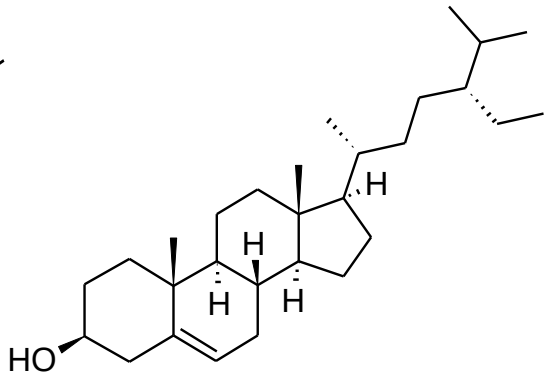
(i)



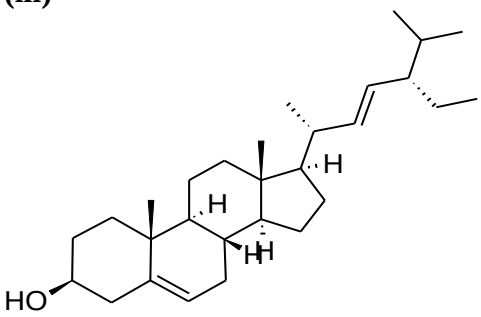
(ii)



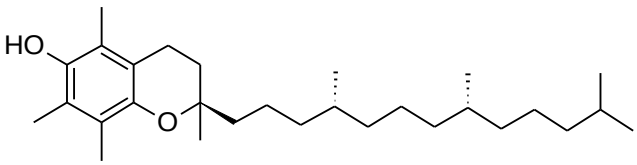
(iii)



(iv)

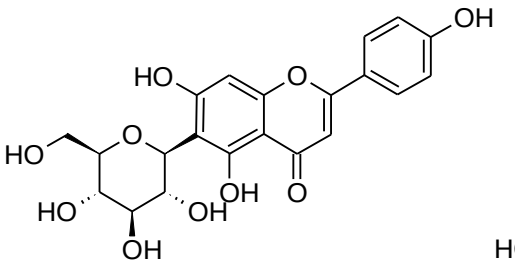


(v)

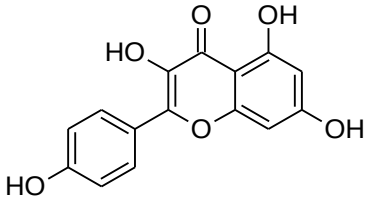


(vi)

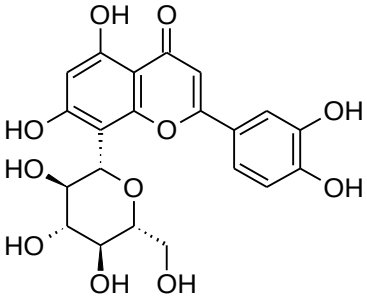
Structure 4: Compounds Identified using HPLC methods: **(i**). Campesterol (**ii)** Cholestanol (**iii**). Cholesterol (**iv**). ß-sitosterol **(v)** Stigmasterol **(vi)** Tocopherol



(i)



(ii)



(iii)

Structure 5: Compounds Identified using HPLC methods: **(i**) Isovitexin **(ii)** Kaempferol **(iii**) Orientin

Fractions from hexane and ethyl acetate (oily) extracts were initially coded "J" (e.g. J1, J2, J3), the fractions from precipitate of the ethyl acetate extract were labelled "JL" (e.g. JL1, JL2, JL3), and the fractions from methanol extract "JML" (e.g. JML1, JML2, JML3) (Table 4.11 and 4.12). Fractions from the saponified and re-extracted ethyl acetate fraction were coded "JSEA" (e.g. JSEA1, JSEA2, JSEA3). Samples were re-coded "Ja" (e.g. Ja1, Ja2, Ja3) prior to NMR spectroscopic analysis.

Fractions and updated corresponding codes are presented in Table 4.11. Fractions (Table 4.11) were also grouped using a range of physical properties (odour, taste and texture).

Table 4.11: Sample coding for NMR analyses based on VLC

Hexane Extract		Ethyl acetate Extract		Ethyl acetate precipitate		Ethyl acetate (Saponified)	
J29	*Ja75*	J59	*Ja83*	JL3	*Ja64*	JSEA2	
J31	*Ja76*	J62	*Ja84*	JL22	*Ja65*	JSEA30	
J35	*Ja77*	J67	*Ja85*	JL33	*Ja66*	JSEA46	
J39	*Ja78*	J69	*Ja86*	JL43	*Ja67*		
J42	*Ja79*	J73	*Ja87*	JL12	*Ja68*	**Methanol Extract**	
J45	*Ja80*	J75	*Ja88*	JL53	*Ja69*	JML40	*Ja96*
J48	*Ja82*	J77	*Ja89*	JL60	*Ja70*	JML44	*Ja97*
		J83	*Ja90*	JL76	*Ja71*	JML62	*Ja98*
		J86	*Ja91*	JL71	*Ja72*	JML69	*Ja99*
		J84	*Ja92*	JL73	*Ja73*	JML68	*Ja100*
		J88	*Ja93*	JL79	*Ja74*		
		J92	*Ja94*				
		J89	*Ja95*				

Italicised codes = Final codes (code on spectra); Non-italicised codes = Initial coding

Smoky vegetable oil taste	Intense sweetness bordering on bitternes
Bitter burnt taste	Waxy taste
Bitter plastic taste	Slightly bitter
Very bitter	Plastic smell
Very sweet with plastic smell	Garlic smell
Soft Waxy solids	Sour and gummy
	Soapy taste

Table 4.12: Chromatographic process

Stage	Petroleum Ether extract	Ethyl acetate Extract	Methanol Extract	Methanol Extract
Stage 1	VLC, 93 fractions	VLC, 70 fractions	VLC, 84 fractions	Saponin rich extract was obtained using variation of the Wall's Procedure (Rothman *et al*., 1952; Rothman *et al*., 1952; Krider and Wall, 1952; Wall and Eddy, 1952)
Stage 2	similar fractions J 25 to J 30 combined based on TLC and run on VLC again	Similar fractions JL 19 – JL 25, JL 28 – JL 35, combined based on TLC and run again on VLC	Fractions JML 55 to JML 60 were combined and run again. JMLA 53 to JMLA 60 were combined= Seph	VLC of saponin rich extract; 62 fractions.
Stage 3	Presented for NMR analysis	Presented for NMR analysis	The fraction "Seph" was further purified by gel filteration to obtain four fractions.	Similar (3) combined on the basis of TLC further purified by VLC and Gel filteration (Sephadex LH 20)
Stage 4		Extract is hydrolised using KOH and Non-"Saponifiables" are extracted and run on VLC; 55 fractions	13 fractions, nine from JML series and four from "Seph" series,	15 fractions still awaiting spectral studies
Stage 5		Spectral studies (NMR) (JSEA 2, JSEA 30, JSEA 46; JSEA 30 is β-sitosterol; JSEA 2 and JSEA 46 are fatty acid methyl esters)	Presented for NMR analysis	

4.11 Antimicrobial assay for isolated compounds

The results of antimicrobial assay (antimicrobial sensitivity and diameter of zone of inhibition) for isolated compounds are presented in **Tables 4.13**; for minimum inhibitory concentration in **Tables 4.14 - 4.16** and minimum bactericidal/fungicidal concentration in **Tables 4.17 – 4.19.**

4.11.1 Antimicrobial sensitivity and diameter of zone of inhibition for isolated compounds

For the antimicrobial sensitivity and diameter of zone of inhibition tests (Table **4.13**), fraction Ja83 was the most effective (30 mm) against *S. aureus* while the pathogen was resistant to the drug Fulcin®. Ja100 was the least effective (22 mm) fraction against *S. aureus.* Ja 83 had the largest (31 mm) diameter of zone of inhibition against *K. pneumoniae* while Ja 82 was least effective (23 mm) against the same pathogen. Ja 83 had yet the highest value against *S. dysenteriae*; Ja 97 (32 mm) was least effective (22 mm). Ja 64 and Ja 82 performed best (28 mm) against *S. typhi* JSEA 30 and Ja 100 performed rather poorly, here having the least (24 mm) values against *S. typhi.* For *P. aeruginosa*, Ja 65 was highest (28 mm); Ja 100 was the least (21 mm). The trio of Ja 77, Ja 82 and Ja 97 were completely ineffective against *P. aeruginosa* at the experimental concentrations. Ja 65 was most effective (27 mm) against *C. albicans* while Ja 100 had the smallest value (20 mm).

In summary, Ja 100 was the least active extract in the tests while Ja 83 shored up the highest values for against clinical isolates. *Aspergillus* species were immune to the activities of isolated compounds.

4.11.2 Minimum inhibitory concentration of isolated compounds

Detailed results for Minimum inhibitory concentration are presented in **Tables 4.14 - 4.16.** Ja 66 and Ja 68 performed similarly against test microbes having their most effective MIC (6.25 µ/mL) against *K. pneumoniae*. Ja 65 had its lowest MIC value (12.5 µ/mL) against *S. typhi, Microsporum spp*. and *T. rubrum* while its best activity (6.25 µ/mL) was against the *S. aureus, E. coli, K. pneumoniae, S. dysenteriae, P. aeruginosa* and *C. albicans*. Ja 75 (**Table 4.15**) had the lowest values for MIC (6.25 µ/mL), on average, showing better activity than any other extract; Ja 65 followed it closely. Ja 77 had a highly chequered activity profile against microbes; it was ineffective against *S. dysenteriae, P. aeruginosa* and *T. rubrum* while showing good activity against (6.25 µ/mL) against *K. pnuemoniae*. Ja 83 **(Table 4.16)** showed uniform activity (6.25 µ/mL) against *S. areus, E. coli, K. pneumoniae, S. dysenteriae* and *S. typhi*; also uniform but less effective (12.5 µ/mL) was their activity against *P. aeruginosa, C. albicans, Microsporum spp*. and *T. rubrum.* But for its activity against *S. dysenteriae,* Ja 100 showed the same activity (12.5 µ/mL) against *S. aureus, E. coli, K. pneumoniae, S. typhi, P. aeruginosa, C. albicans, Microsporum spp*. and *T. rubrum.*

4.11.3 Minimum Bactericidal/Fungicidal concentration

Minimum bactericidal/fungicidal concentration (MBC/MFC) (**Tables 4.17 – 4.19)** for the fractions mostly clustered around 25 μ/mL and 6.25 μ/mL. A few cases (Ja 82, Ja 100, Ja 97, JSEA 30) however had values around 50 μ/mL. Above higher concentrations were required to kill *Microsporum spp.* and *T. rubrum* than other parasites. Ja 66 showed a similar pattern of reactivity to Ja 68 but was in addition to being ineffective against *T. rubrum* and the *Aspergilli* also not effective against *S. dysenteriae*.

The same concentration (50 μ/mL) of fractions Ja 69, Ja 75, Ja 77 and Ja 82 were required to kill *Microsporum spp*.; and 12.5 μ/mL for *T. rubrum*, *C. albicans* and *E. coli*. Ja 77 was however ineffective against *T. rubrum*, *P. aeruginosa* and *S. dysenteriae* (Table 4.18).

Fractions (Ja 83, Ja 97, JSEA 30 and Ja 100) displayed a similar activity profile against pathogens. Ja 100 was an oddity as it showed a realtively impressive MBC/MFC considering that it had shown a relatively weak MIC profile.

Table 4.13: Antimicrobial Sensitivity and Diameter of Zone of Inhibition (mm) for fractions from *Raphia africana*

Pathogen	**Ja 64**	**Ja 65**	**Ja 66**	**Ja 68**	**Ja 69**	**Ja 75**	**Ja 77**	**Ja 82**	**Ja 83**	**Ja 97**	**JSEA 30**	**Ja 100**	Fulcin®
Staphylococcus aureus	S(28)	S(29)	S(26)	S(25)	S(26)	S(24)	S(26)	S(28)	S(30)	S(25)	S(24)	S(22)	R
Escherichia coli	S(29)	S(28)	S(24)	S(23)	S(28)	S(25)	S(24)	S(26)	S(27)	S(26)	S(28)	S(26)	R
Klebsiella pneumoniae	S(26)	S(27)	S(28)	S(27)	S(29)	S(24)	S(27)	S(23)	S(31)	S(28)	S(26)	S(24)	R
Shigella dysenteriae	S(29)	S(27)	R	S(24)	S(27)	S(26)	R	S(27)	S(32)	S(22)	R	S(27)	R
Salmonella typhi	S(28)	S(26)	S(25)	S(26)	S(25)	S(26)	S(26)	S(28)	S(27)	S(27)	S(24)	S(24)	R
P. aeruginosa	S(27)	S(28)	S(24)	S(24)	S(24)	S(25)	R	R	S(24)	R	S(22)	S(21)	R
Candida albicans	S(26)	S(27)	S(26)	S(22)	S(26)	S(24)	S(24)	S(24)	S(24)	S(25)	S(25)	S(20)	R
A. fumigatus	R	R	R	R	R	R	R	R	R	R	R	R	S(32)
A. niger	R	R	R	R	R	R	R	R	R	R	R	R	S(30)
Microsporum sp	S(25)	S(24)	S(24)	S(23)	S(23)	S(21)	S(24)	S(23)	S(21)	S(22)	S(20)	S(21)	S(37)
T. rubrum	S(26)	S(24)	R	R	S(21)	S(22)	R	S(20)	S(24)	S(23)	S(25)	S(20)	S(34)

Key: S = Sensitivity; R = Resistance; (n) = Diameter of Zone of Inhibition in millimetres

Table 4.14: MIC of Ja 64, Ja 65, Ja 66 and Ja 68

Test Microbes	Concentration (µg/mL)																			
	Ja 64					Ja 65					Ja 66					Ja 68				
	50	*25*	*12.5*	*6.25*	*3.125*	*50*	*25*	*12.5*	*6.25*	*3.125*	*50*	*25*	*12.5*	*6.25*	*3.125*	*50*	*25*	*12.5*	*6.25*	*3.125*
S. aureus	_	_	_	µ	+	_	_	_	µ	+	_	_	µ	+	++	_	_	µ	+	++
E. coli	_	_	_	µ	+	_	_	_	µ	+	_	_	µ	+	++	_	_	µ	+	++
K. pnuemoniae	_	_	µ	+	++	_	_	_	µ	+	_	_	_	µ	+	_	_	_	µ	+
S. dysenteriae	_	_	_	µ	+	_	_	_	µ	+	Я	Я	Я	Я	Я	_	_	µ	+	++
S. typhi	_	_	_	µ	+	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++
P. aeruginosa	_	_	_	µ	+	_	_	_	µ	+	_	_	µ	+	++	_	_	µ	+	++
C. albicans	_	_	µ	+	++	_	_	_	µ	+	_	_	µ	+	++	_	_	µ	+	++
A. fumigatus	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я
A. niger	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я
Microsporum sp	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++
T. rubrum	_	_	µ	+	++	_	_	µ	+	++	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я

Key: µ = MIC; Key: - = No turbidity (no growth); + = Turbidity (light growth); ++ = Moderate turbidity; Я = pathogen is resistant to isolated compound

Table 4.15: MIC of Fractions Ja 69, Ja 75, Ja 77 and Ja 82

Pathogens	Concentration (µg/mL)																			
	Ja 69					Ja 75					Ja 77					Ja 82				
	50	*25*	*12.5*	*6.25*	*3.125*	*50*	*25*	*12.5*	*6.25*	*3.125*	*50*	*25*	*12.5*	*6.25*	*3.125*	*50*	*25*	*12.5*	*6.25*	*3.125*
S. aureus	_	_	µ	+	++	_	_	_	µ	+	_	_	µ	+	++	_	_	_	µ	+
E. coli	_	_	_	µ	+	_	_	_	µ	+	_	_	µ	+	++	_	_	µ	+	++
K. pnuemoniae	_	_	_	µ	+	_	_	_	µ	+	_	_	_	µ	+	_	_	µ	+	++
S. dysenteriae	_	_	_	µ	+	_	_	_	µ	+	Я	Я	Я	Я	Я	_	_	_	µ	+
S. typhi	_	_	µ	+	++	_	_	_	µ	+	_	_	µ	+	++	_	_	_	µ	+
P. aeruginosa	_	_	µ	+	++	_	_	_	µ	+	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я
C. albicans	_	_	µ	+	++	_	_	_	µ	+	_	_	µ	+	++	_	_	µ	+	++
A. fumigatus	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я
A. niger	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я
M. sp	_	_	µ	+	++	_	_	_	µ	+	_	_	µ	+	++	_	_	µ	+	++
T. rubrum	_	_	µ	+	++	_	_	_	µ	+	Я	Я	Я	Я	Я	_	_	µ	+	++

Key: µ = MIC; Key: - = No turbidity (no growth); + = Turbidity (light growth); ++ = Moderate turbidity; Я = pathogen is resistant to isolated compound

Table 4.16: MIC of fractions Ja 83, Ja 97, JSEA 30 and Ja 100

Pathogens	Concentration (µg/mL)																			
	Ja 83					Ja 97					JSEA 30					Ja 100				
	50	*25*	*12.5*	*6.25*	*3.125*	*50*	*25*	*12.5*	*6.25*	*3.125*	*50*	*25*	*12.5*	*6.25*	*3.125*	*50*	*25*	*12.5*	*6.25*	*3.125*
S. aureus	_	_	_	µ	+	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++
E. coli	_	_	_	µ	+	_	_	µ	+	++	_	_	_	µ	+	_	_	µ	+	++
K. pnuemoniae	_	_	_	µ	+	_	_	_	µ	+	_	_	µ	+	++	_	_	µ	+	++
S. dysenteriae	_	_	_	µ	+	_	_	µ	+	++	Я	Я	Я	Я	Я	_	_	_	µ	+
S. typhi	_	_	_	µ	+	_	_	_	µ	+	_	_	µ	+	++	_	_	µ	+	++
P. aeruginosa	_	_	µ	+	++	Я	Я	Я	Я	Я	_	_	µ	+	++	Я	Я	µ	+	++
C. albicans	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++
A. fumigatus	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я
A. niger	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я
Microsporum sp	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++
T. rubrum	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++	_	_	µ	+	++

Key: µ = MIC; Key: - = No turbidity (no growth); + = Turbidity (light growth); ++ = Moderate turbidity; +++ = heavy turbidity; Я = pathogen is resistant to isolated compound

Table 4.17: **MBC and MFC of Ja 64, Ja 65, Ja 66 and Ja 68**

Pathogens	**Concentration (µg/mL)**																			
	Ja 64					**Ja 65**					**Ja 66**					**Ja 68**				
	50	***25***	***12.5***	***6.25***	***3.125***	***50***	***25***	***12.5***	***6.25***	***3.125***	***50***	***25***	***12.5***	***6.25***	***3.125***	***50***	***25***	***12.5***	***6.25***	***3.125***
S. aureus	_	_	µ	+	++	_	_	µ	+	++	_	µ	+	++	+++	_	µ	+	++	+++
E. coli	_	_	µ	+	++	_	_	µ	+	++	_	µ	+	++	+++	µ	+	++	+++	++++
K. pnuemoniae	_	µ	+	++	+++	_	µ	+	++	+++	_	_	µ	+	++	_	µ	+	++	+++
S. dysenteriae	_	_	µ	+	++	_	_	µ	+	++	Я	Я	Я	Я	Я	_	µ	+	++	+++
S. typhi	_	_	µ	+	++	_	µ	+	++	+++	_	µ	+	++	+++	_	µ	+	++	+++
P. aeruginosa	_	µ	+	++	+++	_	_	µ	+	++	_	µ	+	++	+++	_	µ	+	++	+++
C. albicans	_	µ	+	++	+++	_	µ	+	++	+++	_	µ	+	++	+++	µ	+	++	+++	++++
A. fumigatus	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я
A. niger	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я
Microsporum sp	_	_	µ	+	++	_	µ	+	++	+++	_	µ	+	++	+++	µ	+	++	+++	++++
T. rubrum	_	_	µ	+	++	_	µ	+	++	+++	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я

KEY: *µ* =MBC/MFC; - = No Colony growth; + = Scanty colony growth; ++ = moderate colony growth; +++ = heavy colony growth; ++++ = very heavy colony growth; Я = pathogen is resistant to isolated compound

Table 4.18: **MBC and MFC of Ja 69, Ja 75, Ja 77 and Ja 82**

Pathogens	**Concentration (µg/mL)**																			
	Ja 69					**Ja 75**					**Ja 77**					**Ja 82**				
	50	***25***	***12.5***	***6.25***	***3.125***	***50***	***25***	***12.5***	***6.25***	***3.125***	***50***	***25***	***12.5***	***6.25***	***3.125***	***50***	***25***	***12.5***	***6.25***	***3.125***
S. aureus	_	**µ**	+	++	+++	_	**µ**	+	++	+++	_	**µ**	+	++	+++	_	_	**µ**	+	++
E. coli	_	_	**µ**	+	++	_	**µ**	+	++	+++	_	**µ**	+	++	+++	_	**µ**	+	++	+++
K. pnuemoniae	_	_	**µ**	+	++	_	**µ**	+	++	+++	_	**µ**	+	++	+++	**µ**	+	++	+++	++++
S. dysenteriae	_	**µ**	+	++	+++	_	**µ**	+	++	+++	**Я**	**Я**	**Я**	**Я**	**Я**	_	**µ**	+	++	+++
S. typhi	_	**µ**	+	++	+++	_	**µ**	+	++	+++	_	**µ**	+	++	+++	_	_	**µ**	+	++
P. aeruginosa	_	**µ**	+	++	+++	_	**µ**	+	++	+++	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**
C. albicans	_	**µ**	+	++	+++	_	**µ**	+	++	+++	_	**µ**	+	++	+++	_	**µ**	+	++	+++
A. fumigatus	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**
A. niger	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**	**Я**
Microsporum sp	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	_	**µ**	+	++	+++	**µ**	+	++	+++	++++
T. rubrum	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	**Я**	**Я**	**Я**	**Я**	**Я**	**µ**	+	++	+++	++++

KEY: *µ* = MBC/MFC, - = No Colony growth, + = Scanty colony growth, ++ = moderate colony growth, +++ = heavy colony growth; ++++ = very heavy colony growth; Я = pathogen is resistant to isolated compound

Table 4.19: MBC and MFC of Ja 83, Ja 97, JSEA 30 and Ja 100

Pathogens	Concentration (µg/mL)																			
	Ja 83					Ja 97					JSEA 30					Ja 100				
	50	*25*	*12.5*	*6.25*	*3.125*	*50*	*25*	*12.5*	*6.25*	*3.125*	*50*	*25*	*12.5*	*6.25*	*3.125*	*50*	*25*	*12.5*	*6.25*	*3.125*
S. aureus	_	_	µ	+	++	_	µ	+	++	+++	_	µ	+	++	+++	µ	+	++	+++	++++
E. coli	_	µ	+	++	+++	_	µ	+	++	+++	_	_	µ	+	++	_	µ	+	++	+++
K. pnuemoniae	_	_	µ	+	++	_	_	µ	+	++	_	µ	+	++	+++	_	µ	+	++	+++
S. dysenteriae	_	_	µ	+	++	µ	+	++	+++	++++	Я	Я	Я	Я	Я	_	µ	+	++	+++
S. typhi	_	_	µ	+	++	_	µ	+	++	+++	_	µ	+	++	+++	_	µ	+	++	+++
P. aeruginosa	_	µ	+	++	+++	Я	Я	Я	Я	Я	µ	+	++	+++	++++	µ	+	++	+++	++++
C. albicans	_	µ	+	++	+++	_	µ	+	++	+++	_	µ	+	++	+++	µ	+	++	+++	++++
A. fumigatus	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я
A. niger	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я	Я
Microsporum sp	µ	+	++	+++	++++	µ	+	++	+++	++++	µ	+	++	+++	++++	µ	+	++	+++	++++
T. rubrum	_	µ	+	++	+++	µ	+	++	+++	++++	_	µ	+	++	+++	µ	+	++	+++	++++

KEY: µ = MBC/MFC, - = No Colony growth, + = Scanty colony growth, ++ = moderate colony growth, +++ = heavy colony growth; ++++ = very heavy colony growth; Я = pathogen is resistant to isolated compound

4.11.4 ^{1}H NMR Spectrum of Ja 64

Ja 64 was obtained as a white waxy solid: The proton NMR data were as follows: ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.34 (t, *J* = 7.5 Hz, 2H), 1.68 – 1.54 (m, 2H), 1.41 – 1.11 (m, 26H), 0.88 (t, *J* = 6.9 Hz, 3H) (*Spectrum detail:* Appendix III.

4.11.5 ^{1}H NMR Spectrum of Ja 65

Ja 65 was obtained as a white waxy solid: The proton NMR data were as follows: ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.34 (t, *J* = 7.5 Hz, 2H), 1.68 – 1.54 (m, 2H), 1.41 – 1.11 (m, 26H), 0.88 (t, *J* = 6.9 Hz, 3H) (*Spectrum detail:* Appendix IV).

4.11.6 NMR Spectra of Ja 66

Ja 66 was obtained as a white waxy solid. The proton NMR data were as follows: ^{1}H NMR: (500 MHz, $CDCl_3$) δ 5.40 – 5.18 (m, 3H), 4.34 – 4.00 (m, 4H), 2.35 (d, *J* = 7.6 Hz, 2H), 2.02 (d, *J* = 5.6 Hz, 4H), 1.65 – 1.60 (m, 2H), 1.28 (d, *J* = 21.9 Hz, 50H), 0.88 (t, *J* = 6.8 Hz, 3H) (*Spectrum detail:* Appendix V-A).

HSQC and HMBC experiment details (*spectral details*: Appendix V-C –Appendix V-C2).

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.18, 130.04, 129.85, 77.16, 68.61, 65.20, 34.27, 32.08, 29.93, 29.85, 29.84, 29.82, 29.79, 29.75, 29.68, 29.64, 29.61, 29.59, 29.52, 29.47, 29.43, 29.40, 29.35, 29.28, 29.25, 29.22, 24.86, 22.85, 14.28, 14.27.

4.11.7 ^{1}H NMR Spectrum of Ja 67

Ja 67 was obtained as a white waxy solid with a strong woody smell (freshly cut wood). It had the following spectral data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.40 – 5.26 (m, 2H), 4.26 – 4.07 (m, 2H), 3.97 – 3.87 (m, 1H), 3.65 (ddd, *J* = 17.2, 11.5, 4.9 Hz, 2H), 2.33 (dt, *J* = 19.7, 7.5 Hz, 2H), 2.04 – 1.95 (m, 2H), 1.63 (dt, *J* = 14.7, 7.4 Hz, 2H), 1.43 – 1.09 (m, 21H), 0.88 (t, *J* = 6.9 Hz, 3H) (*Spectrum detail*).

4.11.8 ^{1}H NMR Spectrum of Ja 68

Ja 68 was obtained as a white waxy solid. Its ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.35 (t, *J* = 7.5 Hz, 2H), 1.70 – 1.56 (m, 2H), 1.28 (d, *J* = 21.3 Hz, 26H), 0.88 (t, *J* = 6.9 Hz, 3H) (*Spectrum detail:* Appendix VII).

4.11.9 NMR Spectra for Ja69

Ja 69 was obtained as a white waxy solid with the following spectral data:^{1}H NMR (500 MHZ, $CDCl_3$) δ 2.35 (t, *J* = 7.5 Hz, 2H), 1.63 (dt, *J* = 14.9, 7.4 HZ, 2H), 1.38 – 1.10 (m, 24H), 0.88 (t, *J* = 6.9 HZ, 3H) (*Spectrum detail:* Appendix VIII).

4.11.9.1 NMR Spectra for Ja69

^{13}C NMR (126 MHz, $CDCl_3$) δ 178.24, 77.16, 33.87, 32.08, 29.88, 29.87, 29.85, 29.84, 29.82, 29.81, 29.79, 29.74, 29.59, 29.51, 29.39, 29.22, 24.86, 22.85, 14.27.

4.11.10 ^{1}H NMR Spectrum of Ja75

Ja 75 was obtained as a white waxy solid. Its ^{1}HNMR (Table 30) showed it to be a triglyceride. Its proton NMR data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.41 – 5.21 (m, 3H), 4.21 (dd,dd, *J* = 17.7, 11.8, 4.9 Hz, 4H), 2.37 – 2.24 (m, 2H), 2.09 – 1.95 (m, 4H), 1.67 – 1.52 (m, 2H), 1.28 (dd, *J* = 23.2, 14.5 Hz, 24H), 0.87 (t, *J* = 6.8 Hz, 3H) (*Spectrum detail:* Appendix X).

4.11.11 ^{1}H NMR Spectrum of Ja76

Ja 76 was obtained a pale yellow semi-liquid. Its proton NMR data [^{1}H NMR (500 MHz, $CDCl_3$) δ 5.41 – 5.23 (m, 2H), 2.37 – 2.25 (m, 2H), 2.08 – 1.96 (m, 2H), 1.63 (dt, *J* = 14.4, 7.1 Hz, 2H), 1.40 – 1.07 (m, 19H), 0.88 (t, *J* = 6.8 Hz, 3H)] (*Spectrum detail:* Appendix XI; Table 4.31).

4.11.12 NMR Spectra for Ja77

Ja 77 was obtained as a white waxy substance with a bland "candle-like" smell. Its proton NMR data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.42 – 5.26 (m, 2H), 4.21 – 4.05 (m, 5H), 2.34 (t, *J* = 7.4 Hz, 2H), 2.07 – 1.96 (m, 4H), 1.63 (dt, *J*= 7.43, 7.43, 15.04 Hz, 2H),1.36 – 1.20 (m, 28H), 0.88 (t, *J* = 6.8 Hz, 6H) (*Spectrum detail:* Appendix XII). Its carbon 13 data:

^{13}C NMR (126 MHz, $CDCl_3$) δ 179.21, 174.09, 130.16, 129.85, 68.53, 65.18 34.26, 34.06, 32.07, 32.05, 31.93, 31.76, 31.67, 29.91, 29.84, 29.80, 29.74, 29.67, 29.60, 29.58, 29.51, 29.47, 29.39, 29.27, 29.24, 29.22, 27.37, 25.78, 25.04, 24.85, 22.84, 22.83, 22.72, 21.23, 14.26 (Spectrum details: Appendix XII – Appendix XII-E).

4.11.13 NMR Spectra for Ja78

Ja 78 was obtained as a whitish waxy substance with a bland "candle-like" smell. Its proton NMR data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.45 – 5.18 (m, 3H), 4.38 – 4.01 (m, 4H), 2.76 (t, *J* = 6.6 Hz, 2H), 2.39 – 2.19 (m, 2H), 2.12 – 1.96 (m, 2H), 1.66 – 1.52 (m, 2H), 1.40 – 1.11 (m, 64H), 0.87 (t, *J* = 6.9 Hz, 9H) (*Spectrum detail:* Appendix XIII; Table 4.33). The total degree of unsaturation was found to be 1. 10

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.07, 130.33, 130.04, 128.18, 128.02, 68.47, 65.15, 34.24, 34.18, 34.07, 32.05, 32.03, 31.65, 29.90, 29.87, 29.83, 29.78, 29.74, 29.72, 29.65, 29.61, 29.56, 29.49, 29.44, 29.40, 29.37, 29.30, 29.28, 29.25, 29.20, 29.17, 29.10, 27.35, 27.30, 25.76, 22.82, 22.70, 14.38, 14.24, 14.19 (spectrum details: Appendix VIII –Appendix VIX).

4.11.14 ^{1}H NMR Spectrum of Ja79

Ja 79 was obtained as a pale yellow waxy substance with a bland "candle-like" smell. Its proton NMR data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.40 – 5.21 (m, 3H), 4.34 – 4.04

(m, 4H), 2.78 – 2.72 (m, 2H), 2.30 (qd, *J* = 12.9, 7.0 Hz, 6H), 2.09 – 1.95 (m, 2H), 1.60 (d, *J* = 6.4 Hz, 6H), 1.45 – 0.98 (m, 44H), 0.86 (t, *J* = 6.7 Hz, 9H) (*Spectrum detail:* Appendix XIV).

4.11.15 ^{1}H NMR Spectrum of Ja80

Ja 80 was also obtained as a yellowish gummy substance with a sweet woody smell. Its proton NMR data:^{1}H NMR (500 MHz, $CDCl_3$) δ 5.39 – 5.28 (m, 2H), 4.18 (ddd, 2H), 3.96 – 3.89 (m, 1H), 3.64 (ddd, 2H), 2.31 (dd, *J* = 24.1, 7.5 Hz, 2H), 2.00 (dd, *J* = 21.7, 9.9 Hz, 1H), 1.60 (d, *J* = 6.3 Hz, 3H), 1.45 – 1.04 (m, 16H), 0.86 (t, *J* = 6.8 Hz, 3H) (*Spectrum detail:* Appendix XV).

4.11.16 1H NMR Spectrum of Ja81

Ja 81 was obtained as a yellowish gummy substance with a sweet woody smell. It had the following ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.39 – 5.26 (m, 2H), 4.18 (ddd, 1H), 3.97 – 3.90 (m, 1H), 3.65 (ddd, 2H), 2.35 (t, *J* = 7.3, 3.4 Hz, 2H), 2.06 – 1.96 (m, 2H), 1.61 (p, *J* = 6.3 Hz, 2H), 1.41 – 1.01 (m, 18H), 0.87 (t, *J* = 6.5 Hz, 3H).

4.11.17 ^{1}H NMR Spectrum of Ja82

Ja 82 was obtained as an amber coloured gummy substance with the following ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.28 (td, *J* = 13.6, 7.5 Hz, 1H), 4.20 – 3.96 (m, 2H), 3.93 – 3.74 (m, 1H), 3.71 – 3.53 (m, 1H), 2.32 – 2.19 (m, 6H), 2.01 (dd, *J* = 31.9, 14.1 Hz,

2H), 1.64 – 1.46 (m, 6H), 1.44 – 1.05 (m, 28H), 0.96 – 0.72 (m, 7H) (*Spectrum detail:* Appendix XVII). Average chain length and total degree of unsaturation were calculated as described by Crowther, (2008) and found to be 12.05 and 0.24 respectively (*Spectrum detail:* Appendix XXIX-J).

4.11.18 ^{1}H NMR Spectrum of Ja83

Ja 83 was obtained as an off-white amorphous substance with the following data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99 (d, *J* = 11.0 Hz, 1H), 6.87 (d, *J* = 8.7 Hz, 1H), 6.01 (d, *J* = 16.9 Hz, 1H), 4.91 (d, *J* = 4.6 Hz, 1H), 4.92 – 4.90 (m, 1H), 4.19 (s, 1H), 4.16 (d, *J* = 4.6 Hz, 2H), 4.14 (dd, *J* = 4.2, 2.0 Hz, 3H), 4.12 (d, *J* = 6.2 Hz, 2H), 3.93 (d, *J* = 8.5 Hz, 4H), 3.82 (d, *J* = 5.0 Hz, 3H), 3.70 (dd, *J* = 4.1, 2.0 Hz, 2H), 3.69 (d, *J* = 2.2 Hz, 2H), 3.60 (d, *J* = 9.4 Hz, 3H), 3.59 (d, *J* = 8.0 Hz, 2H).

4.11.19 ^{1}H NMR Spectrum of Ja95

Ja 95 had the following NMR data: ^{1}H NMR (500 MHz, $cdcl_3$) δ 4.42 (d, *J* = 7.8 Hz, 1H), 4.20 (dd, *J* = 12.1, 4.5 Hz, 1H), 4.16 (d, *J* = 6.1 Hz, 1H), 4.10 (dd, *J* = 15.3, 8.3 Hz, 2H), 3.95 (dd, *J* = 13.3, 5.9 Hz, 3H), 3.92 – 3.86 (m, 8H), 3.81 (d, *J* = 5.9 Hz, 3H), 3.68 (dd, *J* = 11.2, 5.6 Hz, 7H), 3.61 (dd, *J* = 11.3, 6.0 Hz, 1H), 3.58 – 3.31 (m, 3H), 2.39 – 2.32 (m, 4H), 2.31 – 2.25 (m, 1H), 2.17 (s, 4H), 1.85 (dd, *J* = 26.7, 12.8 Hz, 1H), 1.65 (dd, *J* = 15.3, 8.5 Hz, 5H), 1.14 (d, *J* = 6.6 Hz, 2H), 0.97 (dd, *J* = 6.7, 2.5 Hz, 2H), 0.92 (d, *J* = 3.4 Hz, 2H), 0.69 (d, *J* = 8.8 Hz, 2H).

4.11.20 ^{1}H NMR Spectrum of Ja96

Ja 96 was obtained as a white waxy substance with the following ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.34 (t, *J* = 7.4 Hz, 1H), 2.10 (d, *J* = 9.7 Hz, 1H), 1.62 (dd, *J* = 14.3, 7.0 Hz, 1H), 0.88 (t, *J* = 6.7 Hz, 3H), (*Spectrum detail:* Appendix XXII).

4.11.21 ^{1}H NMR Spectrum of Ja 97

Ja 97 was obtained as a white semi-liquid substance. ^{1}H NMR data [^{1}H NMR (500 MHz, $CDCl_3$) δ 4.18 (ddd, 2H), 3.97 – 3.76 (m, 1H), 3.65 (ddd, 2H), 2.33 (t, *J* = 16.8, 7.8 Hz, 2H), 2.16 – 2.02 (m, 2H), 1.68 – 1.56 (m, 2H), 1.40 – 1.13 (m, *J* = 11.6 Hz, 12H), 0.88 (t, 3H)]. ^{1}H NMR spectrum for Ja 97 is shown in Appendix XXII.

4.11.22 ^{1}H NMR Spectrum of Ja 98

Ja 98 was obtained as a white waxy solid with the following NMR data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.22 – 4.10 (m, 2H), 3.98 – 3.86 (m, 1H), 3.67 (ddd, *J* = 8.0 Hz, 1H), 2.35 (t, 2H), 1.70 – 1.47 (m, *J* = 14.8 Hz, 2H), 1.50 – 1.07 (m, 16H), 0.88 (t, *J* = 7.0 Hz, 3H). ^{1}H NMR spectrum for Ja 98 is shown in Appendix XXIII.

4.11.23 ^{1}H NMR Spectrum of JSEA2

JSEA2 was obtained as a clear transparent thin oil. It had a pleasant smell, faintly reminiscent, of coconut oil. It was the second fraction from the saponified ethyl acetate extract. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.35 – 5.21 (m, 1H), 3.60 (s, 3H), 2.24

(t, *J* = 7.5 Hz, 2H) 2.04 – 1.90 (m, 2H), 1.57 (t, *J* = 6.6 Hz, 2H), 1.23 (d, *J* = 22.9 Hz, 21H), 0.82 (t, *J* = 6.8 Hz, 3H). *Spectrum detail*: (Appendix XXVI-XXVI-E6).

4.11.24 ^{1}H NMR Spectrum of JSEA30

JSEA30 was obtained a mass of white needle-shaped crystals with a waxy feel. Its spectral data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.66, 5.29, 5.26, 4.32, 3.58, 3.41, 3.40, 3.38, 3.31, 3.29, 3.27, 3.23, 2.83, 2.70, 2.69, 2.68, 2.65, 2.64, 2.63, 2.51, 2.50, 2.49, 2.47, 2.38, 2.37, 2.35, 2.27, 2.23, 2.22, 2.21, 2.18, 2.16, 1.99, 1.98, 1.96, 1.95, 1.94, 1.93, 1.91, 1.90, 1.89, 1.82, 1.81, 1.80, 1.78, 1.77, 1.74, 1.71, 1.69, 1.68, 1.65, 1.60, 1.59, 1.53, 1.47, 1.44, 1.39, 1.38, 1.35, 1.23, 1.18, 1.12, 0.94, 0.90, 0.89, 0.87, 0.86, 0.81, 0.80, 0.79, 0.77, 0.75, 0.73, 0.71, 0.70. ^{1}H NMR spectrum for JSEA30 is shown in Appendix XXVII.

4.11.25 ^{1}H NMR spectrum for JSEA46

^{1}H NMR (500 MHz, $CDCL_3$) δ 2.40 – 2.18 (m, 2H), 1.61 (dt, *J* = 14.2, 7.1 Hz, 2H), 1.38 – 1.12 (m, 16H), 0.87 (t, *J* = 6.9 Hz, 3H)**.** Details for calculation of average chain length and detailed spectrum are provided in appendices XXIX-R and XXVII respectively.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Phytochemistry

The phytochemistry *Raphia* genus is scantily annotated; very few studies have reported on preliminary phytochemical screening of any member of the genus. In fact, no study was found on preliminary phytochemical screening of the mesocarp of any member of the genus; this is an initial report. Okwu and Nnamdi (2008) have reported on alkaloids, carbohydrates, flavonoids, lipids, phenolic compounds, proteins, saponins, and tannins in the exudates of *Raphia hookeri.*

Flavonoids have been reported to exhibit anti-diarrhoeal, anti-dysentery and antifungal activities (Cowan, 1999). Alkaloids have been implicated in a number of anti-tumour and cytotoxic activities (Wansi *et al*., 2013; Kittakoop *et al.*, 2014; Nwodo *et al.,* 2015). Steroids have been used for their cardiotonic activity with promise for anticancer therapy (Rudkowska *et al*., 2008). Bioactive triterpenes like lupeol and betulin have shown promising anti-inflammatory and anti-cancer activity in studies (Saleem *et al.*, 2004, 2005; Saleem, 2009; Wal *et al.*, 2011; Dikko *et al.*, 2016). Saponins have been demonstrated to possess antiproliferative and antioxidant behaviour Hostettmann and Marston, 1995; (Francis *et al.*, 2002; Skene and Sutton, 2006; Tapondjou *et al*., 2015).

5.2 Antimicrobial activity of crude extracts

Activity of extracts against the clinical isolates is no doubt due to presence of phytochemicals in the plant. Distribution of secondary metabolites in the plant extracts has a direct effect on activity against test microbes; presence of free anthraquinones, steroid/triterpenes, saponins, alkaloids, cardiac glycosides and flavonoids in extracts is positively correlated with antimicrobial properties (Cowan, 1999).

Flavonoids are synthesised by plants in response to microbial infection (Havsteen 1983; Cook and Samman 1996). It has been speculated that their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. Alkaloids are reportedly toxic to microbes often showing good anti-diarrhoeal effects (Cowan, 1999).

5.3 *Artemia salina* lethality assay

It must be noted that activities of extracts are considered significant if their LC_{50} are within the range of ≤ 30 mg/L (Meyer *et al*., 1982). Extracts showed very mild toxicity [soxhlet and microwave assisted extraction (269.2 mg/L); cold maceration (467.7 mg/L)] against *Artemia salina;* this finding is puzzling because of the ichthyotoxic nature of the plant (Adeogun *et al*., 2012). Subject to more detailed animal studies the fruit may, therefore, be considered harmless and friendly to the human palate. The brine shrimp lethality assay showed that extraction protocol had a strong effect on activity of the

extracts against test shrimp. This may be due to effectiveness (more exhaustive) nature of soxhlet and microwave assisted extractions as against the rather gentle cold maceration method. It must be noted here that the brine shrimp lethality assay was carried out to discriminate and select extracts for further study as the extraction protocols provided twelve extracts.

5.4 GC-MS profile of *R. africana* and profiles of some raphia species

Based on the GCMS results (Tables 4.9 and 4.10), *R. africana* shows similar fatty acid content with other members of the Raphia genus (Tables 2.1). On the average the fatty acids profiles tend to cluster around palmitoyl, stearoyl, linoleoyl groups; in this regard *R. africana* very closely resembles *R. vinifera* differing mostly on the linolenoyl content (*R. africana*: 9.6/ *R. vinifera*: trace). In all *R. africana* is unusual in having roughly double the amount (66.9 %) of palmitoyl groups when compared to other *raphia* species [*R. hookeri* (32.3%), *R. sudanica* (29.7 %), *R. regalis* (17 %) and *R. vinifera* (66.9 %)] previously studied by Opute (1978).

5.5 HPLC Profile of *R. africana*

The HPLC profile indicated the presence of eight sterols. Of these only β-sitosterol has been previously isolated by Yapna *et al.* (2013) from seed coats of *Raphia farinifera* (Structure 9 and 10).

5.6 NMR Studies

The discussion on NMR results is interspersed extensively with tables. Where the NMR data obtained was insufficient to completely characterise a compound to a name due to limitations in method an attempt was made to confer a "maximum unique character profile" or characterisation has been aided by simulations using the predict and highlight feature of MestReNova 9®.

The available NMR literature (Simova *et al*., 2003; Vlahov *et al*., 2010; Nieva-echevarría *et al*., 2014, 2015) on glycerolipids involves whole oils. Therefore, such whole-oil signals when reported sometimes show a dilution effect, a variation from those obtained from pure compounds, due to concentration differences. In such a situation, references have been made to older but seminal works by Emeritus Prof. F. D. Gunstone (Gunstone, 1990, 1993; Gunstone *et al.*, 1977); who clearly used ^{13}C NMR and ^{1}H NMR for assignments. Scheme 2 presents the sequence in the approaches that were used in structure elucidation.

5.7 Characterisation of compounds isolated from *R. africana* using NMR methods

Signal areas of all spectra were determined using the Mnova 9 ® software. To avoid inclusion of side bands, special attention (careful phasing) was paid to the cut-off points in all spectral signal integrals for protons.

The mole ratio of components for triglycerides (saturated protons, unsaturated protons, acyl chain-end methyl group, and glycerol backbone), were determined using the following general procedure as previously described by (Crowther, 2008); a slight modification of the method by (Johnson and Shoolery, 1962). Integrals were divided by the number of like substituents in the triglyceride (i.e., one glycerol backbone or three acyl chains), then divided by the number of protons in the substituent (say, three protons per methyl group or two protons per methylene group), and finally divided by a constant (value of one proton) to normalise the integrals. From the normalised integrals, average chain length and average degree of unsaturation were determined.

Most triglyceride shifts for the methine proton on the glycerol backbone (5.26 ppm) and the acyl chain olefinic protons (5.35 ppm) are usually not fully baseline resolved and were integrated together. Since there are four methylene protons in the glycerol backbone (4.10-4.32 ppm) and only one methine, the methine proton integral is a quarter of the methylene integral. The difference between the total area at 5.26 - 5.35 ppm and the area due to the methine proton (taken as a quarter of the glyceryl methylene integral) was therefore attributed to the olefinic protons (Johnson and Schoolery, 1962; Crowther, 2008).

Normalised integrals for acyl chain-end methyl (0.88 ppm), methylene beta to the carbonyl (1.63 ppm), methylene alpha to the carbonyl (2.34 ppm), and methylene in the glycerol backbone (4.10-4.32 ppm) all represent one proton integral in the triglyceride;

normalised proton integrals with values far less than one were taken to be artifacts (Table 5.1 - 5.2).

The average proton integral for triglyceride spectra [an average of adjusted integrals for chain-end methyls (0.88 ppm), methylenes beta to the carbonyl (1.63 ppm), methylenes alpha to the carbonyl (2.34 ppm), and methylenes in the glycerol backbone (4.10-4.32 ppm)] was used to derive the normalised integrals (Crowther, 2008).

Methylene protons of the glyceryl group were, in some cases (were careful phasing was difficult), used for the relative intensity because these group of protons have the shortest T1 (longitudinal relaxation, or relaxation in the z-direction) relaxation times compared to the other protons of glycerides. The normalised proton of the terminal CH_3 group, in this instance, derived as: (the area of peak G/9)/(the area of peak B/4) (Miyake *et al.*, 1998a, 1998b).

The total degree of unsaturation was given by normalised integrals at 5.35 ppm. The bisallylic methylene group at 2.76 ppm (linoleic or other polyunsaturate) was used as an additional identifier (polyunsaturation). The average chain length for triglycerides was calculated by using a linear sum of the carbons in the chain with a factor of two used in

the event there were two carbons represented by the proton integral (Nieva-Echevarría *et al*., 2015).

A spreadsheet using Microsoft Excel 2013® was constructed to repeat these calculations on various spectra (Table 5.3 and 5.4).

Discussion on NMR results was grouped. Fatty acids were treated first, followed by glycerides and finally compounds whose character could only be approximately acertained from the data obtained in this work.

5.7.1 Characterization of Ja 64, Ja 65, Ja 68, and Ja 76 as Heptadecanoic acid

Ja 64's NMR data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.34 (t, *J* = 7.5 Hz, 2H), 1.68 – 1.54 (m, 2H), 1.41 – 1.11 (m, 26H), 0.88 (t, *J* = 6.9 Hz, 3H) Ja 64 was identified as heptadecanoic acid (Margaric acid) based on similar results by Johnson and Shoolery (1962); Shahidi and Wanasundara (2009). The number of carbons was approximated thus: the methylene resonances from peak range 1-3 ppm were taken as two protons per carbon and one carbon per the three methyl protons (0.88 ppm); adding together integrated areas from the spectrum (*Spectrum detail:* Appendix III, Table 5.5), gives [(2.06/2) + (2.15/2) + (26.26/2) + (3.00/3) ≈ 16. Adding the carbon from the carbonyl gave 17 carbon atoms.

Table 5.1: Chemical shift assignments and multiplicities of ^{1}H NMR signals in $CDCl_3$ of protons of glycerides and fatty acids of natural origin (1) [Nieva-echevarría *et al*. (2014)]

Shift (ppm)	Multiplicity	Functional group	
		Type of protons	**Compound**
0.88	t	-C**H**$_3$	Saturated, monounsaturated ω-9 and/or ω-7 acyl groups and FA
0.89	t	-C**H**$_3$	Unsaturated ω-6 acyl groups and FA
0.97	t	-C**H**$_3$	Unsaturated ω-3 acyl groups and FA
1.19–1.42	m[a]	-(C**H**$_2$)n-	Acyl groups and FA
1.61	m	-OCO-CH$_2$-C**H**$_2$-	Acyl groups: TG, except for DHA, EPA and ARA acyl groups
1.62	m	-OCO-CH$_2$-C**H**$_2$-	Acyl groups: 1,2-DG, except for DHA, EPA and ARA acyl groups
1.63	m	-OCO-CH$_2$-C**H**$_2$-, -COOH-CH$_2$-C**H**$_2$-	Acyl groups: 1,3-DG, 1-MG and FA, except DHA, EPA and ARA
1.64	m	-OCO-CH$_2$-C**H**$_2$-	Acyl groups in 2-MG, except for DHA, EPA and ARA acyl groups
1.69	m	-OCO-CH$_2$-C**H**$_2$-	EPA and ARA acyl groups in TG
1.72	m	-COOH-CH$_2$-C**H**$_2$-	EPA and ARA acids
1.92–2.15	m[b]	-C**H**$_2$-CH=CH-	Acyl groups and FA, except for -CH$_2$- of DHA acyl group in β-position in relation to carbonyl group
2.26–2.36	dt	-OCO-C**H**$_2$-	Acyl groups in TG, except for DHA acyl groups
2.33	m	-OCO-C**H**$_2$- , -COOH-C**H**$_2$-	Acyl groups in 1,2-DG, except for DHA acyl groups
2.35	t	-OCO-C**H**$_2$-CH$_2$-	Acyl groups in 1,3-DG, 1-MG and FA, except for DHA acyl groups
2.38	t	-OCO-C**H**$_2$-	Acyl groups in 2-MG, except for DHA acyl groups
2.37–2.41	m	-OCO-C**H**$_2$-	DHA acyl groups in TG
2.39–2.44	m	-COOH-C**H**$_2$-	DHA acid
2.77	t	=HC-C**H**$_2$-CH=	Diunsaturated ω-6 acyl groups and FA
2.77–2.90	m	=HC-C**H**$_2$-CH=	Polyunsaturated ω-6 and ω-3 acyl groups and FA

Key: t: triplet; m: multiplet. a Overlapping of multiplets of methylenic protons in the different acyl groups either in β-position, or further, in relation to double bonds, or in γ-position, or further, in relation to the carbonyl group; TG: triglycerides; DHA: docosahexaenoate; EPA: eicosapentaenoate; ARA: arachidonate; 1,3-DG: 1,3-diglyceride; 1-MG: 1-monoglyceride; FA: fatty acid; 1,2-DG: 1,2-diglyceride; 2-MG: 2-monoglyceride. b Overlapping of multiplets of the α-methylenic protons in relation to a single double bond of the different unsaturated acyl groups; d: doublet. c This signal shows different multiplicity if the spectrum is acquired from the pure compound or taking part in the mixture.

Table 5.2: Chemical shift assignments and multiplicities: ^{1}H NMR signals in $CDCl_3$ of main protons of glycerides and fatty acids of natural origin (2)[Nieva-echevarría *et al*. (2014)]

Shift (ppm)	Multiplicity	Functional group	
		Type of protons	**Compound**
3.73	m[c]	ROC**H**$_2$-CH(OR´)-C**H**$_2$OH	Glyceryl group in 1,2-DG
3.84	m[c]	ROC**H**$_2$-CHOR-C**H**$_2$OH	Glyceryl group in 2-MG
3.94	m	ROCH$_2$-C**H**OR-CH$_2$OH	Glyceryl group in 1-MG
4.05–4.21	m	ROC**H**$_2$-C**H**OH-C**H**$_2$OH´	Glyceryl group in 1,3-DG
4.18	ddd	ROC**H**$_2$-CHOH-CH$_2$OH	Glyceryl group in 1-MG
4.22	dd,dd	ROC**H**$_2$-CH(OR´)-C**H**$_2$OR″	Glyceryl group in TG
4.28	ddd	ROC**H**$_2$-CH(OR´)-CH$_2$OH	Glyceryl group in 1,2-DG
4.93	m	ROCH$_2$-C**H**(OR)-C**H**$_2$OH	Glyceryl group in 2-MG
5.08	m	ROCH$_2$-C**H**(OR)-CH$_2$OH	Glyceryl group in 1,2-DG
5.27	m	ROCH$_2$-C**H**(OR´)-CH$_2$OR″	Glyceryl group in TG
5.28–5.46	m	-C**H**=C**H**-	Acyl groups and FA

Key: t: triplet; m: multiplet. a: Overlapping of multiplets of methylenic protons in the different acyl groups either in β-position, or further, in relation to double bonds, or in γ-position, or further, in relation to the carbonyl group; TG: triglycerides; DHA: docosahexaenoate; EPA: eicosapentaenoate; ARA: arachidonate; 1,3-DG: 1,3-diglyceride; 1-MG: 1-monoglyceride; FA: fatty acid; 1,2-DG: 1,2-diglyceride; 2-MG: 2-monoglyceride. b: Overlapping of multiplets of the α-methylenic protons in relation to a single double bond of the different unsaturated acyl groups; d: doublet. c: This signal shows different multiplicity if the spectrum is acquired from the pure compound or taking part in the mixture.

Table 5.3: Spreadsheet format and formulas used for elucidation of proton NMR spectra

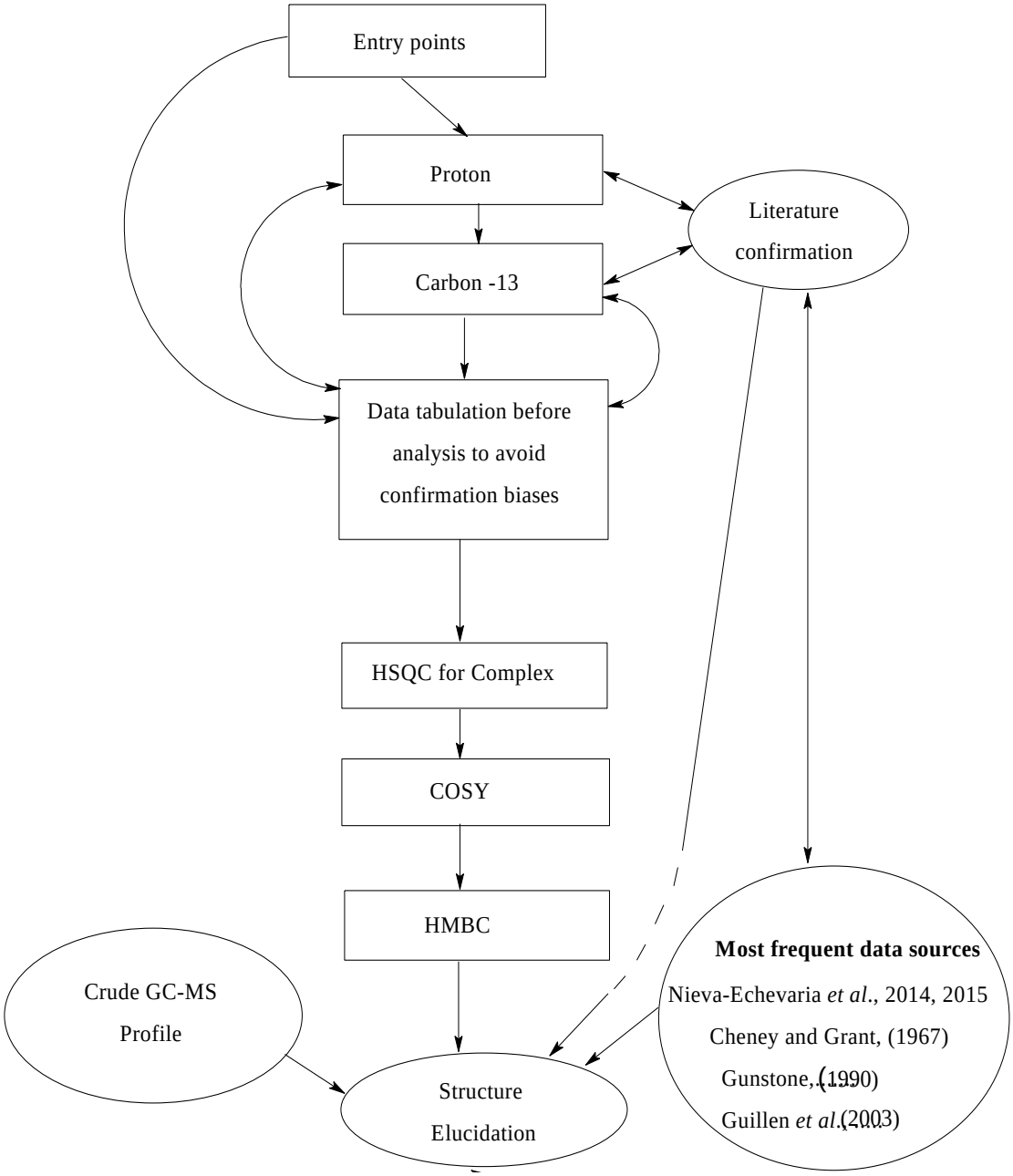
Assignment	Position (ppm)	† Ja 100 Integrals	Ja 100 Adjusted Integral	Normalized Integral	Total Degree of Unsaturation	Mest.Norm .Int
C*H*$_3$ *(G)*	0.9	2369.18	=C3/3/3	=D3/223.8475694	=SUM(E6,E8,E11)	0.98
(C*H*$_2$)n *(F)*	1.3	7865.94	=C4/3/2	=D4/223.8475694		3.7
C*H*$_2$CH$_2$(CO) *(E)*	1.6	1787.32	=C5/3/2	=D5/223.8475694		0.86
CH$_2$CH=CHCH$_2$ *(D)*	2	573.55	=C6/3/4	=D6/223.8475694		0.44
C*H*$_2$(CO) *(C)*	2.3	1461.35	=C7/3/2	=D7/223.8475694		0.71
CHC*H*$_2$CH= *(H)*	2.8	242.08	=C8/3/2	=D8/223.8475694		0.12
C*H*$_2$O *(B)*	4.3	764.66	=C9/1/4	=D9/223.8475694		0.27
C*H*O *(B/4)*	5.3	=C9/4	=C10/1/1	=D10/223.8475694		0.27
C*H*=C*H (A)*	5.4	=314.35-(C9/4)	=C11/3/2	=D11/223.8475694		0.15

Key: **Mest. Norm. Int**. = Normalised integrals as read directly from Mestrenova 9®; C, D and E = Microsoft Excel® cell labels; A, B, C, D, E F, G and H (italicised) = Distinct signal area labels (fatty acid and triglyceride). † Spectral details of fraction Ja 100 were used for this example.

Table 5.4: Spreadsheet format and formulas used for elucidation of proton NMR elucidation

Saturated Presence Test		Total Adjusted Proton	Average Proton Integral	Average Chain Length	Ave. Chain Lgth. Mest
	=STDEV(D3, D5,D7,D10)	=SUM(D3,D5 ,D7,D10)	=D15/4	=SUM(E3,E4,E5,(E6*2),E7 ,E8,E9,E10,(E11*2),1)	=SUM(H3,H4,H5,(H6*2),H 7,H8,H9,H10,(H11*2),1)
ratio of 2.0: 0.8 = 0.24 <1.3333		=D3-E15	=POWER(D16,2)	=STDEV(E3,E5,E7,E10)	
		=D5-E15	=POWER(D17,2)		
Therefore Saturated chain present		=D7-E15	=POWER(D18,2)		
		=D10-E15	=POWER(D19,2)		
			=AVERAGE(E1 6:E19)		
			=SQRT(E20)		

Key: Ave. Chain Lgth. Mest = Average chain length as read directly from Mestrenova 9® ; D and E = Microsoft Excel® cell labels; H(n)=proton and its position on fatty acid or glyceride.



Scheme 2: Organa for Structure Elucidation of Isolated compounds

Ja 65's NMR data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.34 (t, $J = 7.5$ Hz, 2H), 1.68 – 1.54 (m, 2H), 1.41 – 1.11 (m, 26H), 0.88 (t, $J = 6.9$ Hz, 3H). was identified as heptadecanoic acid (Margaric acid) based on similar results by Johnson and Shoolery (1962); Guillén and Ruiz (2003b); Martínez-Yusta *et al.* (2014). As in the analyses Ja 64 the number of carbons was approximated thus: the methylene resonances from peak range 1-3 ppm were taken as two protons per carbon and one carbon per the three methyl protons (0.88 ppm); summing integrated areas from the spectrum (*Spectrum detail:* Appendix IV); (Table 5.6), we get $[(1.93/2) + (2.10/2) + (25.53/2) + (3.00/3) \approx 16$. Adding the carbon from the carbonyl gives 17 carbon atoms.

Ja68's NMR data [^{1}H NMR (500 MHz, $CDCl_3$) δ 2.35 (t, $J = 7.5$ Hz, 2H), 1.70 – 1.56 (m, 2H), 1.28 (d, $J = 21.3$ Hz, 26H), 0.88 (t, $J = 6.9$ Hz, 3H)] showed it to be a saturated fatty acid its normalised integral values suggest it has a 17 carbon chain (Heptadecanoic acid)(Margaric acid) (Table 5.7) (*Spectrum detail:* Appendix VII).

Analyses of spectral data for **Ja 76** (compare discussion on Ja 64) [^{1}H NMR (500 MHz, $CDCL_3$) δ 5.41 – 5.23 (m, 2H), 2.37 – 2.25 (m, 2H), 2.08 – 1.96 (m, 2H), 1.63 (dt, *J* = 14.4, 7.1 Hz, 2H), 1.40 – 1.07 (m, 19H), 0.88 (t, *J* = 6.8 Hz, 3H)] revealed it was a monounsaturated fatty acid; specifically heptadecenoic acid (Table 5.8), (*Spectrum detail:* Appendix XI).

5.7.2 Characterization of Ja69 as hexadecanoic acid

Ja 69's ^{1}HNMR (Table 5.9) showed it to be a saturated fatty acid and that it had a chain length of fifteen carbons. The spectral data [^{1}H NMR (500 MHZ, $CDCl_3$) δ 2.35 (T, $J = 7.5$ Hz, 2H), 1.63 (DT, $J = 14.9, 7.4$ Hz, 2H), 1.38 – 1.10 (M, 24H), 0.88 (T, $J = 6.9$ HZ, 3H)] is consistent with literature reports for hexadecanoic acid (palmitic acid) (Aboaba *et al.*, 2011), (*Spectrum detail:* Appendix VIII).

The carbon signal at δ 178.24 was not consistent with literature reports for free fatty acids (δ 179.60) (Vlahov *et al.*, 2010). The unknown signal may be due to concentration effects as most of the authors worked on whole oils.

5.7.3 Characterization of JSEA2 methyl oleate (9-Z-Octadecenoic acid methyl ester)

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.35 – 5.21 (m, 1H) **(Olefinic)**, 3.60 (s, 3H) **(methoxy)**, 2.24 (t, *J* = 7.5 Hz, 2H) **(H-2)**, 2.04 – 1.90 (m, 2H)**(Allylic)**, 1.57 (t, *J* = 6.6 Hz, 2H) (H-3), 1.23 (d, *J* = 22.9 Hz, 21H) **(Methylene envelope)**, 0.82 (t, *J* = 6.8 Hz, 3H) **(Methylic)**. (Spectrum detail Table 5.10a, Table 5. 10b and Table 5.10c: Appendix XXIX-Q).

Table 5.5: **Signal Characteristics for Ja 64**

	Multiplet Label	Shift	Range	H's	Class	*J's*	Normalised	Absolute Integral	Functional group
1	C (m)	1.63	1.67.. 1.59	2	m		2.15	41075.39	-OCO-CH_2-C$\mathbf{H}_2$- (acyl chains)
2	A (t)	0.88	0.91.. 0.85	3	t	6.85, 6.85	3.00	57338.86	-C$\boldsymbol{H}_3$
3	B (d)	1.28	1.40.. 1.20	26	d	21.20	26.26	501811.07	-(C$\mathbf{H}_2$)$_n$- (acyl chains)
4	D (t)	2.34	2.38.. 2.31	2	t	7.49, 7.49	2.06	39318.25	-OCO-C$\boldsymbol{H}_2$- (acyl chains)

Key: *J* = coupling constant; m = multiplet; t = triplet; d = doublet

Table 5.6: Signal Characteristics for Ja 65

	Multiplet Label	Shift	Range	H's	Class	*J's*	Normalised	Absolute Integral	Functional group
1	C (m)	1.63	1.68.. 1.54	2	m		2.10	11403.52	-OCO-CH_2-C**H**$_2$- (acyl chains)
2	A (t)	0.88	0.91.. 0.81	3	t	6.94, 6.94	3.00	16294.62	-C***H***$_3$
3	B (m)	1.29	1.41.. 1.11	26	m		25.53	138654.99	-(C**H**$_2$)$_n$- (acyl chains)
4	D (t)	2.34	2.37.. 2.32	2	t	7.54, 7.54	1.93	10477.55	-OCO-C***H***$_2$- (acyl chains)

Key: *J* = coupling constant; m = multiplet; t = triplet

Table 5.1: Signal Characteristics for Ja 68

	Name	Shift	Range	H's	Class	*J's*	Absolute Integral	Normalised	Functional group
1	C (m)	1.64	1.70.. 1.56	2	m		46202.11	2.12	-OCO-CH_2-C**H**$_2$- (acyl chains)
2	A (t)	0.88	0.92.. 0.84	3	t	6.94, 6.94	65299.53	3.00	-C***H***$_3$
3	B (d)	1.28	1.41.. 1.09	26	d	21.29	573488.73	26.35	-(C**H**$_2$)$_n$- (acyl chains)
4	D (t)	2.35	2.41.. 2.30	2	t	7.52, 7.52	46231.68	2.12	-OCO-C***H***$_2$- (acyl chains)

Key: *J* = coupling constant; m = multiplet; t = triplet; d = doublet

Table 5.8: **Signal Characteristics for Ja 76**

	Multiplet	Shift	Range	H's	Signal Class	J's	Normalised Integral	Absolute Integral	Functional group
1	C (dt)	1.63	1.67.. 1.55	2	dt	7.09, 7.09, 14.36	2.01	56158.87	-OCO-CH_2-C**H**$_2$- (acyl chains)
2	A (t)	0.88	0.94.. 0.76	3	t	6.78, 6.78	3.00	83616.67	-C***H***$_3$
3	E (m)	2.31	2.37.. 2.25	2	m		1.83	51132.07	-OCO-C***H***$_2$- (acyl chains)
4	D (m)	2.03	2.08.. 1.96	2	m		1.59	44313.10	-C**H**$_2$-CH=CH- (acyl chains)
5	B (m)	1.32	1.40.. 1.07	19	m		18.56	517334.84	-(C**H**$_2$)$_n$- (acyl chains)
6	F (m)	5.32	5.41.. 5.23	2	m		0.95	26553.21	-C**H**=C**H**- (acyl chains)

Key: *J* = coupling constant; m = multiplet; t = triplet; d = double

Table 5.9: Signal Characteristics for Ja 69

	Multiplet Label	Shift	Range	H's	Class	*J's*	Absolute Integral	Normalised Integral	Functional group
1	C (m)	1.29	1.38.. 1.10	24	m		79444655.43	24.33	-(C**H**$_2$)$_n$- (acyl chains)
2	A (t)	2.35	2.38.. 2.31	2	t	7.53, 7.53	7013519.92	2.15	-OCO-C***H***$_2$- (acyl chains)
3	B (dt)	1.63	1.67.. 1.57	2	dt	7.42, 7.42, 14.89	9117845.33	2.79	-OCO-CH$_2$-C**H**$_2$- (acyl chains)
4	D (t)	0.88	0.91.. 0.85	3	t	6.87, 6.87	9795372.78	3.00	-C***H***$_3$

Key: *J* = coupling constant; m = multiplet; t = triplet; d = doublet

Table 5.10a: Prominent COSY correlations and characterisations for JSEA2

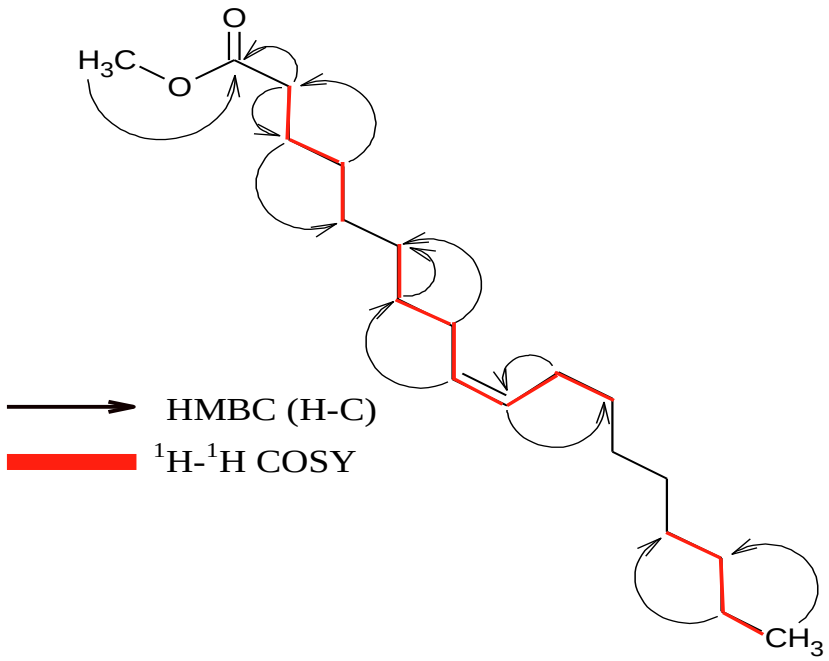
	f1 (ppm)	f2 (ppm)	Coordinate character (f1)	Coordinate character (f2)
1	1.26	1.57	Methylene envelope	H-3
2	2.00	5.29	Allylic	Olefinic
3	2.22	1.57	H-2	H-3
4	0.81	1.21	Methylic	Methylene envelope

Table 5.10b: Prominent HSQC correlations and characterisations for JSEA2

	f1 (ppm)	f2 (ppm)	Coordinate character (f1)	Coordinate character (f2)
1	51.16	3.60	Methoxyl carbon	Methoxyl proton
2	24.72	1.57	C-3 Carbon	C-3 proton
3	29.51	1.25	Methylene	Methylene proton
4	27.00	2.00	Allylic carbon	Allylic proton
5	129.68	5.27	Olefinic carbon	Olefinic proton
6	14.39	0.82	Methylic carbon	Methylic proton
7	33.83	2.24	C-2 carbon	C-2 proton

Table 5.10c: Prominent HMBC correlations and characterisations for JSEA2

	f1 (ppm)	f2 (ppm)	Coordinate character (f1)	Coordinate character (f2)	Reference
1	129.69	1.26	Olefinic carbon	Methylene proton	(Vlahov *et al.*, 2010)
2	129.69	2.00	Olefinic carbon	Allylic proton	
3	28.96	1.57	Methylene	C-3 proton	
4	27.00	1.25	Allylic carbon	Methylene proton	
5	24.72	1.25	C-3 Carbon	Methylene proton	
6	24.72	2.24	C-3 Carbon	C-2 proton	
7	174.10	2.24	Ester (Carbonyl)	C-2 proton	
8	33.83	1.57	C-2 carbon	C-3 proton	
9	31.84	1.25	Methylene (C-16 of Oleate)	C-2 proton	
10	27.00	5.27	Allylic carbon	Olefinic proton	
11	13.87	1.20	Methylic Carbon	Methylene proton	
12	28.94	2.22	Methylene	Methylene proton	
13	29.06	1.24	Methylene	Methylene proton	
14	33.83	1.24	C-2 carbon	Methylene proton	
15	31.84	0.82	Methylene (C-16 of Oleate)	Methylic proton	(Vlahov *et al.*, 2010)
16	29.02	2.00	Methylene	Allylic proton	
17	174.10	3.60	Ester (Carbonyl)	Methoxyl proton	
18	29.56	5.27	Methylene	Olefinic proton	



Structure 6: JSEA2's NMR is consonant with structural features for methyl oleate (9-Z-Octadecenoic acid methyl ester)

5.7.4 Characterization of JSEA46

^{1}H NMR (500 MHz, $CDCL_3$) δ 2.40 – 2.18 (m, 2H) **(H-2)**, 1.61 (dt, *J* = 14.2, 7.1 Hz, 2H) **(H-3)**, 1.38 – 1.12 (m, 16H) **(Methylenes)**, 0.87 (t, *J* = 6.9 Hz, 3H) **(Methyl)**. Integration of signal areas 2.31 ppm, 1.61 ppm, 1.12-1.38 ppm and 0.87 ppm, with signal 0.87 ppm normalised to three protons, showed 2 H, 2 H, 16 H (eight methylene groups) and 3 H (one methyl group) respectively (Table 5.11). JSEA46 is demonstrably, Dodecanoic acid (Lauric acid), a saturated fatty acid (Figure 5.1).

Table 5.11: Signal Characteristics for JSEA46

Name	Shift	H's	Class	J's	Functional group
1 D (m)	1.29	16	m		-(C***H***$_2$)$_n$- (acyl chains)
2 B (m)	2.31	2	m		-OCO-C***H***$_2$- (acyl chains)
3 C (dt)	1.61	2	dt	7.10, 14.25	-OCO-CH$_2$-C***H***$_2$- (acyl chains)
4 E (t)	0.87	3	t	6.92, 6.92	-C***H***$_3$

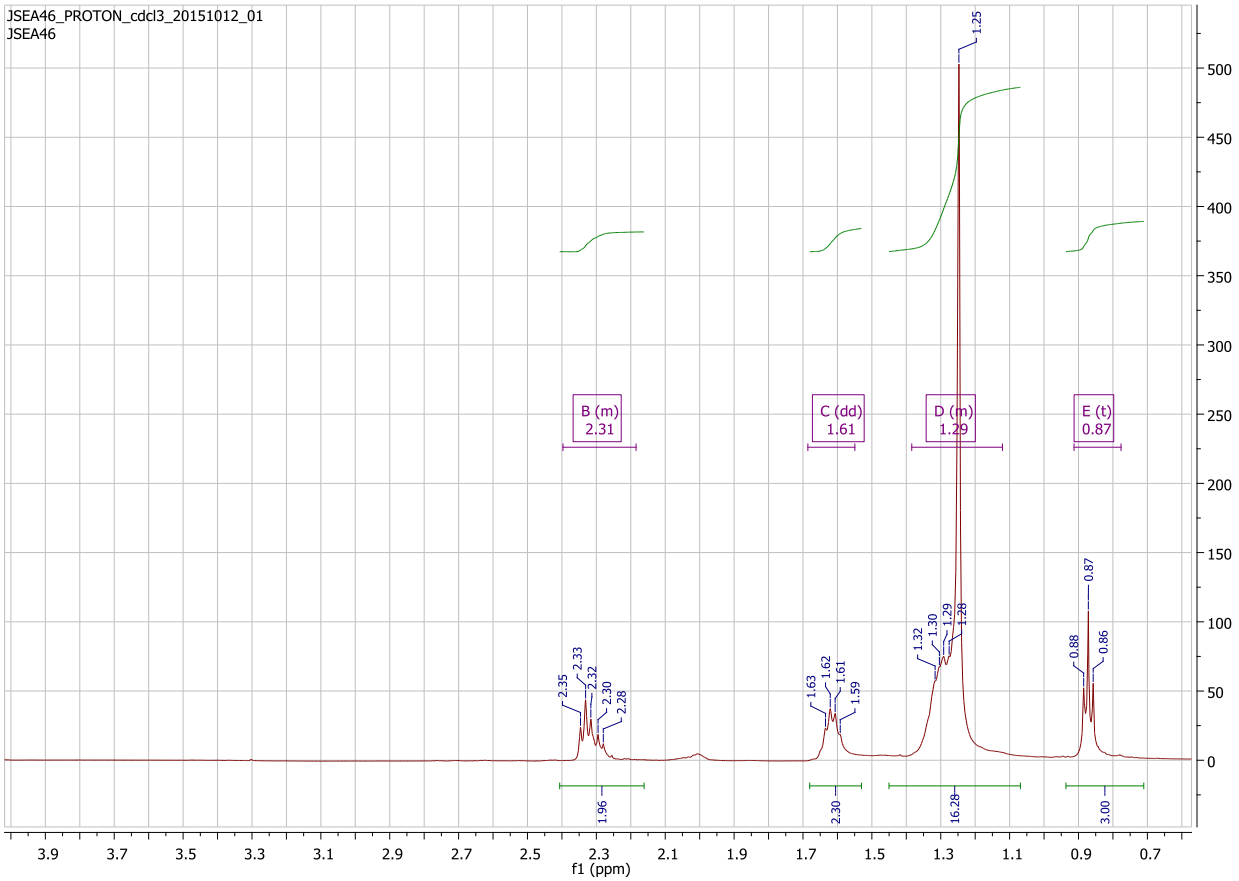


Figure 5.1 1H NMR spectrum for JSEA46

5.7.5 Characterization of Ja96

Ja 96 had the following NMR data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.34 (t, *J* = 7.4 Hz, 1H), 2.10 (d, *J* = 9.7 Hz, 1H) [was ignored in chain-length calculation on account of

its small integral size], 1.62 (dd, *J* = 14.3, 7.0 Hz, 1H), 0.88 (t, *J* = 6.7 Hz, 3H), (*Spectrum detail:* Appendix XXII) (Johnson and Shoolery, 1962). Its ^{1}HNMR showed it to be a saturated fatty acid with a chain length of fourteen carbons (compare elucidation for Ja64).

5.7.6 Identification of Ja 66 as a Triglyceride

Ja 66's ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.40 – 5.18 (m, 3H), 4.34 – 4.00 (m, 4H), 2.35 (d, *J* = 7.6 Hz, 2H), 2.02 (d, *J* = 5.6 Hz, 4H), 1.65 – 1.60 (m, 2H), 1.28 (d, *J* = 21.9 Hz, 50H), 0.88 (t, *J* = 6.8 Hz, 3H) showed it to be a triglyceride; its normalised integral values suggest it has an average chain length of 20. The ratio of signals at 2.02 ppm: 0.88 ppm (allylic proton area to methylic proton area) was found to be (0.13), a figure less than 4/3 but close to 0.4 therefore two acyl chains are saturated (Guillén and Ruiz, 2003). Characteristic signals at 4.22 and normalised integral values suggest it is a triglyceride (Guillén and Ruiz, 2003a; Nieva-echevarría *et al.*, 2015), (*Spectrum detail:* Appendix V-A). Shifts at δ 5.33 ppm (olefinic) and δ 2.02 ppm (allylic) indicate a single double bond in the triglyceride (Table 5.12) (Guillén and Ruiz, 2003).

Data from the HSQC experiment showed interactions between olefinic protons (δ 5.35) and olefinic carbons (δ 130.17); the glyceryl protons interacted with carbons at δ 68.57 and δ 65.19 (Vlahov *et al.*, 2010). The signal at δ 68.57 corresponds to acyl groups in position two on the glyceryl backbone while the signal at δ 65.19 position correspond to acyl groups at position 1 and 3 on the glyceryl backbone.

The HMBC experiment showed interaction between olefinic protons (δ 5.35) and the allylic carbons (δ 27.55). The glyceryl protons [(sn-1) stereospecific numbering]

interacted with glyceryl carbon at sn-3 position (δ 68.57); in like manner, the protons at sn-3 interacted with the carbon at sn-1 position (δ 68.57) (*spectral details*: Appendix V-C –Appendix V-C2).

A COSY interaction between glyceryl protons (δ 5.24; sn-2) and δ 4.14 and 4.21 (sn-1, 3) confirmed the triglyceride nature of Ja66.

The carbon signal at δ 24.86 indicates an oleate group at position two while the signal at δ 32.08 indicates saturated acyl groups at position sn-1 and 3. In summary Ja66 is a triglyceride with two saturated acyl groups at position sn-1, 3 and an oleate group at position sn-1.

5.7.7 Characterization of Ja 67

Ja 67's spectral data [^{1}H NMR (500 MHz, $CDCl_3$) δ 5.40 – 5.26 (m, 2H), 4.26 – 4.07 (m, 2H), 3.97 – 3.87 (m, 1H), 3.65 (ddd, *J* = 17.2, 11.5, 4.9 Hz, 2H), 2.33 (dt, *J* = 19.7, 7.5 Hz, 2H), 2.04 – 1.95 (m, 2H), 1.63 (dt, *J* = 14.7, 7.4 Hz, 2H), 1.43 – 1.09 (m, 21H), 0.88 (t, *J* = 6.9 Hz, 3H)]indicated an acyl chain length (number of carbons) of 16. It was approximated thus: the methylene resonances from peak range 1-3 ppm were taken as two protons per carbon, one carbon per the three methyl protons (0.88 ppm) and one carbon per proton for the olefinic protons (5.34 ppm); adding together integrated areas from the spectrum, gave: [(2.29/2) + (2.08/2) + (20.82/2) + (3.00/3) + (0.67/1) + (0.99/2) ≈ 15] (Crowther, 2008) (Table 5.13). Adding the carbon from the carbonyl gave 16 carbon atoms. Characteristic 1-monoglyceride resonances at 3.93 ppm for the glyceryl proton at sn-2 position, 3.65 ppm for the glyceryl protons at sn-3 position and 4.18 ppm for protons at sn-1 position show that Ja 67 is indeed a monoglyceryl ester of hexadecenoic acid (Palmitoleic acid) where the acyl group is at

the sn-1 position (Nieva-Echevarría *et al*., 2015), (*Spectrum detail:* Appendix VI). Shifts at δ 5.34 ppm (olefinic) and δ 2.02 ppm (allylic) indicate a single double bond in the monoglyceride (Guillén and Ruiz, 2003).

5.7.8 Characterization of Ja75

Ja 75's ^{1}HNMR showed it to be a triglyceride. Its proton NMR data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.41 – 5.21 (m, 3H), 4.21 (dd,dd, *J* = 17.7, 11.8, 4.9 Hz, 4H), 2.37 – 2.24 (m, 2H), 2.09 – 1.95 (m, 4H), 1.67 – 1.52 (m, 2H), 1.28 (dd, *J* = 23.2, 14.5 Hz, 24H), 0.87 (t, *J* = 6.8 Hz, 3H) showed one of its chains is saturated and that its average chain length is 15 carbon atoms. The ratio of signals at 2.01 ppm: 0.88 ppm (allylic proton area to methylic proton area) was found to be (0.34), a figure less than 4/3 but close to 0.4 therefore two acyl chains are saturated (Guillén and Ruiz, 2003). Characteristic signals at 4.21 and normalised integral values suggested it is a triglyceride (Guillén and Ruiz, 2003; Crowther, 2008; Nieva-echevarría *et al.,* 2014) (*Spectrum detail:* Appendix X). Shifts at δ 5.30 ppm (olefinic) and δ 2.01 ppm (allylic) indicate a single double bond in the triglyceride (Knothe and Kenar, 2004; Vlahov, 1999; Soininen *et al*., 2012).

Table 5.12: Signal Characteristics for Ja 66

	Multiplet label	Shift	Range	H's	Class	*J's*	Normalised	Absolute Integral	Functional group
1	C (m)	1.63	1.65.. 1.60	6	m		5.94	7927121.44	-OCO-CH_2-C**H**$_2$- (acyl chains)
2	A (t)	0.88	0.96.. 0.75	9	t	6.84, 6.84	9.00	12007159.95	-C***H***$_3$
3	F (m)	4.22	4.34.. 4.00	4	m		3.70	4932655.98	C**H**$_2$OCOR (glyceryl group)
4	E (d)	2.35	2.37.. 2.32	6	d	7.56	6.31	8418500.68	-OCO-C***H***$_2$- (acyl chains)
5	D (d)	2.02	2.09.. 1.92	4	d	5.58	3.57	4768702.74	-C**H**$_2$-CH=CH- (acyl chains)
6	B (d)	1.28	1.43.. 1.11	57	d	21.95	57.25	76383961.58	-(C**H**$_2$)$_n$- (acyl chains)
7	G (m)	5.33	5.40.. 5.18	3	m		2.88	3837683.86	>C**H**OCOR (glyceryl group)/ -C**H**=C**H**- (acyl chains)

Key: *J* = coupling constant; m = multiplet; t = triplet; d = doublet

Table 5.13: Signal Characteristics for Ja 67

	Multiplet Label	Shift	Range	H's	*J*'s	Normalised	Absolute Integral	Functional group
1	C (m)	3.93	3.97.. 3.87	1		0.45	11444.65	$ROCH_2$-C**H**OH-CH_2OH Glyceryl group in 1-MG
2	A (m)	5.34	5.40.. 5.26	2		0.67	17066.42	>C**H**OCOR (glyceryl group)/ -C**H**=C**H**- (acyl chains)
3	H (m)	1.32	1.43.. 1.09	21		20.82	531837.73	-$(CH_2)_n$- (acyl chains)
4	G (dt)	1.63	1.67.. 1.57	2	7.39, 7.39, 14.71	2.29	58408.59	-OCO-CH_2-C**H**$_2$- (acyl chains)
5	F (m)	2.02	2.04.. 1.95	2		0.99	25351.14	-C**H**$_2$-CH=CH- (acyl chains)
6	E (dt)	2.33	2.38.. 2.24	2	7.47, 7.47, 19.70	2.08	53191.63	-OCO-C***H***$_2$- (acyl chains)
7	D (ddd)	3.65	3.74.. 3.51	2	4.89, 11.47, 17.23	0.72	18412.82	$ROCH_2$-CHOH-C**H**$_2$OH Glyceryl group in 1-MG
8	B (m)	4.18	4.26.. 4.07	2		1.56	39759.11	ROC**H**$_2$-CHOH-CH_2OH Glyceryl group in 1-MG
9	I (t)	0.88	0.96.. 0.77	3	6.90, 6.90	3.00	76625.57	-C***H***$_3$

Key: *J* = coupling constant; m = multiplet; t = triplet; d = doublet

Table 5.14: Signal Characteristics for Ja 96

	Shift Range (ppm)	Absolute Integral	H's	*J's*	Functional group
1	1.74 .. 1.51	25304.19	2		-OCO-CH_2-C***H***$_2$- (acyl chains)
2	1.00 .. 0.73	35482.75	3	6.7 Hz	-C***H***$_3$
3	2.43 .. 2.23	29181.21	2	7.4	-OCO-C***H***$_2$- (acyl chains)
4	2.18 .. 1.85	14029.88	0	9.7	-C***H***$_2$-CH=CH- (acyl chains)
5	1.47 .. 1.11	249063.91	20		-(C***H***$_2$)$_n$- (acyl chains)
6	5.37 .. 5.29	1194.83	0		-C***H***=C***H***- (acyl chains)

Table 5.15: Signal Characteristics for Ja 75

	Multiplet	Shift	Range	H's	*J's*	Absolute Integral	Normalised Integral	Functional group
1	C (m)	2.33	2.37.. 2.24	2		13172.08	6.09	-OCO-C***H***$_2$- (acyl chains)
2	A (m)	5.30	5.41.. 5.21	3		5713.85	2.64	>C**H**OCOR (glyceryl group)/ -C**H**=C**H**- (acyl chains)
3	F (dd)	1.28	1.50.. 1.09	24	14.46, 23.23	136184.17	62.95	-(C**H**$_2$)$_n$- (acyl chains)
4	E (m)	1.62	1.67.. 1.52	2		15686.03	7.25	-OCO-CH$_2$-C**H**$_2$- (acyl chains)
5	D (m)	2.01	2.09.. 1.95	4		8351.29	3.86	-C**H**$_2$-CH=CH- (acyl chains)
6	B (dd,dd)	4.21	4.32.. 4.11	4	4.94, 11.82, 17.70	6281.62	2.90	C**H**$_2$OCOR (glyceryl group)
7	H (t)	0.87	0.93.. 0.80	3	6.84, 6.84	19469.95	9.00	-C***H***$_3$

Key: *J* = coupling constant; m = multiplet; t = triplet; d = doublet

5.7.9 Characterization of Ja77

A study of proton NMR data for **Ja 77**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.42 – 5.26 (m, 2H), 4.21 – 4.05 (m, 5H), 2.34 (t, *J* = 7.4 Hz, 2H), 2.07 – 1.96 (m, 4H), 1.63 (dt, *J*= 7.43, 7.43, 15.04 Hz, 2H),1.36 – 1.20 (m, 28H), 0.88 (t, *J* = 6.8 Hz, 6H) (Table 5.16). Characteristic signals at 4.16 and normalised integral values suggested it to be a 1,3-diglyceride (Guillén *et al.*, 2003; Majid and Pihillagawa, 2014; Bulama *et al.*, 2015), (*Spectrum detail:* Appendix XII). Signals at δ 5.34 (olefinic) and δ 2.01 (allylic) indicate a single double bond in the 1,3-diglyceride (Guillén and Ruiz, 2003). The ratio of signals at 2.01 ppm: 0.88 ppm (allylic proton area to methylic proton area) was found to be (0.32), a figure less than 4/3 bespeaking that one of the acyl chains is saturated (Vlahov *et al*., 1999), (*Spectrum detail:* Appendix XII). Its normalised integral values suggest it has an average chain length of 22. It carbon NMR data:

^{13}C NMR (126 MHz, $CDCl_3$) δ 179.21 (Free fatty acid), 174.09 (glyceryl ester), 130.16, 129.85(olefinic carbon), 68.53 (Glyceryl backbone: monoglyceride), 65.18 (glyceryl backbone,monoglyceride) 34.26, 34.06 (oleate or linoleate group at position Sn-2), 32.07, 32.05, 31.93, 31.76, 31.67, 29.91, 29.84, 29.80, 29.74, 29.67, 29.60, 29.58, 29.51, 29.47, 29.39, 29.27, 29.24, 29.22 (methylenic carbons), 27.37 (allylic carbons), 25.78, 25.04, 24.85 (oleate or linoleate group at position Sn-2), 22.84, 22.83, 22.72, 21.23, 14.26 (methylic carbon) (Vlahov *et al*., 2010)(Spectrum details: Appendix XII – Appendix XII-E). Prominent HSQC correlations showed interactions between olefinic protons and olefinic carbons (δ 5.35: δ 130.16 - δ129.85); also between glyceryl carbons and glyceryl protons (δ 68.53, δ 65.18: δ 4.21.. 4.05). The

Table 5.26: Signal Characteristics for Ja 77

	Name	Shift	Range	H's	Multiplet Class	*J's*	Normalised Integral	Functional group
1	C (t)	2.34	2.37.. 2.32	*2*	**t**	7.43, 7.43	3.13	-OCO-C**H**$_2$- (acyl chains)
2	A (m)	5.34	5.42.. 5.26	*2*	**m**		2.01	>C**H**OCOR (glyceryl group)/ -C**H**=C**H**- (acyl chains)
3	E (m)	1.28	1.36.. 1.20	*28*	**m**		28.30	-(C**H**$_2$)$_n$- (acyl chains)
4	G (dt)	1.63	1.70.. 1.52	*2*	**dt**	7.43, 7.43, 15.04	1.96	-OCO-CH$_2$-C**H**$_2$- (acyl chains)
5	D (m)	2.01	2.07.. 1.96	*4*	**m**		2.46	-C**H**$_2$-CH=CH- (acyl chains)
6	B (m)	4.16	4.21.. 4.05	*5*	**m**		2.39	C**H**$_2$OCOR (glyceryl group)
7	F (t)	0.88	0.90.. 0.85	*6*	**t**	6.82, 6.82	6.00	-C**H**$_3$

Key: *J* = coupling constant; m = multiplet; t = triplet; d = doublet; dt = doublet of triplets

carbon-13 data however showed a significant impurity (δ 179.21); a free fatty acid (oleic) (Vlahov *et al.*,2010).

5.7.10 Characterization of Ja78

Ja 78's proton NMR data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.45 – 5.18 (m, 3H), 4.38 – 4.01 (m, 4H), 2.76 (t, *J* = 6.6 Hz, 2H), 2.39 – 2.19 (m, 2H), 2.12 – 1.96 (m, 2H), 1.66 – 1.52 (m, 2H), 1.40 – 1.11 (m, 64H), 0.87 (t, *J* = 6.9 Hz, 9H).

It was determined from proton NMR data that **Ja 78** is a triglyceride with a twenty-carbon atom average chain length based on normalised proton integrals (Table 5.17). The ratio of signals at 2.0 ppm: 0.88 ppm (allylic proton area to methylic proton area) was found to be (0.6), a figure less than 4/3 indicating that two of the acyl chains are saturated (*Spectrum detail:* Appendix XIII). The total degree of unsaturation was found to be 1.10 (the sum of normalised proton integrals for allylic, bisallylic and olefinic protons). From its ^{13}C NMR data:

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.07 (glyceryl ester), 130.33, 130.04, 128.18, 128.02 (olefinic carbons), 68.47, 65.15 (glyceryl backbone carbons: sn-2 and sn1,2 respectively), 34.24, 34.18 (linoleate group in position sn-2), 34.07 (saturated group in position sn-1 and 3), 32.05, 32.03 (saturated), 31.65 (linoleate), 29.90, 29.87, 29.83, 29.78, 29.74, 29.72, 29.65, 29.61, 29.56, 29.49, 29.44, 29.40, 29.37, 29.30, 29.28, 29.25, 29.20, 29.17, 29.10 (methylenes), 27.35, 27.30, 25.76, 22.82, 22.70, 14.38, 14.24, 14.19 (methylic) (spectrum details: Appendix VIII –Appendix VIX).

Ja78 is a triglyceride with two saturated acyl groups (positions sn-1 and sn-3) and a linoleate group in position sn-2.

5.7.11 Characterization of Ja79

Ja 79's proton NMR data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.40 – 5.21 (m, 3H), 4.34 – 4.04 (m, 4H), 2.78 – 2.72 (m, 2H), 2.30 (qd, *J* = 12.9, 7.0 Hz, 6H), 2.09 – 1.95 (m, 2H), 1.60 (d, *J* = 6.4 Hz, 6H), 1.45 – 0.98 (m, 44H), 0.86 (t, *J* = 6.7 Hz, 9H); showed it to be a triglyceride (Table 5.18) thus:

Its average chain length was 15 carbon atoms based on normalised proton integrals. The ratio of signals at 2.0 ppm: 0.88 ppm (allylic proton area to methylic proton area) was found to be 0.4, less than 4/3, showing that two of the acyl chains are saturated (*Spectrum detail:* Appendix XIV). The total degree of unsaturation was found to be 0.56 (the sum of normalised proton integrals for allylic, bisallylic and olefinic protons).

5.7.12 Characterization of Ja80

Ja 80's proton NMR data:^{1}H NMR (500 MHz, $CDCl_3$) δ 5.39 – 5.28 (m, 2H), 4.18 (ddd, 2H), 3.96 – 3.89 (m, 1H), 3.64 (ddd, 2H), 2.31 (dd, *J* = 24.1, 7.5 Hz, 2H), 2.00 (dd, *J* = 21.7, 9.9 Hz, 1H), 1.60 (d, *J* = 6.3 Hz, 3H), 1.45 – 1.04 (m, 16H), 0.86 (t, *J* = 6.8 Hz, 3H) showed it was a 1-monoglyceride with a single double bond and a 17 carbon chain (*Spectrum detail:* Appendix XV).

5.7.13 Characterization of Ja81

NMR spectral analysis of **Ja 81** provided the following data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.39 – 5.26 (m, 2H), 4.18 (ddd, 1H), 3.97 – 3.90 (m, 1H), 3.65 (ddd, 2H),

Table 5.17: Signal Characteristics for Ja 78

	Name	Shift	Range	H's	Normalised Integral	Class	*J's*	Functional group
1	C (t)	2.76	2.81.. 2.68	2	0.34	t	6.58, 6.58	-CH=CH-C**H**$_2$-CH=CH- (acyl chains) bisallylic
2	A (m)	5.33	5.45.. 5.18	3	1.00	m		>C**H**OCOR (glyceryl group)/ -C**H**=C**H**- (acyl chains)
3	G (m)	1.27	1.40.. 1.11	64	11.93	m		-(C**H**$_2$)$_n$- (acyl chains)
4	F (m)	1.62	1.66.. 1.52	2	1.68	m		-OCO-CH$_2$-C**H**$_2$- (acyl chains)
5	E (m)	2.03	2.12.. 1.96	2	1.16	m		-C**H**$_2$-CH=CH- (acyl chains)
6	D (m)	2.31	2.39.. 2.19	2	1.57	m		-OCO-C**H**$_2$- (acyl chains)
7	B (m)	4.23	4.38.. 4.01	4	1.25	m		C**H**$_2$OCOR (glyceryl group)
8	H (t)	0.87	0.89.. 0.85	9	1.55	t	6.89, 6.89	-C**H**$_3$

Key: *J* = coupling constant; m = multiplet; t = triplet; d = doublet

Table 5.18: **Signal Characteristics for Ja 79**

	Mult. label/Class	Shift	Range	H's	Integral	*J's*	Functional group
1	C (m)	2.74	2.78.. 2.72	2	0.18		=HC-C**H**$_2$-CH= (acyl chains)
2	A (m)	5.32	5.40.. 5.21	3	1		>C**H**OCOR (glyceryl group)/ -C**H**=C**H**- (acyl chains)
3	G (m)	1.25	1.45.. 0.98	44	23.97		-(C**H**$_2$)$_n$- (acyl chains)
4	F (d)	1.6	1.67.. 1.51	6	3.08	6.44	-OCO-CH$_2$-C**H**$_2$- (acyl chains)
5	E (m)	2.01	2.09.. 1.95	2	1.74		-C**H**$_2$-CH=CH- (acyl chains)
6	D (qd)	2.3	2.36.. 2.22	6	2.5	6.95, 12.89, 12.54, 12.54	-OCO-C**H**$_2$- (acyl chains)
7	B (m)	4.19	4.34.. 4.04	4	1.6		-C**H**$_2$OCOR (glyceryl group)
8	H (t)	0.86	0.95.. 0.65	9	4.96	6.73, 6.73	-C**H**$_3$

Key: *J* = coupling constant; m = multiplet; t = triplet; d = doublet; qd =

2.35 (t, *J* = 7.3, 3.4 Hz, 2H), 2.06 – 1.96 (m, 2H), 1.61 (m, *J* = 6.3 Hz, 2H), 1.41 – 1.01 (m, 18H), 0.87 (t, *J* = 6.5 Hz, 3H). This showed it was the same compound as Ja 80. The acyl chain was found to be 17 carbons long extrapolating from proton signal areas after normalising the methyl signal to three. Distinguishing signals at δ 4.18 (ddd, 1H) [sn-1], 3.97 – 3.90 (m, 1H) [sn-2], 3.65 (ddd, 2H) [sn-3] for glyceryl protons and δ 2.35 for H-2 protons on the acyl moiety in 1-monoglyceride showed Ja 81 to be a 1-monoglyceride (Nieva-echevarría *et al.*, 2015), (*Spectrum detail:* Appendix XVI). Signals at δ 5.34 (olefinic) and δ 2.02 (allylic) indicate a single double bond in the 1-monoglyceride (Guillén and Ruiz, 2003).

5.7.14 Characterization of Ja82

Ja 82 had the following ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.28 (td, *J* = 13.6, 7.5 Hz, 1H), 4.20 – 3.96 (m, 2H), 3.93 – 3.74 (m, 1H), 3.71 – 3.53 (m, 1H), 2.32 – 2.19 (m, 6H), 2.01 (dd, *J* = 31.9, 14.1 Hz, 2H), 1.64 – 1.46 (m, 6H), 1.44 – 1.05 (m, 28H), 0.96 – 0.72 (m, 7H). Characteristic signals at 3.62 ppm, 3.84 ppm, 4.08 ppm and 4.28 ppm for glyceryl signals of 1-monoglyceride (sn-3), 2-monoglyceride (sn-1 and sn-3), 1,3-diglyceride (sn-1, sn-2 and sn-3) and 1,2- diglyceride (sn-1) respectively indicating Ja 82 to be a mixture (Nieva-echevarría *et al.*, 2015). A ratio of signal areas at 2.0 ppm: 0.8 ppm was found to be less than 4:3 indicating presence of at least one saturated acyl group in the mixture [Since every mole of monoenoic acid or polyunsaturated fatty acid has four allylic protons, the ratio of peak areas of allylic methylenes (δ = 2.05) ppm and terminal methyls of all chains (δ = 0.8 – 1.0 ppm) lower than 4:3 (1.3333) indicates at least one acyl chain is saturated; the ratio is zero for glycerides

containing only saturated chains] (Vlahov, 1999) (*Spectrum detail:* Appendix XVII). Average chain length and total degree of unsaturation were calculated as described by Crowther (2008) and found to be 12.05 and 0.24 respectively (*Spectrum detail:* Appendix XXIX-J).

5.7.15 Characterization of Ja83 as a digalactosyldiacylglyceride

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99 (d, *J* = 11.0 Hz, 1H), 6.87 (d, *J* = 8.7 Hz, 1H), 6.01 (d, *J* = 16.9 Hz, 1H), 4.91 (d, *J* = 4.6 Hz, 1H) **(anomeric sugar proton, probably galactosylic**), 4.92 – 4.90 (m, 1H), 4.19 (s, 1H), 4.16 (d, *J* = 4.6 Hz, 2H**)**, 4.14 (dd, *J* = 4.2, 2.0 Hz, 3H), 4.12 (d, *J* = 6.2 Hz, 2H), **(H-1a and H-1b)** 3.93 (d, *J* = 8.5 Hz, 4H)(**H-6a)**, 3.82 (d, *J* = 5.0 Hz, 3H)**(H-6b)**, 3.70 (dd, *J* = 4.1, 2.0 Hz, 2H) **(H-3b)**, 3.69 (d, *J* = 2.2 Hz, 2H) **(H-2)**, 3.60 (d, *J* = 9.4 Hz, 3H) **(H-5)**, 3.59 (d, *J* = 8.0 Hz, 2H). The NMR data showed that it had glyceryl chains [0.95 - 0.85 **(methyls**) and **methylenes** (2.64, 2.32, 2.30, 2.29, 2.08, 1.59, 1.46, 1.37, 1.29, 1.24,), and a **sugar moiety** (6.01, 5.98, 5.71, 5.33, 4.87, 4.34, 4.29, 4.27, 4.16, 4.13, 4.11, 3.91, 3.87, 3.77, 3.68, 3.67, 3.66, 3.60, 3.57, 3.55, 3.44). Integration of the sugar region showed that at least 20 protons were involved at which point at least 2 sugar units were assumed (Appendix XVIII) (Sato, 2015**).**

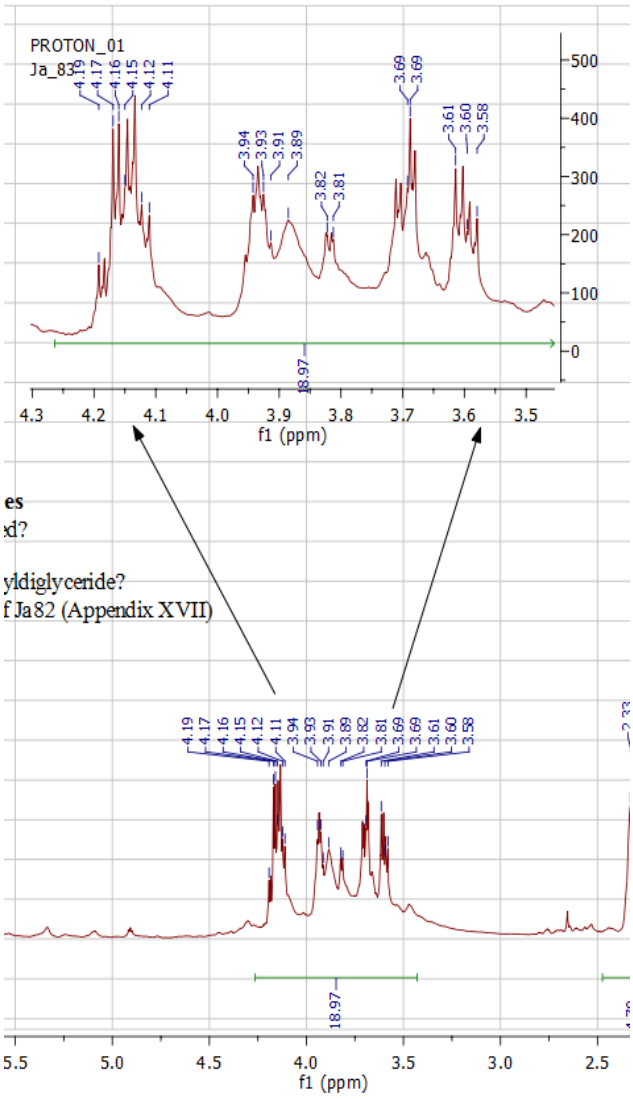


Figure 5.2: Analysis of Sugar moiety of Ja83

5.7.16 Characterization of Ja95

^{1}H NMR (500 MHz, cdcl$_3$) δ 4.42 (d, *J* = 7.8 Hz, 1H) **(galactosylic anomeric proton)**, 4.20 (dd, *J* = 12.1, 4.5 Hz, 1H), 4.16 (d, *J* = 6.1 Hz, 1H), 4.10 (dd, *J* = 15.3, 8.3 Hz, 2H), 3.95 (dd, *J* = 13.3, 5.9 Hz, 3H), 3.92 – 3.86 (m, 8H), 3.81 (d, *J* = 5.9 Hz, 3H), 3.68 (dd, *J* = 11.2, 5.6 Hz, 1H), 3.61 (dd, *J* = 11.3, 6.0 Hz, 1H), 3.58 – 3.31 (m, 1H), 2.39 – 2.32 (m, 4H), 2.31 – 2.25 (m, 1H), 2.17 (s, 4H), 1.85 (dd, *J* = 26.7, 12.8

Hz, 1H), 1.65 (dd, *J* = 15.3, 8.5 Hz, 5H), 1.14 (d, *J* = 6.6 Hz, 2H), 0.97 (dd, *J* = 6.7, 2.5 Hz, 2H), 0.92 (d, *J* = 3.4 Hz, 2H), 0.69 (d, *J* = 8.8 Hz, 2H).

Analysis of its spectral data showed to be probably a steroidal saponin (Figure 5.3 – 5.4).

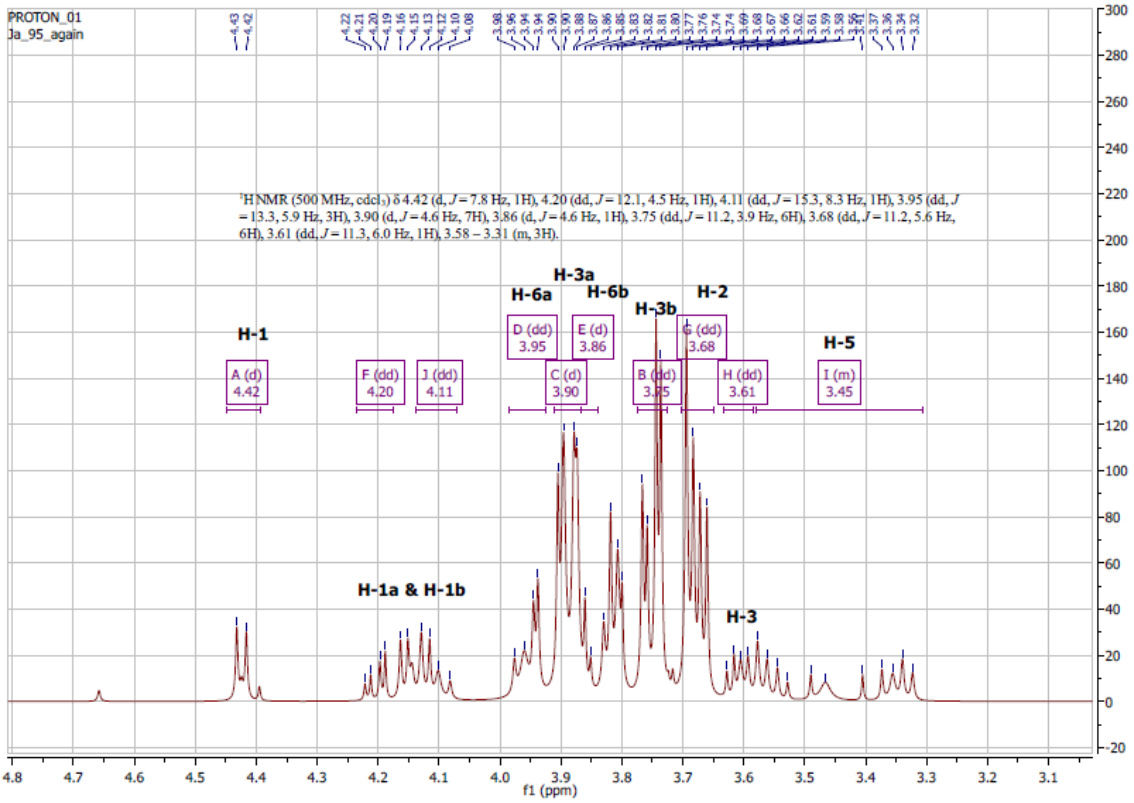


Figure 5.3: Analysis of galactosyl moiety of Ja95: H-1 = anomeric proton

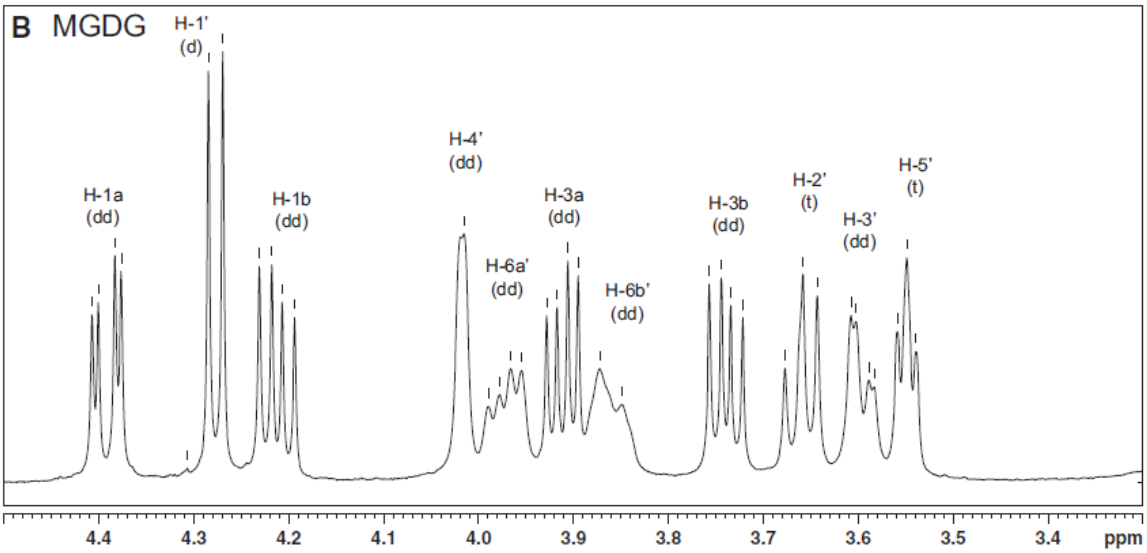


Figure 5.4 ^{1}H NMR spectrum of galactosyl region of a monogalactosyl diglyceride (Sato, 2015)

The signal at 4.42 is typical of galactosylic anomeric proton of saponins as exemplified with the spectrum of galactosyl region of a monogalactosyl diglyceride (Sato, 2015)(Figure 5.4).Other features that point to a galactose moiety are labelled H-1 to H-6 in the spectrum for Ja 95 which are also evident in the literature.

5.7.17 Characterization of Ja97

Ja 97's NMR data: ^{1}H NMR data [^{1}H NMR (500 MHz, cdcl$_3$) δ 4.18 (ddd, 2H), 3.97 – 3.76 (m, 1H), 3.65 (ddd, 2H), 2.33 (t, *J* = 16.8, 7.8 Hz, 2H), 2.16 – 2.02 (m, 2H), 1.68 – 1.56 (m, 2H), 1.40 – 1.13 (m, *J* = 11.6 Hz, 12H), 0.88 (t, 3H)] indicated a monounsaturated 1-Monoglyceride based on characteristic glyceryl backbone signals at 3.65 ppm [Stereospecific numbering (sn-3), 3.94 ppm (sn-2) and 4.18 ppm (sn-1) (Nieva-echevarría *et al* ., 2015); other signals at 5.35 ppm, 2.33 ppm, 2.16 – 2.02 ppm, 1.63 ppm, 1.13-1.40 ppm and 0.88 ppm indicated olefinic protons, H-2 acyl moiety, allylic methylenes, H-3 acyl moiety, other methylenes (methylene envelope)

and methyl group respectively (Johnson and Shoolery, 1962). Integration of signal areas 2.35 ppm, 1.63 ppm, 1.13-1.40 ppm and 0.88 ppm, with signal 0.88 ppm normalised to three protons, showed 2 H, 2 H, 14 H and 3 H respectively consistent with a monoglyceryl ester of pentadecenoic acid. ^{1}H NMR spectrum for Ja97 is shown in (*Spectrum detail:* Appendix XXII2).

5.7.18 Characterization of Ja98

It was determined that **Ja 98** is a 1-Monoglyceride based on ^{1}H NMR data using characteristic glyceryl backbone signals at 3.65 ppm [Stereospecific numbering (sn)-3)], 3.94 ppm(sn-2) and 4.18 ppm (sn-1) (Nieva-Echevarría *et al*., 2015); other signals at 2.35 ppm, 1.63 ppm, 1.07-1.50 ppm and 0.88 ppm indicated H-2 acyl moiety, H-3 acyl moiety, other methylenes (methylene envelope) and methyl group respectively (Johnson and Shoolery, 1962) (Table 4.19). Integration of signal areas 2.35 ppm, 1.63 ppm, 1.07-1.50 ppm and 0.88 ppm, with signal 0.88 ppm normalised to three protons, showed 2 H, 2 H, 24 H and 3 H respectively; specifically, consistent with pentadecanoic acid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.34 (t, J = 7.5 Hz, 2H), 1.70 – 1.55 (m, 2H), 1.41 – 1.17 (m, 22H), 0.88 (t, J = 6.8 Hz, 3H). ^{1}H NMR spectrum for Ja 98 is shown in (*Spectrum detail:* Appendix XXIII).

5.7.19 Characterization of JSEA30

JSEA30 was deemed to be probably a mixture of a β-Sitosterol and fatty acid methyl ester. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.66, 5.29, 5.26, 4.32, 3.58, 3.41, 3.40, 3.38,

3.31, 3.29, 3.27, 3.23, 2.83, 2.70, 2.69, 2.68, 2.65, 2.64, 2.63, 2.51, 2.50, 2.49, 2.47, 2.38, 2.37, 2.35, 2.27, 2.23, 2.22, 2.21, 2.18, 2.16, 1.99, 1.98, 1.96, 1.95, 1.94, 1.93, 1.91, 1.90, 1.89, 1.82, 1.81, 1.80, 1.78, 1.77, 1.74, 1.71, 1.69, 1.68, 1.65, 1.60, 1.59, 1.53, 1.47, 1.44, 1.39, 1.38, 1.35, 1.23, 1.18, 1.12, 0.94, 0.90, 0.89, 0.87, 0.86, 0.81, 0.80, 0.79, 0.77, 0.75, 0.73, 0.71, 0.70. In the absence of key data like ^{13}C NMR and other NMR experiments, it is difficult to characterise JSEA30 further. The methylic region has been compared in Figure 5.4 with that of β-Sitosterol from literature (Wang, 2014).

5.10 Antimicrobial Activity of Isolated Compounds

Isolated compounds (Ja 64, Ja 65, Ja 66, Ja 68, Ja 69, Ja 75, Ja 77, Ja 82, Ja 97, Ja 100 and JSEA30), which are lipids, showed a remarkably similar pattern of sensitivity to test microbes as the crude extracts. They were however more active at even lower concentrations [Diameter of zone of inhibition (20-31 mm); MIC (6 25 μ/mL - 12.5 μ/mL)] (Table 4.13 - 4.19). *Shigella dysenteriae, Pseudomonas aeruginosa, Aspergillus fumigatus, Aspergillus niger* and *Trichophyton rubrum* were however resistant to all isolated compounds (Table 4.13 - 4.19). Lipids are believed to possess a stimulatory effect on the immune system and for most the anti-pathogenic effects are modulated through a host's immune response (De Pablo, 2000; He *et al.*, 2007; Federico *et al.*, 2010; Su *et al.*, 2013; Damiani and Ullrich, 2016; Szostak *et al.*, 2016).

Table 5.19: Signal characteristics for Ja98

S/N	Signal (ppm)	Annotations
1	0.88	Methylic
2	0.89	Methylic
3	7.26	Chloroform-d
4	2.34	H-2
5	2.33	H-2
6	1.64	H-3
7	1.63	H-3
8	1.61	H-3
9	1.30	Methylene
10	1.28	Methylene
11	1.25	Methylene
12	0.86	Methylic
13	2.36	H-2

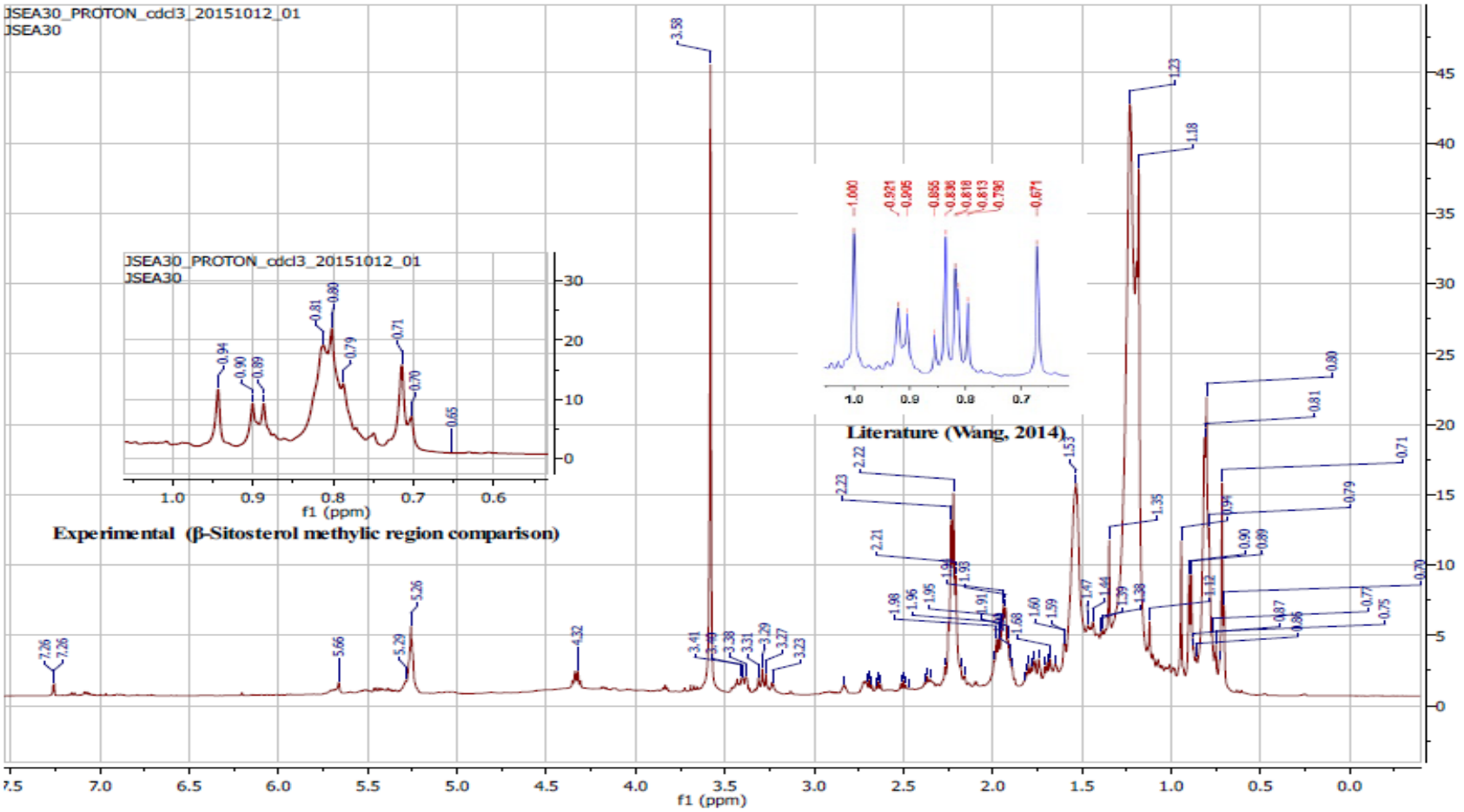
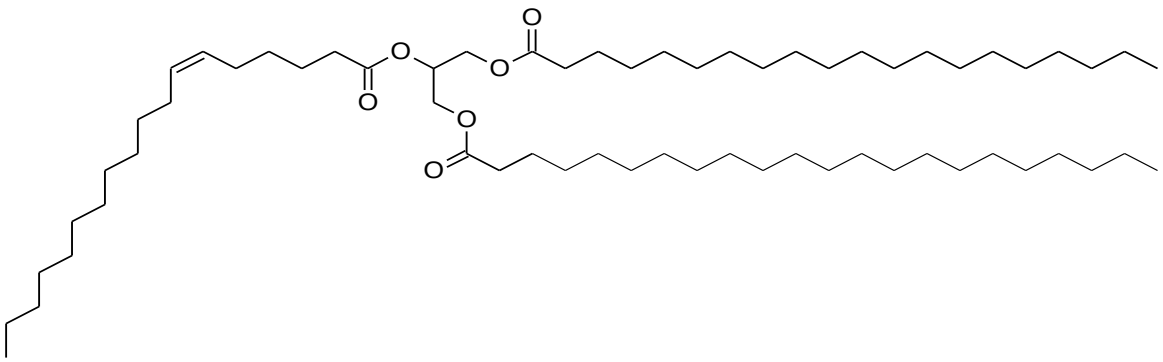
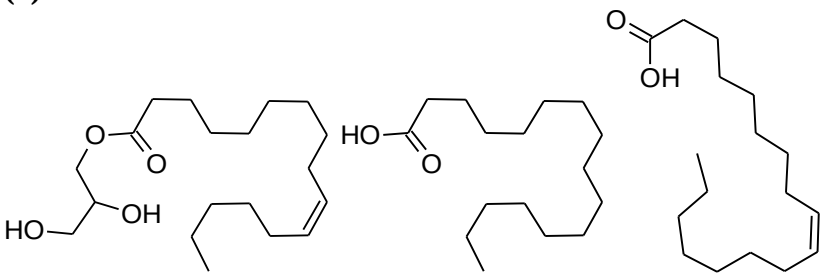


Figure 5.4 Comparison of Methylic regions for JSEA30 and that of β-Sitosterol literature



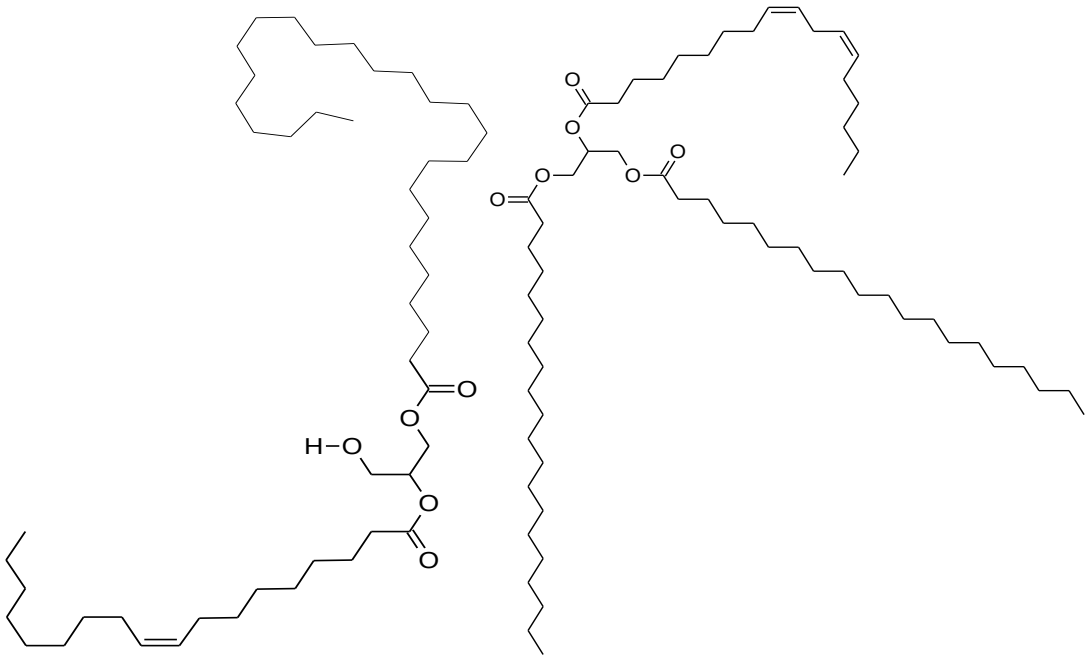
(a)



(b)

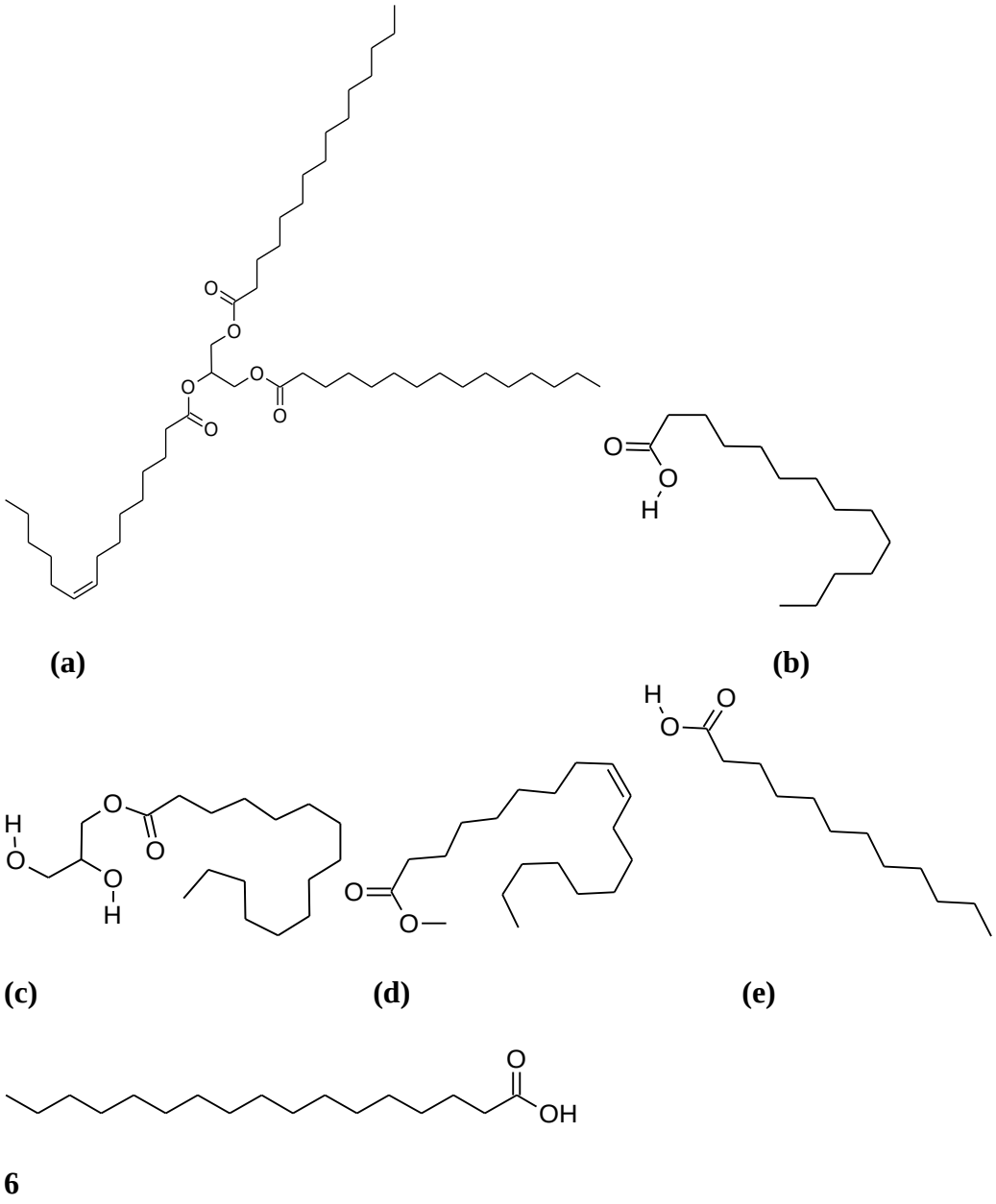
(c)

(d)



(e) **(f)**

Structure 7: Structures of compounds Characterised using NMR methods: **(a)** (Ja66) Propyl-1- docosanoate -3- eicosanoate-2- (6Z) octadec-6-enoate **(b)** (Ja67) 2,3-dihydroxypropyl (xZ)-hexadec-x-enoate **(c)** (Ja69) Hexadecanoic acid **(d)** (Ja76) Heptadecenoic acid **(e)** (Ja77)1,2-diglyceride of oleic and cerotic acid **(f)** (Ja78) Propyl -1,3-eicosanoate-2-linoleate



Structure 8: Structures of compounds Characterised using NMR methods: **(a)** (Ja79) a triglyceryl ester of Pentadecenoic acid and two saturated acids **(b)** (Ja96) Myristic acid Teteradecanoic acid **(c)** (Ja98) 2,3-dihydropropyl pentadecanoate **(d)** (JSEA2) Methyl- (9Z)-octadec-9-enoate **(e)** (JSEA46) Dodecanoic acid (Lauric acid) **(f)** (Ja64, Ja65) Heptadecanoic acid (Margaric acid)

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Raphia africana is an important plant in Nigerian ethno-medicine. The fruit mesocarp is used in treating mouth sores among other ailments. Motivated by the numerous applications of *Raphia africana* and the lack of commensurate investigations thereof, fruit mesocarp of the plant was investigated thereby revealing several bioactive compounds.

Extraction of the fruit mesocarp using different solvents (pet-ether, chloroform, ethyl acetate and methanol) revealed that the plant under study contains an abundance of oil with the pet-ether extract yielding the highest (11.3 %) amount followed by chloroform (8.5 %), ethyl acetate (6.4 %) and methanol (3.8 %). Phytochemical screening of the extracts showed that it contained anthraquinones, steroids, triterpenes, alkaloids, cardiac glycosides and flavonoids with the ethyl acetate extract having the widest range of metabolites but contained no saponins, the methanol contained no anthraquinones in addition to having no saponins, the chloroform extract contained but had no anthraquinones and flavonoids while the pet-ether extract contained only steroids and triterpenes.

. Pre-isolation studies using GCMS and HPLC showed that extracts of *Raphia africana* contain flavonoids (isovitexin, kaempferol, orientin, isoorientin, and orientin) and steroids (stigmasterol, sitosterol, campesterol, cholesterol, cholestanol and tocopherol). The GCMS study revealed numerous lipids including lauric acid, hexadecanoic acid, octadecanoic acid, hexadecenoic acid, octadecenoic acid and some galactosylglycerides in addition to phytol. From this phytochemical investigation, the

bulk of chemical constituents of *Raphia africana* fruit mesocarp can be listed as diterpene alcohols (3,7,11,15-Tetramethyl-2-hexadecen-1-ol), fatty acid methyl/ethyl esters (methyl oleate, ethyl oleate), monoglycerides (Hexadecanoic acid 2-monoglyceryl ester, heptadecenoic acid 1-monoglyceryl ester, pentadecenoic acid 1-monoglyceryl ester, pentadecanoic acid 1-monoglyceryl ester), diglycerides (1,2-diacylglyceride), triglycerides, monogalactosyldiacylglycerides, steroidal glycosides (diosgenin type) and free fatty acids (heptadecanoic acid, hexadecanoic acid, pentadecanoic acid, dodecanoic acid). These compounds may be responsible for the observed activity on the pathogens used in this work hence lending credence to ethno-medicinal applications of this plant. The is also an initial report of the isolation of these compounds from this plant and therefore this work is of chemotaxonomic relevance to the plant.

Antimicrobial analysis of the fractions using well diffusion method showed that they were active against a range of bacteria (*S. aureus, E. coli, K. pneumoniae* and *S. typhi*) and fungi (*C. albicans* and *Microsporum sp*) but were not effective against *Aspergillus fumigatus* and *Aspergillus niger* . The isolated compounds showed higher but similar activity compared to the crude extracts. Ja 83 (a monogalactosyldiacylglyceride) showed the largest zone of inhibition against *Staphylococcus aureus* (30 mm), against *Klebsiella pneumoniae* (31 mm) and 32 mm against *Shigella dysenteriae* while showing the least zone against *Microsporum sp* [Ranges: diameter of zone of inhibition (20-31 mm); MIC (6 25 μ/mL - 12.5 μ/mL)]. Isolated compounds were inactive towards *Aspergillus fumigatus* and *Aspergillus niger*. Several fractions (Ja 69, Ja 75, Ja 77 and Ja 82) were similarly active (MIC) against *Microsporum spp*. at 50 μ/mL, *T. rubrum*, *C. albicans* and *E. coli* at 12.5 μ/mL. Ja 77 (1,3-diglyceride) was however ineffective against *T. rubrum, P.*

aeruginosa and *S. dysenteriae.* Minimum bactericidal/fungicidal concentration (MBC/MFC) for the fractions mostly clustered around 25 µ/mL and 6.25 µ/mL. A few cases (Ja 82, Ja 100, Ja 97, JSEA 30) however had values around 50 µ/mL. Above all, higher concentrations (50 µ/mL) were required to kill *Microsporum spp.* and *T. rubrum* than other parasites. Ja 66 (triglyceride) showed a similar pattern of reactivity to Ja 68 (heptadecanoic acid) but was in addition to being ineffective against *T. rubrum* and the *Aspergilli* also not effective against *S. dysenteriae*.

Lethality assay using brine shrimp (*Artemia salina*) showed that the ethyl acetate extract obtained via microwave assisted extraction and maceration to exhibit mild toxicity (269.2 - 467.7 µg/mL). The extract obtained via microwave assisted extraction and soxhlet extraction had the lowest LC50 values (269.2 µg/mL) against brine shrimp.

6.1 Recommendations

Further studies (*in vivo*) should be carried out on the extracts of the plant in order to ascertain the effects of the fruit mesocarp on mouth sores. Since there are various causative agents (bacterial, viral, fungal, cancer, stress) for various ulcers results from such a study would impact potential treatment of mouth ulcers and related ailments.

In vivo toxicity studies need to be carried out on animal models since the *raphia* plants are also used as fish poison in South-Eastern Nigeria (Igbo land).

The methanol extract revealed some glycosides and galactocyldiacylglycerides that could not be completely characterized because of lack of required equipment. These

need to be further characterised. While efforts are being made to characterise using the necessary spectroscopic tools, it is recommended that further work be done using a combination of spectral studies like high resolution mass spectrometry, quadruple liquid chromatography mass spectrometry in order characterise them.

There is a need for conservation of this species (Raphia palms). Most of the giant leaved species (*Raphia hookeri*) are all but gone from many habitats where they were found in large numbers. The shorter leaved varieties like *Raphia africana* are also endangered because of indiscriminate destruction of the plants for other agricultural purposes.

The consumption of this fruit is encouraged since the compounds are more beneficial than harmful to human health as can be adjudged from the low toxicity and beneficial nature of all the compounds identified.

The seed and other parts of the plant need to be studied for possible medicinal uses.

REFERENCES

Aboaba, S., Oladosu, I. & Ekundayo, O. (2011). Chemical composition, acetyl cholinesterase inhibitory properties and brine shrimp toxicity of essential oils from *Ficus capensis* Thunb from Nigeria. *International Journal of Biological and Chemical Sciences*, *4*(5), 1494–1500. https://doi.org/10.4314/ijbcs.v4i5.65555

Adeogun, A., Alaka, O. & Taiwo, V. (2012). Some Pathological Effects of Sub-lethal concentrations of the Methanolic Extracts of *Raphia hookeri* on *Clarias gariepinus*. *African Journal of*. Retrieved from https://www.ajol.info/index.php/ajbr/article/view/95285

Adetutu, A., Morgan, W. A., Corcoran, O. & Chimezie, F. (2012). Antibacterial activity and in vitro cytotoxicity of extracts and fractions of *Parkia biglobosa* (Jacq.) Benth. Stem bark and *Ageratum conyzoides* Linn. Leaves. *Environmental Toxicology and Pharmacology*, *34*(2), 478–483. https://doi.org/10.1016/j.etap.2012.06.008

Afolayan, A. O., Borokini, T. I. & Afolayan, G. O. (2014). Sublethal Effects of Methanolic Extract of *Raphia hookeri* on the Reproductive Capacity of *Clarias gariepinus*. *Advances in Zoology*, *2014*(Article ID 615908), 10. https://doi.org/10.1155/2014/615908

Ailhaud, G., Massiera, F., Weill, P., Legrand, P., Alessandri, J. M. & Guesnet, P. (2006). Temporal changes in dietary fats: Role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. *Progress in Lipid Research*, *45*(3), 203–236. https://doi.org/10.1016/j.plipres.2006.01.003

Alkadi, H. O. (2007). Antimalarial drug toxicity: A review. *Chemotherapy*, *53*(6), 385–391. https://doi.org/10.1159/000109767

Andre, C. M., Hausman, J.-F. & Guerriero, G. (2016). *Cannabis sativa*: The Plant of the Thousand and One Molecules. *Frontiers in Plant Science*, *7*(February), 19. https://doi.org/10.3389/fpls.2016.00019

Anyam, J. V. (2011). *Isolation and Characterisation of Medicinal Principles of Pouteria alnifolia Baker Roberty (Sapotaceae)*. Ahmadu Bello University, Zaria, Nigeria. Retrieved from http://scholar.google.com/scholar?hl=en&q=john+anyam&btnG=&as_sdt=1%2C5&as_sdtp=#5

Anyam, J. V., Okoro, I. S., Tor-Anyiin, T. A., Asar, S. T. & Anyam, J. N. (2015). On a

Facile Method for Enhanced Throughput in Isolation of Lupeol from *Maranthes polyandra*. In *Op-14* (p. 54). Antalya: 11th International Symposium on The Chemistry of Natural Compounds. Retrieved from https://www.researchgate.net/profile/Khamid_Khodjaniyazov/publication/292988265_Alkylation_of_p-cresol_by_polyprenols/links/56b4874508ae2f32011277b6.pdf#page=54

Apak, L. & Olila, D. (2006). The *in-vitro* antibacterial activity of *Annona senegalensis*, *Securidacca longipendiculata* and *Steganotaenia araliacea* - Ugandan medicinal plants. *African Health Sciences*, 6(1), 31–35. https://doi.org/10.5555/afhs.2006.6.1.31

Asmussen, C. B., Dransfield, J., Deickmann, V., Barfod, A. S., Pintaud, J. C. & Baker, W. J. (2006). A new subfamily classification of the palm family (Arecaceae): Evidence from plastid DNA phylogeny. *Botanical Journal of the Linnean Society*, *151*(1), 15–38. https://doi.org/10.1111/j.1095-8339.2006.00521.x

Augustin, J. M., Kuzina, V., Andersen, S. B. & Bak, S. (2011). Molecular activities, biosynthesis and evolution of triterpenoid saponins. *Phytochemistry*, *72*(6), 435–457. https://doi.org/10.1016/j.phytochem.2011.01.015

Becker, M., Staab, D. & Von Bergmann, K. (1993). Treatment of severe familial hypercholesterolemia in childhood with sitosterol and sitostanol. *Journal of Pediatrics*, *122*(2), 292–296. https://doi.org/10.1016/S0022-3476 (06)80136-8

Ben-Menachem, G., Kubler-Kielb, J., Coxon, B., Yergey, A. & Schneerson, R. (2003). A newly discovered cholesteryl galactoside from *Borrelia burgdorferi*. *Proceedings of the National Academy of Sciences*, *100*(13), 7913–7918. https://doi.org/10.1073/pnas.1232451100

Bessong, P. O., Rojas, L. B., Obi, L. C., Tshisikawe, P. M. & Igunbor, E. O. (2006). Further screening of Venda medicinal plants for activity against HIV type 1 reverse transcriptase and integrase. *African Journal of Biotechnology*, *5*(6), 526–528. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-33645277788&partnerID=40&md5=4e511101508a829ce63cb3f04a471398

Bhuvaneswari, S., Aravind, K. R., Ramkumar, B., Vinod Raja, N., Neelakandan, A., Mukesh Kumar, P. & Udaya Prakash, N. K. (2014). Studies on the phytochemistry and bioactivity of leaves of trees in Chennai - I. *International Journal of ChemTech Research*, *6*(9), 4078–4083. Retrieved from http://www.researchgate.net/profile/Udaya_Prakash_Nyayiru_Kannaian/publication/267098056_Studies_on_the_Phytochemistry_and_Bioactivity_of_Leaves_of_

Trees_in_Chennai_-I/links/544572940cf2d62c304d8064.pdf

Bode, H. B., Zeggel, B., Silakowski, B., Wenzel, S. C., Reichenbach, H. & Müller, R. (2003). Steroid biosynthesis in prokaryotes: Identification of myxobacterial steroids and cloning of the first bacterial 2, 3(S)-oxidosqualene cyclase from the myxobacterium *Stigmatella aurantiaca*. *Molecular Microbiology*, *47*(2), 471–481. https://doi.org/10.1046/j.1365-2958.2003.03309.x

Boutanaev, A. M., Moses, T., Zi, J., Nelson, D. R., Mugford, S. T., Peters, R. J. & Osbourn, A. (2015). Investigation of terpene diversification across multiple sequenced plant genomes. *Proceedings of the National Academy of Sciences*, *112*(1), E81–E88. https://doi.org/10.1073/pnas.1419547112

Bowden, F. (1986). The Application of Vacuum Liquid Chromatography to the Separation of Terpene Mixtures. *Journal of Natural Products*, *49*(5), 934–936. https://doi.org/10.1021/np50047a033

Brkljača, R. & Urban, S. (2011). Recent Advancements in HPLC-NMR and Applications for Natural Product Profiling and Identification. *Journal of Liquid Chromatography & Related Technologies*, *34*(13), 1063–1076. https://doi.org/10.1080/10826076.2011.587748

Brkljača, R. & Urban, S. (2014). Chemical Profiling (HPLC-NMR & HPLC-MS), Isolation, and Identification of Bioactive Meroditerpenoids from the Southern Australian Marine Brown Algae *Sargassum paradoxum*. *Marine Drugs*, *13*(12), 102–127. https://doi.org/10.3390/md13010102

Broendegaard, V. J. (1985). Ethnobotany: Plants in traditions, history and popular medicine-Tree felling and moon phases: superstition or folk-visdom. *In: Contributions to Ethnomedicine, Ethnobotany and Ethnozoology. Verl. Mensch und Leben, Berlin, Bd*, *6*, 82-92.

Bucar, F., Wube, A. & Schmid, M. (2013). Natural product isolation - how to get from biological material to pure compounds. *Natural Product Reports*, *30*(4), 525–545. https://doi.org/10.1039/C3NP20106F

Bulama, J., Dangoggo, S. & Mathias, S. (2015). Isolation and characterization of palmitic acid from ethyl acetate extract of root bark of *Terminalia glaucescens*. *International Journal of Scientific and Research Publications*, *5*(3), 1–3. Retrieved from http://www.ijsrp.org/research-paper-0315/ijsrp-p3925.pdf

Burkill, H. M. (1985). *The Useful Plants of West Tropical Africa vol. 4. The useful plants of west tropical Africa* (2nd Ed.). London: Royal Botanic Gardens, Kew.

Butler, M. S., Robertson, A. A. B., Cooper, M. A., Hay, M., Thomas, D. W., Craighead, J. L., … Sturm, K. (2014). Natural product and natural product derived drugs in clinical trials. *Natural product reports*, *31*(11), 1612–1661. https://doi.org/10.1039/C4NP00064A

Capone, A., Giannella, M., Fortini, D., Giordano, A., Meledandri, M., Ballardini, M., … Petrosillo, N. (2013). High rate of colistin resistance among patients with carbapenem-resistant *Klebsiella pneumoniae* infection accounts for an excess of mortality. *Clinical Microbiology and Infection*, *19*(1), 23–30. https://doi.org/10.1111/1469-0691.12070

Cazarolli, L., Zanatta, L., Alberton, E., Bonorino Figueiredo, M. S., Folador, P., Damazio, R., … Barreto Silva, F. R. (2008). Flavonoids: Prospective Drug Candidates. *Mini-Reviews in Medicinal Chemistry*, *8*(13), 1429–1440. https://doi.org/10.2174/138955708786369564

Chaieb, K., H. H., Tarek, Z., Kahla-Nakbi, A. B. & Rouabhia, K. M. (2007). The Chemical Composition and Biological Activity of Clove Essential Oil, *Eugenia caryophyllata*. *Phytotherapy Research*, *21*, 501–506. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/ptr.2124/full

Chakraborty, S., Deokule, J. S., Garg, P., Bhattacharya, S. K., Nandy, R. K., Nair, G. B., … Ramamurthy, T. (2001). Concomitant infection of enterotoxigenic *Escherichia coli* in an outbreak of cholera caused by *Vibrio cholerae* O1 and O139 in Ahmedabad, India. *Journal of Clinical Microbiology*, *39*(9), 3241–3246. https://doi.org/10.1128/JCM.39.9.3241-3246.2001

Chan, C. H., Yusoff, R. & Ngoh, G. C. (2014). Optimization of microwave-assisted extraction based on absorbed microwave power and energy. *Chemical Engineering Science, 111*, 41–47. https://doi.org/10.1016/j.ces.2014.02.011

Chan, C. H., Yusoff, R., Ngoh, G. C. & Kung, F. W. L. (2011). Microwave-assisted extractions of active ingredients from plants. *Journal of Chromatography A*. https://doi.org/10.1016/j.chroma.2011.07.040

Christie, W. W. (2012). *Sterols 3. Sterols and Their Conjugates from Plants and Lower Organisms Structure, Occurrence, Biochemistry and Analysis*. In: Harwood, J. (2012). *Lipids in plants and microbes*. *Springer Science & Business Media*. 1–8. *Lipidlibrary.Aocs.Org*, Retrieved from http://lipidlibrary.aocs.org/lipids/plant_st/file.pdf

Cook, N. C. & Samman, S. (1996). Flavonoids - Chemistry, metabolism, cardioprotective effects, and dietary sources. *Journal of Nutritional Biochemistry*, *7*(2), 66–76. https://doi.org/10.1016/0955-2863 (95)00168-9

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, *12*(4), 564–582. https://doi.org/0893-8512/99/$04.00□ 0

Crawford, A. D., Liekens, S., Kamuhabwa, A. R., Maes, J., Munck, S., Busson, R., de Witte, P. A. M. (2011). Zebrafish bioassay-guided natural product discovery: Isolation of angiogenesis inhibitors from East African medicinal plants. *PLoS ONE*, *6*(2), e14694. https://doi.org/10.1371/journal.pone.0014694

Crowther, M. W. (2008). NMR and IR Spectroscopy for the Structural Characterization of Edible Fats and Oils. An Instrumental Analysis Laboratory. *Journal of Chemical Education*, *85*(11), 1550. https://doi.org/10.1021/ed085p1550

Cruz-Vega, D., Verde-Star, M. J., Salinas-Gonzalez, N. R., Rosales-Hernandez, B., Estrada-Garcia, I., Mendez-Aragon, P., Castro-Garza, J. (2009). Review of pharmacological effects of *Glycyrrhiza radix* and its bioactive compounds. *Zhongguo Zhong Yao Za Zhi = Zhongguo Zhongyao Zazhi = China Journal of Chinese Materia Medica*, *22*(April 2008), 557–559. https://doi.org/10.1002/ptr

Cushnie, T. P. T. & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, *26*(5), 343–356. https://doi.org/10.1016/j.ijantimicag.2005.09.002

Cushnie, T. P. T., Cushnie, B. & Lamb, A. J. (2014). Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International Journal of Antimicrobial Agents*, *44*(5), 377–386. https://doi.org/10.1016/j.ijantimicag.2014.06.001

Da Mota, S. D. P., Mancio, A. A., Lhamas, D. E. L., De Abreu, D. H., Da Silva, M. S., Dos Santos, W. G., … Machado, N. T. (2014). Production of green diesel by thermal catalytic cracking of crude palm oil (*Elaeis guineensis* Jacq) in a pilot plant. *Journal of Analytical and Applied Pyrolysis*, *110*(1), 1–11. https://doi.org/10.1016/j.jaap.2014.06.011

Da Silva, B. P. & Parente, J. P. (2010). Chemical properties and antiinflammatory activity of a galactomannoglucan from *Arecastrum romanzoffianum*. *Phytochemistry Letters*, *3*(3), 109–112. https://doi.org/10.1016/j.phytol.2010.02.007

Damiani, E. & Ullrich, S. E. (2016). Understanding the connection between platelet-activating factor, a UV-induced lipid mediator of inflammation, immune suppression and skin cancer. *Progress in Lipid Research*, *63*, 14–27. https://doi.org/10.1016/j.plipres.2016.03.004

Das, A. K., Mandal, V. & Mandal, S. C. (2013). Design of experiment approach for the

process optimisation of microwave assisted extraction of lupeol from *Ficus racemosa* leaves using response surface methodology. *Phytochemical Analysis : PCA*, *24*(3), 230–47. https://doi.org/10.1002/pca.2403

De Pablo, M. A., Ángeles Puertollano, M. & Álvarez De Cienfuegos, G. (2000). Immune cell functions, lipids and host natural resistance. *FEMS Immunology and Medical Microbiology*, *29*(4), 323–328. https://doi.org/10.1016/S0928-8244 (00)00224-8

Dias, D. A., Urban, S. & Roessner, U. (2012). A historical overview of natural products in drug discovery. *Metabolites*, *2*(2), 303–336. https://doi.org/10.3390/metabo2020303

Dikko, Y. J., Khan, M. E., Tor-Anyiin, T. A. & Anyam, J. V. (2016). In vitro Antimicrobial Activity of Fruit Pulp Extracts of *Azanza garckeana* (F. Hoffm.) Exell & Hillc. and Isolation of One of its Active Principles, Betulinic Acid. *British Journal of Pharmaceutical Research*, *14*(1), 1–10. https://doi.org/10.9734/BJPR/2016/30152

Dinan, L. (1998). Natural Products Isolation. *Natural Product Isolation*, *864*, 279–327. https://doi.org/10.1007/978-1-59259-256-2_10

Djouossi, M. G., Tamokou, J.-D., Ngnokam, D., Kuiate, J.-R., Tapondjou, L. A., Harakat, D. & Voutquenne-Nazabadioko, L. (2015). Antimicrobial and antioxidant flavonoids from the leaves of *Oncoba spinosa* Forssk. (Salicaceae). *BMC Complementary and Alternative Medicine*, *15*(1), 134. https://doi.org/10.1186/s12906-015-0660-1

Douglas, M. (1958). Raffia Cloth Distribution in the Lele Economy. *Africa Journal of the International African Institute*, *28*(2), 109–122. https://doi.org/10.2307/1157129

Dubey, V. S., Bhalla, R. & Luthra, R. (2003). An overview of the non-mevalonate pathway for terpenoid biosynthesis in plants. *Journal of biosciences*, *28*(5), 637.https://doi.org/10.1007/BF02703339

Edem, D. O., Eka, O. U. & Ifon, E. T. (1984). Chemical evaluation of the nutritive value of the raffia palm fruit (*Raphia hookeri*). *Food Chemistry*, *15*(1), 9–17. https://doi.org/10.1016/0308-8146 (84)90034-7

EFSA. (2010). Scientific Opinion on the substantiation of health claims related to various food(s)/food constituent(s) and protection of cells from premature aging, antioxidant activity, antioxidant content and antioxidant properties, and protection of DNA, proteins an. *EFSA Journal*, *8*(2), 1–63. https://doi.org/10.2903/j.efsa.2010.1489.

Eyres, G. T., Urban, S., Morrison, P. D., Dufour, J. P. & Marriott, P. J. (2008). Method for small-molecule discovery based on microscale-preparative multidimensional gas chromatography isolation with nuclear magnetic resonance spectroscopy. *Analytical Chemistry*, *80*(16), 6293–6299. https://doi.org/10.1021/ac8007847

Falgenhauer, L., Waezsada, S. E., Yao, Y., Imirzalioglu, C., Käsbohrer, A., Roesler, U., Chakraborty, T. (2016). Colistin resistance gene mcr-1 in extended-spectrum β-lactamase-producing and carbapenemase-producing Gram-negative bacteria in Germany. *The Lancet Infectious Diseases*, *16*(3), 282–283. https://doi.org/10.1016/S1473-3099 (16)00009-8

Faparusi, S. I. (1981). Sugars identified in Raphia palm wine. *Food Chemistry*, *7*(2), 81–86. https://doi.org/10.1016/0308-8146 (81)90052-2

Farag, S. & Kayser, O. (2015). Cannabinoids Production by Hairy Root Cultures of *Cannabis sativa* L. *American Journal of Plant Sciences*, *6*(July), 1874–1884. https://doi.org/10.4236/ajps.2015.611188

Federico, A., D'Aiuto, E., Borriello, F., Barra, G., Gravina, A. G., Romano, M. & de Palma, R. (2010). Fat: A matter of disturbance for the immune system. *World Journal of Gastroenterology*, *16*(38), 4762–4772. https://doi.org/10.3748/wjg.v16.i38.4762

Flores-Sanchez, I. J., Ortega-Lopez, J., Montes-Horcasitas, M. del C. & Ramos-Valdivia, A. C. (2002). Biosynthesis of sterols and triterpenes in cell suspension cultures of *Uncaria tomentosa*. *Plant and Cell Physiology*, *43*(12), 1502–1509. https://doi.org/10.1093/pcp/pcf181

Fongang, Y. S. F., Bankeu, J. J. K., Ali, M. S., Awantu, A. F., Zeeshan, A., Assob, C. N., … Tsamo, E. (2015). Flavonoids and other bioactive constituents from *Ficus thonningii* Blume (Moraceae). *Phytochemistry Letters*, *11*, 139–145. https://doi.org/10.1016/j.phytol.2014.11.012

Foss, S. M. (1978). Urhobo mats in praise of daughters. *African Arts*, *12*(1), 60–62,108. https://doi.org/10.2307/3335383

Francis, G., Kerem, Z., Makkar, H. P. S. & Becker, K. (2002). The biological action of saponins in animal systems: a review. *British Journal of Nutrition*, *88*(6), 587–605. https://doi.org/doi:10.1079/BJN2002725

Friedman, M. (2007). Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. *Molecular Nutrition and Food Research*, *51*(1), 116–134. https://doi.org/10.1002/mnfr.200600173

Ganzler, K., Szinai, I. & Salgó, A. (1990). Effective sample preparation method for extracting biologically active compounds from different matrices by a microwave technique. *Journal of Chromatography*, *520*, 257–262. Retrieved from http://www.sciencedirect.com/science/article/pii/0021967390851099

Gao, S., You, J., Wang, Y., Zhang, R. & Zhang, H. (2012). On-line continuous sampling dynamic microwave-assisted extraction coupled with high performance liquid chromatographic separation for the determination of lignans in Wuweizi and naphthoquinones in Zicao. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *887–888*, 35–42. https://doi.org/10.1016/j.jchromb.2012.01.005

Geetha, T. & Varalakshmi, P. (2001). Anti-inflammatory activity of lupeol and lupeol linoleate in rats. *Journal of Ethnopharmacology*, *76*(1), 77–80. https://doi.org/10.1016/S0378-8741 (01)00175-1

Gerber, F., Krummen, M., Potgeter, H., Roth, A., Siffrin, C. & Spoendlin, C. (2004). Practical aspects of fast reversed-phase high-performance liquid chromatography using 3µm particle packed columns and monolithic columns in pharmaceutical development and production working under current good manufacturing practice. *Journal of Chromatography A*, *1036*(2), 127–133. https://doi.org/10.1016/j.chroma.2004.02.056

German, P., Greenhouse, B., Coates, C., Dorsey, G., Rosenthal, P. J., Charlebois, E., Lindegardh, N., Havlir, D. & Aweeka, F. T. (2007). Hepatotoxicity Due to a Drug Interaction between Amodiaquine plus Artesunate and Efavirenz. *Clinical Infectious Diseases*, *44*(15 MARCH), 889–891. https://doi.org/10.1086/513706

Gidado, A., Ameh, D. A., Atawodi, S. E. & Ibrahim, S. (2008). Hypoglycaemic activity of *Nauclea latifolia* sm. (Rubiaceae) in experimental animals. *African Journal of Traditional, Complementary and Alternative Medicines*, *5*(2), 201–208. https://doi.org/10.1089/ast.2007.0153

Goozner, M. & Moore, J. P. (2004). *The $ 800 million pill - The truth behind the cost of new drugs*. *The Journal of Clinical Investigation*. https://doi.org/10.1172/JCI23540

Gruca, M., van Andel, T. R. & Balslev, H. (2014). Ritual uses of palms in traditional medicine in sub-Saharan Africa: a review. *Journal of Ethnobiology and Ethnomedicine*, *10*(1), 60. https://doi.org/10.1186/1746-4269-10-60

Gu, Q., Yi, X., Zhang, Z., Yan, H., Shi, J., Zhang, H. & Shao, J. (2016). A facile method for simultaneous analysis of phytosterols, erythrodiol, uvaol, tocopherols and lutein in olive oils by LC-MS. *Analytical Methods*, *8*(6), 1373–1380.

https://doi.org/10.1039/C5AY02193F

Guillén, M. & Ruiz, A. (2003). Edible oils: discrimination by 1H nuclear magnetic resonance. *Journal of the Science of Food and …*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/jsfa.1317/pdf

Guillén, M. D. & Cabo, N. (1997a). Characterization of edible oils and lard by Fourier transform infrared spectroscopy. Relationships between composition and frequency of concrete bands in the fingerprint region. *Journal of the American Oil Chemists' Society*, *74*(10), 1281–1286. https://doi.org/10.1007/s11746-997-0058-4

Guillén, M. D. & Cabo, N. (1997b). Infrared spectroscopy in the study of edible oils and fats. *Journal of the Science of Food and Agriculture*, *75*(1), 1–11. https://doi.org/10.1002/ (SICI) 1097-0010(199709)75:1<1:: AID-JSFA842>3.0.CO;2-R

Guillén, M. D. & Ruiz, A. (2001). High-resolution 1H nuclear magnetic resonance in the study of edible oils and fats. *Trends in Food Science & Technology*, *12*(9), 328–338. https://doi.org/10.1016/S0924-2244 (01)00101-7

Guillen, M. D. & Ruiz, A. (2003). Rapid simultaneous determination by proton NMR of unsaturation and composition of acyl groups in vegetable oils. *European Journal of Lipid Science and Technology*, *105*(11), 688–696. https://doi.org/10.1002/ejlt.200300866

Guillén, M. D. & Ruiz, A. (2003a). 1H nuclear magnetic resonance as a fast tool for determining the composition of acyl chains in acylglycerol mixtures. *European Journal of Lipid Science and Technology*, *105*(9), 502–507. https://doi.org/10.1002/ejlt.200300799

Guillén, M. D. & Ruiz, A. (2003b). Edible oils: Discrimination by 1H nuclear magnetic resonance. *Journal of the Science of Food and Agriculture*, *83*(4), 338–346. https://doi.org/10.1002/jsfa.1317

Guillen, M. D., Ruiz, A., Cabo, N., Chirinos, R. & Pascual, G. (2003). Characterization of *sacha inchi* (*Plukenetia volubilis* L.) oil by FTIR spectroscopy and 1H NMR. Comparison with linseed oil. *Journal of the American Oil Chemists' Society*, *80*(8), 755–762. https://doi.org/10.1007/s11746-003-0768-z

Gunstone, F. D. & Herslöf, B. G. (2012). *B. Lipid Glossary 2*. https://doi.org/10.1016/B978-0-9531949-2-6.50007-8

Gunstone, F. D. & Seth, S. (1994). A study of the distribution of eicosapentaenoic acid and docosahexaenoic acid between the alpha and beta glycerol chains in fish oils by ^{13}C-NMR spectroscopy. *Chemistry and Physics of Lipids*, *72*(2), 119–126. Retrieved from http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=emed3&AN=1994286332http://sfx.ucl.ac.uk/sfx_local?sid=OVID:embase&id=pmid:&id=doi:10.1016%2F0009-3084%252894%252990095-7&issn=0009-3084&isbn=&volume=72&issue=2&spage=119&pages=119-126&da

Gunstone, F. D. (1990). 13C-NMR spectra of some synthetic glycerol esters alone and as mixtures. *Chemistry and Physics of Lipids*, *56*(2–3), 195–199. https://doi.org/10.1016/0009-3084 (90)90102-W

Gunstone, F. D. (1993). Information on the Composition of Fats from Their High-Resolution 13C Nuclear Magnetic Resonance Spectra. *Journal of the American Oil Chemists' Society*, *70*(4), 361–366.

Gunstone, F. D. (2011). *Vegetable Oils in Food Technology*. *Food Technology*. https://doi.org/10.1002/9781444339925

Gunstone, F. D., Polard, M. R., Scrimgeour, C. M. & Vedanayagam, H. S. (1977). Fatty acids. Part 50. 13C nuclear magnetic resonance studies of olefinic fatty acids and esters. *Chemistry and Physics of Lipids*, *18*(1), 115–29. https://doi.org/10.1016/0009-3084 (77)90031-7

Halket, J. M., Waterman, D., Przyborowska, A. M., Patel, R. K. P., Fraser, P. D. & Bramley, P. M. (2005). Chemical derivatization and mass spectral libraries in metabolic profiling by GC/MS and LC/MS/MS. *Journal of Experimental Botany*, *56*(410), 219–243. https://doi.org/10.1093/jxb/eri069

Hanson, J. R. (2010). Natural products from the hallucinogenic sage. *Science Progress*, *93*(2), 171–180. https://doi.org/10.3184/003685010X12626983776947

Hardman, R. & Sofowora, E. A. (1972). Antimony Trichloride as A Test Reagent for Steroids, Especially Diosgenin and Yamogenin, in Plant Tissues. *Stain Technology*, *47*(4), 205–208. https://doi.org/10.3109/10520297209116486

Harris, R. (2003). *Trease and Evans' pharmacognosy*. *International Journal of Aromatherapy* (Vol. 13). Elsevier Health Sciences. https://doi.org/10.1016/S0962-4562 (03)00051-1

Havsteen, B. (1983). Flavonoids, a class of natural products of high pharmacological potency. *Biochemical Pharmacology*, *32*(7), 1141–1148. https://doi.org/10.1016/0006-2952 (83)90262-9

He, X., Zhang, H., Yang, X., Zhang, S., Dai, Q., Xiao, W. & Ren, G. (2007). Modulation of immune function by conjugated linoleic acid in chickens. *Food and Agricultural Immunology*, *18*(3–4), 169–178. https://doi.org/10.1080/09540100701718419

Helm, R. (2000). *Invitation to Organic Chemistry (Johnson, A. William)*. *Journal of Chemical Education* (Vol.77). Jones and Bartlett Learning. https://doi.org/10.1021/ed077p449

Hesse, M. (2002). *Alkaloids: Nature's curse or blessing*. Verlag Helvetica Chimica Acta. https://doi.org/http://doi.wiley.com/10.1002/anie.200385928

Hockings, K. J., Bryson-Morrison, N., Carvalho, S., Fujisawa, M., Humle, T., McGrew, W. C., Matsuzawa, T. (2015). Tools to tipple: ethanol ingestion by wild chimpanzees using leaf-sponges. *Royal Society Open Science*, *2*(6), 150150. https://doi.org/10.1098/rsos.150150

Hoffman, S. J., Rottingen, J. A. & Frenk, J. (2015). International Law Has a Role to Play in Addressing Antibiotic Resistance. *Journal of Law, Medicine and Ethics*, *43*(S3), 65–67. https://doi.org/10.1111/jlme.12276

Hokkanen, M. (2012). Imperial networks, colonial bioprospecting and Burroughs Wellcome & co.: The case of *Strophanthus kombe* from Malawi (1859-1915). *Social History of Medicine*, *25*(3), 589–607. https://doi.org/10.1093/shm/hkr167

Horner, T. G. & Klufers, P. (2016). The Species of Fehling's Solution. *European Journal of Inorganic Chemistry*, *2016*(12), 1798–1807. https://doi.org/10.1002/ejic.201600168

Hostettmann, K. & Marston, A. (1995). *Saponins*. Cambridge: Cambridge Univ. Press.

Hostettmann, K., Marston, A. & Hostettmann, M. (1998). Special Column Chromatography. *Preparative Chromatography Techniques*, 33–49. https://doi.org/10.1007/978-3-662-03631-0_4

Husain, S. Z., Malik, R. N., Javaid, M. & Bibi, S. (2008). Ethonobotanical properties and uses of medicinal plants of Morgah Biodiversity Park, Rawalpindi. *Pakistan Journal of Botany*, *40*(5), 1897–1911.

Huxley, A. J. & Griffiths, M. (1992). *Dictionary of gardening*. .Royal Horticultural Society (Great Britain). Macmillan Press. Retrieved from http://tropical.theferns.info/viewtropical.php?id=Raphia+africana

Ibargoitia, M. L., Sopelana, P. & Guillén, M. D. (2014). 1H Nuclear Magnetic Resonance monitoring of the degradation of margarines of varied compositions when heated to high temperature. *Food Chemistry*, *165*, 119–128. https://doi.org/10.1016/j.foodchem.2014.05.065

Ibegbulem, C. O., Igwe, C. U., Okwu, G. N., Ujowundu, C. O., Onyeike, E. N. & Ayalogu, E. O. (2013). Total amino acid profiles of heat-processed fresh *Elaeis guineensis* and *Raphia hookeri* wines. *Food Chemistry*, *138*(2–3), 1616–1620. https://doi.org/10.1016/j.foodchem.2012.11.110

Igietseme, J. U., Omosun, Y. & Black, C. M. (2014). *Bacterial Sexually Transmitted Infections (STIs): A Clinical Overview*. *Molecular Medical Microbiology: Second Edition* (Vol. 3). Elsevier Ltd. https://doi.org/10.1016/B978-0-12-397169-2.00078-0

Igoli, O. J. & Gray, A. I. (2008). Friedelanone and other triterpenoids from *Hymenocardia acida*. *International Journal of Physical Sciences*, *3*(June), 156–158. Retrieved from http://strathprints.strath.ac.uk/19442/

Ikeda, I. & Sugano, M. (1983). Some aspects of mechanism of inhibition of cholesterol absorption by□β-sitosterol. *BBA - Biomembranes*, *732*(3), 651–658. https://doi.org/10.1016/0005-2736 (83)90243-2

Ilesanmi, F. F., Balogun, E. A. & Ilesanmi, O. S. (2011). Comparative effect of chronic administration of chloroquine phosphate and methanol extract of *Landolphia owariensis* on the activities of rat enzymes. *Journal of Applied Pharmaceutical Science*, *1*(6), 72–74. Retrieved from http://imsear.hellis.org/handle/123456789/150852

Imogie, A., Ugbah, M. & Udosen, C. (2007). Seedling to Various Rates of Organic and Inorganic Fertilizer. *Journal of Agronomy*, *6*(4), 597–600. Retrieved from http://www.docsdrive.com/pdfs/ansinet/ja/2007/590-593.pdf

Inchingolo, R., Cardenia, V. & Rodriguez-Estrada, M. T. (2014). Analysis of phytosterols and phytostanols in enriched dairy products by fast gas chromatography with mass spectrometry. *Journal of Separation Science*, *37*(20), 2911–2919. https://doi.org/10.1002/jssc.201400322

Ismail, A. A., van de Voort, F. R., Emo, G. & Sedman, J. (1993). Rapid quantitative determination of free fatty acids in fats and oils by Fourier transform infrared spectroscopy. *Journal of the American Oil Chemists' Society*, *70*(4), 335–341. https://doi.org/10.1007/BF02552703

Jacomet, S. (2009). Plant economy and village life in Neolithic lake dwellings at the time

of the Alpine Iceman. *Vegetation History and Archaeobotany*, *18*(1), 47–59. https://doi.org/10.1007/s00334-007-0138-2

Jeraj, M., Velušček, A. & Jacomet, S. (2009). The diet of Eneolithic (Copper Age, Fourth millennium cal B.C.) pile dwellers and the early formation of the cultural landscape south of the Alps: A case study from Slovenia. *Vegetation History and Archaeobotany*, *18*(1), 75–89. https://doi.org/10.1007/s00334-008-0144-z

Johnson, L. F. & Shoolery, J. N. (1962). Determination of Unsaturation and Average Molecular Weight of Natural Fats by Nuclear Magnetic Resonance. *Analytical Chemistry*, *34*(9), 1136–1139. Retrieved from http://pubs.acs.org/doi/abs/10.1021/ac60189a033

Kakar, M. E., Khan, M. A., Khan, M. S., Ashraf, K., Kakar, M. A. & Hamdullah, R. A. (2017). Prevalence of tick infestation in different breeds of cattle in balochistan. *Journal of Animal and Plant Sciences* (Vol. 27). https://doi.org/10.1017/CBO9781107415324.004

Karger, B. L. (1997). HPLC: Early and Recent Perspectives. *Journal of Chemical Education*, *74*(1), 45. https://doi.org/10.1021/ed074p45

Kayser, O., Kiderlen, A. F. & Croft, S. L. (2003). Natural products as antiparasitic drugs. *Parasitology Research*, *90* (2), 779–848. https://doi.org/10.1007/s00436-002-0768-3

Keay, R., Onochie, C. & Stanfield, D. (1960). *Nigerian Trees*. Vol. 1. Retrieved from https://scholar.google.com/scholar?q=Keay+Trees+of+Nigeria&btnG=&hl=en&as_sdt=0%2C5#3

Keeler, J. (2002). *Relaxation*. *SpringerReference* (2nd ed.). John Wiley and Sons. https://doi.org/10.1007/SpringerReference_67582

Kittakoop, P., Mahidol, C. & Ruchirawat, S. (2014). Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation. *Current Topics in Medicinal Chemistry*, *14*(2), 239–52. https://doi.org/10.2174/1568026613666131216105049

Knothe, G. & Kenar, J. A. (2004). Determination of the fatty acid profile by 1H-NMR spectroscopy. *European Journal of Lipid Science and Technology*, *106*(2), 88–96. https://doi.org/10.1002/ejlt.200300880

Komba, E. V. G., Mdegela, R. H., Msoffe, P. L. M. & Ingmer, H. (2013). Human and animal Campylobacteriosis in Tanzania: A review. *Tanzania Journal of Health*

Research, *15*(1) 1-13. https://doi.org/10.4314/thrb.v15i1.6

Krider, M. M. & Wall, M. E. (1952). Steroidal sapogenins. V. 1 Enzymatic hydrolysis of steroidal saponins. *Journal of the American Chemical Society*, *74*(12), 3201-3201.https://doi.org/10.1021/ja01132a538

Kuwahara, Y. & Suzuki, H. (1983). Pheromone Study on Acarid Mites XI. Function of Mite Body as Geometrical Isomerization and Reduction of Citral (the Alarm Pheromone). *Chemical and Pharmaceutical Bulletin*, *18*(1), 30–39. https://doi.org/10.1248/cpb.37.3229

Lawal, I. O., Uzokwe, N. E., Igboanugo, A. B. I., Adio, A. F., Awosan, E. A., Nwogwugwu, J. O., … Adesoga, A. A. (2010). Ethno medicinal information on collation and identification of some medicinal plants in Research Institutes of South-west Nigeria. *African Journal of Pharmacy and Pharmacology*, *4*(1), 1–7. Retrieved from www.academicjournals.org/ajpp

Lee, K. H. (1999). Anticancer drug design based on plant-derived natural products. *Journal of Biomedical Science*, *6*(4), 236–50. https://doi.org/25392

Leopold, E. J. (1982). Vacuum Dry Column Chromatography. *Journal of Organic Chemistry*, *47*(23), 4592–4594. https://doi.org/10.1021/jo00144a042

Li, J., Nation, R. L., Turnidge, J. D., Milne, R. W., Coulthard, K., Rayner, C. R. & Paterson, D. L. (2006). Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. *Lancet Infectious Diseases*, *6*(9), 589–601. https://doi.org/10.1016/S1473-3099 (06)70580-1

Liu, Y. Y., Wang, Y., Walsh, T. R., Yi, L. X., Zhang, R., Spencer, J., Shen, J. (2016). Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. *The Lancet Infectious Diseases*, *16*(2), 161–168. https://doi.org/10.1016/S1473-3099 (15)00424-7

Luo, Y., Wang, Q. & Zhang, Y. (2016). A systems pharmacology approach to decipher the mechanism of danggui-shaoyao-san decoction for the treatment of neurodegenerative diseases. *Journal of Ethnopharmacology*, *178*, 66–81. https://doi.org/10.1016/j.jep.2015.12.011

Majekodunmi, S. O. (2016). Physicochemical and binding properties of *Raphia africana* gum in Paracetamol Tablet Formulations. *Academia Journal of Biotechnology*, *4*(May), 177–185. https://doi.org/10.15413/ajb.2015.0229

Majid, A. & Pihillagawa, I. (2014). Potential of NMR Spectroscopy in the Characterization of Nonconventional Oils. *Journal of Fuels*, *2014*, 1–7. https://doi.org/10.1155/2014/390261

Majinda, R. R. T. (2012). Extraction and isolation of saponins. In *Methods in Molecular Biology* (Vol. 864, pp. 415–426). Humana Press. https://doi.org/10.1007/978-1-61779-624-1_16

Malhotra-Kumar, S., Xavier, B. B., Das, A. J., Lammens, C., Butaye, P. & Goossens, H. (2016). Colistin resistance gene mcr-1 harboured on a multidrug resistant plasmid. *The Lancet Infectious Diseases*, *16*(3), 283–284. https://doi.org/10.1016/S1473-3099 (16)00012-8

Mann, A., Ibrahim, K., Oyewale, A. O., Amupitan, J. O., Fatope, M. O. & Okogun, J. I. (2011). Antimycobacterial friedelane-terpenoid from the root bark of Terminalia avicennioides. *American Journal of Chemistry*, *1*(2), 52-55. https://doi.org/10.5923/j.chemistry.20110102.11

Martínez-Yusta, A., Goicoechea, E. & Guillén, M. D. (2014). A review of thermo-oxidative degradation of food lipids studied by ^{1}H NMR spectroscopy: Influence of degradative conditions and food lipid nature. *Comprehensive Reviews in Food Science and Food Safety*, *13*(5), 838–859. https://doi.org/10.1111/1541-4337.12090

Martins, R. C., Filgueiras, T. D. S. & Albuquerque, U. P. (2014). Use and diversity of palm (arecaceae) resources in central Western Brazil. *The Scientific World Journal*, *2014*. https://doi.org/10.1155/2014/942043

Meetei, P. A., Singh, P., Nongdam, P., Prabhu, N. P., Rathore, R. & Vindal, V. (2012). NeMedPlant: a database of therapeutic applications and chemical constituents of medicinal plants from northeast region of India. *Bioinformation*, *8*(4), 209–11. https://doi.org/10.6026/97320630008209

Mesfin, F., Demissew, S. & Teklehaymanot, T. (2009). An ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, *5*(28), 28. https://doi.org/10.1186/1746-4269-5-28

Meyer, B., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. & McLaughlin, J. L. (1982). Brine Shrimp: A Convinient General Bioassay for Active Plant Consyituents. *Kournal of Medicinal Plant Research*, *45*, 31–34. https://doi.org/10.1055/s-2007-971236

Michael, A. S., Thompson, C. G. & Abrahamovitz, M. (1956). Artemia salina as a Test Organism for Bioassay. *Science*, *123*(3194), 464–464.

https://doi.org/10.1126/science.123.3194.464

Middlesworth, V. F. & Cannell, R. J. P. (1998). Dereplication and Partial Identification of Natural Products. *Natural Product Isolation*, *864*, 279–327. https://doi.org/10.1007/978-1-59259-256-2_10

Mitsch, W. J., Tejada, J., Nahlik, A., Kohlmann, B., Bernal, B. & Hernández, C. E. (2008). Tropical wetlands for climate change research, water quality management and conservation education on a university campus in Costa Rica. *Ecological Engineering*, *34*(4), 276–288. https://doi.org/10.1016/j.ecoleng.2008.07.012

Miyake, Y., Yokomizo, K. & Matsuzaki, N. (1998a). Determination of unsaturated fatty acid composition by high-resolution nuclear magnetic resonance spectroscopy. *Journal of the American Oil Chemists' Society*, *75*(12), 1091–1094. https://doi.org/10.1007/s11746-998-0295-1

Miyake, Y., Yokomizo, K. & Matsuzaki, N. (1998b). Rapid determination of iodine value by 1H nuclear magnetic resonance spectroscopy. *Journal of the American Oil Chemists' Society*, *75*(1), 15–19. https://doi.org/10.1007/s11746-998-0003-1

Moore, H. E. (1975). The major groups of palms and their distribution. *The Quaterly Review of Biology*, *50*(1), 94. Retrieved from http://kbd.kew.org/kbd/detailedresult.do?id=40791

Moore, J. C. (1964). Gel permeation chromatography. I. A new method for molecular weight distribution of high polymers. *Journal of Polymer Science Part A: General Papers*, *2*(2), 835–843. https://doi.org/10.1002/pol.1964.100020220

Morton, J. F. (1968). Medicinal plants--old and new. *Bulletin of the Medical Library Association*, *56*(2), 161–167. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=199148&tool=pmcentrez&rendertype=abstract

Muafor, F. J., Levang, P. & Le Gall, P. (2014). A Crispy Delicacy: *Augosoma* Beetle as Alternative Source of Protein in East Cameroon. *International Journal of Biodiversity*, , 1–7. https://doi.org/10.1155/2014/214071

Murphy, R. C. (2015). *Fatty Acids. New developments in mass spectrometry No 4.Tandem Mass Spectrometry of Lipids: Molecular Analysis of Complex Lipids*. Retrieved from www.rsc.org

Musso, D. & Gubler, D. J. (2016). Zika virus. *Clinical Microbiology Reviews*, *29*(3), 487–524. https://doi.org/10.1128/CMR.00072-15

Nandagopal, B., Sankar, S., Ramamurthy, M., Sathish, S. & Sridharan, G. (2011). Could the products of Indian medicinal plants be the next alternative for the treatment of infections? *Indian Journal of Medical Microbiology*, *29*(2), 93–101. https://doi.org/10.4103/0255-0857.81775

Nasaruddin, M. L., Hölscher, C., Kehoe, P., Graham, S. F. & Green, B. D. (2016). Wide-ranging alterations in the brain fatty acid complement of subjects with late Alzheimer's disease as detected by GC-MS. *American Journal of Translational Research*, *8*(1), 154–165.

Ndon, B. A. (2003). The Raphia Palm: Economic Palm Series. *Concept Publication, Lagos, Nigeria*, 1-279.

Nicklin, K. (1980). Annang Ibibio raphia weaving. *Textile History*, *11*, 142–162. https://doi.org/10.1179/004049680793691248

Nieva-Echevarría, B., Goicoechea, E., Manzanos, M. J. & Guillén, M. D. (2014). A method based on 1H NMR spectral data useful to evaluate the hydrolysis level in complex lipid mixtures. *Food Research International*, *66*, 379–387. https://doi.org/10.1016/j.foodres.2014.09.031

Nieva-Echevarría, B., Goicoechea, E., Manzanos, M. J. & Guillén, M. D. (2015). Usefulness of 1H NMR in assessing the extent of lipid digestion. *Food Chemistry*, *179*, 182–190. https://doi.org/10.1016/j.foodchem.2015.01.104

Nieva-Echevarría, B., Goicoechea, E., Manzanos, M. J. & Guillén, M. D. (2016). The influence of frying technique, cooking oil and fish species on the changes occurring in fish lipids and oil during shallow-frying, studied by 1H NMR. *Food Research International*, *84*, 150–159. https://doi.org/10.1016/j.foodres.2016.03.033

Ninh, P. H., Honda, K., Sakai, T., Okano, K. & Ohtake, H. (2015). Assembly and multiple gene expression of thermophilic enzymes in *Escherichia coli* for *in vitro* metabolic engineering. *Biotechnology and Bioengineering*, *112*(1), 189–196. https://doi.org/10.1002/bit.25338

Nwodo, J. N., Ibezim, A., Simoben, C. V & Ntie-Kang, F. (2015). Exploring cancer therapeutics with natural products from African medicinal plants, part II: alkaloids, terpenoids and flavonoids. *Anti-Cancer Agents in Medicinal Chemistry*, *50*(August), 1–58. https://doi.org/10.2174/1871520615666150520143827

O'Toole, P. W. & Jeffery, I. B. (2015). Gut microbiota and aging. *Science*, *350*(6265), 1214–1216. https://doi.org/10.1126/science.aac8469

Obahiagbon, F. (2009). A review of the origin, morphology, cultivation, economic products, health and physiological implications of raphia palm. *African Journal of Food Science* Vol 3 (13). *Academic Journals*. Retrieved from http://www.academicjournals.org/app/webroot/article/article1380644347_Obahiagbon.pdf

Obahiagbon, F. I. & Osagie, A. U. (2007). Sugar and macrominerals composition of sap produced by *Raphia hookeri* palms. *African Journal of Biotechnology, 6*(6), 744–750. Retrieved from http://www.ajol.info/index.php/ajb/article/view/56896

Oboutor, E. M., Onajobi, F. D., Olugbade, T. A. & Adesanya, S. A. (2006). Raphiasaponin1, the major ichthyotoxic principle in *Raphia hookeri* fruit. *Nigerian Journal of Natural Products and Medicine*, *10*(1), 104-107. https://doi.org/10.4314/njnpm.v10i1.11863

Odebiyi OO, S. E. (1978). Phytochemical screening of Nigerian medicinal plants II. *Lloydia.*, *41*(3), 234–246. Retrieved from http://europepmc.org/abstract/med/672462

Odeh, I. C., Tor-Anyiin, T. A., Igoli, J. O. & Anyam, J. V. (2016). In vitro antimicrobial properties of friedelan-3-one from *Pterocarpus santalinoides* LHerit, ex Dc. *African Journal of Biotechnology*, *15*(14), 531–538. https://doi.org/10.5897/AJB2015.15091

Ogbuagu, M. N. (2008). Vitamins, phytochemicals and toxic elements in the pulp and seed of raphia palm fruit (*Raphia hookeri*). *Fruits*, *63*(5), 297–302. https://doi.org/10.1051/fruits:2008025

Ogungbe, I. V., Singh, M. & Setzer, W. N. (2012). Antileishmanial natural products from plants. *Studies in Natural Products Chemistry*, *36*, 331–382. https://doi.org/10.1016/B978-0-444-53836-9.00027-X

Okwu, D.E. & Nnamdi, F.U. (2008). Evaluation Of The Chemical Composition Of *Dacryodes edulis* and *Raphia hookeri* Mann And Wendl Exudates Used In Herbal Medicine In South Eastern Nigeria. *African Journal of Traditional, Complementary and Alternative Medicines*, 5 (2) 194-200 doi:10.4314/ajtcam.v5i2.31273

Onawunmi, G. O. (1989). Evaluation of the antimicrobial activity of citral. *Letters in Applied Microbiology*, *9*(3), 105–108. https://doi.org/10.1111/j.1472-765X.1989.tb00301.x

Onyeike, E. N., Ayalogu, E. O. & Okaraonye, C. C. (2005). Nutritive value of the larvae of raphia palm beetle (*Oryctes rhinoceros*) and weevil (*Rhyncophorus pheonicis*).

Journal of the Science of Food and Agriculture, *85*(11), 1822–1828. https://doi.org/10.1002/jsfa.2054

Opute, F. I. (1978). Palmae lipids: Status in chemotaxonomy. *Journal of Experimental Botany*, *29*(5), 1259–1264. https://doi.org/10.1093/jxb/29.5.1259

Osseo-Asare, A. D. (2008). Bioprospecting and resistance: Transforming poisoned arrows into strophantin pills in colonial Gold Coast, 1885-1922. *Social History of Medicine*, *21*(2), 269–290. https://doi.org/10.1093/shm/hkn025

Otedoh, M. (1977). Large-scale seed germination in Raphia palms. *Nigerian Field*. *1*(2), 69–79 Retrieved from http://agris.fao.org/agris-search/search.do?recordID=US201302399995

Otedoh, M. O. (1981). Raphia Palm and Other Wine Producing Palms in Nigeria. *J. Niger. Institute for Oil Palm Res*, *6*(21), 92-99.

Pan, X., Niu, G. & Liu, H. (2003). Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chemical Engineering and Processing*, *42*(2), 129–133. https://doi.org/10.1016/S0255-2701 (02)00037-5

Parker, T., Limer, E., Watson, A. D., Defernez, M., Williamson, D. & Kemsley, E. K. (2014). 60MHz 1H NMR spectroscopy for the analysis of edible oils. *TrAC - Trends in Analytical Chemistry*, *57*(100), 147–158. https://doi.org/10.1016/j.trac.2014.02.006

Pascal, S., Taton, M. & Rahier, A. (1993). Plant sterol biosynthesis. Identification and characterization of two distinct microsomal oxidative enzymatic systems involved in sterol C4- demethylation. *Journal of Biological Chemistry*, *268*(16), 11639–11654.

Pednekar, S., Joshi, N., Menon, S., Shailjan, S. & Khairnar, B. (2011). Microwave-assisted extraction of gymnemic acid: A potent antidiabetic agent from *Gymnema sylvestre* R.B. and its comparison with conventional extraction technique. *Asian Journal of Chemistry*, *23*(10), 4380–4384.

Pépin, J., Milord, F., Khonde, A. N., Niyonsenga, T., Loko, L., Mpia, B. & De Wals, P. (1995). Risk factors for encephalopathy and mortality during melarsoprol treatment of *Trypanosoma brucei gambiense* sleeping sickness. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *89*(1), 92–97. https://doi.org/10.1016/0035-9203 (95)90673-8

Phillips, J. A. & Neyland, A. (2016). Zika Virus. *Workplace Health Saf*, *64*, 396.

https://doi.org/10.1177/2165079916657109

Piironen, V., Lindsay, D. G., Miettinen, T. A., Toivo, J. & Lampi, A. M. (2000). Plant sterols: Biosynthesis, biological function and their importance to human nutrition. *Journal of the Science of Food and Agriculture*, *80*(7), 939–966. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<939::AID-JSFA644>3.0.CO;2-C

Pitchai, D., Manikkam, R., Rajendran, S. R. & Pitchai, G. (2010). Database on pharmacophore analysis of active principles, from medicinal plants. *Bioinformation*, *5*(2), 43–5. https://doi.org/10.6026/9732063000543

Pollan, M. (2013). Say Hello to the 100 Trillion Bacteria That Make Up Your Microbiome. *The New York Times*. Retrieved from http://www.nytimes.com/2013/05/19/magazine/say-hello-to-the-100-trillion-bacteria-that-make-up-your-microbiome.html%5Cnhttp://www.nytimes.com/2013/05/19/magazine/say-hello-to-the-100-trillion-bacteria-that-make-up-your-microbiome.html?pagewanted=all&_r=0

Prasain, J. K., Wilson, L., Hoang, H. D., Moore, R. & Miller, M. A. (2015). Comparative lipidomics of *Caenorhabditis elegans* metabolic disease models by SWATH non-targeted tandem mass spectrometry. *Metabolites*, *5*(4), 677–696. https://doi.org/10.3390/metabo5040677

Prescott, A. B. (1903). *A Textbook of Organic Chemistry. Journal of the American Chemical Society* (4th ed., Vol. 25). New Age International. https://doi.org/10.1021/ja02009a018

Rainey, H. J., Iyenguet, F. C., Malanda, G.-A. F., Madzoké, B., Santos, D. Dos, Stokes, E. J., Strindberg, S. (2009). Survey of Raphia swamp forest, Republic of Congo, indicates high densities of Critically Endangered western lowland gorillas *Gorilla gorilla gorilla*. *Oryx*, *44*(1), 124. https://doi.org/10.1017/s003060530999010x

Robacker, D. C. & Hendry, L. B. (1977). Neral and geranial: Components of the sex pheromone of the parasitic wasp, *Itoplectis conquisitor*. *Journal of Chemical Ecology*, *3*(5), 563–577. https://doi.org/10.1007/BF00989077

Rothman, E. S., Wall, M. E. & Eddy, C. R. (1952). Steroidal Sapogenins. III. Structure of Steroidal Saponins2. *Journal of the American Chemical Society*, *74*(16), 4013-4016. Retrieved from http://pubs.acs.org/doi/abs/10.1021/ja01136a012

Rouessac, F. Rouessac, A. (2007). *Chemical Analysis: Modern Instrumental Methods adn Techniques* (Engl.). Chichester [u.a.]: Wiley. Retrieved from

http://books.google.co.uk/books/about/Chemical_analysis.html?id=xUpCAQAAIAAJ&redir_esc=y

Routray, W. & Orsat, V. (2012). Microwave-assisted extraction of flavonoids: a review. *Food and Bioprocess Technology*, *5*(2), 409-424. https://doi.org/10.1007/s11947-011-0573-z

Rubin, D. C., Shaker, A. & Levin, M. S. (2012). Chronic intestinal inflammation: Inflammatory bowel disease and colitis-associated colon cancer. *Frontiers in Immunology*, *3*, 107. https://doi.org/10.3389/fimmu.2012.00107

Rubin, E. J., Herrera, C. M., Crofts, A. A. & Stephen, M. (2015). PmrD Is Required for Modifications to *Escherichia coli* Endotoxin. *Antimicrobial Agents and Chemotherapy*, *59*(4), 2051–2061. https://doi.org/10.1128/AAC.05052-14

Rudkowska, I., AbuMweis, S. S., Nicolle, C. & Jones, P. J. H. (2008). Cholesterol-lowering efficacy of plant sterols in low-fat yogurt consumed as a snack or with a meal. *Journal of the American College of Nutrition*, *27*(5), 588–595. https://doi.org/10.1080/07315724.2008.10719742

Russel, T. (1965). The raffia palms of West Africa. *Kew Bulletin*, *19*(2), 173. https://doi.org/10.2307/4108027

Saeidnia, S., Manayi, A., Gohari, A. R. & Abdollahi, M. (2014). The story of beta-sitosterol-a review. *European Journal of Medicinal Plants*, *4*(5), 590–609.

Saleem, M. (2009). Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. *Cancer Letters*, *285*(2), 109–15. https://doi.org/10.1016/j.canlet.2009.04.033

Saleem, M., Afaq, F., Adhami, V. M. & Mukhtar, H. (2004). Lupeol modulates NF-kappaB and PI3K/Akt pathways and inhibits skin cancer in CD-1 mice. *Oncogene*, *23*, 5203–5214. https://doi.org/10.1038/sj.onc.1207641

Saleem, M., Kweon, M. H., Yun, J. M., Adhami, V. M., Khan, N., Syed, D. N. & Mukhtar, H. (2005). A novel dietary triterpene lupeol induces fas-mediated apoptotic death of androgen-sensitive prostate cancer cells and inhibits tumor growth in a xenograft model. *Cancer Research*, *65*(23), 11203–11213. https://doi.org/10.1158/0008-5472.CAN-05-1965

San-Blas, G. & Calderone, R. A. (2008). *Pathogenic Fungi: Insights in Molecular Biology*. *Washington: Caister Academic Press*. Caister Academic Press. Retrieved from http://books.google.com/books?id=BxYFKB6sVhoC&pgis=1

Sandy, M. & Bacon, L. (2008). A tensile testing method for monocotyledon leaves with parallel venation. In *15th triennial conference, New Delhi, 22-26 September 2008: preprints (ICOM Committee for Conservation)* (pp. 198–205).

Sarker, S. D. & Nahar, L. (2012). Hyphenated techniques and their applications in natural products analysis. In *Natural Products Isolation* (pp. 301-340). Humana Press.. https://doi.org/10.1007/978-1-61779-624-1_12 [doi]

Sato, N. (2015). Is monoglucosyldiacylglycerol a precursor to monogalactosyldiacylglycerol in all cyanobacteria? *Plant and Cell Physiology*, *56*(10), 1890–1899. https://doi.org/10.1093/pcp/pcv116

Saxton, J. E. (1971). The Alkaloids. A Specialist Periodical Report. *London: The Chemical Society*, 169.

Schaller, H. (2010). Sterol and Steroid Biosynthesis and Metabolism in Plants and Microorganisms. *Comprehensive Natural Products II*, 755–787. https://doi.org/http://dx.doi.org/10.1016/B978-008045382-8.00008-3

Schardl, C. L., Grossman, R. B., Nagabhyru, P., Faulkner, J. R. & Mallik, U. P. (2007). Loline alkaloids: Currencies of mutualism. *Phytochemistry*, *68*(7), 980–996. https://doi.org/10.1016/j.phytochem.2007.01.010

Sedman, J., Gao, L., lez, D. G. G., Ehsan, S. & van de Voort, F. R. (2010). Determining nutritional labeling data for fats and oils by 1H NMR. *European Journal of Lipid Science and Technology*, *112*(4), 439–451. https://doi.org/10.1002/ejlt.200900185

Shahidi, F. & Wanasundara, U. N. (2009). Plant-Environment Interactions. In C. C. Akoh & D. B. Min (Eds.), *Food Lipids: Chemistry, Nutrition, and Biotechnology*, (2nd ed., p. 175). New York: Marcel Dekker, Inc. https://doi.org/10.1007/978-3-540-89230-4

Shen, J., Obin, M. S. & Zhao, L. (2013). The gut microbiota, obesity and insulin resistance. *Molecular Aspects of Medicine*. https://doi.org/10.1016/j.mam.2012.11.001

Shimbe, R. Y., Tor-Anyiin, T. A., Khan, M. E. & Anyam, J. V. (2016). β-sitosterol from *Hymenocardia acida* root extract and its antimicrobial activity. *Journal of Chemical Society of Nigeria*, *41*(1), 76–81. Retrieved from http://journals.chemsociety.org.ng/index.php/jcsn/article/view/51

Shinwari, Z. K. (2010). Medicinal plants research in Pakistan. *Journal of medicinal plants research*, *4*(3), 161-176.

Shukla, S. & Gupta, S. (2010). Apigenin: A promising molecule for cancer prevention. *Pharmaceutical Research*, *27*(6), 962–978. https://doi.org/10.1007/s11095-010-0089-7

Siciliano, C., Belsito, E., De Marco, R., Di Gioia, M. L., Leggio, A. & Liguori, A. (2013). Quantitative determination of fatty acid chain composition in pork meat products by high resolution 1H NMR spectroscopy. *Food Chemistry*, *136*(2), 546–554. https://doi.org/10.1016/j.foodchem.2012.08.058

Simova, S., Ivanova, G. & Spassov, S. L. (2003). Alternative NMR method for quantitative determination of acyl positional distribution in triacylglycerols and related compounds. *Chemistry and Physics of Lipids*, *126*(2), 167–176. https://doi.org/10.1016/j.chemphyslip.2003.08.003

Skene, C. D. & Sutton, P. (2006). Saponin-adjuvanted particulate vaccines for clinical use. *Methods*, *40*(1), 53–59. https://doi.org/10.1016/j.ymeth.2006.05.019

Sofowora, A. (1993). Recent Trends in Research into African Medicinal-Plants. *Journal of Ethnopharmacology*, *38*(2–3), 209–214.

Soininen, T. H., Jukarainen, N., Julkunen-Tiitto, R., Karjalainen, R. & Vepsäläinen, J. J. (2012). The combined use of constrained total-line-shape 1H NMR and LC-MS/MS for quantitative analysis of bioactive components in yellow onion. *Journal of Food Composition and Analysis*, *25*(2), 208–214. https://doi.org/10.1016/j.jfca.2011.09.001

Song, F. L., Gan, R. Y., Zhang, Y., Xiao, Q., Kuang, L. & Li, H. Bin. (2010). Total phenolic contents and antioxidant capacities of selected Chinese medicinal plants. *International Journal of Molecular Sciences*, *11*(6), 2362–2372. https://doi.org/10.3390/ijms11062362

Sopelana, P., Arizabaleta, I., Ibargoitia, M. L. & Guillén, M. D. (2013). Characterisation of the lipidic components of margarines by 1H Nuclear Magnetic Resonance. *Food Chemistry*, *141*(4), 3357–3364. https://doi.org/10.1016/j.foodchem.2013.06.026

Springob, K. & Kutchan, T. M. (2009). Introduction to the different classes of natural products. In *Plant-Derived Natural Products* (pp. 3-50). Springer, New York, NY. https://doi.org/10.1007/978-0-387-85498-4_1

Sticher, O. (2008). Natural product isolation. *Natural Product Reports*, *25*(3), 517–54. https://doi.org/10.1039/b700306b

Su, K.-P., Wang, S.-M. & Pae, C.-U. (2013). Omega-3 polyunsaturated fatty acids for major depressive disorder. *Expert Opinion on Investigational Drugs*, *22*(12), 1519–34. https://doi.org/10.1517/13543784.2013.836487

Sundström, M., Pelander, A., Angerer, V., Hutter, M., Kneisel, S. & Ojanperä, I. (2013). A high-sensitivity ultra-high performance liquid chromatography/high- resolution time-of-flight mass spectrometry (UHPLC-HR-TOFMS) method for screening synthetic cannabinoids and other drugs of abuse in urine. *Analytical and Bioanalytical Chemistry*, *405*(26), 8463–8474. https://doi.org/10.1007/s00216-013-7272-8

Szostak, A., Ogłuszka, M., te Pas, M. F. W., Poławska, E., Urbański, P., Juszczuk-Kubiak, E., Pierzchała, M. (2016). Effect of a diet enriched with omega-6 and omega-3 fatty acids on the pig liver transcriptome. *Genes & Nutrition*, *11*(1), 9. https://doi.org/10.1186/s12263-016-0517-4

Tan, W., Lu, J., Huang, M., Li, Y., Chen, M., Wu, G., Wang, Y. (2011). Anti-cancer natural products isolated from Chinese medicinal herbs. *Chinese Medicine*, *6*(1), 27. https://doi.org/10.1186/1749-8546-6-27

Tapondjou, L. A., Siems, K. J., Böttger, S. & Melzig, M. F. (2015). Steroidal Saponins from the Mesocarp of the Fruits of *Raphia farinifera* (Arecaceae) and their Cytotoxic Activity. *Natural Product Communications*, *10*(11), 1941–4. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/26749832

Thimmappa, R., Geisler, K., Louveau, T., O'Maille, P. & Osbourn, A. (2014). Triterpene biosynthesis in plants. *Annual Review of Plant Biology*, *65*, 225–57. https://doi.org/10.1146/annurev-arplant-050312-120229

Throne, J. E., Weaver, D. K., Chew, V. & Baker, J. E. (1995). Probit analysis of correlated data: multiple observations over time at one concentration. *Journal of Economical Entomology*, *88*(5), 1510–1512. https://doi.org/10.1093/jee/88.5.1510

Todd, J. J., Pourshahidi, L. K., McSorley, E. M., Madigan, S. M. & Magee, P. J. (2015). Vitamin D: Recent Advances and Implications for Athletes. *Sports Medicine*, *45*(2), 213–229. https://doi.org/10.1007/s40279-014-0266-7

Tor-Anyiin, T. A., Igoli, J., Anyam, J. N. & Anyam, J. V. (2015). Isolation and Antimicrobial Activity of Sarracenin from Root Bark of *Strychnos spinosa*. *Journal of Chemical Society of Nigeria*, *40*(1), 71–75. Retrieved from http://journals.chemsociety.org.ng/index.php/jcsn/article/view/13

Trathnigg, B. (1995). Determination of MWD and chemical composition of polymers by chromatographic techniques. *Progress in Polymer Science*, *20*(4), 615–650.

https://doi.org/10.1016/0079-6700 (95)00005-Z

Udofia, P. G., Eyen, N. O. & Ekanem, M. C. (2009). Optimization of functional and sensory attributes of raphia palmwine with central component design (CCD). *Advances in Natural and Applied Sciences*, *3*(1), 49-56. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-73249127023&partnerID=40&md5=f9e02f2e6e67a6da2a3e5899d2f3cb77

Umoren, S. A. & Ebenso, E. E. (2008). Studies of the anti-corrosive effect of *Raphia hookeri* exudate gum-halide mixtures for aluminium corrosion in acidic medium. *Pigment and Resin Technology*, *37*(3), 173–182. https://doi.org/10.1108/03699420810871020

Usman, A., Abdulrahman, F. I. & Usman, A. (2009). Qualitative phytochemical screening and *in vitro* antimicrobial effects of methanol stem bark extract of *Ficus thonningii* (Moraceae). *African Journal of Traditional, Complementary and Alternative Medicines*, *6*(3), 289–295. https://doi.org/10.4314/ajtcam.v6i3.57178

Van Olden, C., Groen, A. K. & Nieuwdorp, M. (2015). Role of Intestinal Microbiome in Lipid and Glucose Metabolism in Diabetes Mellitus. *Clinical Therapeutics*, *37*(6), 1172–1177. https://doi.org/10.1016/j.clinthera.2015.03.008

Van Walbeek, W., Moodie, C. A., Scott, P. M., Harwig, J. & Grice, H. C. (1971). Toxicity and excretion of ochratoxin A in rats intubated with pure ochratoxin A or fed cultures of Penicillium viridicatum. *Toxicology and Applied Pharmacology*, *20*(3), 4239–41. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/5132783

Vanhaecke, P., Persoone, G., Claus, C. & Sorgeloos, P. (1981). Proposal for a short-term toxicity test with Artemia nauplii. *Ecotoxicology and Environmental Safety*, *5*(3), 382–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7297475

Vlahov, G. (1999). Application of NMR to the study of olive oils. *Progress in Nuclear Magnetic Resonance Spectroscopy*, *35*(79), 341–357. https://doi.org/10.1016/S0079-6565 (99)00015-1

Vlahov, G., Giuliani, A. A. & Del Re, P. (2010). 13C NMR spectroscopy for determining the acylglycerol positional composition of lampante olive oils. Chemical shift assignments and their dependence on sample concentration. *Analytical Methods*, *2*(7), 916–923. https://doi.org/10.1039/c0ay00028k

Vlahov, G., Schiavone, C. & Simone, N. (1999). Triacylglycerols of the olive fruit (*Olea europaea* L.): Characterization of mesocarp and seed triacylglycerols in different cultivars by liquid chromatography and ^{13}C NMR spectroscopy. *European Journal of Lipid Science and Technology*, *101*(4), 146-150.

https://doi.org/10.1002/ (SICI) 1521-4133(199904)101:4<146: AID-LIPI146>3.0.CO; 2-3

Vollekova, A., Košťálová, D. & Sochorova, R. (2001). Isoquinoline alkaloids from *Mahonia aquifolium* stem bark are active against *Malassezia spp*. *Folia microbiologica*, *46*(2), 107. Retrieved from http://link.springer.com/article/10.1007/BF02873586

Wal, A., Rai, A., Wal, P. & Sharma, G. (2011). Biological activities of lupeol. *Systematic Reviews in Pharmacy*, *2*(2), 96. https://doi.org/10.4103/0975-8453.86298

Wal, A., Srivastava, R. ., Wal, P., Rai, A. & Sharma, S. (2015). Lupeol as a magical drug. *Pharmaceutical and Biological Evaluations*, *2*(5), 142–151. Retrieved from http://onlinepbe.com/index.php/PBE/article/view/40

Wall, M. E., Eddy, C. R., McClennan, M. L. & Klumpp, M. E. (1952). Detection and estimation of steroidal sapogenins in plant tissue. *Analytical Chemistry*, *24*(8), 1337-1341. Retrieved from http://pubs.acs.org/doi/abs/10.1021/ac60068a018

Wang, F. (2014). 1H-NMR spectra of common steroids. Retrieved June 12, 2017, from http://www.wangfei.ac.cn/nmrspectra/7/1/39

Wang, J., Zhang, J., Zhao, B., Wang, X., Wu, Y. & Yao, J. (2010). A comparison study on microwave-assisted extraction of *Potentilla anserina* L. polysaccharides with conventional method: Molecule weight and antioxidant activities evaluation. *Carbohydrate Polymers*, *80*(1), 84–93. https://doi.org/10.1016/j.carbpol.2009.10.073

Wansi, J. D., Devkota, K. P., Tshikalange, E. & Kuete, V. (2013). Alkaloids from the Medicinal Plants of Africa. In *Medicinal Plant Research in Africa: Pharmacology and Chemistry* (pp. 557–605). https://doi.org/10.1016/B978-0-12-405927-6.00014-X

Wells, T. N. (2011). Natural products as starting points for future anti-malarial therapies: going back to our roots? *Malaria Journal*, *10*(Suppl 1), S1–S3. https://doi.org/10.1186/1475-2875-10-S1-S3

Wilt, T. J., Ishani, A., MacDonald, R., Stark, G., Mulrow, C. D. & Lau, J. (1999). Beta-sitosterols for benign prostatic hyperplasia. *Cochrane Database of Systematic Reviews*, (2), CD001043. https://doi.org/10.1002/14651858.CD001043

Winschel, G. A., Everett, R. K., Coppola, B. P., Shultz, G. V. & Lonn, S. (2015). Using Jigsaw-Style Spectroscopy Problem-Solving to Elucidate Molecular Structure

through Online Cooperative Learning. *Journal of Chemical Education*, *92*(7), 1188–1193. https://doi.org/10.1021/acs.jchemed.5b00114

Wu, C. (2014). An important player in brine shrimp lethality bioassay: The solvent. *Journal of Advanced Pharmaceutical Technology & Research*, *5*(1), 57–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24696818

Xiao, W., Han, L. & Shi, B. (2008). Microwave-assisted extraction of flavonoids from *Radix astragali*. *Separation and Purification Technology*, *62*(3), 614–618. https://doi.org/10.1016/j.seppur.2008.03.025

Xu, R., Zhao, W., Xu, J., Shao, B. & Qin, G. (1996). Studies on bioactive saponins from Chinese medicinal plants. *Advances in Experimental Medicine and Biology*, *404*, 371–382. https://doi.org/10.1007/978-1-4899-1367-8_30

Yang, L., Moreno, A., Fieber, W., Brauchli, R. & Sommer, H. (2015). Spectroscopic separation of 13C NMR spectra of complex isomeric mixtures by the CSSF-TOCSY-INEPT experiment. *Magnetic Resonance in Chemistry*, *53*(4), 304–308. https://doi.org/10.1002/mrc.4188

Yapna, D., Talontsi, F., Kouam, S., Hussain, H., Ngadjui, B., Green, I. & Krohn, K. (2013). Chemical Constituents of the seeds of *Raphia farinifera*. *Syllabus Review*, *4*(11), 9–11. Retrieved from http://www.researchgate.net/profile/Simeon_Kouam/publication/277005996_Chemical_Constituents_of_the_seeds_of_Raphia_farinifera/links/5589468d08ae273b2875c4e8.pdf

Young, J. C. (1995). Microwave-Assisted Extraction of the Fungal Metabolite Ergosterol and Total Fatty-Acids. *Journal of Agricultural and Food Chemistry*, *43*(11), 2904–2910. https://doi.org/Doi 10.1021/Jf00059a025

Yu, G. F., Mulabagal, V., Diyabalanage, T., Hurtada, W. A., DeWitt, D. L. & Nair, M. G. (2008). Non-nutritive functional agents in rattan-shoots, a food consumed by native people in the Philippines. *Food Chemistry*, *110*(4), 991–996. https://doi.org/10.1016/j.foodchem.2008.03.015

Zangger, K. (2015). Pure shift NMR. *Progress in Nuclear Magnetic Resonance Spectroscopy*, *86–87*, 1–20. https://doi.org/10.1016/j.pnmrs.2015.02.002

Zenk, M. H. & Juenger, M. (2007). Evolution and current status of the phytochemistry of nitrogenous compounds. *Phytochemistry*, *68*(22–24), 2757–2772. https://doi.org/10.1016/j.phytochem.2007.07.009

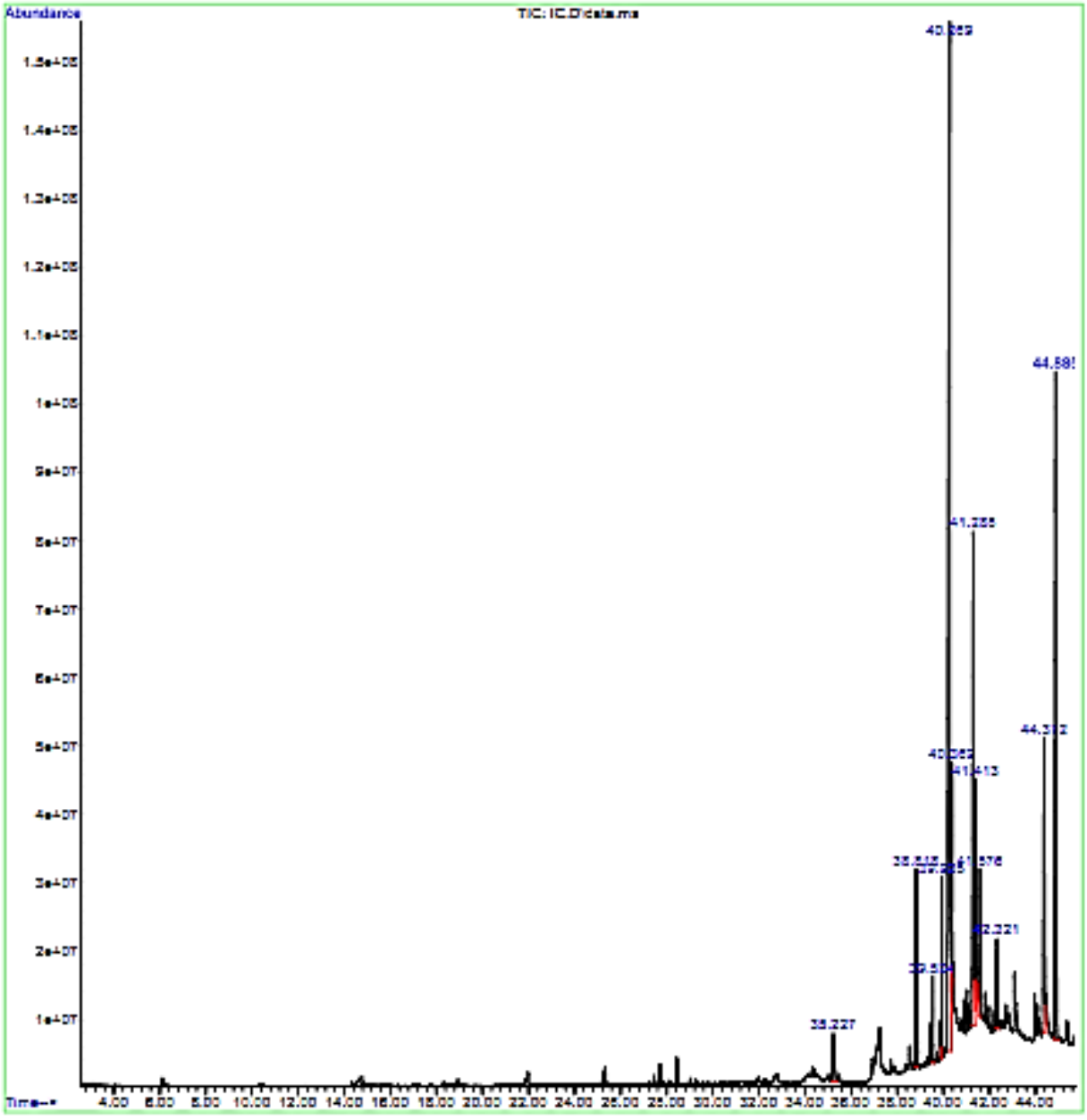
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Zou, T., Wang, D., Guo, H., Zhu, Y., Luo, X., Liu, F. & Ling, W. (2012). Optimization of microwave-assisted extraction of anthocyanins from mulberry and identification of anthocyanins in extract using HPLC-ESI-MS. *Journal of Food Science*, *77*(1), C46–C50. https://doi.org/10.1111/j.1750-3841.2011.02447.x

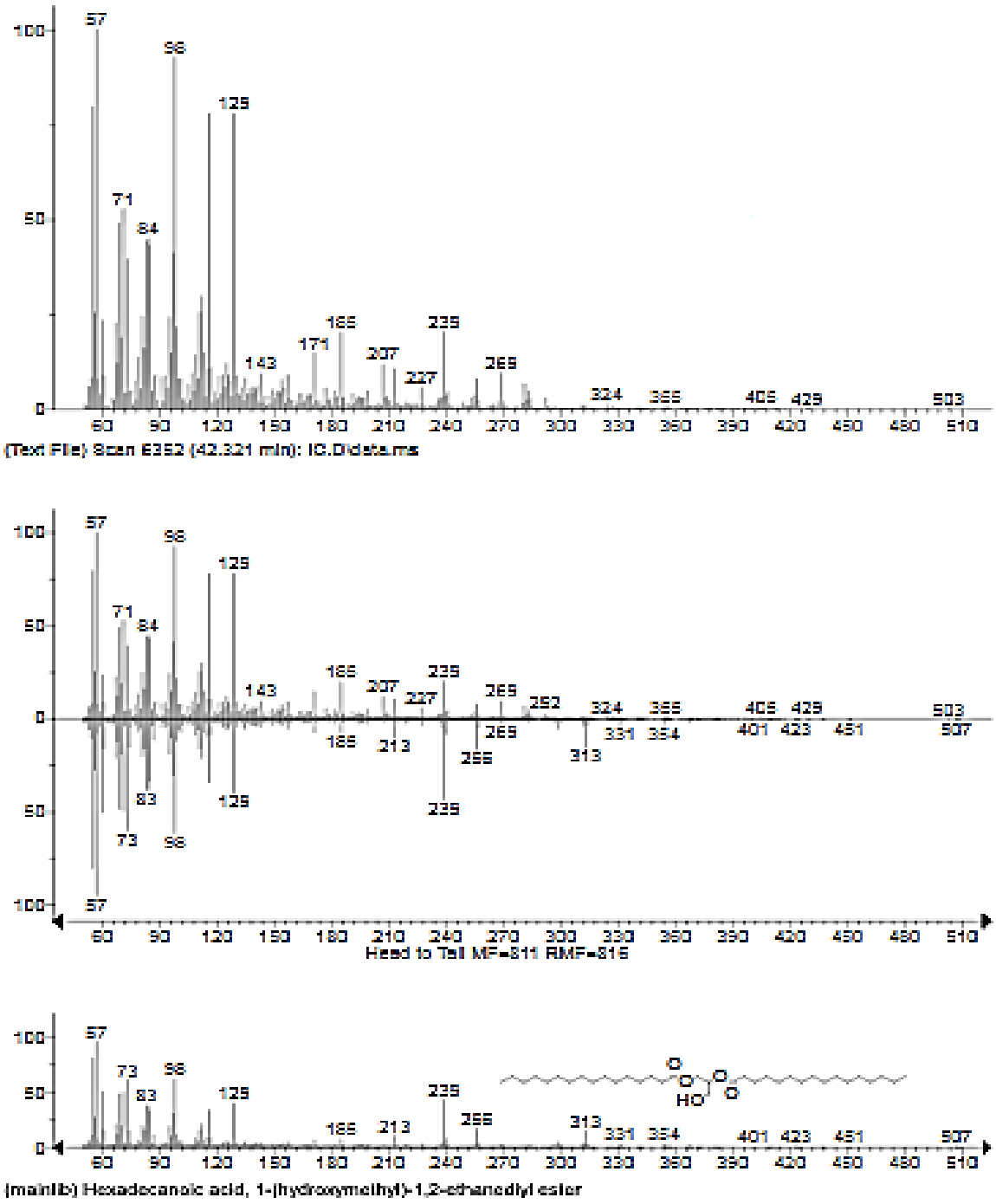
APPENDICES

Appendix I. GC profile for Ethyl acetate Crude Extract (IC) of *Raphia africana*:

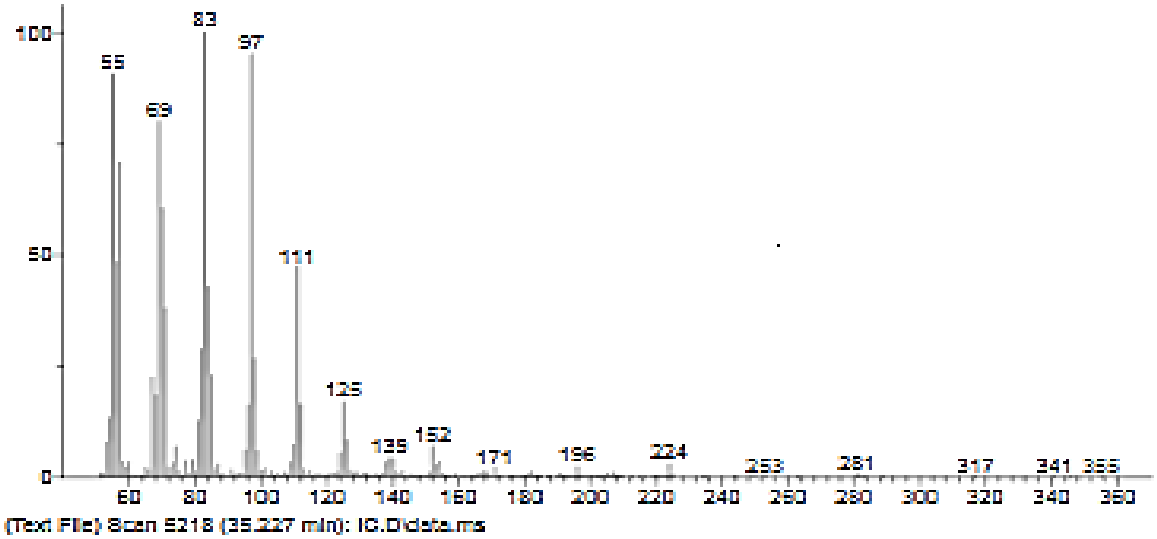
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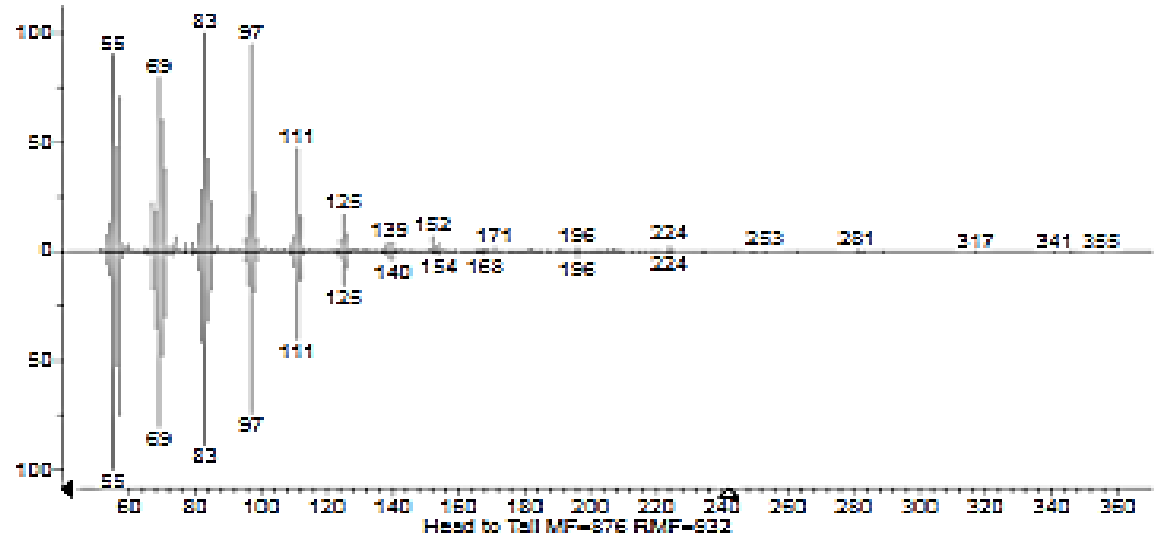


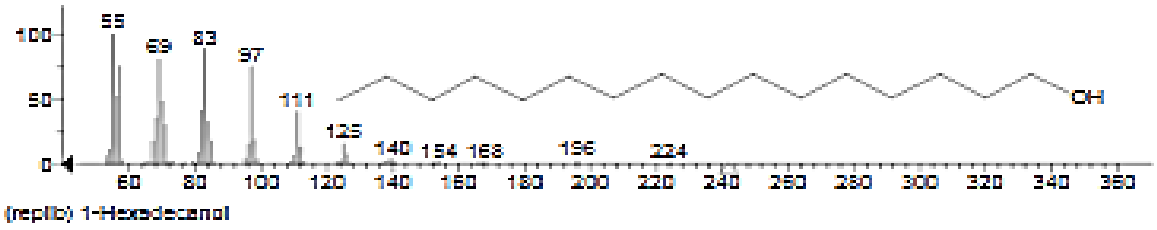
Appendix I-A. GC-MS profile for Ethyl acetate Crude Extract (IC) of *Raphia africana*: A Pre-Isolation Study.



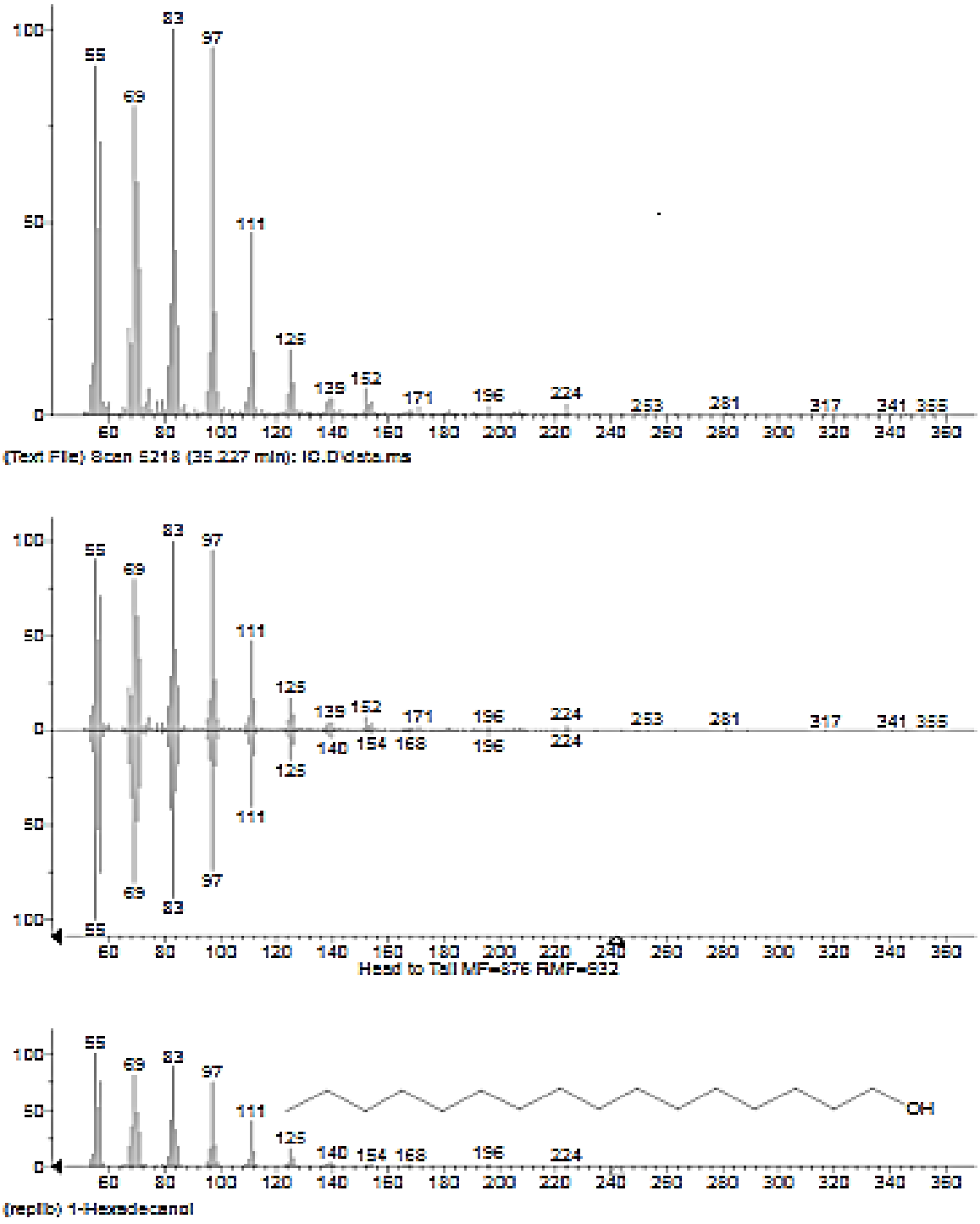
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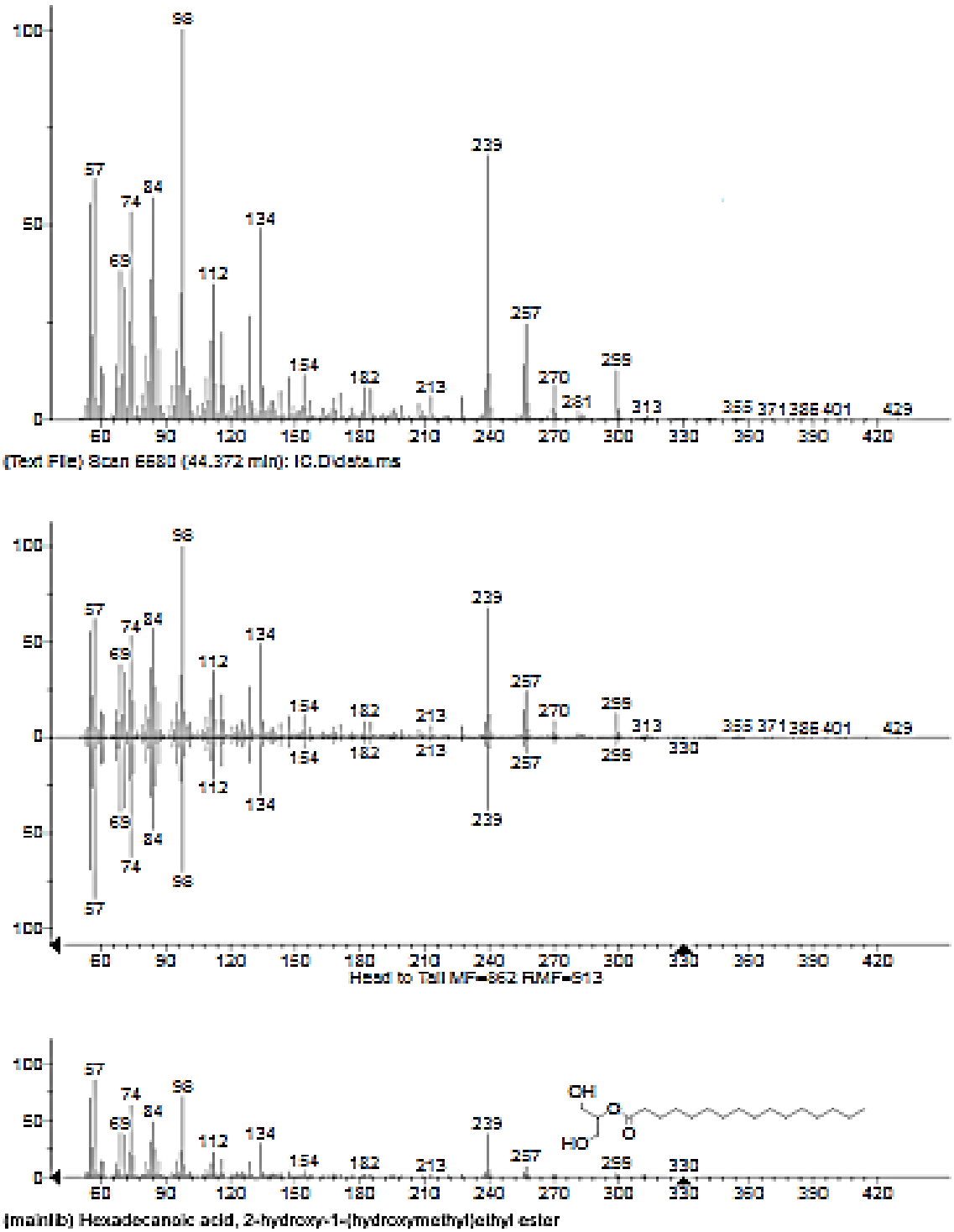




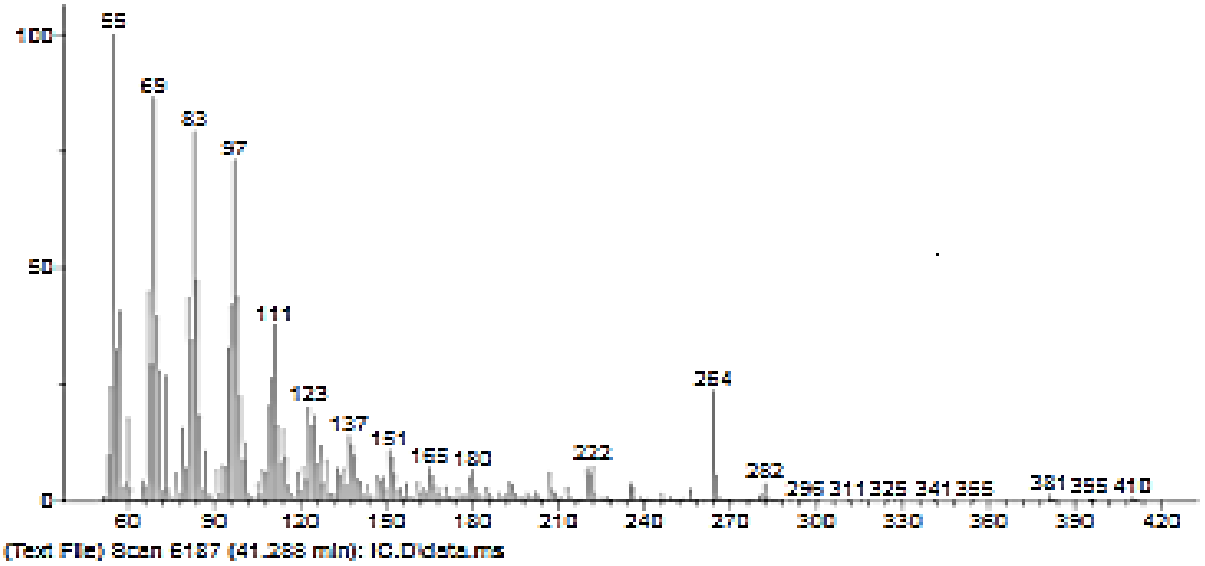
Appendix I-C. GC-MS profile for Ethyl acetate Crude Extract (IC) of *Raphia africana*: A Pre-Isolation Study

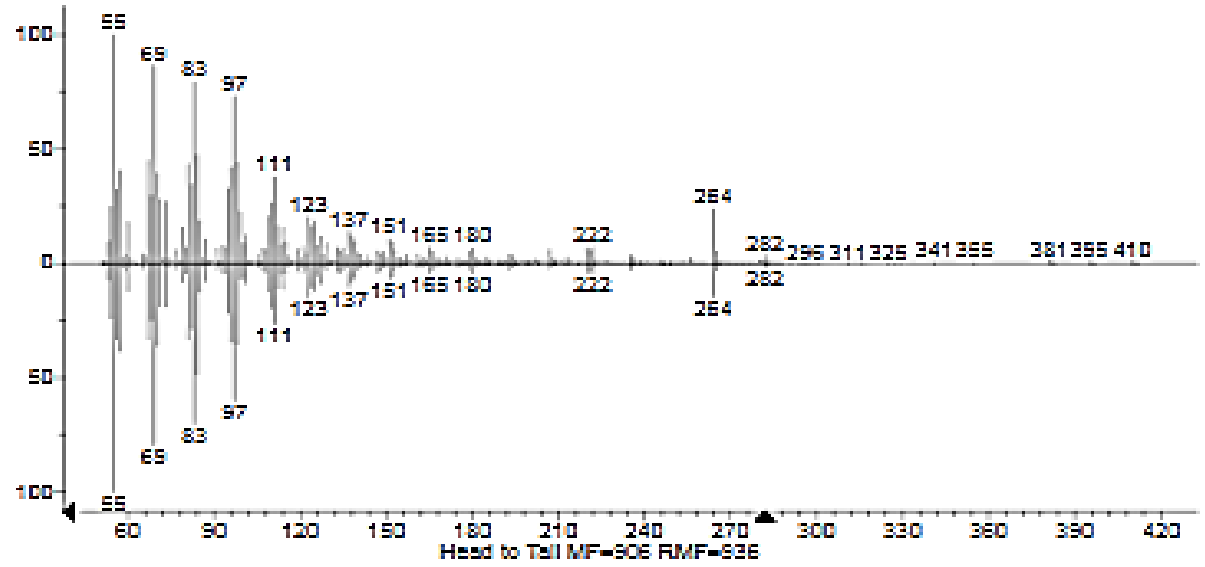


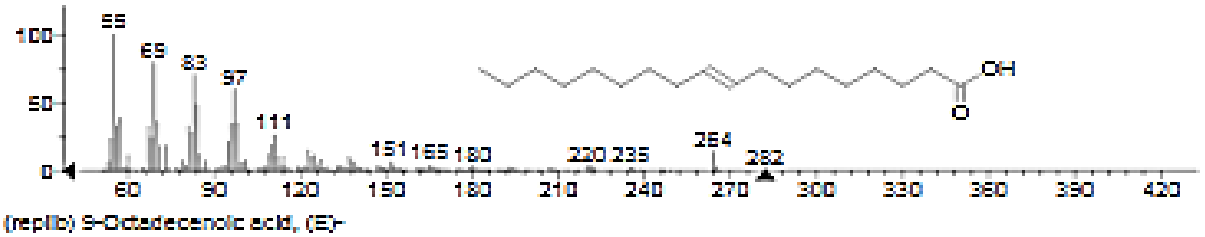
Appendix I-D. GC-MS profile for Ethyl acetate Crude Extract (IC) of *Raphia africana*: A Pre-Isolation Study



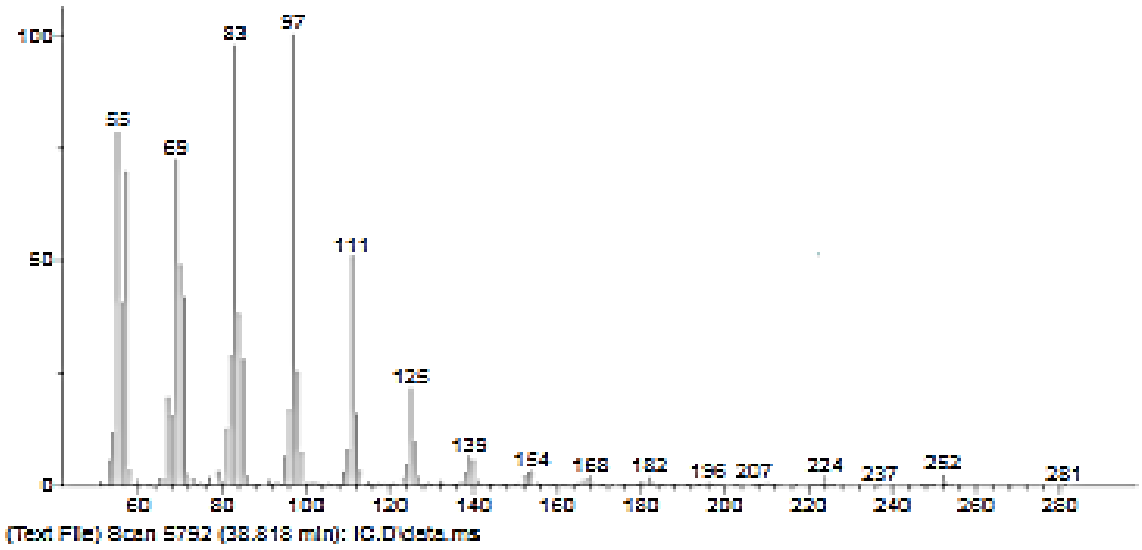
Appendix I-E. GC-MS profile for Ethyl acetate Crude Extract (IC) of *Raphia africana*: A Pre-Isolation Study

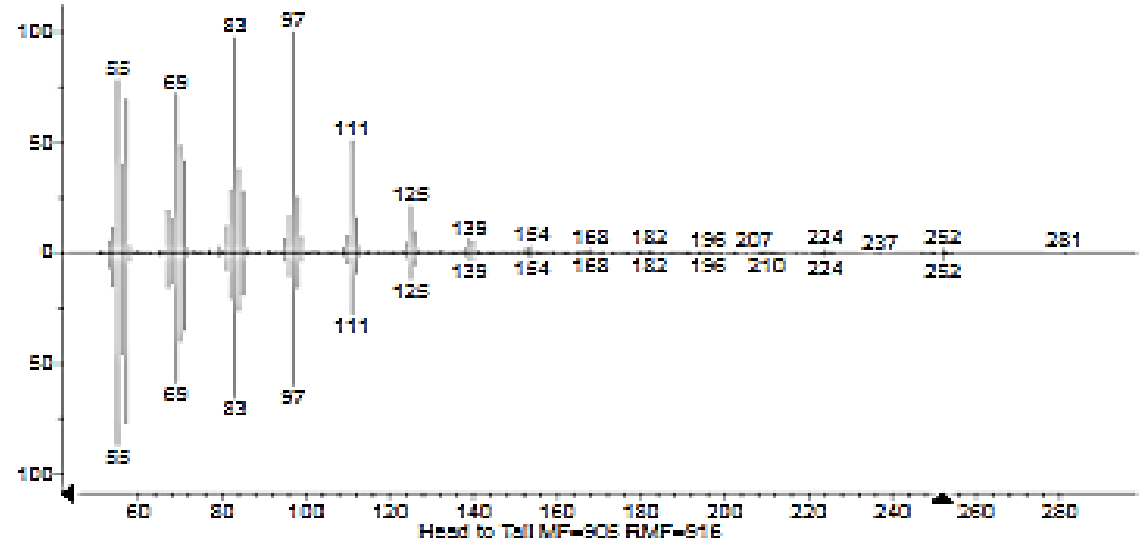


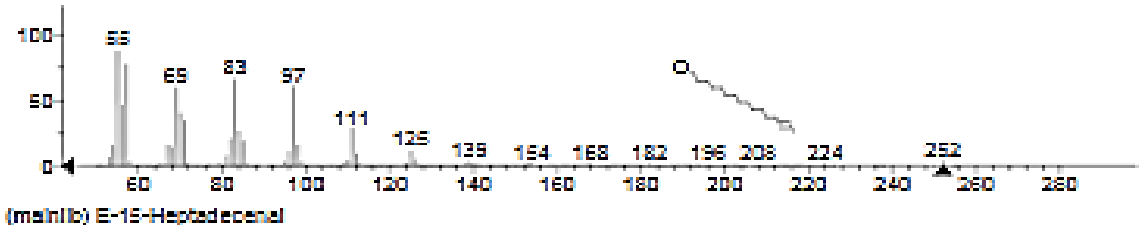




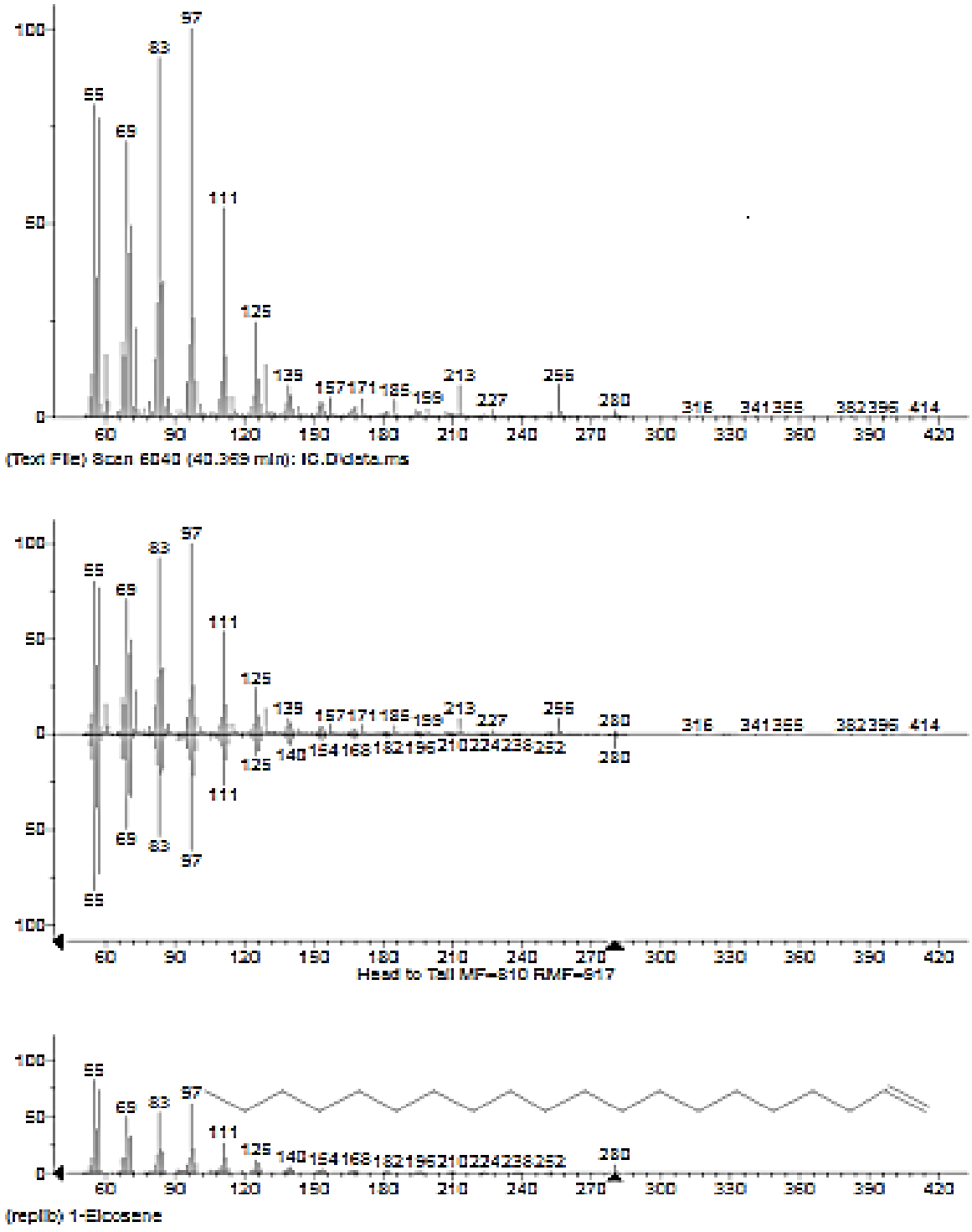
Appendix I-F. GC-MS profile for Ethyl acetate Crude Extract (IC) of *Raphia africana*: A Pre-Isolation Study



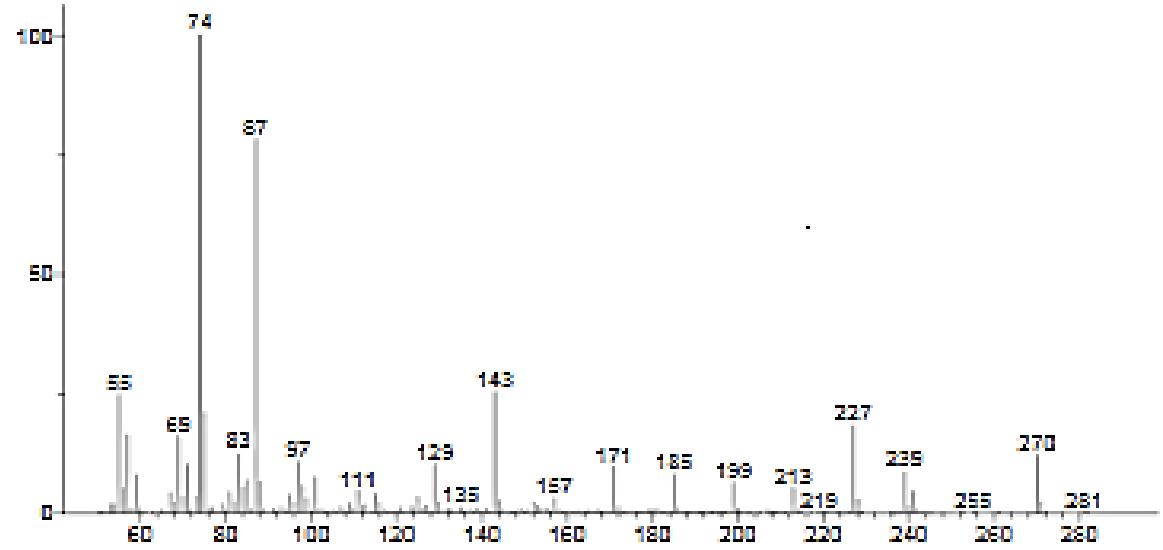


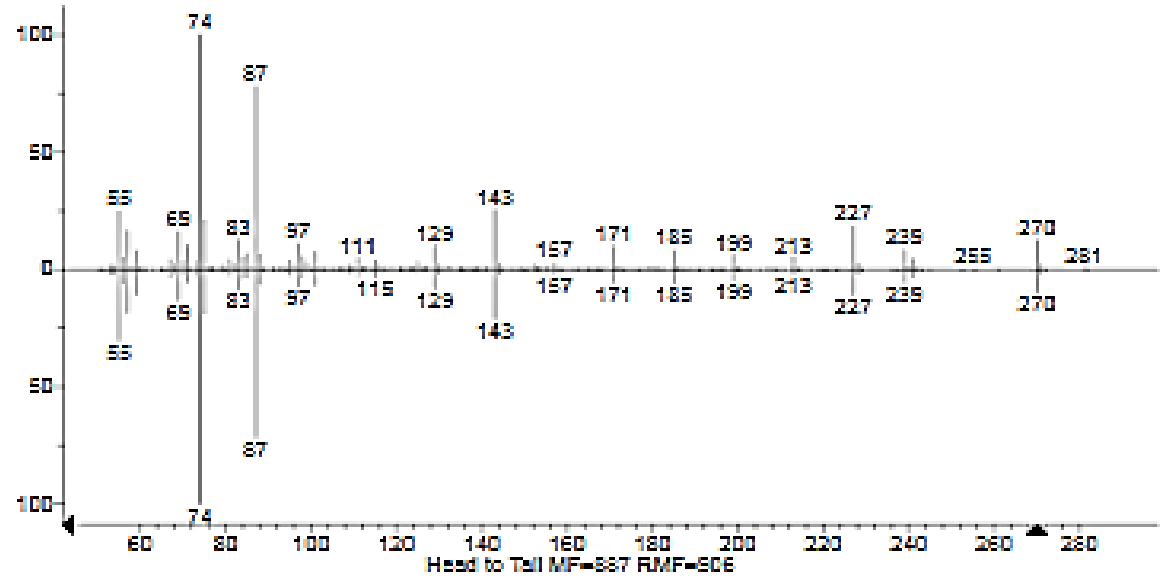


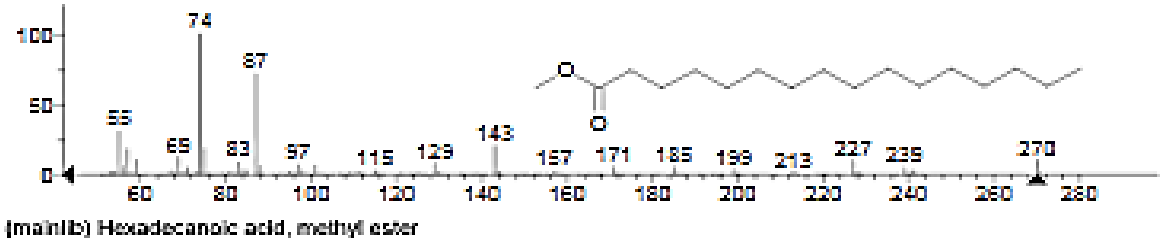
Appendix I-G. GC-MS profile for Ethyl acetate Crude Extract (IC) of *Raphia africana*: A Pre-Isolation Study



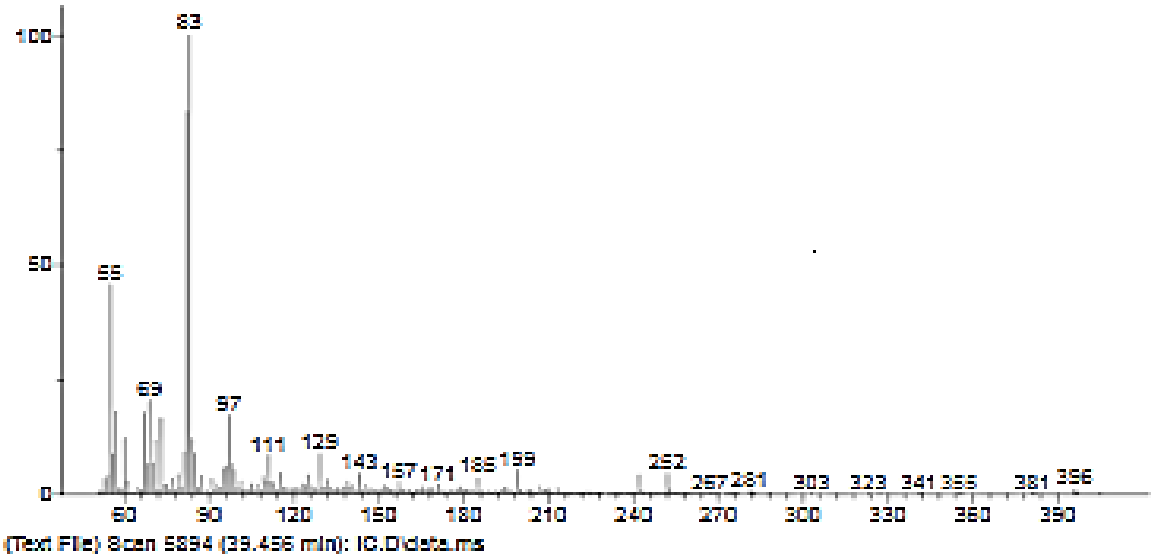
Appendix I-H. GC-MS profile for Ethyl acetate Crude Extract (IC) of *Raphia africana*: A Pre-Isolation Study

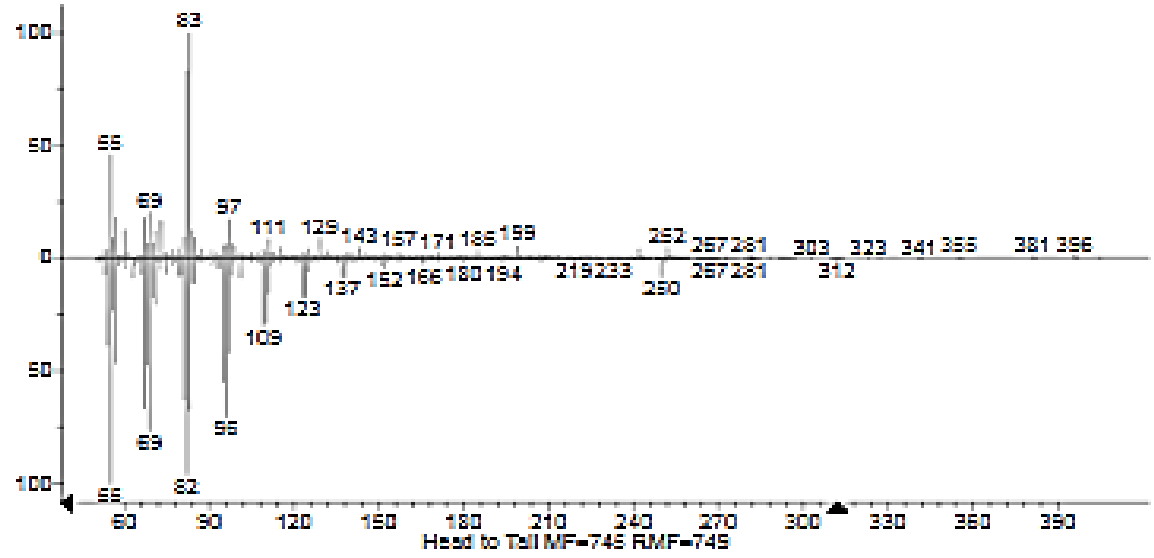


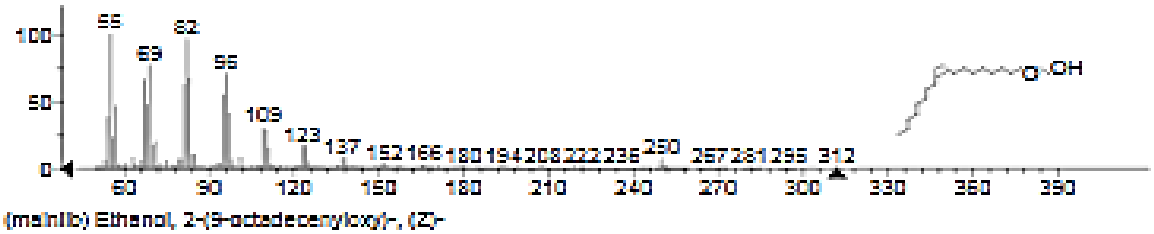




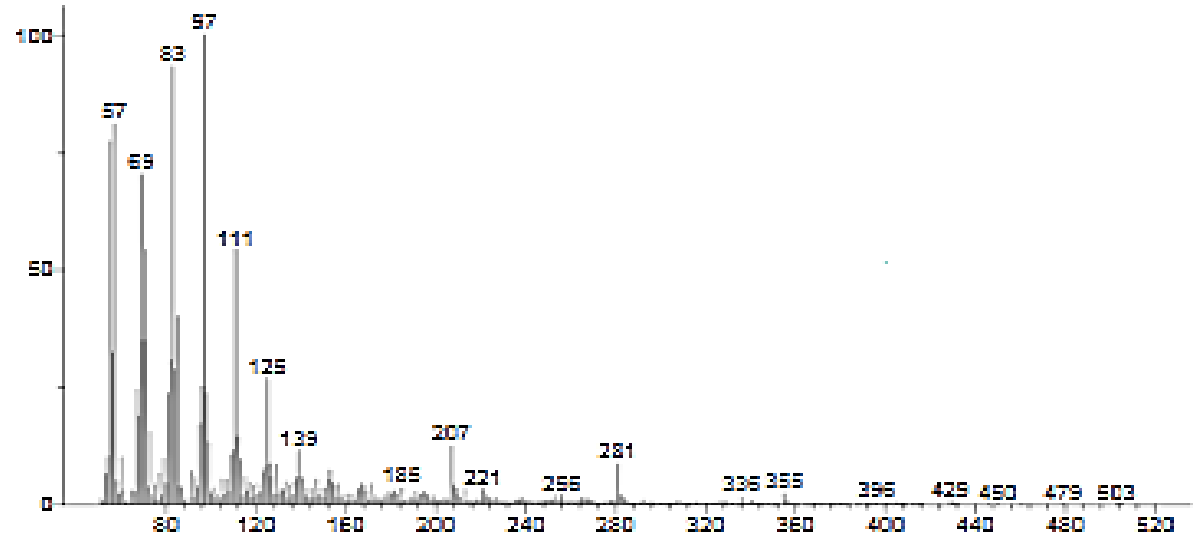
Appendix I-I. GC-MS profile for Ethyl acetate Crude Extract (IC) of *Raphia africana*: A Pre-Isolation Study

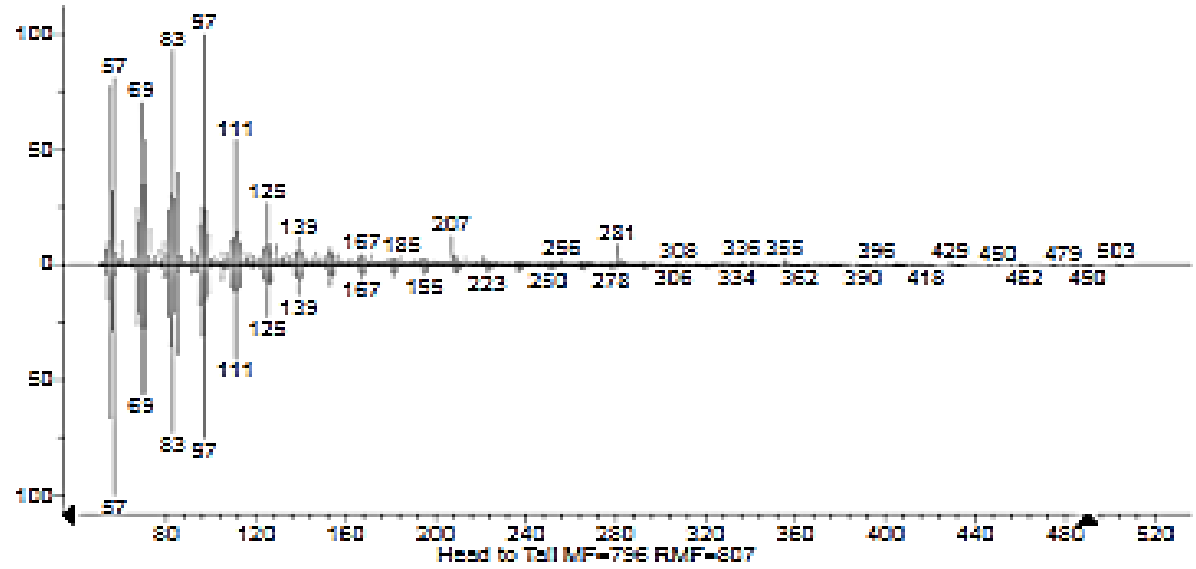


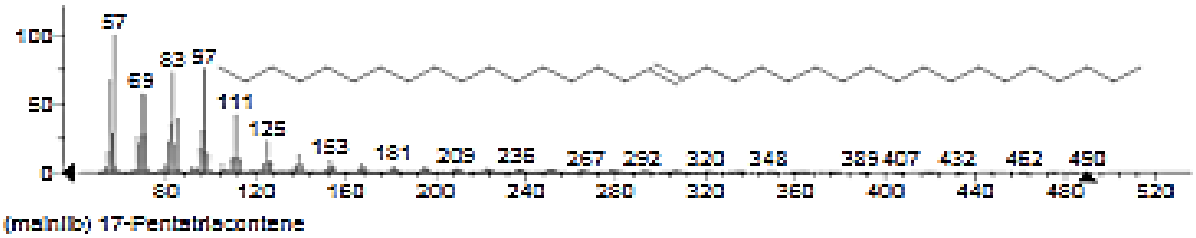




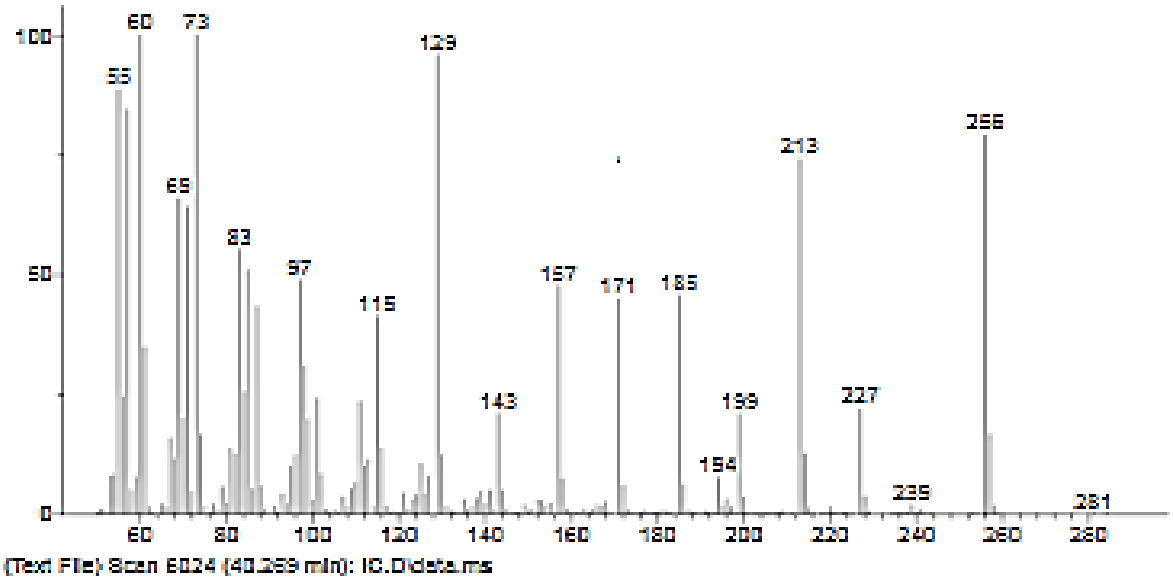
Appendix I-J. GC-MS profile for Ethyl acetate Crude Extract (IC) of *Raphia africana*: A Pre-Isolation Study

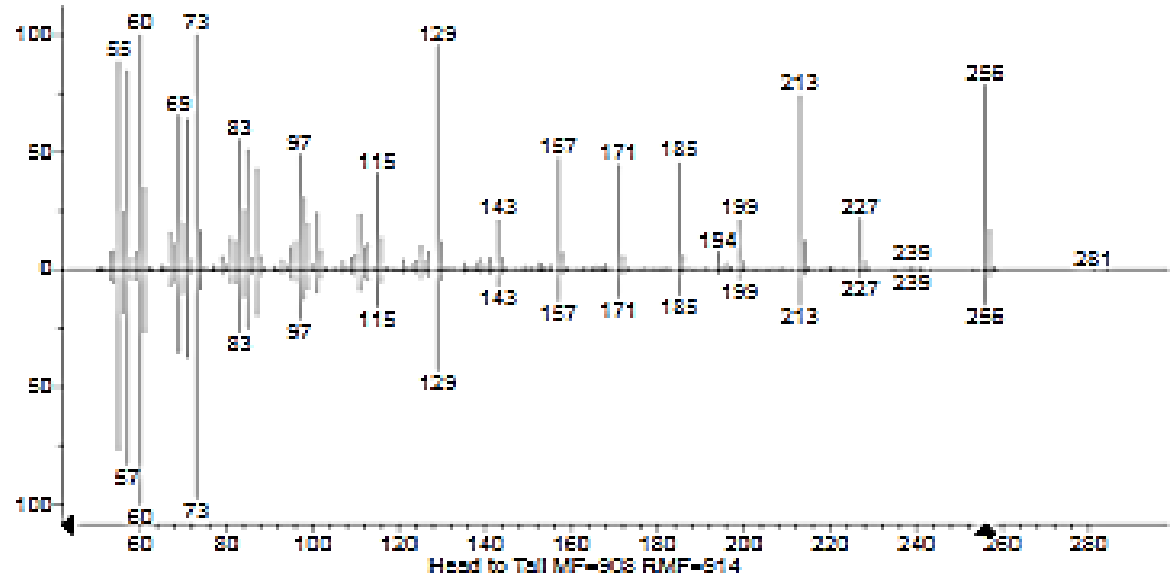


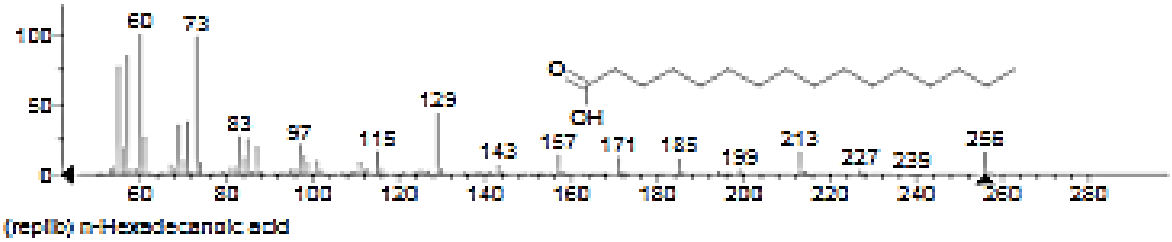




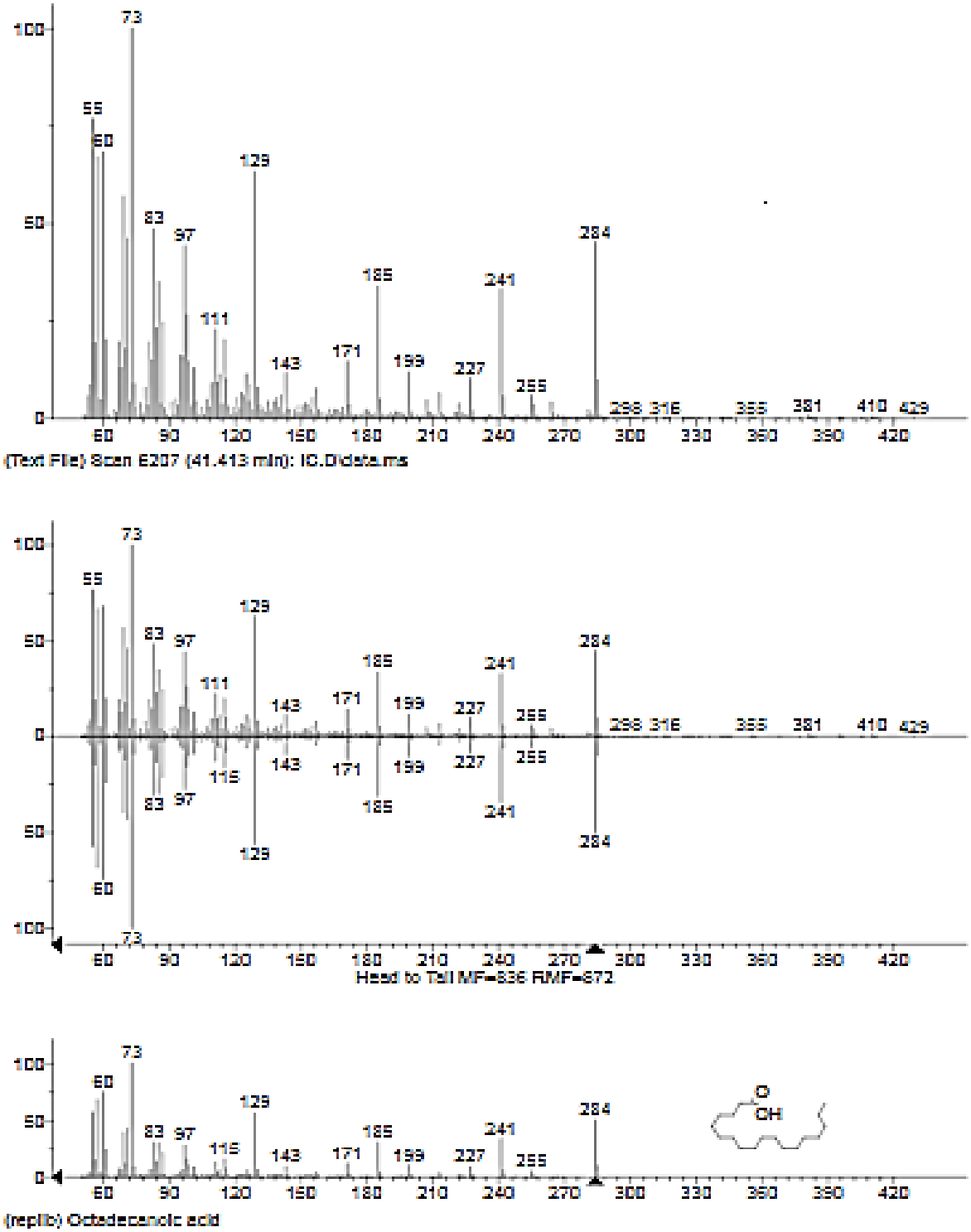
Appendix I-K. GC-MS profile for Ethyl acetate Crude Extract (IC) of *Raphia africana*: A Pre-Isolation Study



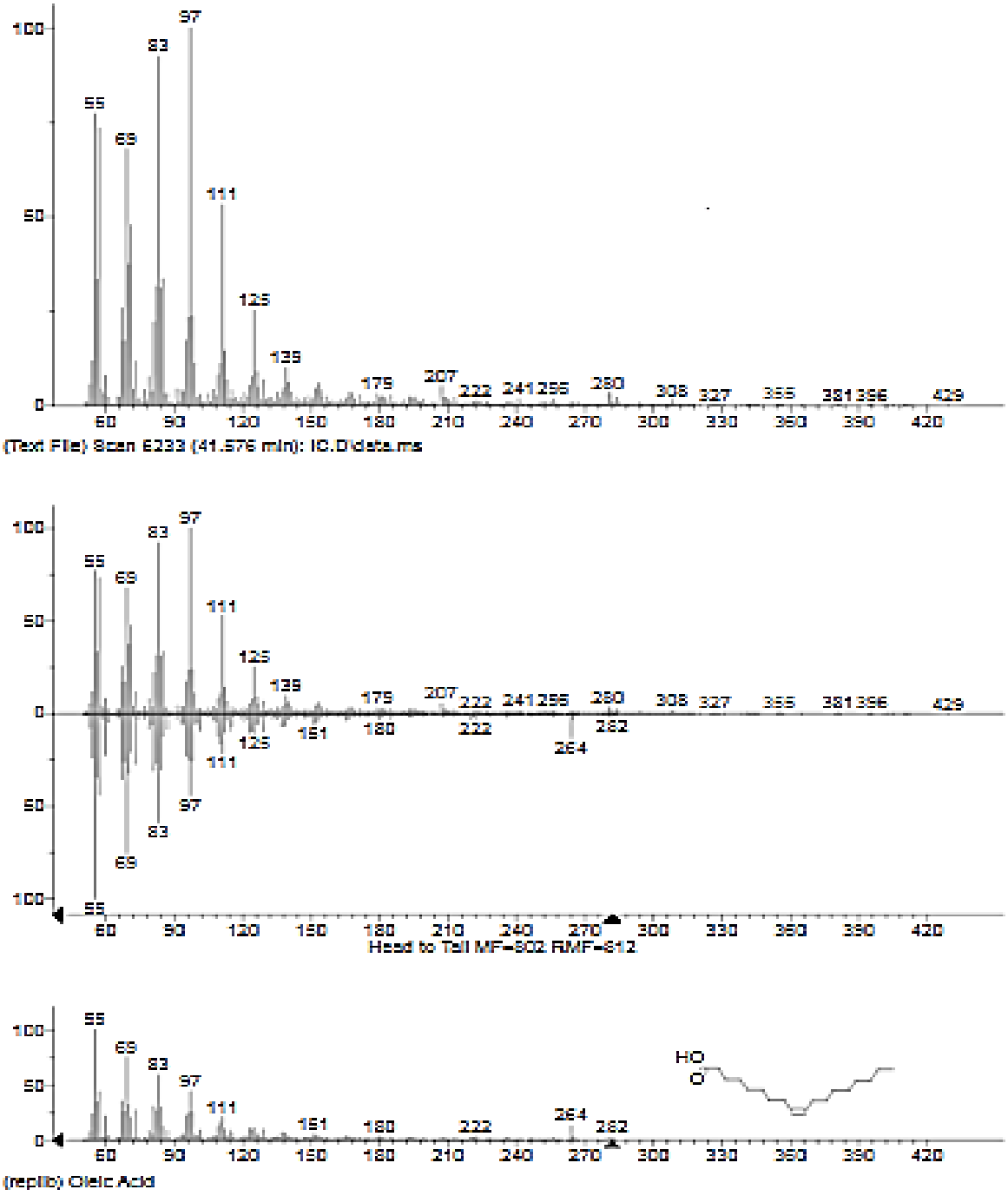




Appendix I-L. GC-MS profile for Ethyl acetate Crude Extract (IC) of *Raphia africana*: A Pre-Isolation Study

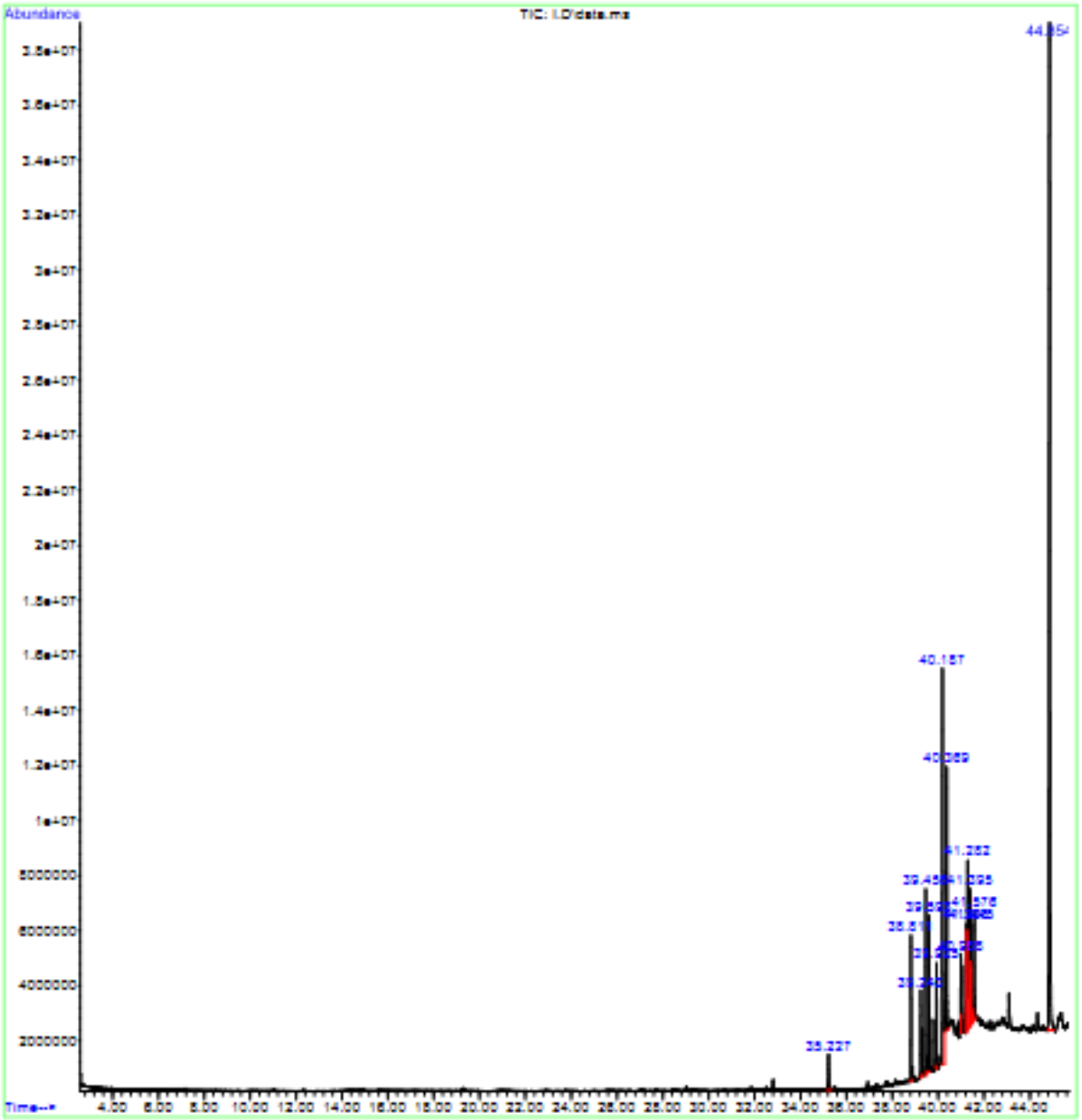


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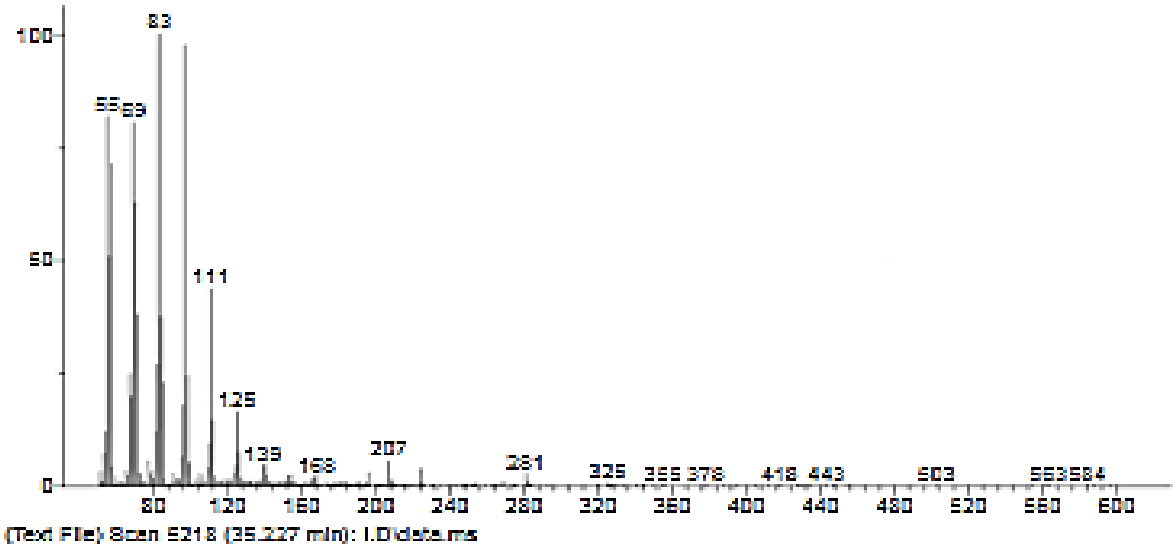


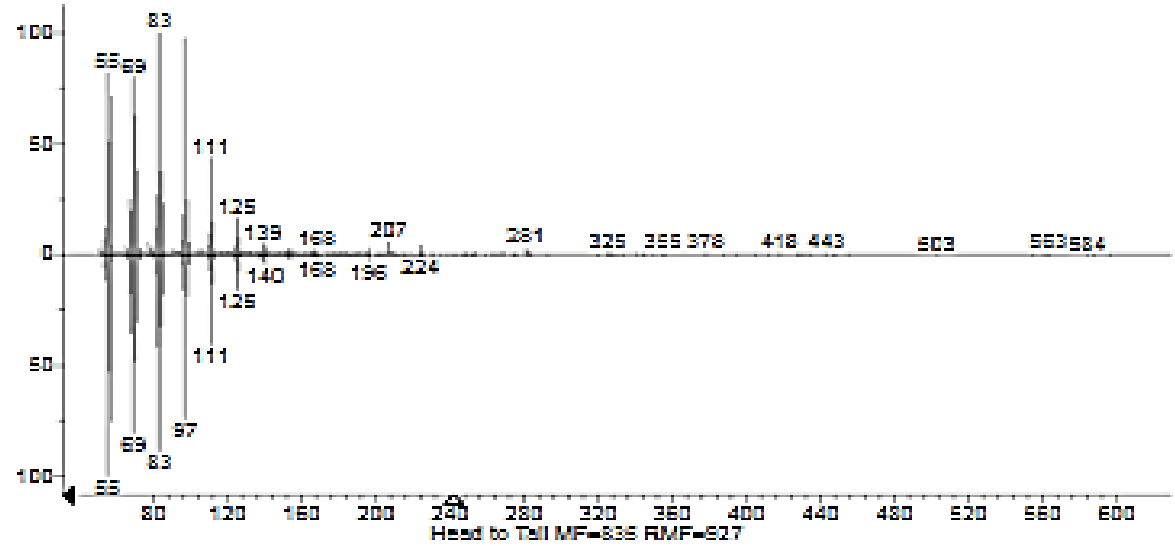
Appendix II-A. GC profile for Methanol Crude Extract (ID) of *Raphia africana*: A Pre-Isolation Study

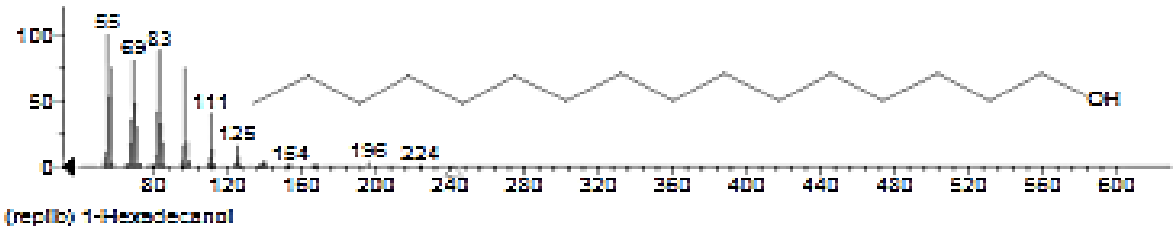
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Instrument : MSD
Sample Name:
Misc Info :
Vial Number: 8



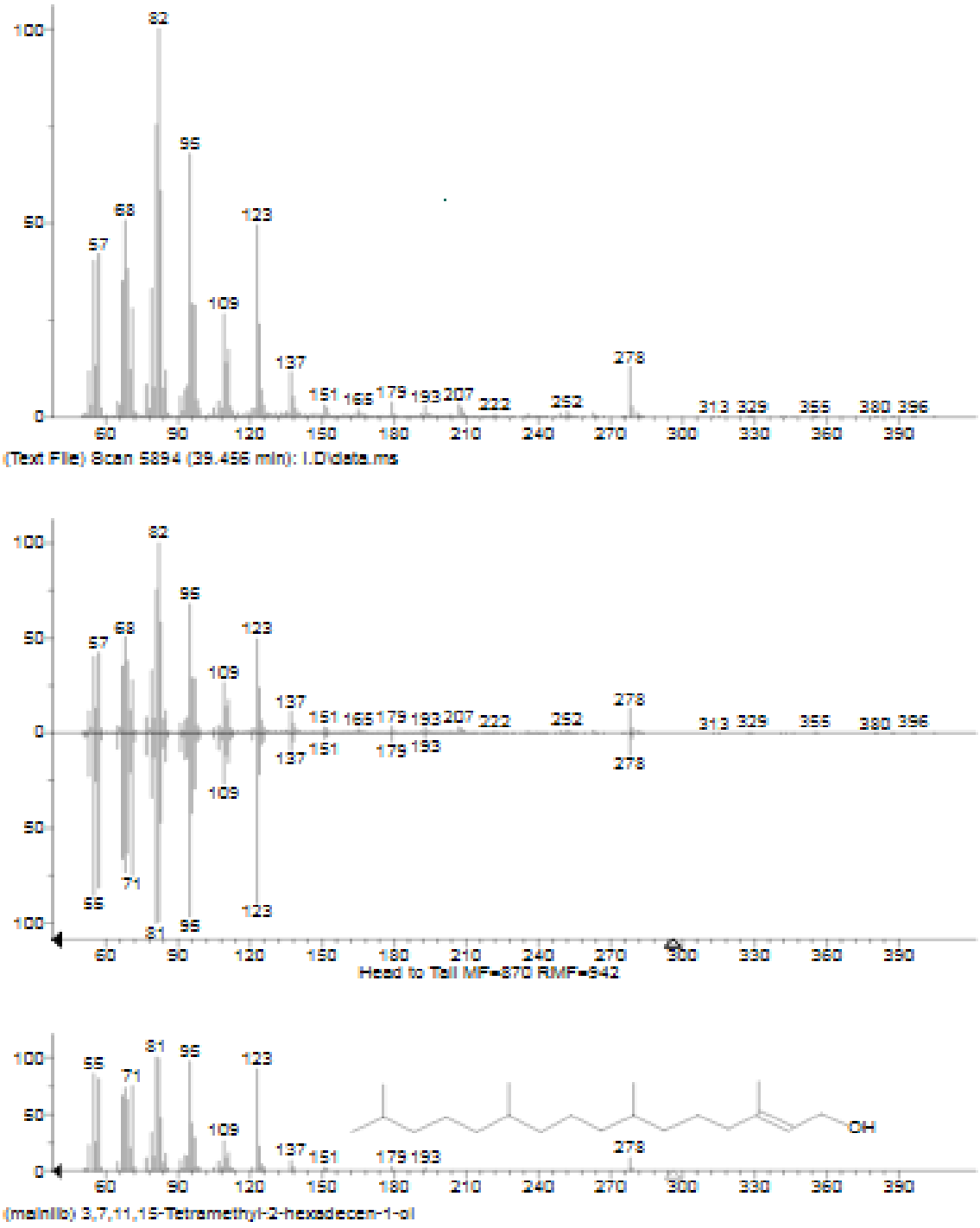
Appendix II-B. GC-MS profile for Methanol Crude Extract (ID) of *Raphia africana*: A Pre-Isolation Study



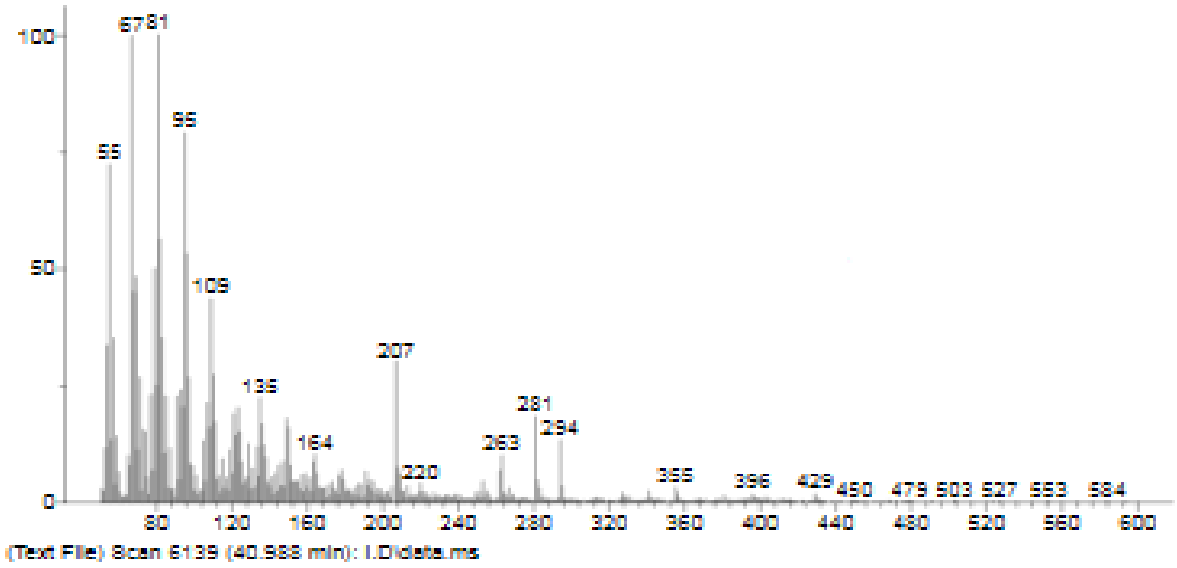


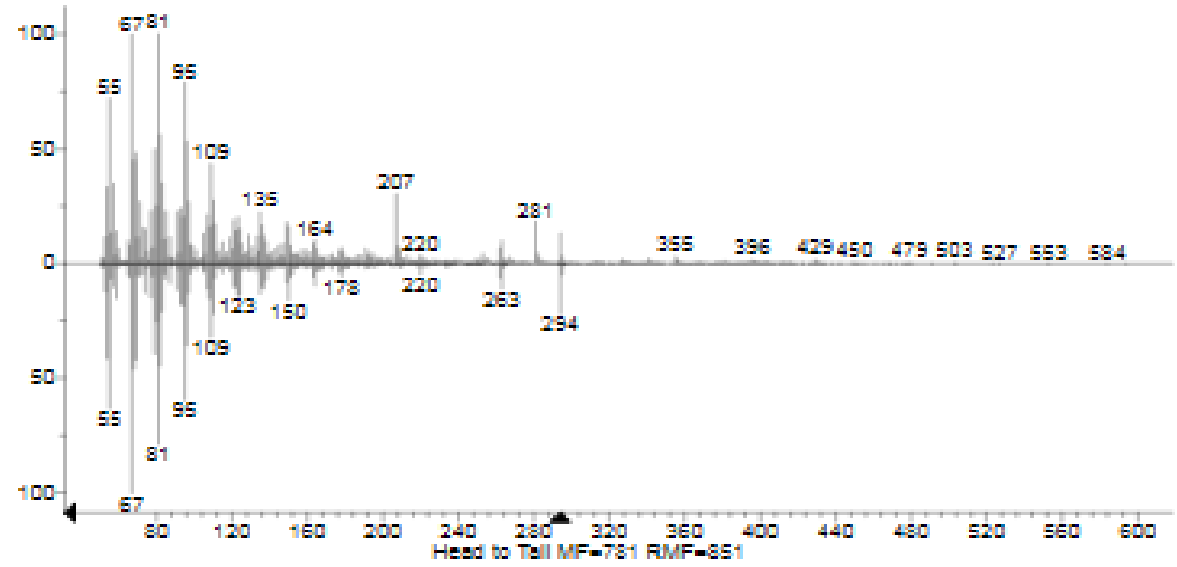


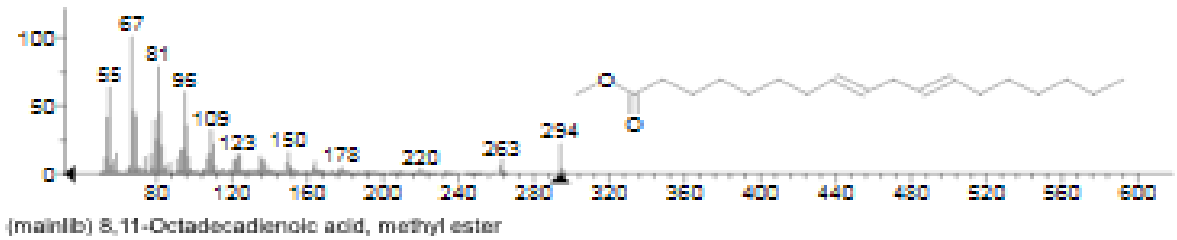
Appendix II-C. GC-MS profile for Methanol Crude Extract (ID) of *Raphia africana*: A Pre-Isolation Study



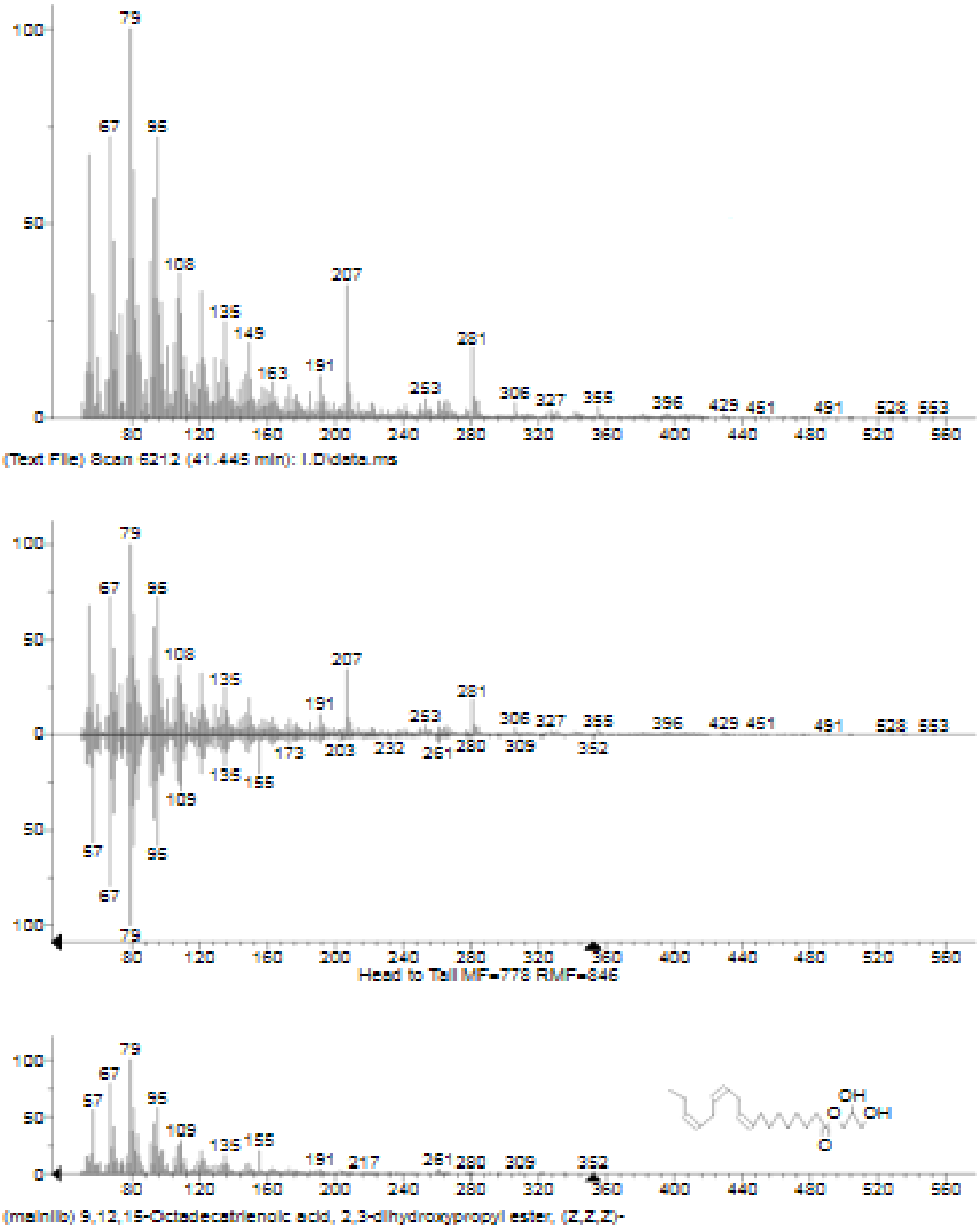
Appendix II-D. GC-MS profile for Methanol Crude Extract (ID) of *Raphia africana*: A Pre-Isolation Study



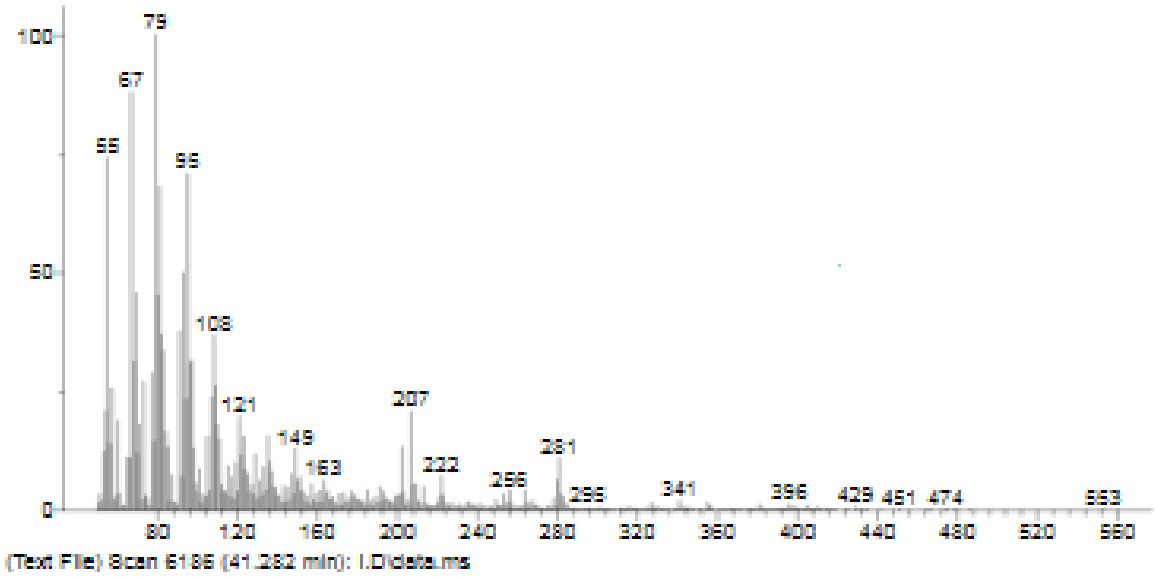


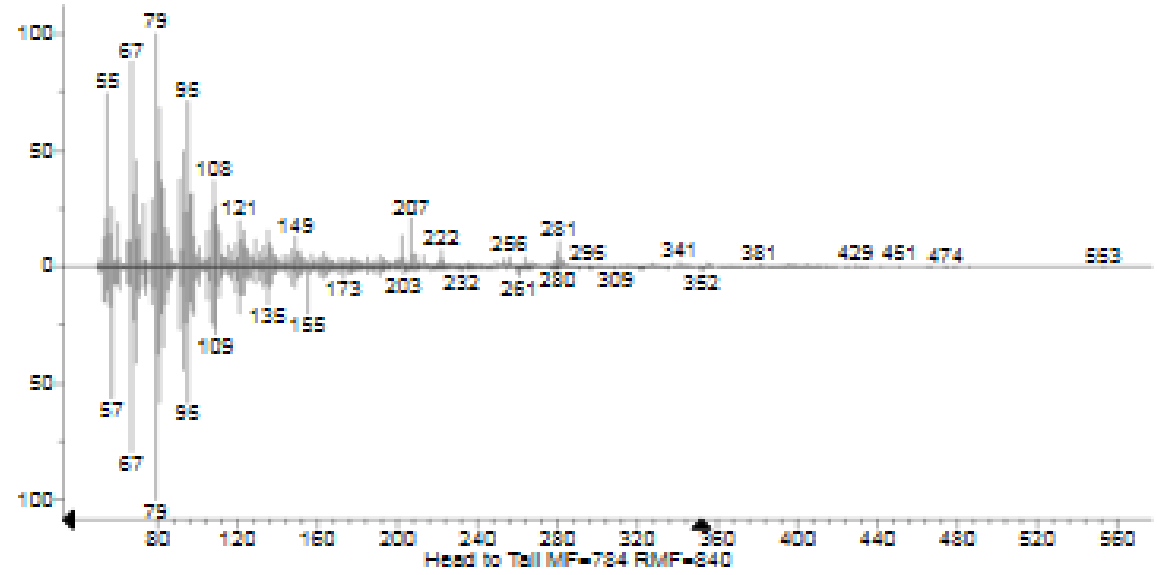


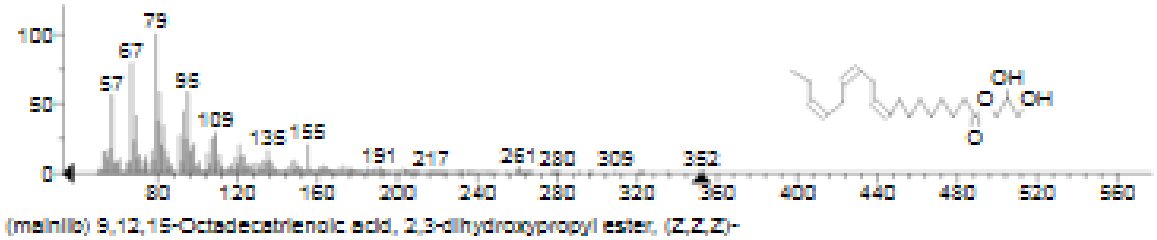
Appendix II-E. GC-MS profile for Methanol Crude Extract (ID) of *Raphia africana*: A Pre-Isolation Study



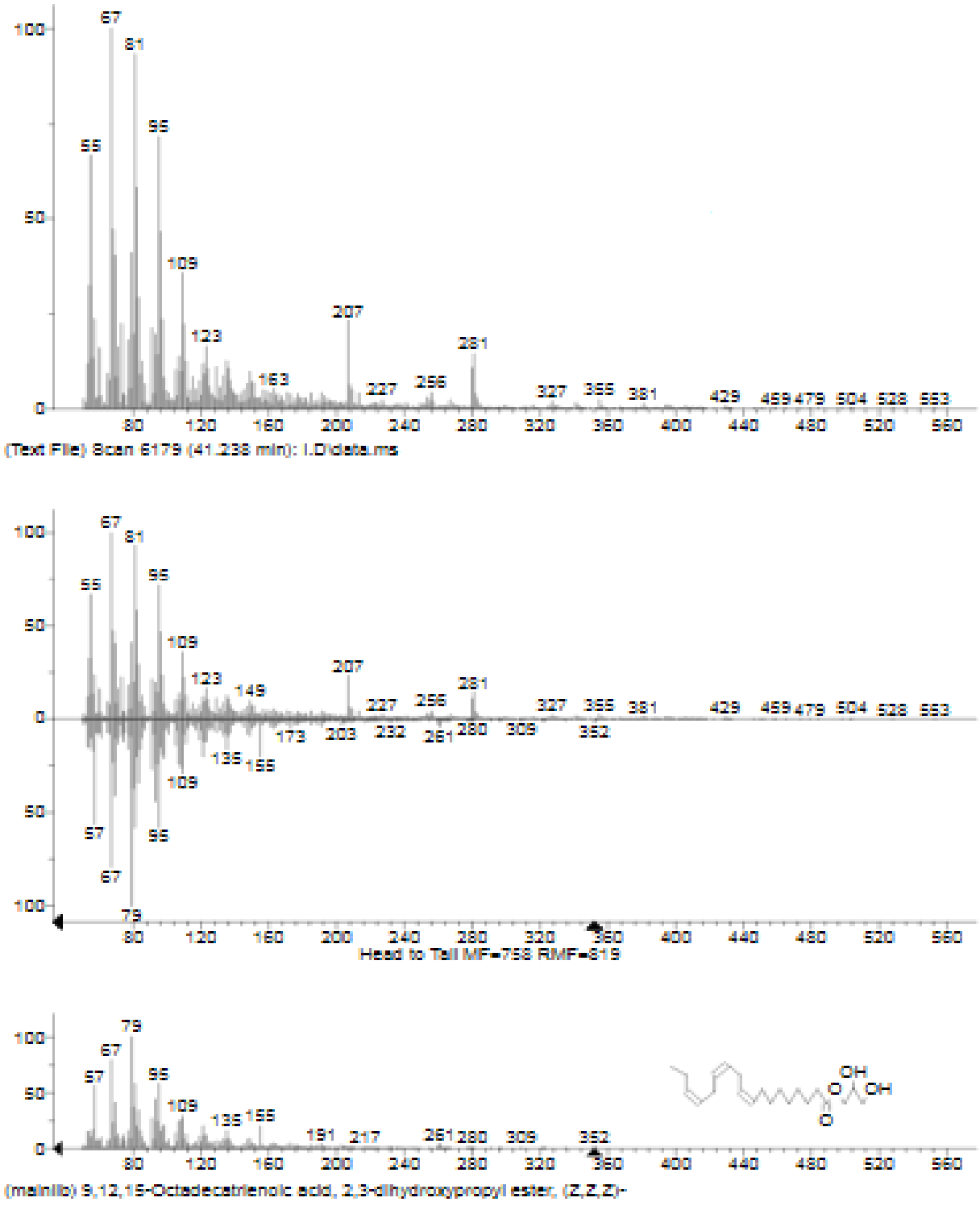
Appendix II-F. GC-MS profile for Methanol Crude Extract (ID) of *Raphia africana*: A Pre-Isolation Study



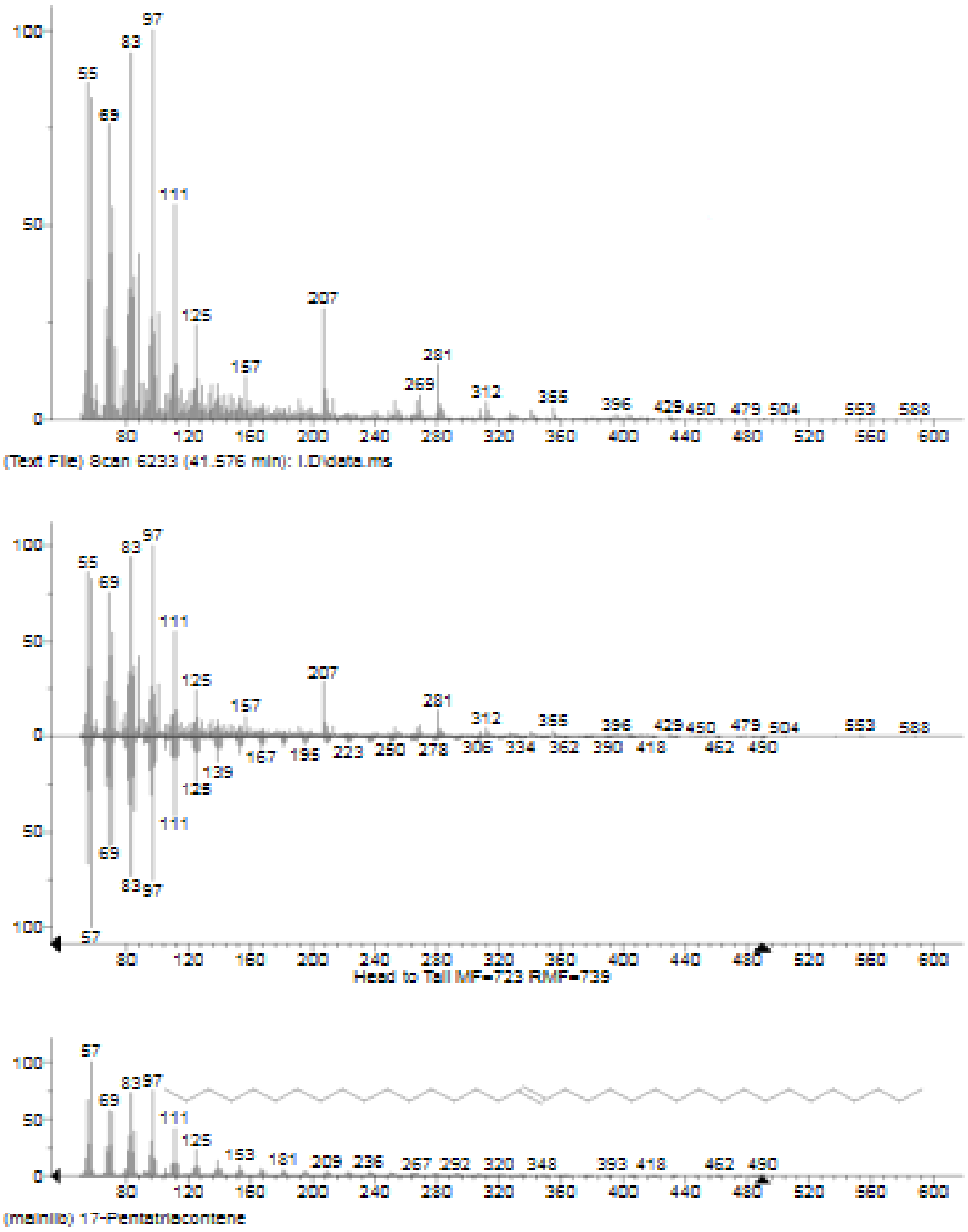




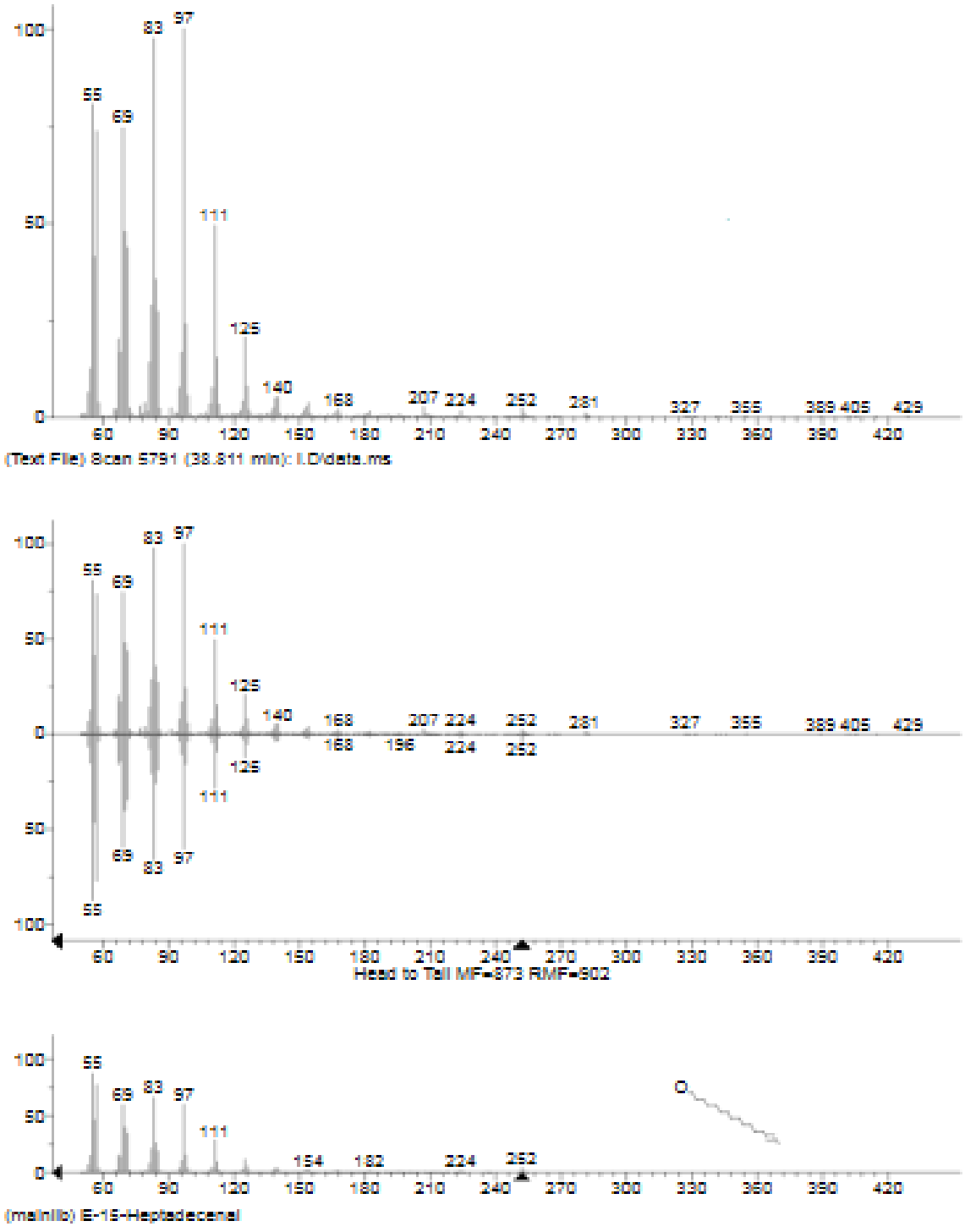
Appendix II-G. GC-MS profile for Methanol Crude Extract (ID) of *Raphia africana*: A Pre-Isolation Study



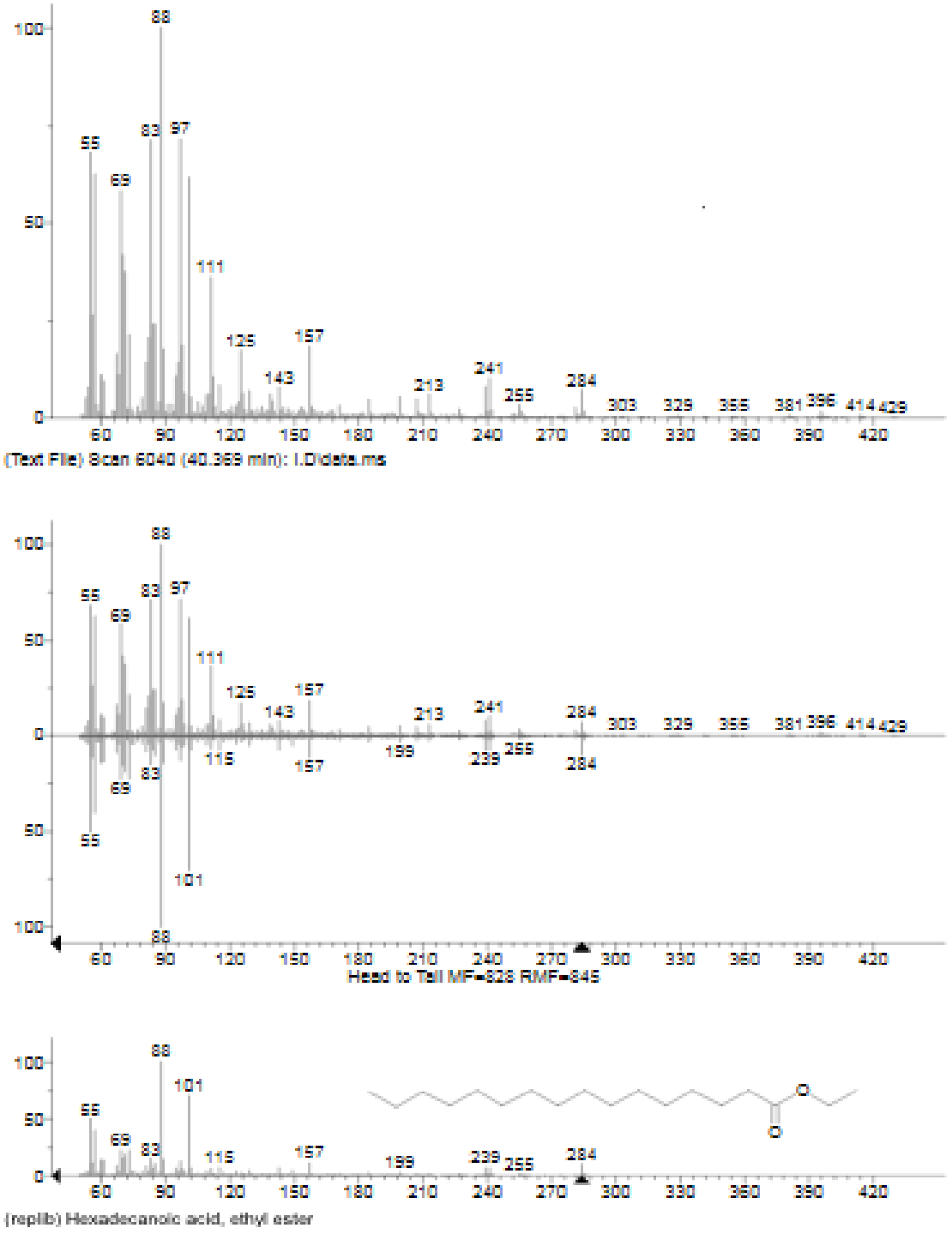
Appendix II-H. GC-MS profile for Methanol Crude Extract (ID) of *Raphia africana*: A Pre-Isolation Study



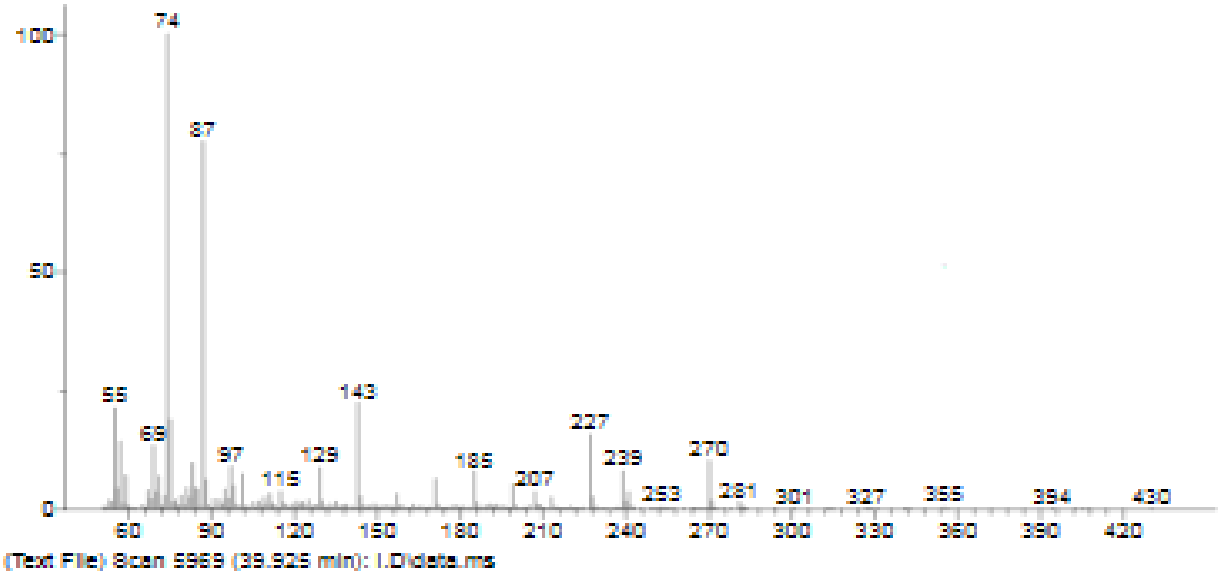
Appendix II-I. GC-MS profile for Methanol Crude Extract (ID) of *Raphia africana*: A Pre-Isolation Study

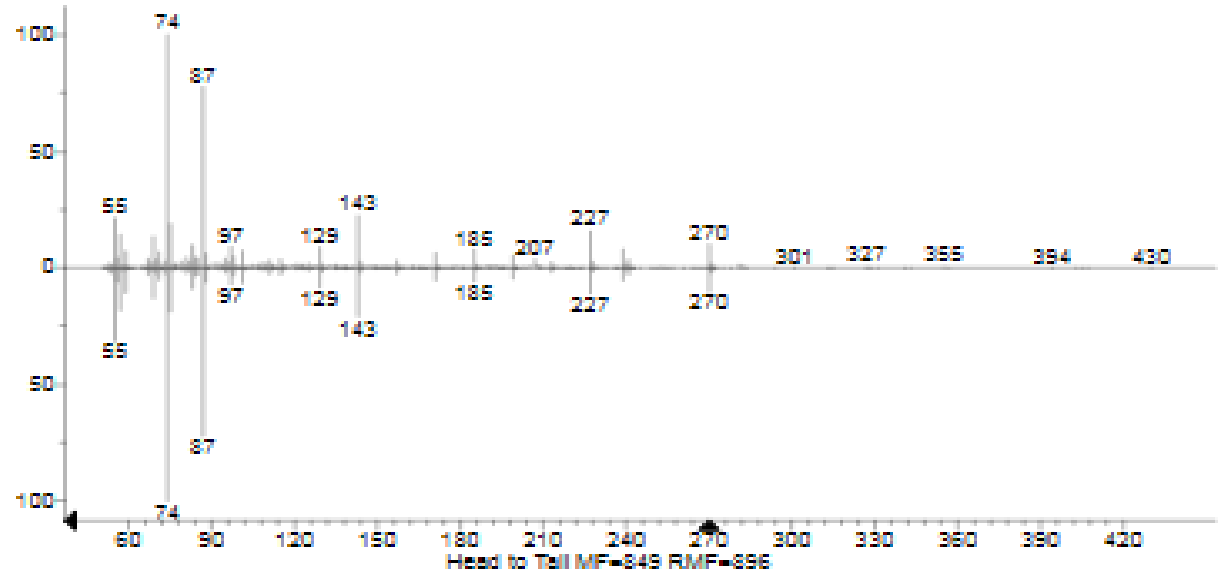


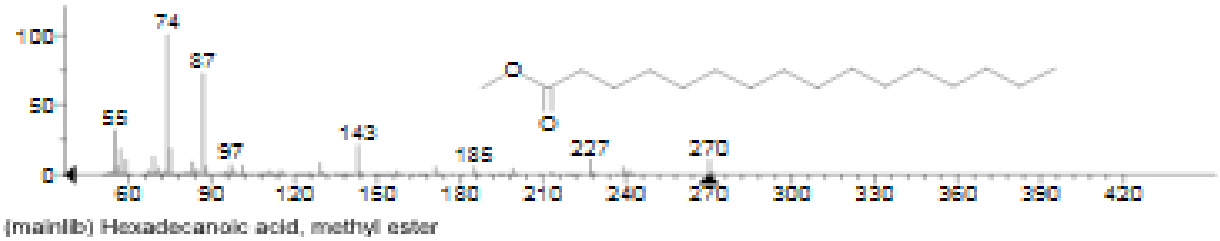
Appendix II-J. GC-MS profile for Methanol Crude Extract (ID) of *Raphia africana*: A Pre-Isolation Study



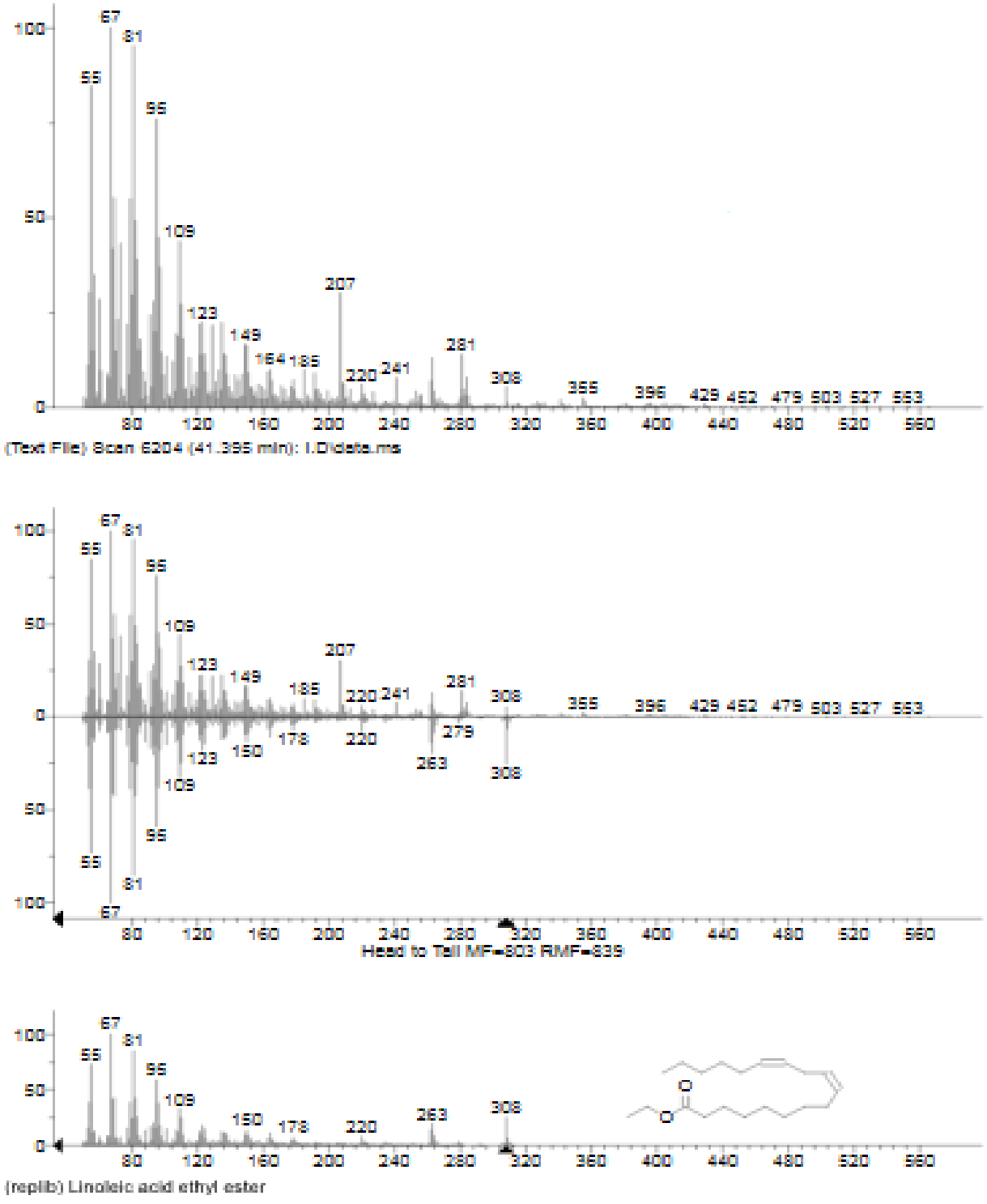
Appendix II-K. GC-MS profile for Methanol Crude Extract (ID) of *Raphia africana*: A Pre-Isolation Study



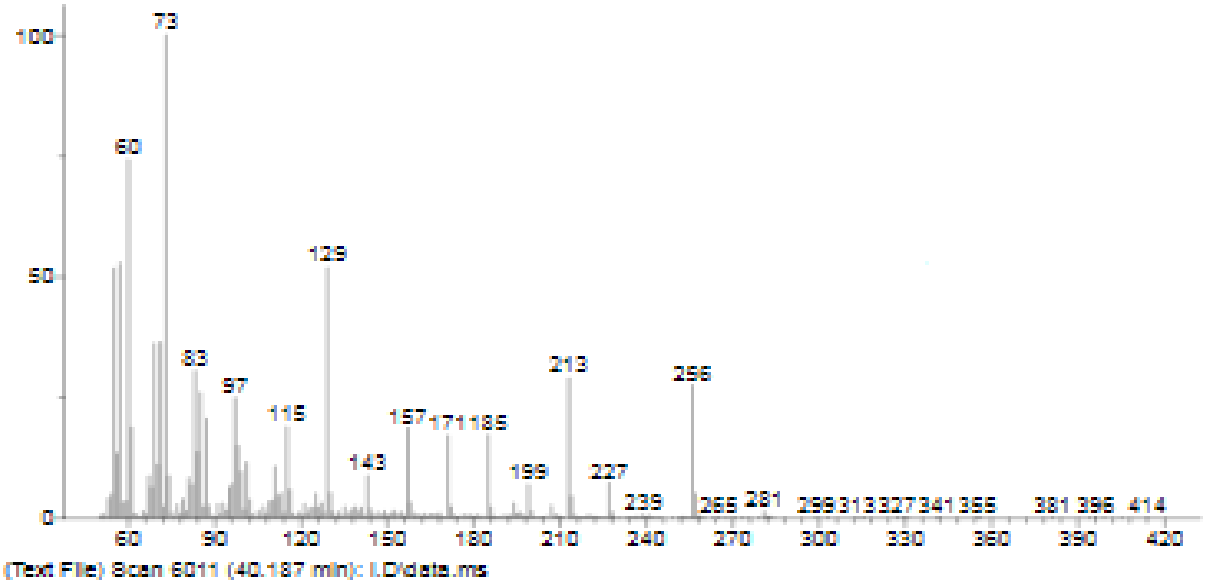


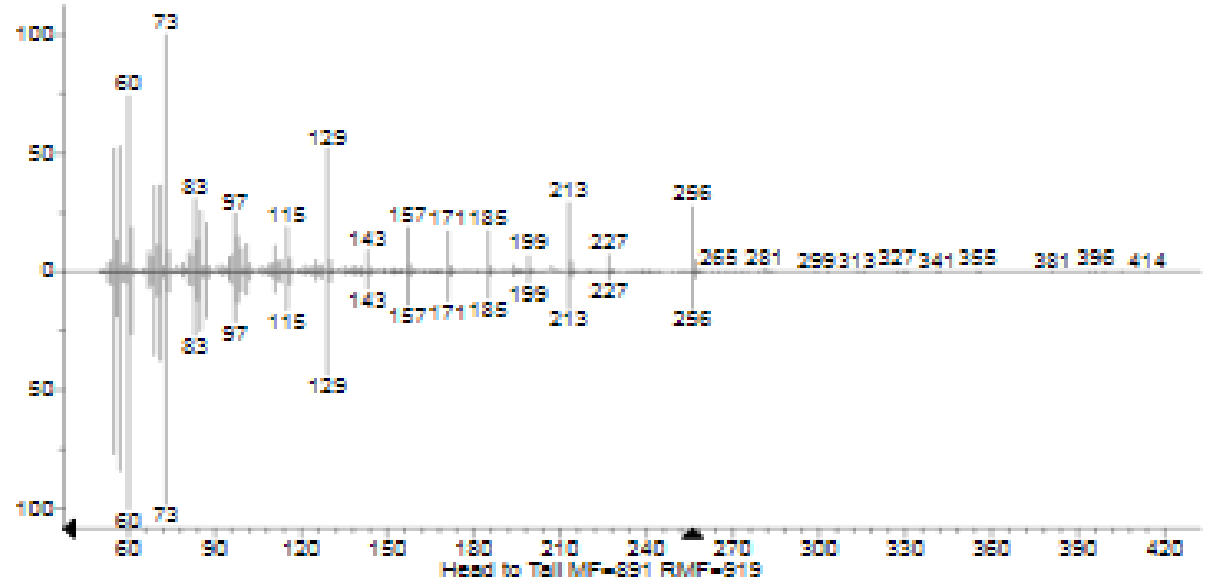


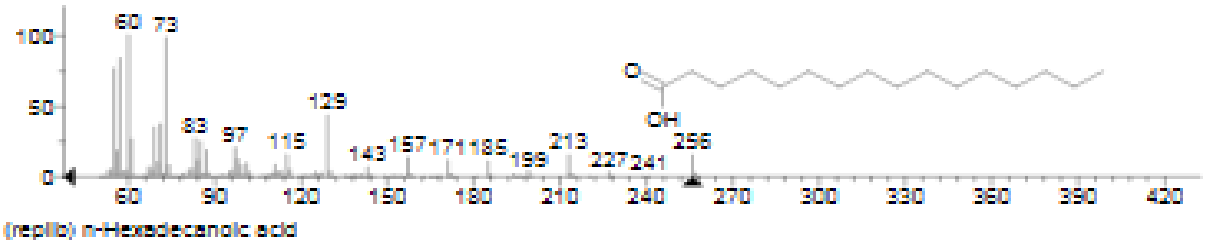
Appendix II-L. GC-MS profile for Methanol Crude Extract (ID) of *Raphia africana*: A Pre-Isolation Study



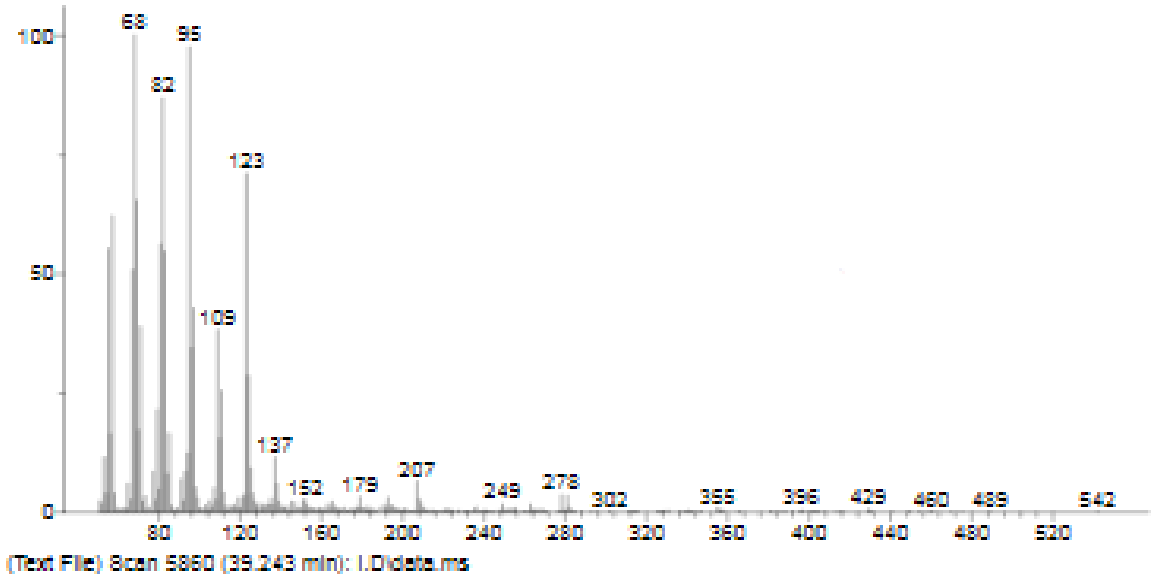
Appendix II-M. GC-MS profile for Methanol Crude Extract (ID) of *Raphia africana*: A Pre-Isolation Study

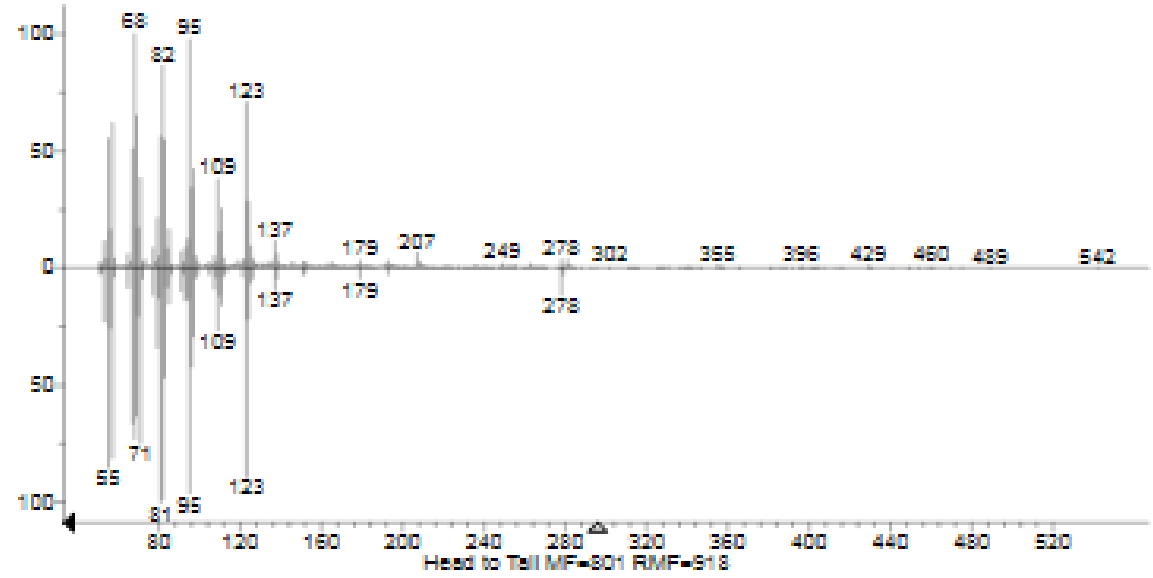


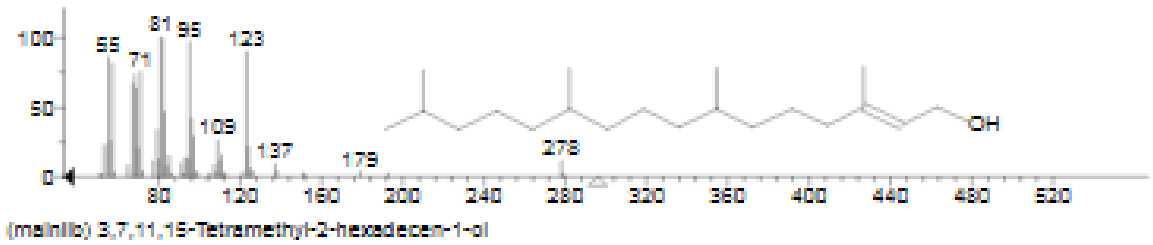




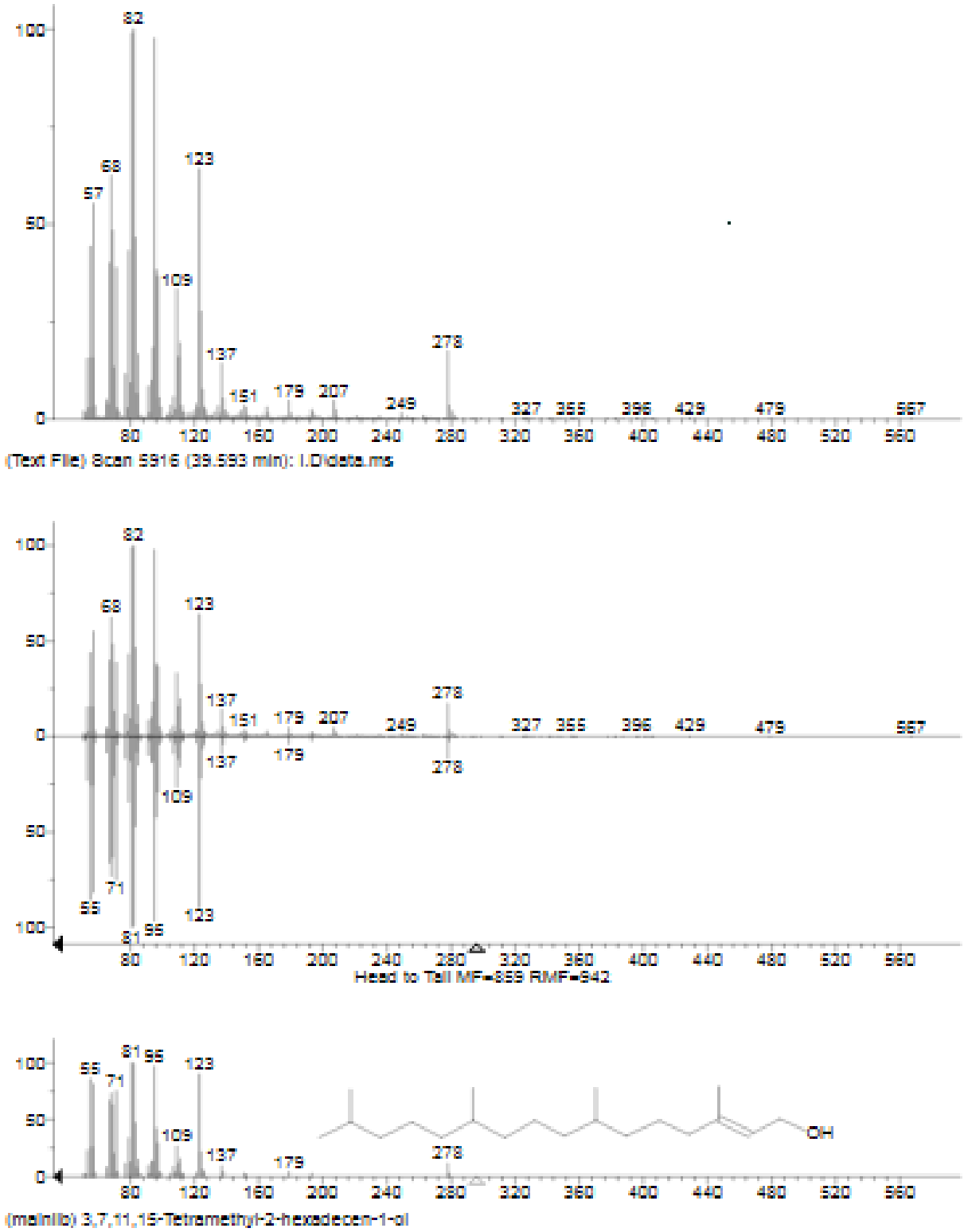
Appendix II-N. GC-MS profile for Methanol Crude Extract (ID) of *Raphia africana*: A Pre-Isolation Study

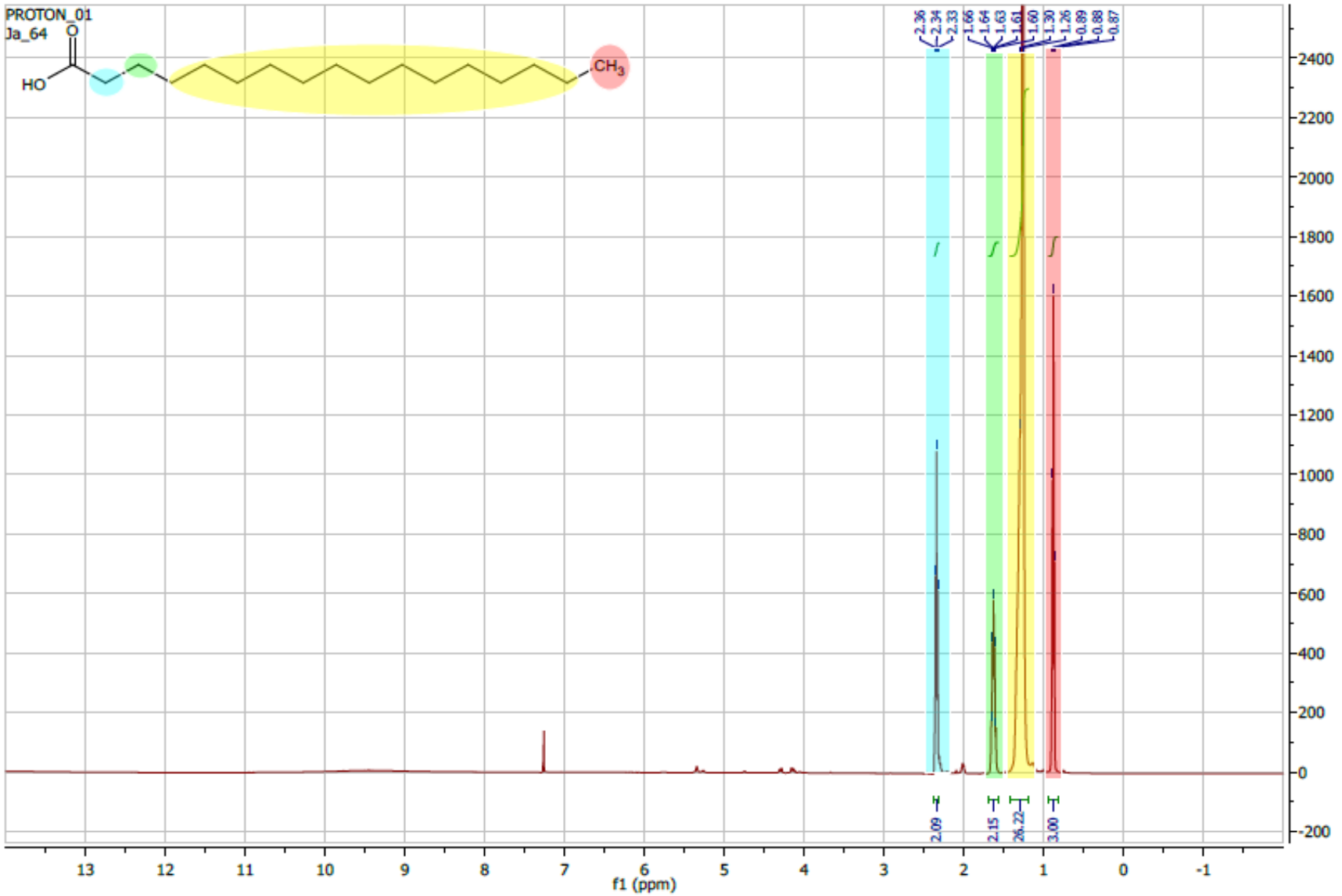




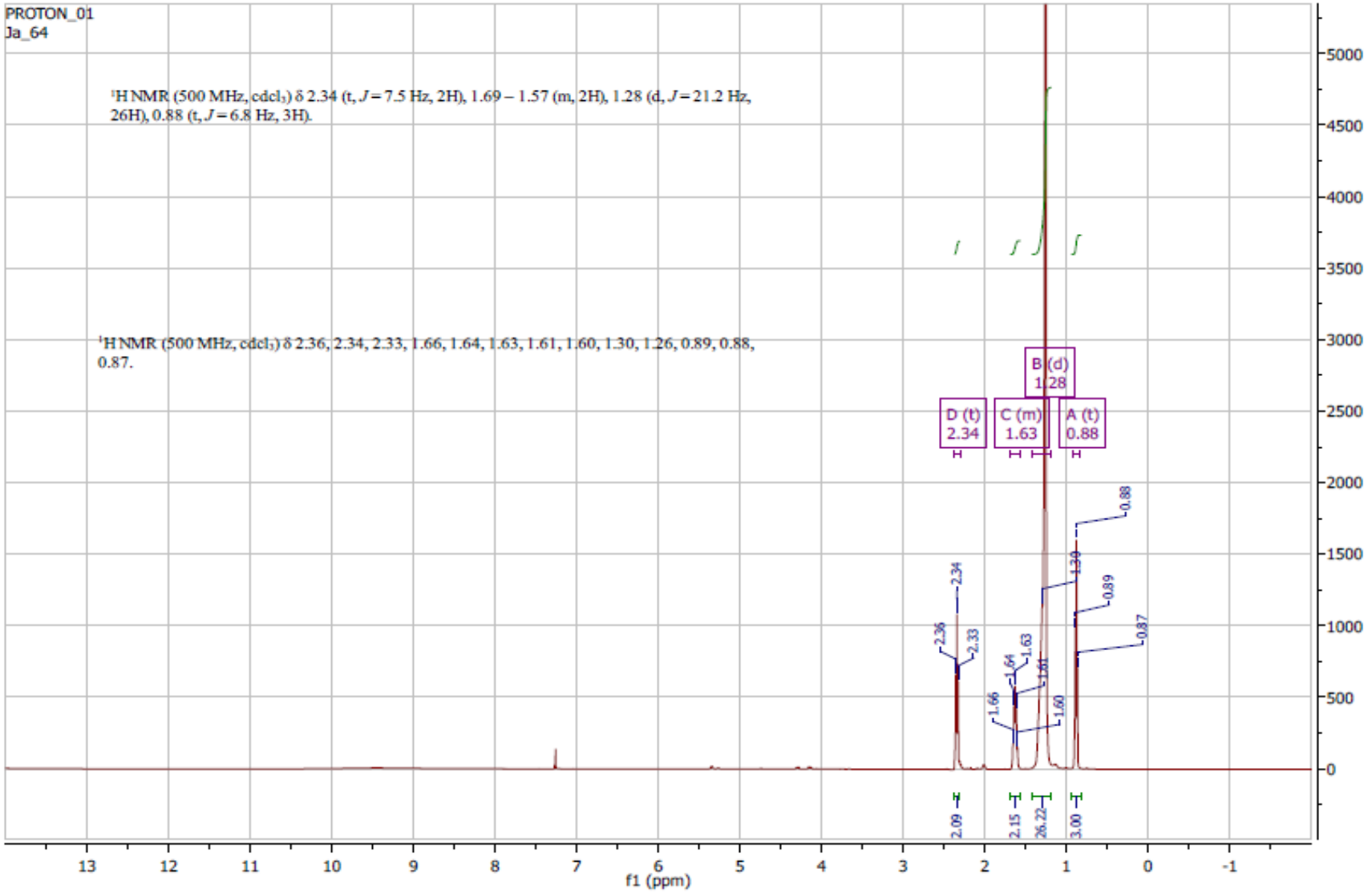


Appendix II-O. GC-MS profile for Methanol Crude Extract (ID) of *Raphia africana*: A Pre-Isolation Study

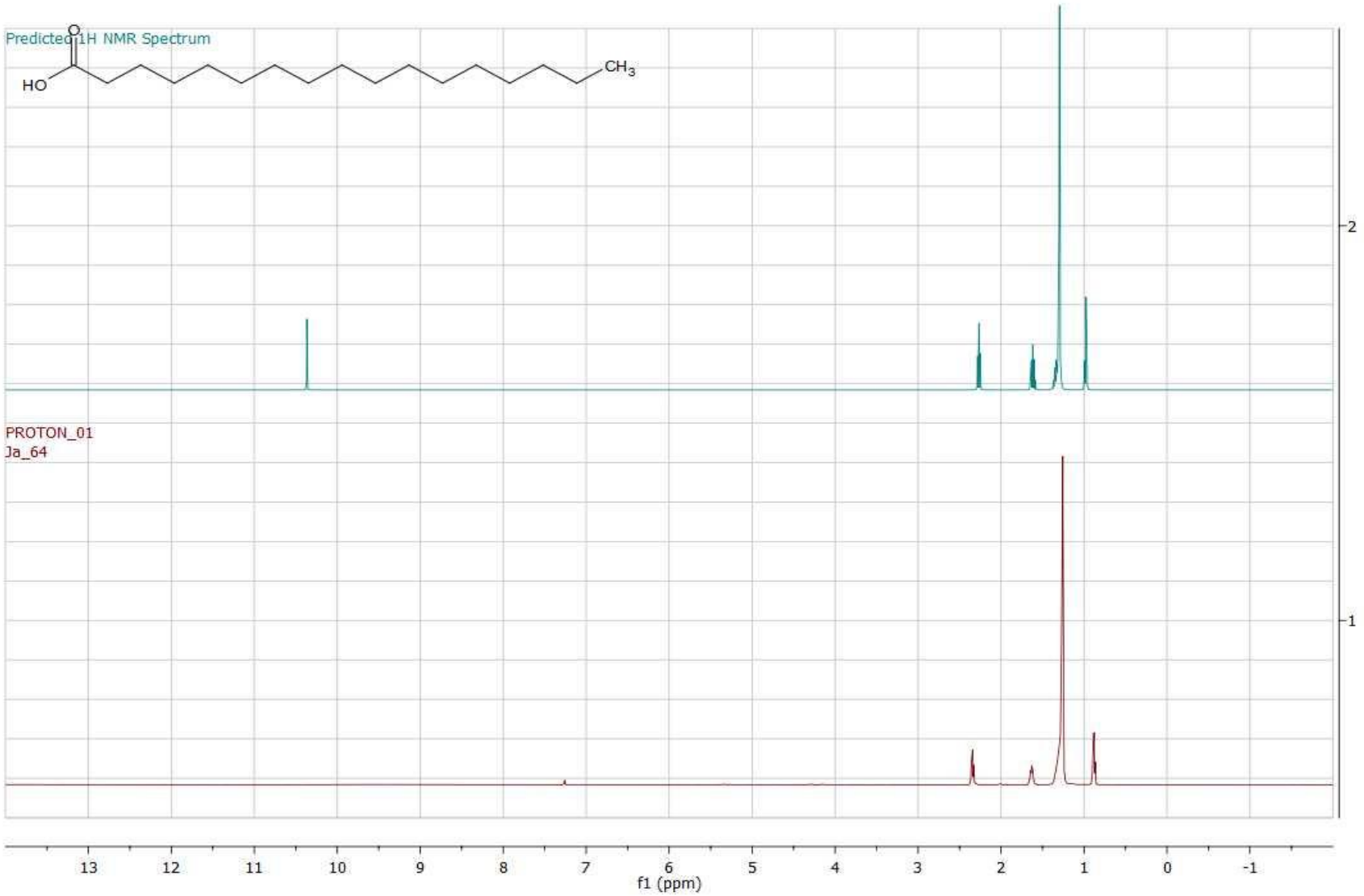




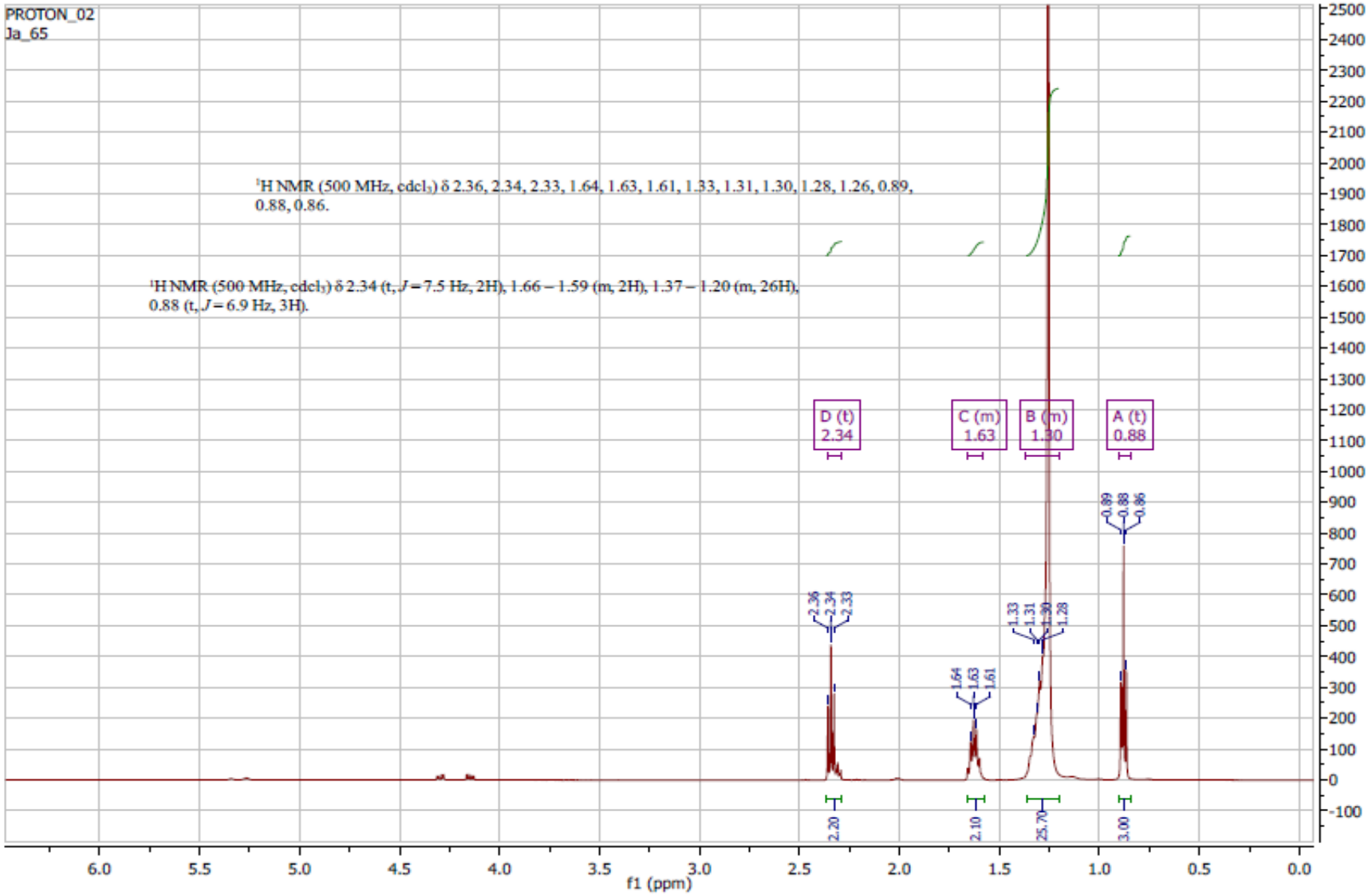
Appendix III. [1]HNMR Spectrum of Ja64



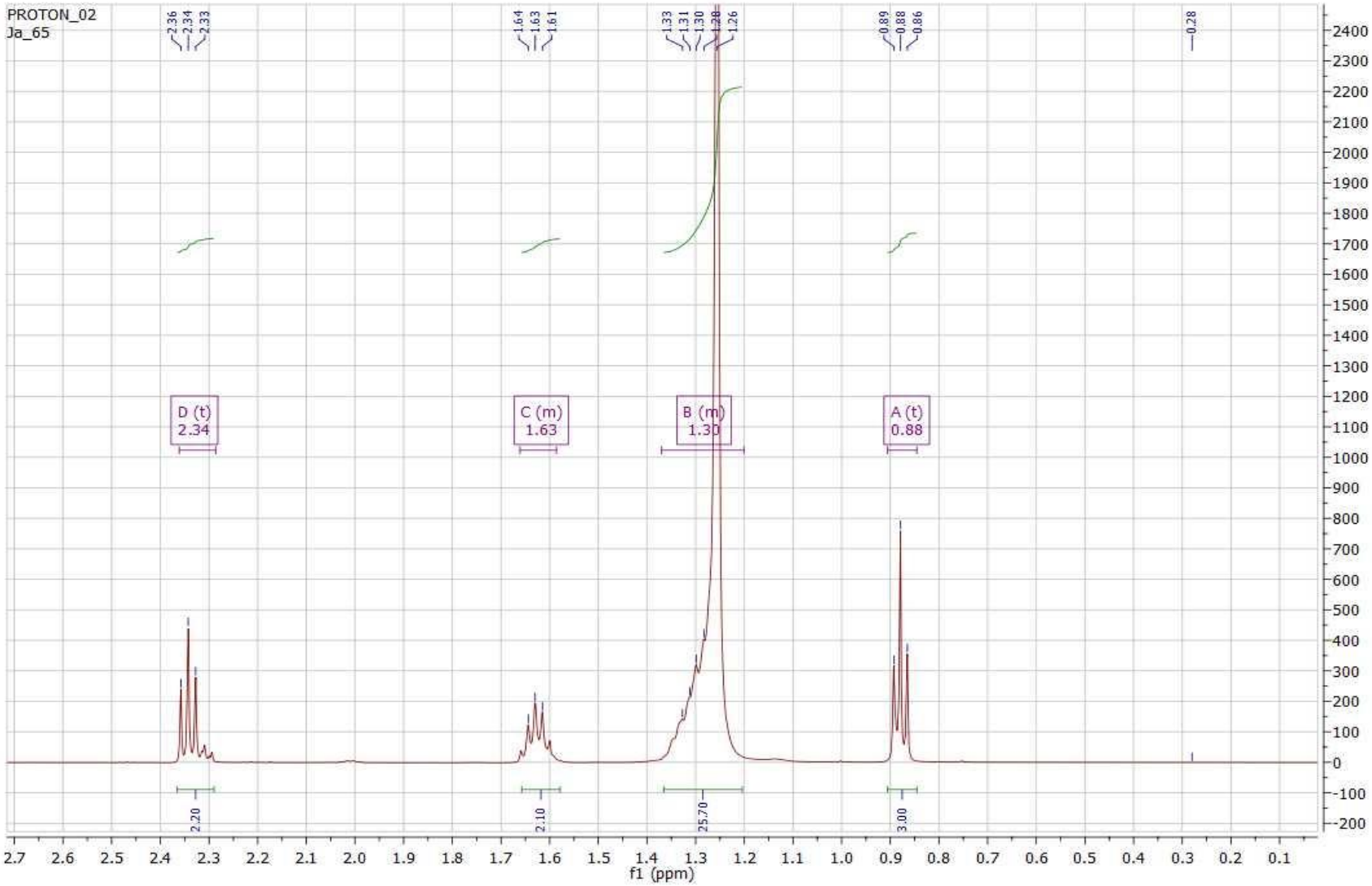
Appendix III-A. [1]HNMR Spectrum of Ja64 (coupling constants)



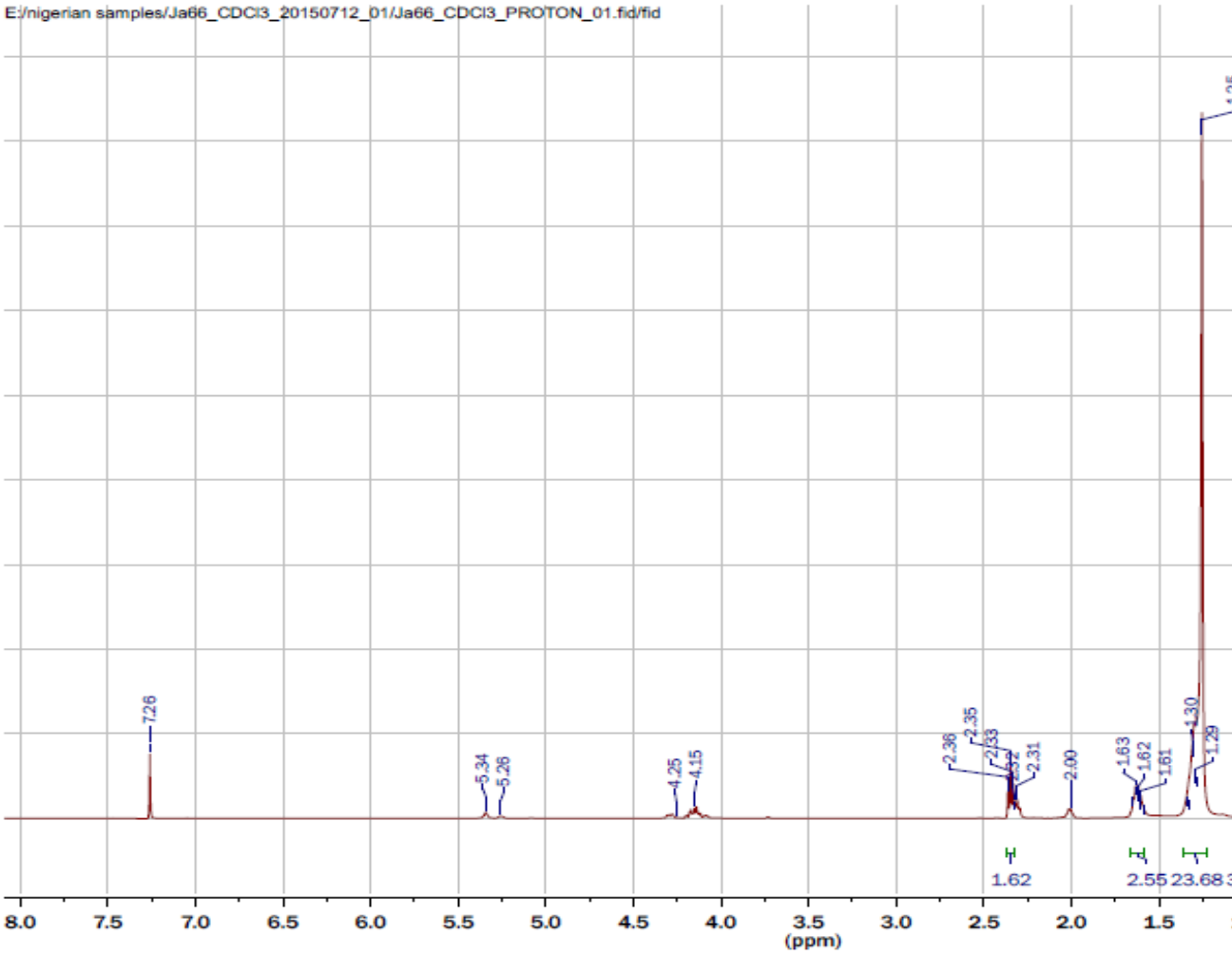
Appendix III-A. [1]HNMR Spectrum of Ja64 (Compared with Margaric acid).



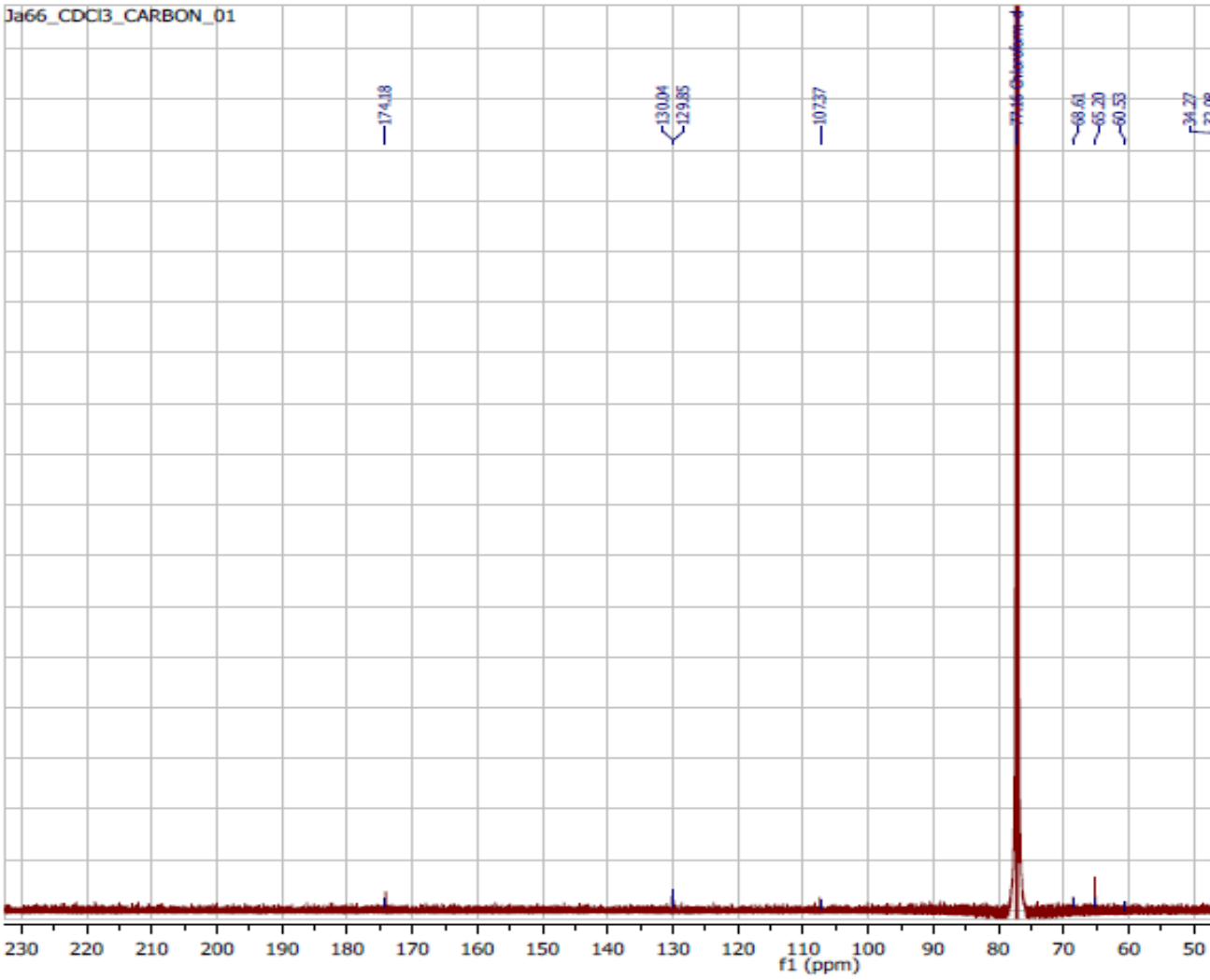
Appendix IV. [1]HNMR Spectrum of Ja65



Appendix IV-A. [1]HNMR Spectrum of Ja65 (Expansions around methylic and methylene regions)

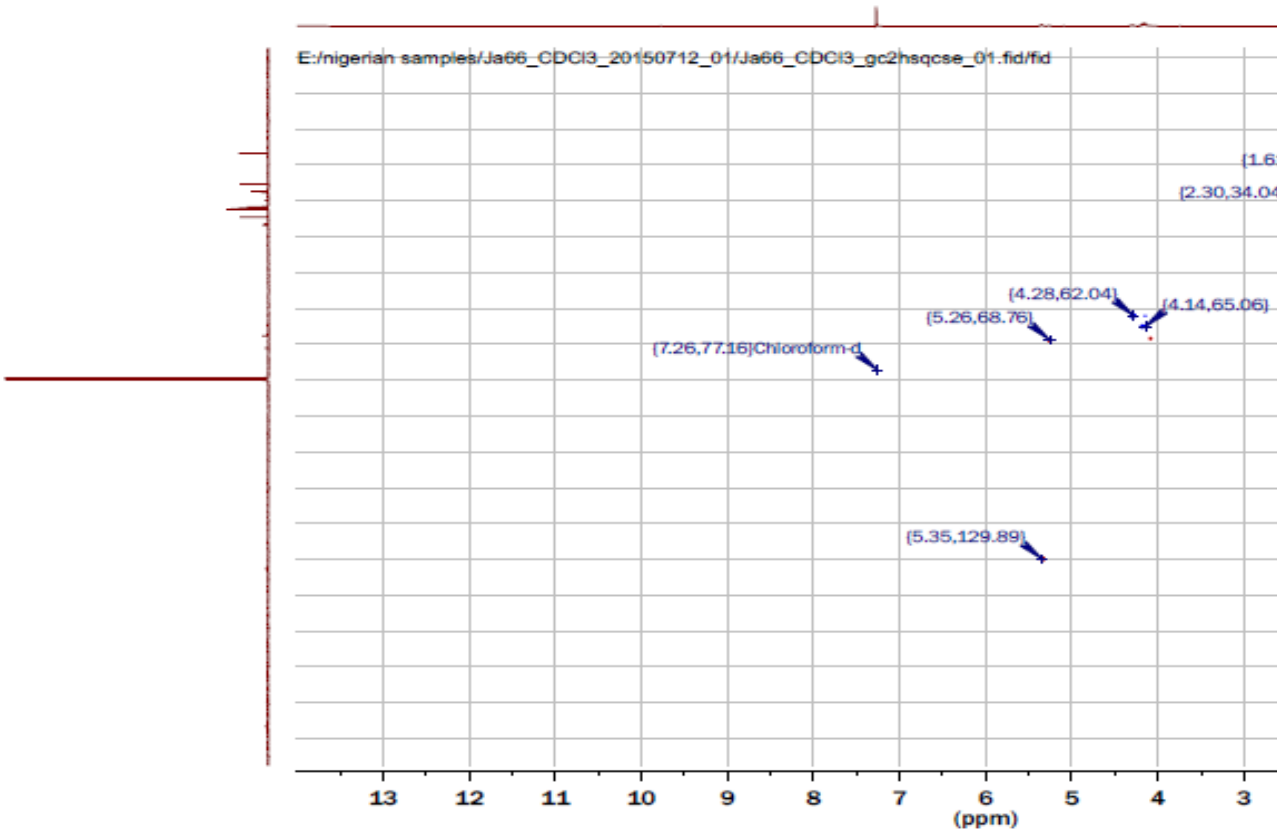


Appendix V-A. ^{1}HNMR Spectrum of Ja66

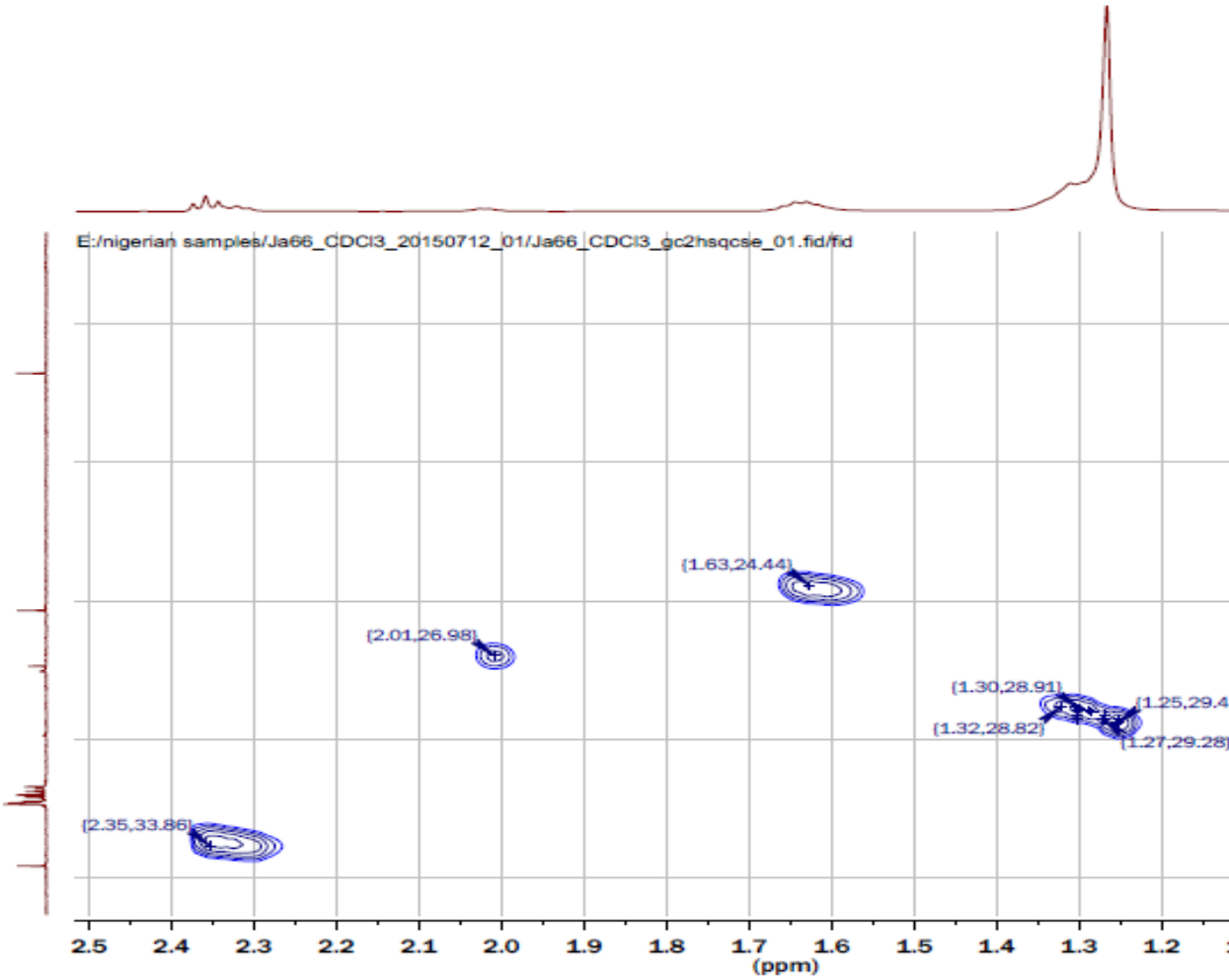


Appendix V-B. 13CNMR Spectrum of Ja66

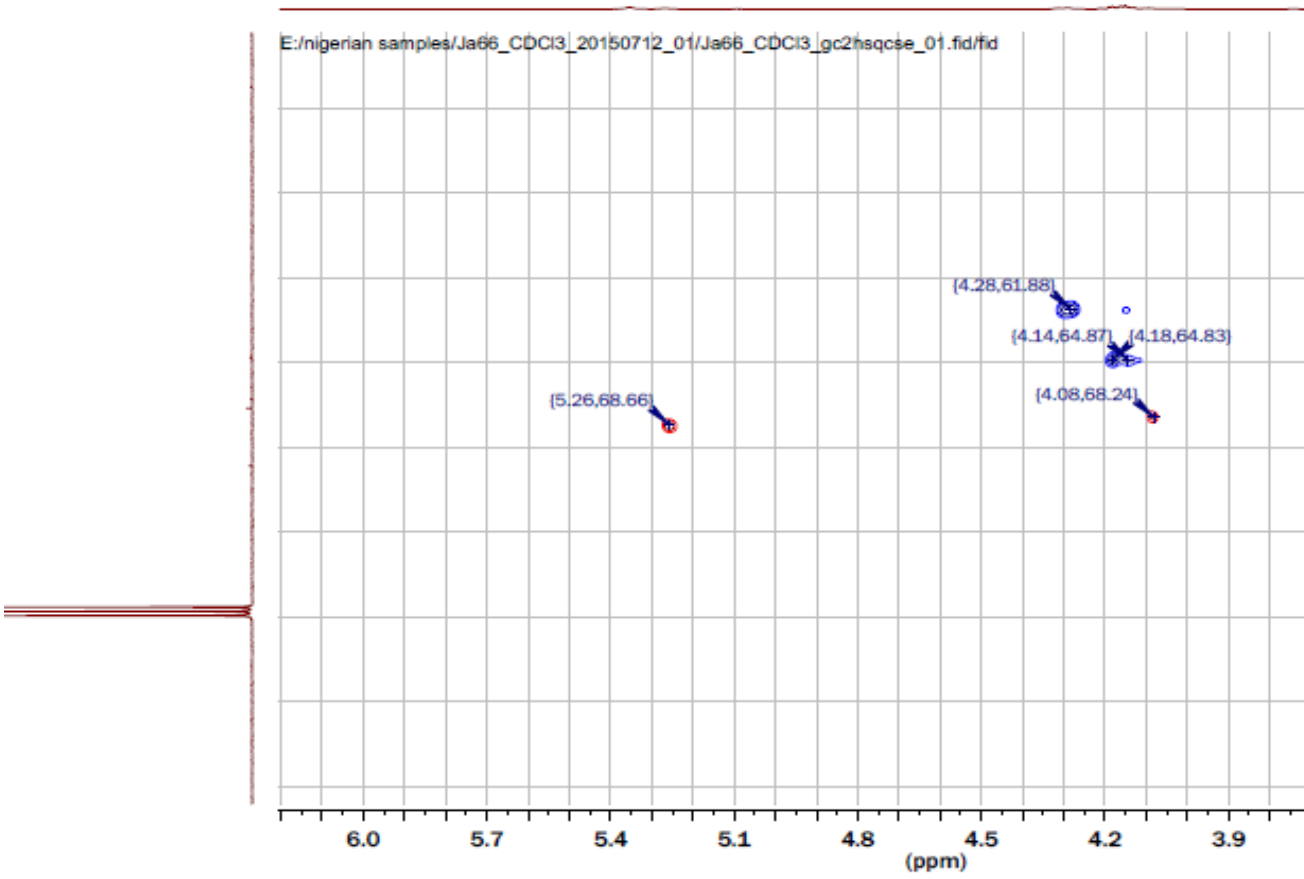
^{13}C NMR (500 MHz, cdcl$_3$) δ 7.26, 5.35, 5.26, 4.28, 4.14, 2.30, 2.01, 2.01, 2.01, 2.01, 1.61, 1.25, 1.25, 0.88.



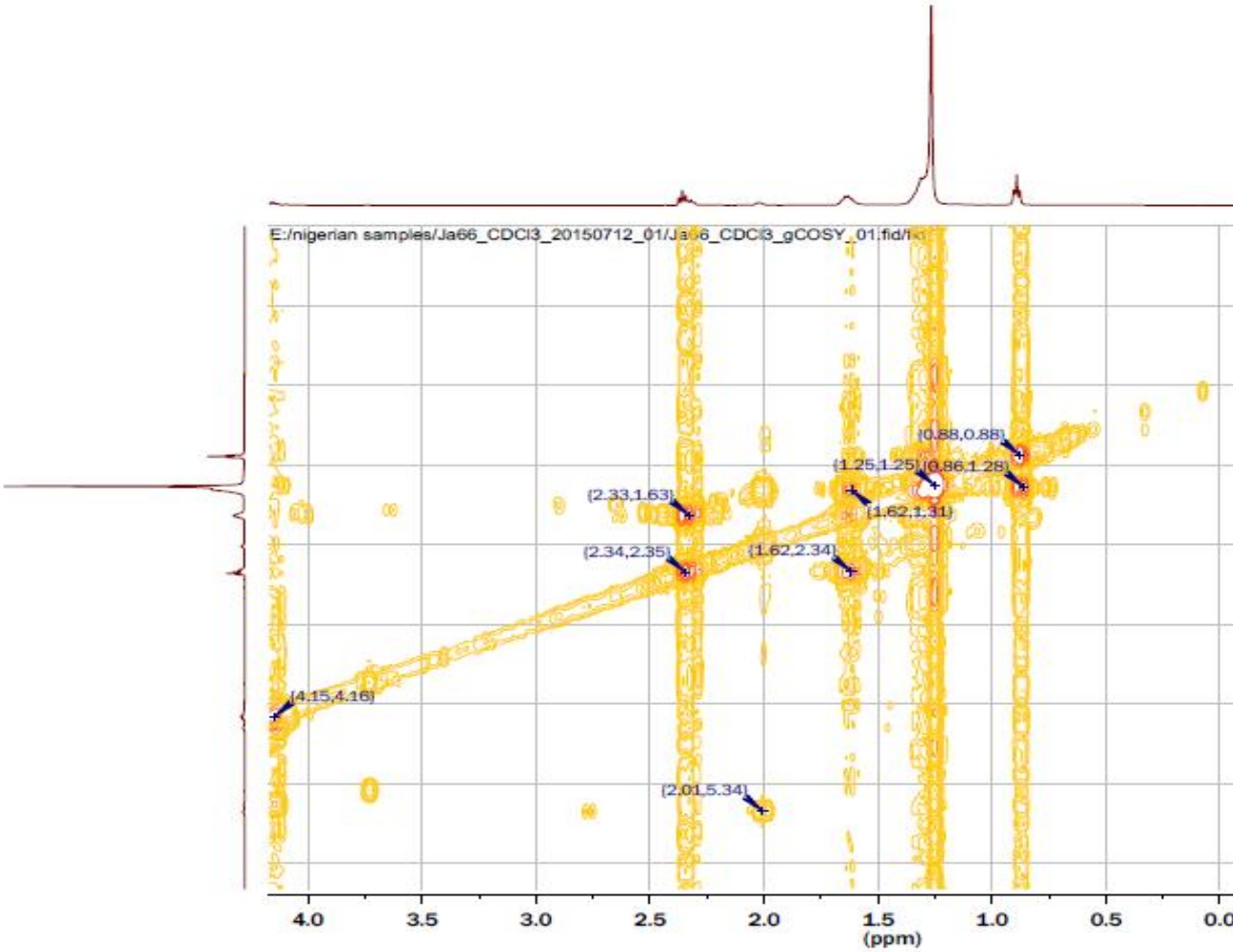
Appendix V-C. HSQC Spectrum of Ja66



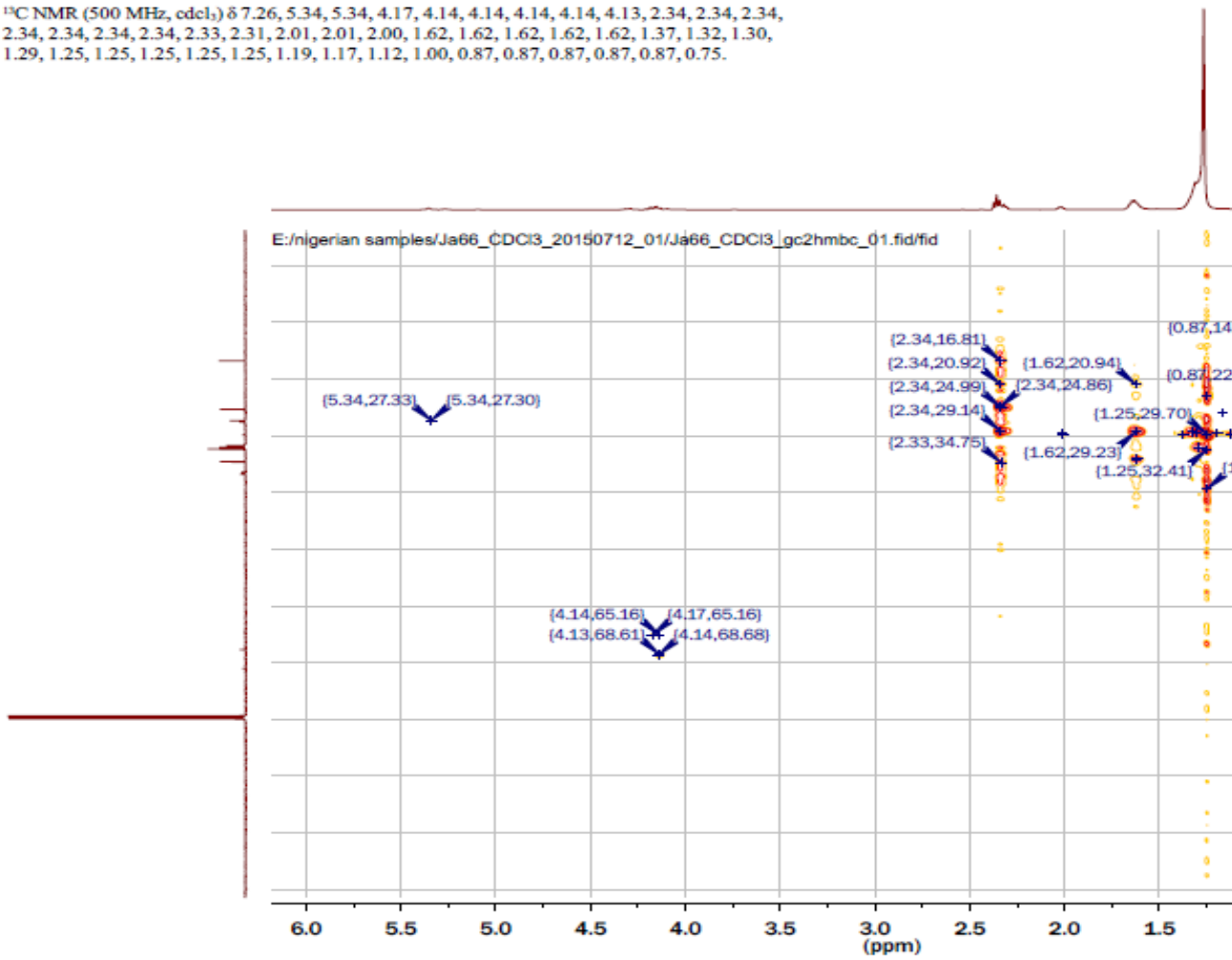
Appendix V-C1. SOME RELEVANT EXPANSIONS- HSQC Spectrum of Ja66



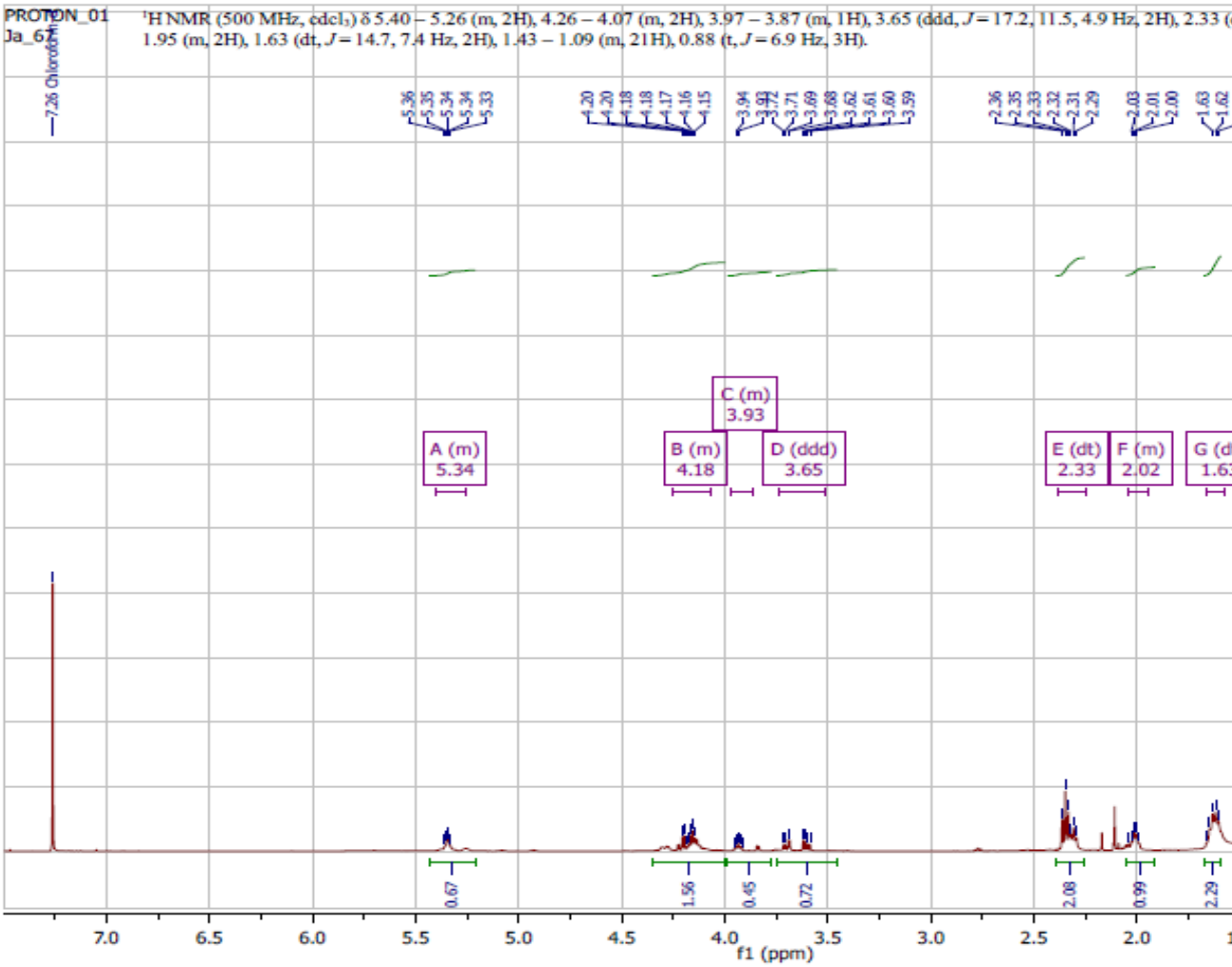
Appendix V-C2. SOME RELEVANT EXPANSIONS- HSQC Spectrum of Ja66



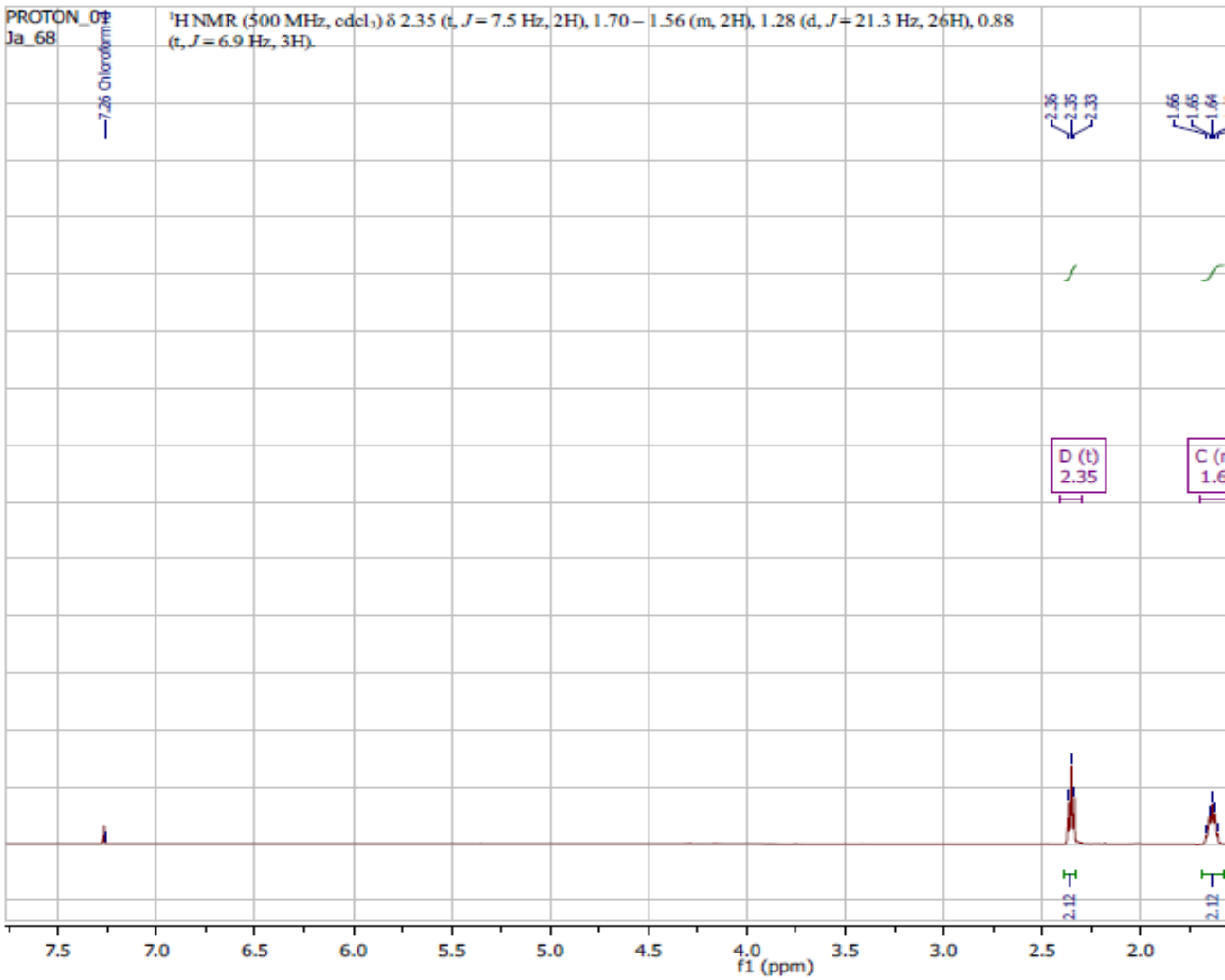
Appendix V-D. COSY Spectrum of Ja66



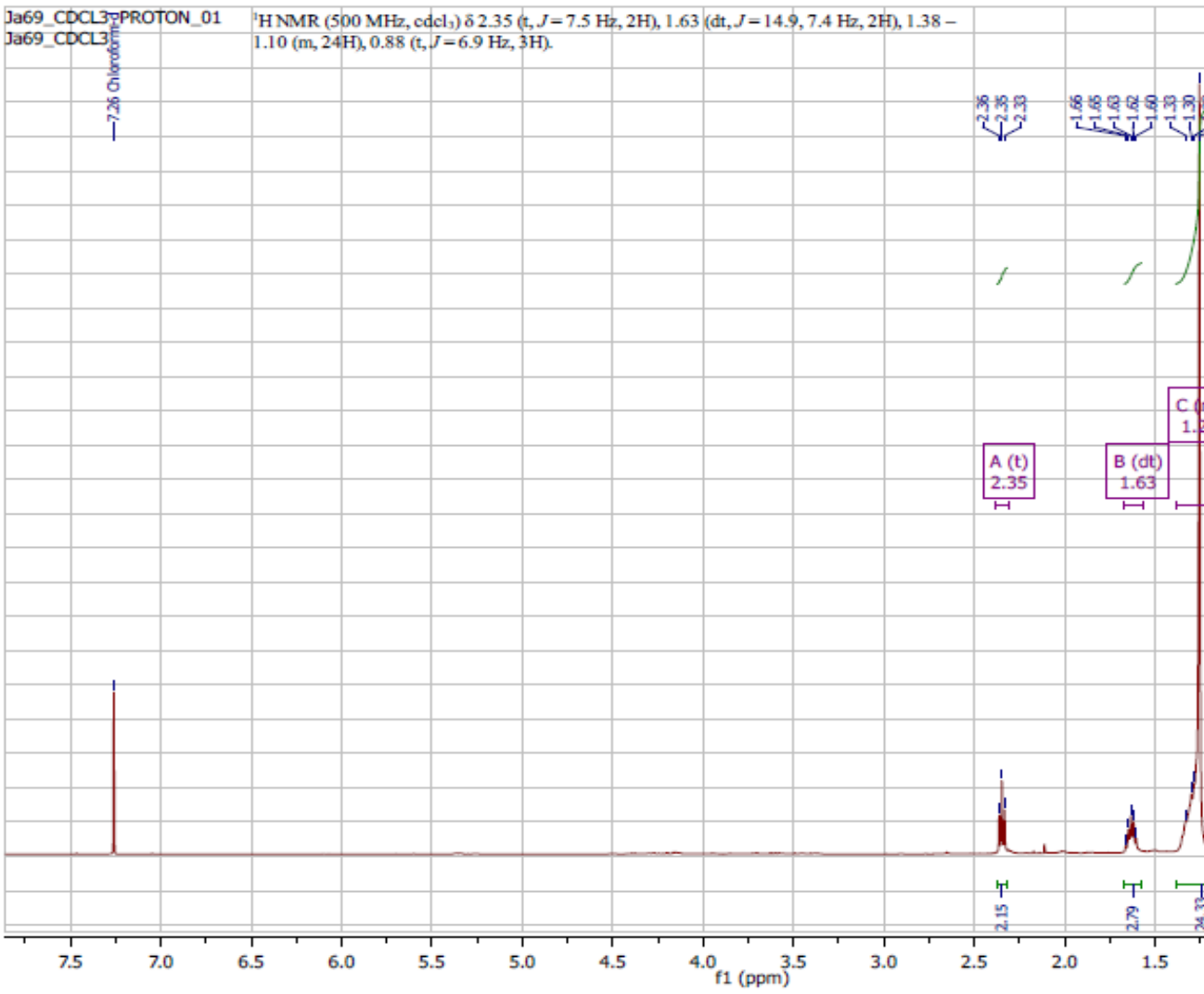
Appendix V-E. HMBC Spectrum of Ja66



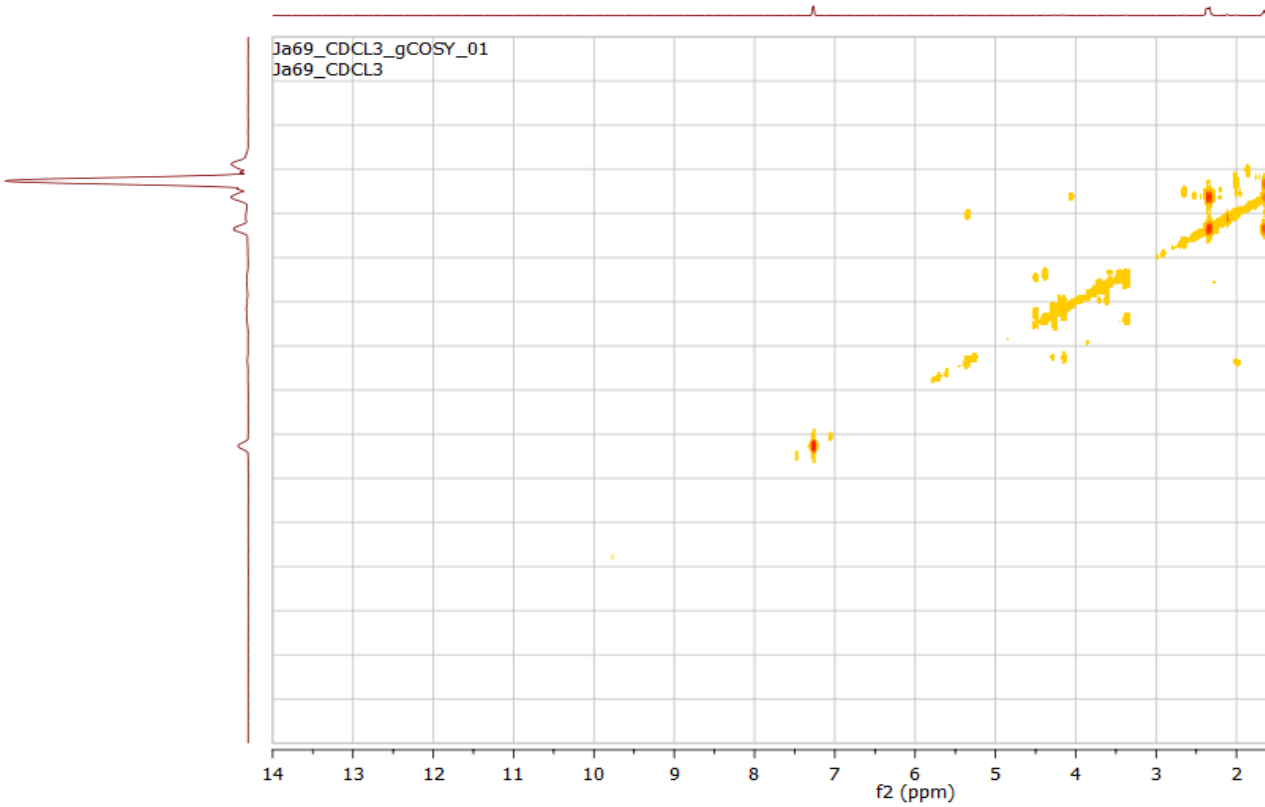
Appendix VI. [1]HNMR Spectrum of Ja67



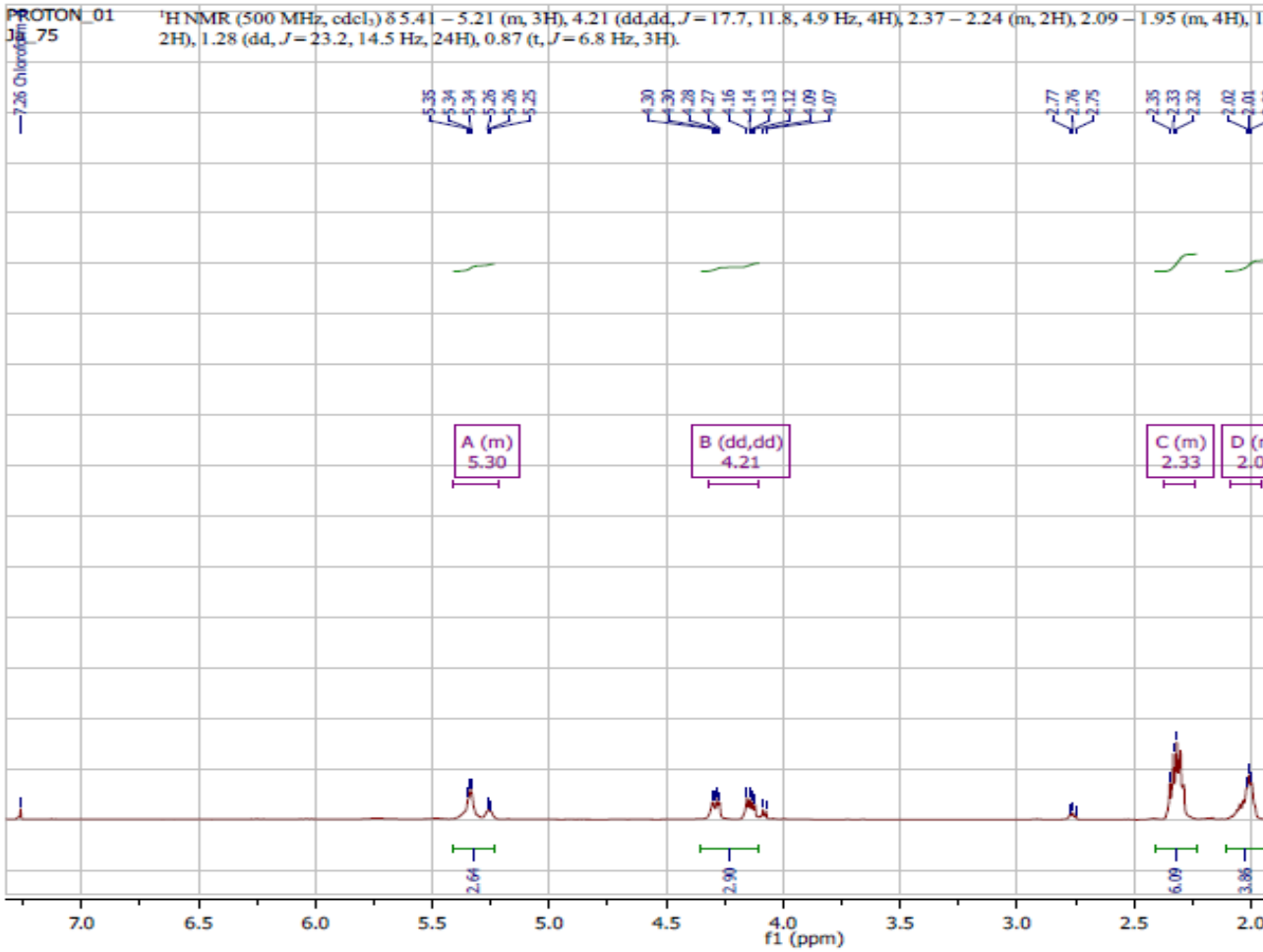
Appendix VII. [1]HNMR Spectrum of Ja68



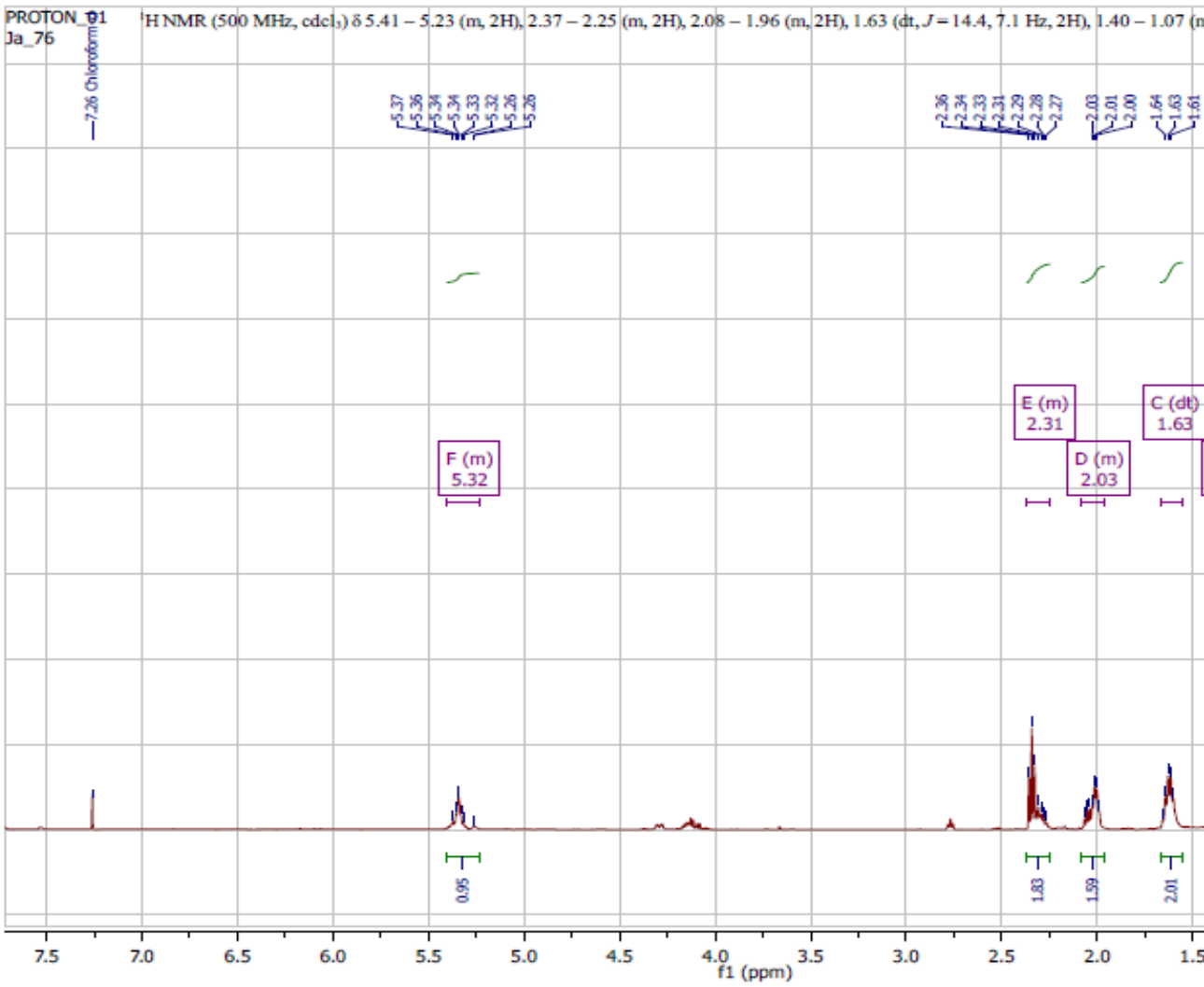
Appendix VIII. [1]HNMR Spectrum of Ja69



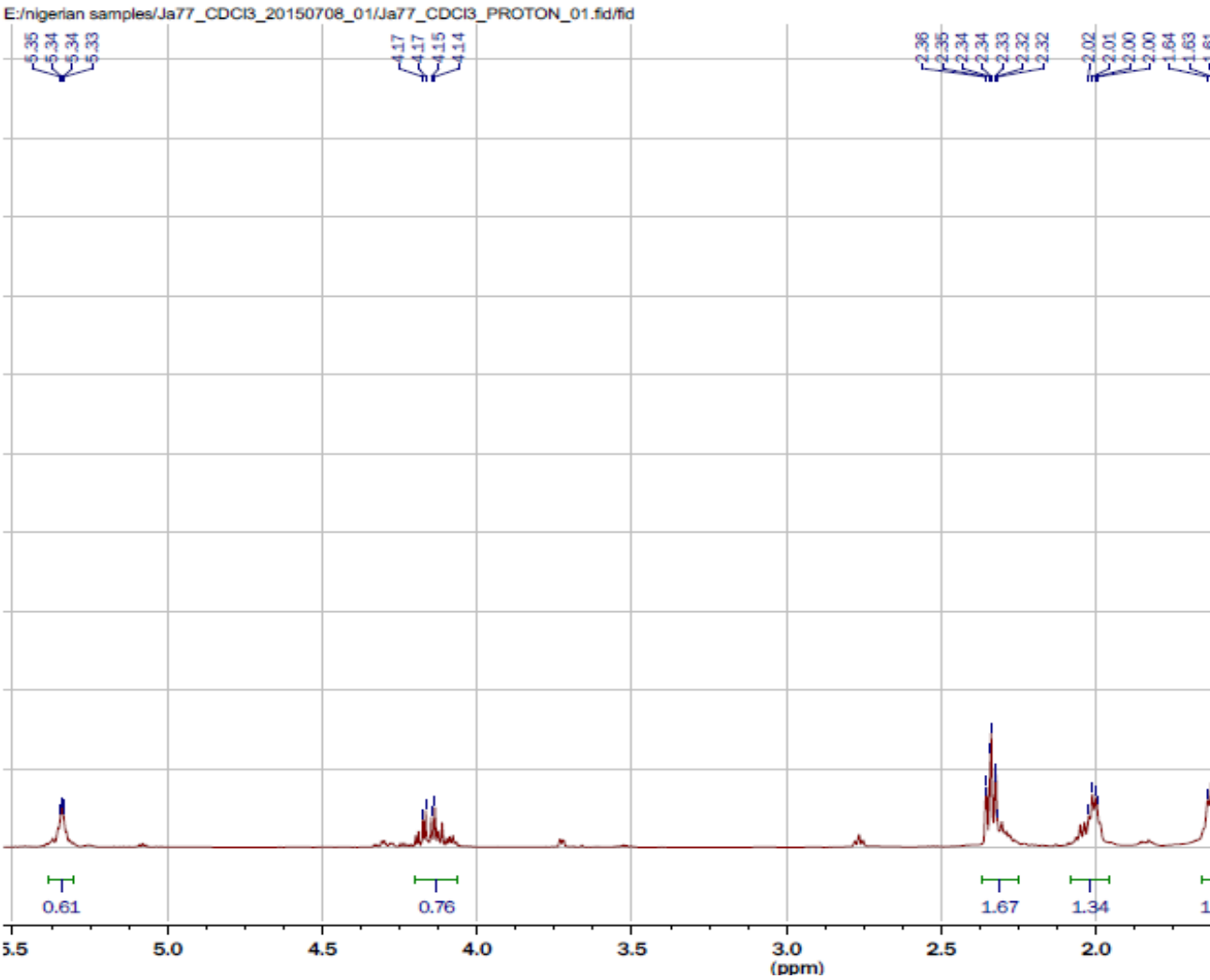
Appendix IX. COSY Spectrum of Ja69



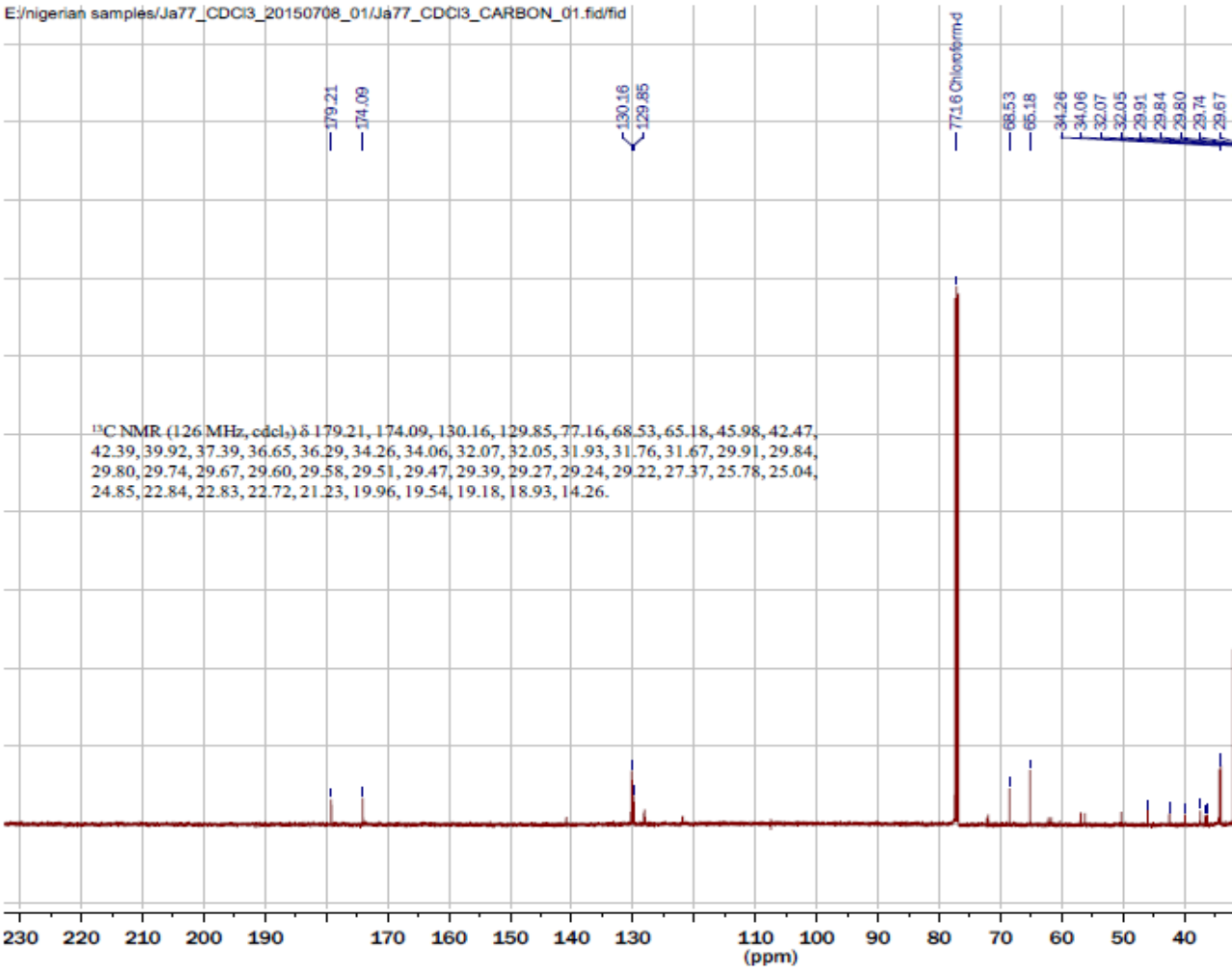
Appendix X. [1]HNMR Spectrum of Ja75



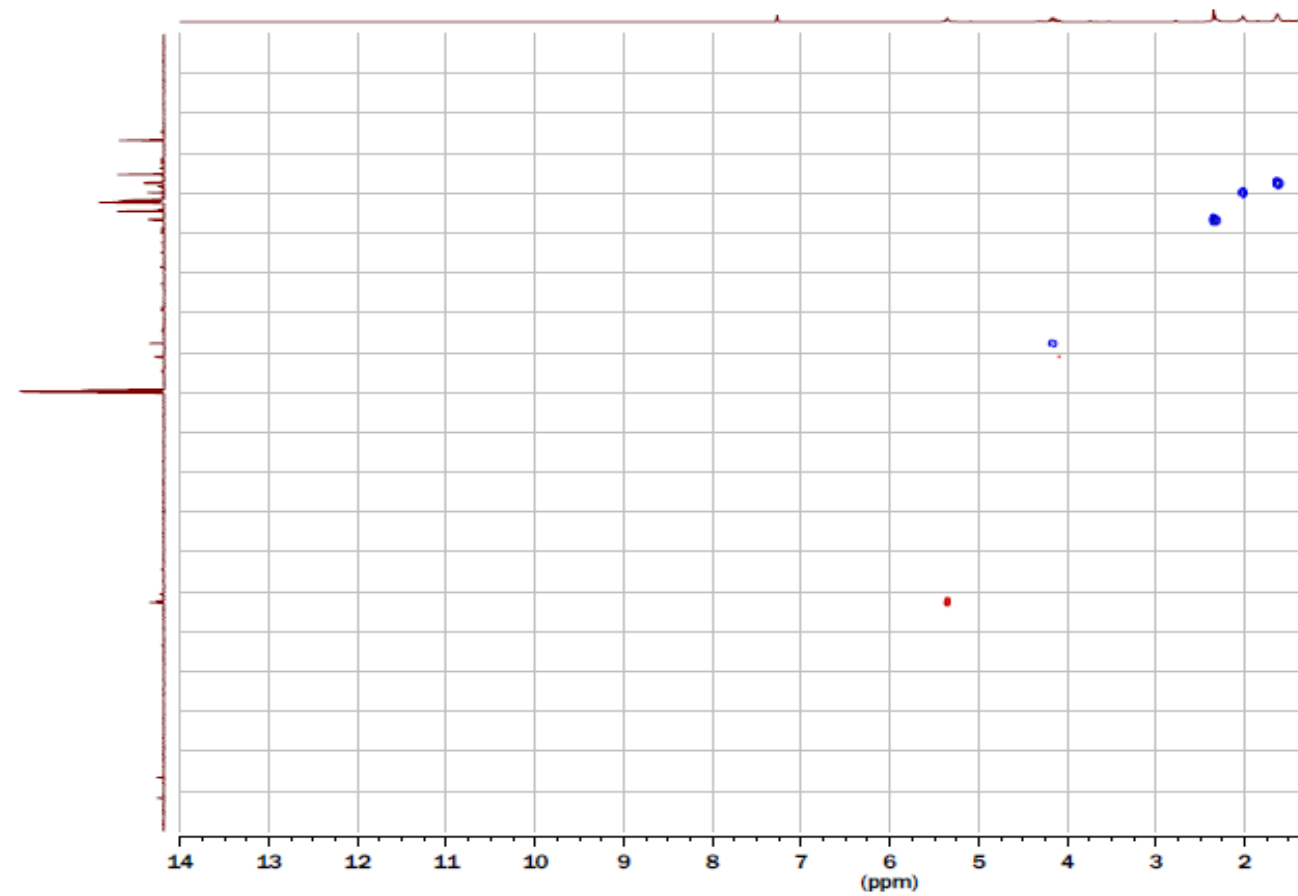
Appendix XI. ^{1}HNMR Spectrum of Ja76



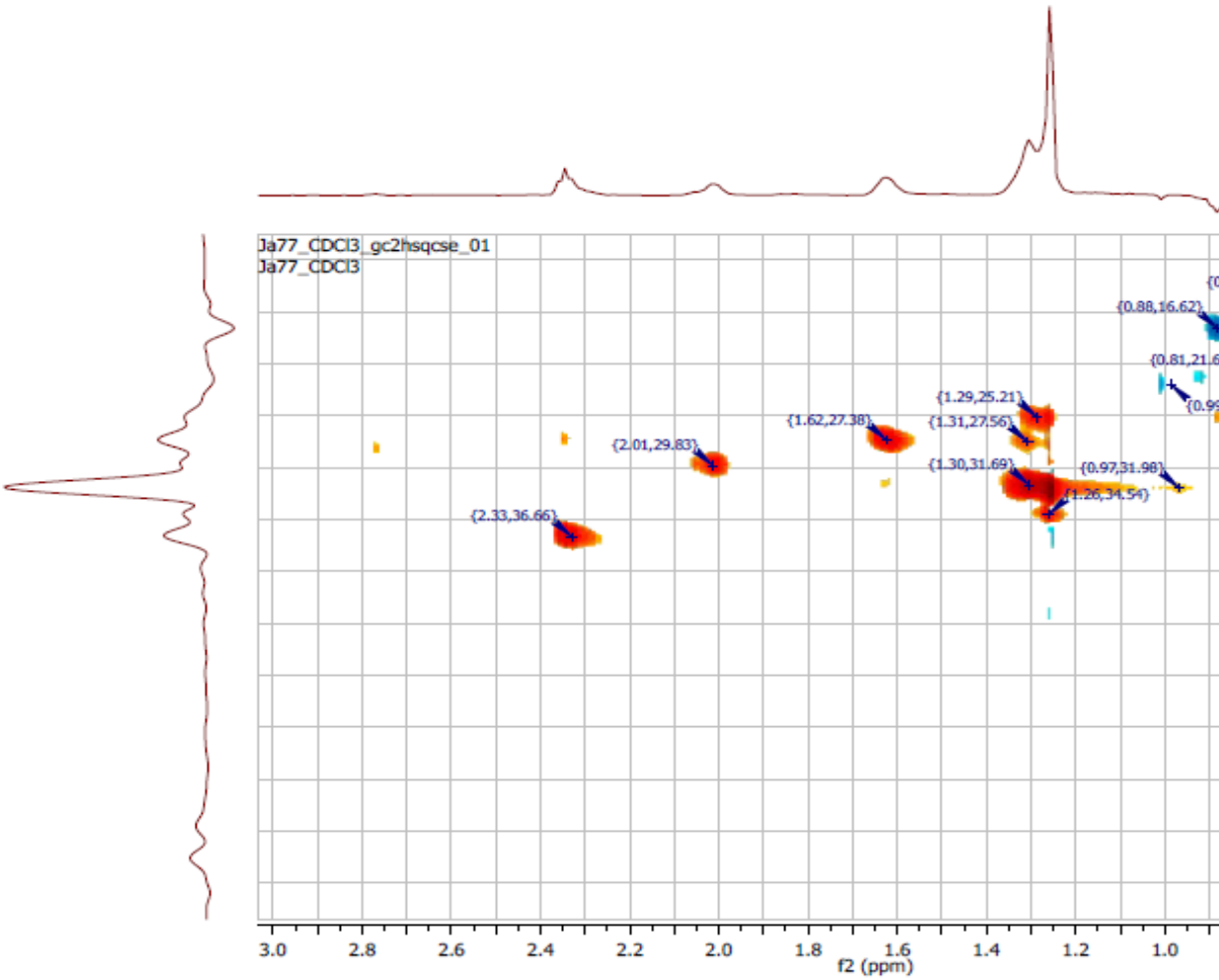
Appendix XII. [1]HNMR Spectrum of Ja77



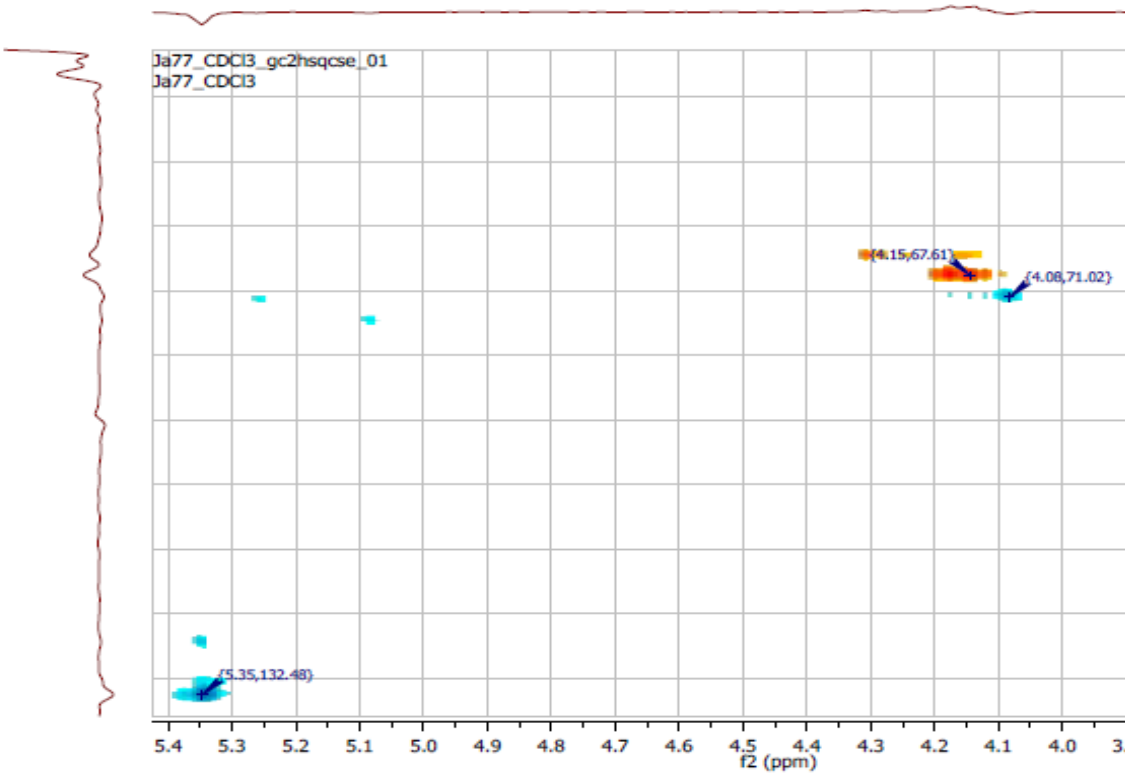
Appendix XII-A. ^{13}CNMR Spectrum of Ja77



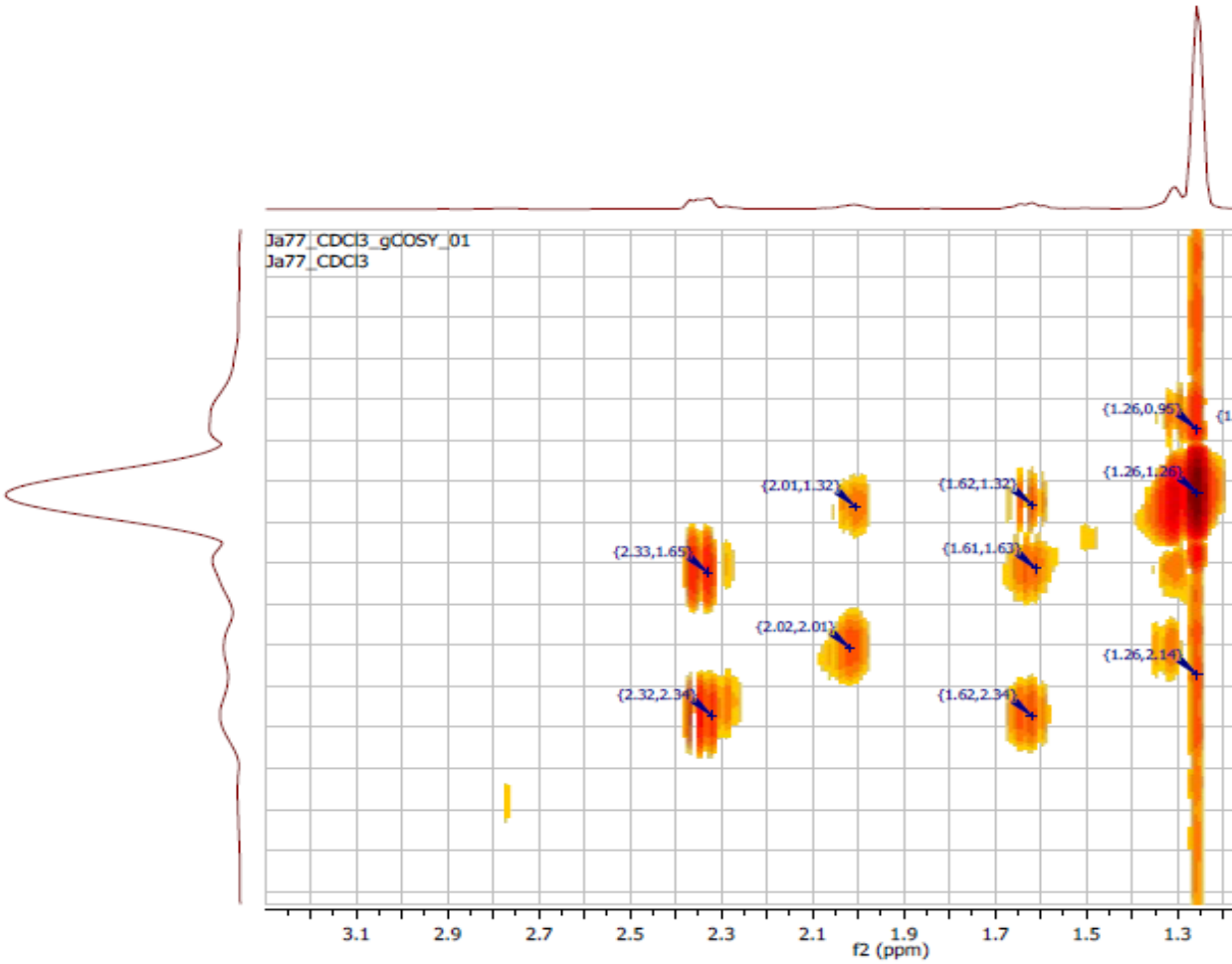
Appendix XII-B. HSQC Spectrum of Ja77



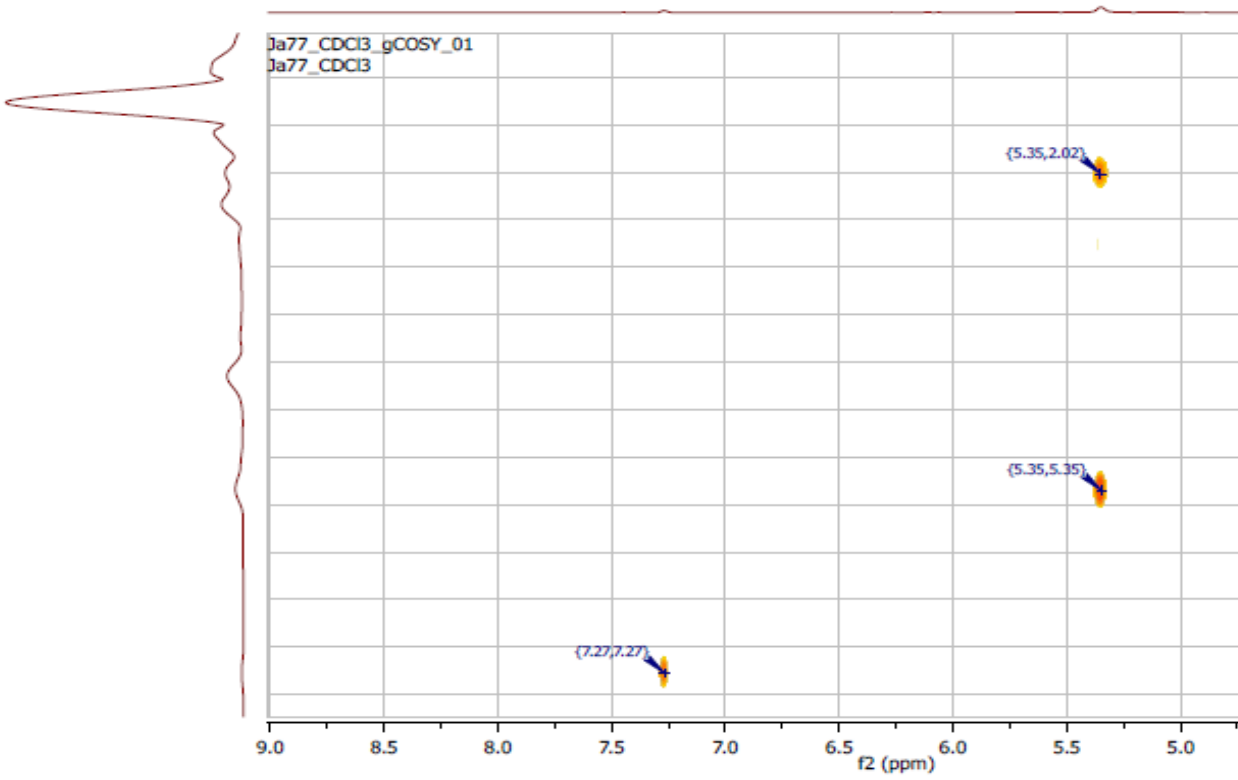
Appendix XII-C. SOME RELEVANT EXPANSIONS- HSQC Spectrum of Ja77



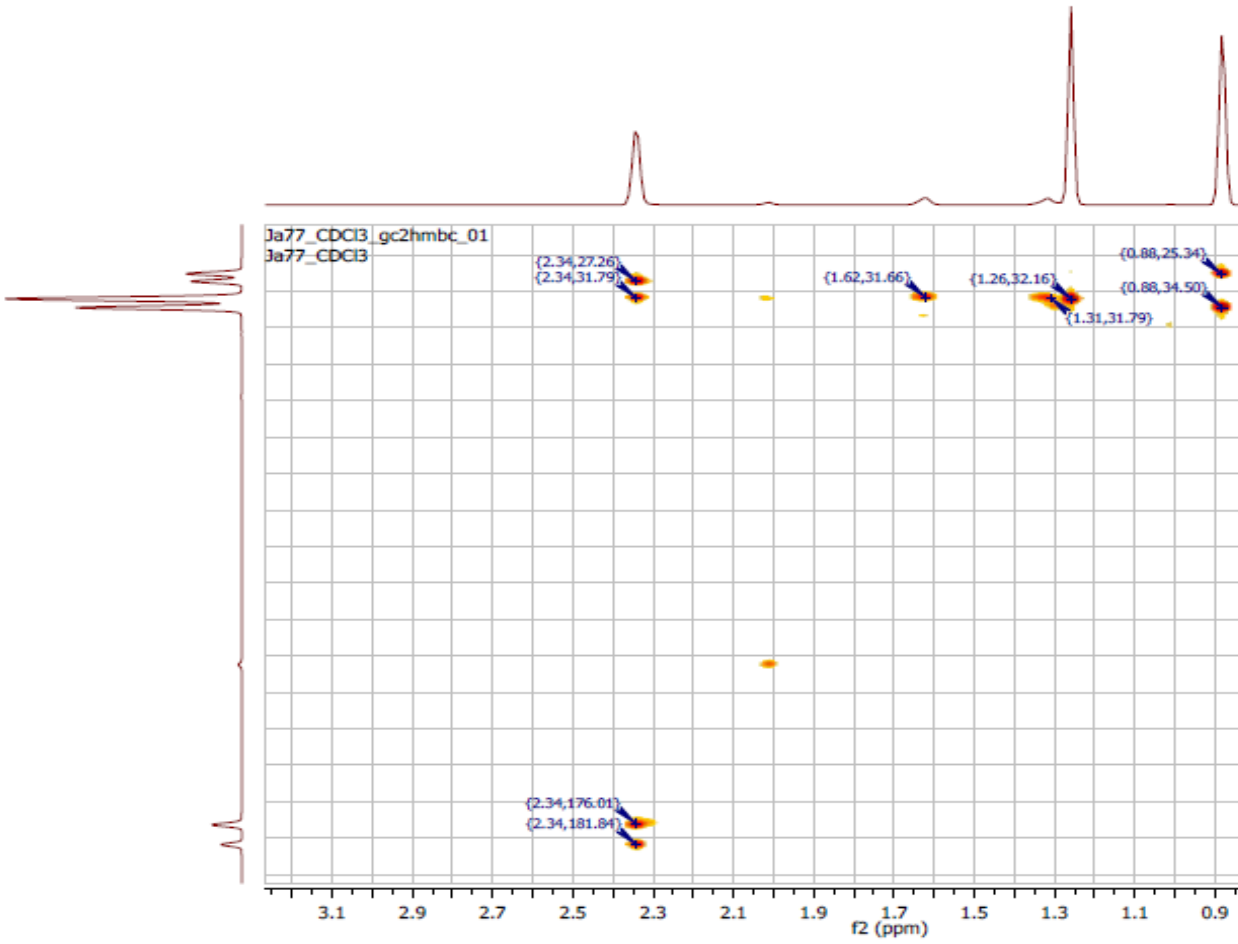
Appendix XII-C1. SOME RELEVANT EXPANSIONS- HSQC Spectrum of Ja77



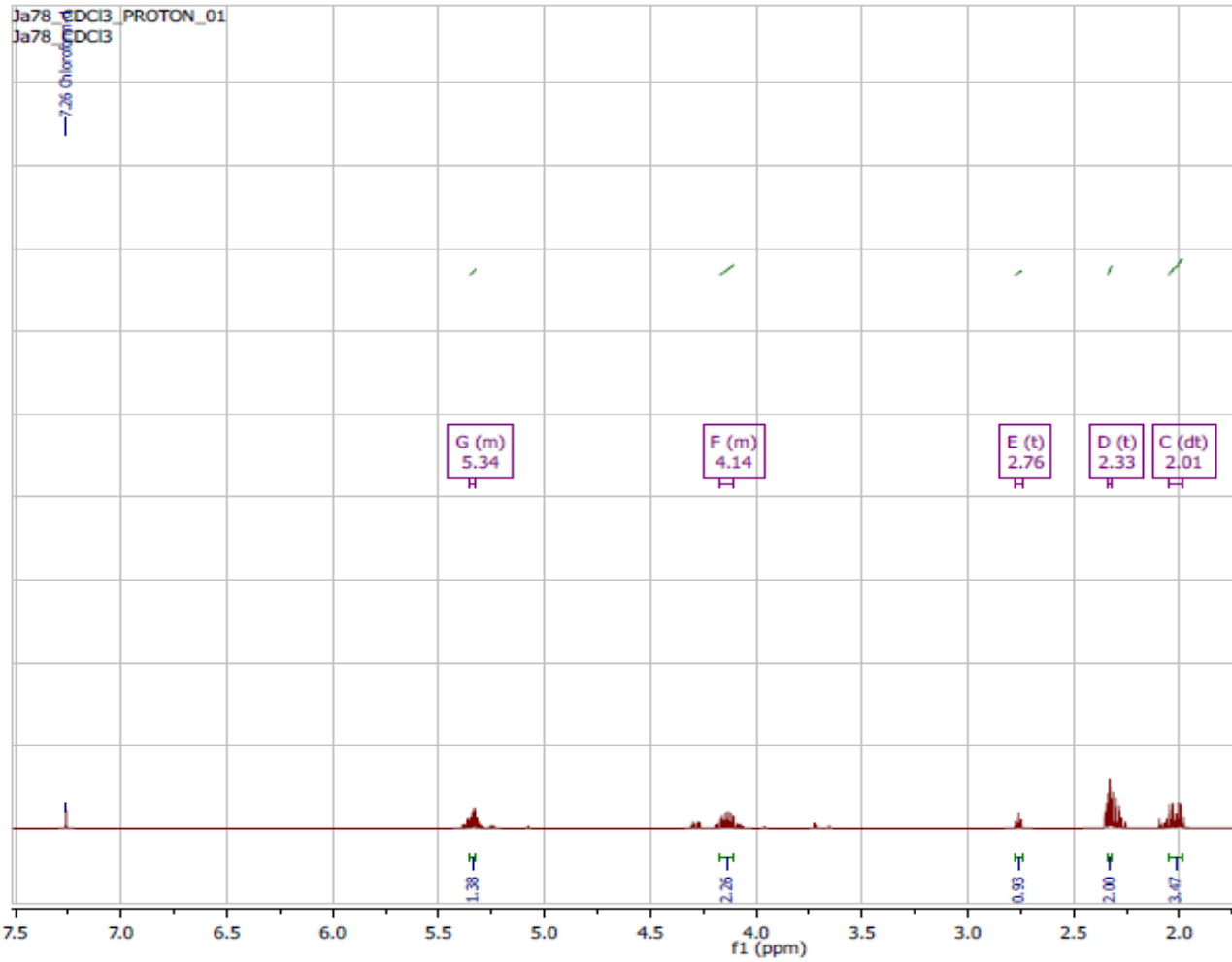
Appendix XII-D. COSY Spectrum of Ja77



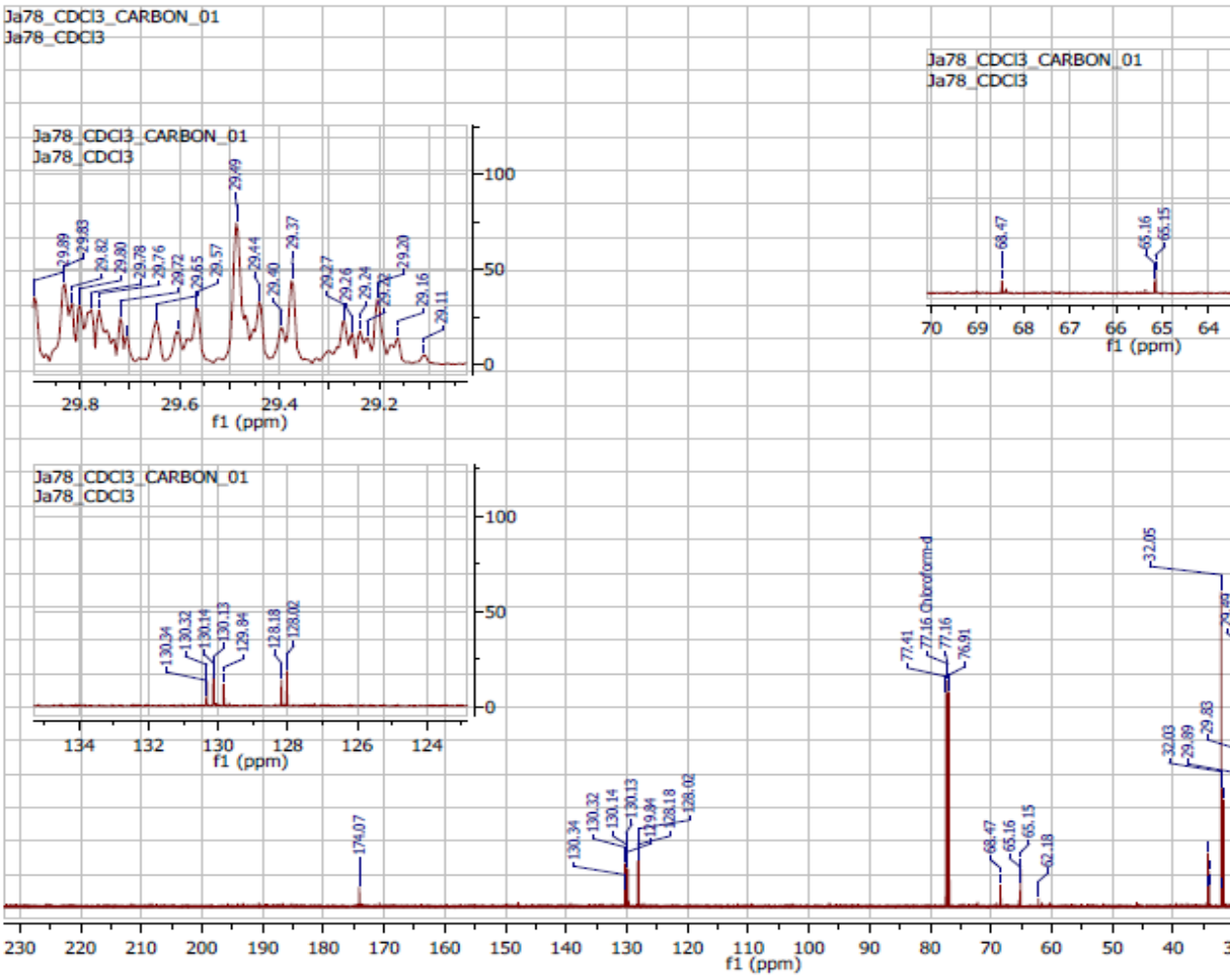
Appendix XII-D1. COSY Spectrum of Ja77



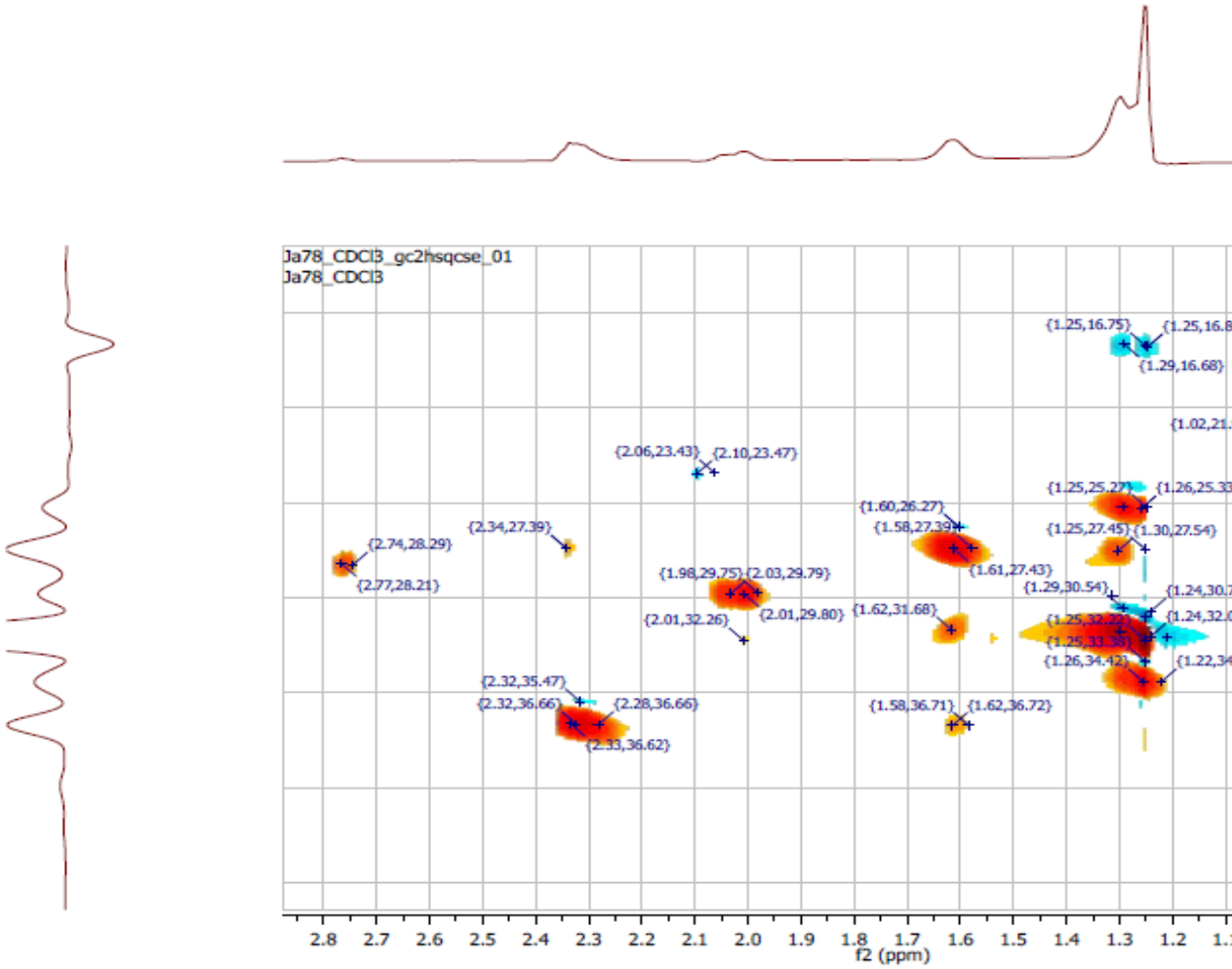
Appendix XII-E. HMBC Spectrum of Ja77



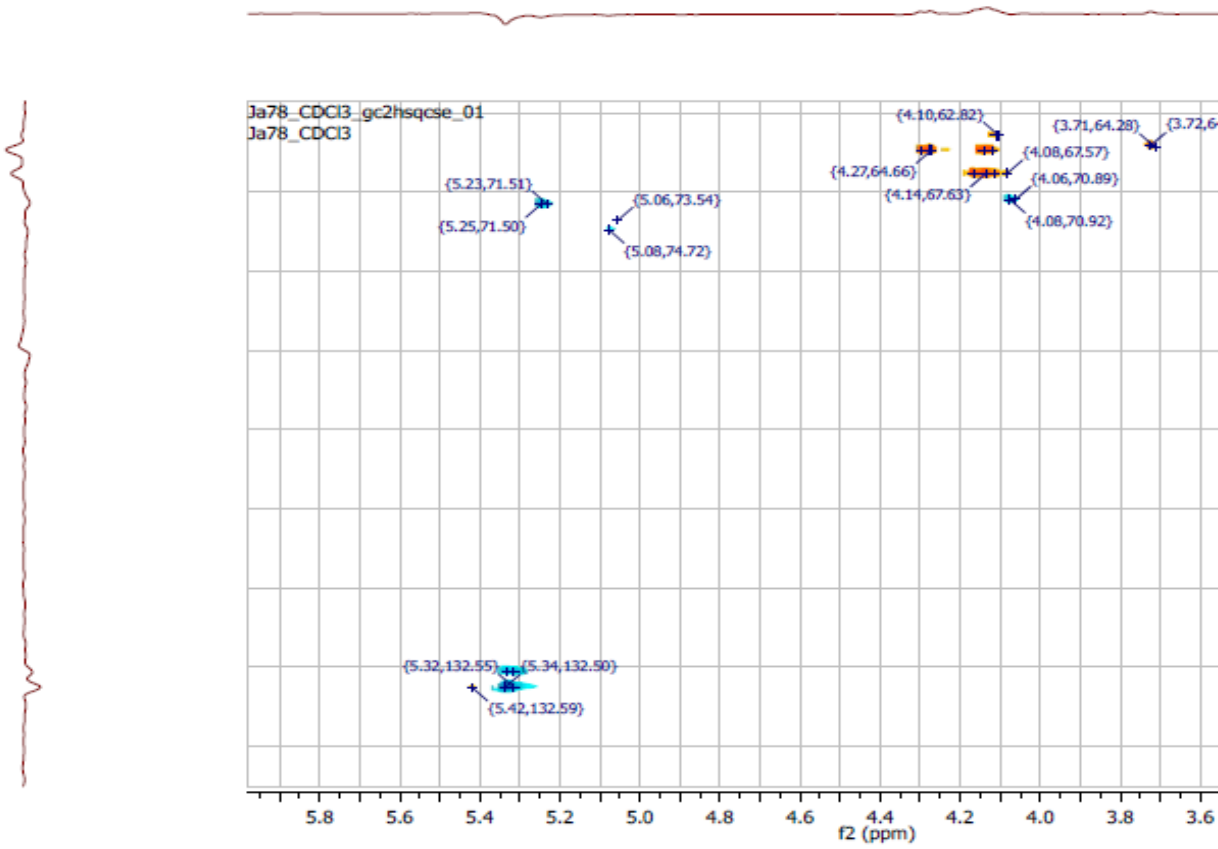
Appendix XIII. ^{1}HNMR Spectrum of Ja78



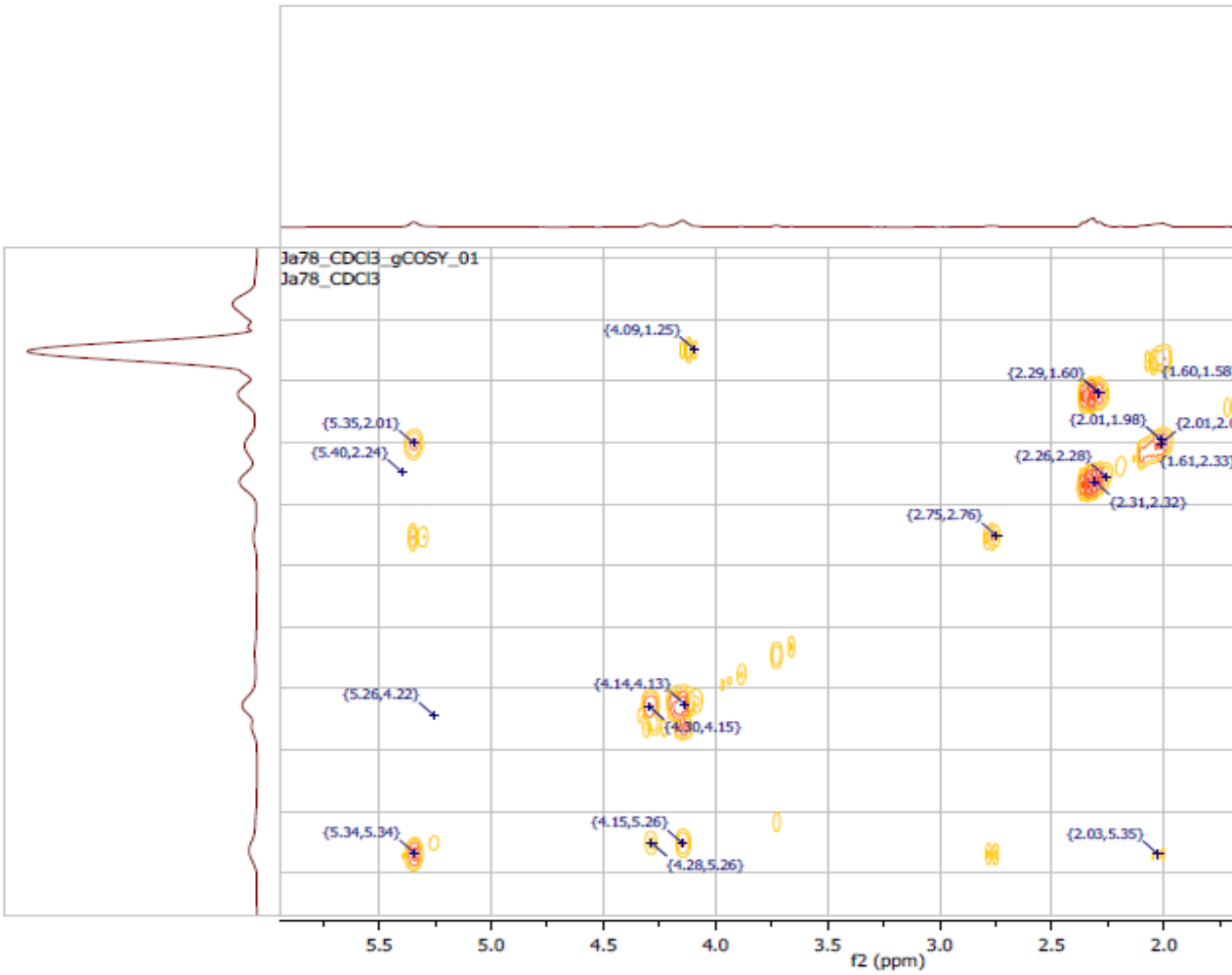
Appendix XIII-A. ^{13}CNMR Spectrum of Ja78



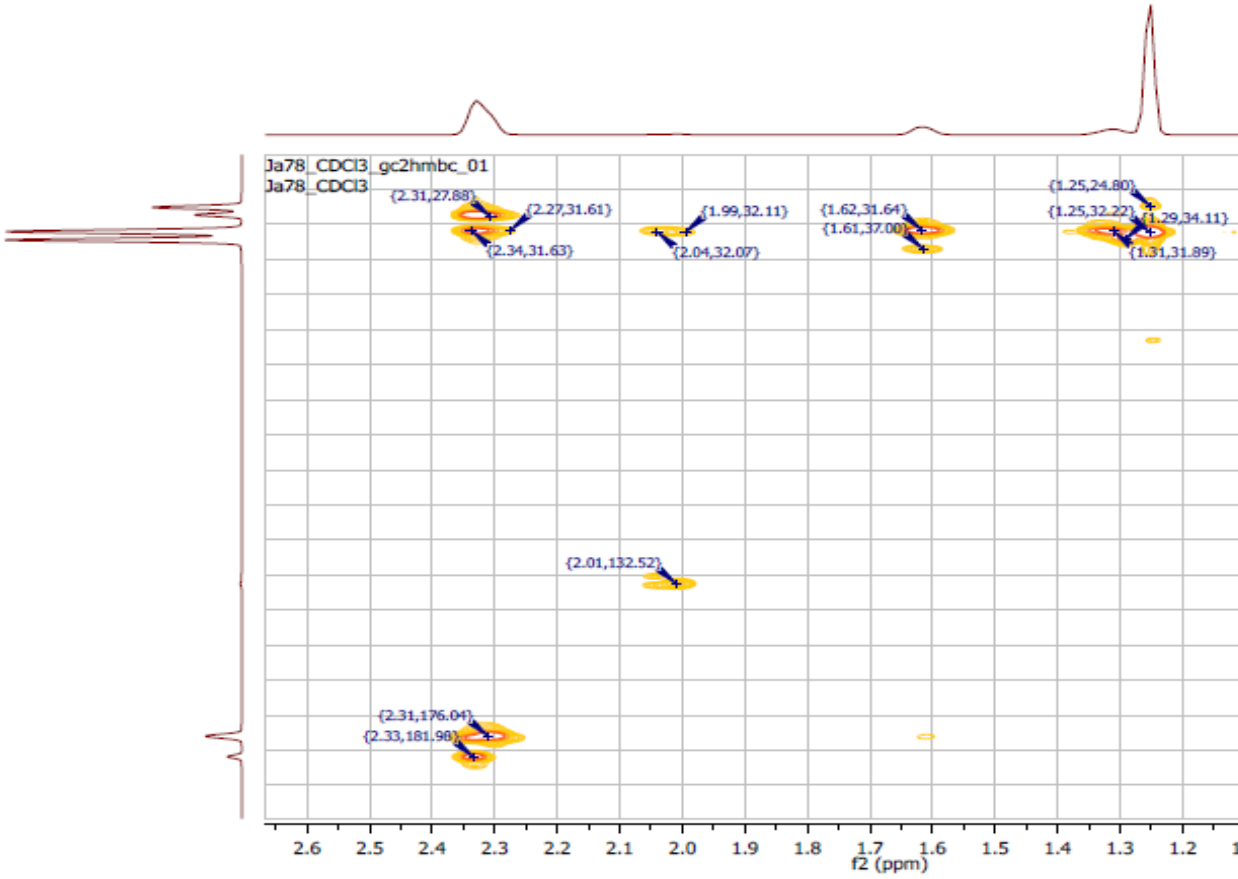
Appendix XIII-B. HSQC Spectrum of Ja78



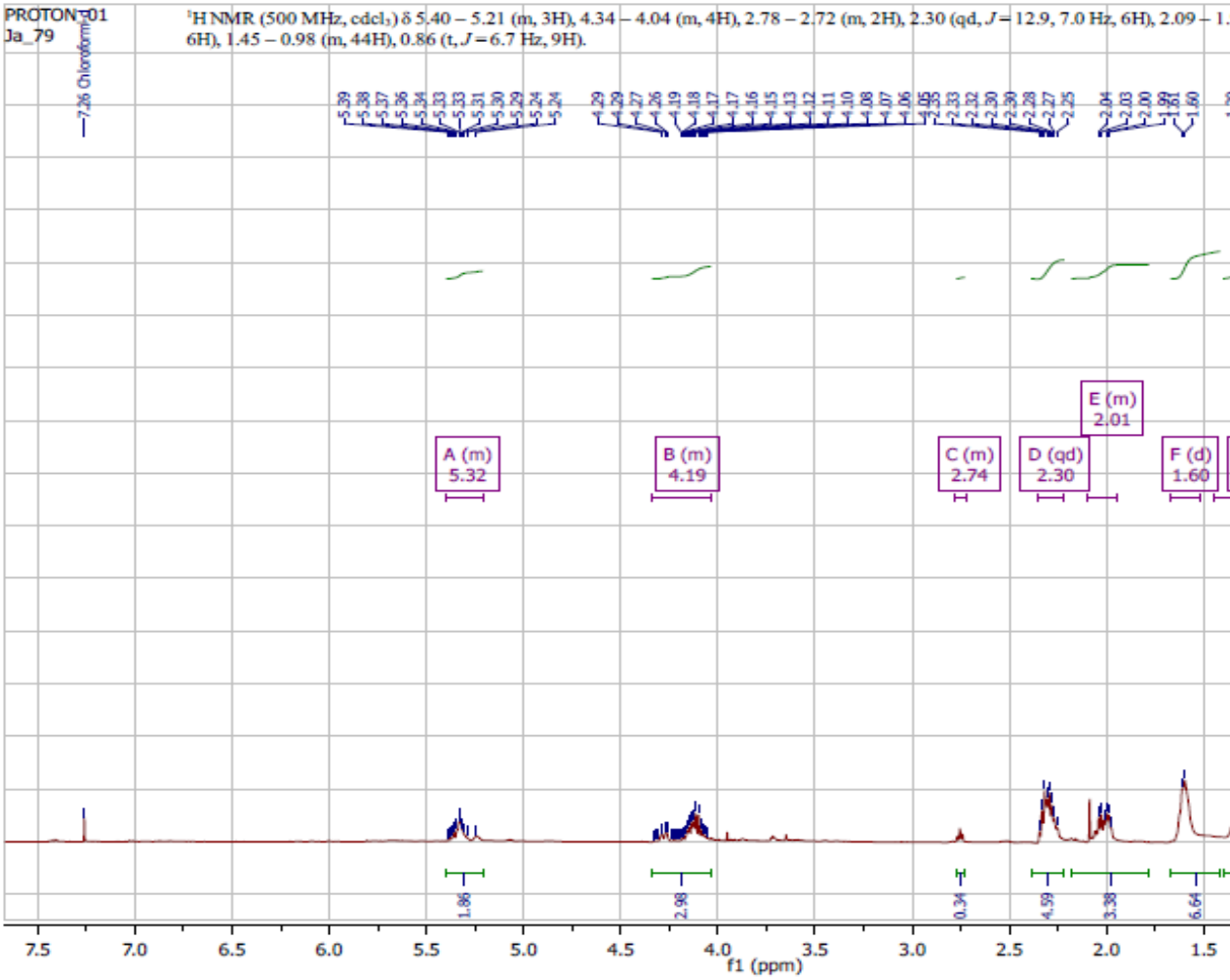
Appendix XIII-C. SOME RELEVANT EXPANSIONS-HSQC Spectrum of Ja78



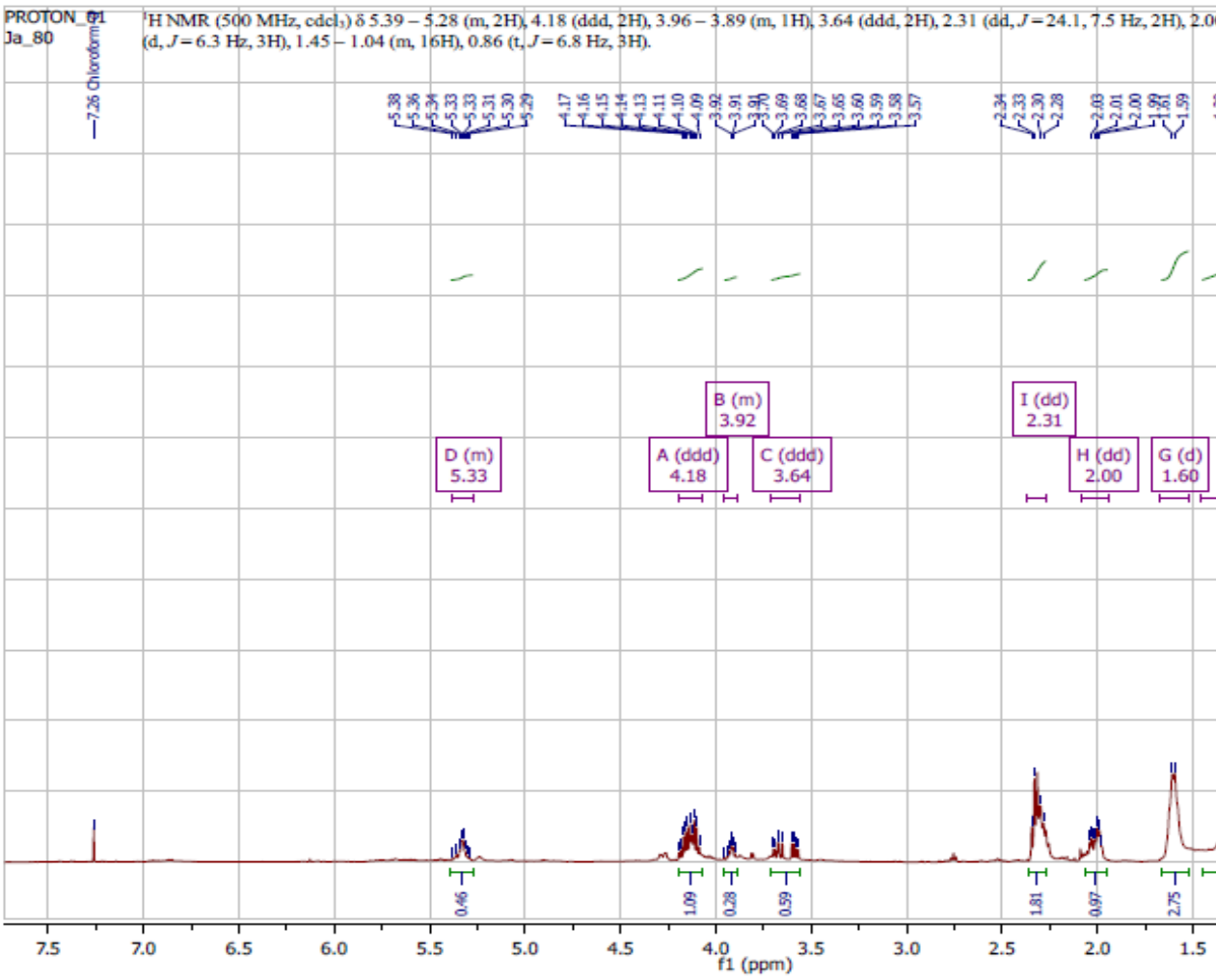
Appendix XIII-D. COSY Spectrum of Ja78



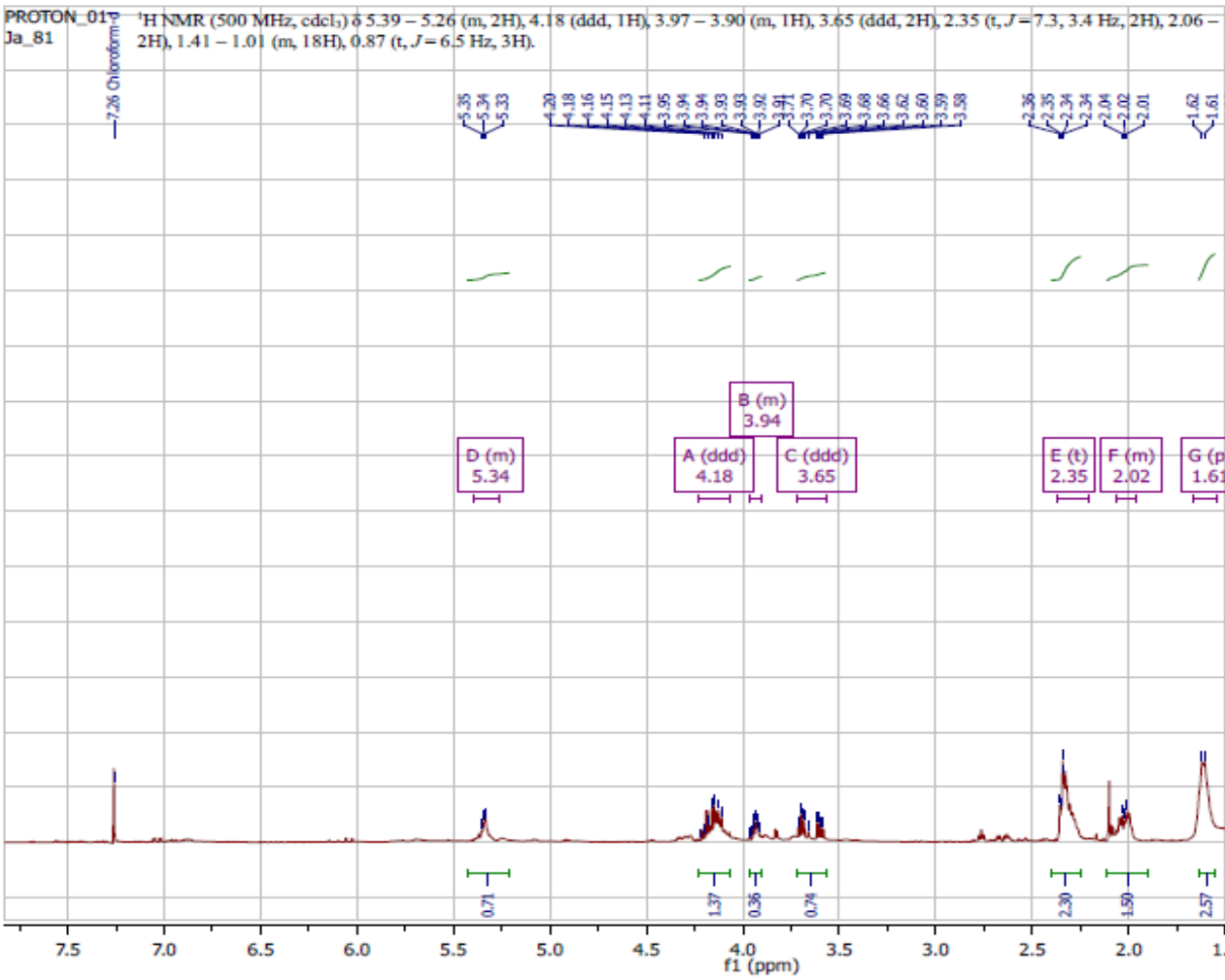
Appendix XIII-E. HMBC Spectrum of Ja78



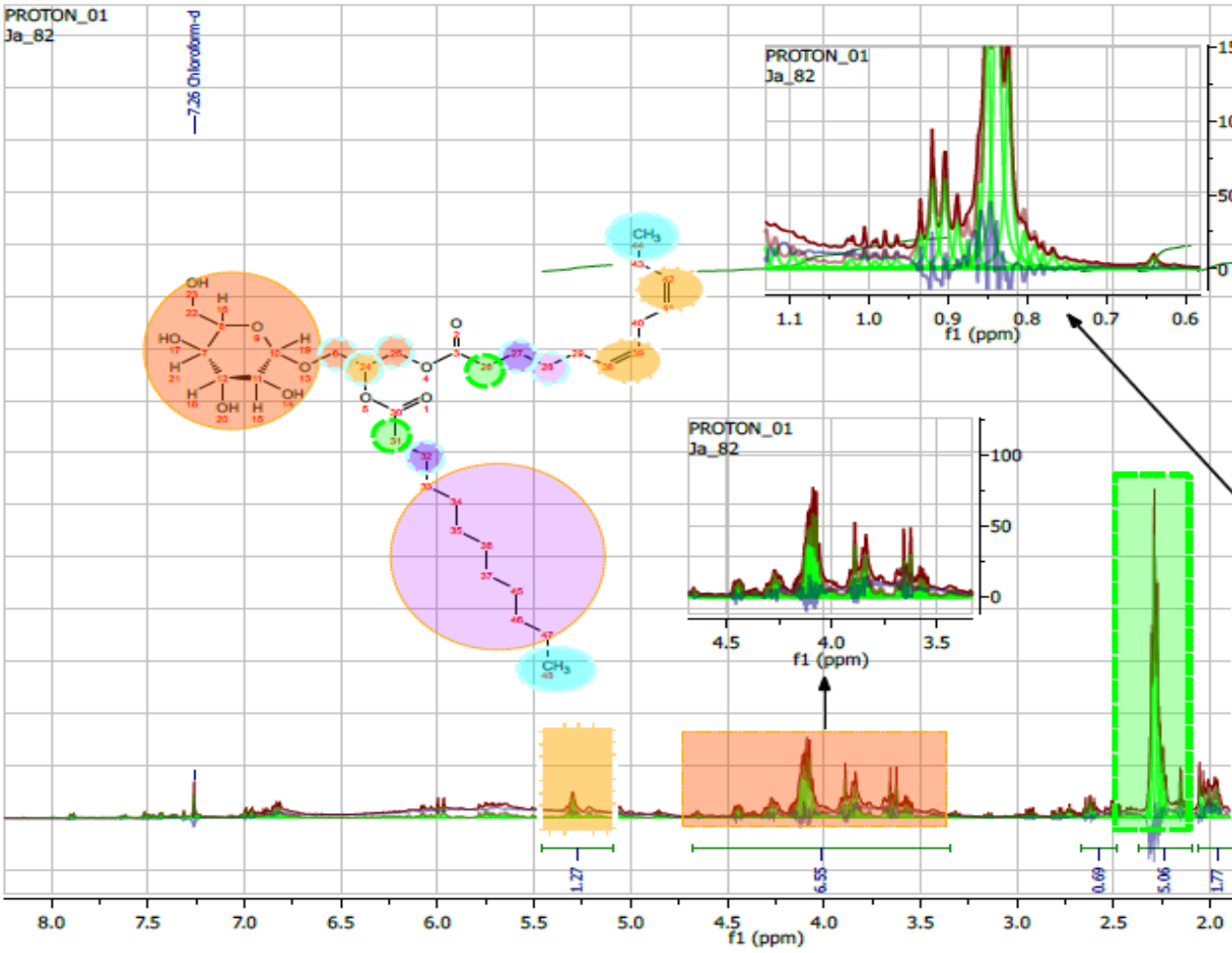
Appendix XIV. [1]HNMR Spectrum of Ja79



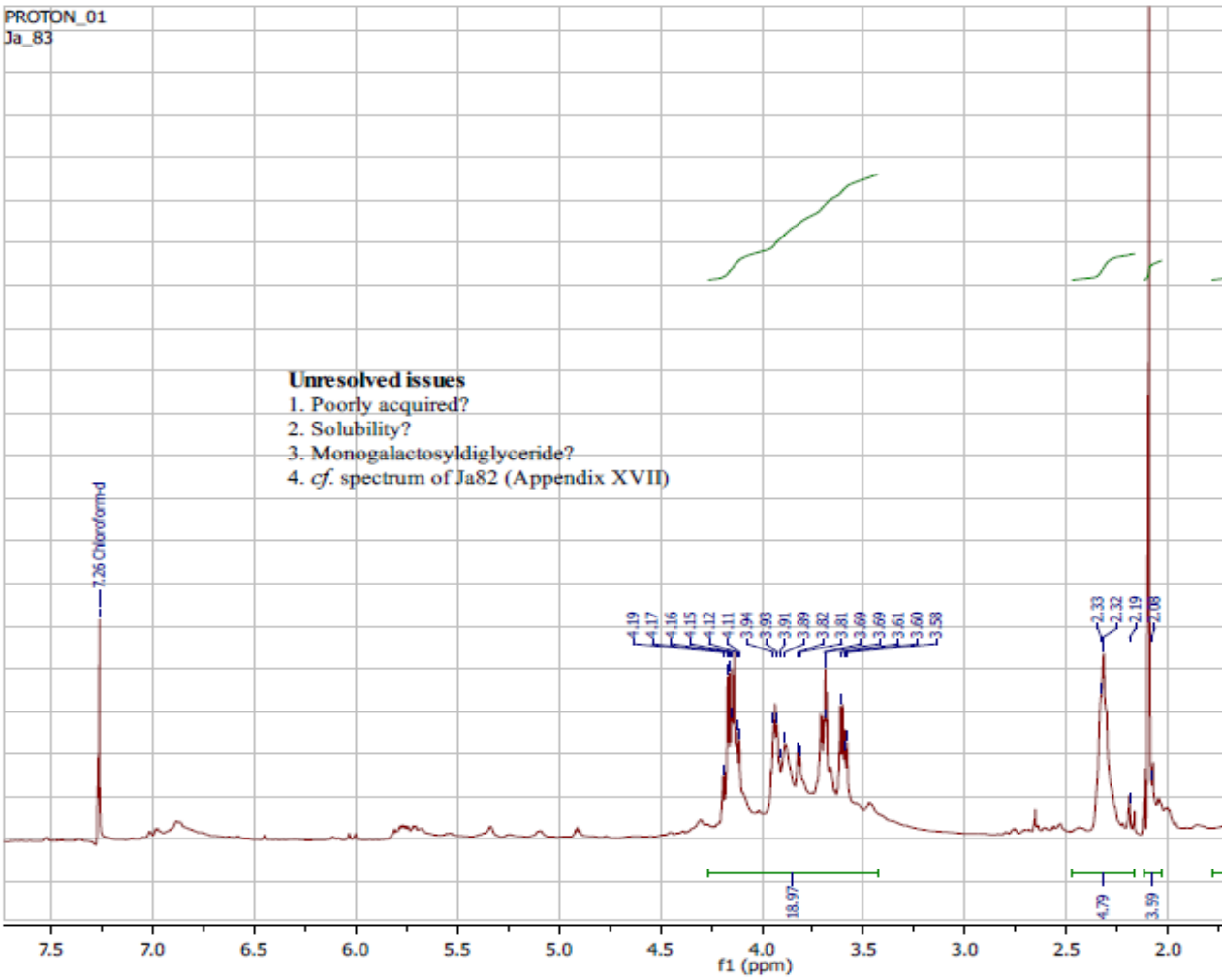
Appendix XV. [1]HNMR Spectrum of Ja80



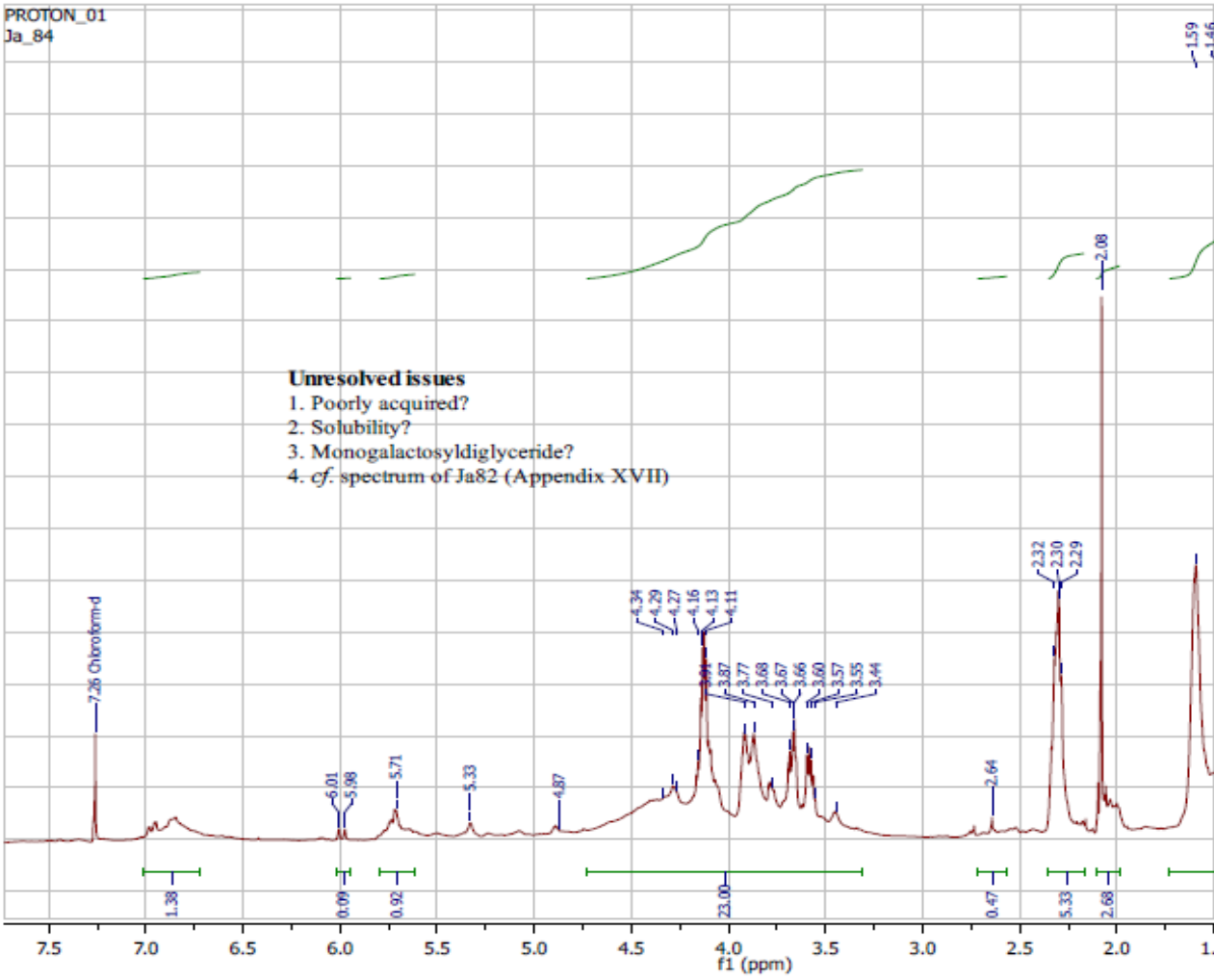
Appendix XVI. [1]HNMR Spectrum of Ja81



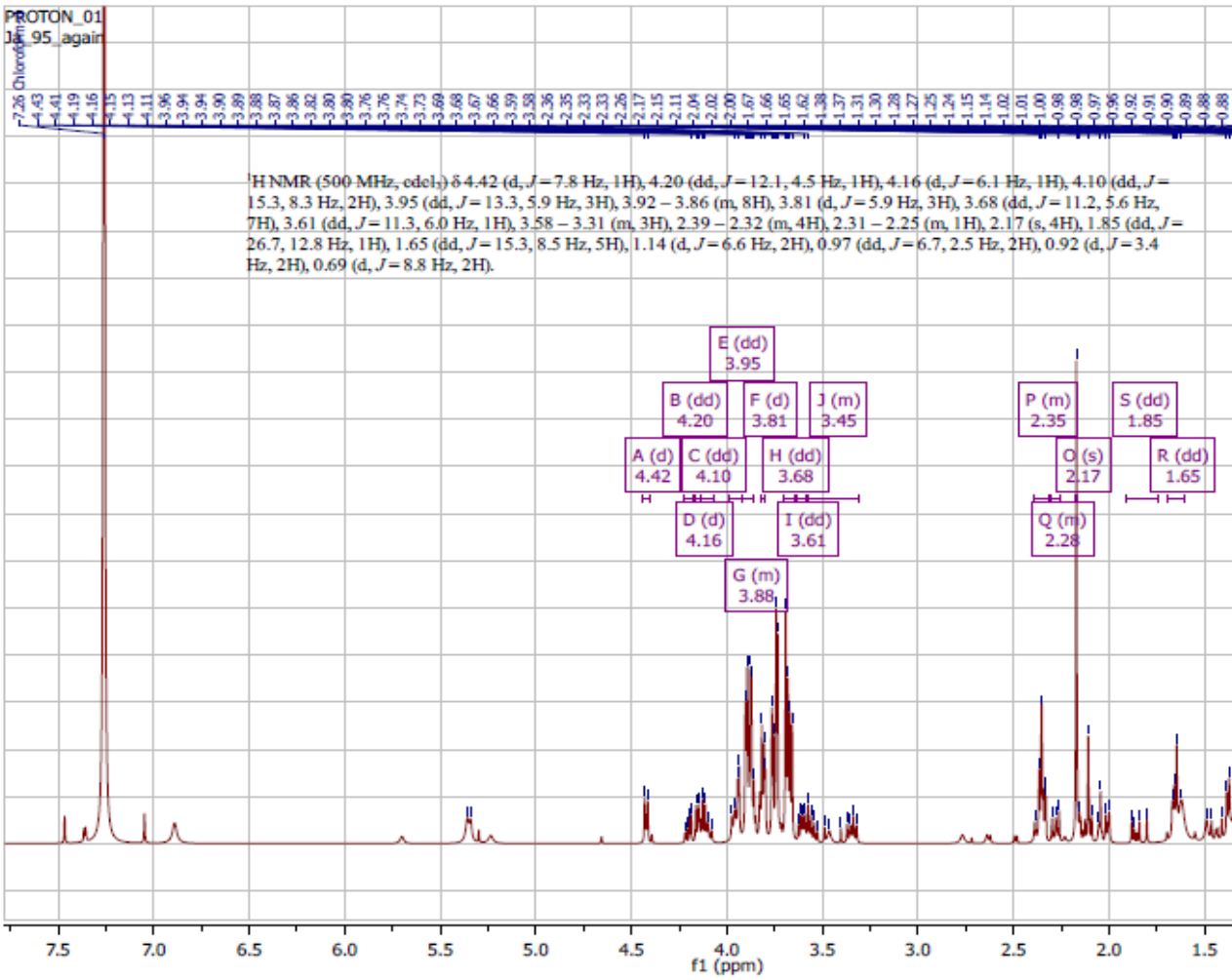
Appendix XVII. ^{1}HNMR Spectrum of Ja82



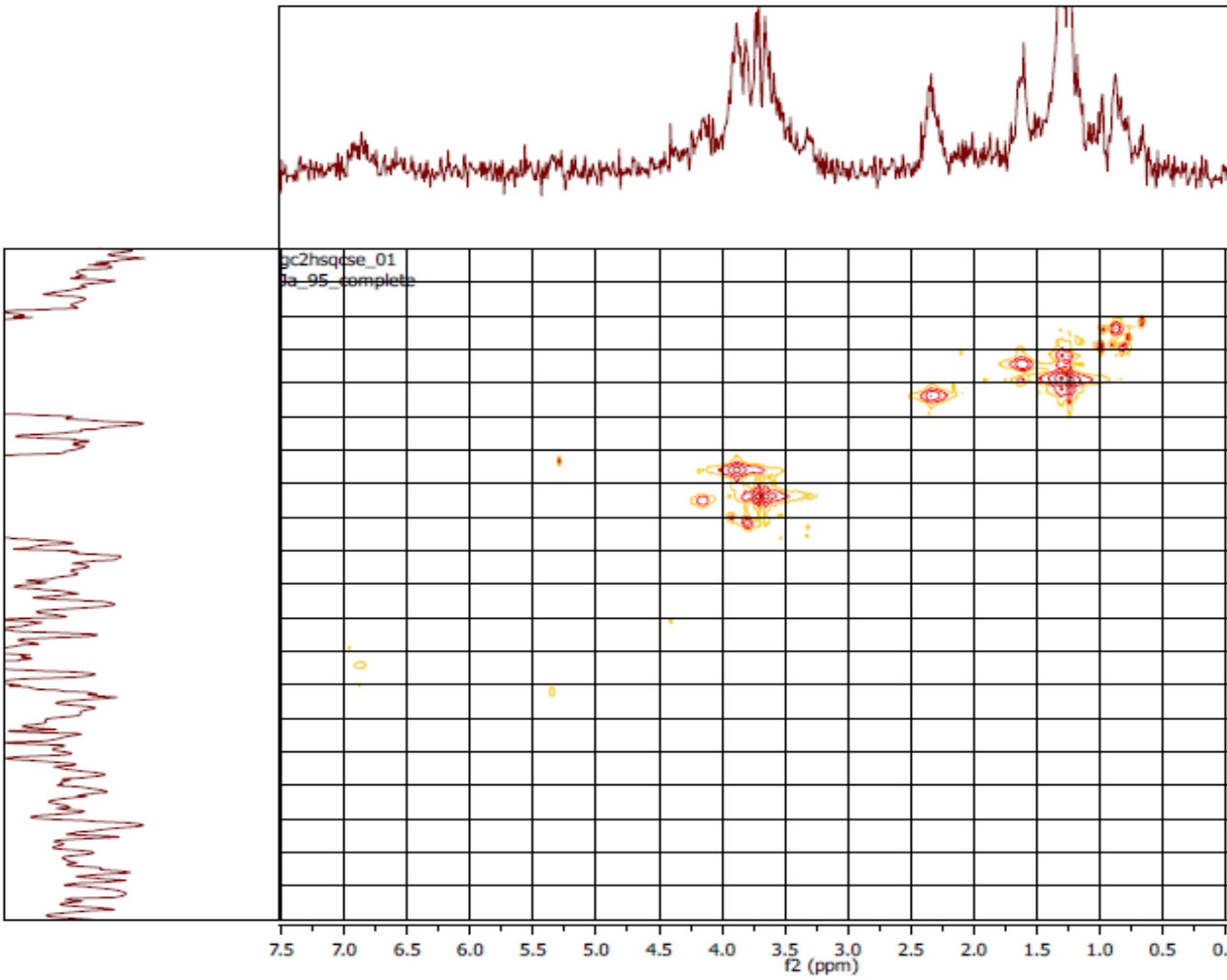
Appendix XVIII. ^{1}HNMR Spectrum of Ja83



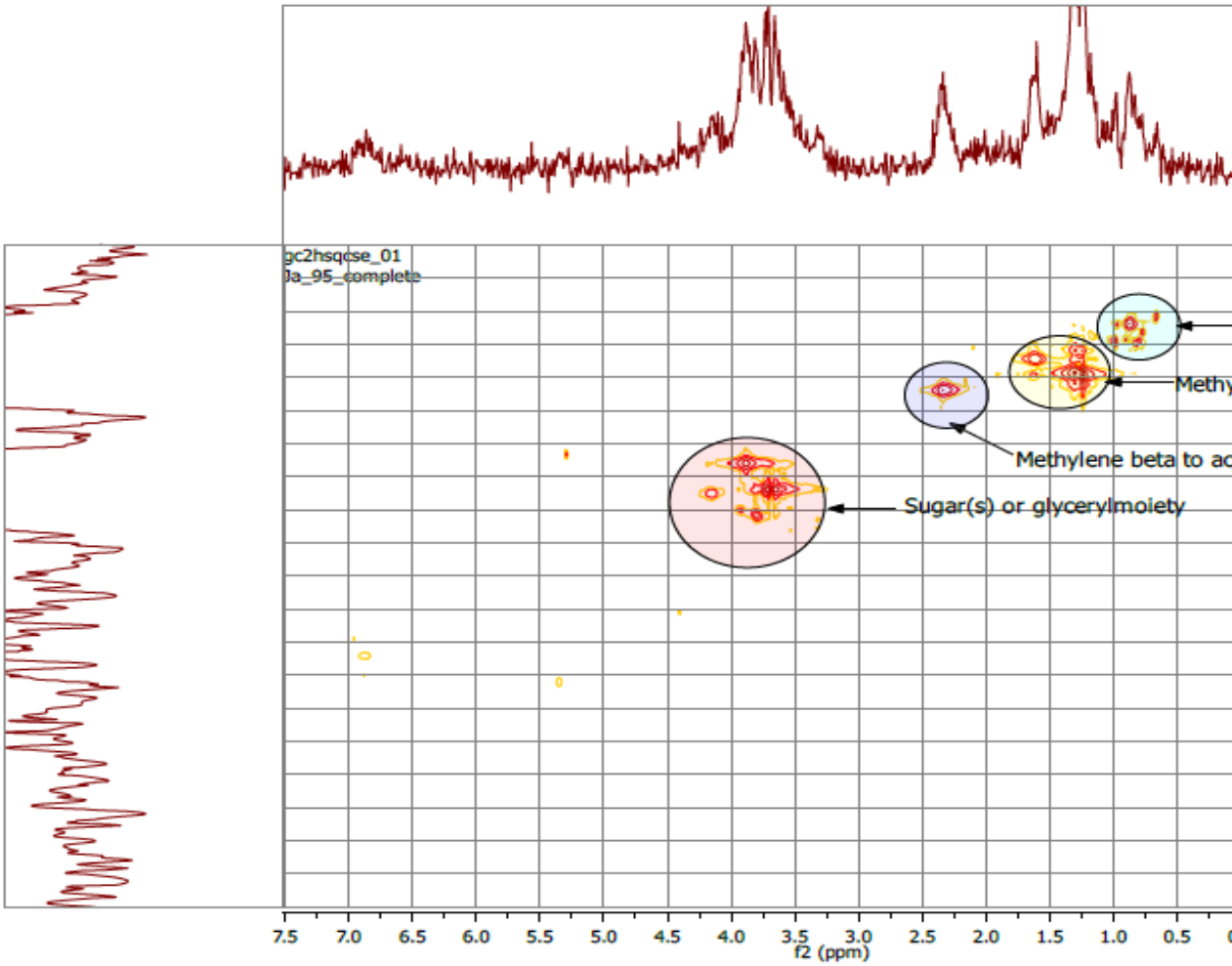
Appendix IXX. ^{1}HNMR Spectrum of Ja84



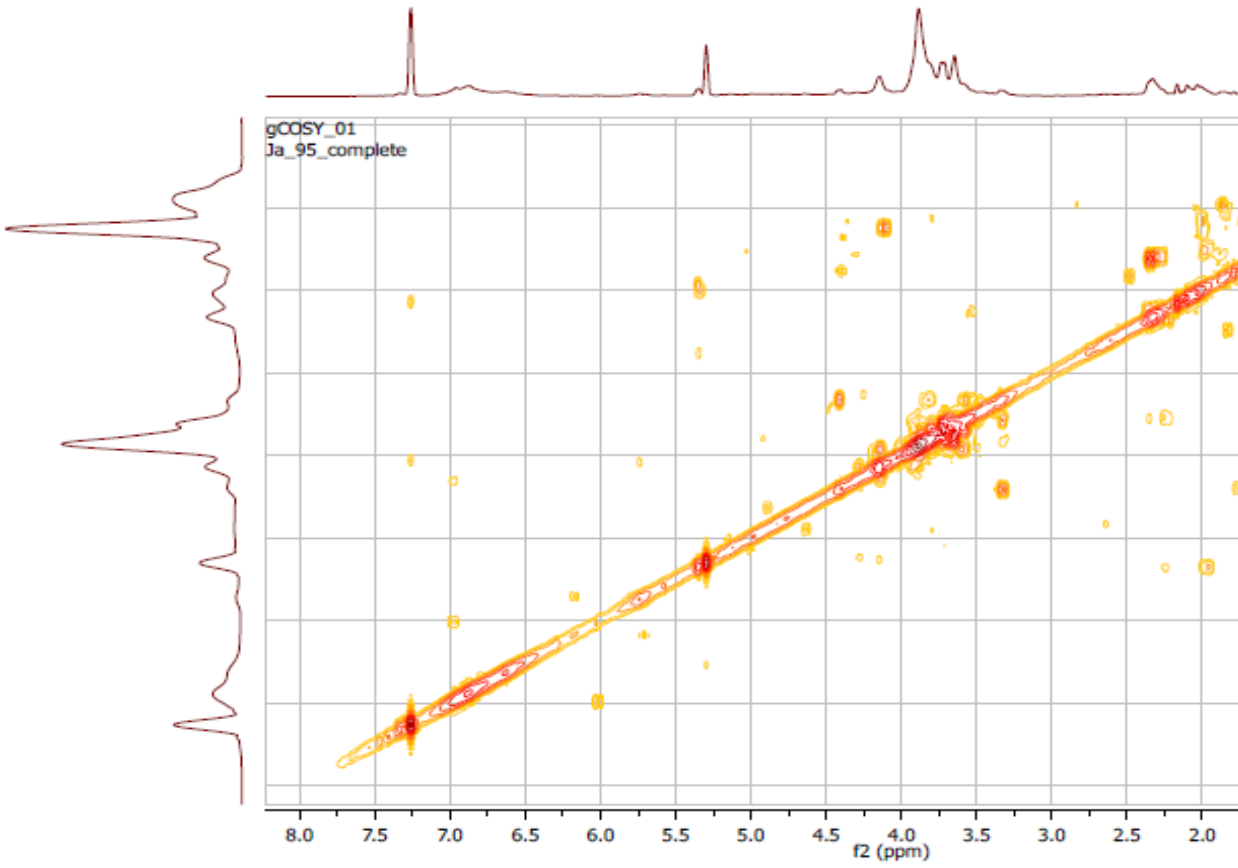
Appendix XX. ^{1}HNMR Spectrum of Ja95



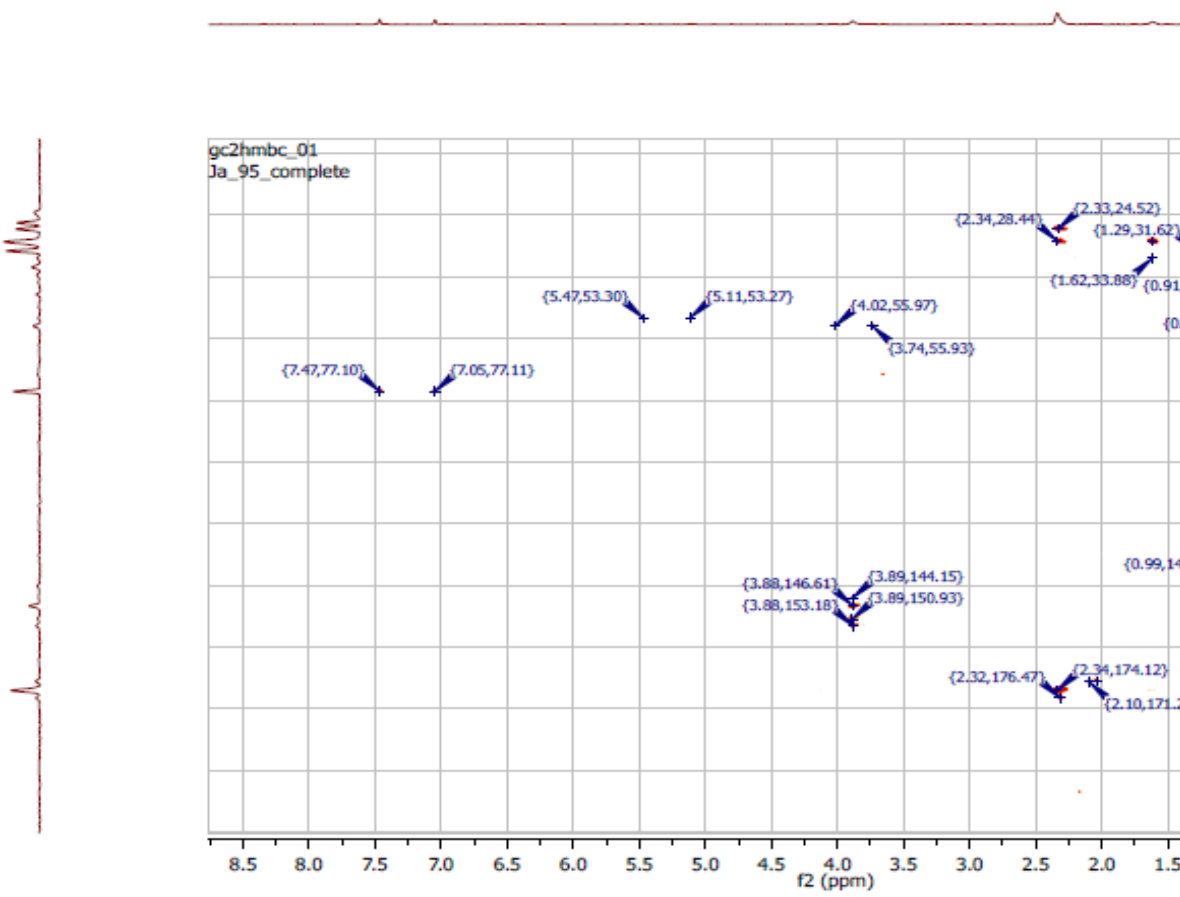
Appendix XXI. HSQC Spectrum of Ja95



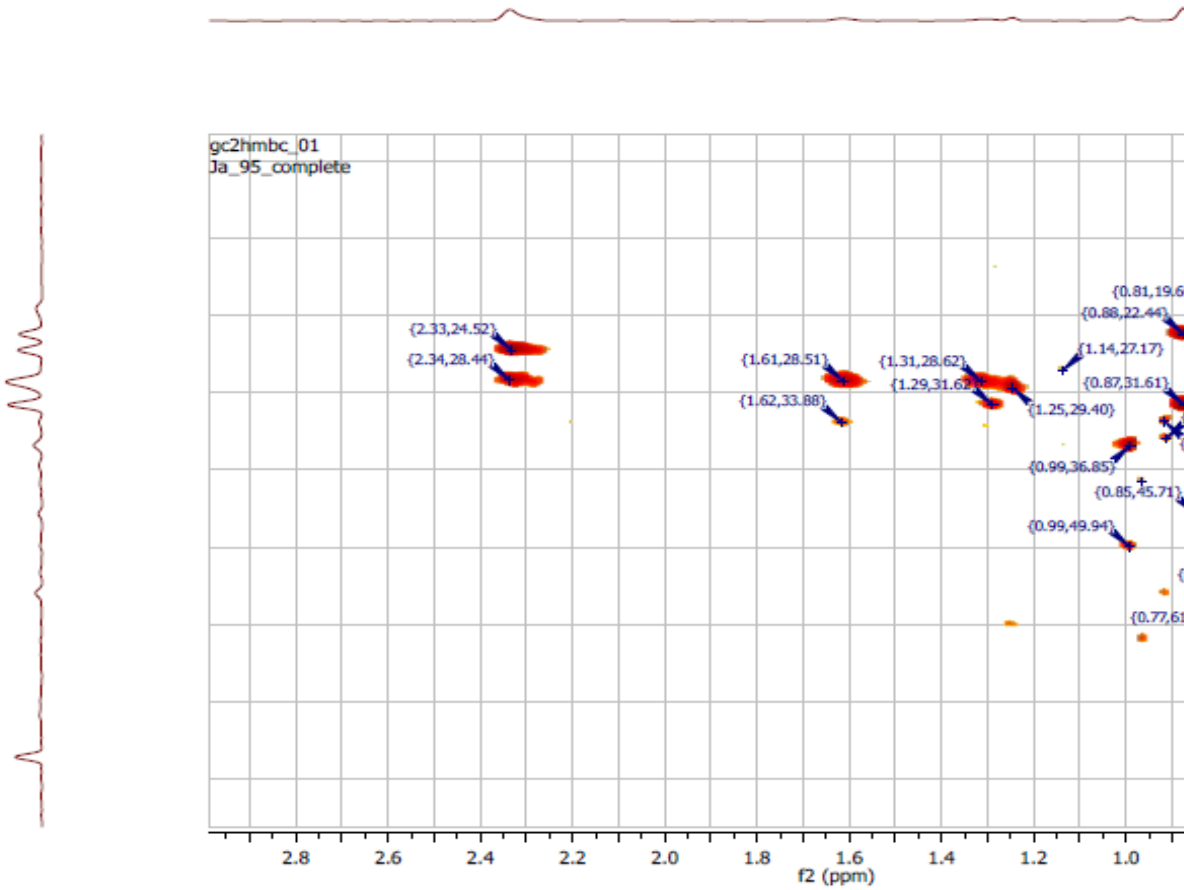
Appendix XXI. SOME RELEVANT EXPANSIONS-HSQC Spectrum of Ja95



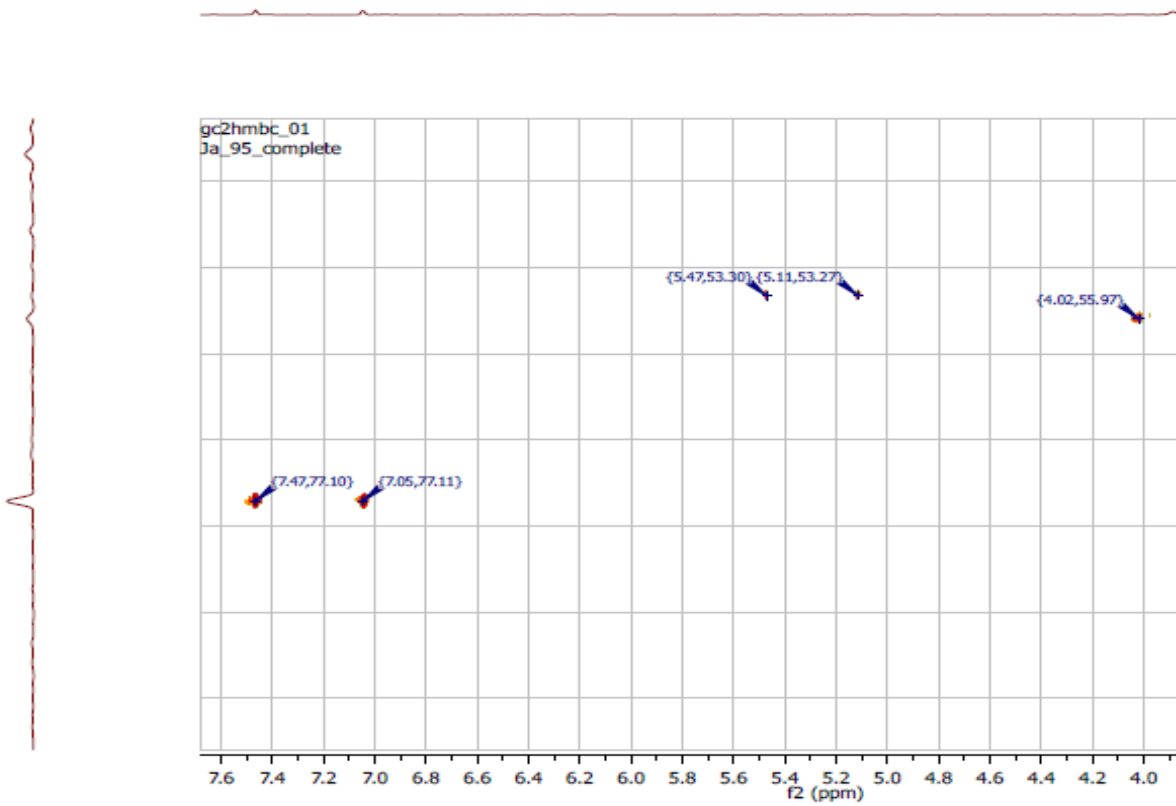
Appendix XXI. COSY NMR Spectrum of Ja95



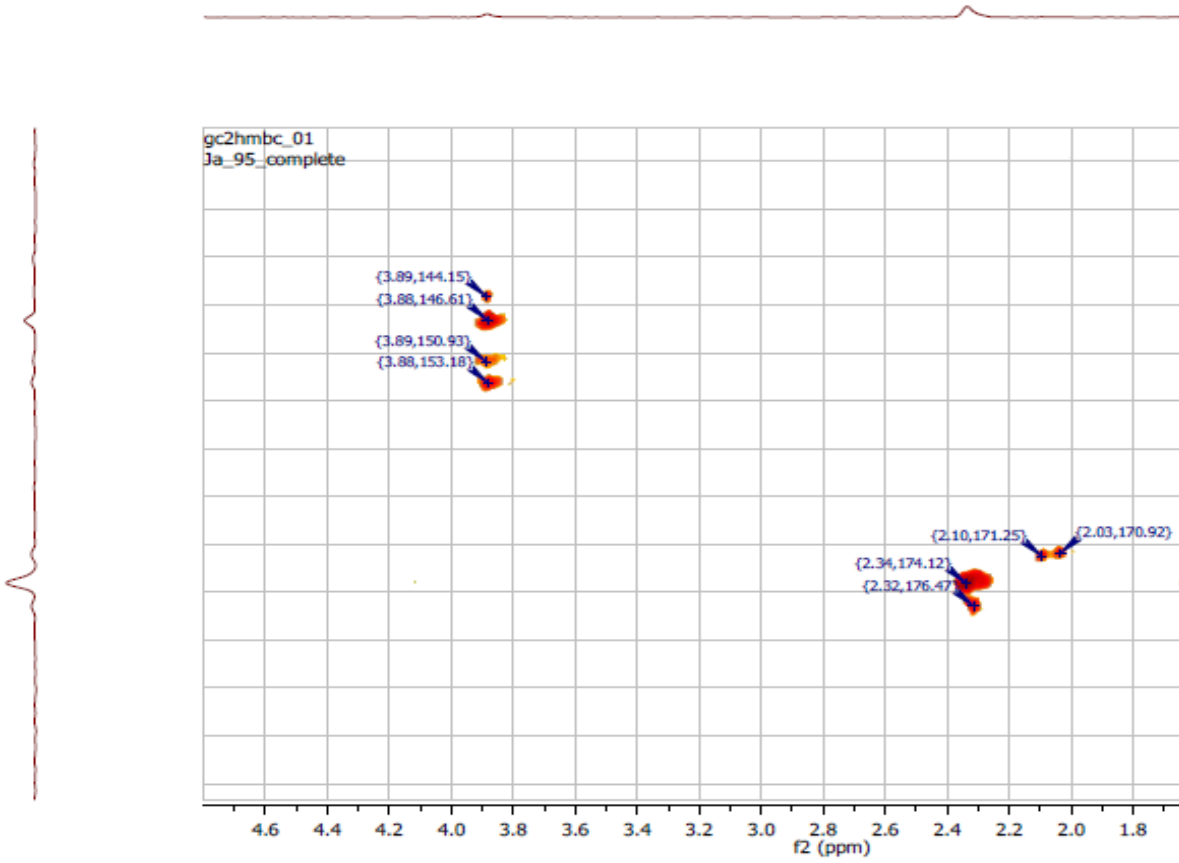
Appendix XXI. HMBC Spectrum of Ja95



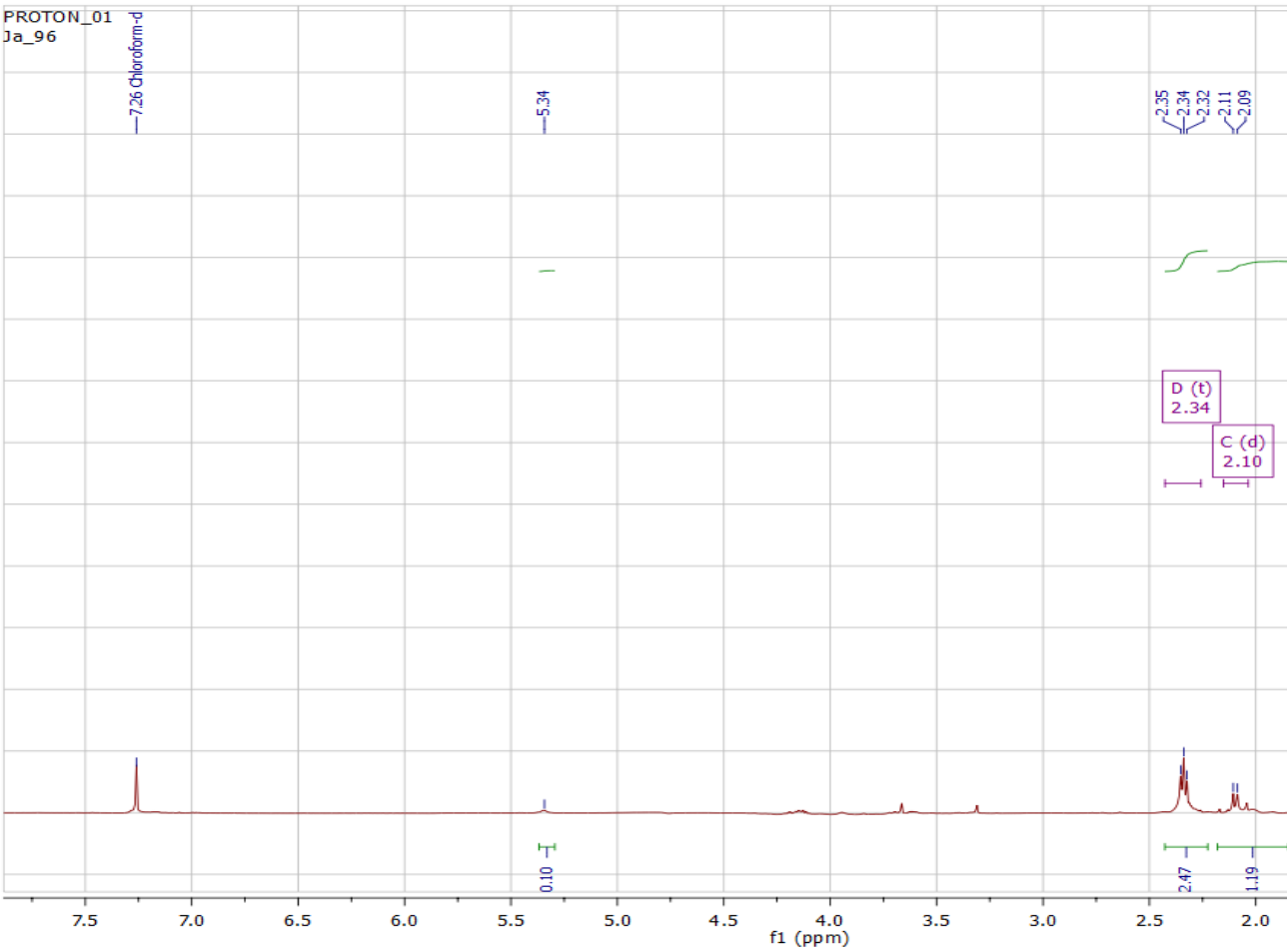
Appendix XXI-A. SOME RELEVANT EXPANSIONS-HMBC-Spectrum of Ja95



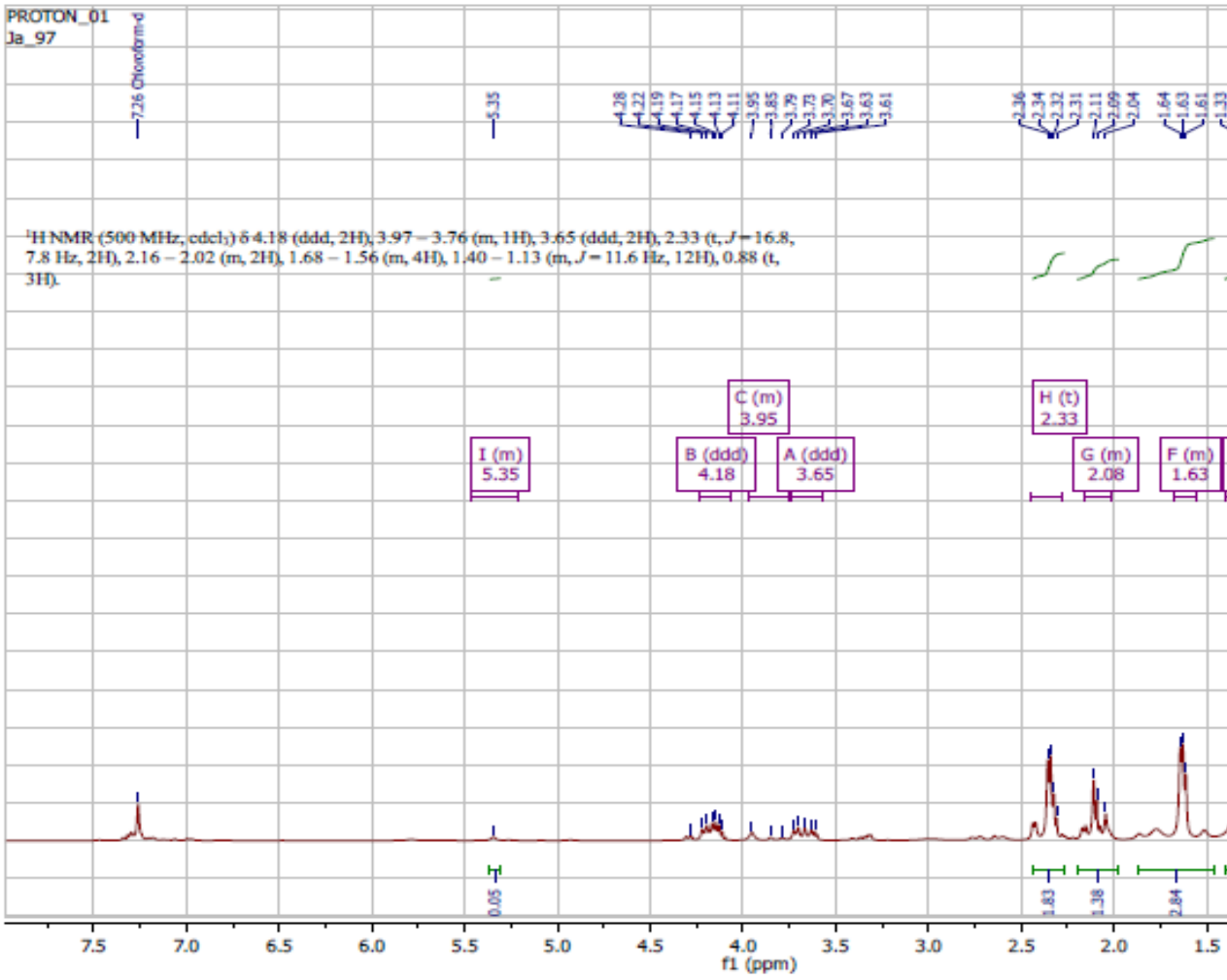
Appendix XXI-B. SOME RELEVANT EXPANSIONS HMBC Spectrum of Ja95



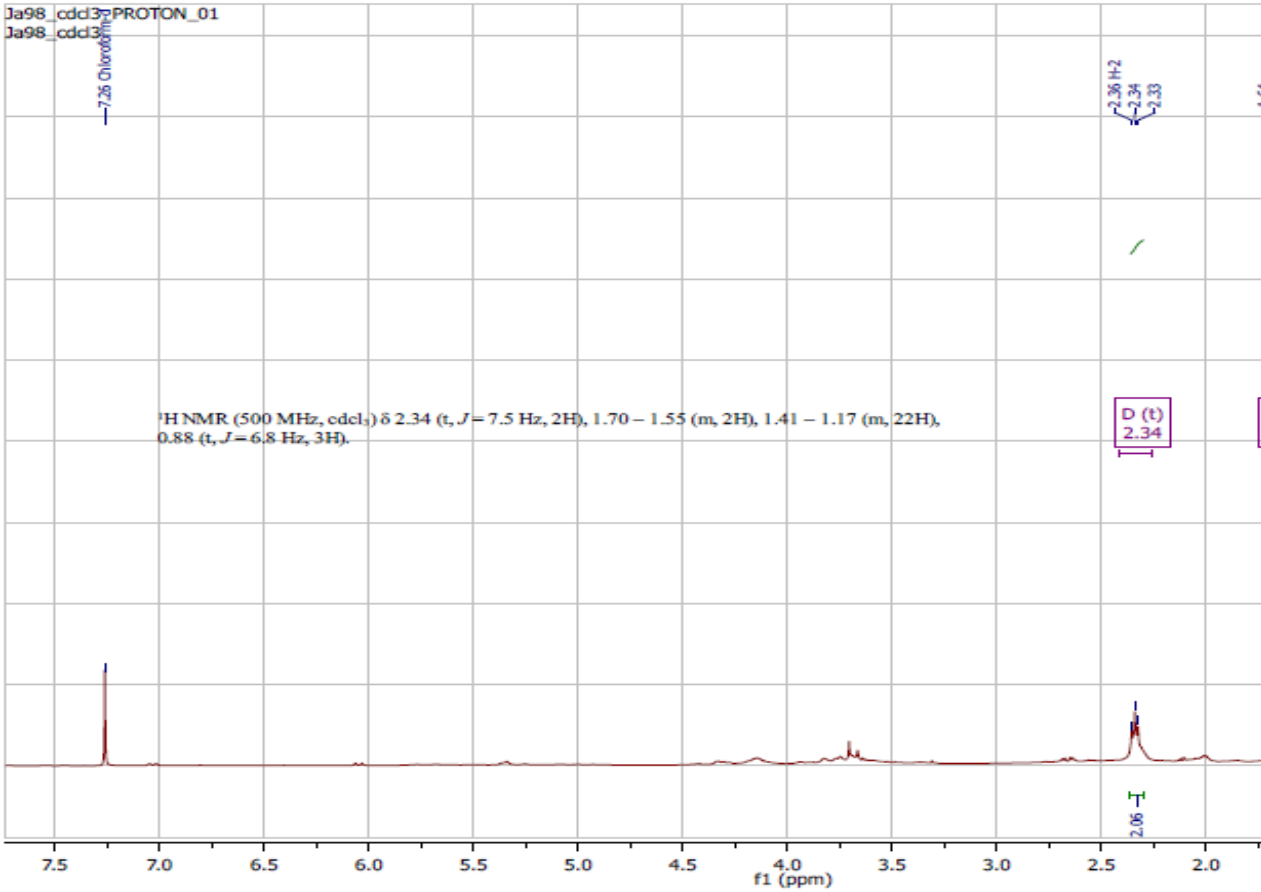
Appendix XXI-C. SOME RELEVANT EXPANSIONS HMBC Spectrum of Ja95



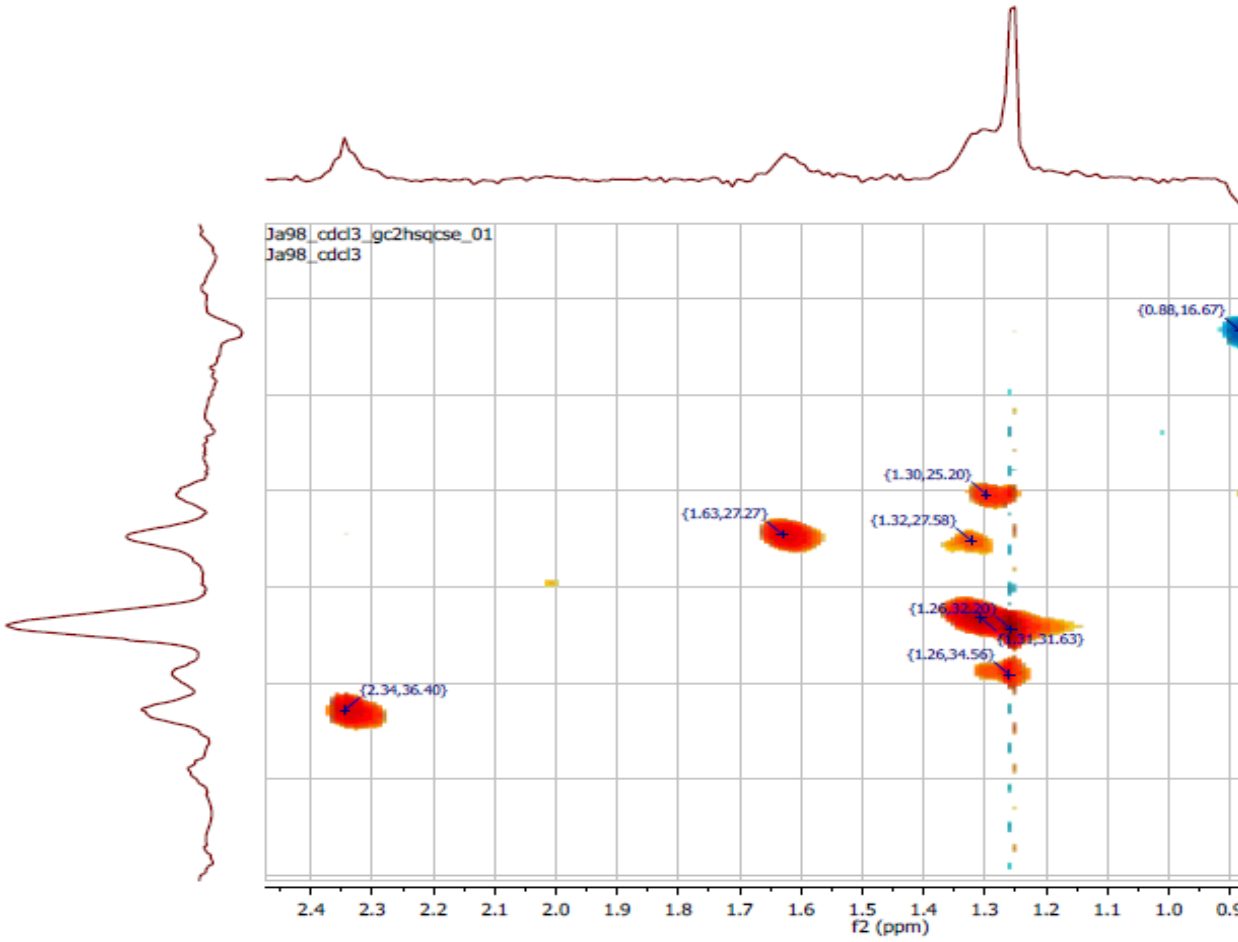
Appendix XXII. [1]HNMR Spectrum of Ja96



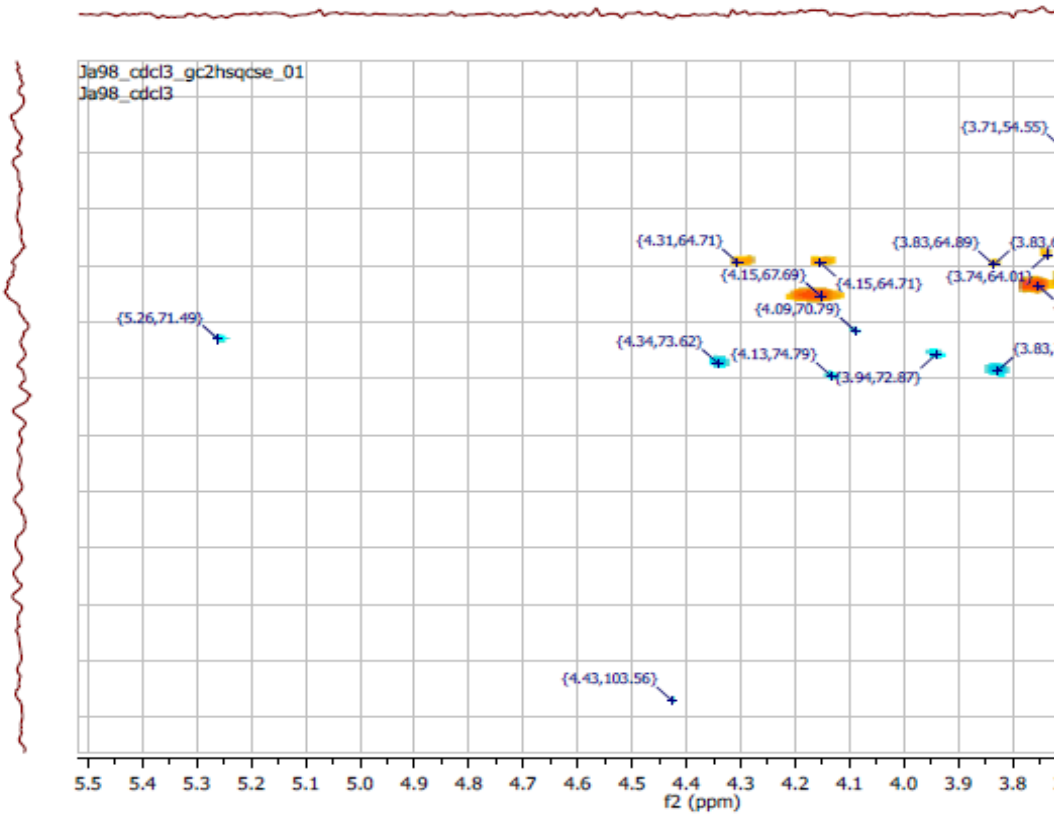
Appendix XXII2. [1]HNMR Spectrum of Ja97



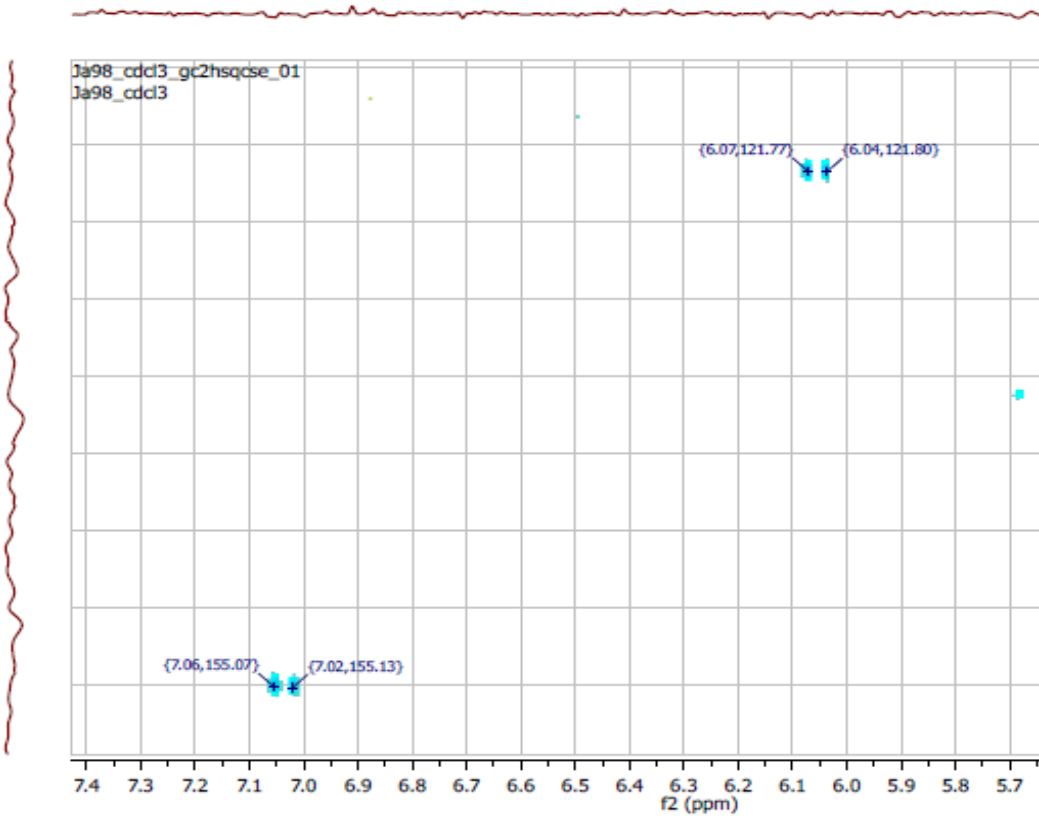
Appendix XXIII. 1HNMR Spectrum of Ja98



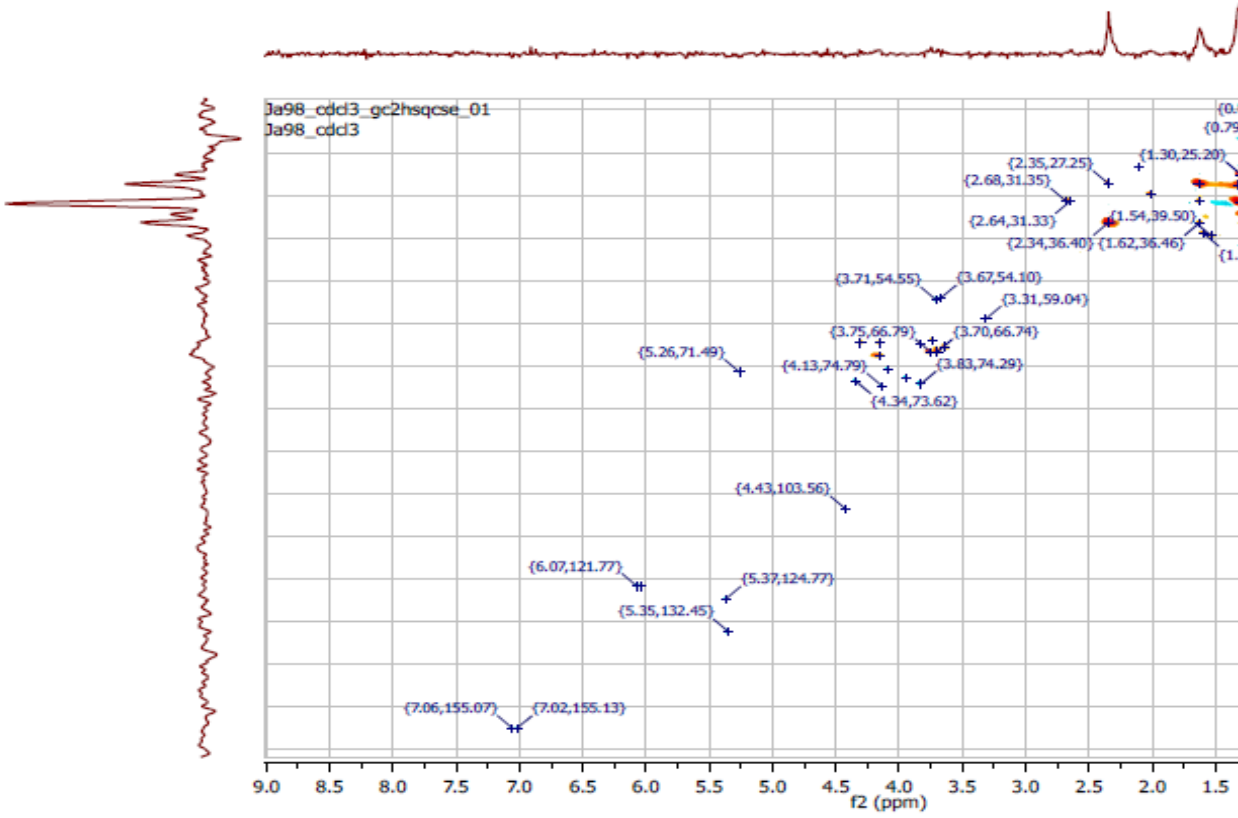
Appendix XXIII-A. SOME RELEVANT EXPANSIONS-HSQC Spectrum of Ja98



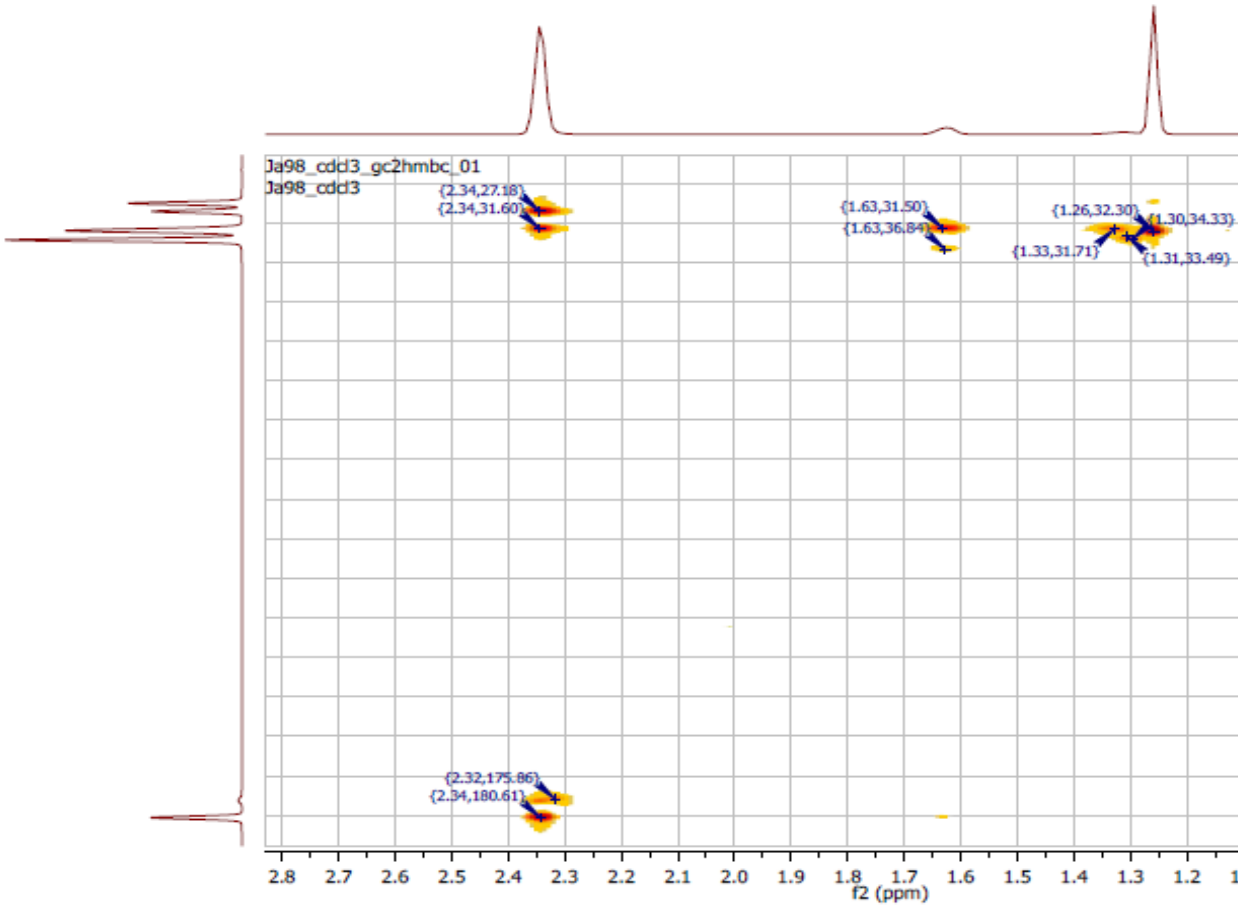
Appendix XXIII-A1. SOME RELEVANT EXPANSIONS-HSQC Spectrum of Ja98



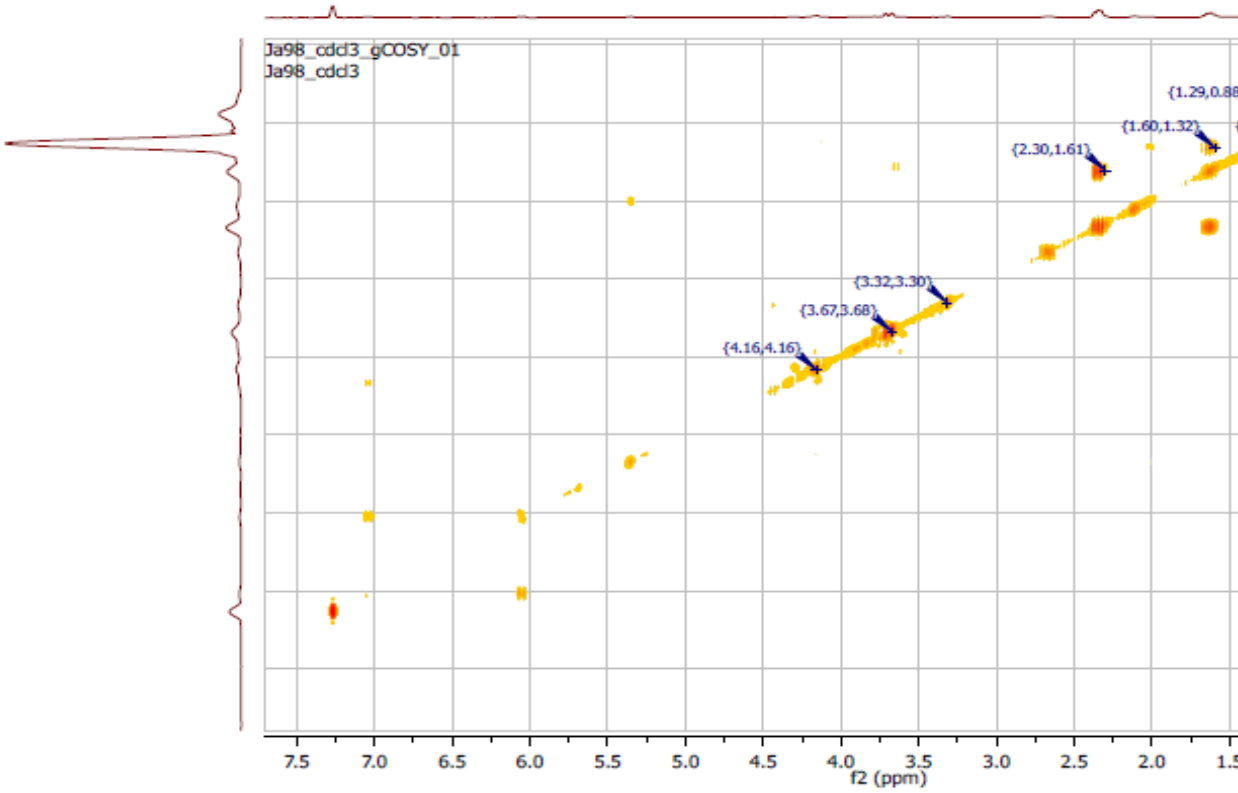
Appendix XXIII-A2. SOME RELEVANT EXPANSIONS-HSQC Spectrum of Ja98



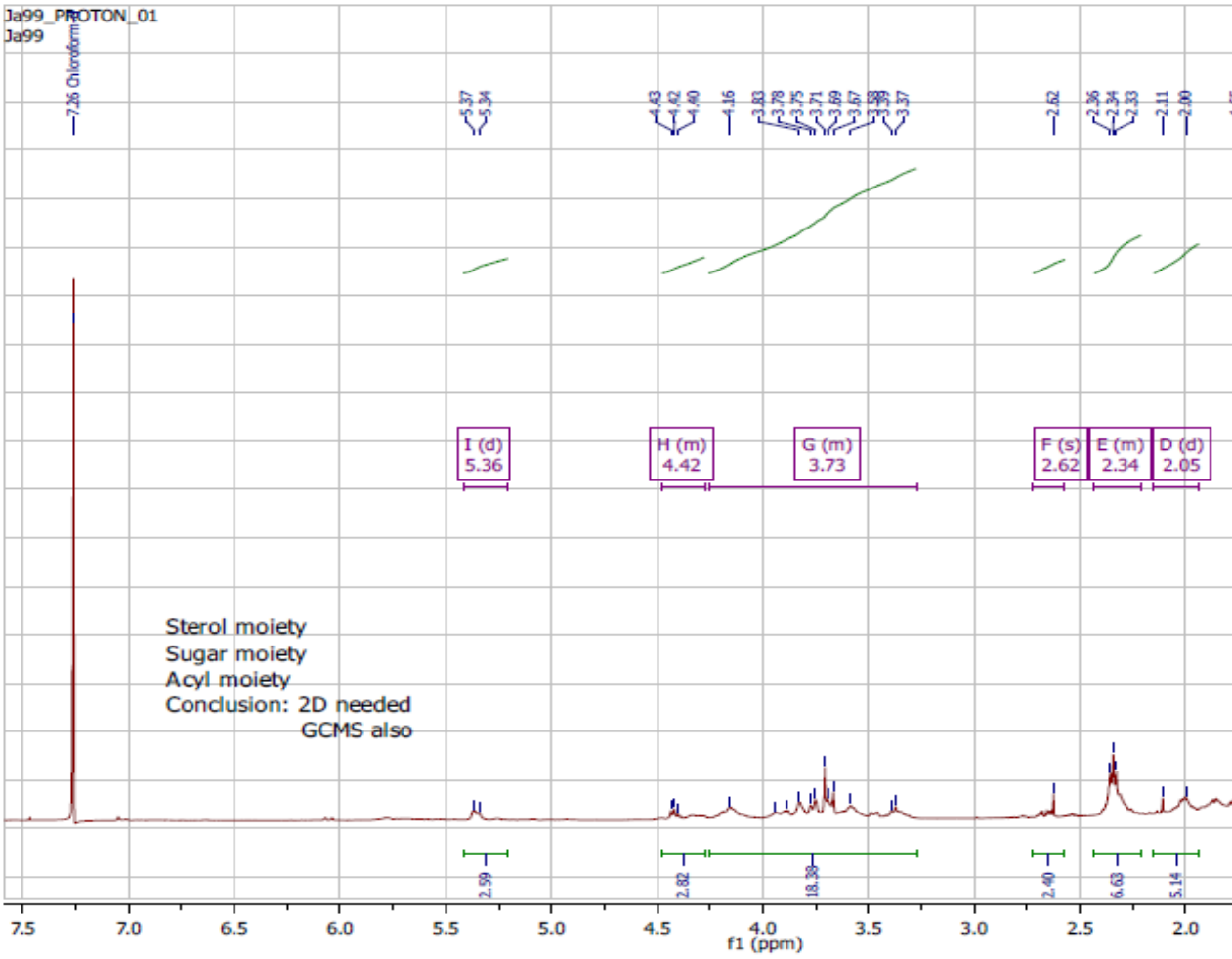
Appendix XXIII-B. HSQC Spectrum of Ja98



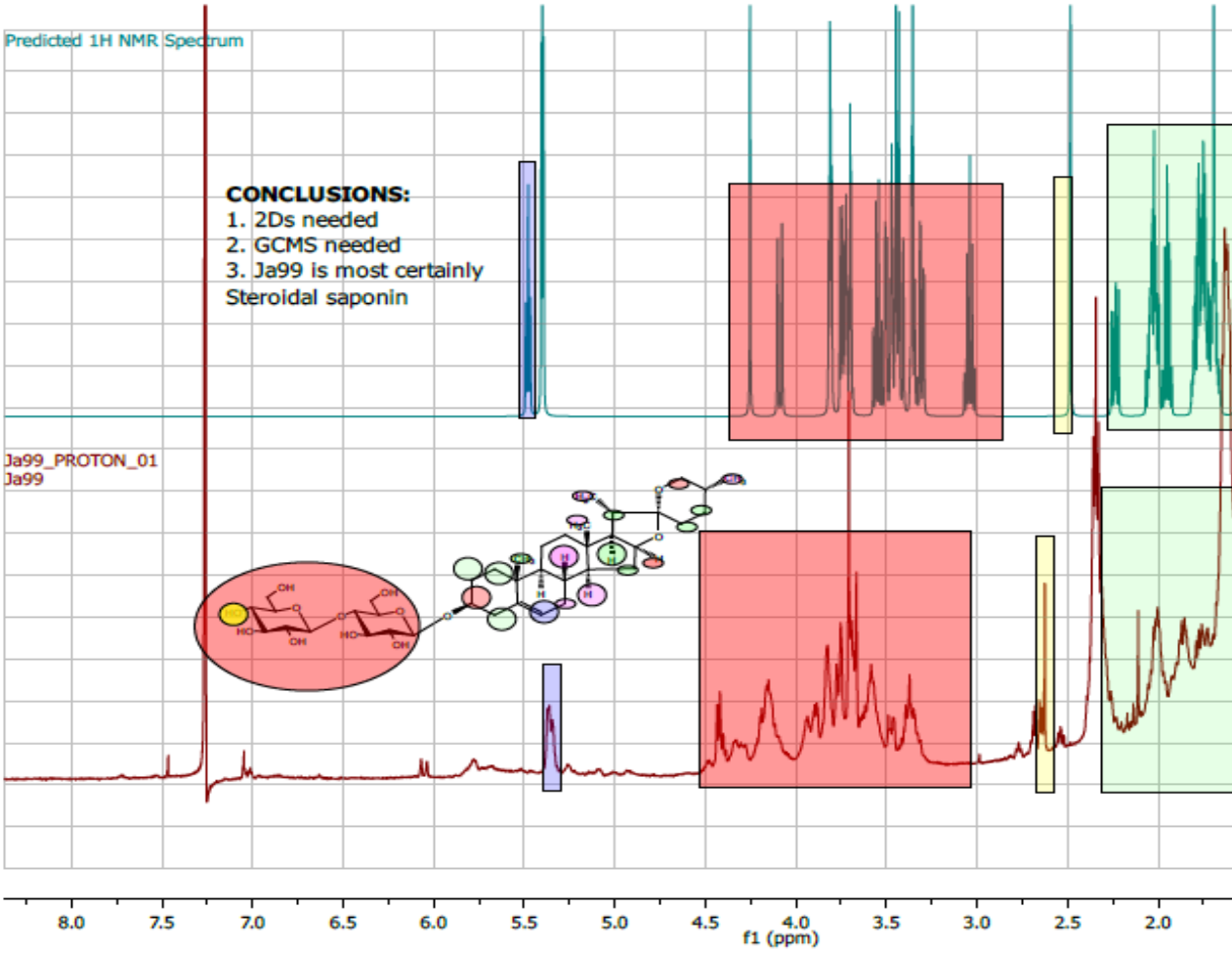
Appendix XXIII-C. HMBC Spectrum of Ja98



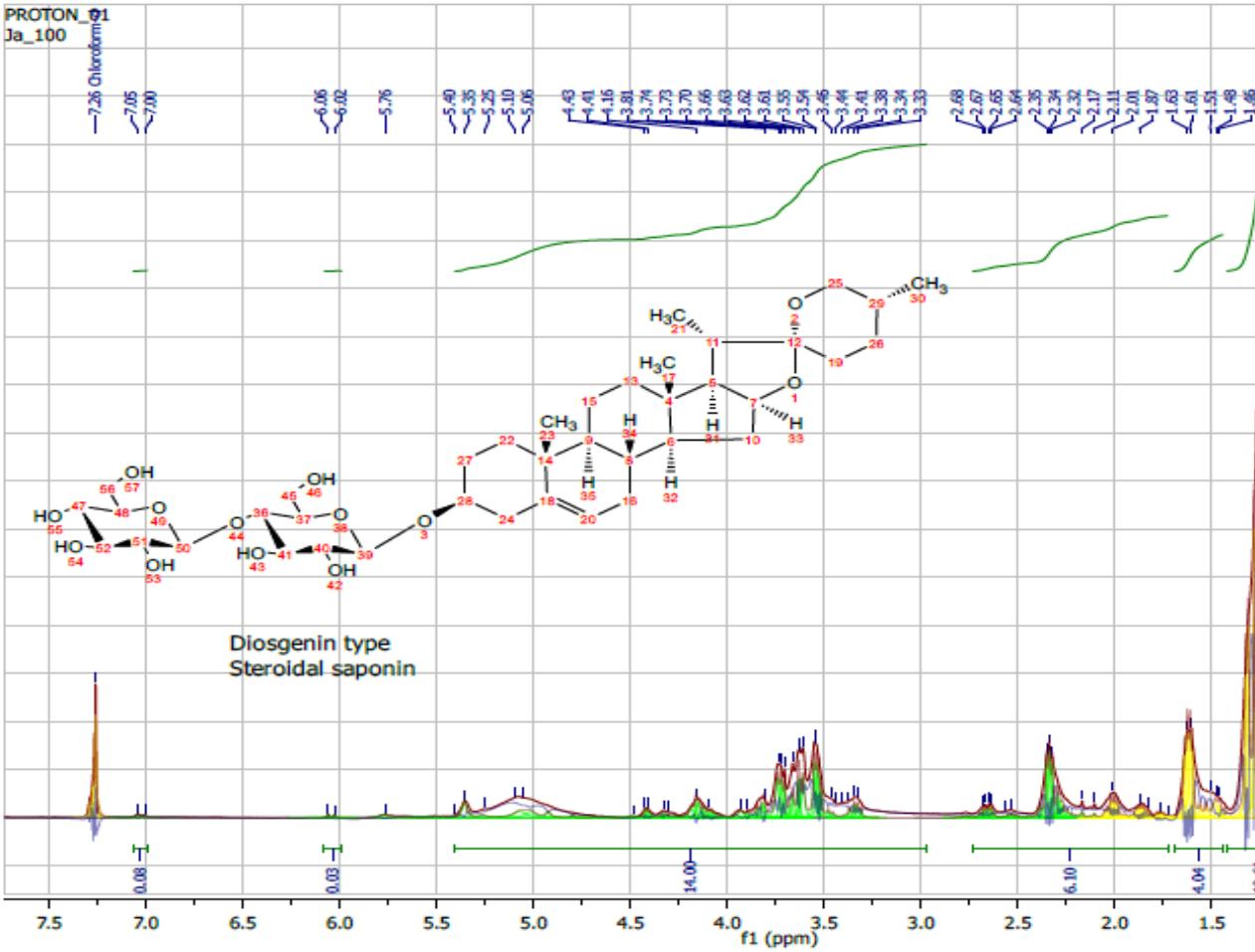
Appendix XXIII-D. COSY Spectrum of Ja98



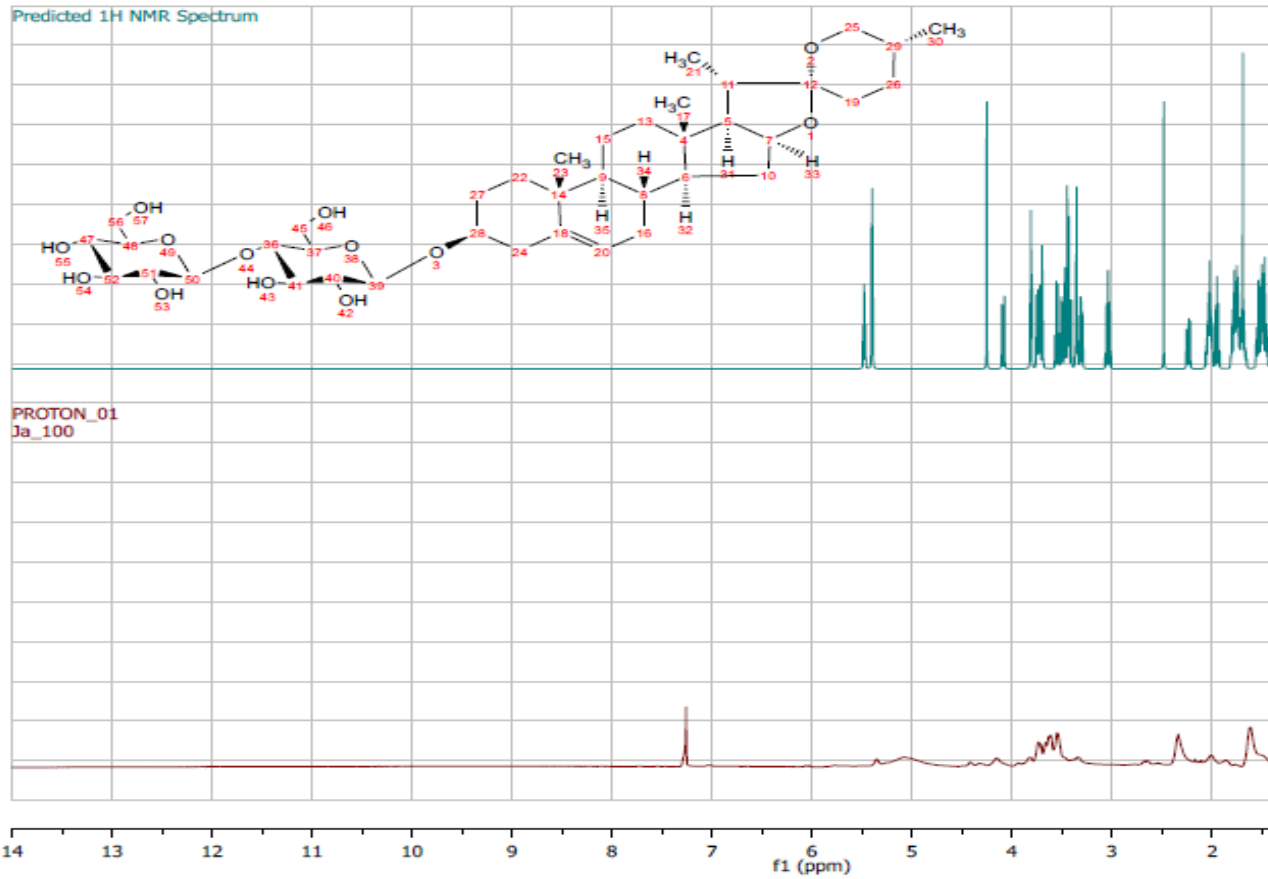
Appendix XXIV. [1]HNMR Spectrum of Ja99



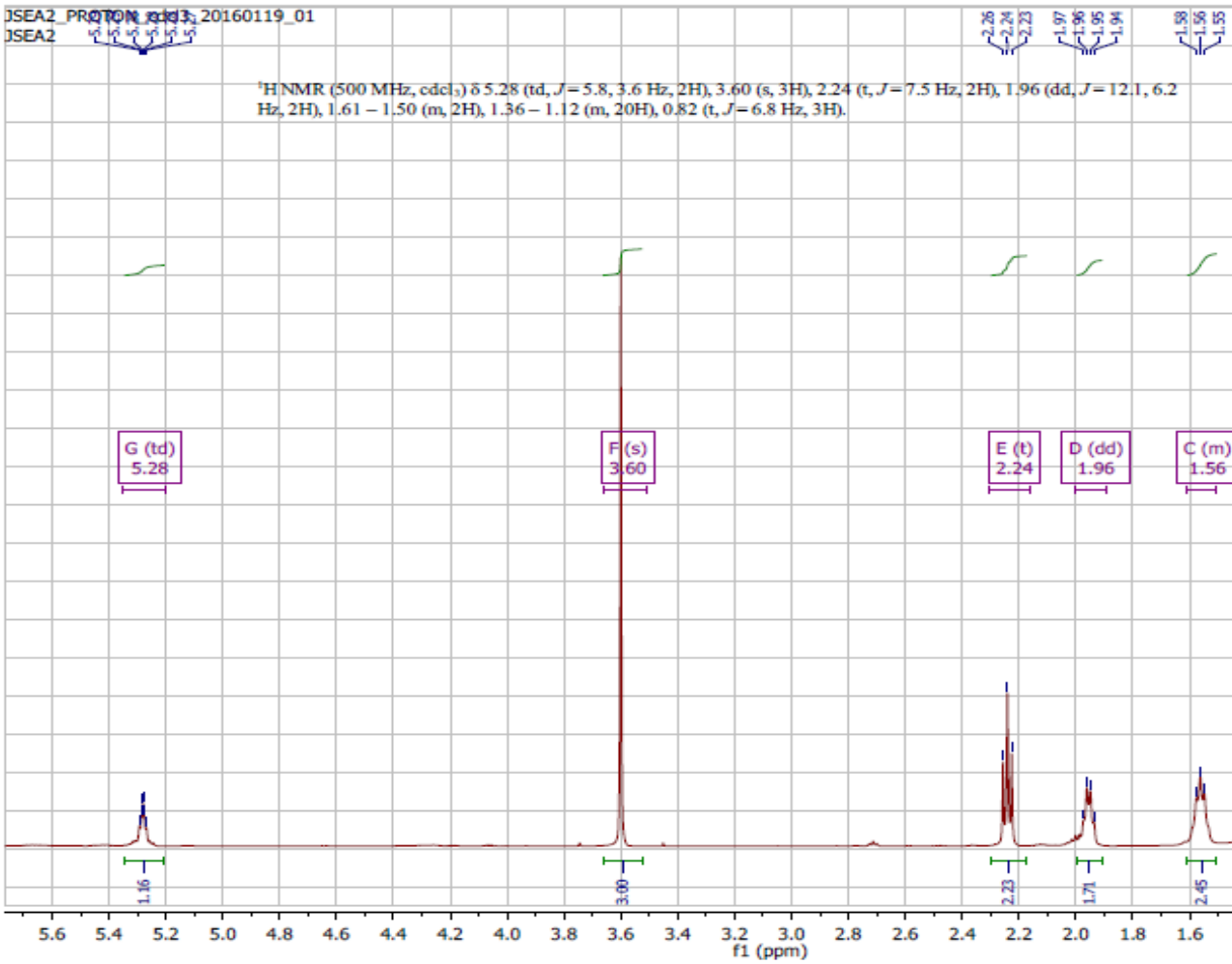
Appendix XXIV-A. STEROIDAL SAPONIN PREDICTION ^{1}HNMR Spectrum of Ja99



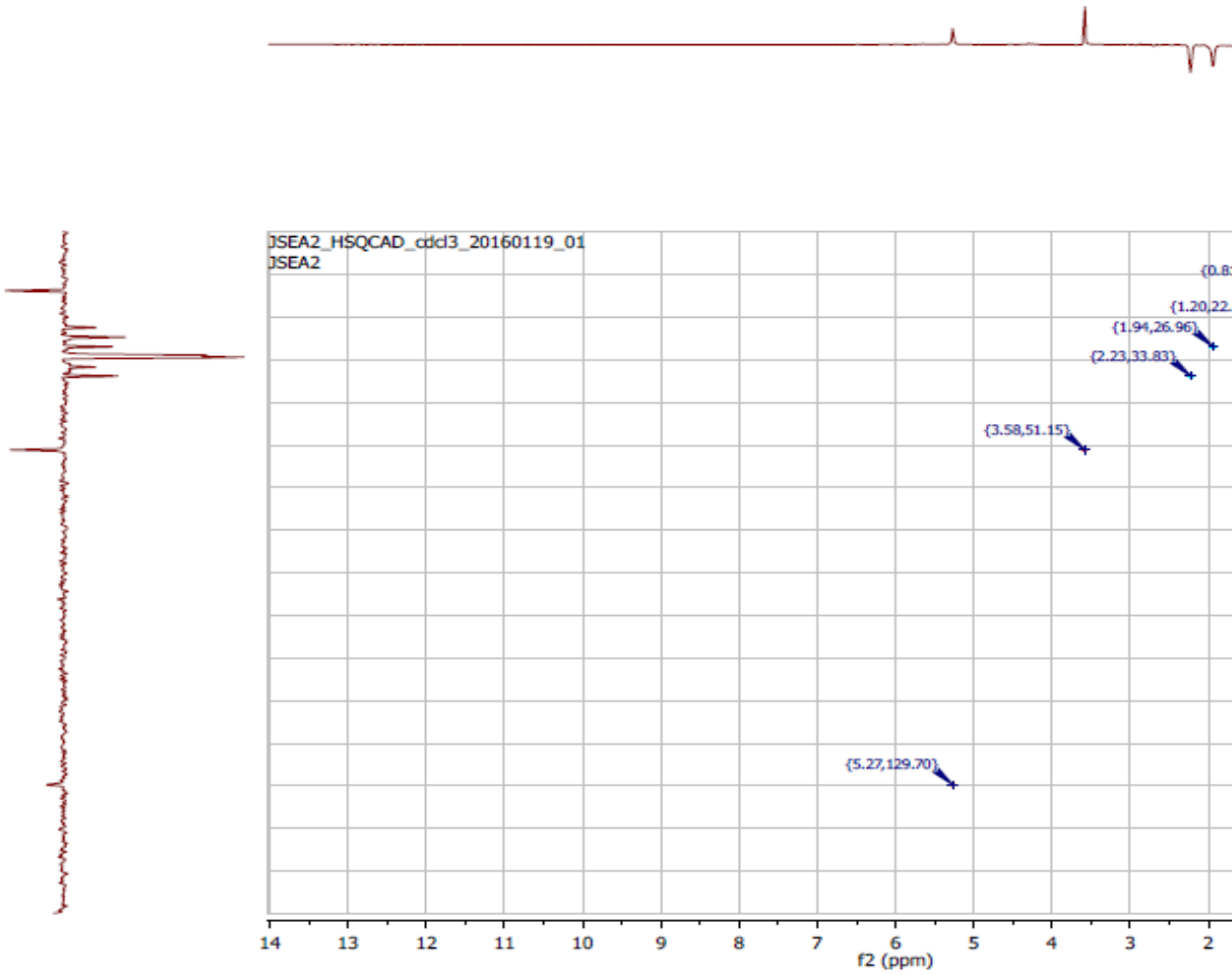
Appendix XXV. STACKED STEROIDAL SAPONIN PREDICTION ^{1}HNMR Spectrum of Ja100



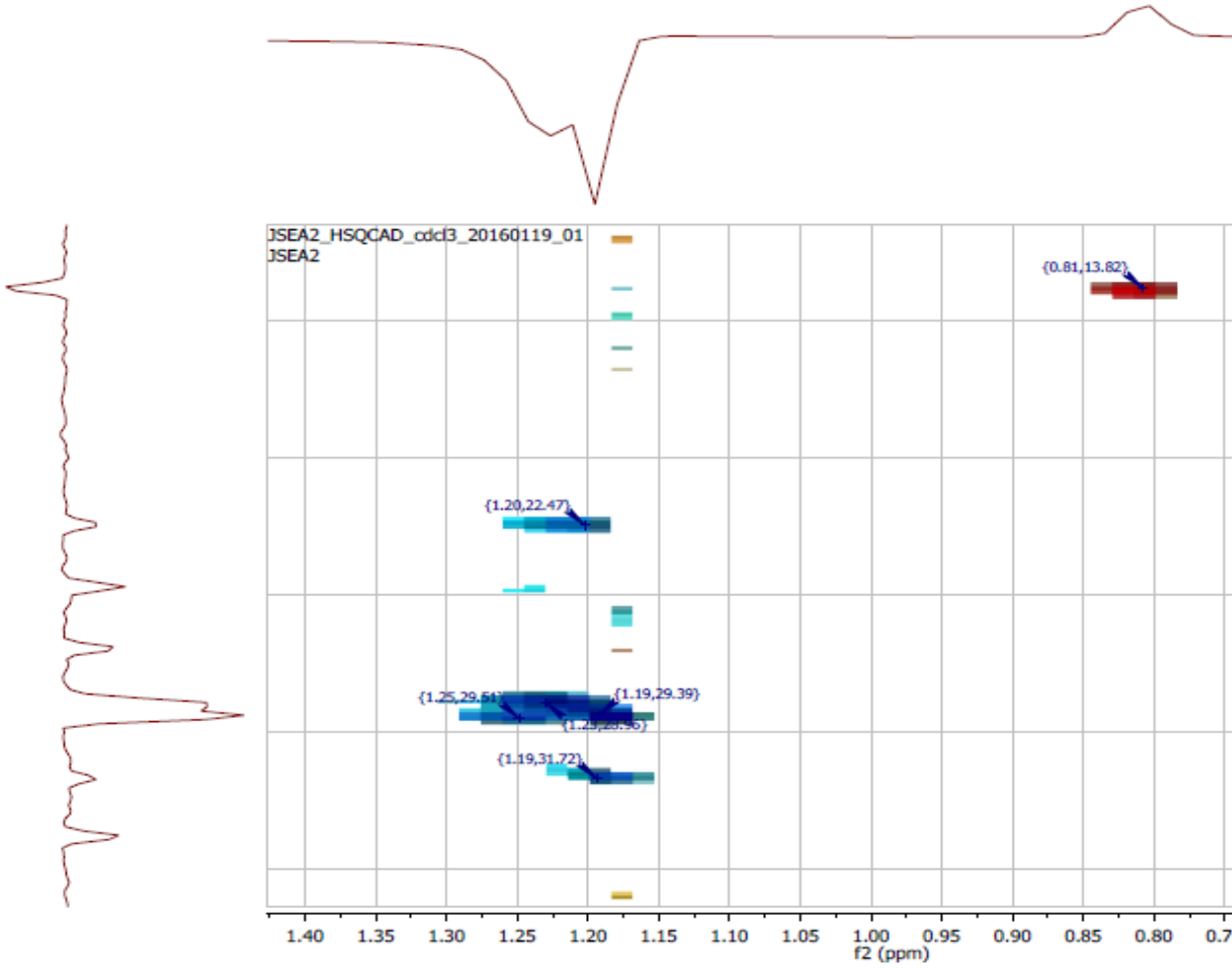
Appendix XXV-A. STACKED STEROIDAL SAPONIN PREDICTION ^{1}HNMR Spectrum of Ja100



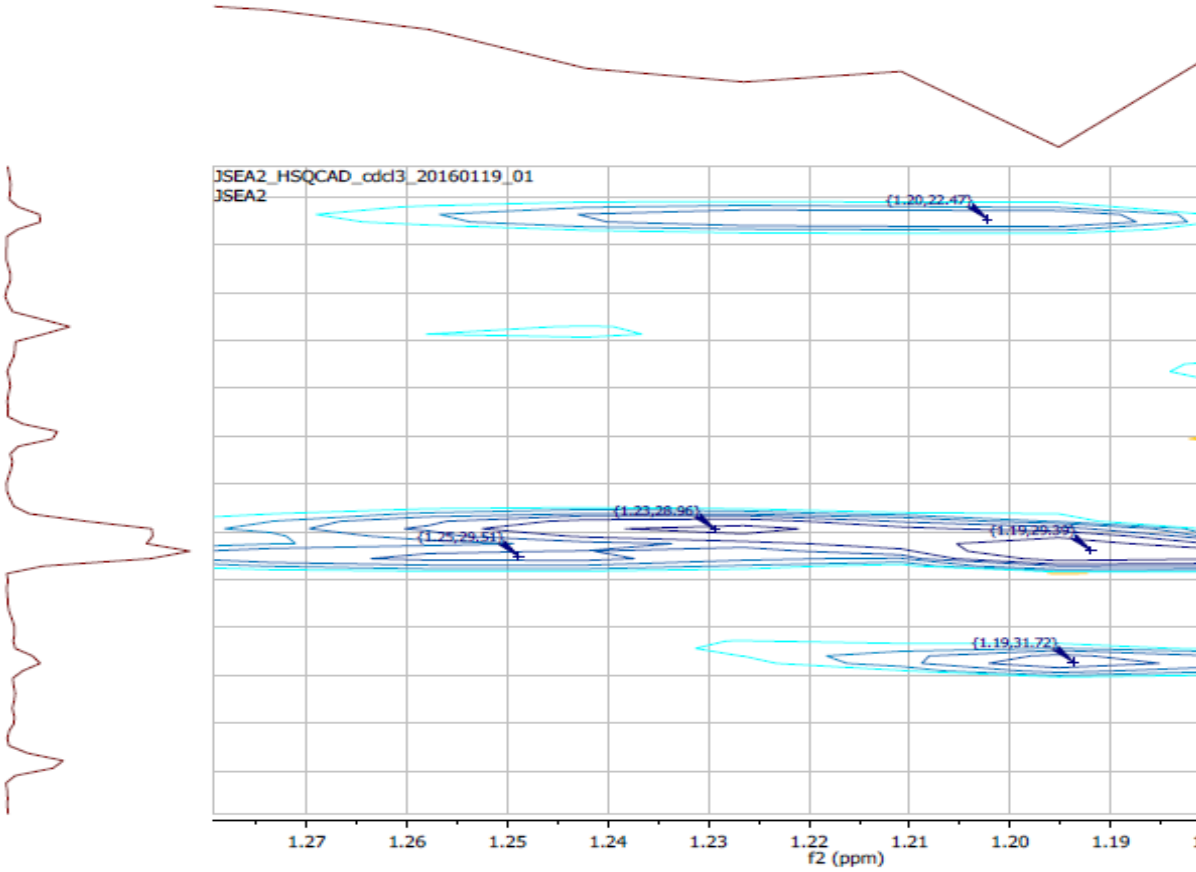
Appendix XXVI. ^{1}HNMR Spectrum of JSEA2



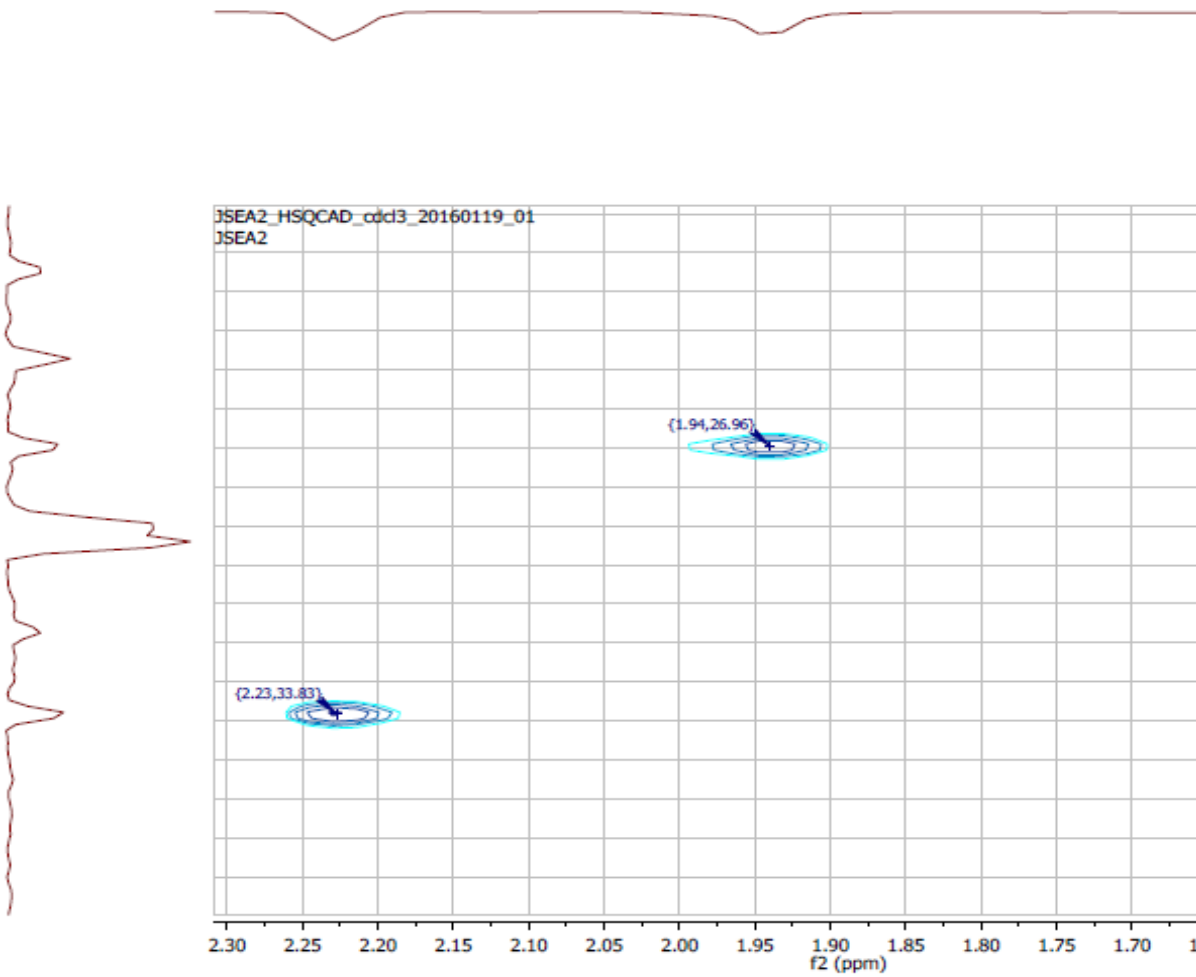
Appendix XXVI-A. HSQC Spectrum of JSEA2



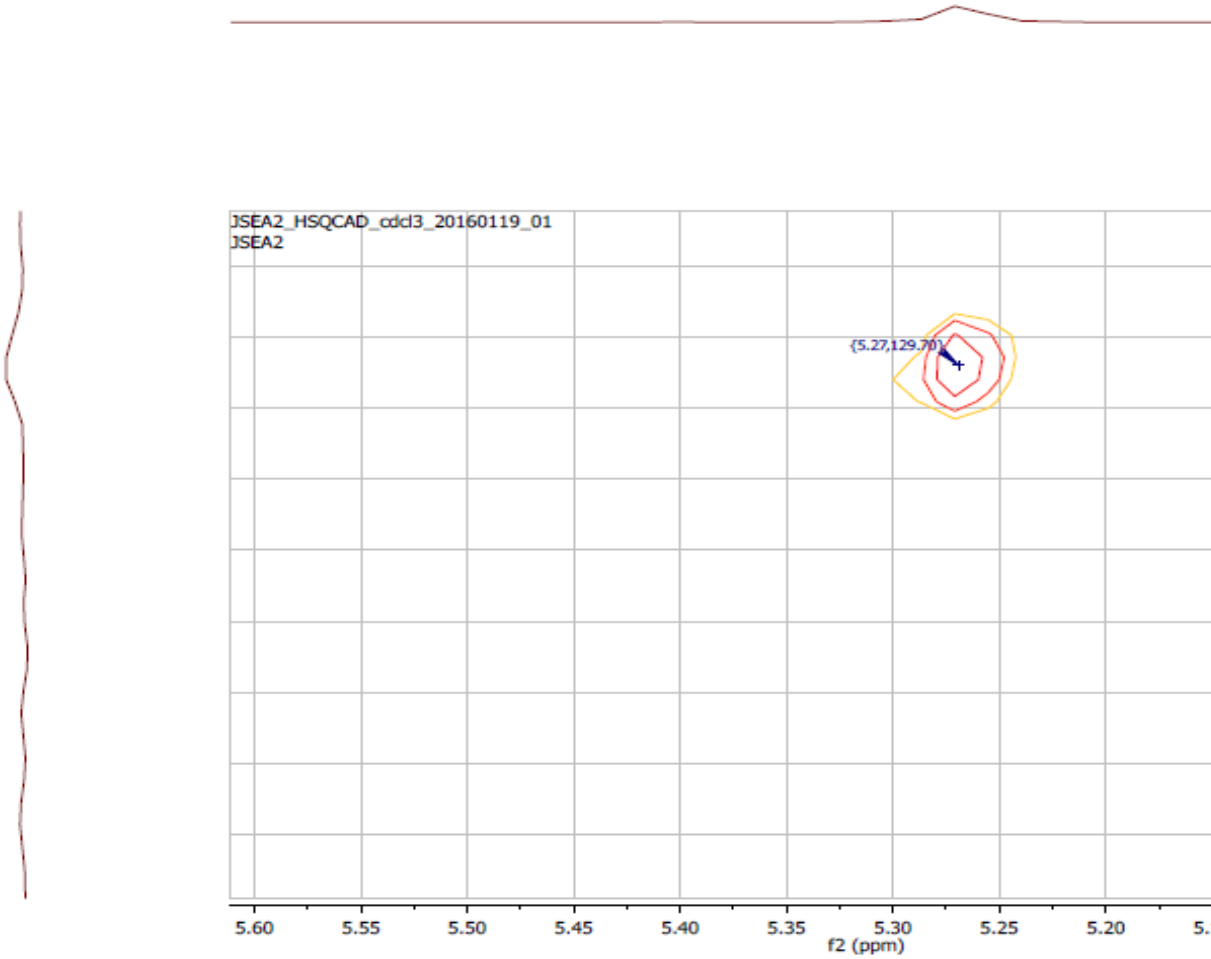
Appendix XXVI-B. SOME RELEVANT EXPANSIONS-HSQC Spectrum of JSEA2



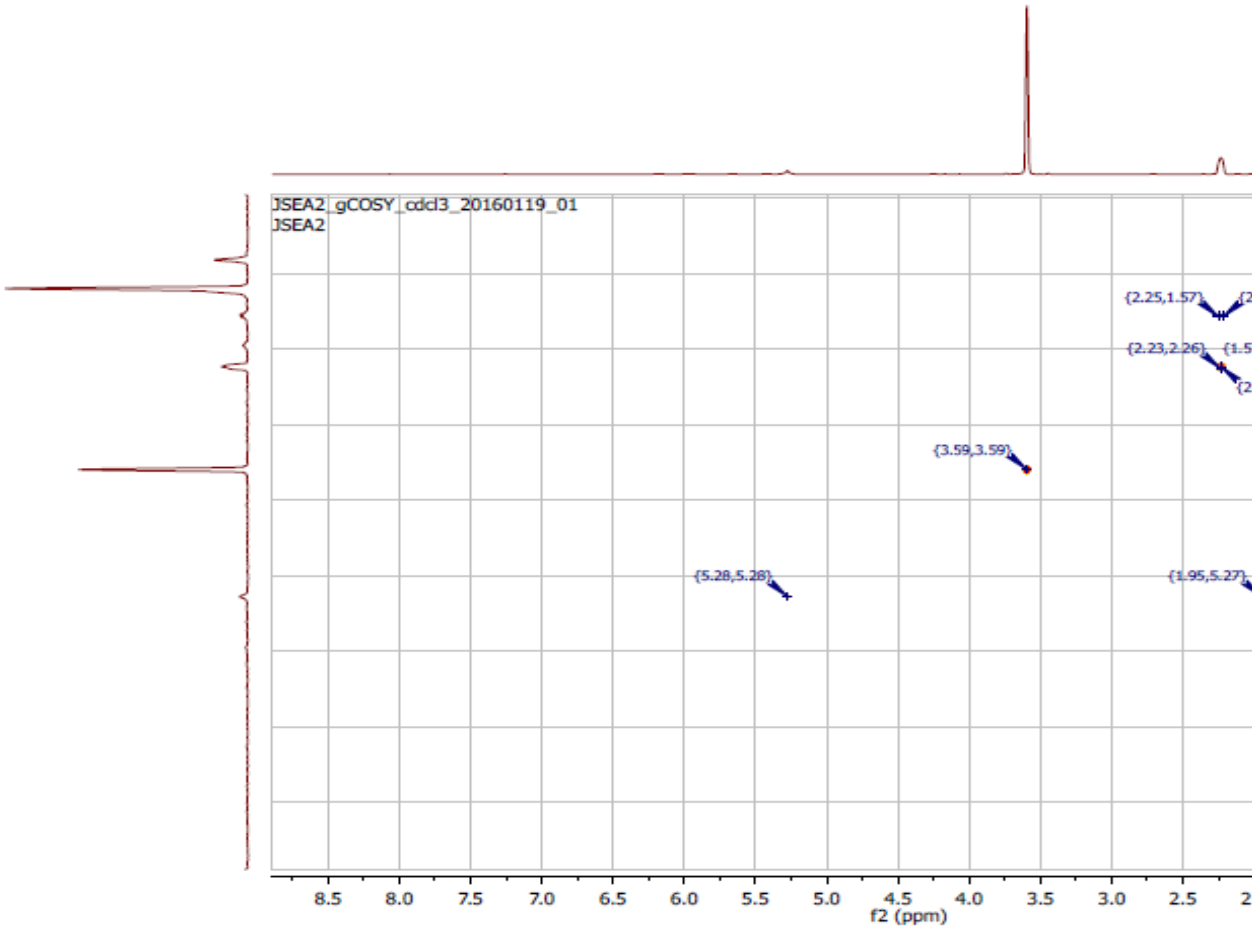
Appendix XXVI-B1. SOME RELEVANT EXPANSIONS-HSQC Spectrum of JSEA2



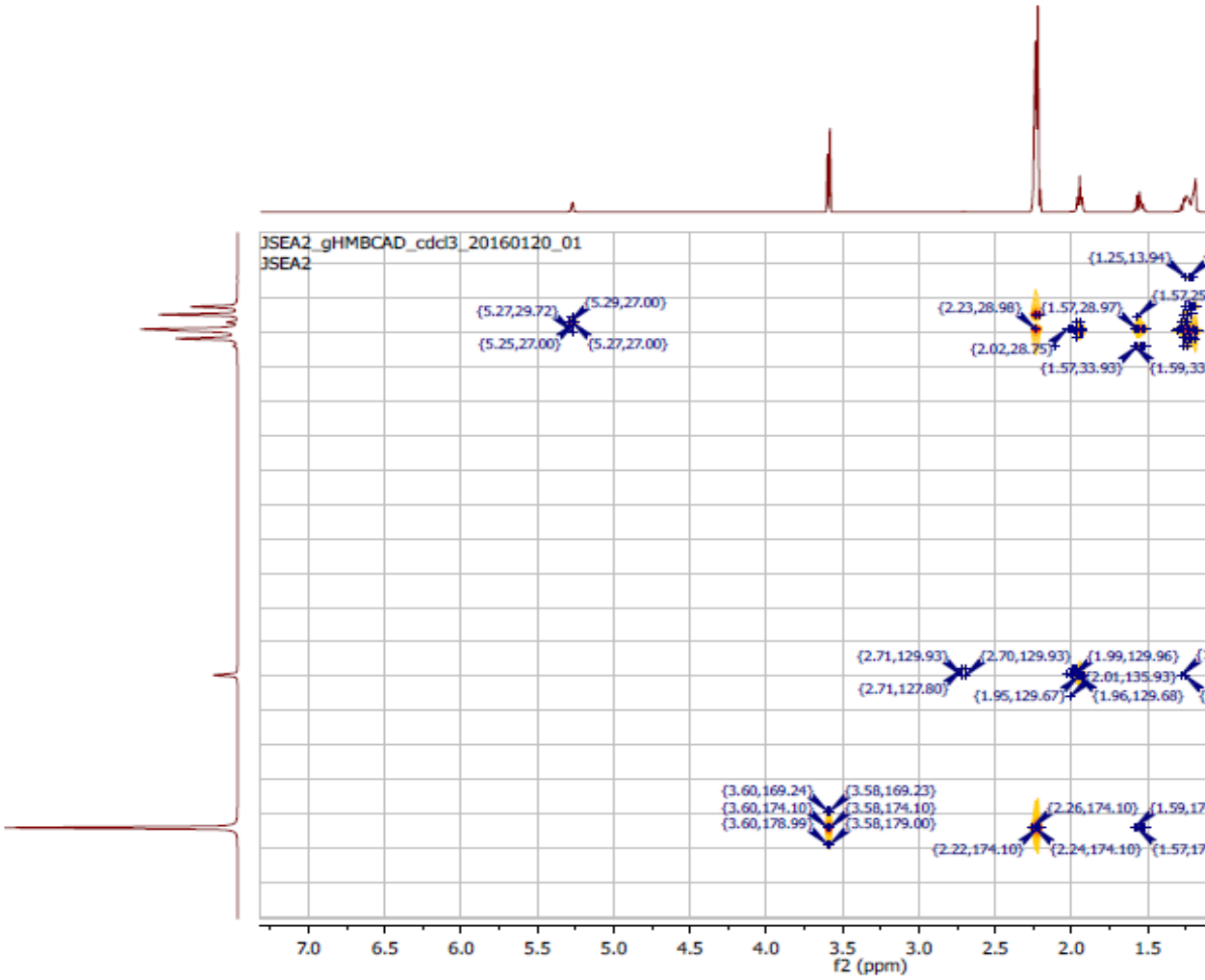
Appendix XXVI-B2. SOME RELEVANT EXPANSIONS-HSQC Spectrum of JSEA2



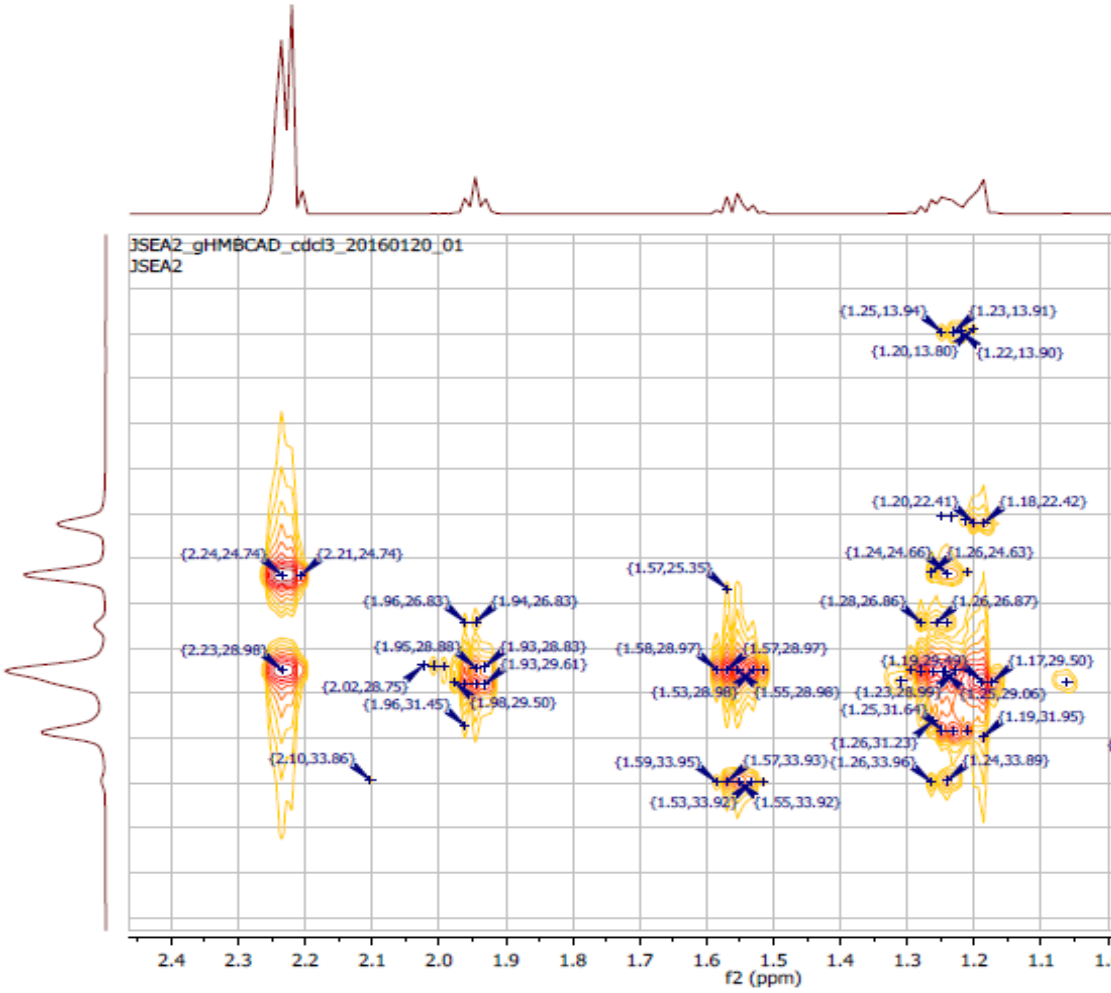
Appendix XXVI-B3. SOME RELEVANT EXPANSIONS-HSQC Spectrum of JSEA2



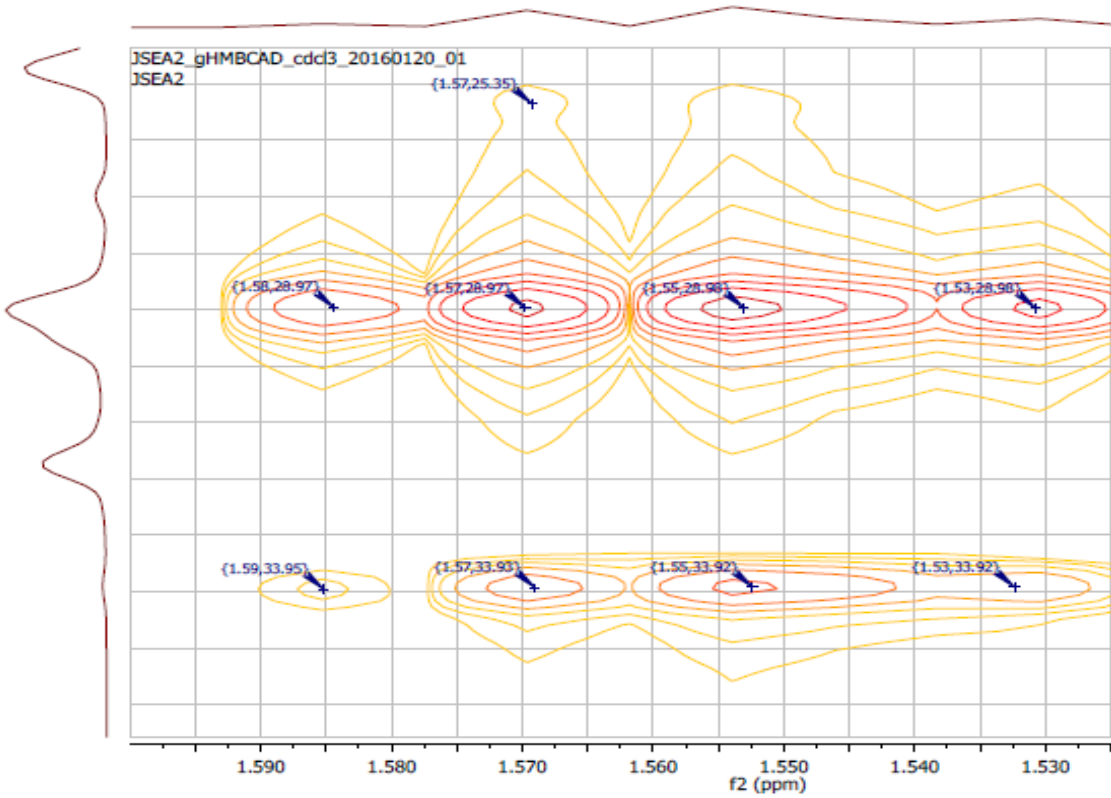
Appendix XXVI-C. COSY Spectrum of JSEA2



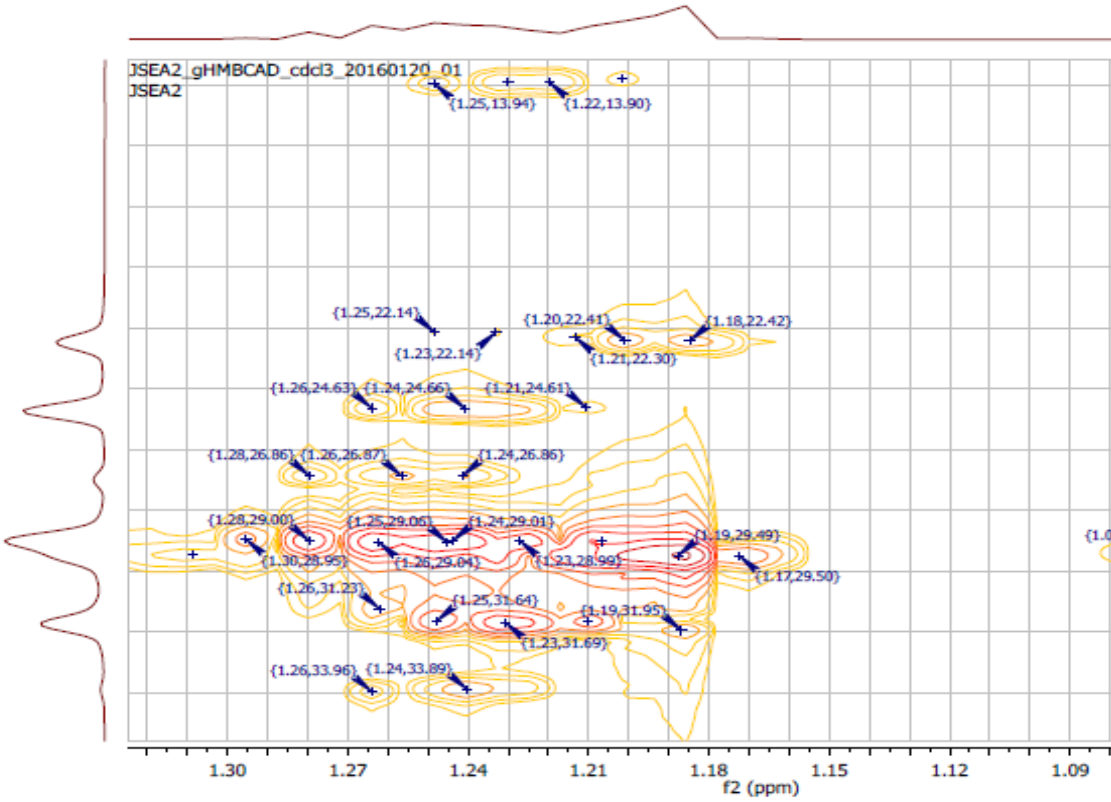
Appendix XXVI-D. HMBC Spectrum of JSEA2



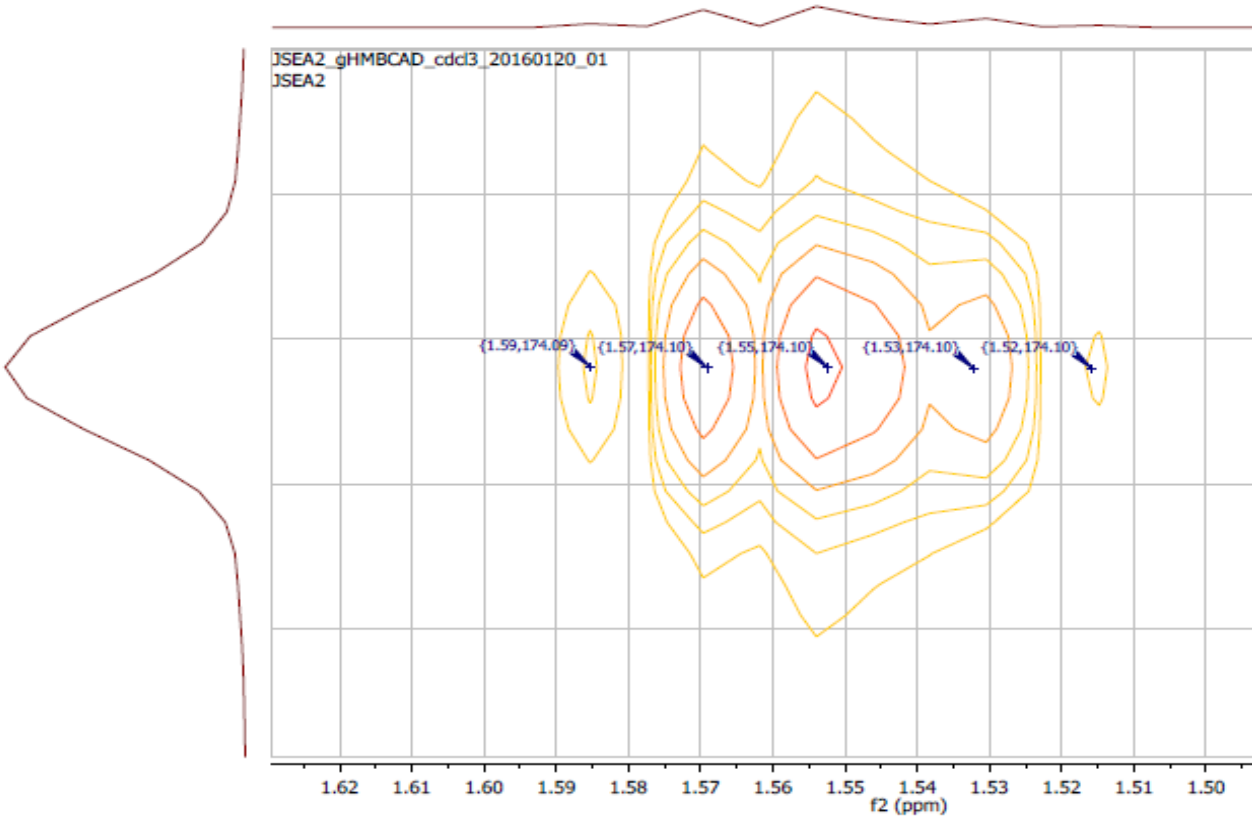
Appendix XXVI-E1. SOME RELEVANT EXPANSIONS-HMBC Spectrum of JSEA2



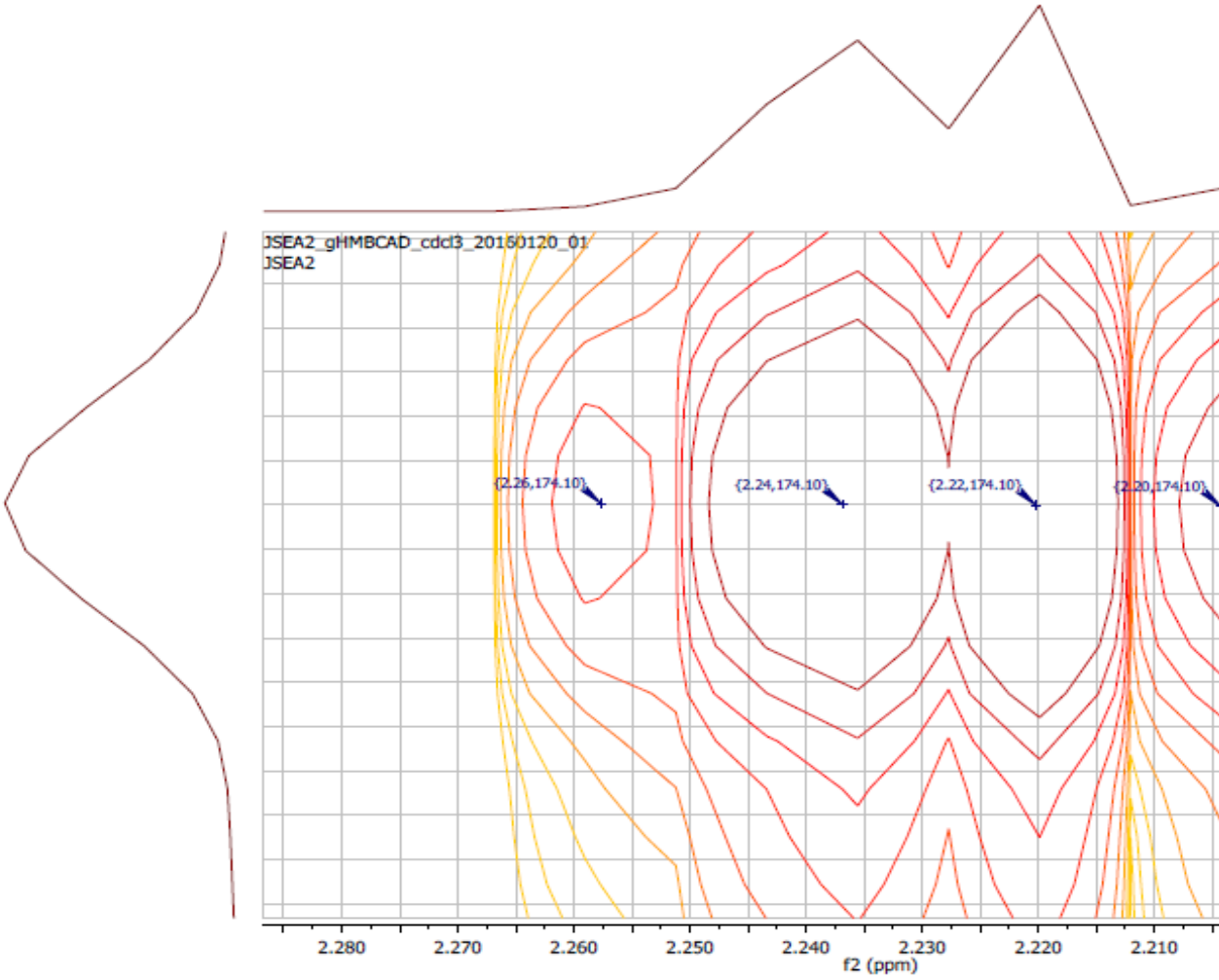
Appendix XXVI-E2. SOME RELEVANT EXPANSIONS-HMBC Spectrum of JSEA2



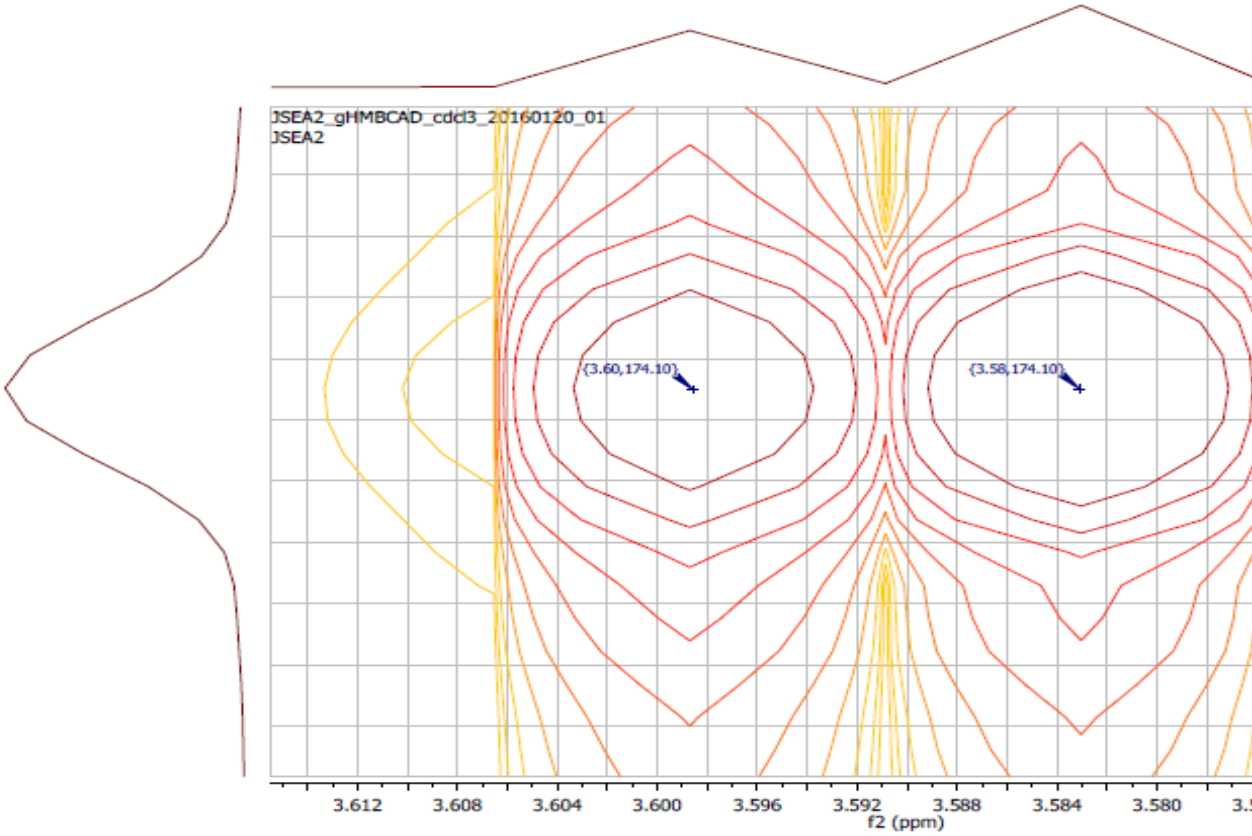
Appendix XXVI-E3. SOME RELEVANT EXPANSIONS-HMBC Spectrum of JSEA2



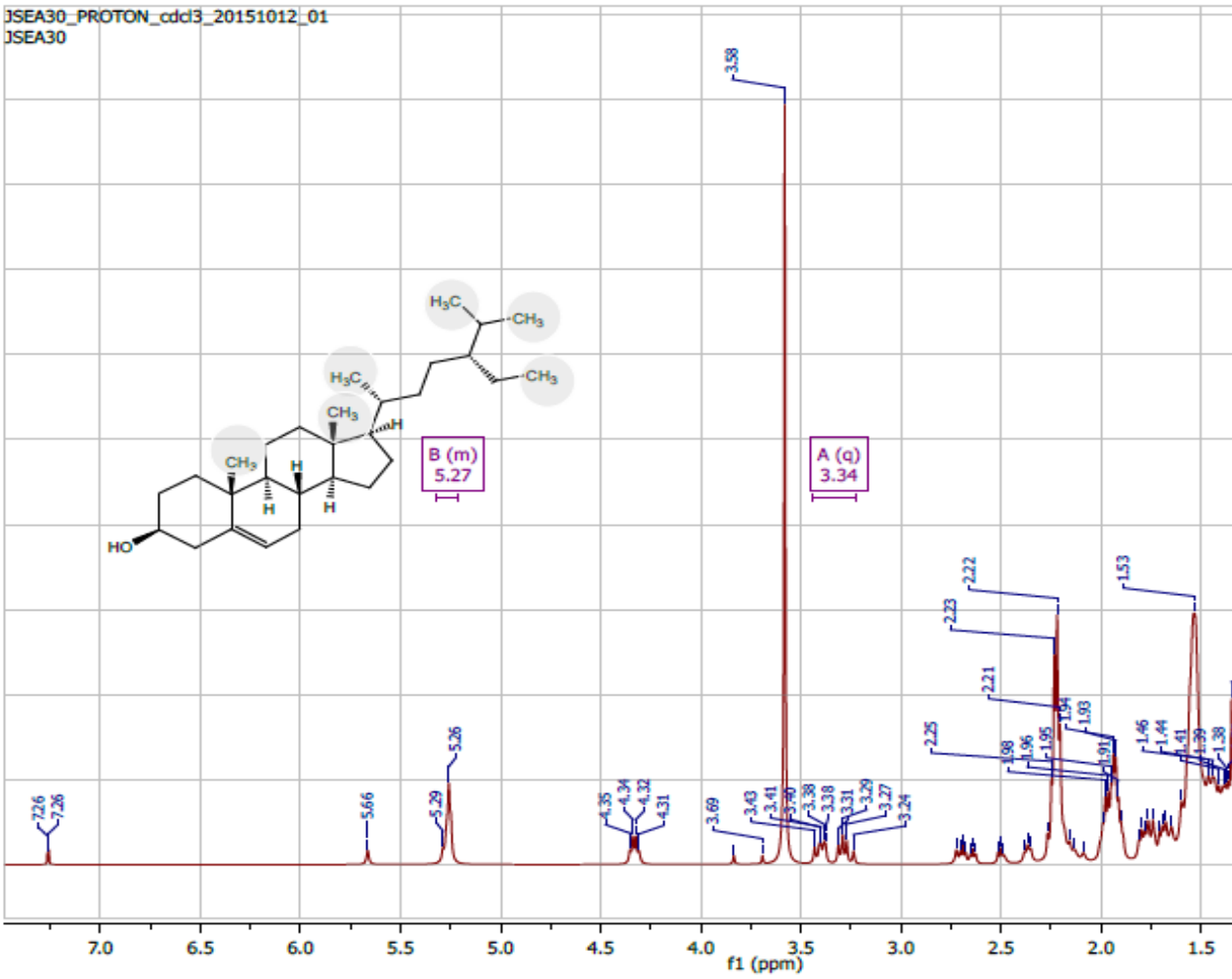
Appendix XXVI-E4. SOME RELEVANT EXPANSIONS-HMBC Spectrum of JSEA2



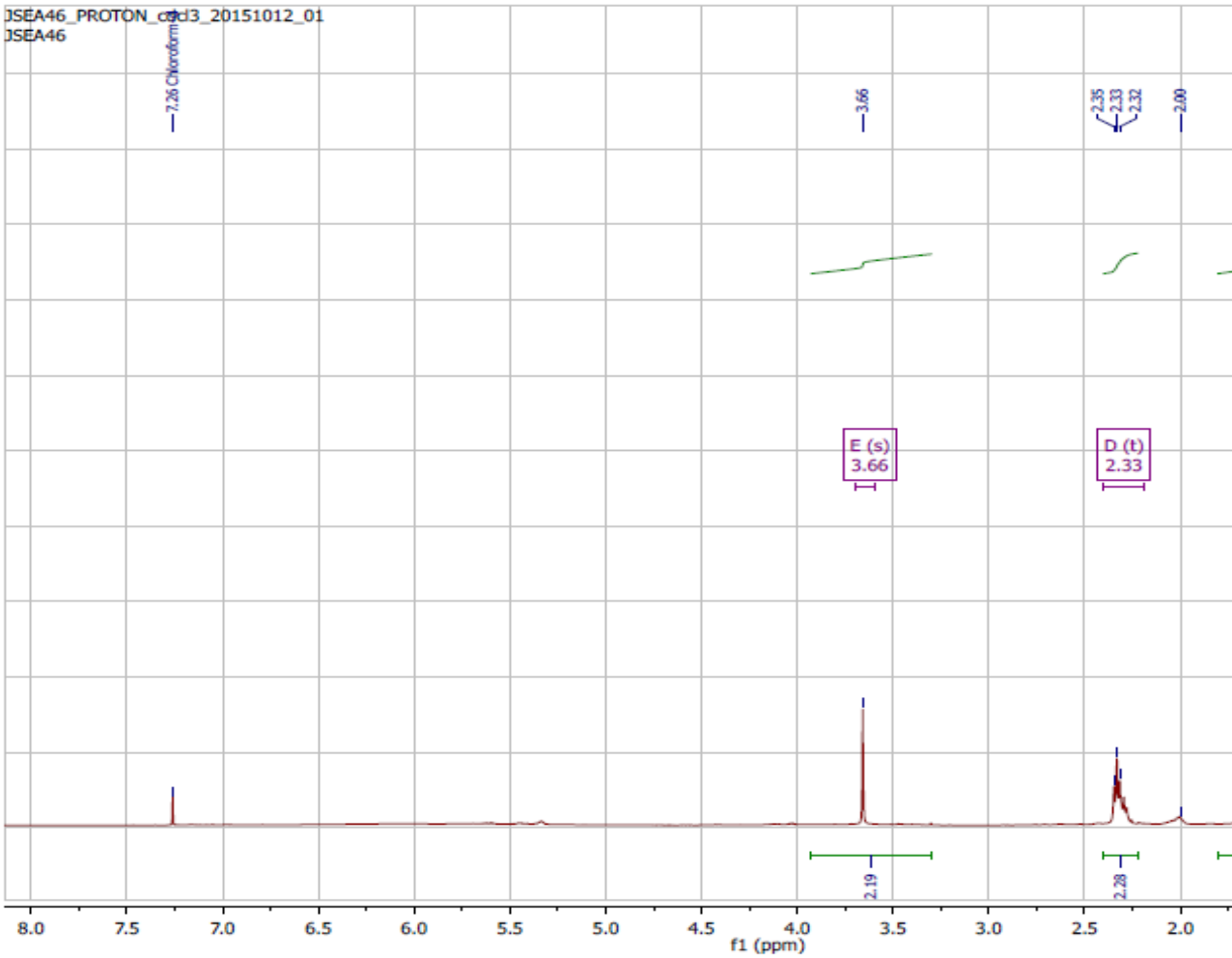
Appendix XXVI-E5. SOME RELEVANT EXPANSIONS-HMBC Spectrum of JSEA2



Appendix XXVI-E6. SOME RELEVANT EXPANSIONS-HMBC Spectrum of JSEA2



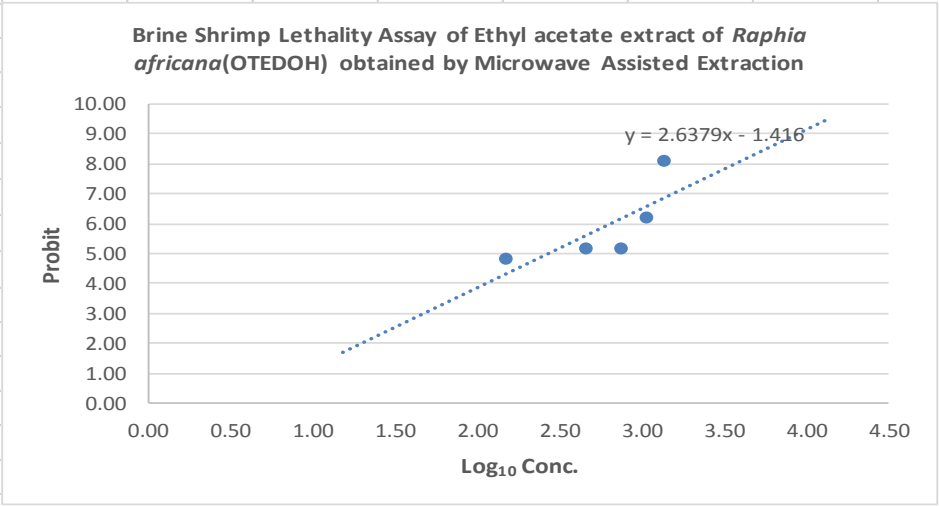
Appendix XXVII. [1]HNMR Spectrum of JSEA30



Appendix XXVII. ^{1}HNMR Spectrum of JSEA46

Brine Shrimp Lethality Assay of Ethyl acetate extract of *Raphia africana* (OTEDOH) obtained by M

Conc (mg/L)	Start No. of nauplii	End No. of nauplii	No. of dead nauplii	Log_{10} Conc.	% Mortality	Corrrected % Mortality	Probit		PLOTTIN
0	10	9	1	---	10	0	-		Log_{10} Co Pro
150	10	5	5	2.18	50	44	4.85		2.18
450	10	4	6	2.65	60	56	5.15		2.65
750	10	4	6	2.88	60	56	5.15		2.88
1050	10	1	9	3.02	90	89	6.23		3.02
1350	10	0	10	3.13	100	100	8.09		3.13

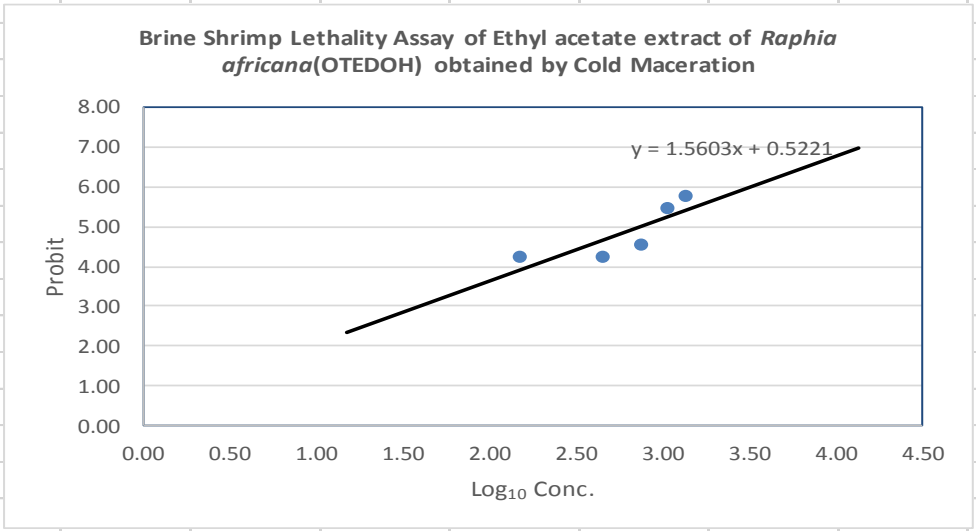


y = 2.6379
Where
x = 2.43
LC50 = Antil
269.153

Appendix XXVIII-A. Brine Shrimp Lethality Assay for obtained via Microwave Assisted Extraction

Brine Shrimp Lethality Assay of Ethyl acetate extract of *Raphia africana* (OTEDOH) obtained b

Conc (mg/L)	Start No. of nauplii	End No. of nauplii	No. of dead nauplii	Log_{10} Conc.	% Mortality	Corrrected % Mortality	Probit		PLOT
0	10	9	1	---	10	0	-		Log_{10} Co
150	10	7	3	2.18	30	22	4.23		2.18
450	10	7	3	2.65	30	22	4.23		2.65
750	10	6	4	2.88	40	33	4.56		2.88
1050	10	3	7	3.02	70	67	5.44		3.02
1350	10	2	8	3.13	80	78	5.77		3.13



y = 1.5603x + 0

Where y =

then

x = 2.67

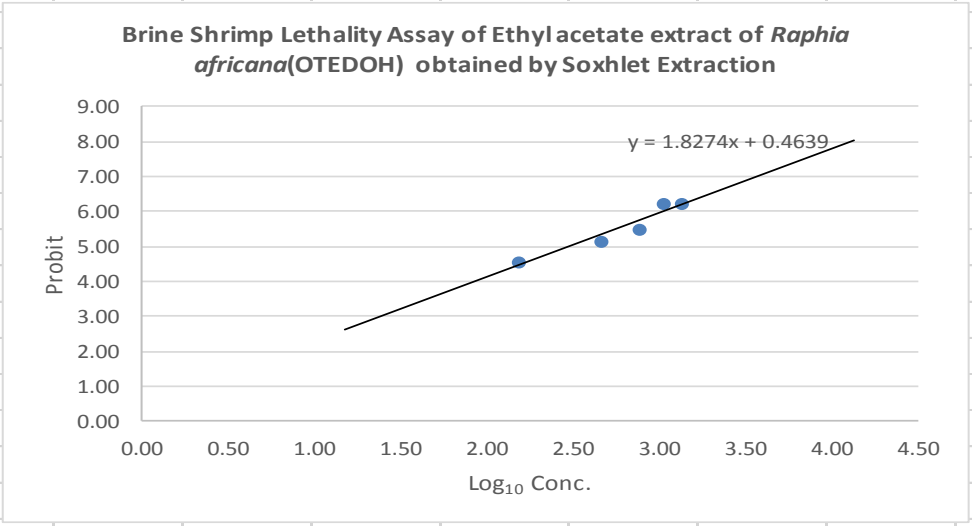
LC50 = A

467.735

Appendix XXVIII-B. Brine Shrimp Lethality Assay for extract obtained via Cold Maceration

Brine Shrimp Lethality Assay of Ethyl acetate extract of *Raphia africana* (OTEDOH) obtained by

Conc (mg/L)	Start No. of nauplii	End No. of nauplii	No. of dead nauplii	Log_{10} Conc.	% Mortality	Corrrected % Mortality	Probit
0	10	9	1	---	10	0	-
150	10	6	4	2.18	40	33	4.56
450	10	4	6	2.65	60	56	5.15
750	10	3	7	2.88	70	67	5.47
1050	10	1	9	3.02	90	89	6.23
1350	10	1	9	3.13	90	89	6.23



PLOTT

Log_{10} Co

2.18

2.65

2.88

3.02

3.13

y = 1.8274

Where y =

then

x = 2.43

LC50 = A

269.153

Appendix XXVIII-C. Brine Shrimp Lethality Assay for extract obtained by Soxhlet Extraction

Proton NMR Integrals and Normalization for Determination of Average Chain Length (Ja 64)					
Assignment	Position (ppm)	Ja 64 Integral	Ja 64 Adjusted Integral	Normalized Integral	Total Degree of Unsatura
CH3 (G)	0.9	63863.8	21287.93	1.01	
(CH2)n (F)	1.3	382101.43	191050.72	9.03	
CH2CH2(CO) (E)	1.6	36876.21	18438.11	0.87	
CH2CH=CHCH2 (D)	2.0	0	0.00	0.00	
CH2(CO) (C)	2.3	47478.07	23739.04	1.12	
CHCH2CH= (H)	2.8	0	0.00	0.00	
CH2O (B)	4.3	0	0.00	0.00	
CHO (B/4)	5.3	0	0.00	0.00	
CH=CH (A)	5.4	0	0.00	0.00	
Saturated Presence Test			Total Adjusted Proton	Average Proton Integr	Average Chain Length
			63465.07	21155.02444	13.0
ratio of 2.0: 0.8 <	1.333333333		2652.963116	0.125405817	
0	Saturated chain only		132.91	17664.77275	
			-2716.92	7381651.268	
			2584.01	6677110.551	
				4692142.197	
				2166.135314	

Appendix XXIX. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF Ja64

Proton NMR Integrals and Normalization for Determination of Average Chain Length (Ja 65)					
Assignment	Position (ppm)	Ja 65 Integral	Ja 65 Adjusted Integral	Normalized	Total Degree of Ur
CH3 (G)	0.9	16294.62	5431.54	0.96	
(CH2)n (F)	1.3	138654.99	69327.50	12.28	
CH2CH2(CO) (E)	1.6	11403.52	5701.76	1.01	
CH2CH=CHCH2 (D)	2.0	0	0.00	0.00	
CH2(CO) (C)	2.3	11610.65	5805.33	1.03	
CHCH2CH= (H)	2.8	0	0.00	0.00	
CH2O (B)	4.3	0	0.00	0.00	
CHO (B/4)	5.3	0	0.00	0.00	
CH=CH (A)	5.4	0	0.00	0.00	
Saturated Presence Test			Total Adjusted Proton	Average Pro	Average Chain Len
			16938.63	5646.208333	
ratio of 2.0: 0.8 <	1.333333333		192.9852256	0.034179615	
0	Saturated chain only		-214.67	46082.49334	
			55.55	3085.987669	
			159.12	25318.11361	
				24828.86487	
				157.5717769	

Appendix XXIX-A. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF Ja65

Proton NMR Integrals and Normalization for Determination of Average Chain Length (Ja 66)					
Assignment	Position (ppm)	Ja 66 Integral	Ja 66 Adjusted Integral	Normalized Integral	Total Degree of Unsatur
CH3 (G)	0.9	10530736.23	1170081.80	1.10	
(CH2)n (F)	1.3	55511292.05	9251882.01	8.70	
CH2CH2(CO) (E)	1.6	5833859.13	972309.86	0.91	
CH2CH=CHCH2 (D)	2.0	1385136.34	115428.03	0.11	
CH2(CO) (C)	2.3	7295798.13	1215966.36	1.14	
CHCH2CH= (H)	2.8	0	0.00	0.00	
CH2O (B)	4.3	3579773.93	894943.48	0.84	
CHO (B/4)	5.3	894943.4825	894943.48	0.84	
CH=CH (A)	5.4	163710.4975	27285.08	0.03	
Saturated Presence Test			Total Adjusted Proton	Average Proton Integral	Average Chain Length
			4253301.50	1063325.374	14.8
ratio of 2.0: 0.8 <	1.333333333		154199.5356	0.145016323	0.14
0.13	Saturated chain present		106756.43	11396935213	
			-91015.52	8283824691	
			152640.98	23299269093	
			-168381.89	28352461371	
				17833122592	
				133540.7151	

Appendix XXIX-B. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF Ja66

Proton NMR Integrals and Normalization for Determination of Average Chain Length (Ja 68)					
Assignment	Position (ppm)	Ja 68 Integral	Ja 68 Adjusted Integral	Normalized Integral	Total Degree of Unsatu
CH3 (G)	0.9	66146.61	7349.62	0.96	
(CH2)n (F)	1.3	573393.3	95565.55	12.47	
CH2CH2(CO) (E)	1.6	46162.15	7693.69	1.00	
CH2CH=CHCH2 (D)	2.0	0	0.00	0.00	
CH2(CO) (C)	2.3	47671.61	7945.27	1.04	
CHCH2CH= (H)	2.8	0	0.00	0.00	
CH2O (B)	4.3	0	0.00	0.00	
CHO (B/4)	5.3	0	0.00	0.00	
CH=CH (A)	5.4	0	0.00	0.00	
Saturated Presence Test			Total Adjusted Proton	Average Proton Integral	Average Chain Length
			22988.58	7662.861111	16
ratio of 2.0: 0.8 <	1.333333333		299.0169458	0.03902158	
0	Saturated chain only		-313.24	98117.90543	
			30.83	950.5231559	
			282.41	79753.83916	
				59607.42258	
				244.1463139	

Appendix XXIX-C. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF Ja68

Proton NMR Integrals and Normalization for Determination of Average Chain Length (Ja 69)					
Assignment	Position (ppm)	Ja 69 Integral	Ja 69 Adjusted Integral	Normalized Integral	Total Degree of Unsatu
CH3 (G)	0.9	11401846.32	1266871.81	0.86	
(CH2)n (F)	1.3	80436580.76	13406096.79	9.11	
CH2CH2(CO) (E)	1.6	10008391.53	1668065.26	1.13	
CH2CH=CHCH2 (D)	2.0	0	0.00	0.00	
CH2(CO) (C)	2.3	8870154.75	1478359.13	1.00	
CHCH2CH= (H)	2.8	0	0.00	0.00	
CH2O (B)	4.3	0	0.00	0.00	
CHO (B/4)	5.3	0	0.00	0.00	
CH=CH (A)	5.4	0	0.00	0.00	
Saturated Presence Test			Total Adjusted Proton	Average Proton Integral	Average Chain Length
			4413296.19	1471098.731	13.
ratio of 2.0: 0.8 <	1.333333333		200695.2401	0.136425405	
0	Saturated chain only		-204226.92	41708633945	
			196966.52	38795811533	
			7260.39	52713319.42	
				26852386266	
				163866.9773	

Appendix XXIX-D. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM 1HNMR OF Ja69

	Proton NMR Integrals and Normalization for Determination of Average Chain Length (Ja 75)			
Position (ppm)	Ja 75 Integral	Ja 75 Adjusted Integral	Normalized Integral	Total Degree of Unsaturatio
0.9	21,856.40	2428.49	1.10	0
1.3	136541.38	22756.90	10.29	
1.6	14,741.11	2456.85	1.11	
2.0	8389.65	699.14	0.32	
2.3	13155.93	2192.66	0.99	
2.8	634.42	105.74	0.05	
4.3	7056.98	1764.25	0.80	
5.3	1764.245	1764.25	0.80	
5.4	3896.435	649.41	0.29	
Saturated Presence Test		Total Adjusted Proton	Average Proton Integral	Average Chain Length
		8842.24	2210.560139	17.360652
ratio of 2.0: 0.8 <	1.333333333	320.2448303		0.144870
0.38	Saturated chain present	217.93	47492.94008	
		246.29	60659.51666	
		-17.91	320.5939986	
		-446.32	199197.2032	
			76917.56348	
			277.3401584	

Appendix XXIX-E. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF Ja75

Proton NMR Integrals and Normalization for Determination of Average Chain Length (Ja 78)					
Assignment	Position (ppm)	Ja 78 Integral	Ja 78 Adjusted Integral	Normalized Integral	Total Degree
C***H3 (G)***	0.9	14982029.9	1664669.99	0.85	
(C***H2***)n ***(F)***	1.3	85013612.31	14168935.39	7.26	
C***H2***CH2(CO) ***(E)***	1.6	12911893.47	2151982.25	1.10	
C***H2***CH=CHCH2 ***(D)***	2.0	9018157.42	751513.12	0.39	
C***H2***(CO) ***(C)***	2.3	11023277.06	1837212.84	0.94	
CHC***H2***CH= ***(H)***	2.8	2618837.85	436472.98	0.22	
C***H2***O ***(B)***	4.3	8609156.62	2152289.16	1.10	
C***H***O ***(B/4)***	5.3	2152289.155	2152289.16	1.10	
C***H***=C***H*** ***(A)***	5.4	5779948.935	963324.82	0.49	
Saturated Presence Test			Total Adjusted Proton	Average Proton Integral	Average Cha
			7806154.23	1951538.558	
ratio of 2.0: 0.8 <	1.333333333		242103.5721	0.124057796	
0.6	Saturated chain present		-286868.57	82293575976	
			200443.69	40177671636	
			-114325.71	13070369047	
			200750.60	40300802174	
				43960604708	
				209667.8438	
				1	1.70 .. 1.50
				2	0.90 .. 0.83
				3	4.37 .. 4.00
				4	2.83 .. 2.68
				5	2.40 .. 2.19
				6	2.13 .. 1.90
				7	1.47 .. 1.09
				8	5.52 .. 5.16

Appendix XXIX-F. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF Ja78

Proton NMR Integrals and Normalization for Determination of Average Chain Length (Ja 79)						
Assignment	Position (ppm)	Ja 79 Integral	Ja 79 Adjusted Integral	Normalized Integral	Total Degree of Unsaturation	
.	0.9	104552.17	11616.91	1.13	0.56	0.2823
(C***H2***)n ***(F)***	1.3	498567.63	83094.61	8.11		
CH2CH2(CO) ***(E)***	1.6	72605.14	12100.86	1.18		
CH2CH=CH***C***H2 ***(D)***	2.0	36424.07	3035.34	0.30		
CH2(CO) ***(C)***	2.3	53066.23	8844.37	0.86		
CHC***H2***CH= ***(H)***	2.8	3611.47	601.91	0.06		
CH2O ***(B)***	4.3	33621.36	8405.34	0.82		
CHO ***(B/4)***	5.3	8405.34	8405.34	0.82		
CH=***CH*** ***(A)***	5.4	12876.08	2146.01	0.21		
Saturated Presence Test			Total Adjusted Proton	Average Proton Integral	Average Chain Length	
			40967.48	10241.86903	15.00447881	
ratio of 2.0: 0.8 <	1.333333333		1886.125159	0.184158297	1.00	
0.35	Saturated chain present		1375.04	1890731.564	0.13	0.0180
			1858.99	3455835.042	0.18	0.0329
			-1397.50	1952998.874	-0.14	0.0186
			-1836.53	3372838.87	-0.18	0.0321
				2668101.087		0.0254
				1633.432303		0.1594

Straight from Mnova

	Name	Shift	Range	H's	Integral	Class
1	C (m)	2.74	2.78 .. 2.72	2	0.18	m
2	A (m)	5.32	5.40 .. 5.21	3	1	m
3	G (m)	1.25	1.45 .. 0.98	60	23.97	m
4	F (d)	1.6	1.67 .. 1.51	6	3.08	d
5	E (m)	2.01	2.09 .. 1.95	2	1.74	m
6	D (qd)	2.3	2.36 .. 2.22	6	2.5	qd
7	B (m)	4.19	4.34 .. 4.04	4	1.6	m
8	H (t)	0.86	0.95 .. 0.65	9	4.96	t

Appendix XXIX-G. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF Ja79

Proton NMR Integrals and Normalization for Determination of Average Chain Length (Ja 80)					
Assignment	Position (ppm)	Ja 80 Integral	Ja 80 Adjusted Integral	Normalized Integral	Total Degree of Unsatura
.	0.9	87235.91	9692.88	0.86	
(CH2)n (F)	1.3	470724.71	78454.12	6.95	
CH2CH2(CO) (E)	1.6	88296.21	14716.04	1.30	
CH2CH=CHCH2 (D)	2.0	30735.65	2561.30	0.23	
CH2(CO) (C)	2.3	62516.77	10419.46	0.92	
CHCH2CH= (H)	2.8	2648.87	441.48	0.04	
CH2O (B)	4.3	41413.34	10353.34	0.92	
CHO (B/4)	5.3	10353.335	10353.34	0.92	
CH=CH (A)	5.4	6263.695	1043.95	0.09	
Saturated Presence Test			Total Adjusted Proton	Average Proton Integral	Average Chain Length
			45181.71	11295.42764	13.53
ratio of 2.0: 0.8 <	1.333333333		2303.878768	0.203965608	
0.35	Saturated chain present		-1602.55	2568162.496	
			3420.61	11700554.72	
			-875.97	767316.3845	
			-942.09	887538.5402	
				3980893.035	
				1995.217541	

Appendix XXIX-H. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF Ja80

Proton NMR Integrals and Normalization for Determination of Average Chain Length (Ja 81) Sugar formulars!					
Assignment	Position (ppm)	Ja 81 Integral	Ja 81 Adjusted Integral	Normalized Integral	Total Degree of Unsatur
.	0.9	107457.55	17909.59	0.90	
(CH2)n (F)	1.3	578607.31	144651.83	7.30	
CH2CH2(CO) (E)	1.6	111875.47	27968.87	1.41	
CH2CH=CHCH2 (D)	2.0	41308.02	5163.50	0.26	
CH2(CO) (C)	2.3	77022.96	19255.74	0.97	
CHCH2CH= (H)	2.8	5504.26	1376.07	0.07	
CH2O (B)	4.3	56381.97	14095.49	0.71	
CHO (B/4)	5.3	14095.4925	14095.49	0.71	
CH=CH (A)	5.4	8371.1275	1046.39	0.05	
Saturated Presence Test			Total Adjusted Proton	Average Proton Integral	Average Chain Length
			79229.69	19807.42292	13.7
ratio of 2.0: 0.8 <	1.333333333		5863.487138	0.296024736	
0.38	Saturated chain present		-1897.83	3601763.453	
			8161.44	66609177.69	
			-551.68	304354.0405	
			-5711.93	32626149.08	
				25785361.07	
				5077.928817	

Appendix XXIX-I. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF Ja81

Proton NMR Integrals and Normalization for Determination of Average Chain Length (Ja 82)					
Assignment	Position (ppm)	Ja 82 Integral	Ja 82 Adjusted Integral	Normalized Integral	Total Degree of Unsatur
CH3 (G)	0.9	25259.62	2806.62	1.12	
(CH2)n (F)	1.3	96188.66	16031.44	6.38	
CH2CH2(CO) (E)	1.6	19283.31	3213.89	1.28	
CH2CH=CHCH2 (D)	2.0	5274.24	439.52	0.17	
CH2(CO) (C)	2.3	21336.48	3556.08	1.41	
CHCH2CH= (H)	2.8	0	0.00	0.00	
CH2O (B)	4.3	1907.32	476.83	0.19	
CHO (B/4)	5.3	476.83	476.83	0.19	
CH=CH (A)	5.4	1013.54	168.92	0.07	
Saturated Presence Test			Total Adjusted Proton	Average Proton Integral	Average Chain Length
			10053.42	2513.354861	12.0
ratio of 2.0: 0.8 <	1.333333333		1391.816409	0.553768364	
0.21	Saturated chain present		293.27	86007.04851	
			700.53	490742.4755	
			1042.73	1087275.715	
			-2036.52	4147433.51	
				1452864.687	
				1205.348368	

Appendix XXIX-J. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF Ja82

Proton NMR Integrals and Normalization for Determination of Average Chain Length (Ja 83)					
Assignment	Position (ppm)	Ja 83 Integral	Ja 83 Adjusted Integral	Normalized Integral	Total Degree of Unsatura
CH3 (G)	0.9	43019.58	4779.95	0.72	
(CH2)n (F)	1.3	126037.7	21006.28	3.15	
CH2CH2(CO) (E)	1.6	42043.2	7007.20	1.05	
CH2CH=CHCH2 (D)	2.0	51247.42	4270.62	0.64	
CH2(CO) (C)	2.3	34931.09	5821.85	0.87	
CHCH2CH= (H)	2.8	0	0.00	0.00	
CH2O (B)	4.3	36262.26	9065.57	1.36	
CHO (B/4)	5.3	9065.565	9065.57	1.36	
CH=CH (A)	5.4	0	0.00	0.00	
Saturated Presence Test			Total Adjusted Proton	Average Proton Integral	Average Chain Length
			26674.57	6668.641667	10.7
ratio of 2.0: 0.8 <	1.333333333		1838.846176	0.275745237	
1.19	Saturated chain present		-1888.69	3567143.62	
			338.56	114621.7451	
			-846.79	717058.9494	
			2396.92	5745241.466	
				2536016.445	
				1592.487502	

Appendix XXIX-K. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF Ja83

Proton NMR Integrals and Normalization for Determination of Average Chain Length (Ja 84)					
Assignment	Position (ppm)	Ja 84 Integral	Ja 84 Adjusted Integral	Normalized Integral	Total Degree of Unsatur
.	0.9	39595.47	6599.25	0.69	
(CH2)n (F)	1.3	152109.82	38027.46	4.00	
CH2CH2(CO) (E)	1.6	46887.72	11721.93	1.23	
CH2CH=CHCH2 (D)	2.0	28061.08	3507.64	0.37	
CH2(CO) (C)	2.3	36048.32	9012.08	0.95	
CHCH2CH= (H)	2.8	864.92	216.23	0.02	
CH2O (B)	4.3	42668.33	10667.08	1.12	
CHO (B/4)	5.3	10667.0825	10667.08	1.12	
CH=CH (A)	5.4	2077.88	259.74	0.03	
Saturated Presence Test			Total Adjusted Proton	Average Proton Integral	Average Chain Length
			38000.34	9500.084375	10.
ratio of 2.0: 0.8 <	1.333333333		2232.450071	0.234992657	
0.71	Saturated chain present		-2900.84	8414869.08	
			2221.85	4936597.981	
			-488.00	238148.27	
			1167.00	1361884.624	
				3737874.989	
				1933.358474	

Appendix XXIX-L. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF Ja84

Proton NMR Integrals and Normalization for Determination of Average Chain Length (Ja 96)					
Assignment	Position (ppm)	Ja 96 Integral	Ja 96 Adjusted Integral	Normalized Integral	Total Degree of Uns
C***H3 (G)***	0.9	41102.7	13700.90	1.00	
(C***H2***)n ***(F)***	1.3	181136.76	90568.38	6.60	
C***H2***CH2(CO) ***(E)***	1.6	24714.54	12357.27	0.90	
C***H2***CH=CHCH2 ***(D)***	2.0	12959.67	3239.92	0.24	
C***H2***(CO) ***(C)***	2.3	30269.09	15134.55	1.10	
CHC***H2***CH= ***(H)***	2.8	0	0.00	0.00	
C***H2***O ***(B)***	4.3	0	0.00	0.00	
C***H***O ***(B/4)***	5.3	0	0.00	0.00	
C***H***=C***H*** ***(A)***	5.4	1844.09	922.05	0.07	
Saturated Presence Test			Total Adjusted Proton	Average Proton Integral	Average Chain Leng
			41192.72	13730.905	
ratio of 2.0: 0.8 <	1.333333333		6958.479066	0.101149968	
0.32 Saturated chain present			-30.00	900.300025	
			-1373.64	1886873.113	
			1403.64	1970205.25	
				1285992.888	
				1134.016264	

Appendix XXIX-M. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF Ja96

Proton NMR Integrals and Normalization for Determination of Average Chain Length (Ja 97)					
Assignment	Position (ppm)	Ja 97 Integral	Ja 97 Adjusted Integral	Normalized Integral	Total Degree of Uns
CH3 (G)	0.9	34931.55	3881.28	0.88	
(CH2)n (F)	1.3	147869.89	24644.98	5.58	
CH2CH2(CO) (E)	1.6	33101.93	5516.99	1.25	
CH2CH=CHCH2 (D)	2.0	16102.29	1341.86	0.30	
CH2(CO) (C)	2.3	21340.82	3556.80	0.80	
CHCH2CH= (H)	2.8	0	0.00	0.00	
CH2O (B)	4.3	18900.83	4725.21	1.07	
CHO (B/4)	5.3	4725.2075	4725.21	1.07	
CH=CH (A)	5.4	0	0.00	0.00	
	Saturated Presence Test		Total Adjusted Proton	Average Proton Integral	Average Chain Leng
			17680.28	4420.070625	
	ratio of 2.0: 0.8 <	1.333333333	881.6384622	0.199462528	
	0.46	Saturated chain present	-538.79	290291.7457	
			1096.92	1203228.459	
			-863.27	745230.4169	
			305.14	93108.51248	
				582964.7835	
				763.5213052	

Appendix XXIX-N. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF Ja97

Assignment	Position (ppm)	Ja 98 Integral	Ja 98 Adjusted Integral	Normalized Integral	Total Degree of Unsatura
CH3 (G)	0.9	9582593.75	3194197.92	0.69	
(CH2)n (F)	1.3	48771852.73	24385926.37	5.24	
CH2CH2(CO) (E)	1.6	12495357.94	6247678.97	1.34	
CH2CH=CHCH2 (D)	2.0	0	0.00	0.00	
CH2(CO) (C)	2.3	9062721.16	4531360.58	0.97	
CHCH2CH= (H)	2.8	0	0.00	0.00	
CH2O (B)	4.3	0	0.00	0.00	
CHO (B/4)	5.3	0	0.00	0.00	
CH=CH (A)	5.4	0	0.00	0.00	
Saturated Presence Test			Total Adjusted Proton	Average Proton Integral	Average Chain Length
			13973237.47	4657745.822	9.235
ratio of 2.0: 0.8 <	1.333333333		1530658.864		
0	Saturated chain only		-1463547.91	2.14197E+12	
			1589933.15	2.52789E+12	
			-126385.24	15973229452	
				1.56194E+12	
				1249777.729	

Appendix XXIX-O. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF Ja98

Proton NMR Integrals and Normalization for Determination of Average Chain Length (Ja 100)				
Assignment	Position (ppm)	Ja 100 Integral	Ja 100 Adjusted Integral	Normalized Integra
CH3	0.9	1999.99	222.22	
(CH2)n	1.3	7560.19	1260.03	
CH2CH2(CO)	1.6	1751.02	291.84	
CH2CH=CHCH2	2.0	897.46	74.79	
CHCH2CH=	2.3	1458.42	243.07	
CH2(CO)	2.8	242.08	40.35	
CH2O	4.3	553.05	138.26	
CHO	5.3	138.2625	138.26	
CH=CH	5.4	176.0875	29.35	
Saturated Presence Test			Total Adjusted Proton	Average Proton Int
			895.39	
ratio of 2.0: 0.8 <	1.333333333	829.1929183	64.08189723	
0.5	Saturated chain present			

Appendix XXIX-P. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF Ja100

Proton NMR Integrals and Normalization for Determination of Average Chain Length (JSEA2)					
Assignment	Position (ppm)	JSEA2 Integral	JSEA2 Adjusted Integral	Normalized Integral	Total Degree of Unsatur
CH3 (G)	0.9	4233.52	1411.17	0.91	
(CH2)n (F)	1.3	27939.59	13969.80	9.02	
CH2CH2(CO) (E)	1.6	3629.94	1814.97	1.17	
CH2CH=CHCH2 (D)	2.0	2673	668.25	0.43	
CH2(CO) (C)	2.3	2841.47	1420.74	0.92	
CHCH2CH= (H)	2.8	0	0.00	0.00	
CH2O (B)	4.3	0	0.00	0.00	
CHO (B/4)	5.3	0	0.00	0.00	
CH=CH (A)	5.4	1531.83	765.92	0.49	
Saturated Presence Test			Total Adjusted Proton	Average Proton Integral	Average Chain Length
			4646.88	1548.959444	14.8
ratio of 2.0: 0.8 <	1.333333333		230.421501		
0.63	Saturated chain present		-137.79	18985.01242	
			266.01	70761.61567	
			-128.22	16441.50815	
				35396.04541	
				188.1383677	

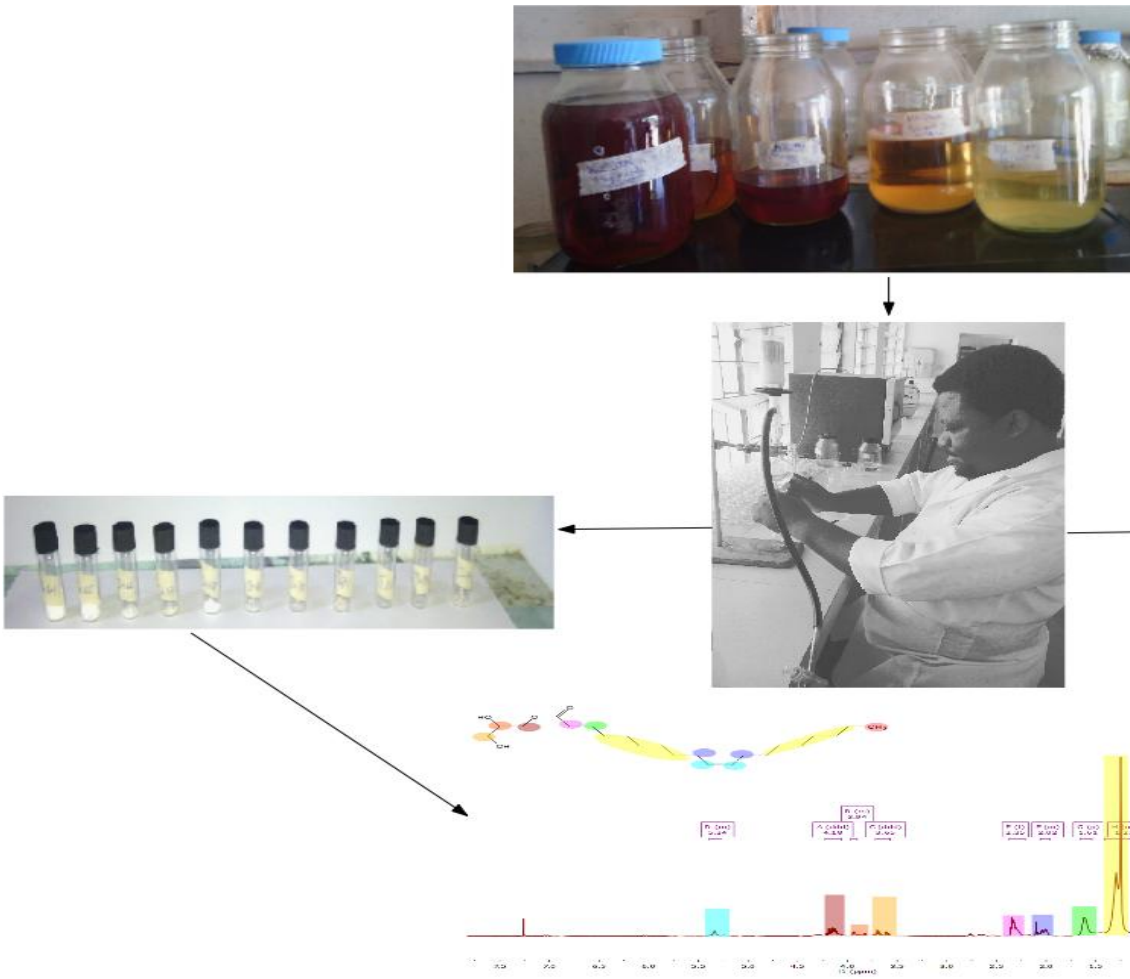
Appendix XXIX-Q. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF JSEA2

Proton NMR Integrals and Normalization for Determination of Average Chain Length (JSEA46)					
Assignment	Position (ppm)	JSEA46 Integra	JSEA46 Adjusted Integral	Normalized Integral	Total Degree of Unsatur
CH3 (G)	0.9	6277.21	697.47	0.32832249	
(CH2)n (F)	1.3	28475.2	14237.60	6.702136548	
CH2CH2(CO) (E)	1.6	4206.38	2103.19	0.990045132	
CH2CH=CHCH2 (D)	2.0	0	0.00	0	
CH2(CO) (C)	2.3	4290.97	2145.49	1.009954868	
CHCH2CH= (H)	2.8	0	0.00	0	
CH2O (B)	4.3	0	0.00	0	
CHO (B/4)	5.3	0	0.00	0	
CH=CH (A)	5.4	0	0.00	0	
Saturated Presence Test			Total Adjusted Proton	Average Proton Integral	Average Chain Length
			4248.68	2124.3375	10.0
ratio of 2.0: 0.8 <	1.333333333		29.90708131	0.01407831	
0	Saturated chain only				
			-21.15	447.2167563	
			21.15	447.2167563	
				447.2167563	
				21.1475	

Appendix XXIX-R. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF JSEA46

Proton NMR Integrals and Normalization for Determination of Average Chain Length (Ja 77)					
Assignment	Position (ppm)	Ja 77 Integral	Ja 77 Adjusted Integral	Normalized	Total Degree of Unsaturation
CH3 (G)	0.9	13520553.85	1502283.76	1.30	0.7
(***CH2***)n ***(F)***	1.3	47135263.29	7855877.22	6.79	
CH2CH2(CO) ***(E)***	1.6	5032625.23	838770.87	0.73	
CH2CH=CH***C***H2 ***(D)***	2.0	4408233.45	367352.79	0.32	
CH2(CO) ***(C)***	2.3	6870171.71	1145028.62	0.99	
CHC***H2***CH= ***(H)***	2.8	602269.48	100378.25	0.09	
CH2O ***(B)***	4.3	4564313.68	1141078.42	0.99	
CHO ***(B/4)***	5.3	1141078.42	1141078.42	0.99	
CH=C***H*** ***(A)***	5.4	2034487.23	339081.21	0.29	
Saturated Presence Test			Total Adjusted Proton	Average Pro	Average Chain Length
			4627161.67	1156790.418	14.085657
ratio of 2.0: 0.8 <	1.333333333		271346.8192	0.234568695	
0.32	Saturated chain present		345493.34	1.19366E+11	
			-318019.55	1.01136E+11	
			-11761.80	138339926.2	
			-15712.00	246866874.2	
				55221822199	
				234993.2386	

Appendix XXIX-S. SPREADSHEETS FOR SOME PERIPHERAL STRUCTURE PARAMETERS FROM ^{1}HNMR OF Ja77



Appendix XXX. SOME KEY STEPS IN A LONG WALK

"*The Universe is under no obligation to make sense to you*".

-Neil deGrasse Tyson