UNIVERSITY OF GHANA

COLLEGE OF BASIC AND APPLIED SCIENCES

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

ESSENTIAL OILS AND COMPOUNDS ISOLATED FROM THE LEAVES AND RHIZOMES OF *AFRAMOMUM ATEWAE*

BY

COFFIE ERIC

(10421123)

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF

MPHIL CHEMISTRY DEGREE

DEPARTMENT OF CHEMISTRY

JULY 2019

DECLARATION

I, Eric Coffie, hereby declare that this thesis was undertaken by me under the supervision and that it has not been submitted to any other university for another degree.

……

ERIC COFFIE

(10421123)

(Candidate)

……

PROF. DORCAS OSEI-SAFO

(Principal Supervisor)

……

PROF. HENOK KINFE

(Co-supervisor)

ABSTRACT

Aframomum species have been reported to be at an alarming rate of decline due to climate change and anthropogenic activities. This work investigated for the first time the chemical composition of *Aframomum atewae*, a less common species of the genus.

Essential oils of the leaves and rhizomes were obtained either through hydrodistillation or solvent extraction followed by chromatographic separation. GC-MS analysis of the constituents revealed that the fresh leaf essential oil was rich in monoterpenes (22.4%) while sesquiterpenes dominated the fresh rhizome essential oil (24.4%). Steroids and long chain hydrocarbons were the major constituents of the dichloromethane-extracted rhizome essential oil. The results for the petroleum ether-extracted rhizome essential oil are pending (due to sample mix up when submitted for GC-MS analysis). The major constituents identified in the three essential oils were 1-methyl-1-(methylamino)isobenzofuran-3-one (17.27%), 2,5-ditertbutylhydroxybenzene (7.80%) and 14β-pregnane (56.95 %) for the leaf, rhizome and DCM-extracted essential oils, respectively. To the best of my knowledge, this is the first time these compounds have been identified in the genus.

The antifungal potential of the 4 essential oils was evaluated against *Candida albicans* and *Saccharomyces cerevisiae* in an Alamar Blue-based broth dilution assay. The fresh rhizome and the DCM-extracted rhizome essential oils exhibited fungicidal activity against *C. albicans* while fungistatic activity was observed for the fresh leaf and the PE-extracted rhizome essential oils. With the exception of the PE-extracted oil which was fungistatic against *S. cerevisiae*, the remaining essential oils did not exhibit any activity against *S. cerevisiae*.

Crude PE, DCM and MeOH extracts of the air-dried pulverized rhizome were prepared by cold percolation. The PE and DCM crude extracts tested positive for terpenoids and steroids while the MeOH contained alkaloids, flavonoids, cardiac glycosides, saponins, and tannins. Through column chromatographic separations of the three extracts, 10 compounds were isolated, out of which 4 compounds were identified to be 1(E)-8-methylundec-8-en-1-yl-3-(cyclohexa-2,5-dien-1-yl)propanoate, 2-(6-oxotetrahydro-2H-pyran-2-yl)ethyl dodec-8-enoate, myristic acid and stigmasterol. Characterization of the compounds was achieved through IR, 1D- and 2D NMR, LC-MS and HR-MS techniques.

DEDICATION

To the glory of Almighty God, my parents, siblings and Ms. Princess Lucy Ocran.

ACKNOWLEDGMENT

All Glory, Honour and Adoration are due to God Almighty, for His guidance and protection throughout this work.

I am grateful to my supervisors, Prof. Dorcas Osei-Safo and Prof. Henok Kinfe for their guidance and assistance throughout this project.

I am very grateful to the South Africa-Ghana flagship program for funding this project.

My deepest appreciation goes to Dr. Patrick K. Arthur and his team especially, Michael Debrah, and Benaiah Abbey of the West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), Department of Biochemistry, Cell and Molecular biology, University of Ghana-Legon for their time and assistance in the antifungal studies.

My utmost appreciation goes to the Chemistry Department, (UG) for providing NMR and IR instruments and to Mr. Samuel Owusu-Atuah for his assistance in obtaining the IR data. My special appreciation also goes to Mr. Bob Essien who was always available to provide me with solvents needed for the work.

I appreciate the efforts of Mr. Henry Onyame and Mr. Samuel Kwain for their guidance in elucidating the structures of the compounds. I also appreciate the students and staff of the Department of Chemistry, (University of Johannesburg-South Africa, UJ) especially, Mr. Bruce Marakalala for welcoming and hosting me during my visit to UJ.

My family supported me both physically and spiritually. My parents, Mr. Simon Coffie and Madam Faustina Armah, to whom I owe all my gratitude in life, I pray for prosperity and good health. You are the best parents one can have. I am grateful to my brothers and sisters for their prayers, encouragement, love and assistance throughout my life.

Special appreciation also goes to the following: Elsie Otumfuo Korkor, Magdalene Dzigbordi Togoh, Maureen Agbagba, Elizabeth Ahiagba, Esther Barnes and the entire 2021 class of chemistry students – UG for their support.

I am very grateful to Ms. Emmanuella Bema Twumasi and my classmates for their assistance and company.

To all others whose names I have not mentioned, I am grateful to you all and God Almighty bless you.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF SCHEMES

LIST OF TABLES

ABBREVIATIONS

DCM	Dichloromethane
MeOH	Methanol
EtOH	Ethanol
PE	Petroleum ether
EtOAc, EA	Ethyl acetate
DMSO	Dimethyl sulfoxide
$CDCl_3$	Deuterated chloroform
ID	One dimensional
2D	Two dimensional
^{1}H NMR	Proton MNR
^{13}C NMR	Carbon 13 NMR
COSY	Correlation Spectroscopy
HSQC	Hetero-nuclear Single Quantum Coherence
HMBC	Hetero-nuclear Multiple Bond Correlation
IR	Infrared
GC-MS	Gas chromatography mass spectrometry
HR-MS	High resolution mass spectrometry
TLC	Thin layer chromatography
LC-MS	Liquid chromatography Mass spectrometry
Br	Broad
NB	Nutrient broth

CHAPTER ONE

1.0 INTRODUCTION

1.1 Medicinal plants

Africa is gifted with rich plant biodiversity including 45,000 species in sub-Saharan Africa[1]. A significant proportion of this natural resource comprises medicinal plants whose therapeutic properties have led to a long time of use as traditional remedies for both human and animal health. Several communities on the continent, as well as in Asia and Latin America, with over 80 % population of the world, still depend heavily on plant-based remedies for their principal healthcare needs. This high patronage is mainly due to accessibility to plant parts and the knowledge of their traditional use, affordability, ease of preparation and perceived safety. Lately, there has been a growing tendency of integrating plant-based medicines into mainstream health systems in many countries[2]. Ghana adopted this policy in 2010[2]. Further, the utilization of medicinal plants for the production of phytopharmaceuticals, food supplements, nutraceuticals, and cosmeceuticals are on the ascendancy. Through scientific and technological advancement, medicinal plants continue to serve as sources of either useful drugs or chemical scaffolds for the semi-synthetic development of new drugs in many drug discovery programs.

The increased interest in medicinal plant material and related products has resulted in a huge global trade at both domestic and international levels, providing a handsome source of income for producers, collectors and practitioners. The trade commodities include extracts, essential oils, phytopharmaceuticals, gums, spices and tannins. In nearly two decades, the total global trade value has more than doubled from $ 2.4 billion to $ 6.2 billion between 1996 and 2013 with a yearly progress percentage of 10.7 % registered in recent years[3].

Continued bioprospecting for new drugs and commercialization has resulted in increased use and high demand for medicinal plants. More often than not, the collection of wild species is conducted indiscriminately and in non-sustainable ways, threatening the future of this vital resource. The situation is worsened by effects of climate change (drought, extreme heat and erratic rainfall patterns) and habitat loss due to surface mining activities, construction and farming. Destruction or degradation of wildlands leaves in its wake loss of unique and precious species containing important cures for diseases we face now and new ones that may emerge in the future. This is critical because the chemical profile and therapeutic properties of many plants remain to be established to define efficacy and safety[4]. Overexploitation for commercialization purposes may also render useful traditional

medicinal plant resources inaccessible and unaffordable to local populations and to the rest of the world.

1.2 *Aframomum* Species

The *Aframomums* constitute a group of diverse plants distributed in West and Central Africa described by Schumann in 1904 to house the African species of *Amomum*. *Aframomum* is the major genus of the family Zingiberaceae, consisting of about 80 species including *A. melegueta, A. giganteum, A. sulcatum, A. danielli and A. longiscapum*. They have long been used ethnobotanically as spices and ornamental plants and are largely collected for a several medicinal applications such as laxative, fever management, inflammatory conditions as well as postpartum haemorrhage[5,6]. In Ghana, *A. melegueta* (peppery spice) was cultivated for many years as a cash crop and was exported to Europe and North America till its importance declined due to export restrictions during World War I and competition from cocoa [7]. The species are reported to be rich in terpenoids, flavonoids and arylalkanoids possessing antiplasmodial, antimicrobial, antioxidant and anticancer activities[5].

1.3 Fungi

A fungus is a collection of eukaryotic organisms that consist of mushrooms, moulds and yeasts. Though several fungi are known to be important and very useful, relatively few fungi species are phytopathogenic and produce toxins that cause diseases (infections and allergies) in man and animals. Harmful fungi present a common threat to the health of humans and agricultural production. These collective few fungi can cause loss of food for consumption, fatal diseases and huge economic losses to agriculture, in humans and animals[8-10]. Examples of infections that are caused by fungi in human are candidiasis, mucormycosis and blastomycosis whiles in plants there are diseases such as smut disease, leaf spot and chlorosis.

1.4 Problem statement

Aframomum species have been cited to be at an alarming rate of decline as a result of climate change effects, habitat destruction from various anthropogenic activities, especially illegal mining, and poor conservation practices. While many of the species have not yet received the requisite taxonomical attention, their pharmacological potential also remains largely under-researched regardless of their wide collection. Literature corroborates this observation with *A. melegueta* and *A. giganteum* characterizing much of the published research on the genus[5]. The rapid loss of this rich biodiversity has necessitated a call for a swift response not only to explore this huge resource for novel drug candidates to demonstrate the full utilization of their myriad of biological activities but also to adopt effective and sustainable measures to conserve the genus.

1.5 Justification

Aframomum atewae Lock & Hall is a less ordinary species of the genus. It is a herb that grows up to about 1.90 m high and has a white flower with indehiscent fruits[14]. It was first identified at the Atewa Forest Range in the Eastern Region of Ghana and was thought to be endemic to that locality. However, in follow-up surveys, it was found in several wetter forests in the country and in one location in Cote d'Ivoire[7]. It is known as '*sensam*' in Ghana, where the rhizome is boiled and drunk for constipation[5].

Several plants can resist infections by fungi present in their environments hence such plants can serve as a source of antifungal compounds. Recently, *Aframomum* plants are being exploited as sources of biodegradable fungicides in the control of several pathogenic fungi[5].

A thorough literature survey on Scifinder, PubChem, Chemspider, and other search engines revealed no documented research information on the species. Against the backdrop of risk of extinction and the need for systematic and targeted research on especially the less common species to unravel their medicinal potential, the current project focuses on the chemical and biological study of the leaves and rhizomes of *A. atewae.* The investigation was conducted as part of a South Africa-Ghana flagship project aimed at identifying gaps in the chemistry and ethnobotany in indigenous selected Ghanaian and South African taxa of the Zingiberaceae.

1.6 Hypothesis

Aframomum atewae may be a source of novel chemical constituents with potent biological activities.

1.7 Aim

To carry out a chemical examination of the leaves and rhizomes of *A. atewae*, evaluate the antifungal potential of the essential oils.

1.8 Objectives

1. To identify and collect samples of *A. atewae* from the Atewa Forest Reserve in the Eastern Region of Ghana.

2. To hydro distil essential oils from the fresh leaves and rhizomes.

3. To prepare nonpolar and polar extracts of the air-dried pulverized rhizomes.

4. To separate the constituents of the different extracts by chromatographic techniques.

5. To carry out GCMS analysis on the essential oils.

6. To elucidate the isolated compounds by means of spectroscopic (IR, NMR) and spectrometric methods (MS).

7. To screen the essential oils for antifungal activity against *Saccharomyces cerevisiae and Candida albicans.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Family Zingiberaceae

Zingiberaceae (Ginger family) is a family of flowering plants with nearly 50 genera and 1600 known species that are found mainly in the tropical parts of the world[3]. Members of the family are mainly used as a spice, ornamental or medicinal plants. Examples of genera that are used as ornamentals are *Alpinia*, *Globba*, *Hedychium* and *Renealmia* while the *Amomum*, *Aframomum*, *Myoga* and *Zingiber* are used as spices. Essential oils obtained from *Alpinia* and *Hedychium* are used in the perfume industry[11]. The species that are endemic to Africa are *Aframomum* and *Zingiber*.

Members of the family are perennial, terrestrial and aromatic herbs with rhizomes which have roots on them. Their stems are short and are replaced by leaf sheaths which form pseudostems. Their leaves are distichous and simple with leaf blade lanceolate to narrowly strap shaped. Flowers are bisexual with tubular calyx and corolla. Stamens are in two whorls with inferior ovaries and placentation is parietal, basal or axial. The style is found at the apex of the ovary and the fruits are capsule, fleshy and sometimes berrylike with few to many seeds[12].

Some members of the family are used in the production of dyes, as well as spices, perfume and medicine.

2.2 Genus *Aframomum*

Aframomum represents the African *Amomum*. *Aframomum* species are dispersed across tropical Africa and some islands in the Indian Ocean (Madagascar, Mauritius and Seychelles). While some species are found in different countries, for example, *A. danielli*, other species such as, *A. longiligulatum* is only found in Cameroon whereas the waterlogged forest of DR Congo River Basin is overshadowed by *A. pseudostipulare*. The genus consists of about 55 species and is one of the biggest genera of the family[12]. They are found in old fields and along roads with most species normally found in light opening and forest borders. They are perennial and aromatic when part is crushed. Their flowers are brightly coloured and have peduncles which are covered with sterile overlapping bracts[5]. Most species are used as toothache, laxatives and stomachache reliever, antidiarrhoea, antihelmintic, fever management, anti-inflammatory, postpartum hemorrhage management and tonic for sexual stimulation[13]. Also, there have been reports of anticancer, antiulcer, antimicrobial, antiplasmodial and hepatoprotective activities of certain species[14].

Several compounds have been isolated from many species since 1970 with their chemistry and biological activities reported[5, 15]. The types of compounds that have been isolated from several species include diterpenoids, sesquiterpenoids, arylalkanoids and flavonoids. About 12 species including *A. melegueta, A. sulcatum, A. danielli, A. giganteum* have extensively been studied chemically with labdane diterpenoids and flavonoids isolated from almost all these species[16, 17]. The less common species includes *A. atewae, A. chrysanthum, A. longiligulatum, A. longiscapum, A. sebericum,* and *A. strabica*. In Ghana, species such as *A. atewae* (Atewa forest range), *A. chrysanthum* (Atewa forest range), *A. stanfieldii* (Atewa forest range), *A. melegueta, A. longiscapum, A. cordifolium* (Bia forest reserve), *A sulcatum, A strobilaceum* (Ankasa forest reserve), and *A. geoscapum* are distributed in various part of the country.

2.3 Chemosystematics of *Aframomum* species

Chemosystematics is a way to group organisms, based on demonstrable similarities and differences through their biochemical compositions[15]. Basically, it is known that plants belonging to the equal family typically produce similar classes of compounds owing to the existence of comparable types of enzymes and thus comparable biosynthetic pathways[16]. The *Aframomum* species, as well as other members of the family Zingiberaceae are best known to produce flavonoids and labdane diterpenoids. Other classes of compounds comprise sesquiterpenoids and arylalkanoids[5]. From the twelve most chemically studied species of *Aframomum*, no less than eleven of them contain diterpenoids[19]. Flavonoids and labdane diterpenes may epitomize as the chemotaxonomic marker of the genus *Aframomum*. Nevertheless, there have been a few reports of sesquiterpenoids in some species of *Aframomum* from which includes *A. arundinaceum* is part of the [20,21].

2.4 Pharmacology of *Aframomum* Extracts

2.4.1 Anticancer activity

The methanolic extracts of *A. arundinaceum* seeds showed milled cytotoxicity against glioblastoma U87MGDEGFR and leukaemia CEM/ ADR5000 cells contrasted their corresponding sensitive equals U87MG and CEM/CEM cell lines[22]. *A. melegueta* chloroform and methanolic seed extracts demonstrate cytotoxicity when tested on PANC-1 pancreatic cancer cells in vitro with IC_{50} equal 50 47.8 µg/mL and 13.8 µg/mL, respectively[23].

2.4.2 Antimicrobial activity

The genus *Aframomum* is reputed to possess antibacterial activities, due to presence of terpenoids such as aframodial [7][27]. The hexane seed extract of *A. sceptrum* was reported to demonstrate the uppermost percentage inhibition of 60.26% against *Hypocrea lixii* (IMI 501885) whereas the ethanolic extracts have exhibited a percentage inhibition of 52.73% against *Fusarium oxysporum f. sp. elaeidis*. The methanol and acetone seed extracts showed the least percentage inhibitions of 42.31% against *H. lixii* and 42.45% against *F. oxysporum f. sp. elaeidis*, respectively [28]. *A. melegueta* has been reported to inhibit *Bacillus cereus* with an MIC of 31.25 mg/mL and was recommended to help in ensuring food safety[29].

2.4.3 Antiestrogenic activity

El-Halawany and Hattori (2012) reported that 100 µg/mL of *A. melegueta* (methanolic seeds extracts) inhibits 56.7 ± 3.4% of estrogenic activity in a yeast assay. Though *A. melegueta* outperformed other herbs in the study, its activity was low compared to the control, tamoxifen (78% inhibition at 10 µM)[27].

2.4.4 Penile erection function

Kamtchouing et al. 2002, reported that when 115 mg/kg of *A. melegueta* (aqueous seeds extract) is administered for eight days, it causes growth in the penile erection, genital sniffing and occurrence of genital grooming, and a rise in mounting frequency by 54% in male rats. There was an increase of 60% in ejaculation latency, whiles a decrease of 32% in intromission latency was observed, and while all these were greater than the control, they were less compared to the activity of *P. guineense*[28]. The same was detected to increase the secretions of epididymis and seminal vesicle, which are accessory sex organs[29].

2.5 Ethnobotanical use and Pharmacological action of selected species of *Aframomum*

2.5.1 *A. melegueta* K. Schum

The leaves, fruits and seeds of *A. melegueta* are normally used as spices in several food delicacies in Africa. Extracts of these plant parts are employed extensively in remedies against diabetes mellitus. In Nigeria, the fruit concoction (mostly done in alcoholic solution) is traditionally used in the curing of diabetes mellitus[30]. Pap containing African giant snail and *A. melegueta* seed is also employed in the treatment of diabetes mellitus. Additionally, a mixture made from *A. melegueta* leaf, pawpaw root and *Allium cepa* dried leaf is applied to cure diabetes mellitus and other metabolic sickness in

Nigeria[31]. A report by Sugita et al (2013) stated that alcoholic seed extract of *A. melegueta* rouses brown adipose tissue and surges body energy outflow in human subjects which is linked with the pathogenesis of type two diabetics (T2D)[32]. In other studies, *A. melegueta* extracts (fruit, leaf and stem) of different solvents established anti-diabetic and anti-oxidative potentials *in vitro*[33-35].

2.5.2 *A. danielli* (Hook. f.) K. Schum

A. danielli is used in flavoring traditional Nigerian dishes, relieving 'postpartum' pain [36] and as an anti-inflammatory agent by rubbing of the alcohol extracts on allergic and eczematous swellings[37]. It also impedes the growth of several bacteria and fungi and relieves thirst during fever[38]. *A. danielli* has been observed to inhibit the growth of *Aspergillus flavus, A. ochraceus, A. parasiticus, Salmonella enteriditis and Staphylococcus aureus*[39]. *A. danielli* have also been reported to prevent the growth of *Listeria monocytogenes* in a dose-dependent manner[40].

2.5.3 *A. citratum* (C. Pereira) K. Schum

A. citratum is used locally for the curing of bacterial infections, malaria, cancers and as an aphrodisiac[41]. Its leafy stem is used as a steam-bath against fever and intercostal pain while the seed is masticated and used as a tonic[42]. In 2011, it was reported that the methanol extract of the back of *A. citratum* is significantly active against several strains of *E. aerogenes, E. coli, K. pneumoniae, E. cloacae, P. aeruginosa* and *P. stuartii* with an MIC range of 256 - 1024 µg/mL[43].

2.5.4 *A. sulcatum* (Oliv. & Hanb.) K. Schum

A decoction of the seeds of *A. sulcatum* is used against umbilical hernia and as a purgative[42]. It is also used traditionally to treat fevers and widely used as anthelmintic in Cameroon[41].

2.5.5 *A. kayserianum* K. Schum

A. kayserianum is used in local medicine as a vermifuge, an anti-mumps and for menstrual cramps[45]. The methanol fraction was reported to have activity against *E. coli* AG100, ATCC 10536, W3110 and AG100 Atet; *P. stuartii* ATCC 29914, *K. pneumoniae* K24 and *E. aerogenes* EA289 (MIC of 64 µg/mL)[44]. Nonpolar acetone extract from the plant was stated to be the potent against various pathogens, such as *Schizosaccharomyces pombe, Saccharomyces cerevisiae, Sclerotinia libertiana, Candida utilis, Hansenula anomala, Penicillium crustosum, Rhizopus chinensis, Mucor mucedo, B. Subtilis, Aspergillus niger, S. aureus, P. aeruginosa* and *E. coli*[46].

2.6 Chemical components of *Aframomum* and their biological activities.

The *Aframomum* species are known to produce terpenoids, arylalkanoids and flavonoids.

2.6.1 Terpenoids

Terpenoids are naturally occurring compounds that occur as functionalized terpenes. Their functionalization is usually as a result of the presence of oxygen. Most are multicyclic structures which obey the isoprene rule. About 60% of known secondary metabolites are terpenoids[47]. Terpenoids consist of hemiterpenoids (C_5), monoterpenoids (C_{10}), sesquiterpenoids (C_{15}), diterpenoids (C_{20}), sesterterpenoids (C_{25}), triterpenoids (C_{30}) and tetraterpenoids (C_{40}).

The leaves and rhizomes of many *Aframomum* species are rich in diterpenoids and relatively few sesquiterpenoids. The essential oils however are reported to contain monoterpenes and sesquiterpenes. The main constituents of the essential oil from the flower of *A. danielli* are α-terpineol [1] (21.2%), 1,8-cineole [2] (18.6%), caryophyllene oxide [3] (14.4%), β-caryophyllene [4] (11.2%), l-linalool [5] (6.1%), and α-humulene [6] (5.6%)[36]. The monoterpenes of *A. danielli* have been reported to show antimicrobial potency on some microorganisms. For example, α-terpineol is said to reduce the population of *Candida tropicalis*[39]. Additionally, (+)-limonene, also found in the essential oil of the plant has a MIC of 78 µg/mL against *A. parasiticus*. The essential oils from the leaf and flower of *A. danielli* have antibrowning effect when used for cereal preservation[47].

From *A. citratum*, the seed essential oil contains compounds such as α-thujene [7], camphene [8], phellandrene [9], terpinen-4-ol [10], myrtenol [11], 6-methyl-5-hepten-2-one [12] and trans-sabinyl acetate6 [13][41]. (**Figure 2.1**).

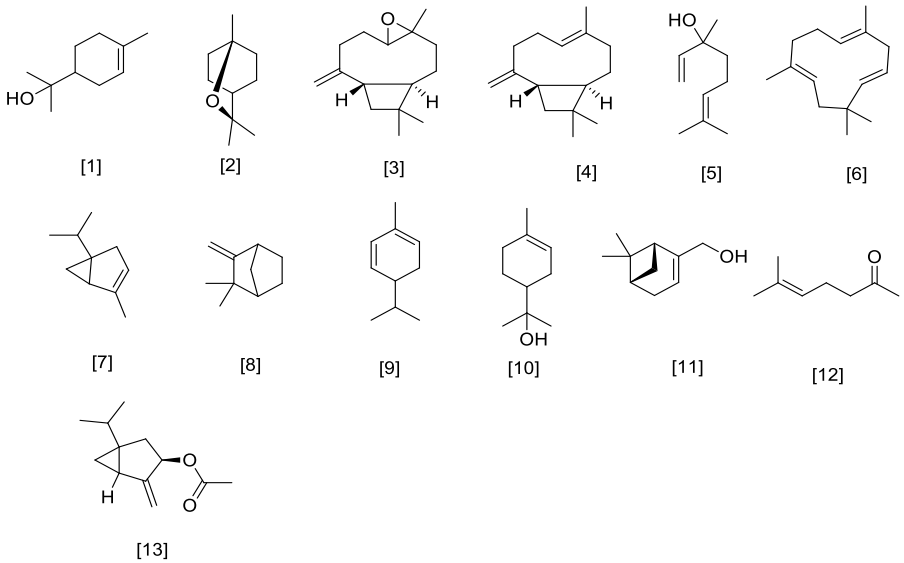


Figure 2.1: Some essential oil constituents of *Aframomum* species

A few sesquiterpenes have been isolated from some species of *Aframomum*, these are (β)-S-nerolidol [14] isolated from *A. sceptrum* and *A. escapum*, (-)-α–bisabolol [15] and 6,7-epoxy-3(15)-caryophyllene [16] found in *A. arundinaceum*, [49-51] (**Figure 2.2)**.

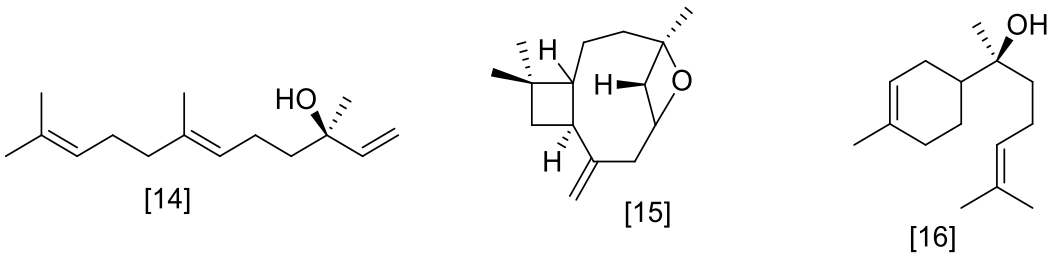
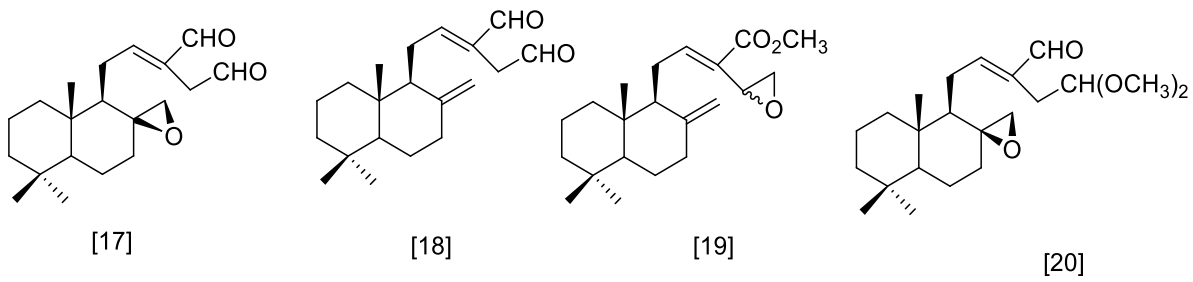


Figure 2.2: Sesquiterpenes from some species of *Aframomum*

Diterpenes containing acid and aldehyde functionalities have also been reported in several species of *Aframomum* including *A. danielli*, *A. melegueta and A. sulcatum*. They include aframodial [17], 8β-17-epoxy-12E-labdene-15,16-dial [18], methyl-14,15-epoxy-8(17),12(E)-labdadiene-16-oate [19] and 8β(17)-epoxy-15,15-dimethoxylabd-12(E)-en-16-al [20]. Aframodial [17] was first isolated from *A. danielli* and it is known to be cytotoxic[32], the same compound from the seed of *A. sulcatum* was tested to be antihypercholesterolemic[5]. The aldehyde, 8β-17-epoxy-12E-labdene-15,16-dial [18] was reported to exhibit strong antifungal activity against *Candida albicans*[48,49]. Other diterpenoids include aulacocarpinolide [21], aulacocarpin A [22] and aulacocarpin B [23] from *A. aulacocarpos.* Also, galanolactone [24], sulcanal [25], galanal A [26], galanal B [27] and 11,15-epoxy-15-hydroxy-8(17),12-labdandien-16-al [28] have been isolated from *A. sulcatum*. Galanolactone, galanal A and B have been demonstrated to possess antifungal, cytotoxic and antiplasmodial activities[52]. Also, galanal B shows activity towards breast adenocarcinoma MDA-MB-231/BCRP cells but are less active towards resistant cancer cells[19]. There have also been report of moderate cytotoxicity of galanals A and B with IC_{50} of 18 µM and 32 µM, respectively to human T lymphoma Jurkat cells has also been reported[55] (**Figure 2.3)**.



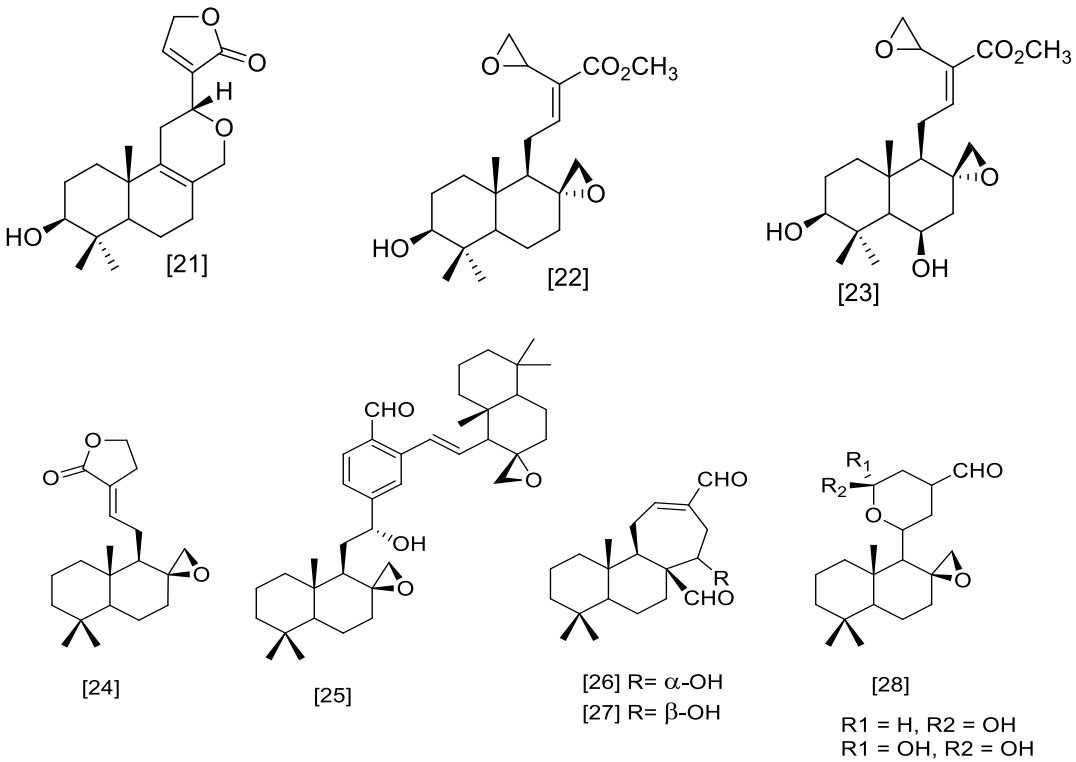


Figure 2.3: Diterpenes from some species of *Aframomum*

Diterpenoids and sesquiterpenoids are known to show activity against fungi, bacteria, protozoa and virus. 1(10)E,5(E)-germacradien-4α-ol, 5E,10(14)-germacradien-1 β,4β-diol and sesquiterpenoids oplodiol with respective IC_{50} values of and 1.54, 1.63 4.17 µM are some active metabolites that have been isolated from *Aframomum species*[53,54].

2.6.2 Flavonoids

Flavonoids are metabolites in plants and are accountable for the most important plant pigments for flower coloration. Antioxidant activity of plant extracts tends to increase when there are high concentrations of flavonoids and phenolics present and some vital structural feature, for instance, the arrangement and the number of hydroxyl groups, the presence of electron-donating/accepting substituents on the ring and the extent of structural conjugation of the ring[58]. Due to their effectiveness, inhibition of several oxidative trauma linked ailments such as cancer, plant phenols have gained increasing attention[5]. Flavonoids are hydroxylated phenolic compounds that have exhibited good potential in the stoppage of cardiovascular disease and potent against several microbes such as polio type 1 and Coxsackie B4 viruses[56,57]. Compounds such as paradol [29], shogaol [30] and gingerol [31] identified in *A. melegueta* EtOH seed extract have been reported to exhibit toxicity after a 4-week treatment in rats against diabetes[59]. In preliminary studies, seed and leaf aqueous extracts of *A. melegueta* have exhibited blood glucose dropping activity in alloxan diabetic rats[60,61].

Kaempferol [32] and quercetin [33] isolated from *A. giganteum* by Vidari et al. 1971, were reported to show antibacterial activities[62]. They also demonstrate potent anti-inflammatory and antiviral activities. Further to that, kaempferol and quercetin prevents the release of histamine in rat mast cell, and are also good radical scavengers[63,64]. Methylated derivatives of quercetin were reported to show activity against Coxsackie B4 viruses and polio type 1, both *in vivo* and *in vitro* [44] (**Figure 2.4**).

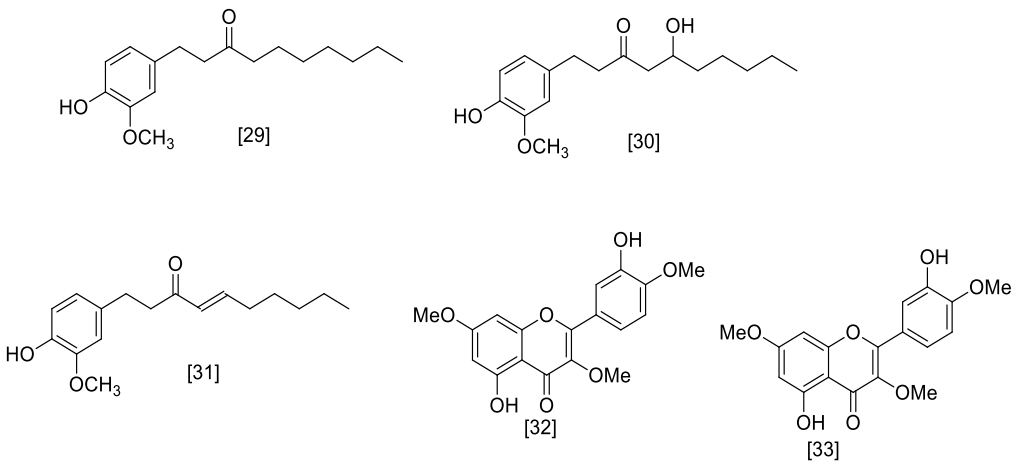


Figure 2.4: Flavonoids from some species of *Aframomum*

2.6.3 Arylalkanoids

Diarylheptanoids are groups of secondary metabolites which bear a 1,7-diphenylheptane skeleton as a unique character[5]. Haining and She (2012) grouped them into linear and cyclic diarylheptanoids[65]. Diarylheptanoids shows wide bioactivity such as estrogenic, anti-inflammatory, antioxidant, leishmanicidal, antihepatoxic, neuroprotective, melanogenesis, antitumour, antibacterial and recently a report on their inhibitory potency against NO production in activated murine macrophages[66].

Tane et al, 2005 isolated (4Z,6E)-5-hydroxy-1,7-bis(4-hydroxyphenyl)hepta-4,6-dien-3-one [34], letestuianin A [35], B [36] and C [37] from the seed of *A. letestuianum* (**Figure 2.5**).

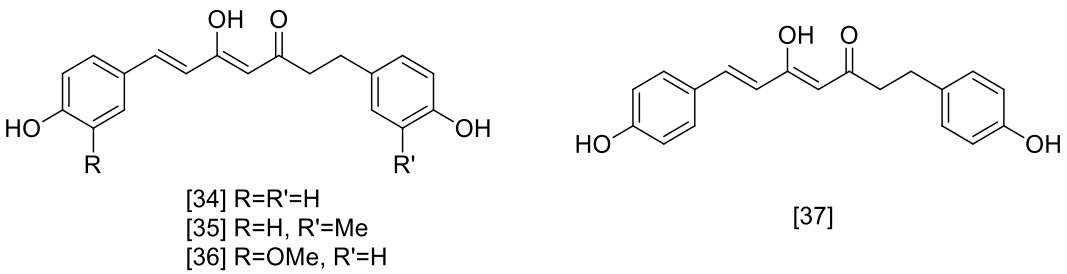


Figure 2.5: Arylalkanoids from some species of *Aframomum*

A summary of compounds that have been isolated from various species of *Aframomum* with their biological activity is presented in **Table 2.1** below.

Table 2.1: Summary of *Aframomum* species, isolated compounds and their biological activities.

SPECIES	COMPOUNDS	BIOLOGICAL ACTIVITY
	Arylalkanoids	
A. letestuianum	(4Z,6E)-5-hydroxy-1,7-bis-(4-hydroxyphenyl)hepta-4,6-dien-3-one [32] Letestuianin A [33] Letestuianin B [34] Letestuianin C [35]	Anti-inflammatory Antihepatoxic Antifungal, Antibacterial Antitumour
A. melegueta	Gingerdione 6-Paradol [31] 6-Shogaol [30] trihydroxyphenyl)heptanes 3-(s)-Acetyl-1–(49-hydroxy-3',5'-dimethoxyphenyl) -7-(3'',4'',5''- (4Z,6E)-5-Hydroxy-1,7-bis-(4-hydroxyphenyl)hepta-4,6-dien-3-one 6-Gingerol [29]	Termite antifeedant Antibacterial Antifungal
	Flavonoids/phenols	
A. handburyii, *A. sceptrum*	3,5,7,4-Tetrahydroflavone	
A. pruinosum	3-Acetoxy-5,4-dihydroxy-4-methoxyflavone 3,5-Dihydroxy-7,4-dimethoxyflavone	
A. letestuianum *A. pruinosum* *A. handburyii*	3-Acetoxy-5,4-dihydroxy-7-methoxyflavone	
A. sceptrum *A. pruinosum* *A. handburyii* *A. letestuianum*	3-Acetoxy-5,7,4-trihydroxyflavone	
A. giganteum	kaempferol-3,7,4-trimethylether Quercetin-3,7,30,4-tetramethylether Quercetin-3,7,4-trimethylether	Antibacterial, Antiviral, Anti-inflammatory
	Terpenoids	
A. arundinaceum	6,7-Epoxy-3(15)-caryophyllene [30] (-)-α-Bisabolol	Antiplasmodial
A. sceptrum *A. escapum*	(+)-S-Nerolidol	
A. zambesiacum	5E,10(14)-germacradien-1β,4β-diol	
A. longifolius	Hemiacetal 15-hydroxy-15- methoxylabda-8(17),12(E)-dien-16-al	
A. arundinaceum *A. danielli*	Methyl 14,15-epoxy-8(17), 12(E)-labdadiene-16-oate [9] Acetal 8β(17)-epoxy-15,15-dimethoxylabd-12(E)-en-16-al	
A. latifolium	Galanals A [26] Galanals B [27] Narigenin 1E,5E-germacradien-4α-ol Norbislabdane sulcanal 12(E),8β(17)-Epoxy-11-hydroxy-12-labden-15,16-dial-11,15-hemiacetal	Antiplasmodial
A. arundinaceum *A. sulcatum*	Galanolactone	Antifungal, cytotoxic

A. sceptrum	8β(17)-Epoxy-3 β,7β-dihydroxylabde-12(E)-en-16,15-olide Methyl 8 β (17)-epoxy-3 β,7 β,15-trihydroxylabd-12(E)-en-16-oate 3 β,7 β,8 β,12γ,17-pentahydroxylabdan-16,15-olide Coronarin B	Antiplasmodial Tripanosomial
A. sceptrum *A. longifolius* *A. danielli*	Labda-8(17),12-dien-15,16-dial	Antifungal, Cytotoxic Antiplasmodial
A. aulacocarpos	Aulacocarpinolide Aulacocarpin A Aulacocarpin B	Antibacterial Antiplasmodial
A. polyanthum *A. keysenanum* *A. masuianum* *A. arundinaeum* *A. sulcatum* *A. longifolius* *A. latifolium*	Aframodial [14]	Cytotoxic Antihypercholesterolemic

2.7 Fungal infections

Infectious diseases are caused by microorganism. Infectious diseases are one of the principal causes of illness and death in the emerging countries [67].

Fungal infections are very common in plants and humans. Some fungal infections do not threaten life but distress the value of life while invasive fungal infections (IFIs) are life-threatening. Serious fungal infections (SFIs), including significant chronic infections, complicated mucocutaneous infections and IFIs influence the quality of life[67]. It has been reported that 4% of Ghanaians are plagued from SFIs annually, with over 35,000 affected by life-threatening IFIs.

The common symptoms of fungal infections include red and possibly cracking, peeling skin or itching. Examples of infectious fungal diseases in human include *Tinea pedis*, ringworm, yeast infection (candida), etc. *Candida albicans* is the dominant causative organism for fungal infections such as oropharyngeal candidiasis[67].

In plants, fungal infections can lead to loss of vegetation which can result in huge economic losses and loss of food for consumptions. There are several plant infectious diseases caused by fungi in Africa and the world.

2.7.1 Control

Even though there is a consensus about the best strategy to control fungal disease, prevention and control strategies are based on improving one's lifestyle and being educated on the extent and effect of some fungi found in our surroundings.

2.7.2 Treatment of fungal infections

Treatment of fungal infections depends on their harshness. Normal preventions include tablets, creams, or suppositories, which are obtained via prescription. In Ghana, plant-based creams such as mercy cream, joy ointment, etc. are used for fungal infections such as ringworm and vaginal candidiasis. Also, antifungal treatment drugs such as fluconazole [38], itraconazole [39], topical miconazole [40], topical nystatin [41], griseofulvin [42], and terbinafine [43] (**Figure 2.6**) are prescribed upon diagnosis[67].

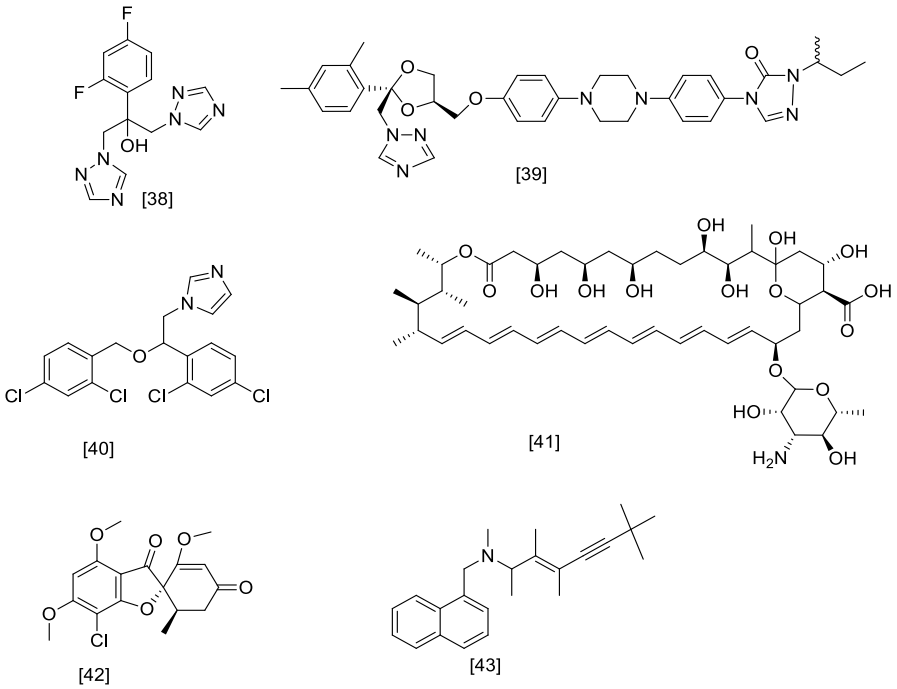


Figure 2.6: Examples of clinically used antifungal agents

2.7.3 Natural products as antifungal agents

Though there are several synthetic fungicides that are used on several plants, these molecules persist in the environment. Hence, they pose several risks to plants, individuals and the environment. Persistent use of synthetic fungicides can cause phytotoxicity among plants, death of aquatic animals, as well as irritation of skin/eyes and respiratory problems of individuals who handle them[68]. These

and other reasons are why there is the need to research into natural products as a source of fungicides due to their biodegradability.

A variety of compounds with antifungal activity against several strains of fungi have been isolated from plants and are vital to humans in the prevention of diseases. These compounds can be used directly or as a forerunner for developing better molecules[68].

3-hydroxy-4-geranyl-5-methoxybiphenyl [44], obtained from the fruits *Garcinia mangostana* was reported to have strong antifungal activity[69]. Steroidal saponins isolated from the roots of *Smilax medica*, showed antifungal activity when tested against the human pathogenic yeasts *C. tropicalis*, *C. albicans*, and *C. glabrata* in the range of 25 – 50 mg/mL[70]. Phytolaccosides B and E [45] from *Phytolacca tetramera* exhibited antifungal activities on a panel of human pathogenic opportunistic fungi[71]. 2',4'-dihydroxychalcone [46] and 2',4'-Dihydroxy-3'-methoxychalcone [47] isolated from the DCM extract of *Zuccagnia punetata* ecchibited minimal antifungal activities against the yeasts *C. albicans*, *C. neoformans* and *S. cerevisiae* having MIC values of 62.5–250 mg/mL[72].
Antifungal activities of several compounds that have been isolated from *Aframomum* have been examined. Examples include galanolactone, labda-8(17),12-dien-15,16-dial, aframodial, 6-gingerol, as well as reports on antifungal activities of essential oils obtained from *A. melegueta* and *A danielli*[5]. Due to many terpenes found in *Aframomum* species, their antifungal activity has been attributed to these compounds[5].

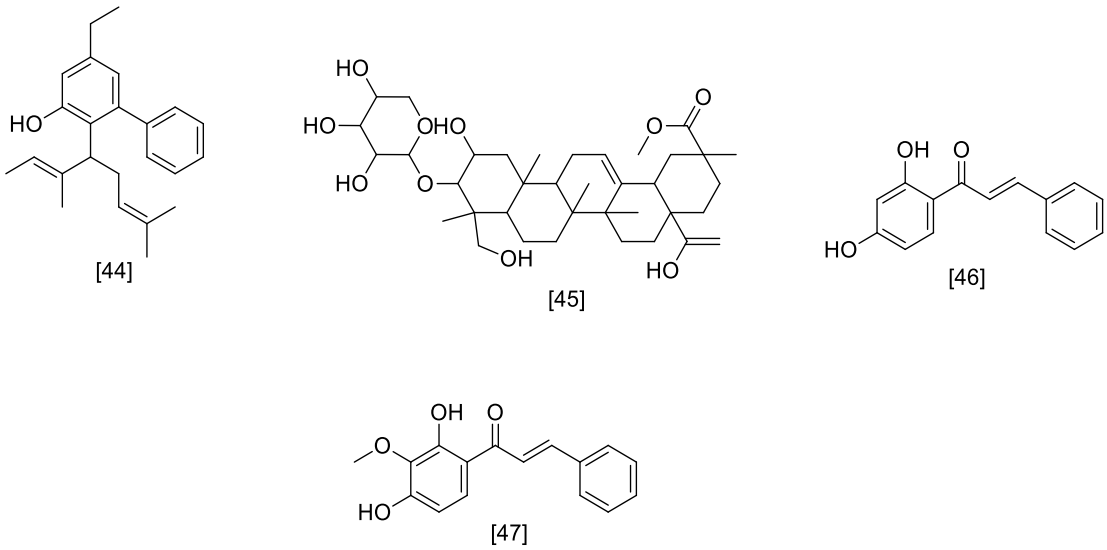


Figure 2.7: Natural products with antifungal activity

CHAPTER THREE

3.0 METHODOLOGY

3.1 Plant collection

Samples of *Aframomum atewae* (**Figures 3.1a and 3.1b)** were collected from the Atewa range Forest Reserve, Sekyemase in the Eastern region of Ghana (**Figure 3.1c)** on Saturday, October 20, 2018. It was identified by Mr. Jonathan Dabo of Ghana Forestry Commission. Its white flower, with a purple tinge, and glabrous leaves distinguished it from *A. chrysanthum* which is more abundant at lower altitudes of the range. *A. chrysanthum* bears yellow flowers and the leaves are slightly rough on both sides. A voucher specimen of *A. atewae* (DJ2018-29) has been kept at the Forestry Research Institute of Ghana, Centre for Scientific and Industrial Research (CSIR-FORIG) Herbarium, Kumasi.

Figure 3.1a: Flower of *Aframomum atewae*

Figure 3.2b: Leaf and rhizome of *Aframomum atewae*

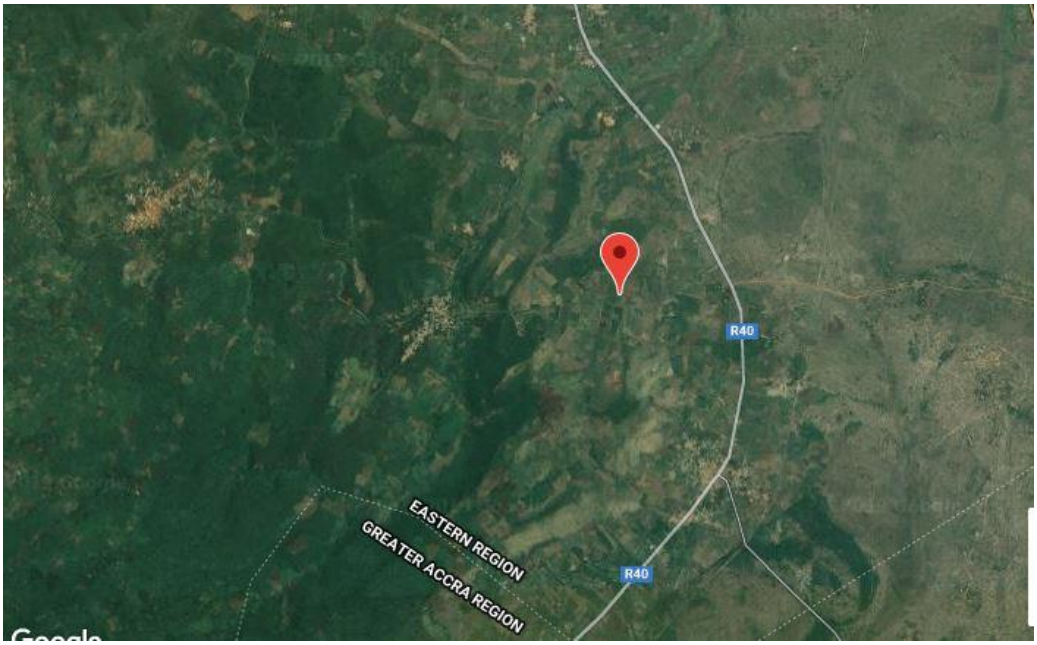


Figure 3.1c: Map showing site of sampling of *A. atewae*

3.2 General experimental methods

Essential oils (EO) were hydrodistilled for 12 hours from fresh rhizomes and leaves using the Clevenger apparatus. Other constituents were obtained by successive exhaustive percolation of the

air-dried pulverized rhizome using petroleum ether (PE), dichloromethane (DCM) and methanol (MeOH). Each extraction was done at least four times at 24 hours interval. The extracts after each cycle were filtered and concentrated to dryness under reduced pressure on G3 Heidolph Rotary Vacuum Evaporator. The recovered solvent was passed through magnesium sulfate ($MgSO_4$) to get rid any traces of water present in the recovered solvent before it was reused for the next extraction. Each extract obtained was column chromatographed (CC) using silica gel 60 (sigma – aldrich; 130-270 mesh) as the stationary phase. Elution was done with PE and the polarity of the mobile phase increased gradually using ethyl acetate (EtOAc). Thin layer chromatography (TLC) using aluminum foil pre-coated with silica gel (0.2 mm thick kieselgel $60F_{254}$ Merck type) was employed to check the progress of the column chromatography.

Visualization of TLC spots was done by ultraviolet (UV) light (Spectroline model ENF-240C/ FE UV lamp; 254-365 nm), acid stain and anisaldehyde spray reagent. The melting point of pure isolates was determined with Stuart Scientific SMP 10 melting point apparatus. Isolated compounds were analyzed by their appearance and retardation factor (R_F) on TLC in different solvent systems. Nuclear Magnetic Resonance (NMR) spectroscopy 1D and 2D data were acquired on a Brüker Ascend 500 MHz spectrometer at the Department of Chemistry, University of Ghana. The internal reference used was Tetramethylsilane (TMS) and $CDCl_3$ was used as a solvent to obtain spectra of samples. Infrared (IR) spectra were obtained on a Perkin-Elmer FTIR spectrometer by means of Attenuated Total Reflectance which allows the sample to be analyzed directly in the solid-state without further preparation. The masses of the compounds were determined using either LC-MS or HR-MS.

LC separations were carried out on Kinetex Core C18 column (2.6 µM, 3 x 50 mm, 100Å) maintained at 40 °C with 10 mM NH_4OAc in 90% CH_3OH in H_2O. HR-MS data was acquired after separation by LC (AQUITY UPLC H-class Waters Corporation) coupled to a SYNAPT G2-Si High Definition mass detector. Column type was AQUITY UPLC BEH C18 (1.7 µm, 2.1 x 50 mm) employing a flow rate was 0.5 mL/min. The MS was operated under electrospray ionization (ESI) and Atmospheric pressure chemical ionization (APCI).

The constituents of the essential oils were characterized using GC-MS. The separation was achieved on a gas chromatograph (6890N, Agilent technologies network) fixed to an Agilent technologies inert XL EI/CI Mass Selective Detector (MSD) (5975B, Agilent Technologies Inc., Palo Alto, CA). Separation of essential oil volatiles was done on a polar STABILWAX (60 mm, 0.25 mm ID, 0.25 µm film thickness) capillary column. The carrier gas used was helium at a flow rate of 2 mL/min. The injector temperature was maintained at 240 °C. One µL of the samples was injected in splitless mode. The oven temperature was programmed as follows: 45 °C for 3 min and ramped up to 250 °C

at a rate of 6 °C/min held for 5 minutes. The MSD (Mass Spectrometric Detector) was operated in a full scan mode and the source and quad temperatures were maintained at 230 °C and 150 °C, respectively. The transfer line temperature was maintained at 250 °C. The mass spectrometer was operated under electron impact mode at ionization energy of 70 eV, scanning from 25 to 450 m/z. The constituents were characterized by their mass spectra. The mass spectra were compared with Wiley mass spectral library.

The antifungal activity of the EO was evaluated against the common *Saccharomyces cerevisiae* and *Candida albicans*, utilizing an Alamar Blue-based broth dilution assay.

3.3 Chemicals and Reagents

3.3.1 Anisaldehyde spray reagent

About 5 mL of conc. H_2SO_4 was added to 135 mL of absolute ethanol. Glacial acetic acid (1.5 mL) and 3.7 mL *p*-anisaldehyde were added to the solution and shaken dynamically and cooled. It was then preserved in an amber bottle and placed in a fridge. The sprayed TLC plates are heated at 110 ^oC for about 5 minutes.

3.3.2 Acid spray

About 10 mL of H_2SO_4 was dissolved in 90 mL of absolute ethanol. The sprayed TLC plates are heated at 110 ^oC for about 5 minutes.

3.3.3 Wagner's Reagent

A mass of 2.00 g of potassium iodide (KI) and 1.28 g of iodine (I_2) were dissolved in a minimum amount of water in a 100 mL volumetric flask and the solution was shaken and topped up to 100 mL. The formation of a brown precipitate (ppt) when a few drops of the reagent is added to an acidified test solution is a sign of the presence of alkaloids.

3.3.4 Mayer's Reagent

Mercuric iodide (HgI_2) of mass 1.36 g in 60 mL of deionized water was added to a 10 mL solution of 5.01 g of potassium iodide (KI) in a 100 mL flask and topped up to 100 mL. The formation of a

yellow ppt when a few drops of this reagent is added to a test mixture is diagnostic of the presence of alkaloids.

3.3.5 Dragendoff's Reagent

Hydrated bismuth nitrate ($BiNO_3.H_2O$) of mass 8.04 g was liquified in 20 mL of concentrated nitric acid and the resulting mixture was added slowly and gently to a mixture of 27.23 g of KI in 50 mL of deionized water while stirring. The mixture was left to stand and then filtered. The filtrate was transferred to a 100 mL volumetric flask and filled to the 100 mL mark. Two or three drops of this reagent when added to an acidified test solution containing alkaloids results in the formation of a reddish-brown ppt.

3.3.6 Iron (III) chloride solution

A volume of 8.5 mL of $FeCl_3$ was measured into a 100 mL flask and filled to the mark with deionized water. The formation of green colour indicates the presence of tannins and phenolic compounds when two to three drops of the solution are added to about 3 mL of a test solution.

3.3.7 Liebermann-Burchard Reagent

Acetic anhydride of volume 50.0 mL and 5.0 mL of conc. H_2SO_4 were carefully mixed in the fume chamber while cooling with ice. The mixture was added gently and carefully to 50 mL of absolute EtOH while cooling in ice. The presence of terpenoids in an extract is indicated by the observance of pink or red spots when this reagent is sprayed onto a TLC plate after development followed by heating in an oven at a temperature of 110 $^{\circ}$C for 15 minutes and then examined under a UV lamp.

3.4 Essential oil distillation of rhizomes and leaves

About 780 g and 360 g of the fresh rhizomes and leaves, respectively were used for the essential oil distillation. Both plant parts were cut into pieces and placed in a round bottomed flask. A substantial amount of water was added to the material in the round bottomed flask and was placed on a heating mantel. The Clevenger apparatus was fixed on the round bottomed flask, clamped for support and connected to a water recirculating chiller. The distillation was done for 12 hours and the essential oils

that were produced were pipetted using a Pasteur pipette. The essential oils were dried using anhydrous $MgSO_4$.

Approximately 1 mL of DCM was added to the samples, sonicated overnight and analyzed by GC-MS.

3.5 Antifungal activity

The fungicidal and static effects of the EO were determined with the metabolic fluorescent sensor Alamar Blue (resazurin) that measures residual metabolic activity to determine cell viability. Alamar Blue, a blue non-fluorescent dye, is reduced to the pink-colored, highly fluorescent resorufin with metabolic activity of the fungal cells. In the presence of antifungal agents, the fluorescence signal yields are significantly reduced, suggesting a reduction in metabolic activity.

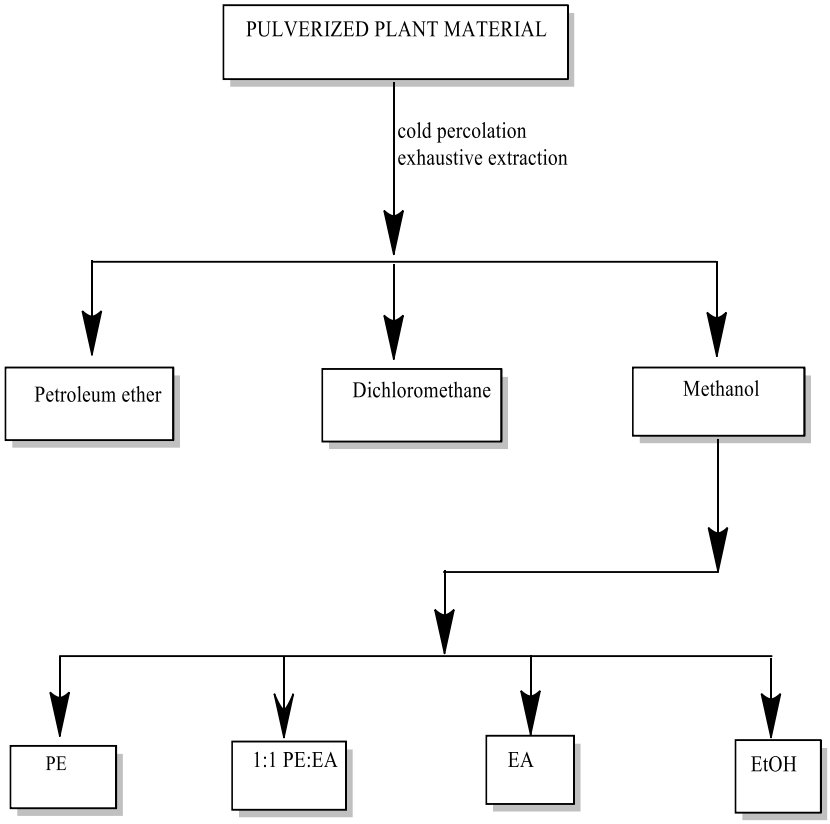
Stock solutions of the essential oils were prepared by carefully weighing about 1 µg of each oil and dissolving it in 100 µL of hexane. Approximately 25 µL of each stock solution was loaded into a 96-well plate and allowed to dry. Ninety µL of nutrient broth (NB) was then added to the plate followed by addition of 10 µL of *S. cerevisiae* and *C. albicans* cells to separate wells. The plates were incubated at 30 °C for 24 hours, after which each well was stained with Alamar Blue and observed for fluorescence intensity mode (excitation = 525 nm, emission = 598 nm) on a Varioskan^{TM} LUX microplate reader.

The negative control consisted of 100 µL of NB only, with neither cells nor essential oil in the 96 well-plates, whereas the positive control contained 90 µL of NB + 10 µL of the cells without the essential oil. Test solutions were determined to be fungistatic when the fluorescent intensity was 50% or less than that of the positive control. On the hand, when the observed fluorescence was less than that of the negative control, the test solution was deemed to be fungicidal to the cells. An observed fluorescent intensity that was greater than that of the positive control implied no antifungal activity.

3.6 Solvent extraction of rhizomes

The air-dried pulverized rhizome of the plant (1.86 kg) was extracted successively with PE, DCM and MeOH by cold percolation to get the corresponding crude extracts. The TLC profile of the MeOH

crude extract indicated many unresolved spots. Therefore, to facilitate separation about 40 g of the extract was fractionated by flash chromatography with PE, EtOAc and EtOH (**Scheme 3.1**).



Scheme 3.1: Preparations of extracts and fractions of *A. atewae*

3.7 Phytochemical screening procedures

Approximately 0.2 g of the PE, DCM and MeOH extracts were weighed into a beaker and dissolved in 80% EtOH for phytochemical screening.

3.7.1 Keller-Kiliani Test for Cardiac Glycosides

About 2 mL of EtOH extract was measured into a test tube and 2 mL of glacial acetic acid was added. A drop of $FeCl_3$ and then 1 mL of conc. H_2SO_4 were added cautiously down the wall of each test tube. Development of a brown ring indicates a positive of cardiac glycosides.

3.7.2 Test for Anthraquinones and Anthracene Derivatives

About 5.0 mL EtOH extract was measured into a boiling tube. The tube was heated in a water bath for 10 min. The test solution was then transferred into a separatory funnel and shaken vigorously with 4 mL of benzene and allowed to equilibrate. The benzene layer was treated with 1.5 mL of conc. NH_3 and allowed to stand. A positive test for anthraquinones and anthracene derivatives was confirmed by a red ppt in the NH_3 layer.

3.7.3 Test for Tannins

About 2 mL of EtOH extract was measured into a test tube. Freshly prepared $FeCl_3$ solution was added to the solution. The formation of a dark greenish colouration signposted the presence of tannins.

3.7.4 Test for Saponins

Deionized water was added to 0.01 g of the extract in a test tube and shaken vigorously. Formation of a persistent foam which disappeared after the solution was left to stand for a while confirmed the presence of saponins.

3.7.5 Test for Terpenoids

TLCs of the crude extracts were developed in 7:3 and 8:2 PE: EtOAc solvent systems. The plates were left to dry and then sprayed with Liebermann-Burchard reagent. They were then heated in an oven at 110 $^{\circ}$C for 15 minutes and examined under a UV lamp. The presence of a pink spot indicated that terpenoids were present in all extracts.

3.7.6 Test for alkaloids

About 4 mL EtOH extract was measured into a boiling tube and 10 mL of 2 M HCl solution was added. The solution was warmed on a water bath and filtered. The filtrate was divided into three test tubes labelled A, B and C. To portion A, B and C Mayer's reagent Dragendoff's and Wagner's reagent were added, respectively. The presence of a brown ppt confirmed the presence of alkaloid.

3.7.7 Test for Flavonoids

About 2 mL of EtOH extract was measured into 3 test tubes; A, B and C. Test tube A was used as control. To test tube B, 3 pieces of boiling chips were added followed by 0.5 mL of conc. HCl and observed for any colour changes after warming. 0.5 mL of conc. HCl was added to test tube C and warmed for 5 minutes on a water bath. The change of colour in test tubes B and C indicated the presence of flavonoids.

3.7.8 Test for steroids

About 2 mL of acetic acid was added to 1 mL of the EtOH extract in a test tube. The solution was boiled and cooled, then drops of conc. H_2SO_4 were added on the wall of the test tube. A brown ring at a junction between the two layers indicated the presence of steroid.

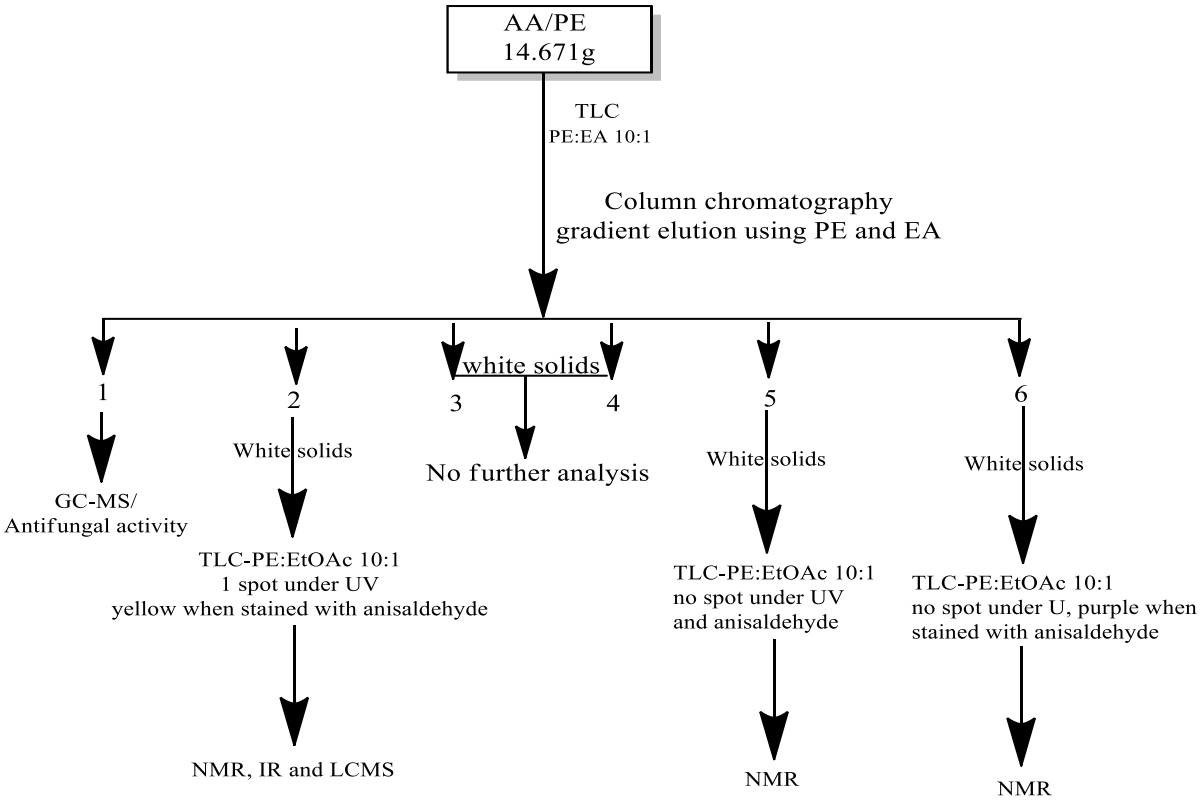
3.7.9 Test for polyphenols

About 2 mL of the EtOH extract was measured into a test tube and 3 drops of 10% aqueous $FeCl_3$ and 3 drops of $K_4[Fe(CN)_6]$ were added. Formation of a blueish colour indicated the presence of polyphenols.

3.8 Investigation of the extracts

3.8.1 Petroleum ether (40-60 ˚C) extract

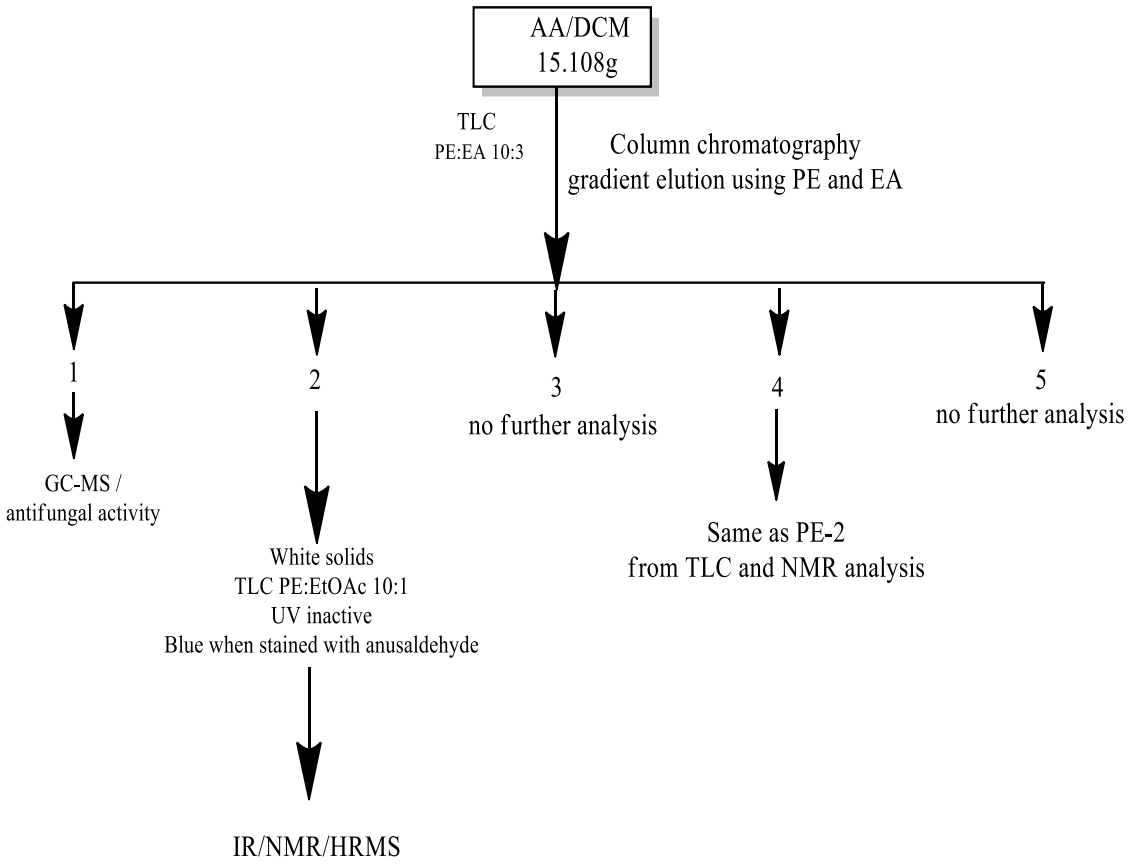
The petroleum ether extract was labelled as AA/PE. A total of 14.671 g was loaded onto a glass column pre-loaded with silica gel (180 g). The column was first eluted with 100% PE, then with PE and EtOAc mixtures until 100% EtOAc was used. The column was lastly washed with chloroform. Eluates were collected in 15 mL volumes and those with alike TLC profiles were added to give 6 subfractions coded AA/PE-1 to AA/PE-6. AA/PE-1 was obtained as an essential oil and was analysed by GC-MS to identify its constituents. It was further tested for its antifungal activity against *S. cerevisiae* and *C. albicans.* The remaining fractions precipitated solids from which AA/PE-2, AA/PE-5 and AA/PE-6 were characterized using NMR, IR spectroscopy and LC-MS. Due to the presence of impurities and paucity of solids from AA/PE-3 and AA/PE-4, no further analysis was performed on them. **Scheme 3.2** present a summary of the work done on AA/PE.



Scheme 3.2: Separation of constituents of AA/PE

3.8.2 Dichloromethane Extract

The dichloromethane extract was labelled as AA/DCM. A total of 15.108 g was column chromatographed, eluting with PE and PE/EtOAc 10:1 and gradually increasing polarity to 100% EtOAc. The column was finally washed with EtOH. Combination of fractions with similar TLC profiles led to 5 subfractions coded AA/DCM-1 to AA/PE-5. AA/DCM-1 was obtained as essential oil which was subjected to GCMS analysis to determine its constituents. It was further tested for its antifungal activity against *S. cerevisiae* and *C. albicans.* The remaining fractions precipitated solids, with which AA/DCM-2 was characterized using NMR, IR spectroscopy and HR-MS. From a comparative TLC and NMR of AA/DCM-4 with AA/PE -6 it was observed that the two precipitates were the same but were kept separate. Due to the presence purity and paucity of solids AA/DCM 3 and AA/DCM 5, no further analysis was performed on them. **Scheme 3.3** presents the work done on the AA/DCM extract.



Scheme 3.3: Separation of constituents of the AA/DCM

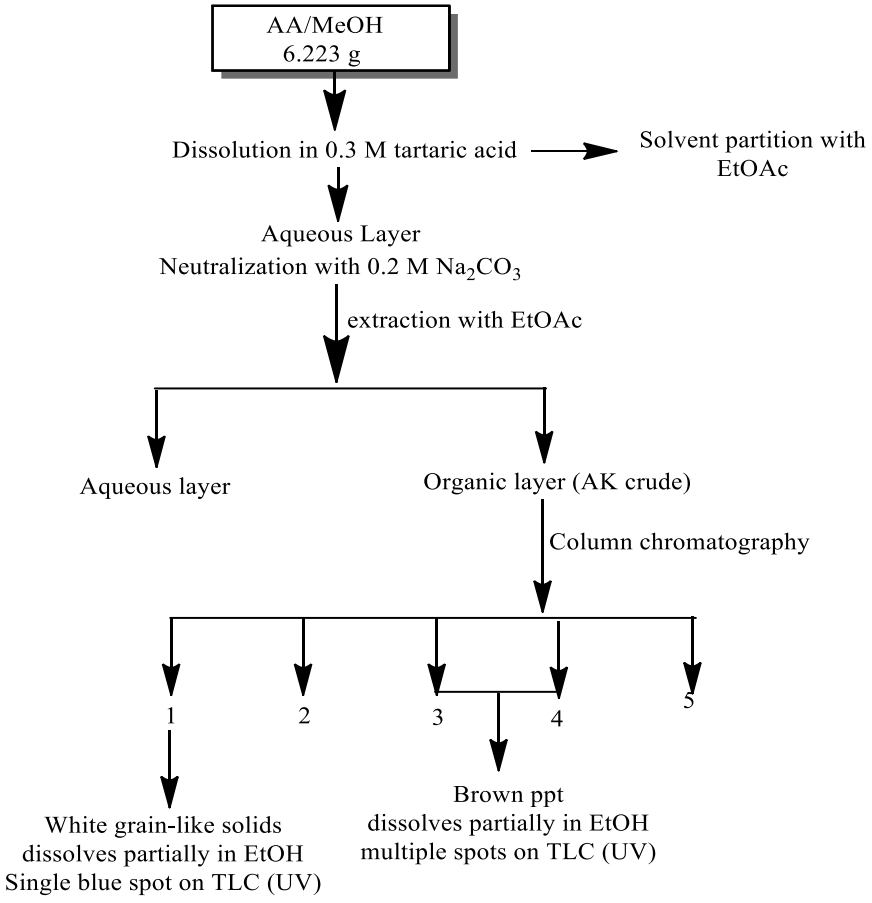
3.8.3. Methanol extract

The methanol fraction was taken through flash chromatography due to difficulties in obtaining good separation when subjected to TLC with various solvents of different polarities. PE, PE:EtOAc 1:1, EtOAc, and EtOH were used for the fractionation. Based on a comparative TLC with isolates from previous extracts, it was observed that some previously isolated compounds were present in fractions PE and PE:EtOAc 1:1. Hence, the EtOAc fraction (2.053 g) was selected for column chromatographic separation whiles the EtOH was kept for future analysis. TLC showed a total of 5 spots under UV and when stained with anisaldehyde spray. About 2.005g of the extract was loaded on a pre-packed silica column. Elution of the column was done starting from 10% EtOAc in PE and polarity was increased to 100% EtOAc. This led to 10 fractions from which solids were obtained from fraction 5, 7 and 9. However, the solids were not soluble in most deuterated solvents hence were not characterised.

From phytochemical screening, it was observed that out of the 3 extracts tested only the MeOH extract confirmed the presence of alkaloid. Hence an attempt was made to isolate the alkaloids present.

Approximately 6.423 g of the extract was dissolved in 150 mL of 0.3 M tartaric acid solution. This was done to convert the alkaloids present into alkaloid salt which will dissolve in the aqueous layer. The aqueous solution of the extract was poured into a separatory funnel and extracted 3 times with EtOAc to free the extract of other class of compounds that could be present. The organic layer was obtained and concentrated to dryness while the aqueous layer was neutralized with 0.2 M Na_2CO_3 to convert the alkaloid salts into free alkaloids. It was also extracted 3 times with EtOAc to obtain a crude alkaloid extract (AK crude).

About 1.876 g of AK crude was separated through column chromatography by eluting with PE and EtOAc to obtain 5 subfractions. Out of the 5 fractions, 3 precipitated solids AK-1, AK-3 and AK-4. Compound AK-1 was obtained as a white grain-like solid which dissolved partially in only EtOH. When subjected to TLC using EtOH: $CHCl_3$ 1:10 as the mobile phase, a blue spot was observed under UV. The remaining solids also dissolved partially in EtOH, but TLC analysis suggested that they were impure (due to the presence of multiple spots when observed under UV). Due to the small nature of these solids, no further analysis could be performed to characterize them. **Scheme 3.4** below presents the work done in an attempt to isolate and characterize an alkaloid from the MeOH extract.



Scheme 3.4: Alkaloid isolation

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Summary of results

The essential oils (EO) obtained by hydrodistillation of the fresh leaves and rhizomes of *A. atewae* were pale yellow in colour and had a pleasant ginger-like disposition. Oil yields were 0.5% and 0.7%, respectively and consisted of monoterpenes, sesquiterpenes, a couple of diterpenes and some non-terpenoid compounds as revealed by GC-MS analysis. The leaf and rhizome essential oils were significantly different in their composition. While 2,5-Di-tert-butylhydroxybenzene (7.80%) formed the major constituent of the rhizome EO, the leaf EO consisted mainly of 1-methyl-1-(methylamino)isobenzofuran-3-one (17.3%). The DCM extracted oil of the rhizome was also analyzed and 14β-pregnane (58.44%) was the major constituent. The chemical composition of the PE-extracted oil is yet to be confirmed. All the four essential oils exhibited activity against *C. albicans* while only the PE-extracted oil was active against *S. cerevisiae.*

On phytochemical analysis, all the three extracts tested positive for terpenoids. Steroids were also present in both the PE and DCM extracts. In addition, flavonoids were identified in the DCM and MeOH extracts while only the MeOH extract gave positive results for alkaloids.

Chromatographic separation of the PE extract led to the isolation of 6 solids out of which 3 were characterized and identified as 1 (E)-8-methylundec-8-en-1-yl-3-(cyclohexa-2,5-dien-1-yl)propanoate, stigmasterol and myristic acid. Out of 5 solids obtained from the DCM extract, only one was characterized as 2-(6-oxotetrahydro-2H-pyran-2-yl)ethyl dodec-8-enoate. Also, the EtOAc fraction of the MeOH extract gave 10 compounds but due to the poor solubility in various NMR solvents including DMSO, none of these compounds has yet been fully characterized.

4.2 Chemical composition of the Essential Oils

4.2.1 Chemical composition of AA-L

A total of 119 constituents, representing 88.42% of the total peak area, were identified by GC-MS analysis of the leaf EO by comparing the mass spectra of the compounds to the Wiley mass spectral library (**Figure 4.1 and Table 4.1**).

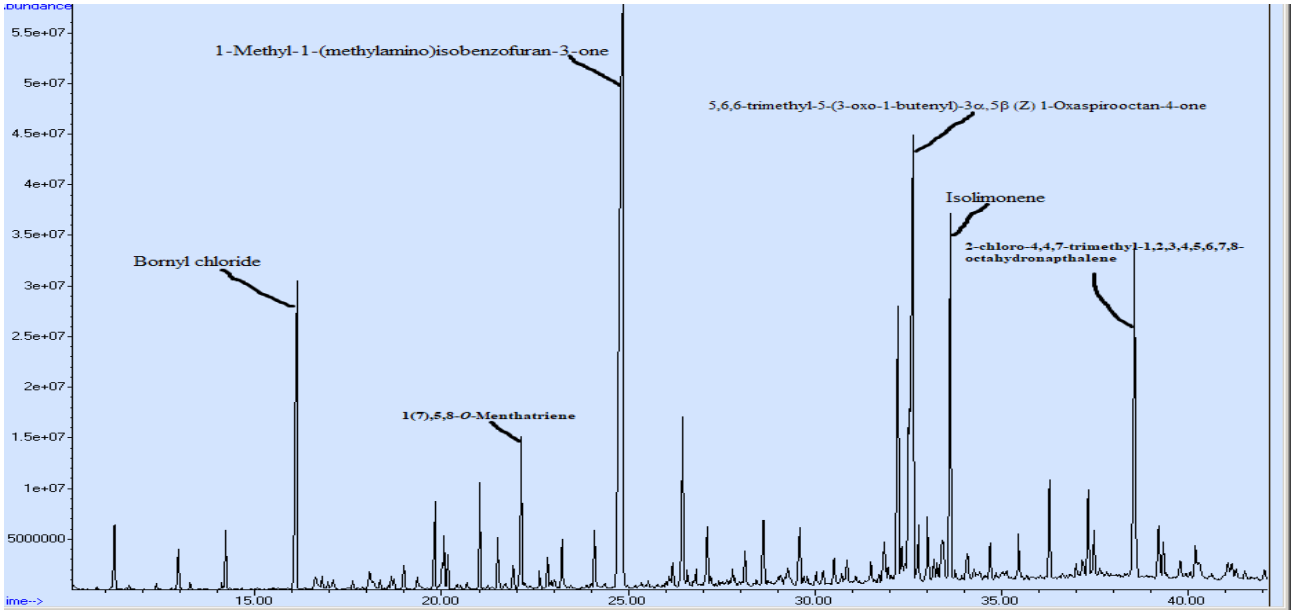


Figure 4.1: Gas chromatogram of leaf essential oil of *A. atewae*

Table 4.1: Retention time, name of constituent, percentage composition, molecular formula and weight of *A. atewae* leaf essential oil as shown by GCMS analysis

Peak No.	Retention time (min)	Compound	MF	Mwt. (g/mol)	Composition (%)
1	10.754	α-Terpinene	$C_{10}H_{16}$	136.238	0.0367
2	11.2363	*l*-Limonene	$C_{10}H_{16}$	136.238	0.797
3	11.5484	1,8-Cineole (Eucalyptol)	$C_{10}H_{18}O$	154.249	0.0263
4	11.6335	(2-Methylprop-1-enyl)-cyclohexa-1,5-diene	$C_{10}H_{14}$	134.218	0.0633
5	12.3569	γ-Terpinene	$C_{10}H_{16}$	136.238	0.0711
6	12.7683	2-methyl-1,3-Dioxolane-2-pentanol	$C_9H_{18}O_3$	174.240	0.0255
7	12.9527	*p*-Cymene	$C_{10}H_{14}$	134.218	0.5945
8	13.2648	terpinolene	$C_{10}H_{16}$	136.238	0.0745
9	14.1159	2-Heptanol	$C_7H_{16}O$	116.204	0.0713
10	14.2152	1-Butylpyrrole	$C_8H_{13}N$	123.196	0.8467
11	14.3287	(*Z*)-7-Pentadecen-5-yne,	$C_{15}H_{26}$	206.370	0.0285
12	14.6549	2-Pentyl-2-cyclopenten-1-one	$C_{10}H_{16}O$	152.237	0.0179
13	15.0663	Irid-2-ene	$C_{10}H_{18}$	138.250	0.0397
14	15.4918	1,4-Dicyclohexylbutane	$C_{10}H_{30}$	222.410	0.0249
15	15.6195	2,4-Hexadiene	C_6H_{10}	82.146	0.0265
16	15.889	*p*-Mentha-1,5,8-triene	$C_{10}H_{14}$	134.218	0.0337
17	**16.116**	**Bornyl chloride**	**$C_{10}H_{17}Cl$**	**172.695**	**4.9307**
18	16.3429	2,3-dimethyl-1,2-pentadiene	C_7H_{12}	92.170	0.0571
19	16.6267	α-Campholene aldehyde	$C_{10}H_{16}O$	152.233	0.6374
20	16.7969	*o*-Allyltoluene	$C_{10}H_{12}$	132.202	0.1582
21	16.9529	Acetic acid	$C_2H_4O_2$	60.052	0.1247
22	17.0948	β-Phellandrene	$C_{10}H_{16}$	136.238	0.1894
23	17.5203	4,5-Bis(hydroxymethyl)-3,6-dimethylcyclohexene	$C_{10}H_{18}O_2$	170.250	0.0502
24	17.6338	*Cis*-Ocimene	$C_{10}H_{16}$	136.238	0.1232
25	17.7473	Hydrocinnamaldehyde	$C_9H_{10}O$	134.175	0.025
26	17.8891	11-Endo-methyltetracyclo[5.4.0.0(2,6).0(4,10)]undec-8-ene	$C_{12}H_{16}$	160.250	0.0403
27	18.3572	α-Pinene	$C_{10}H_{16}$	136.238	0.1424
28	18.57	Nonan-2-ol	$C_9H_{20}O$	144.258	0.048
29	18.6551	α-Terpinolene	$C_{10}H_{16}$	136.238	0.1669
30	18.726	3-Carbomethoxy-4-methylenecyclohex-1-ene	$C_9H_{12}O_2$	152.000	0.1461
31	18.9955	Cyperene	$C_{15}H_{24}$	204.357	0.3681
32	19.3502	3-Pinanone	$C_{10}H_{16}O$	152.233	0.1902
33	19.4353	3-Eicosyne	$C_{20}H_{38}$	278.524	0.0763
34	**19.8183**	**Pinocarvone**	**$C_{10}H_{14}O$**	**150.104**	**1.0947**
35	20.0169	Caryophyllan-2,6-β-oxide	$C_{15}H_{26}O$	222.370	0.3771
36	20.0736	Nopinone	$C_9H_{14}O$	138.210	0.587

37	20.1729	β-Elemene	$C_{15}H_{24}O$	220.351	0.4325
38	20.414	α-Farnesene	$C_{15}H_{24}$	220.351	0.0722
39	20.5133	*E, E, E*-2,4,6-Octatriene	C_8H_{12}	108.184	0.0512
40	20.6836	*p*-Methylstyrene	C_9H_{10}	118.179	0.1112
41	**21.024**	**Myrtenal**	**$C_{10}H_{14}O$**	**150.221**	**1.1560**
42	21.1233	Methyl (*Z*)-5,11,14,17-eicosatetraenoate	$C_{21}H_{34}O_2$	318.500	0.0238
43	**21.251**	***o*-Mentha-1(7),5,8-triene**	**$C_{10}H_{14}$**	**134.218**	**1.9593**
44	21.3644	*p*-Mentha-1,3,8-triene	$C_{10}H_{14}$	134.218	0.0444
45	21.5063	*Cis*-Sabinol	$C_{10}H_{16}O$	152.230	0.6221
46	21.6907	*trans*-3-methyl-2-(1-methyl-3-butynyl)oxirane	$C_8H_{12}O$	124.000	0.0538
47	21.7191	Safranal	$C_{10}H_{14}O$	150.210	0.0654
48	21.9176	Isodurene	$C_{10}H_{14}$	134.222	1.5121
49	22.2439	δ-3-Carene	$C_{10}H_{16}$	136.238	0.0793
50	22.3858	endo-Borneol	$C_{10}H_{18}O$	154.249	0.0606
51	22.6411	Eucarvone	$C_{10}H_{14}O$	150.221	0.2137
52	22.8397	β-Selinene	$C_{15}H_{24}$	204.357	0.42
53	22.939	Furfural	$C_5H_4O_2$	96.085	0.1142
54	23.0241	Linderol	$C_{10}H_{18}O$	154.249	0.1783
55	23.2369	1-Methyl-1-phenyl-guanidine	$C_8H_{11}N_3$	149.193	0.6842
56	23.478	1,2,3,4,5,7,8,9-Octahydro-6H-benzocyclohepten-6-one	$C_{11}H_{16}O$	164.291	0.3070
57	23.7475	Ethyl anthranilate	$C_9H_{11}NO_2$	165.192	0.0295
58	23.8894	3-Phenylbutanal	$C_{10}H_{12}O$	148.205	0.0447
59	24.0028	6,6-Dimethylcycloocta-2,4-dien-1-one	$C_{10}H_{14}O$	150.221	0.0736
60	24.1022	Myrtenol	$C_{10}H_{16}O$	152.237	0.7569
61	24.3717	2-Valerylfuran	$C_9H_{12}O_2$	152.190	0.0977
62	**24.8398**	**1-Methyl-1-(methylamino)isobenzofuran-3-one**	**$C_{10}H_{11}NO_2$**	**177.20**	**17.2655**
63	24.9816	1-methyladamantane	$C_{11}H_{18}$	150.26	0.0517
64	25.1944	α-Ionone	$C_{13}H_{20}O$	192.30	0.0395
65	25.5348	*Cis*-1,2-diethenyl-4-(1-methylethylidene)-cyclohexane	$C_{13}H_{20}$	176.3	0.1261
66	25.6483	(*Z*)-2,2-Dimethyl 5-decen-3-yne	$C_{12}H_{20}$	164.29	0.0339
67	25.776	2-methoxy-3-ethylpyrazine	$C_7H_{10}N_2O$	138.17	0.064
68	26.0029	*o*-Phenetidine	$C_8H_{11}NO$	137.180	0.0663
69	26.1022	3-ethyl-4,5-dihydro-1H-pyrazole	$C_5H_{10}N_2$	98.146	0.1949
70	**26.2015**	**1(Z)-5(E)-7-Dodecatriene**	**$C_{12}H_{20}$**	**164.29**	**2.6484**
71	26.8115	*o*-Menth-8-ene	$C_{10}H_{18}$	138.250	0.2318
72	27.1236	4-(2,2,4-Trimethylcyclopent-3-en-1-yl)but-2-enol	$C_{12}H_{20}O$	180.34	0.8324
73	**27.2229**	**Caryophyllene oxide**	**$C_{15}H_{24}O$**	**220.356**	**2.1424**
74	27.5349	α-Selinene	$C_{15}H_{24}$	204.357	0.091
75	27.6626	methylpatchenol	$C_{12}H_{20}O$	180.260	0.0952
76	27.7335	Capnellane-8-one	$C_{15}H_{24}O$	220.351	0.0689
77	27.7903	Perillyl alcohol	$C_{10}H_{16}O$	152.237	0.2234
78	28.386	Viridiflorol	$C_{15}H_{26}O$	222.370	0.2962
79	28.4428	2-methyl-2-bornene	$C_{11}H_{18}$	150.260	0.1147
80	**28.6272**	**1-Vinylbenzyl alcohol**	**$C_9H_{10}O$**	**134.178**	**1.0063**
81	28.8399	8,8-dimethyl-9-methylene-1,5-Cycloundecadiene	$C_{14}H_{22}$	190.324	0.057
82	29.0243	Isolongifolene	$C_{15}H_{24}$	204.357	0.8011
83	29.0811	Germacrene B	$C_{15}H_{24}$	204.357	0.2375
84	**29.2797**	**α-Patchoulene**	**$C_{15}H_{24}$**	**204.357**	**1.0430**
85	29.6059	3,4,4a,5,8,8a-Hexahydro-4a-methyl-2(1H)-naphthalenone	$C_{11}H_{16}O$	164.240	0.9775
86	29.7052	*trans*-Caryophyllene	$C_{15}H_{24}$	204.357	0.7922
87	30.0315	Aromadendrene	$C_{15}H_{24}$	204.357	0.8943
88	30.5138	(-)-Camphor	$C_{10}H_{16}O$	152.237	0.4346
89	30.9819	Viridiflorene	$C_{15}H_{24}$	204.357	0.2127
90	31.3649	α-Guaiene	$C_{15}H_{24}$	204.351	0.0838
91	31.8755	β-Selinene	$C_{15}H_{24}$	204.357	0.9387
92	32.0599	Valerenal	$C_{15}H_{22}O$	218.330	0.1644
93	**32.2302**	**Bicyclo(5.3.1)undec-1-en-9-one**	**$C_{11}H_{16}O$**	**164.237**	**3.8698**
94	32.4288	Caryophyllenol I	$C_{15}H_{24}O$	220.351	0.3742
95	32.528	**3,5-Diisopropenyl-1,2-dimethyl-cyclohexane**	**$C_{14}H_{24}$**	**192.340**	**2.4262**
96	32.6273	**5,6,6-trimethyl-5-(3-oxo-1-butenyl)- 1-Oxaspiro[2.5]octan-4-one**	**$C_{14}H_{20}O_3$**	**236.314**	**9.019**
97	32.7692	Caryophylla-3,8(13)-dien-5α-ol	$C_{15}H_{24}O$	220.351	0.913
98	**33.0387**	**2,3-Dimethyl-1,5-divinylcyclohexane**	**$C_{12}H_{20}$**	**164.290**	**1.0356**
99	33.2799	Cuminaldehyde	$C_{10}H_{12}O$	148.205	0.3206

100	**33.4217**	**1,2-Dimethyl-1,4-cyclohexadiene**	**C_8H_{12}**	**108.184**	**1.2823**
101	**33.6345**	**(+)-trans-Isolimonene**	**$C_{10}H_{16}$**	**136.238**	**4.9119**
102	34.3437	Torreferol	$C_{20}H_{34}O_2$	306.490	0.1529
103	34.4288	Junipene	$C_{15}H_{24}$	204.351	0.2619
104	34.5565	Valerenol	$C_{15}H_{24}O$	220.351	0.1292
105	34.9962	3-Thujen-2-one	$C_{10}H_{14}O$	150.22	0.1949
106	35.053	Alloaromadendrene	$C_{15}H_{24}$	204.351	1.1402
107	35.1381	α-Humulene	$C_{15}H_{24}$	204.351	0.2372
108	35.6913	1-(2,3-Dimethylphenyl)ethanol	$C_{10}H_{14}O$	150.22	0.1777
109	35.975	Neoalloocimene	$C_{10}H_{16}$	136.238	0.1028
110	36.5424	Calarene	$C_{15}H_{24}$	204.351	0.1371
111	36.741	Eremophilene	$C_{15}H_{24}$	204.351	0.1159
112	37.6347	β-Caryophyllene	$C_{15}H_{24}$	204.351	0.4406
113	37.8332	Hinesol	$C_{15}H_{26}O$	222.366	0.2793
114	**38.5709**	**3-chloro-1,1,6-trimethyl-1,2,3,4,5,6,7,8-octahydronaphthalene, (3*S*-*cis*)- (9CI)**	**$C_{13}H_{21}Cl$**	**212.76**	**5.6537**
115	39.4361	*Cis*-Caryophyllene	$C_{15}H_{26}$	206.367	0.1741
116	39.8049	(1R,3S)-Cembra-4,7,11,15-tetraen-3-ol	$C_{20}H_{32}O_2$	288.468	0.5288
117	39.961	4α-Isopropenyl-2-carene	$C_{13}H_{20}$	176.312	0.1617
118	40.6419	Vulgarol B	$C_{15}H_{24}O$	220.351	0.1236
119	41.0674	Vulgarol A (Labd-13-ene-8,15-diol)	$C_{20}H_{36}O_2$	308.500	0.5407
120	42.0604	5,6,7-Trimethoxy-1-tetralone	$C_{13}H_{16}O_4$	252.260	0.0322

Monoterpenes occurring in minor quantities formed the major part of the EO (22.42%) while sesquiterpenes consisted of 13.98 %. The identified functional groups were hydrocarbons (57), alcohols (23), ketones (15), aldehydes (8) ester (1) and miscellaneous (15). Eighteen (15.13%) out of the 119 constituents had a composition of more than 1%, the major ones being 1-methyl-1-(methylamino)isobenzofuran-3-one (**17.27%**); 5,6,6-trimethyl-5-(3-oxo-1-butenyl)-1-Oxaspiro[2.5]octan-4-one (**9.02 %**), 2-chloro-4,4,7-trimethyl-1,2,3,4,5,6,7,8-octahydronapthalene (**5.36 %**); bornyl chloride (**4.93%**); (+)-trans-isolimonene (**4.91%**); bicycloundec-1-en-9-one (**4.25%**) and 1,2-dimethyl-3,5-bis(1-methylethenyl)-cyclohexane (**3.51%**). This chemical profile is a marked contrast from the reported chemical composition of many of the extensively researched *Aframomum* plant parts, particularly, the leaf. The isobenzofuran-3-one derivative, identified as the single major constituent, is being reported as an *Aframomum* essential oil constituent for the first time. A literature survey on Scifinder, NIST and PubChem identified the isobenzofuranone moiety as a structural unit of noscapine-type alkaloids which are known for their antitumor and antitussive properties[73]. However, hexahydro-3a-2(3H)-benzofuranone ($C_8H_{12}O_2$, 2.1%), has been identified in the crude extracts of *A. melegueta*[74]. Bicycloundecec-1-en-9-one has not as yet featured in any essential oil of *Aframomum* although the bicycloundecane moiety is a structural feature of several organic compounds including the *Homo*-adamatanyl type compounds isolated from *Hypericum cohaerens*[75]. The other major constituents, 2-chloro-4,4,7-trimethyl-1,2,3,4,5,6,7,8-octahydronapthalene; bornyl chloride and 1,2-dimethyl-3,5-bis(1-methylethenyl)-cyclohexane are also new EO constituents of *Aframomum.* Some reported common and abundant *Aframomum* leaf EO constituents include β-pinene (*A. elloitti*, 44.3%; *A. longiscarpum*, 42.6%; *A. sceptrum*, 15.1%; *A.*

geocarpum, 11.3%; *A. danielli*, 47.6%; *A. citratum*, *A. hanburyi*, *A. letestuanum* and *A. pruinosum* - 30-60%[76]) and β-caryophyllene (*A. danielii*, *A. letestuianum* and *A. pruinosum* - 18.4-82.4%)[76-78].

β-pinene was not identified in essential oil of the leaves of A. atewae but 1.23% of β-caryophyllene was present. The minor constituents including α-humulene, α-ionone, α-pinene, α-selinene, γ-terpinene, 1,8-cineole, 3-eicosyne, myrtenal, terpinolene, valerenal and vulgarol A are common minor EO constituents of many *Aframomum* species[79]. Mass fragmentations and some major compounds are shown in **Figures 4.2-4.6** below

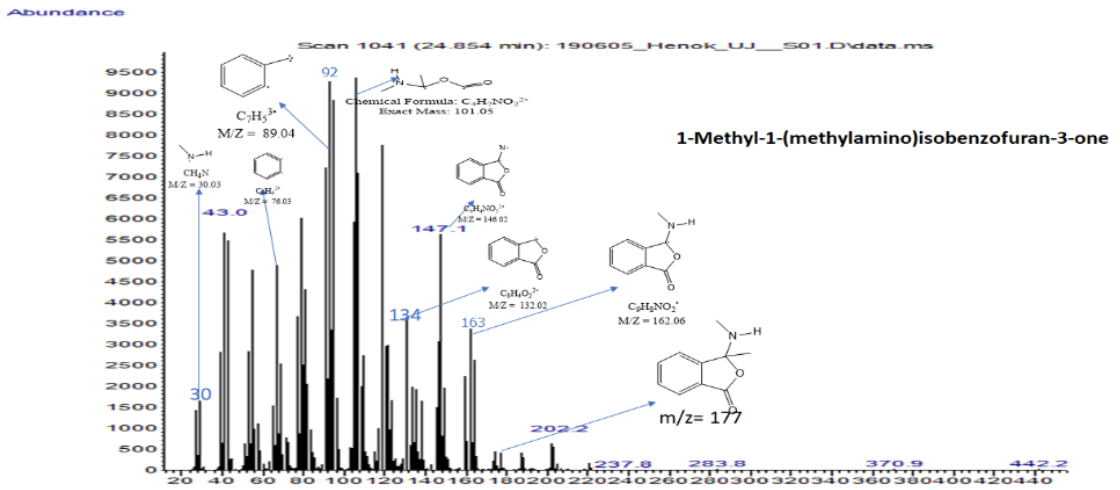


Figure 4.2: MS of 1-methyl-1-(methylamino)isobenzofuran-3-one, (17.66%), MF = $C_{10}H_{11}NO_2$, Rt 24.854

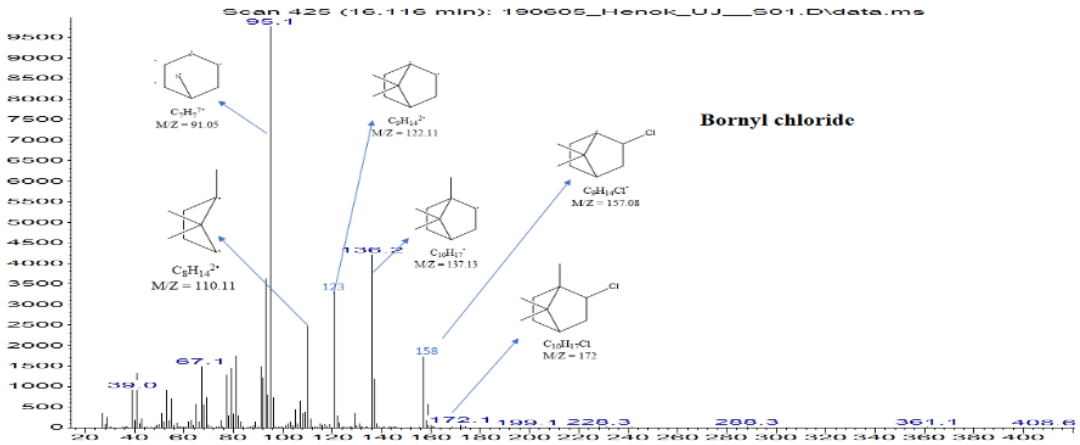


Figure 4. 3: MS of Bornyl chloride, (4.93%), MF = $C_{10}H_{17}Cl$, Rt 16.166

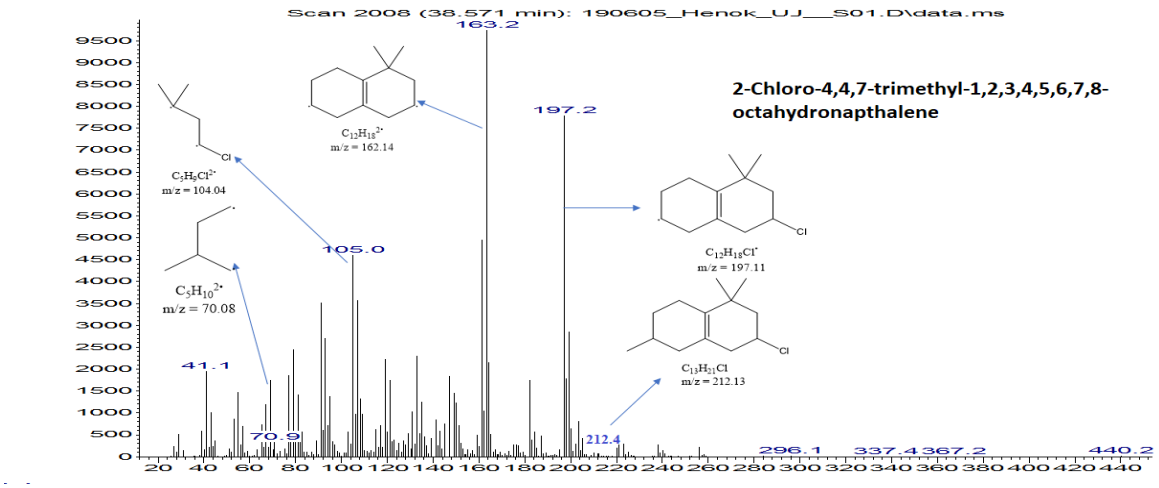


Figure 4.4: MS of 2-chloro-4,4,7-trimethyl-1,2,3,4,5,6,7,8-octahydronapthalene, (5.56%), MF = $C_{13}H_{21}Cl$, Rt 36.671

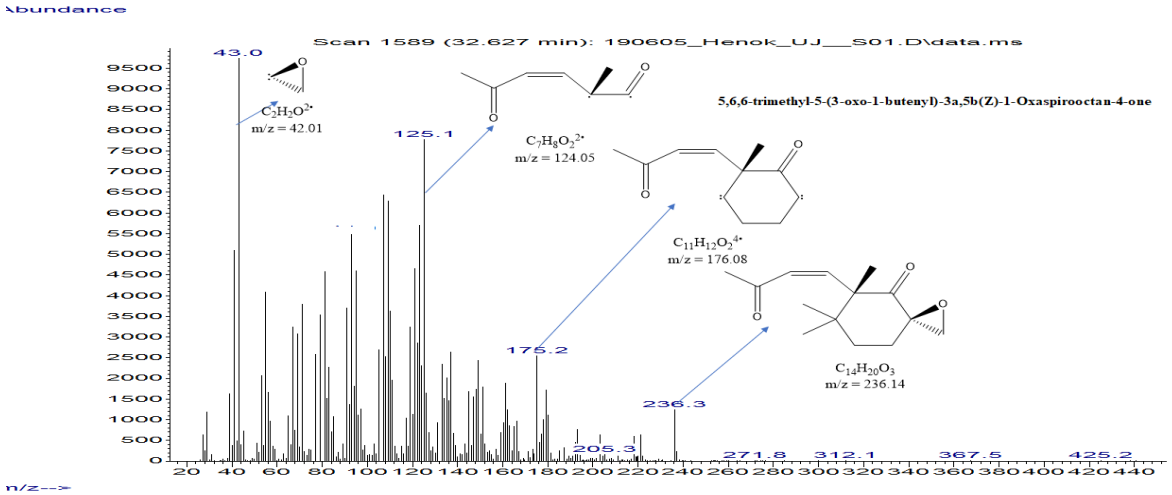


Figure 4.5: Mass fragmentation of 5,6,6-trimethyl-5-(3-oxo-1-butenyl)- 1-Oxaspiro[2.5]octan-4-one, (9.019%), MF = $C_{14}H_{20}O_3$, Rt 32.627

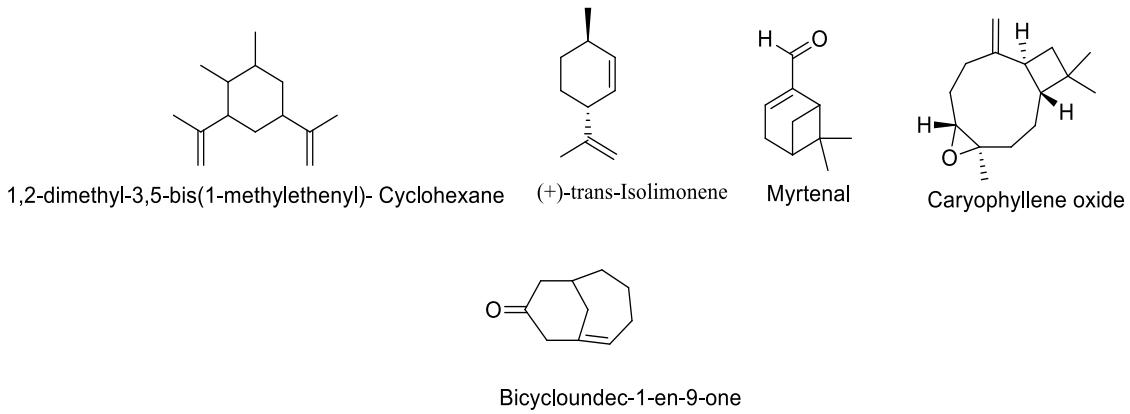


Figure 4.6: Other major constituents of the leaf essential oil

4.2.2 Chemical composition of the rhizome essential oil

The rhizome EO on GC-MS analysis led to the identification of 100 constituents making up 88.43% of the total peak area. (**Figure 4.7** and **Table 4.2**). This oil contained more sesquiterpenes (24.44%)

than monoterpenes (8.08%) as compared to the leaf EO. The functional groups that were identified were significantly different in quantity with respect to the leaf essential oil - hydrocarbons (34), alcohols (10), ketones (16), aldehydes (7) ester (2) and miscellaneous (31).

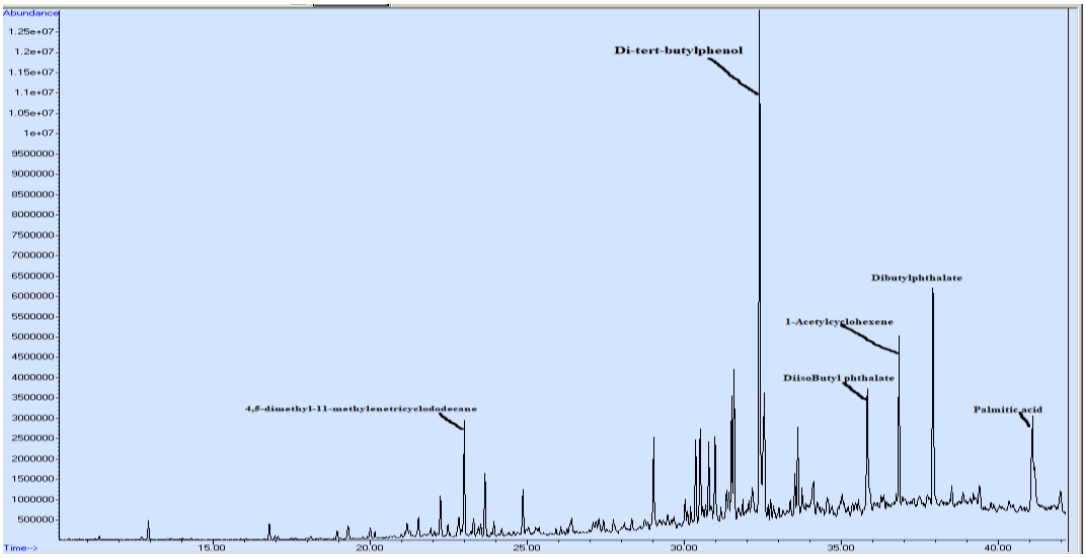


Figure 4.7: Gas chromatogram of rhizome essential oil of *A. atewae*

Table 4.2: Retention time, name of constituent, percentage composition, molecular formula and weight of *Aframomum* rhizome essential oil as shown by GC-MS analysis

Peak No.	Retention time (min)	Compound	MF	Mwt. (g/mol)	Composition (%)
1	12.9385	*p*-Cymene	$C_{10}H_{14}$	134.21	0.3111
2	16.7968	*O*-Allyltoluene	$C_{10}H_{12}$	132.202	0.2579
3	18.9529	8,9-Dehydro-neoisolongifolene	$C_{15}H_{22}$	202.335	0.5782
4	20.0026	1,2,4-trimethyl(1-methylethenyl)- Benzene	$C_{12}H_{16}$	160.25	0.1878
5	20.1586	2,4,6-Octatrienal	$C_8H_{10}O$	122.16	0.1063
6	21.18	α-Gurjunene	$C_{15}H_{24}$	204.357	0.4142
7	21.2651	γ-himachalene	$C_{15}H_{24}$	204.357	0.1833
8	21.9176	γ-Selinene	$C_{15}H_{24}$	204.357	0.2422
9	22.0452	δ-cadinene	$C_{15}H_{24}$	204.357	0.148
10	22.2438	1,2,3,4-tetrahydro-2,3-methano-2,8-dimethoxynaphthalene	$C_{13}H_{16}O_2$	204.26	0.7025
11	22.4708	β-Chamigrene	$C_{15}H_{24}$	204.357	0.2231
12	22.8254	Neoalloocimene	$C_{10}H_{16}$	136.23	0.4703
13	22.8963	α-Selinene	$C_{15}H_{24}$	204.357	0.7009
14	**22.9956**	**4,5-dimethyl-11-methylenetricyclo[7.2.1.0 (4.9)]dodecane**	**$C_{15}H_{24}$**	**204.357**	**1.9799**
15	23.2935	(5R)-2-cyano-5,9-dimethyldeca-2,8-dienenitrile	$C_{13}H_{18}N_2$	202.30	0.3519
16	23.4495	(E)-(3'-oxo-3',4'-dihydro-2'H-1',4'-benzoxazin-2'ylidene)acetic acid	$C_{10}H_7NO_4$	204.17	0.2331
17	23.5205	(2-methylcyclopent-1-enyl)(4,4-dimethyl-3-oxocyclopent-1-enyl)methane	$C_{14}H_{20}O$	204.31	0.189
18	**23.6623**	**Isolongifolene**	**$C_{15}H_{24}$**	**204.357**	**2.0337**
19	23.946	β-Patchoulene	$C_{15}H_{24}$	204.357	0.2967
20	24.1872	*p*-Cresol	C_7H_8O	108.14	0.1512
21	24.5702	(3E)-2,6-Dimethyl-5-isopropyliden-1,3,6,9-decatetraene	$C_{15}H_{22}$	202.34	0.0908
22	24.8539	Calamenene	$C_{15}H_{22}$	202.34	0.7842
23	24.9674	Pentacyclo[7.5.0.0(2,8).0(5,14).0(7,11)]tetradecane	$C_{14}H_{20}$	118.31	0.153
24	25.0525	1-chloro-2,4-dimethoxy-3-methylphenol	$C_9H_{11}ClO_3$	202.63	0.2219
25	25.2794	Cumic acid	$C_{10}H_{12}O_2$	164.204	0.2994

26	25.3787	(E)-2-Methyl-4(2',4',4'-trimethylbicyclo[4.1.0]hept-2'-en-3'-yl)-1,3-butadiene	$C_{15}H_{22}$	202.34	0.2985
27	25.9178	Dimethyl sulfone	$C_2H_6O_2S$	93.13	0.1225
28	26.0738	α-Calacorene	$C_{15}H_{20}$	200.32	0.1437
29	26.4142	1,2,3,6-Tetramethylbicyclo[2.2.2]oct-2-ene	$C_{12}H_{20}$	164.29	0.5323
30	27.0951	α-Terpinene	$C_{10}H_{16}$	134.238	0.2453
31	27.1944	4,5-Dehydro-isolongifolene	$C_{15}H_{22}$	200.352	0.2415
32	27.2795	Nerolidol oxide	$C_{15}H_{26}O_2$	238.37	0.3236
33	27.4355	Methyl allomaltol	$C_6H_6O_3$	126.110	0.1971
34	27.7476	Bisabolol oxide	$C_{15}H_{26}O_2$	238.371	0.3263
35	28.0739	2-endo-(Dichloroamino)norbornane	$C_7H_{11}Cl_2N$	180.07	0.3723
36	**28.7406**	**Ledene**	**$C_{15}H_{24}$**	**204.357**	**1.2532**
37	28.8399	isoobtusadiene	$C_{15}H_{21}BrO$	297.231	0.1255
38	**29.0243**	**δ-Guaiene**	**$C_{15}H_{24}$**	**204.357**	**1.8647**
39	29.2512	4,4-dimethyltricyclo[6.3.2.0(2,5)]trideca-8-ene-1-ol	$C_{15}H_{24}O$	220.351	0.1022
40	29.3222	2-methyl-2-bornene	$C_{11}H_{18}$	150.261	0.2611
41	29.464	1-(2,4-dimethylphenyl)-2-phenylethane	$C_{16}H_{14}$	206.28	0.3806
42	29.6626	2,3-Dihydro-4,5-dimethoxy-6-methyl-3-methyleneinden-1-one	$C_{13}H_{14}O_3$	218.25	0.2747
43	29.8186	(E)-2-methyl-5-[(1α,6 α,7 α)-3-(hydroxymethyl)-7-methylbicyclo[4.1.0]hept-2-en-7-yl]-2-penten-1-ol	$C_{15}H_{24}O_2$	236.35	0.1141
44	30.0314	4-Methyl-1,2,3,4,5,6-hexahydro-1,5-methano-4,1-benzazaphosphocine	$C_{12}H_{16}NP$	205.24	0.5923
45	30.1023	2,5-Dimethoxyacetophenone	$C_{10}H_{12}O_{12}$	180.203	0.3048
46	**30.2158**	**Carvacrol**	**$C_{10}H_{14}O$**	**150.217**	**3.8917**
47	**30.3577**	**1-[2-Hydroxy-4-methyl-3-(1-methylethyl)phenyl]-1-propanone**	**$C_{13}H_{18}O_2$**	**206.26**	**1.5648**
48	30.5846	trans-Caryophyllene	$C_{15}H_{24}$	204.357	0.3864
49	30.6839	3,4-Dihydro-iso-methyl-β-ionone	$C_{10}H_{24}O$	208.34	0.5354
50	**30.7832**	**(R)-4-Methyl-2-(1-ethylethenyl)-1-cyclopentene-1-carboxaldehyde**	**$C_{11}H_{16}O$**	**164.24**	**1.5449**
51	30.8541	N-(Methyl-D2)-aniline	$C_7H_7D_2N$		0.5345
52	31.1662	2-Chloro-4-nitromesitylene	$C_9H_{10}ClNO_2$	199.63	0.4406
53	31.4215	2,3-dihydro-4-oxy-2-prop-2-ylidene-4H-pyrido[2,3-e]1,3-thiazine	$C_{10}H_{10}N_2OS$	206.26	0.6415
54	**31.5066**	**7-methyl- 3,4,5,6,7,8-Hexahydronaphthalen-1(2H)-one**	**$C_{10}H_{16}O$**	**164.24**	**2.2442**
55	**31.5776**	**Cadinene**	**$C_{15}H_{24}$**	**204.357**	**2.6417**
56	31.7194	Mellitene	$C_{12}H_{18}$	162.276	0.6448
57	31.9322	(E,1RS,2RS,6RS)-2-(3'-methyl-1,3'-butadien-1-yl)-2,4,4-trimethylbicyclo[4.1.0]heptan-3-one	$C_{15}H_{22}O$	218.33	0.27
58	31.9748	2-(1-methyl-1-propenyl)-(Z)-Naphthalene	$C_{14}H_{14}$	182.26	0.2849
59	32.0457	Nootkatone	$C_{15}H_{22}O$	218.340	0.534
60	**32.1591**	**Alloaromadendrene**	**$C_{15}H_{24}$**	**204.357**	**1.3663**
61	**32.3861**	**2,5-Di-tert-butylhydroxybenzene**	**$C_{14}H_{22}O$**	**206.32**	**7.8033**
62	**32.5421**	**Ethyl 2-bromopropanoate**	**$C_5H_9BrO_2$**	**181.03**	**3.4835**
63	32.6556	2-(2'-Methyl-2'-nitropropyl)indan-1-one	$C_{13}H_{15}NO_3$	233.26	0.3776
64	32.7407	2,2,4,4-tetramethyl-1-(1-methylethenyl)bicyclo[3.2.1]oct-6-en-3-one	$C_{15}H_{22}O$	218.33	0.449
65	32.84	cis-3a,4,5,6,7,7a-hexahydro-2-(2'-propenyl)-1*H*-inden-1-one	$C_{12}H_{16}O$	176.25	0.5465
66	33.0102	8.9-Epoxiacorenon-B	$C_{15}H_{24}O_2$	236.35	0.4942
67	33.1379	5-Oxo-isolongifolene	$C_{15}H_{22}O$	218.33	0.4792
68	33.294	Dimethanonaphthalen-9-ol, decahydro-2-methyl-, (1α,2β,4 α,4aα,5β, 8 β.,8aα,9R)	$C_{13}H_{20}O$	192.30	0.5578
69	33.3649	8-Allyl-2-amino-6-methylimidazo[1,5-a]-1,3,5-triazin-4(3H)-one	$C_9H_{11}N_5O$	205.22	0.5758
70	**33.5209**	**8,9-Dehydro-neoisolongifolene**	**$C_{15}H_{22}$**	**200.352**	**1.0386**
71	**33.606**	**Ambrial**	**$C_{16}H_{26}O$**	**234.377**	**1.9507**
72	**33.7621**	**2-[(Z)-(3''-Methylbutadien-2''-yl)methylidene]-1,3,3-trimethylcyclohexanol**	**$C_{15}H_{24}O$**	**220.35**	**1.1714**
73	33.8897	Valerenal	$C_{15}H_{22}O$	218.33	0.5864
74	33.9748	Retinal	$C_{20}H_{28}O$	284.436	0.3308
75	**34.1167**	**cis/trans-7-Bicyclo[4.1.0]hept-7-ylidene-bicyclo[4.1.0]heptane**	**$C_{14}H_{20}$**	**188.31**	**1.6085**
76	34.2302	2-Ethyl-3-phenyl-2-butene-1-al	$C_{12}H_{14}O$	174.24	0.9137
77	34.3862	β-Oplopenone	$C_{15}H_{24}O$	220.35	0.4572

78	34.5422	1,3,6-Trimethyl-8-ethyl-2,7-naphthyridine	$C_{13}H_{16}N_2$	200.28	0.9592
79	34.6983	1-(2'-ethenyl-1'-cyclohexenyl)-2-propen-1-ol	$C_{11}H_{16}O$	164.24	0.5498
80	34.8969	β-Caryophyllene	$C_{15}H_{24}$	204.357	0.3587
81	35.0245	1-*O*-Acetylfructose	$C_8H_{14}O_7$	222.19	0.9085
82	35.0813	1,1,2,2-tetramethyl-3-[(methyl)methylene]-8-oxobicyclo[4.3.0]non-4(5)-ene	$C_{15}H_{22}O$	218.33	0.522
83	35.2089	Cyclohexanol, 3-(1,3-butadienyl)-4,4-dimethyl-2-methylene-, [1α,3α (E)]	$C_{13}H_{20}O$	192.30	0.4184
84	35.3508	8,9,10,12-Tetrahydro-7*H*-indolo(1,2β)(2)-benzazepine	$C_{17}H_{17}N$	235.32	0.54
85	35.4501	trichloromethyl- Benzene	$C_7H_5Cl_3$	195.48	0.4867
86	35.5494	2-[(Z)-(3"-Methylbutadien-2"-yl)methylidene]-1,3,3-trimethylcyclohexanol	$C_{15}H_{24}O$	220.35	0.667
87	**35.8189**	**Di-iso-Butyl phthalate**	**$C_{16}H_{22}O_4$**	**275.35**	**3.8654**
88	36.1451	3-Chloro-1-butyne	C_4H_5Cl	88.54	0.6158
89	36.2586	(Z,1'RS,2'SR,4'RS,7'SR)-1-(2',5',5'-trimethyl-3'-oxabicyclo[5.1.0.0(2,4)]oct-4'-yl)-3-methyl-1,3-butadiene	$C_{15}H_{22}O$	218.33	0.5926
90	**36.3437**	**1,4,7,10,13,16-Hexaoxacyclooctadecane**	**$C_{12}H_{24}O_6$**	**264.32**	**1.4553**
91	36.7409	1-(1-cyclohexen-1-yl)-1-Amino-3-hydroxyamino-isoquinoline	$C_9H_9N_3O$	175.19	0.7621
92	**36.826**	**1-Acetylcyclohexene**	**$C_8H_{12}O$**	**124.180**	**3.1646**
93	**37.2799**	**trans-1,2-Cyclobutanedicarbonyl chloride**	**$C_6H_6Cl_2O_2$**	**181.01**	**1.0099**
94	37.7906	Benzene, 1-(chloromethyl)-2,4-dinitro-	$C_7H_5ClN_2O4$	216.58	0.4938
95	**37.9183**	**Dibutyl-phtalate**	**$C_{16}H_{22}O_4$**	**275.35**	**4.5759**
96	39.0956	(S)-(-)-2-[(2-Methoxyethoxy)methoxy]propan-1-al	$C_7H_{11}O_4$	162.18	0.7272
97	39.8333	6-ethyl-1,2,3,4,5,6,7,8-octahydro-6-nitro-5-[(2-nitrophenyl)thio]-,(1*R*,4*S*,5*R*,6*R*)4-Epoxynaphthalene	$C_{18}H_{20}N_2O_5S$	376.43	0.3185
98	40.0318	(18S,19S)-18,19-Dihydroxy-1,4,7,10,13,16-hexaoxocycloencosane	$C_{14}H_{28}O_8$	324.37	0.345
99	40.4574	tricyclo[5.3.2.0(1,7)]dodecan-2-on-11-ene	$C_{12}H_{16}O$	176.26	0.4252
100	**41.0673**	**Palmitic acid**	**$C_{16}H_{32}O_2$**	**256.43**	**4.3375**

Twenty-two constituents had compositions of >1%. Like the leaf EO, the major constituents of the rhizome oil 2,5-ditertbutylhydroxybenzene (7.80%), dibutylphthalate (4.58 %), palmitic acid (4.34%), carvacrol (3.89 %), diisobutylphthalate (3.87 %), ethyl 2-bromopropanoate (3.48%) and 1-acetylcyclohexene (3.16%) - were also uncharacteristic of *Aframomums*. To the best of my knowledge phthalates and palmitic acid have not been previously reported as constituents of *Aframomum* oil. These phthalates however, have been reported in *Pterocarpus marsupium* EtOH extract with antimicrobial and antifouling activities[80]. Other compounds which occurred above 1% are cadinene (2.64%) ambrial (1.95%), δ-guaiene (1.86%), alloaromadendrene (1.37 %), ledene (1.25%), and 8,9-dehydro-neoisolongifolene (1.04%). The work of Zollo et al., (2002) also examined the essential oils of the rhizomes and reported mainly oxygenated monoterpenes and oxygenated sesquiterpenoids. The major monoterpenoid identified was linalool (*A. citratum* and *A. hanburyi)* and the major sesquiterpenoid identified was (E)-nerolidol (*A. pruinosum* and *A. letestuanum*)[79].

Mass spectra and corresponding structures of some of major compounds are presented in the **Figures 4.8 - 4.13**

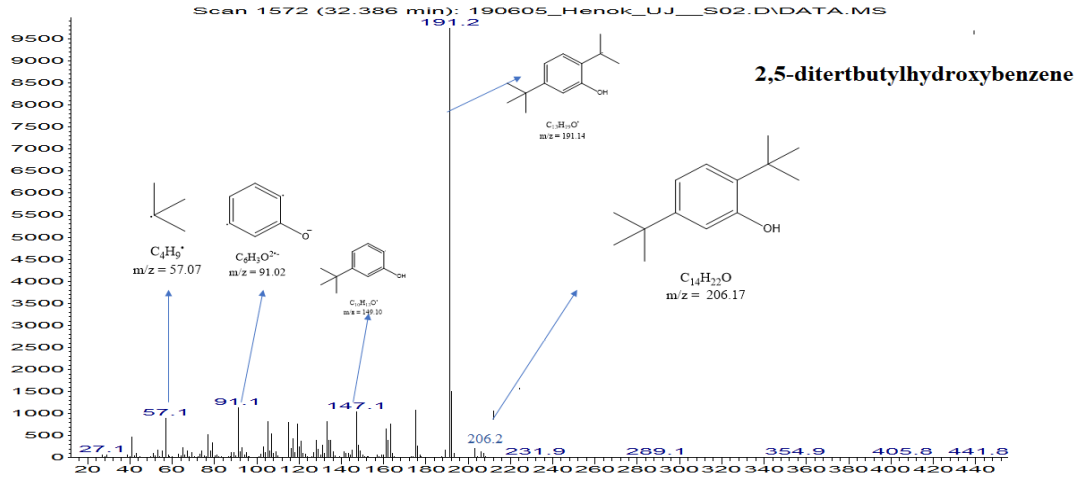


Figure 4. 8: MS of 2,5-ditertbutylhydroxybenzene, (7.80%) MF = $C_{14}H_{22}O$, Rt 32.386

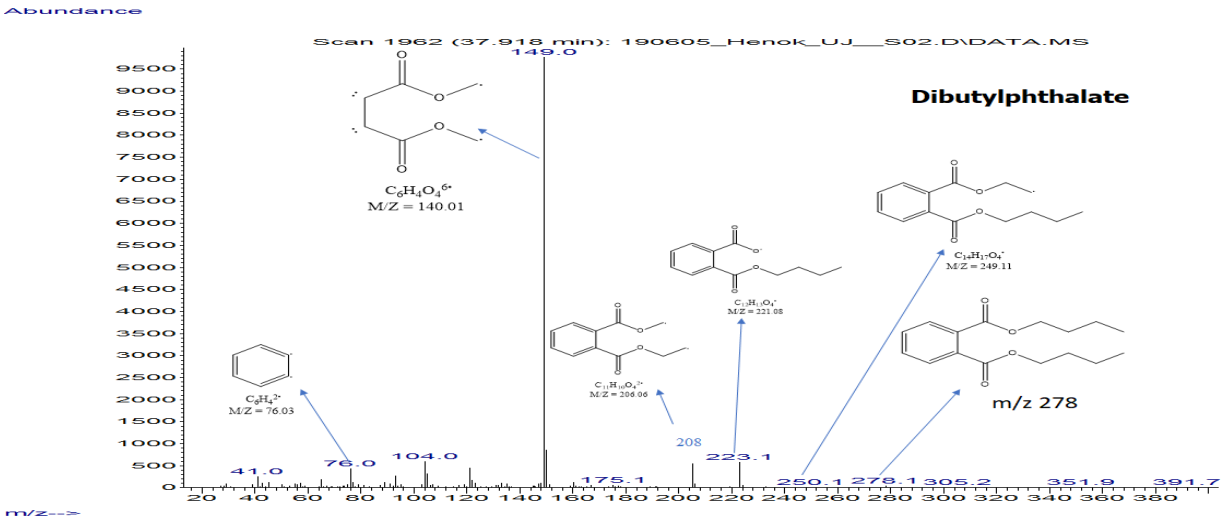


Figure 4. 9: MS of dibutylphthalate, (4.58%), MF = $C_{16}H_{22}O_4$, Rt 37.716

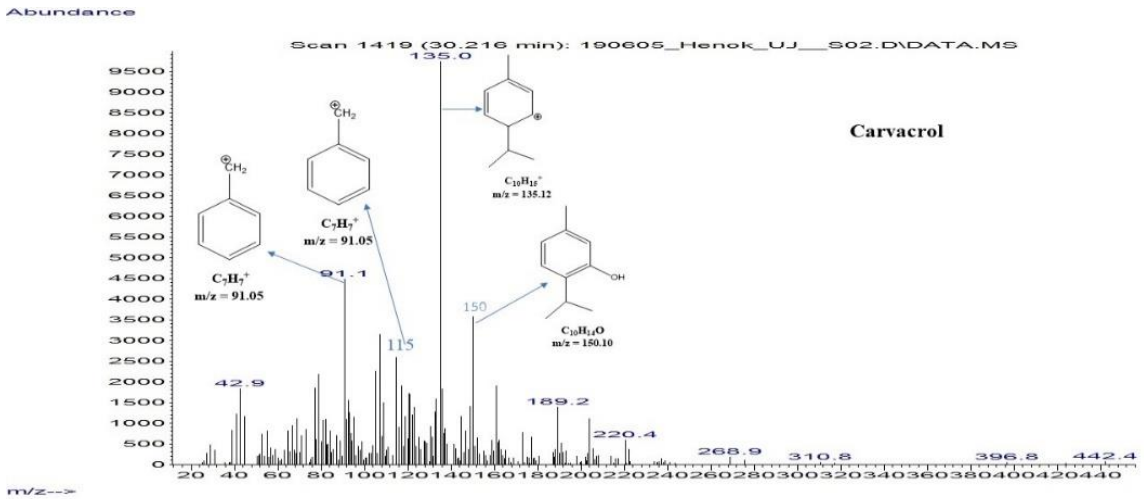


Figure 4.10: MS of carvacrol, (3.89%), MF = $C_{10}H_{14}O$, Rt 30.2158

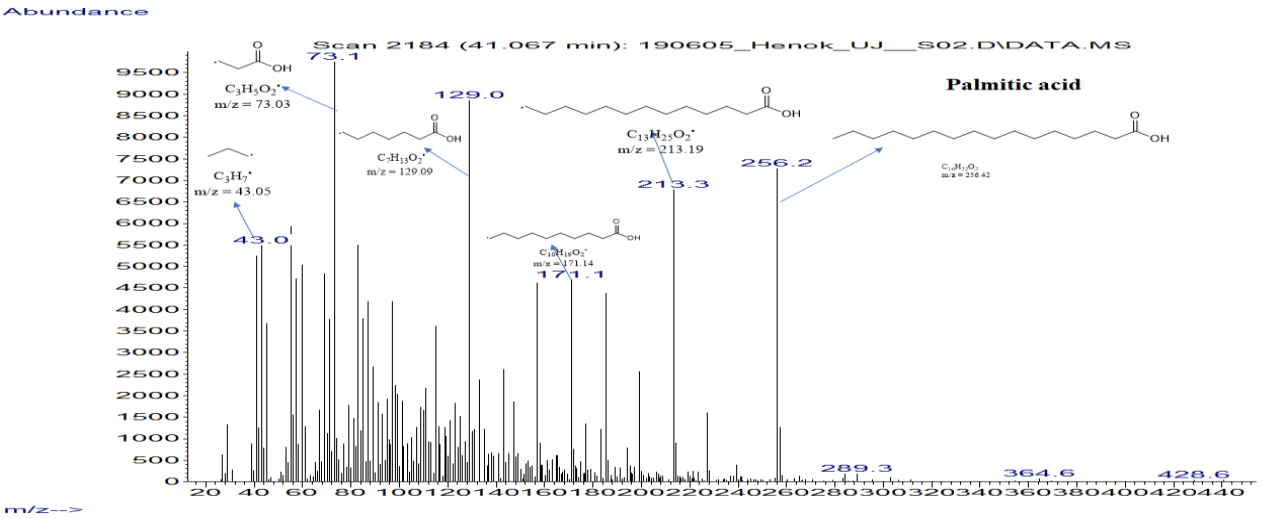


Figure 4.11: MS of palmitic acid, (4.34%), MF = $C_{16}H_{32}O_2$, Rt 41.067

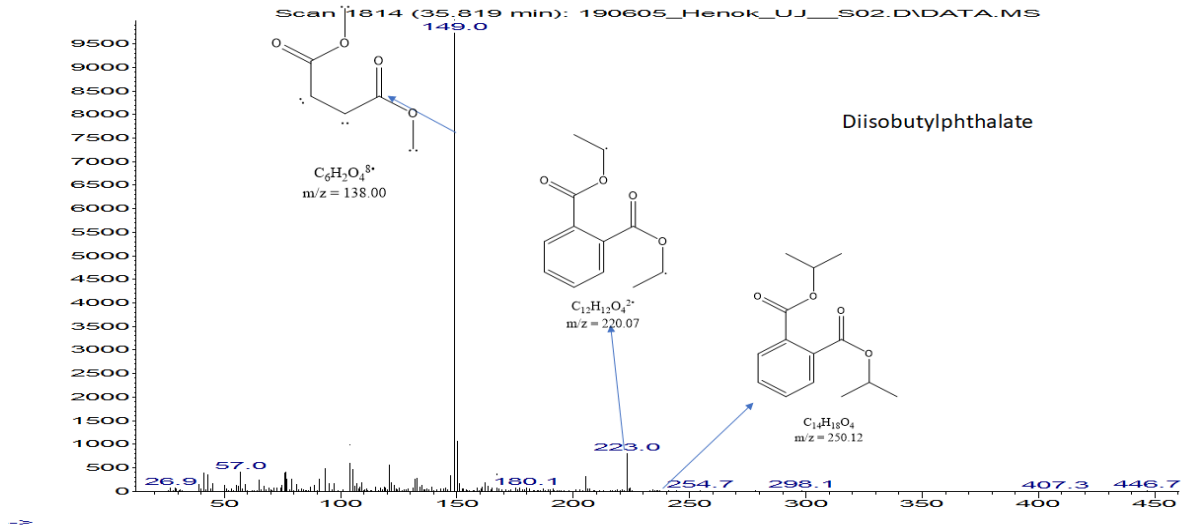


Figure 4.12: MS of diisobutylphthalate, (3.87%), MF = $C_{16}H_{22}O_4$, Rt 35.8189

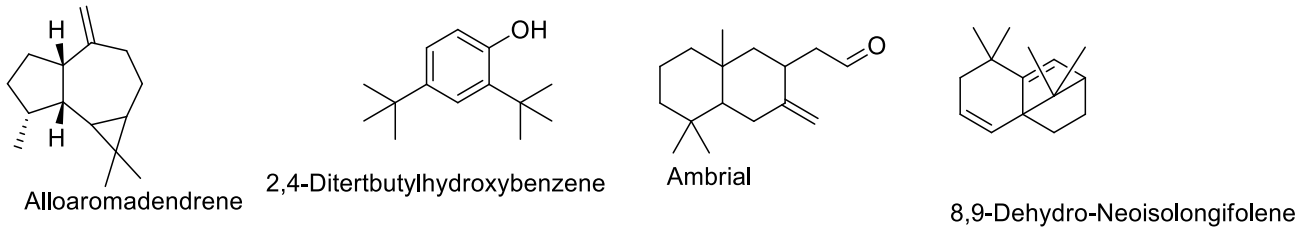


Figure 4.13: Other major constituents of the leaf essential oil

4.2.3 Chemical composition of DCM-extracted essential oil

Fifty compounds, comprising 84.89% of the DCM-extracted EO, were identified in the GC-MS analysis. The major constituents were mainly steroids (59.72%) and long chain hydrocarbons (18.06%), a reflection of the different extraction protocol employed. (**Figure 4.14 and Table 4.3**).

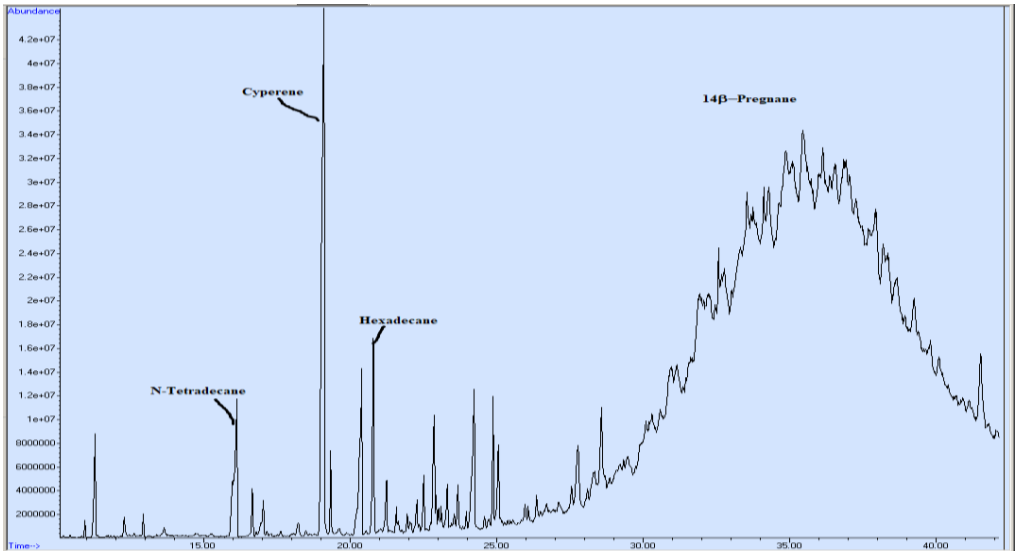


Figure 4.5: Gas chromatogram of DCM-extracted essential oil of *A. atewae*

Table 4.3: Retention time, percentage composition, molecular formula and molecular weight of constituents of *A. atewae* rhizome DCM-extracted essential oil as shown by GCMS analysis

Peak No.	Retention time (min)	Compound	MF	Mwt. (g/mol)	Composition (%)
1	10.9526	4-Methylthiazole	C_4H_5NS	99.16	0.0292
2	11.3072	Dodecane	$C_{12}H_{26}$	170.33	0.2777
3	12.3002	1-Octanol, 2-butyl-	$C_{12}H_{26}O$	186.336	0.0609
4	12.9385	*p*-Cymene	$C_{10}H_{14}$	134.21	0.0457
5	13.6619	Tridecane	$C_{13}H_{28}$	184.37	0.0339
6	16.0024	Tetradecane	$C_{14}H_{30}$	198.39	0.8036
7	16.6691	Egitol	C_2Cl_6	236.7	0.0903
8	16.7968	Benzene, (2-methyl-1-propenyl)-	$C_{10}H_{12}$	132.206	0.0123
9	16.9528	1-Octadecene	$C_{18}H_{36}$	252.486	0.0487
10	17.6337	α–Terpinene	$C_{10}H_{16}$	134.238	0.0168
11	18.2295	Pentadecane	$C_{15}H_{32}$	212.421	0.0524
12	18.499	2-Hydroxy-3-tert-butylacetophenone	$C_{12}H_{16}O_2$	192.254	0.0185
13	**19.109**	**Cyperene**	**$C_{15}H_{24}$**	**204.357**	**2.5054**
14	19.6196	Pentadecane, 3-methyl-	$C_{16}H_{34}$	226.44	0.0461
15	20.3998	Hexadecane	$C_{16}H_{34}$	226.44	0.7518
16	20.797	cyclosativene	$C_{15}H_{24}$	204.357	0.5392
17	21.2509	1-Heptadecene	$C_{17}H_{34}$	238.455	0.2685
18	21.563	Bicyclo[4.1.0]heptane, 4,4-dimethyl-3-(3-methyl-3-butenylidene)-2-methylene	$C_{15}H_{22}$	202.34	0.063
19	21.6339	Pristane	$C_{19}H_{40}$	268.51	0.0329
20	21.946	1-(8-Fluoro-2-hydroxynaphthalen-1-yl)ethanone	$C_{12}H_9FO$	204.20	0.0465
21	22.0452	2,5-dimethoxy-3-methylnaphthalene	$C_{13}H_{14}O_2$	202.25	0.0424
22	22.3573	δ-Gurjunene	$C_{15}H_{24}$	204.357	0.0158
23	22.5134	Ledene	$C_{15}H_{24}$	204.357	0.1324
24	22.7545	Germacrene-D	$C_{15}H_{24}$	204.357	0.0184
25	22.868	Alloaromadendrene	$C_{15}H_{24}$	204.357	0.3563
26	22.9389	4,5-dimethyl-11-methylenetricyclo[7.2.1.0(4.9)]dodecane	$C_{15}H_{24}$	204.357	0.0669
27	23.024	β-Maaliene	$C_{15}H_{24}$	204.357	0.0569
28	23.1233	Azulene	$C_{10}H_8$	128.17	0.0648
29	23.5489	α-Amorphene	$C_{15}H_{24}$	204.357	0.0438
30	23.6765	Selina-3,7(11)-diene	$C_{15}H_{24}$	204.357	0.1188
31	23.9602	α-Gurjunene	$C_{15}H_{24}$	204.357	0.0385
32	24.2297	Octadecane	$C_{18}H_{38}$	254.494	0.8373

33	24.5844	α-Curcumene	$C_{15}H_{24}$	204.357	0.0268
34	24.7262	isospathulenol	$C_{15}H_{24}O$	220.357	0.033
35	24.8822	(1S)-cis-Calamenene	$C_{15}H_{22}$	202.33	0.2985
36	25.9745	Nonadecane	$C_{19}H_{40}$	268.5	0.689
37	26.088	2,3-dicyano-7,7-dimethyl-5,6-benzonorbornadiene	$C_{15}H_{12}N_2$	220.27	0.0382
38	26.3575	α-Calacorene	$C_{15}H_{20}$	220.39	0.0708
39	26.7972	Octahydro-α-camphorene	$C_{20}H_{40}$	280.53	0.0272
40	27.549	Lemonene	$C_{10}H_{16}$	136.24	0.1373
41	**27.776**	**Eicosane**	**$C_{20}H_{42}$**	**282.56**	**3.7822**
42	**28.1023**	**14β-Pregnane**	**$C_{21}H_{36}$**	**288.52**	**58.4384**
43	29.4782	Heneicosane	$C_{21}H_{44}$	296.58	0.6989
44	**31.9322**	**Docosane**	**$C_{22}H_{46}$**	**310.60**	**2.71**
45	**32.2726**	**Docosane, 2,21-dimethyl-**	**$C_{24}H_{50}$**	**338.65**	**1.1801**
46	**33.5351**	**Tricosane**	**$C_{23}H_{48}$**	**324.48**	**2.8882**
47	**36.8544**	**Tetracosane**	**$C_{24}H_{50}$**	**338.65**	**2.5814**
48	**39.2375**	**Aniline, *O*-3-butenyl-**	**$C_{10}H_{13}N$**	**147.22**	**1.7711**
49	**40.0886**	**6-fluoro-4,6-cholestadien-3β-ol**	**$C_{27}H_{43}FO$**	**402.63**	**1.2596**
50	42.0461	Cholesta-3,5-diene	$C_{27}H_{44}$	368.64	0.0298

The monoterpene and sesquiterpene components of the oil were 3.38% and 2.44%, respectively. This observation is not surprising, considering that the plant material was dried and pulverized prior to solvent extraction, which would have resulted in the loss of most of the volatile components. Further, the open column chromatographic separation process would also contribute to evaporation of volatile organic compounds and account for the fewer number of constituents in comparison to the hydrodistilled essential oils. The breakdown for the functional groups identified is as follows: hydrocarbons (40), alcohols (3), ketones (2) and miscellaneous (5).

14β-pregnane (58.44%), formed the bulk of the oil, occurring as a broad peak on the GC chromatogram. It did not feature in either of the hydrodistilled essential oils and it is also being cited for the first time in *Aframomum* species. Pondugula et al (2013) reported that both natural pregnane (pregnenolone, progesterone, 17α-hydroxypregnenolone, 5β-pregnane-3,20-dione and 17α-hydroxyprogesterone) and synthetic (6,16α-dimethyl pregnenolone, dexamethasone *t*-butylacetate and pregnenolone16α-carbonitrile) activate mouse homologue[81]. Naturally occurring pregnane derivatives such as progesterone, 3,20-pregnanediones and 3,20-pregnenediones have been reported to protect against pentylenetetrazole (PTZ)-induced seizures in animals as well as the synthetic A-ring reduced alphaxolone and pregnanes 2*β*-morpholino-5*α*,3*α*-pregnanolone. Metabolites of progesterone, 3*α*,5*α*- pregnanolone and 3*α*,5*β*-pregnanolone, exhibit anticonvulsant activity[82]. Eicosane (3.28), tricosane (2.89%), docosane (2.71%), tetracosane (2.58%), (-)-cyperene (2.51%) and 6-fluoro-4,6-cholestadien-3β-ol (1.26%). The long chain hydrocarbons are commonly-occurring essential oil constituents. Marrufo et al., (2013) reported that *Moringa oleifera* leaf essential oil is

rich in these compounds and showed a strong radical scavenging activity[83]. The mass spectrum of some selected major constituents are shown in **Figure 4.15 – 4.18** below.

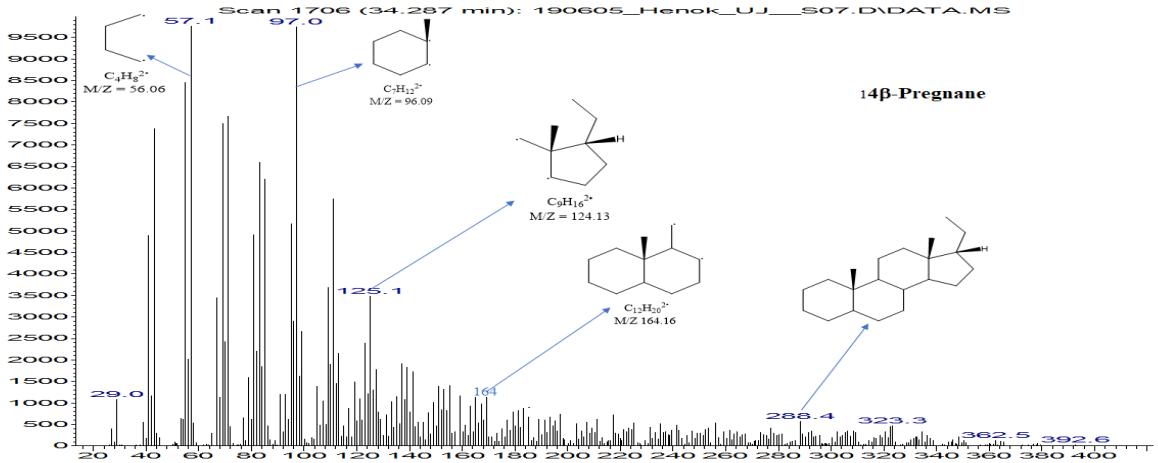


Figure 4. 14: MS of 14β-pregnane, (58.44%), MF = $C_{21}H_{36}$, Rt 34.287

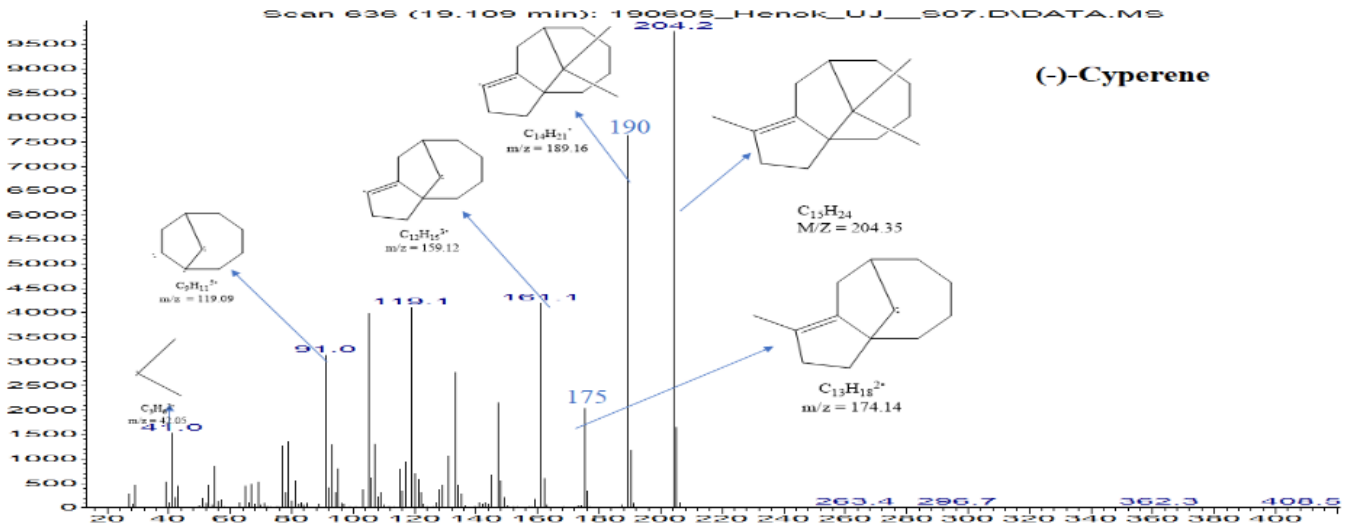


Figure 4.15: MS of (-)-cyprene, (2.51%), MF = $C_{14}H_{24}$, Rt 19.109

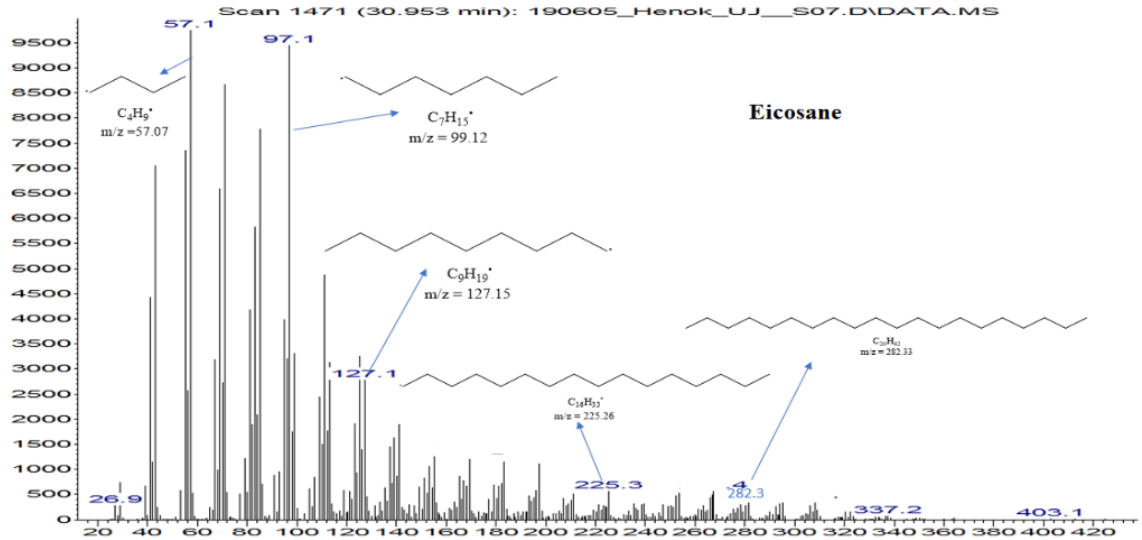


Figure 4.16: MS of diisobutylphthalate, (3.87%), MF = $C_{16}H_{22}O_4$, Rt 35.8189

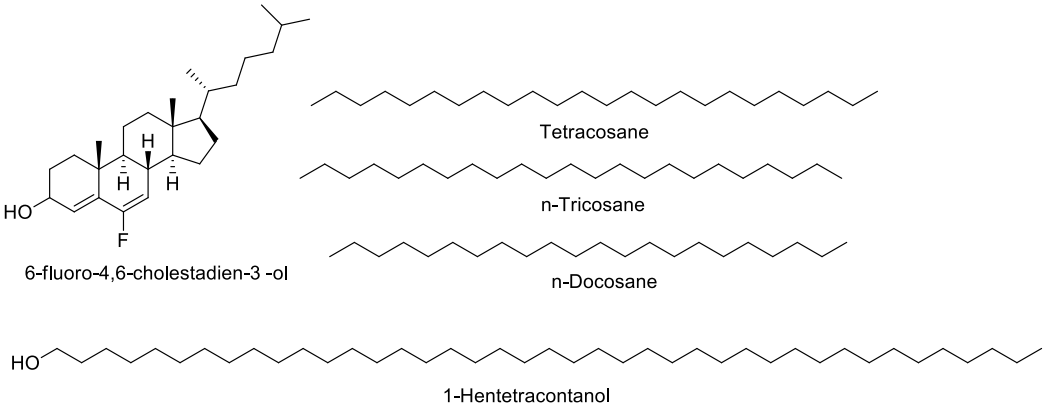


Figure 4.17: Other major constituents of AA/R/DCM 1

Regardless of the major differences in the chemical profile of the three essential oils, they all contained some common minor constituents (**Table 4.4**) The hydrodistilled essential oils were more similar in the respect.

Table 4.4: Classes of terpenes and common constituents of the 3 essential oils

	Leaf (%)	**Rhizome (%)**	**DCM-extracted rhizome (%)**
Classes of terpenes			
Monoterpene hydrocarbons	10.9169	1.2846	3.3845
Oxygenated monoterpenes	11.4998	6.7907	-
Sesquiterpene hydrocarbons	6.9138	17.2087	2.4097
Oxygenated sesquiterpenes	7.0682	7.2345	0.033
Diterpene	0.5407	-	-
Common constituents			
p-cymene	0.5945	0.3111	0.0292
α-Terpinene	0.0367	0.2453	0.0168
α-Selinene	0.091	0.7009	-
o-Allytoluene	0.1582	0.2579	-
Isolongifolene	0.8011	2.0337	-
Valerenal	0.1644	0.5864	-
Ledene	1.2532	-	0.1324
Alloaromandendrene	1.1402	1.3663	-
β-caryophyllene	0.4406	0.3587	-
α-gurjenene	0.4142	-	0.0385
Cyperene	0.3681	-	2.5054

Most essential oil constituents are biologically active molecules and form part of the plants' defence systems and hence are used for crop protection. Due to their biodegradable nature, these botanical pesticides are gradually replacing their synthetic counterparts. They also find wide application as flavoring agents, fragrance, or as medicines used to treat various human and animal ailments. Since the components of essential oils are what provide them with their intrinsic properties, a variation in

the distribution of the components modifies the particular properties of essential oils. This variation is influenced by extrinsic factors such as soil, climate, elevation and geographical origin.

4.3 Antifungal studies of essential oils

As indicated in section 3.5, page 23, the four essential oils were evaluated for their activity against *C. albicans* and *S. cerevisiae*. **Figure 4.19** shows the activity of the 4 essential oils against *C. albicans*. The fluorescence observed for the negative control, the positive control, PE-extracted oil, DCM-extracted oil, rhizome EO and leaf EO were 300, 1450, 600, 250, 250 and 550 nm, respectively. The PE-extracted oil and the rhizome EO exhibited fungistatic activity toward *C. albicans* because the fluorescence emitted was greater than that of the negative control but lower than that of positive control. Conversely, fluorescence emitted from the DCM-extracted oil and the rhizome EO was lower than observed for both the negative and positive controls hence, they were fungicidal to the cells. β-Caryophyllene, caryophyllene oxide and terpinolene have been reported to restrict biofilm development of fungi including *C. albicans* [84]. β-Caryophyllene (0.39%) was identified as a constituent of the fresh leaf EO whiles caryophyllene oxide (2.14%), and terpinolene (0.07%) were also present in the rhizome EO, hence these constituents can be linked to the observed activities of the essential oils against C. albicans. Shin et al (2003) also reported that *p*-cymene showed activity against *C. albicans*. *p*-Cymene was identified as a constituent of the 3 oils with compositions - 0.59%, 0.31% and 0.029% for the fresh leaf EO, fresh rhizome EO and DCM-extracted oil, respectively.

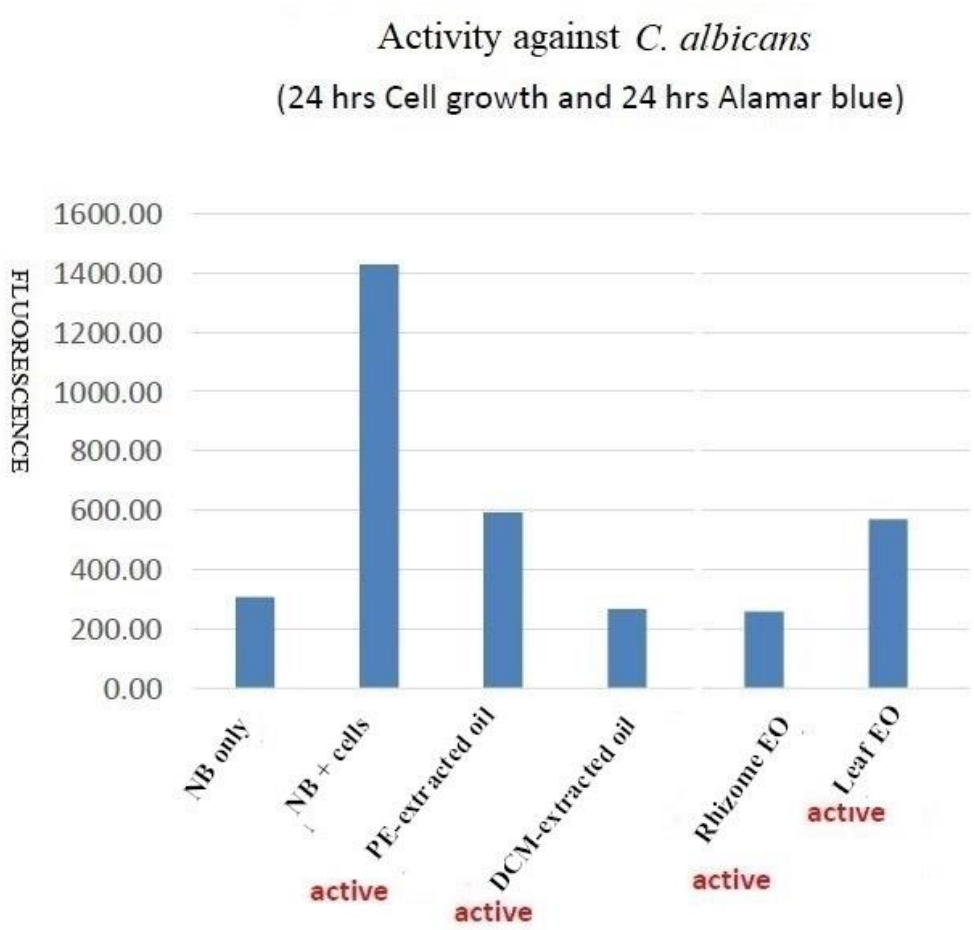


Figure 4.18: Graph of results showing activity of essential oils against *C. albicans*

The activity of the oils against *S. cerevisiae* is captured in **Figure 4.20.** Observed fluorescence values were 100, 1800, 200, 2100, 2250 and 2600 nm for the negative control, positive control, PE-extracted oil, DCM-extracted oil, rhizome EO and leaf EO, respectively. Out of the 4 oils, only the PE-extracted oil emitted fluorescence less than that of the positive control but greater than the negative control, suggestive of the fungistatic nature of the oil to the cells. The 3 remaining oils, however, did not show any activity against *S. cerevisiae*. In contrast to Adegoke et al. (1996) reporting that α-terpinene caused growth reduction of *S. cerevisiae*[36], the 3 EOs tested showed no activity potency due to the low percentage of α-terpinene, (0.017% - 0.037%).

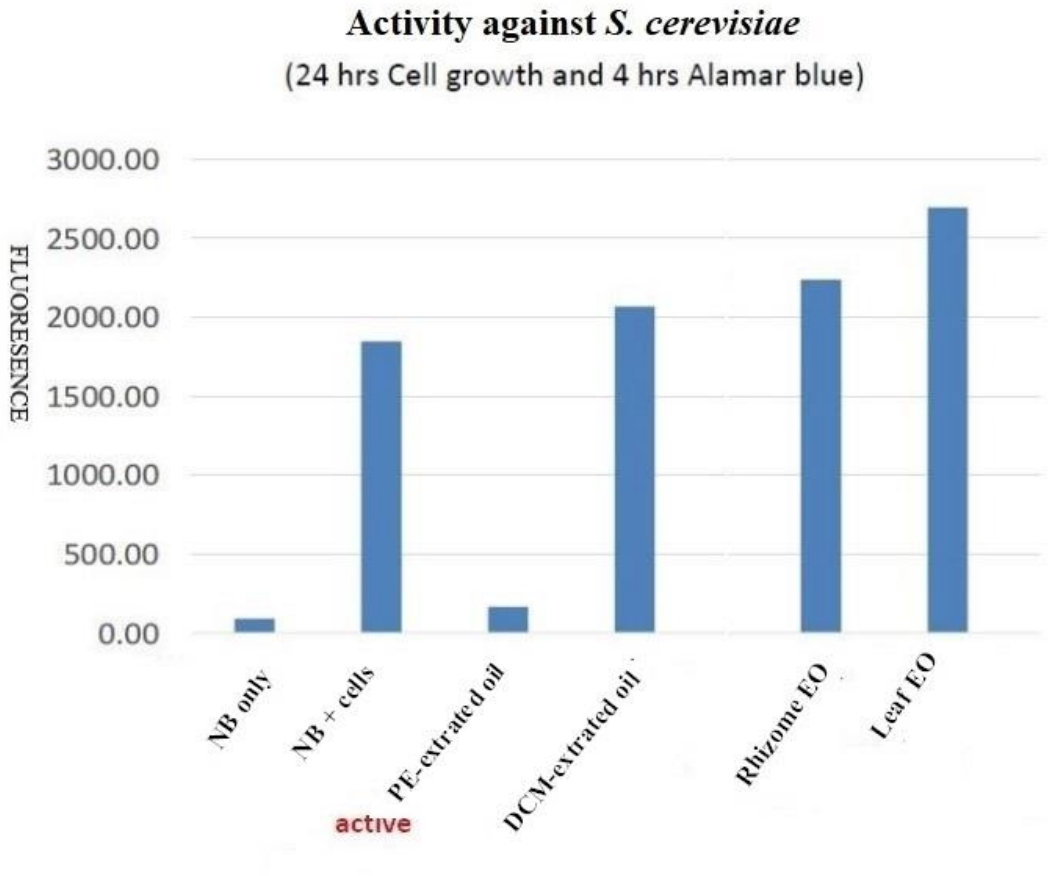
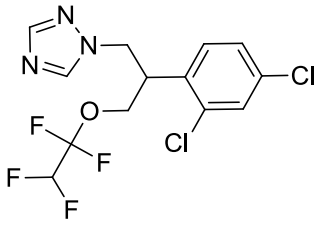


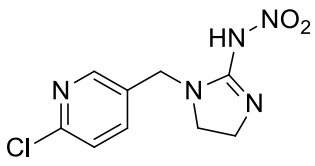
Figure 4.19: Graph of results showing the activity of essential oils against *S. cerevisiae*

Unfortunately, the constituents of the PE-extracted oil could not be determined due to sample mix-up during GC-MS analysis. Hence the fungistatic activity observed could not be attributed to any compound.

The observed activities for the various oils suggest that the essential oils of *Aframomum atewae* have the potential as effective fungicides. In Ghana, especially in the cocoa production sector, farmers use chemicals that contain nonbiodegradable compounds such as tetraconazole [48] and neonicotinoid such as imidacloprid [49] to control plant pathogens. These compounds are able to accumulate in the soil and the fruits of cocoa, and could affect the quality of the cocoa beans and also lead to soil contamination hence destroying of ecosystem and pollution of water bodies. Due to similarities in mechanisms of infection between plant and animal pathogenic fungi[88], essential oil from *Aframomum atewae* could be explored as a biodegradable source of plant pathogens control.



[48]



[49]

4.4 Investigation of extracts from the rhizome of *A. atewae*

4.4.1 Phytochemical Screening of the PE, DCM and MeOH Crude Extracts

From the phytochemical screening, it was observed that the MeOH extract contained all the tested phytochemicals except anthraquinones, anthracenes and steroids. The PE extract contained mainly terpenoids and steroids with the DCM extract containing flavonoids in addition to steroids and terpenoids.

Table 4. 5: Results of phytochemical screening of crude extracts

Class of compounds	Petroleum ether	Dichloromethane	Methanol
Alkaloids	-	-	+
Anthraquinones and anthracenes	-	-	-
Flavonoids	-	+	+
Cardiac glycosides	-	-	+
Saponins	-	-	+
Tannins	-	-	+
Terpenoids	+	+	+
Steroid	+	+	-

Legend: (+) = present and (-) = absent

The mass and percentage yield of crude extracts are summarized in **Table 4.6** below.

Table 4.6: Mass and percentage yields of various extracts

Extract	Mass	Yield (%)
AA/R/PE	0.0164 kg	0.88%
AA/R/DCM	0.0255 kg	1.37%
AA/R/MeOH	0.0441 kg	2.37%
AA/R/MeOH/PE	3.3846 g	8.46%
AA/R/MeOH/PE:EtOAc 1:1	3.401 g	8.50%
AA/R/MeOH/EtOAc	5.053 g	12.63%
AA/R/MeOH/EtOH	8.493 g	21.23%

4.4.2 Investigation of the Petroleum Ether (PE) Extract

Through chromatographic separation, an essential oil and 3 compounds labelled AA/R/PE-1, AA/R/PE-2, AA/R/PE-5 and AA/R/PE-6 were obtained from the PE extract. AA/R/PE-1 was characterized by identifying the constituents of the EO using GC-MS (**Table 4.3**). The antifungal property of the oil was also tested against *C. albicans* and *S. cerevisiae* (**Figures 4.18, 4.19**).

4.4.2.1 Characterization of AA/R/PE-2

AA/R/PE-2 was isolated as a white solid with a melting point of 112-114 °C. When subjected to IR spectroscopic analysis (**Appendix I**), there were absorptions at 2915.15 cm^{-1} and 2849.10 cm^{-1} assigned to C-H_{str} (sp^3 and sp^2, respectively), a C=O_{str} at 1730.01 cm^{-1} and a C=C_{str} at 1630 cm^{-1}. A peak at 1471 cm^{-1} was assigned to a C-$O_{str.}$

From the ^{13}C NMR spectrum (**Figure 4.21**), 17 signals between the range δ_C 14 - 175 were observed. Five signals were due to sp^2 hybridized carbons and the remaining were attributed to sp^3 hybridized carbons. The signal at δ_C 174.1 was assigned to the carbonyl carbon whereas the remaining 4 sp^2 carbons were assigned to alkene carbons. An sp^3 hybridized carbon attached to a heteroatom was observed at δ_C 64.5.

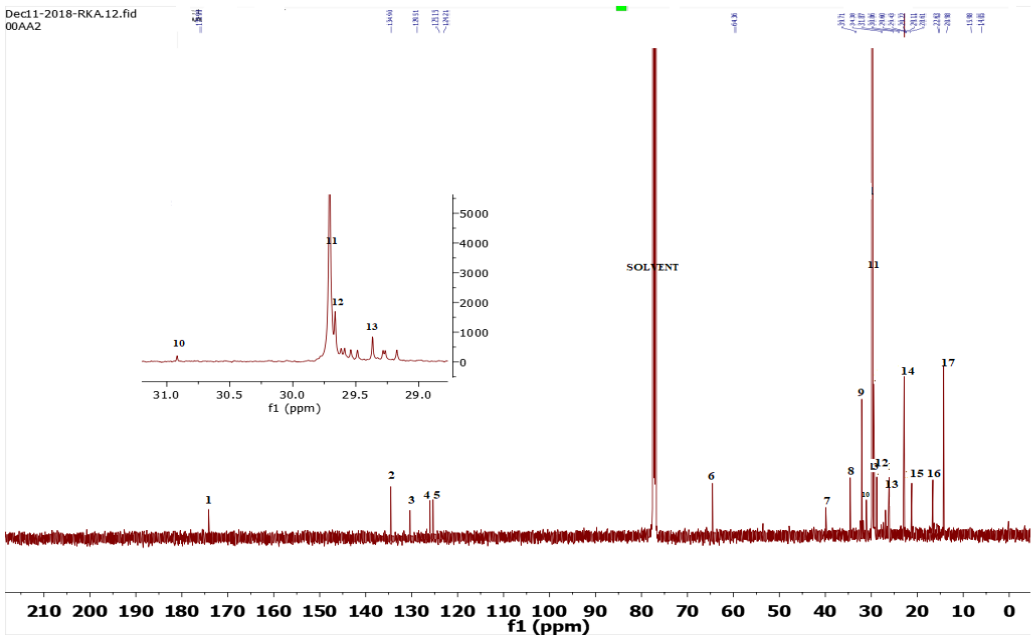


Figure 4.20: Full ^{13}C NMR Spectrum of AA/R/PE-2

The DEPT 135° spectrum (**Figure 4.22**) revealed 2 quaternary carbons identified at δ_C 174.1 (C-1) and δ_C 135.0 (C-2). Methine carbons occurred at δ_C 129.7 (C-3), δ_C 125.1 (C-4,), δ_C 124.4 (C-5) and

δ_C 31.0 (C-10). At δ_C 16.1 (C-16) and δ_C 14.2 (C-17), the signals were assigned to methyl carbons while the remaining 9 signals were identified as methylene carbons.

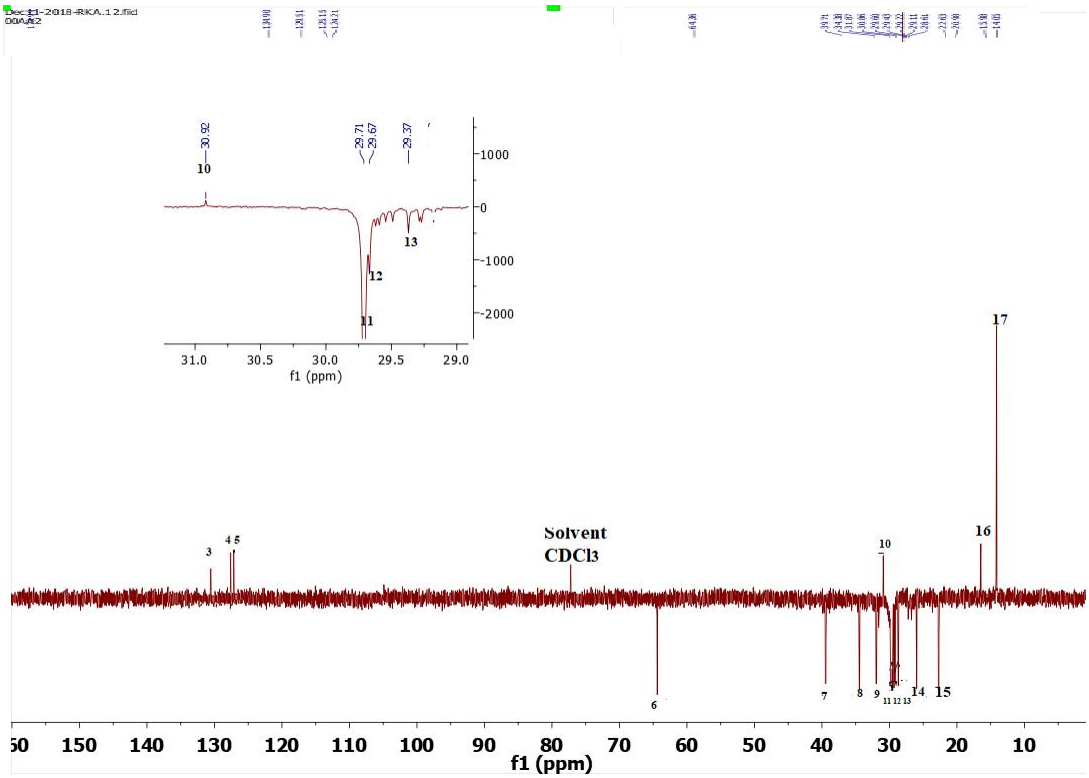


Figure 4.21: Full DEPT 135 ° Spectrum of AA/R/PE-2

All the signals in the ^{1}H NMR spectrum (**Figure 4.23),** were observed within the range δ_H 0.88 - 5.50. The signals at δ_H 0.88 (t, 3H, *J* = 2.4 Hz) and δ_H 1.80 (s, 3H) were assigned to protons on the 2 methyl carbons identified in the ^{13}C NMR spectrum. Methylene protons were observed at δ_H 4.05 (t, 2H, *J* = 3.7 Hz), δ_H 2.28 (t, 2H, *J* = 2.3 Hz), δ_H 2.20 (t, 2H, *J* = 2.6 Hz), δ_H 1.98 (t, 2H, *J* =3.2 Hz), δ_H 1.62 (m, 4H) and δ_H 1.60 (t, 2H, *J* = 2.7 Hz). At δ_H 1.26 (s), the signal integrated for 8 protons suggesting the presence of a continuous methylene chain in the compound. Three olefinic signals occurred at δ_H 5.35 (d, 2H, *J* = 4.1 Hz), δ_H 5.30 (t, 1H, *J* = 3.5 Hz), δ_H 5.12 (dd, 2H, *J* = 4.1, 2.3 Hz) while a methine was identified at δ_H 2.17 (m, 1H).

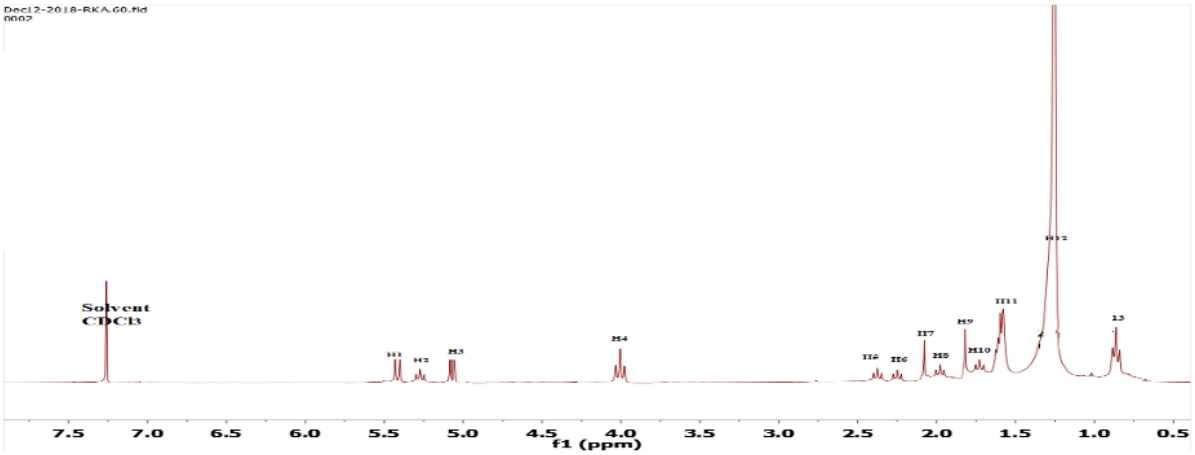
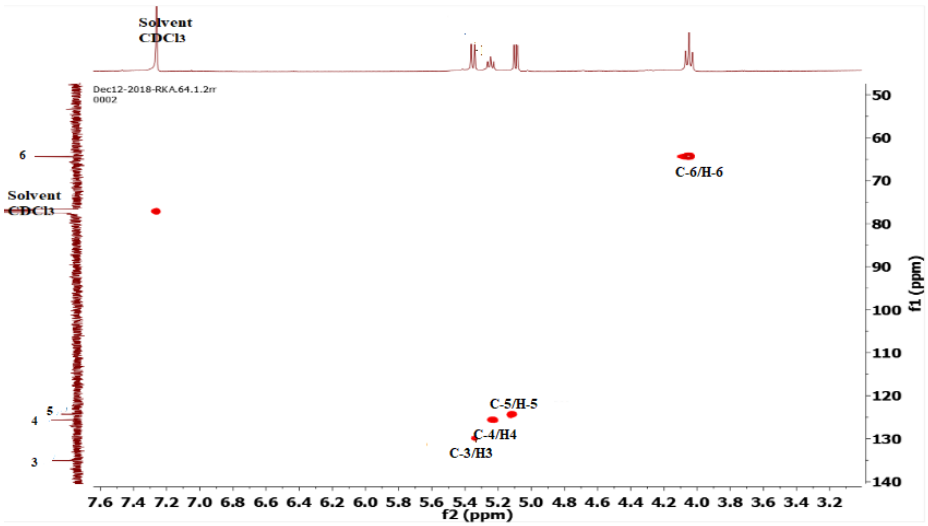


Figure 4.22: Full ^{1}H NMR Spectrum of AA/R/PE-2

From the HSQC spectrum (**Figures 4.24**), the following correlations were made: C-3 (δ_C 129.7) / δ_H 5.35, C-4 (δ_C 125.1) / δ_H 5.30, C-5 (δ_C 124.4) / δ_H 5.12 and C-10 (δ_C 31.0) / δ_H 2.17. In the ^{1}H NMR spectrum, the signals at δ_H 5.35 and δ_H 5.12 integrated for 2 protons each. Hence, their HSQC correlation to C-3 (δ_C 129.7) and C-5 (δ_C 124.4), respectively, which in the DEPT 135 ° experiment were identified as methine carbons, suggests that each of these two signals represents two sets of equivalent carbons. Further correlations were observed for the methyl groups at C-16 (δ_C 16.1)/ δ_H 1.80 and C-17 (δ_C 14.2)/ δ_H 0.88. The oxygenated methylene carbon C-6 (δ_C 64.5) correlated with the protons at δ_H 4.05. There was also a correlation between both C-11 (δ_C 29.8, br) and C-14 (δ_C 28.8) with δ_H 1.26 (br s, 8H). From this observation, it can be deduced that the broad signal at δ_C 29.8 (C-11) represents 3 methylene carbons.



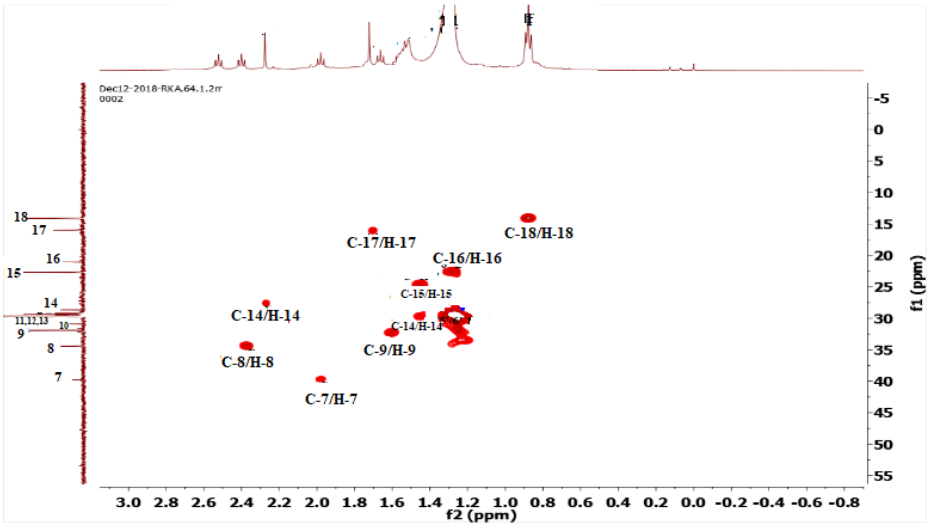
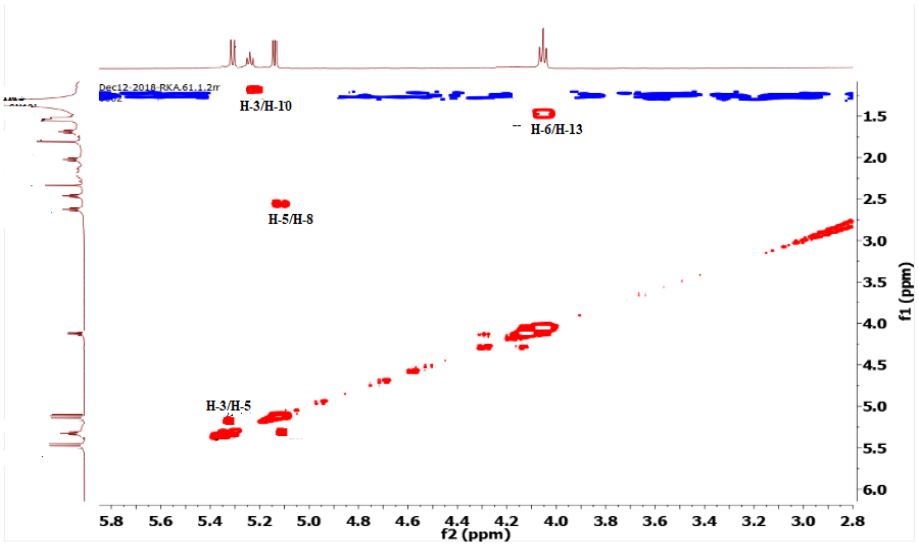


Figure 4.23: Expanded HSQC spectrum of AA/R/PE-2

Four COSY spin systems were observed involving the methine protons H-3 (δ_H 5.35, d, 2H, *J* = 4.1 Hz), H-5 (δ_H 5.12, dd, 2H, *J* = 4.1, 2.3 Hz) and the methylene protons H-8 (δ_H 2.28, t, 2H, *J* = 2.3 Hz); H-9 (δ_H 1.60, 2H, t, *J* = 2.7) and H-13 (δ_H 2.20, 2H, t, *J* = 2.6); H-4 (δ_H 5.30, t, 1H, *J* = 3.5 Hz), H-16 (δ_H 1.26, m) and H-18 (δ_H 0.88, t, 3H, *J* = 2.4). The last spin system consisted of a cluster of contours from which correlations were established at H-6 (δ_H 4.05, 2H, t, *J* = 3.7) / H-14 (δ_H 1.62, m) and H-7 δ_H (1.98, 2H, t, J = 3.2) /H-12 (δ_H 1.26, m) (**Figure 4.25**).



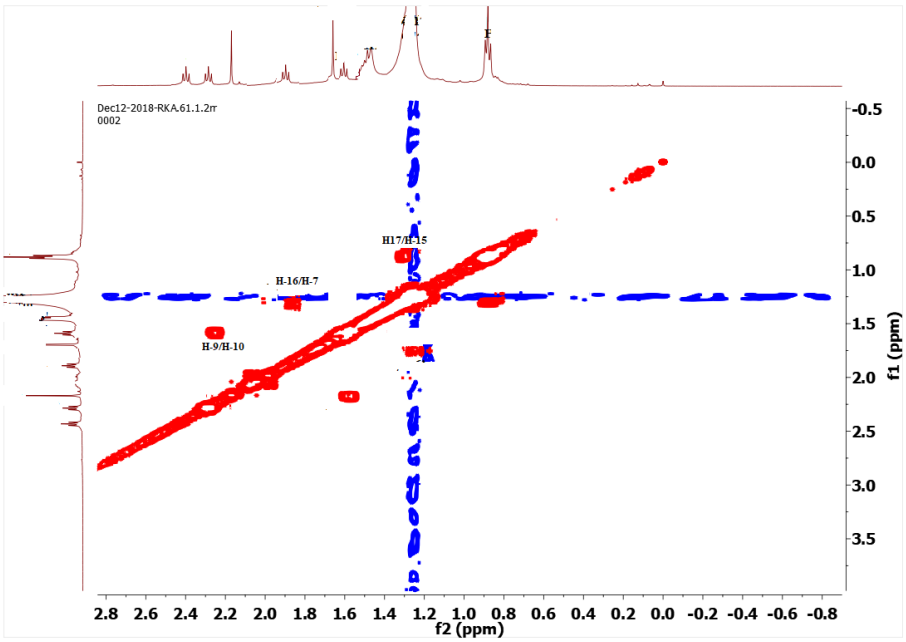
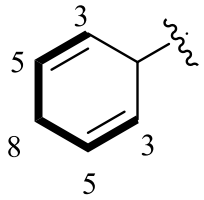
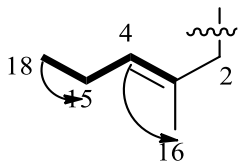


Figure 4.24: Expanded COSY NMR Spectrum of AA/R/PE-2

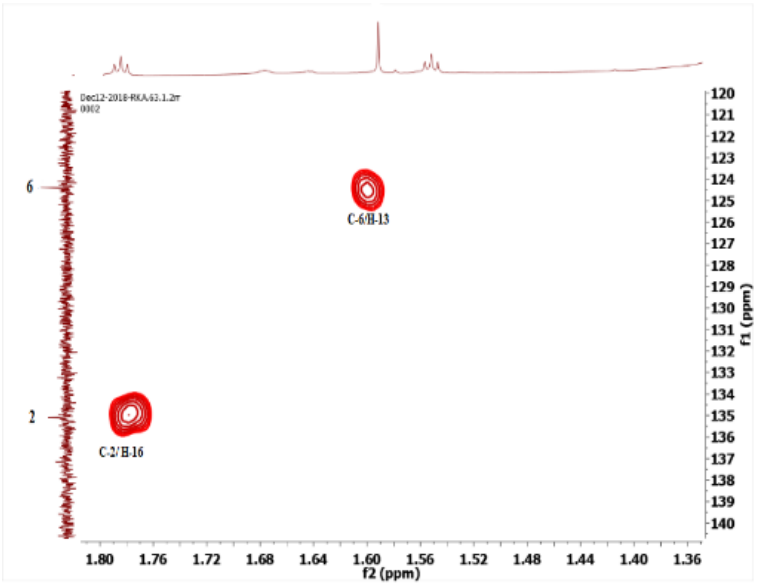
The identification of two sets of equivalent olefinic methine carbons as part of one spin system suggested the presence of a 1,4-cyclohexadienyl moiety in the compound.

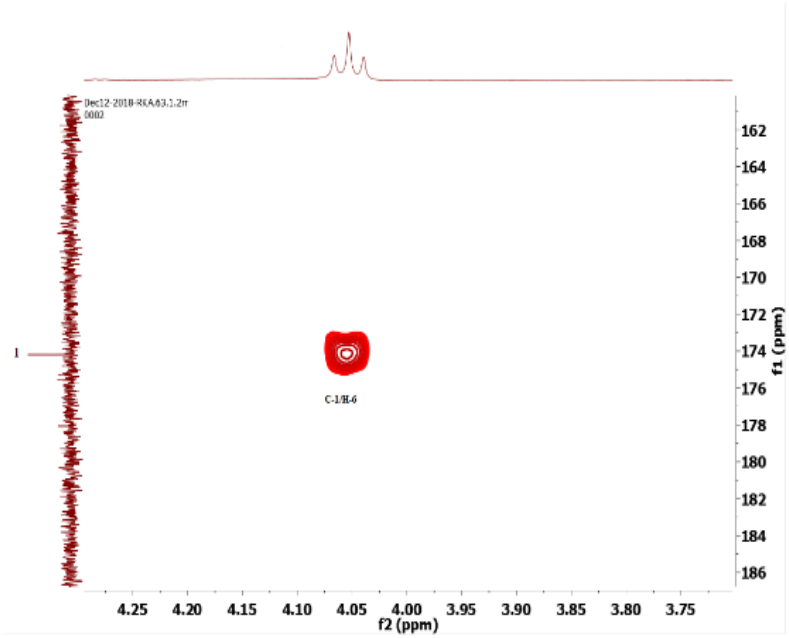


The HMBC correlation C-10 (δ_C 31.0)/ H-13 (δ_H 2.20) indicated the position of attachment of a side chain to the ring. Another correlation from C-1 (δ_C 174.1) to H-6 (δ_H 4.05) buttressed the presence of an ester carbonyl in the compound. C-6 showed correlations with H-14 (identified as ^2J from H-6/H-14 COSY splitting) and H-15 (^3J). The H-17 tertiary methyl protons (δ_H 1.80) correlated with the quaternary carbon C-2 (δ_C 135.0), the methylene carbon C-7 (δ_C 39.9) and the olefinic methine carbon C-4 (δ_C 125.1). The link between C-4 and C-16 was established by the COSY spin system H-4/H-15/H-17 and ^2J coupling H-17 to C-15, which supported a 2-methyl-2-pentenyl moiety in the structure.



The H-7/H-12 spin system also supported another connection between C-7 and C-12 which showed long range coupling to the continuous methylene chain alluded to the proton and ^{13}C NMR spectra. (**Figure 4.26)**





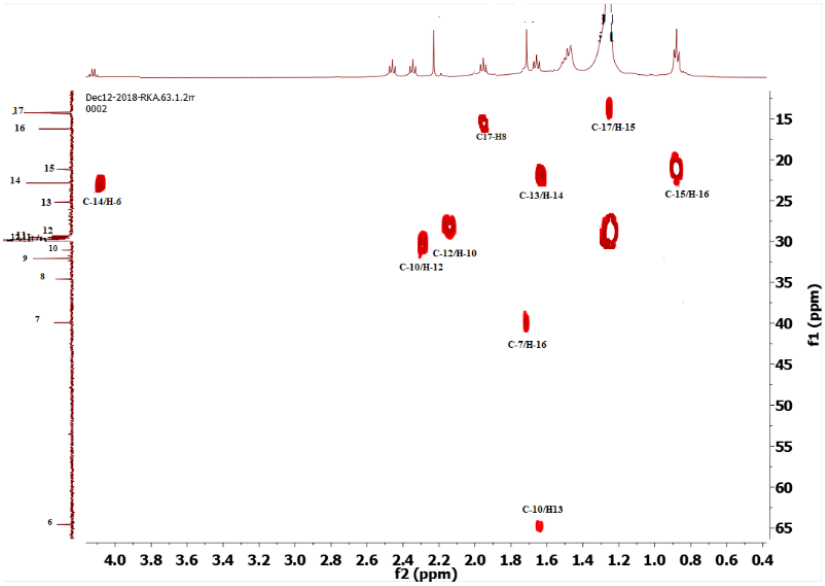
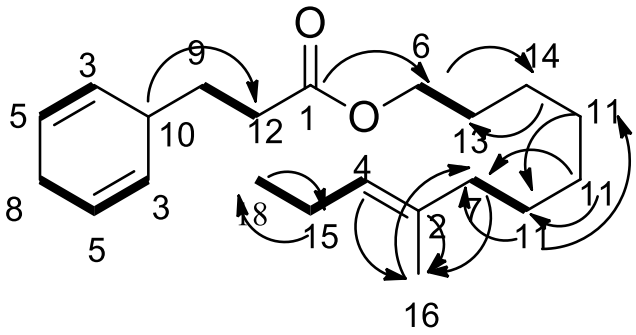


Figure 4.25: Expanded HMBC NMR Spectrum of AA/R/PE-2

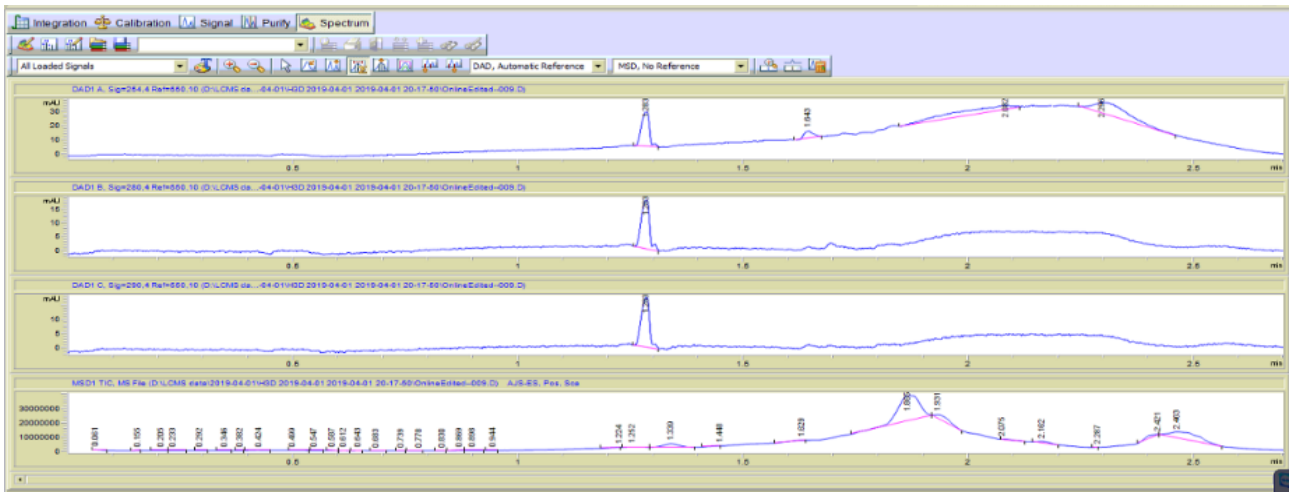
Table 4.7 contains a summary of the NMR data for AA/R/PE-2

Table 4.7: ^{1}H, ^{13}C, COSY and HMBC NMR data for AA/R/PE-2

Signals	δ_C	$^{13}C_{mult}$	δ_H /(*J*/Hz)	COSY	HMBC
1	174.1	C			6
2	135.0	C			16
3	129.7	CH	5.35 (1H, d, *J* = 4.1)	5	
	129.7	CH	5.35 (1H, d, *J* = 4.1)	5	
4	125.1	CH	5.30 (1H, t, *J* = 3.5, 12.1)	15	16
5	124.4	CH	5.12 (1H, dd, *J* = 4.1, 2.3)	3, 8	
	124.4	CH	5.12 (1H, dd, *J* = 4.1, 2.3)	3, 8	
6	64.5	CH_2	4.05 (2H, t, *J* = 3.7)	13	13, 14
7	39.9	CH_2	1.98 (2H, t, *J* = 3.2)	3	16
8	34.6	CH_2	2.28 (2H, t, *J* = 2.3)	5	
9	32.0	CH_2	1.60 (2H, t, *J* = 2.7)	10, 12	
10	31.0	CH	2.17 (1H, m)	9	12
	29.8	CH_2	1.26 (2H, m)		11
11	29.8	CH_2	1.26 (2H, m)		7, 11
	29.8	CH_2	1.26 (2H, m)	7	7, 11
12	29.5	CH_2	2.20 (2H, t, *J* = 2.6)	3	
13	28.8	CH_2	1.62 (2H, m)	6	
14	25.2	CH_2	1.62 (2H, m)		13
15	22.8	CH_2	1.26 (2H, m)	4, 17	17
16	16.1	CH_3	1.80 (3H, s)		7
17	14.2	CH_3	0.88 (3H, t, *J* = 2.4)	15	15



The LC-MS of AA/R/PE-2 (**Figure 4.27**) gave a signal at *m/z* 318.2 at a retention time of 1.291 minutes deduced to be the molecular ion peak with molecular formula $C_{21}H_{34}O_2^{\bullet+}$ (5 degrees of unsaturation).



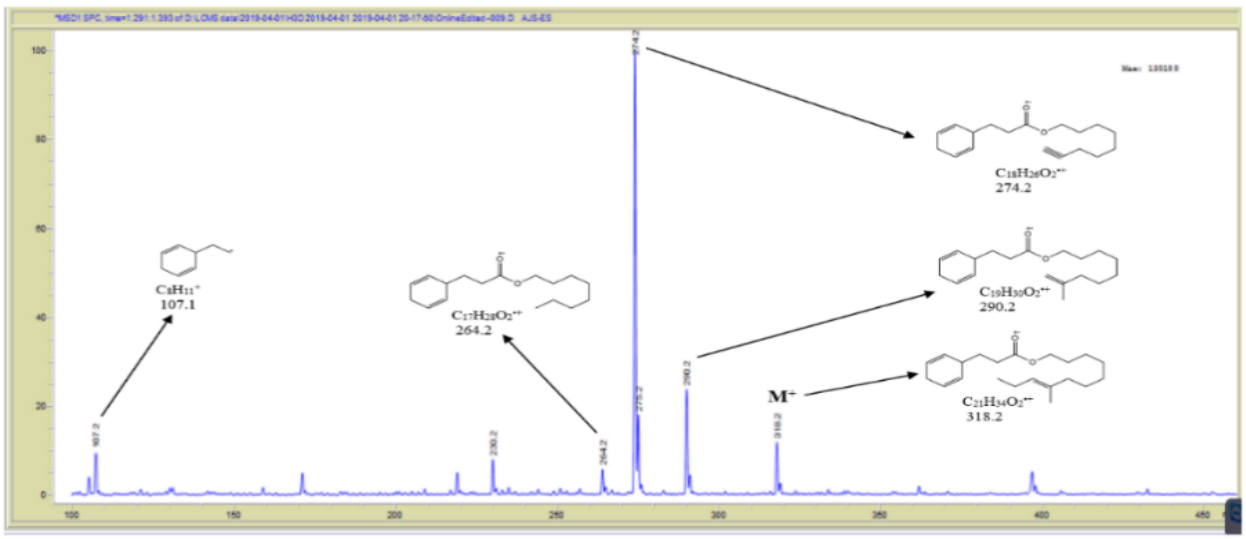


Figure 4.26: LC-MS of AA/R/PE-2

The proposed structure of AA/R/PE-2 was supported by the molecular formula and further confirmed with the daughter ions $C_{18}H_{26}O_2^{\bullet+}$ (*m/z* 274.2, base peak), $C_{19}H_{30}O_2^{\bullet+}$ (*m/z* 290.2), $C_{18}H_{28}O_2^{\bullet+}$ (*m/z* 264.2) and $C_8H_{18}^{\bullet+}$ (*m/z* 107.2) (**Figure 4.28**).

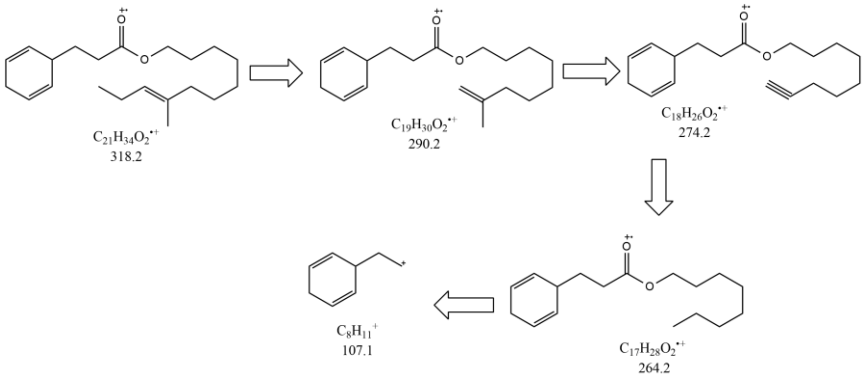


Figure 4.27: Proposed fragmentation pattern for AA/R/PE-2

From the above information, the structure below was proposed and was named as 1 (E)-8-methylundec-8-en-1-yl 3-(cyclohexa-2,5-dien-1-yl)propanoate (**Figure 4.29)**.

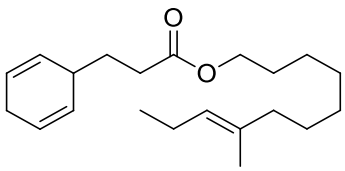


Figure 4.28: Proposed structure of AA/R/PE 1 (E)-8-methylundec-8-en-1-yl 3-(cyclohexa-2,5-dien-1-yl)propanoate

A thorough literature survey on Scifinder, PubChem, Chemspider, and other search engines revealed no compound with the name (E)-8-methylundec-8-en-1-yl 3-(cyclohexa-2,5-dien-1-yl)propanoate have been isolated from any species of *Aframomum*.

4.4.2.3 Identification of AA/R/PE-5

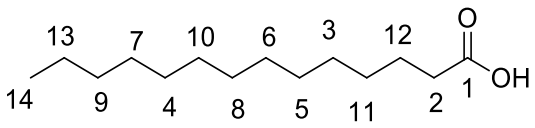
AA/R/PE 5 was identified as myristic acid and was obtained as white amorphous solid. The melting point was measured to be 55.3-55.5 °C, compared to the literature value of 54.4 °C. When subjected to IR spectroscopic **(Appendix II)** analysis, there was an absorption band of 2915.82 cm^{-1} and 2848.62 cm^{-1} was assigned to C-H (stretching) and 1699.00 cm^{-1} assigned to C=O absorption. The stretching peaks at 719.91 and 939.23 cm^{-1} were due to O-H swinging or rocking mode, which are characteristics of the aliphatic chain of myristic acid. This characteristic behaviour of the O-H functionality of myristic acid has been reported by Rama Mohan et al, 2015[80].

The ^{13}C-NMR (**Appendix IIIA and Appendix IIIB**) showed the presence of 16 carbons with peaks occurring between δ_C 14-178. A peak at δ_C 178.4 was assigned to the carbonyl carbon of the compound whiles the remaining peaks between δ_C 14 - 34 were due to sp^3 hybridized carbons. From the DEPT 135° (**Appendix IV**) spectrum, a total of 14 methylene, 1 quaternary and 1 methyl carbons were observed with the methyl carbon at δ_C 14.1. The ^{1}H NMR spectrum (**Appendix V**), 4 distinct

peaks were observed between δ_H 0.89-2.34 which integrated for a total of 27 protons. Peaks at δ_H 0.89 (t, 3H, *J* = 6.8 Hz) were assigned to methyl hydrogens of the methyl carbon. The methylene protons were observed at δ_H 1.28 (4H), δ_H 1.64 (t, 2H, *J* = 7.4 Hz) and δ_H 2.34 (t, 2H, *J* = 7.5 Hz), and δ_H 1.26 (s, 16H). The acidic hydrogen was not observed in the proton NMR and this may be as a result of an exchange between the compound and the solvent. From the HSQC spectrum (**Appendix VI**), protons δ_H 1.64 and δ_H 2.37 were assigned to the methylene carbons at δ_C 24.7 and δ_H 33.8, respectively while proton δ_H 1.28 was assigned to carbons δ_C 22.7 and δ_C 31.9. The HMBC spectrum (**Appendix VII**), showed the correlation between the carbonyl carbon at δ_C 178.4 and proton δ_H 2.34 which is attached to the methylene carbon at δ_C 33.8. The methyl proton at δ_H 0.89 was also coupled to the methylene carbon at δ_C 31.8. From the COSY spectrum (**Appendix VIII**) there was coupling between proton δ_H 2.34 and δ_H 1.64 as well as δ_H 1.28 and δ_H 0.89. A summary of the NMR data is shown in **Table 4.8**.

Table 4.8: ^{1}H, ^{13}C, COSY and HMBC NMR data for AA/R/PE-2

Signals	Atom	$^{1}\delta_C$	δ_H /(*J*/Hz)	COSY	HMBC
1	C	178.3			H2
2	CH_2	33.8	2.34, t (*J* = 7.53H)	H12	
3	CH_2	31.9	1.28		H14
4	CH_2	29.7	1.26		
5	CH_2	29.7	1.26		
6	CH_2	29.7	1.26		
7	CH_2	29.6	1.26		
8	CH_2	29.4	1.26		
9	CH_2	29.4	1.26		
10	CH_2	29.3	1.26		
11	CH_2	29.1	1.26		H2, H12
12	CH_2	24.7	1.64 t (7.38Hz)	H2	H2
13	CH_2	22.7	1.28	H14	H16
14	CH_3	14.1	0.89, t (6.84 Hz)	H13	



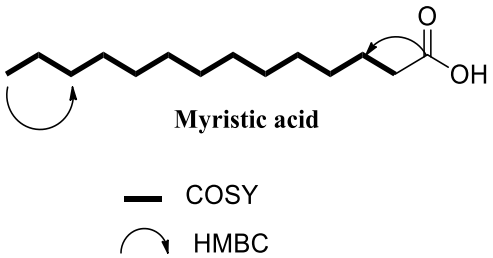


Figure 4.29: Structure of Myristic acid

Myristic acid is found in coconut essential oil, palm kernel essential oil, breast milk and animal fat[85]. It has several uses in the beauty industry. It is also used as surfactant, emulsifier and cleansing agent. One of its primary use is as a lubricant, due to its high rate of absorption by the skin. Myristic acid has been widely used for construction of phase modification materials for heat energy storage applications[85].

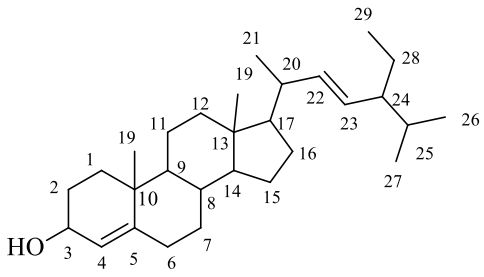
4.4.2.4 Identification of AA/R/PE-6

AA/R/PE-6 was identified as stigmasterol. The melting point was measured to be 156-158 °C, compared to the literature value of 160-162 °C. When subjected to IR spectroscopic **(Appendix IX)** analysis, there was an absorption band of 3430 cm^{-1} which is characteristic of O-H (stretching), 2868 cm^{-1} was assigned to C-H (stretching) and 1667 cm^{-1} assigned to C=C absorption. The ^{13}C-NMR spectrum (**Appendix X**), showed the presence of 29 carbons with peaks occurring between δ_C 11-145. Peaks at δ_C 121.84 and δ_C 140.93 for (C5) and (C6), respectively, which are sp^2 hybridized carbons, a peak at δ_C 71.89 which is due to C3 of stigmasterol was also observed. The angular methyls, C18 and C19 occurred at δ_C 11.8 and δ_C 19.3, respectively. From the DEPT 135 (**Appendix XI**) analysis, the spectrum showed that there were 3 quaternary, 11 methines, 9 methylene and 6 methyl carbons. The ^{1}H NMR (**Appendix XII**) showed characteristic steroidal proton signals between δ_H 0.7-2.4. Peaks at δ_H 0.70 (s, 3H) and δ_H 1.30 (s, 3H), were characteristic of the hydrogens of the angular methyls, C19 and C18, respectively. A signal at δ_H 3.54 (m) is due to the hydroxy proton at C3. The multiplicity of the hydroxy proton is due to coupling with H2 and H4. The signals at δ_H 5.05 (d, 1H), δ_H 5.18 (dd, 1H) and δ_H 5.37 (t, 1H) are the protons on C6, C22 and C23, respectively (**Table 4.9**).

Table 4.9: Comparative ^{13}C-NMR chemical shifts of stigmasterol with literature

Position	Carbon type	PE-6	δC[86]
1	CH_2	37.3	37.3
2	CH_2	29.2	31.6
3	CH	71.8	71.8
4	CH_2	45.8	42.3
5	C	140.8	140.8
6	CH	121.7	121.7
7	CH_2	31.9	31.9
8	CH	31.7	31.9
9	CH	50.2	51.2
10	C	36.2	36.5
11	CH_2	21.1	21.1
12	CH_2	39.8	39.7
13	C	42.3	42.3
14	CH	56.8	59.9
15	CH_2	26.1	24.4
16	CH_2	28.3	28.4
17	CH	56.1	56.3
18	CH_3	11.9	11.0
19	CH_3	19.4	21.2
20	CH	40.5	40.5
21	CH_3	19.8	21.2
22	CH	138.3	138.3
23	CH	129.3	129.3
24	CH	51.2	51.2
25	CH	40.5	31.9
26	CH_3	19.0	21.2
27	CH_3	18.8	19.0
28	CH_2	26.1	25.4
29	CH_3	12.0	12.1

Stigmasterol is a plant sterol that has been isolated from several plants and to the best of my knowledge, this is the first time it has been isolated from a species of *Aframomum.*



Stigmasterol

Stigmasterol is known to inhibit tumor elevation in two stage carcinogenesis in mice[86]. Also, like cholesterol, it has been identified to regulate the activity of Na+/K+-ATPase in plants52. However, a mixture of stigmasterol and sitosterol is known to inhibit the hatching of *N. americanus* egg with an IC_{50} of 9.4 µg/µl[77]. It is also known to have anti-inflammatory activity[87].

4.4.3 Investigation of the Dichloromethane (DCM) Extract

Through chromatographic separation, an essential oil and 2 compounds labelled AA/R/DCM-1 and AA/R/DCM-3, respectively, were obtained from the DCM crude extract. AA/R/DCM-1 was characterized by identifying the constituent using GCMS (**Table: 4.3**). The antifungal property was also tested using *C. albicans* and *S. cerevisiae* (**Figures 4.18, 4.19**)

4.4.3.1 Characterization of AA/R/DCM- 3

AA/R/DCM 3 was obtained as a white solid with melting point 77-79 °C. When subjected to IR spectroscopy (**Appendix XIII**), peaks at 2915.60 cm^{-1}, 2848.81 cm^{-1}, 1734.88 cm^{-1} and 1706.24 cm^{-1} were attributed to C-H_{str} (sp^3), C-H_{str} (sp^2), C=O_{str} and C=C_{str}, respectively. A molecular formula of $C_{19}H_{32}O_4$ was deduced from the HR-ESI-MS (**Figure 4.31**) with an observed m/z 325.2279 $[M+H]^+$ and calculated m/z 325.2376. The double bond equivalence was calculated to be 4.

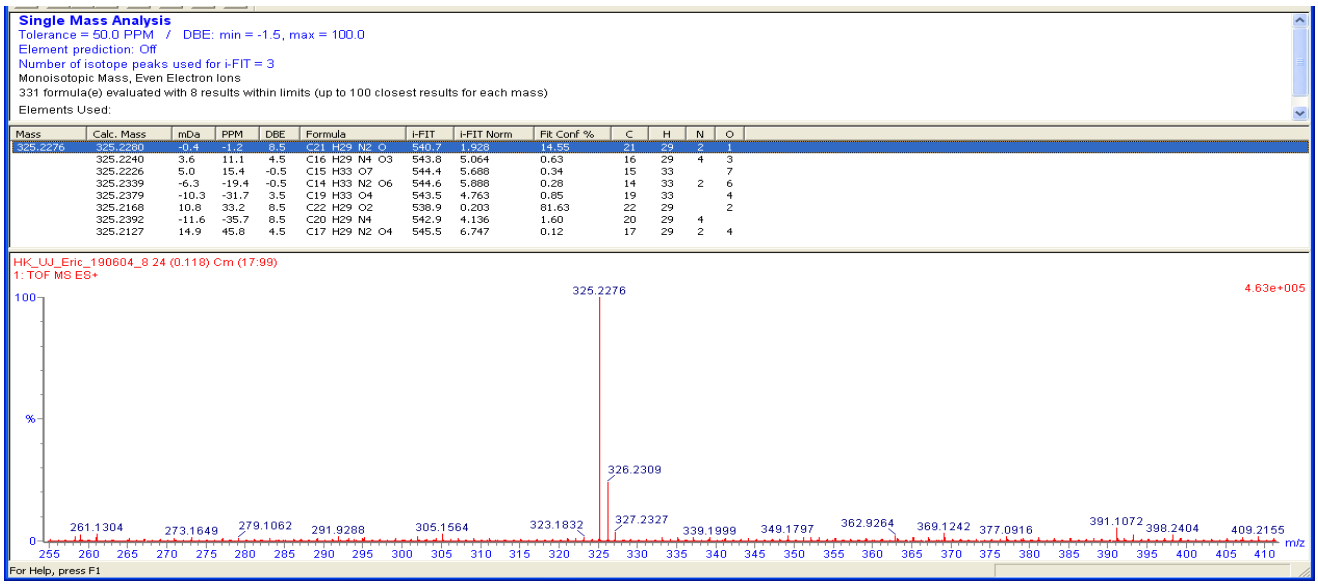


Figure 4.30: HR-MS spectrum of AA/R/DCM-3

The ^{13}C NMR spectrum (**Figure 4.32**) exhibited 18 signals, comprising 4 sp^2 hybridized and 14 sp^3 carbons which occurred between δ_C 14 – 179. The signals at δ_C 179.1 and δ_C 173.3 were assigned to carbonyl carbons whereas the remaining 2 sp^2 were assigned to alkene carbons. Two sp3 hybridized carbons attached to a heteroatom were observed at δ_C 68.9 and δ_C 62.1.

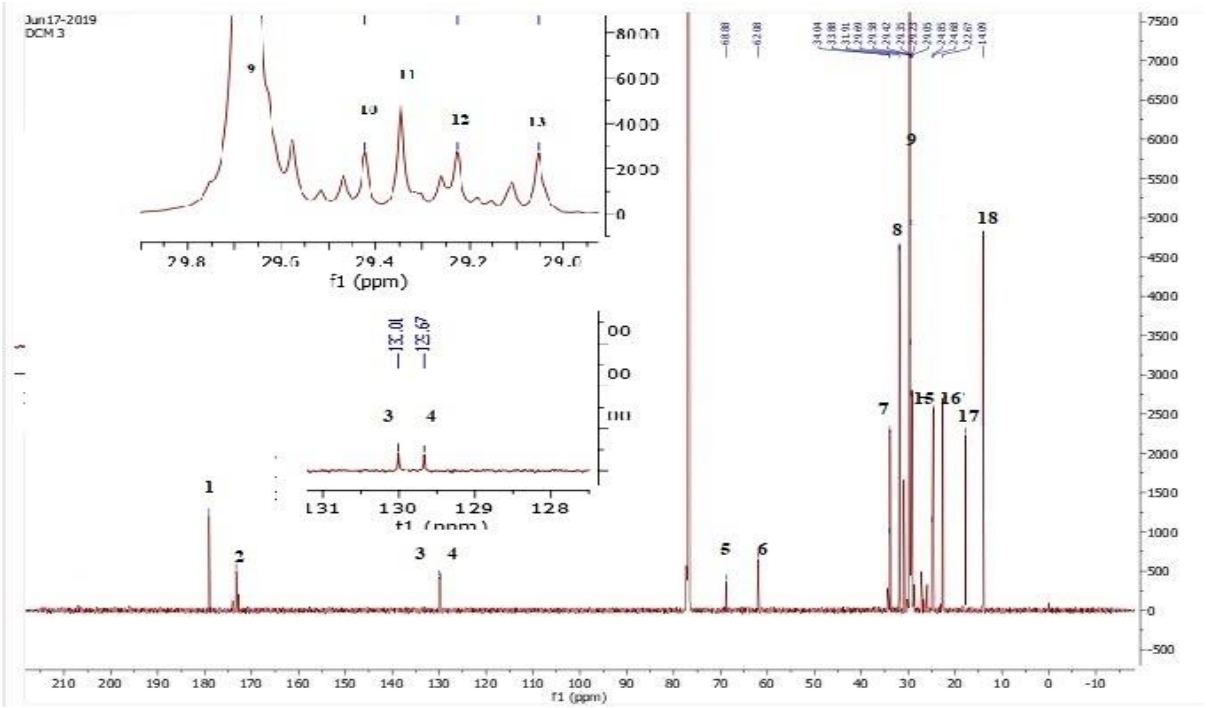


Figure 4.31: ^{13}C NMR spectrum of AA/R/DCM-3

The DEPT 135 ° spectrum (**Figure 4.33)** revealed 2 quaternary carbons observed at δ_C 179.1 (C-1) and δ_C 173.3 (C-2). Methine carbons occurred at δ_C130.0 (C-3), δ_C129 (C-4) and δ_C 68.6 (C-5). At δ_C 14.1(-18), the signal was assigned to a methyl carbon 12 methylene and 1 methyl carbons. A methylene carbon attached to a heteroatom was observed at δ_C 62.1 (C-6). The remaining 14 signals were also identified as methylene carbons.

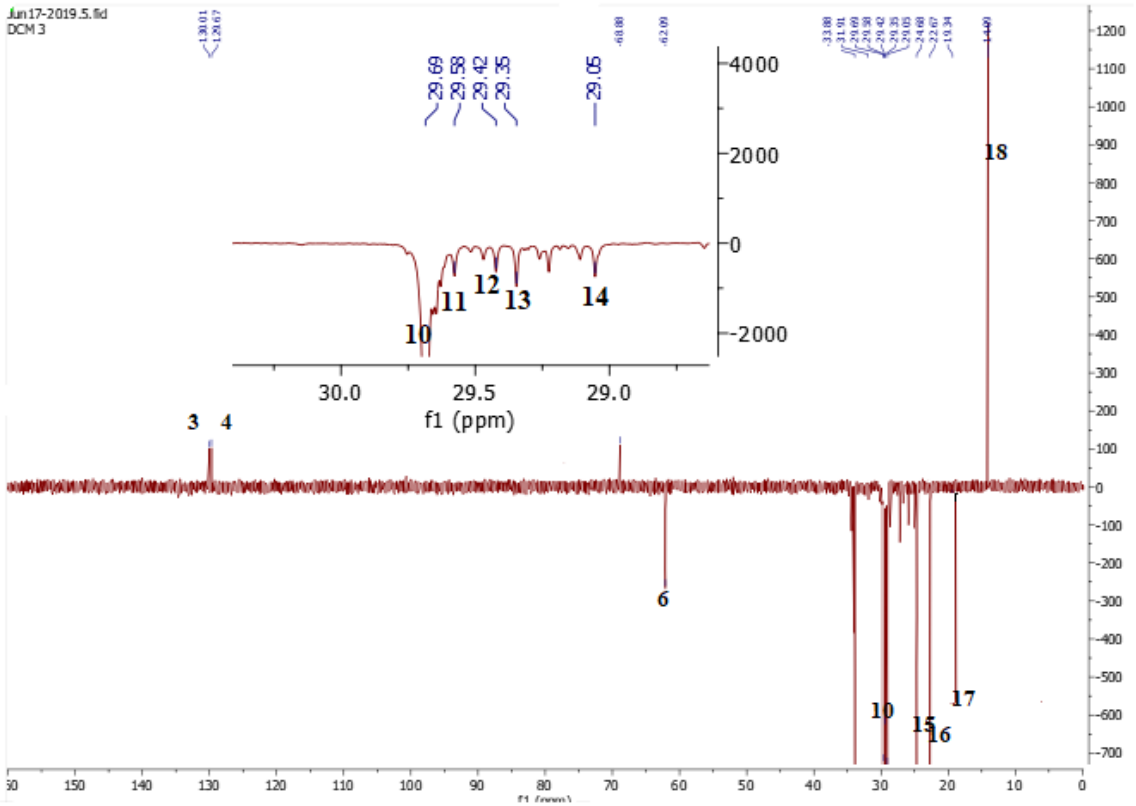


Figure 4.32: DEPT 135 spectrum of AA/R/DCM-3

From the ^{1}H NMR spectrum (**Figure 4.34),** 9 signals were observed between δ_H 0.88 - 5.50. A methyl proton signal was observed at δ_H 0.88 (t, *J* = 6.9 Hz, 3H) whiles the methine signal were observed at δ_H 5.34 (m, 2H) and δ_H 5.26 (td, *J* = 5.1, 4.3, 1.6 Hz, 1H). The methylene protons were observed at δ_H 1.25 (s, 14H), δ_H 1.63 (m, 2H), δ_H 2.01 (m, 2H), δ_H 2.32 (m, 6H), δ_H 4.29 (dd, *J* =11.9, 4.3 Hz, 1H) and δ_H 4.15 (dd, *J* = 11.9, 4.3 Hz, 1H).

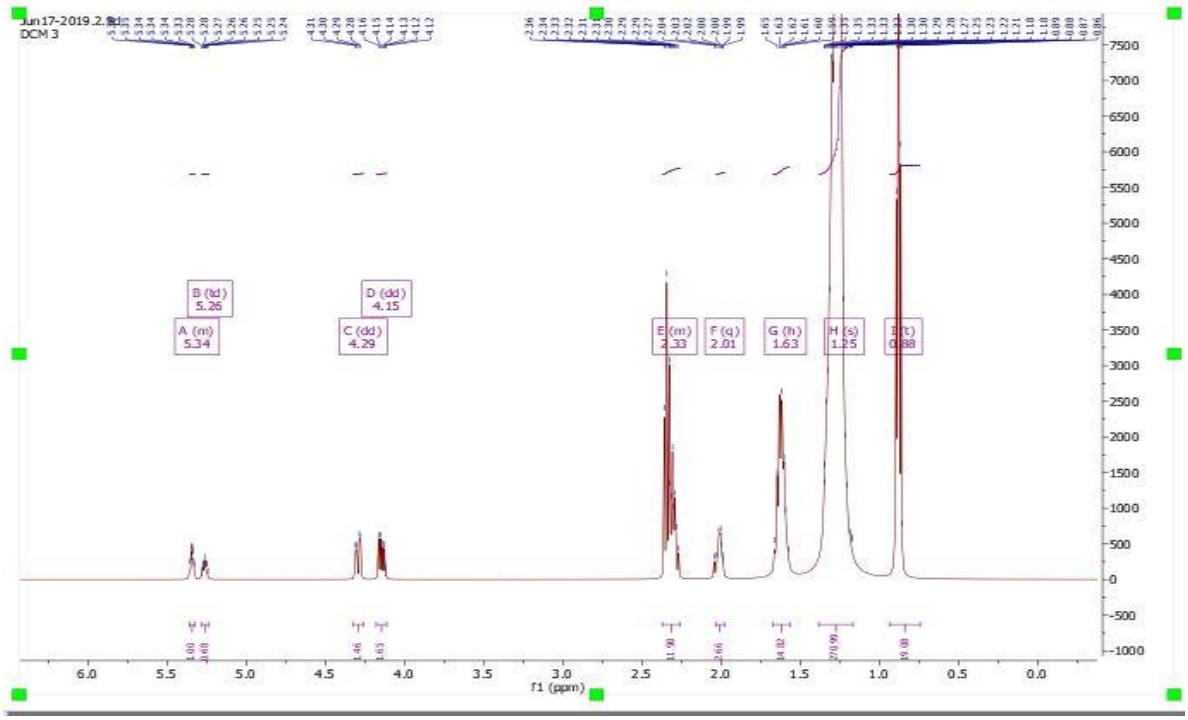
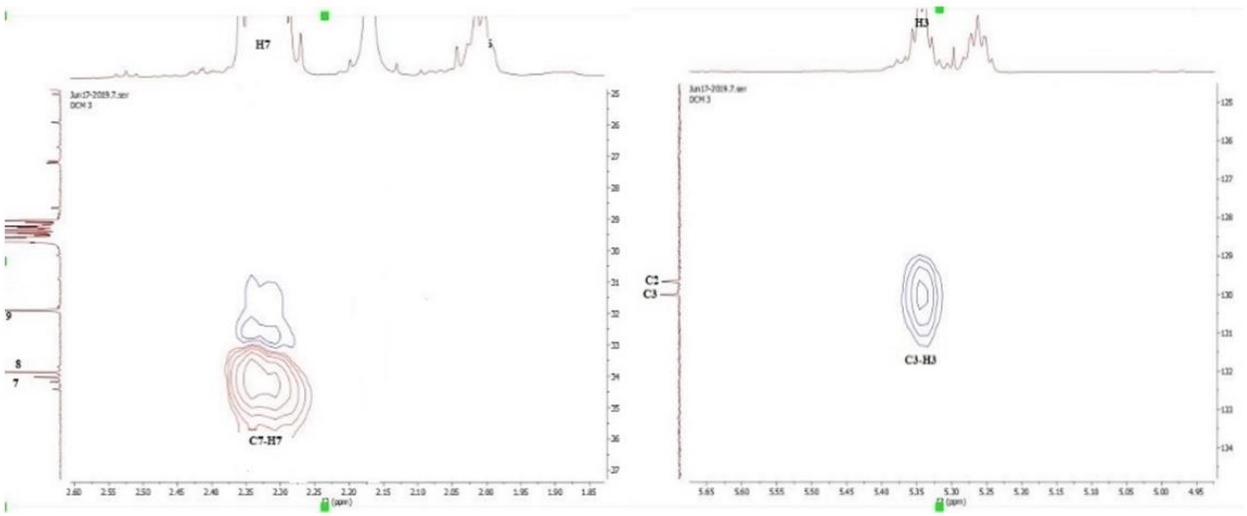


Figure 4.33: ^{1}H NMR of AA/R/DCM-3

From the HSQC spectrum (**Figure 4.35**), the oxygenated methine carbon C-5 (δ_C 68.9) was assigned to proton δ_H 5.26 whereas the remaining 2 methine carbons C-3 (δ_C 130.1) and C-4 (δ_C 129.7) assigned to δ_H 5.34 (m, 2H). The methylene carbon attached to an oxygen at C-7 (δ_C 62.1) was assigned to the protons at δ_H 4.29 and δ_H 4.15. The signal at δ_C 19.1 correlated with the proton at δ_H 2.01, other methylene carbons at δ_C 34.0 (C7), δ_C 33.9 (C8) and δ_C 31.9 (C9) were assigned to proton δ_H 2.32. the proton signal at δ_H 1.25 was assigned to carbons C-10 (δ_C 29.7), C-11 (δ_C 29.4), C-12 (δ_C 29.3), C-13 (δ_C 29.2), C-14 (δ_C 29.1) and C-15 (δ_C 22.7). The proton at δ_H 0.88 were attached to the methyl carbon at δ_C 14.1 (C18).



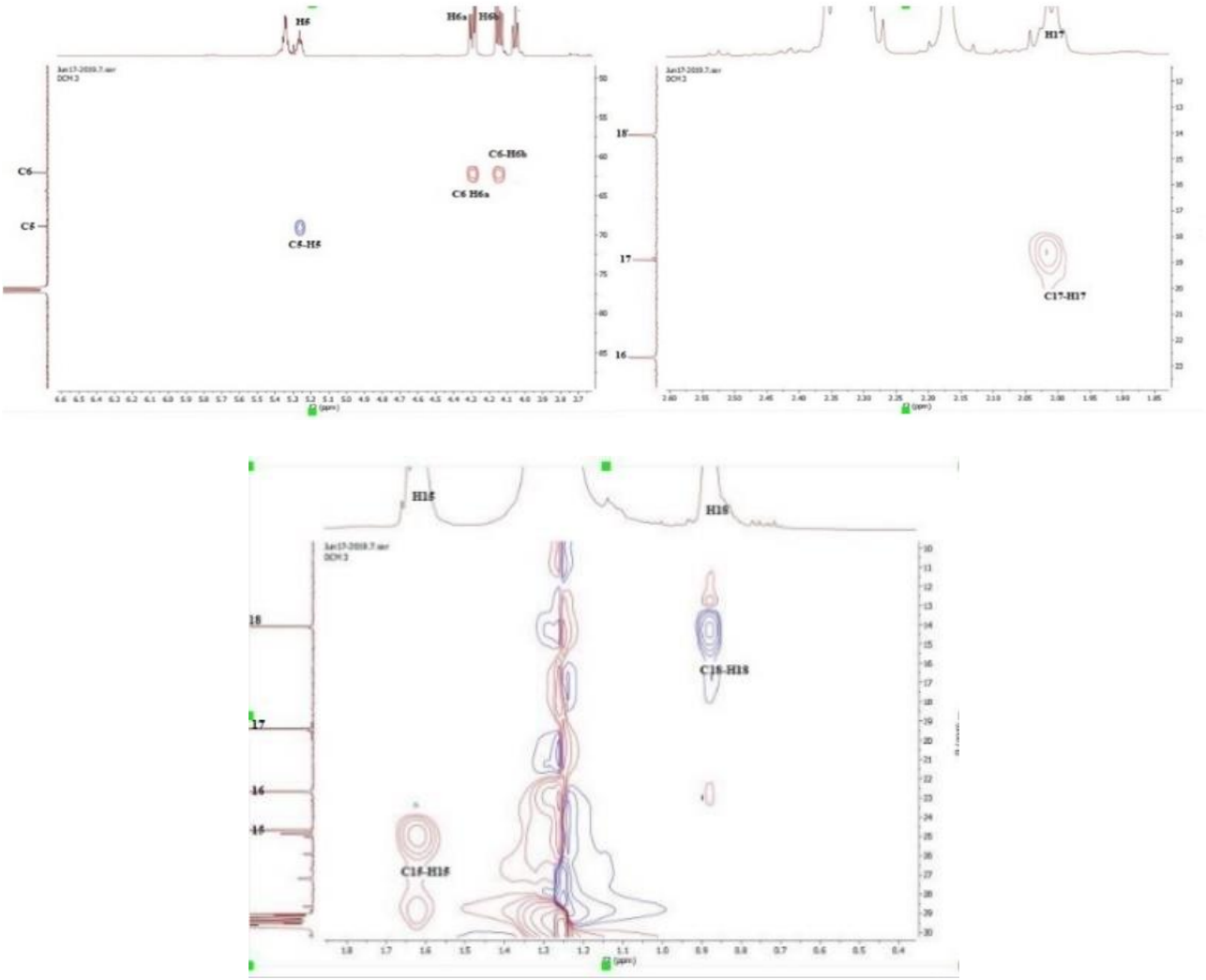


Figure 4.34: HSQC spectrum of AA/R/DCM-3

From the COSY spectrum (**Figure 4.36**), 2 spin systems were observed. One spin system was between H-9 (δ 2.32), H-17 (δ 2.01), H-14 (δ 1.25), H-5 (δ 5.26), H-8 (δ 2.32) and H-6 (δ 4.12 and δ 4.29). The other spin system was identified as the aliphatic system, between protons H-7 (δ 2.32), H-15 (δ 1.62), C-13 (δ 1.25), C-11 (δ 1.25), C-10 (δ 1.25), C-12 (δ 1.25), C-4 (δ 5.32), C-3 (δ 5.32), C-10 (δ 1.25), C-16 (δ 1.25) and C-18 (δ 0.88).

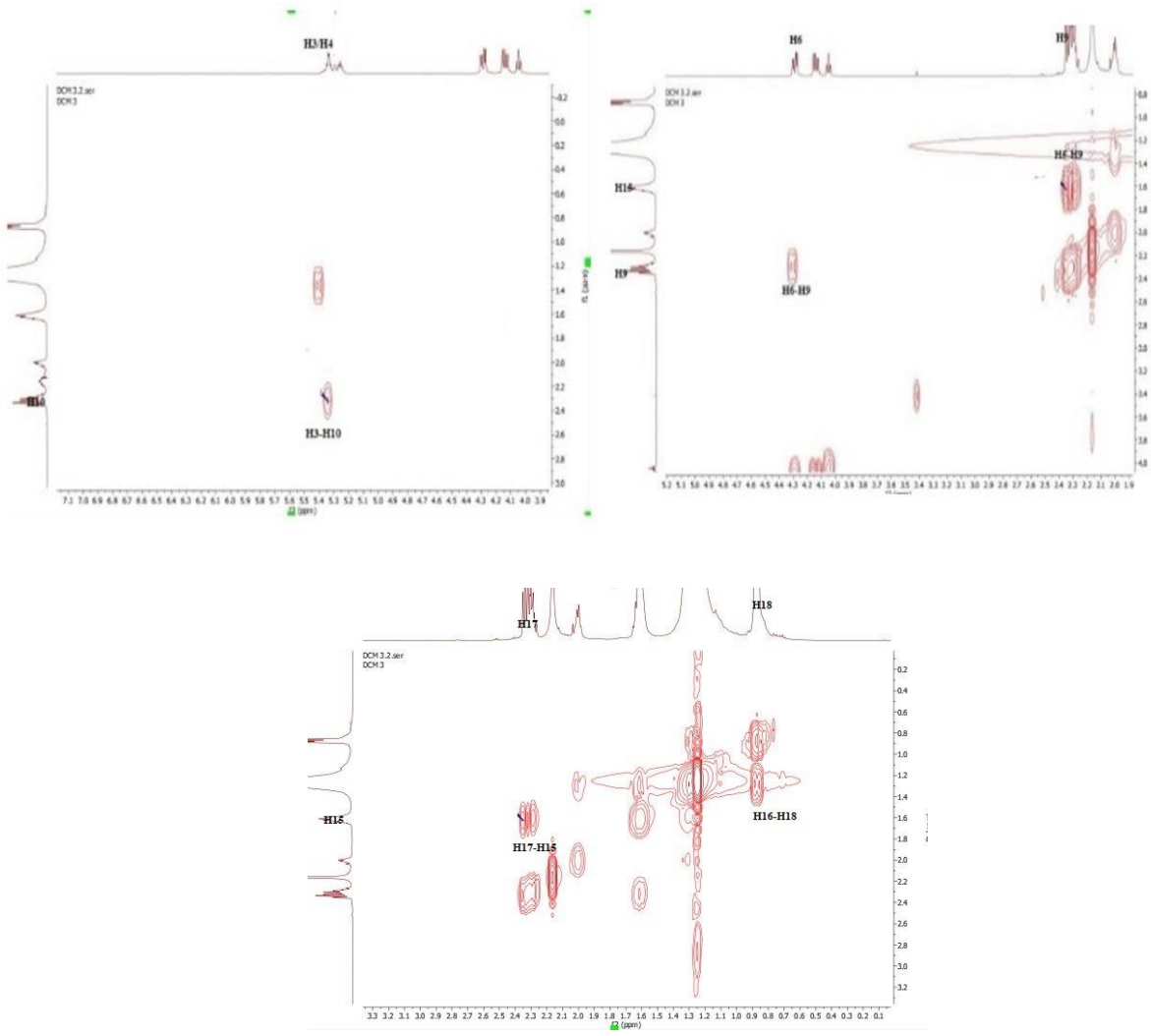
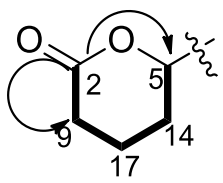
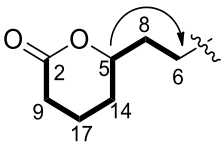


Figure 4.35: COSY spectrum of AA/R/DCM 3

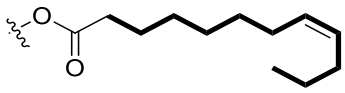
From the HMBC spectrum (**Figure 4.37**), correlations between C-2/H-9 and C-2/H-5 were observed, suggesting a lactone.



Further to this, a correlation between C-5/H-6 supported the first spin system observed in the COSY spectrum.



An HMBC correlation between C-1/H15 was observed coupled with the aliphatic spin system observed in the COSY spectrum was concluded to be an aliphatic ester moiety.



The two structural moieties were connected based on the correlation between C1/H-6.

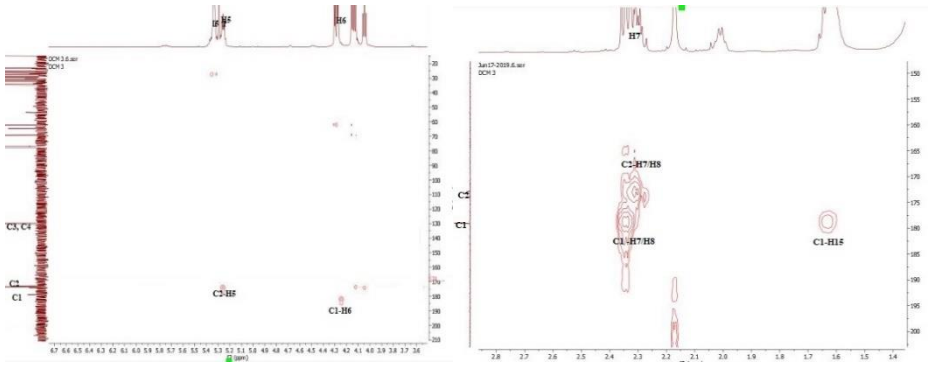


Figure 4. 36: HMBC spectrum of AA/R/DCM 3

The summary of the NMR data is presented in **Table 4.10**

Table 4.10 ^{1}H, ^{13}C, COSY and HMBC NMR data for AA/R/PE-2

	δ_C	DEPT	δ_H	HMBC	COSY
1.	179.1	C		H6, H15, H8	
2.	173.3	C		H5, H9	
3.	130.0	CH	5.35		H10
4.	129.7	CH	5.35		H10
5.	68.9	CH	5.26	H6	H9
6.	62.1	CH_2	4.15,4.29	H5	
7.	34.0	CH_2	2.32		H15
8.	33.9	CH_2	2.32		
9.	31.9	CH_2	2.32	H17	5,6
10.	29.7	CH_2	1.25		
	29.7	CH_2	1.25		H16
11.	29.4	CH_2	1.25		
12.	29.3	CH_2	1.25		
13.	29.2	CH_2	1.25		
14.	29.1	CH_2	1.25		
15.	24.9	CH_2	1.62		H7
16.	22.7	CH_2	1.25	H17	H18, H10
17.	19.1	CH_2	2.01		
18.	14.1	CH_3	0.88		H17

From the spectrometric and spectroscopic information (**Table 4.10**) was deduced to be 2-(6-oxotetrahydro-2H-pyran-2-yl)ethyldodec-8-enoate (**Figure 4.37**).

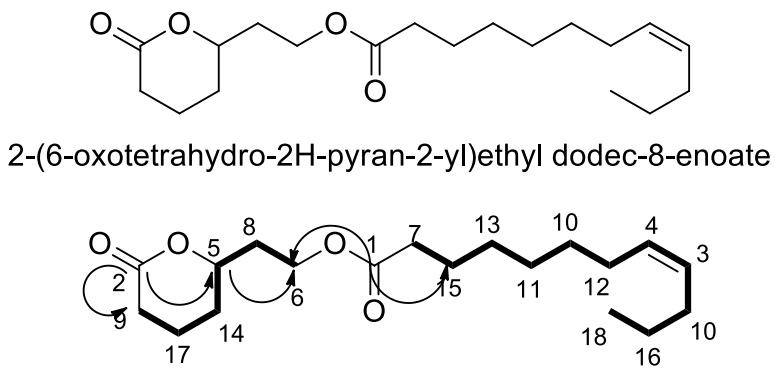


Figure 4.37: Structure of AA/R/DCM 3

Due to the truncated nature of the HR-MS data (**Figure 4.31**), fragmentation patterns of the proposed structure could not be proposed.

From a literature survey, the proposed compound has not been isolated from any species of *Aframomum* and other plant species. Hence for the first time, 2-(6-oxotetrahydro-2H-pyran-2-yl)ethyldodec-8-enoate has been isolated from a plant.

CHAPTER FIVE

5.0 Conclusion

In this current research, the constituents and antifungal activity of *A. atewae* essential oils were studied for the first time as well as characterization of compounds isolated from extracts of the rhizome.

The essential oils were obtained by hydrodistillation of fresh plant parts (leaves and rhizomes) and chromatographic separation of crude extracts. GC-MS analysis indicated that the essential oils of the plant consist of various classes of terpenes and non-terpenes with the leaf EO characterizing 120 compounds followed by the rhizome EO, 100 compounds and the DCM extract oil, 50 compounds. The fresh leaf essential was rich in monoterpenes (22.4%) while sesquiterpenes dominated the fresh rhizome essential oil (24.4%). The DCM-extracted oil was composed mainly of steroids (59.72%) and long chain hydrocarbons (18.06%)

Several *Aframomum* species have been explored for essential oils but for the first time the following major constituents have been identified in the essential oils of *A. atewae* - 1-methyl-1-(methylamino)isobenzofuran-3-one (17.27%), 2,5-ditertbutylhydroxybenzene (7.80%), and 14β-pregnane (56.95%) for fresh leaf essential oil, fresh rhizome essential oil and DCM-extracted essential oil, respectively. This variation in major components identified in *A. atewae* to previously studied *Aframomum* species may be as a result of extrinsic factors such as soil, climate, elevation and geographical origin.

Fungicidal activity was observed for the rhizome EO and the DCM-extracted oil whereas the leaf EO and the PE-extracted oil exhibited a fungistatic activity against *C. albicans*. Apart from the PE-extracted oil which was fungistatic against *S. cerevisiae*, the remaining oils were inactive.

A total of 10 compounds were isolated upon column chromatographic separation of the various extracts obtained from air dried pulverized rhizome of *A. atewae*. Due to poor solubility, only 4 compounds were characterized as 1(E)-8-methylundec-8-en-1-yl-3-(cyclohexa-2,5-dien-1-yl)propanoate, myristic acid, stigmasterol and 2-(6-oxotetrahydro-2H-pyran-2-yl)ethyl dodec-8-enoate using IR, NMR, LC-MS and HR-MS.

5.1 Recommendations

The chemical composition of the PE-extracted oil should be acquired in order to attribute the observed activity against *S. cerevisiae* to a particular compound. Also, the isolated compounds should be tested for their antifungal activities.

References

1. Linder, H. P. (**2014**). The evolution of African plant diversity. *Frontiers in Ecology and Evolution*, 1–14.
2. Abbiw, D., Agbovie, T., Akuetteh, B., Amponsah, K., Dennis, F., Ekpeh, P., Gillet, H., Ofosuhene-Djan, W., and Owusu-Afriyie, G. (**2002**). Conservation and Sustainable Use of Medicinal Plants in Ghana: Conservation Report. (Accessed from http//www.unepwcmc.org/species/plants/ghana/pdfs/ Conservation_report.pdf on 15 May 2010).
3. Tripathi, H., Suresh, R., Kumar, S., Khan, F. (**2017**). International trade in medicinal and aromatics plants: A case study of past 18 years. *Journal of Medicinal and Aromatic Plant Sciences* 39(1), 1–17.
4. Roberson, E. (**2008**). Medicinal Plants at Risk. *Biological Diversity*, *1*(1), 1–16.
5. Amadi, S. W., Zhang, Y., Wu, G. (**2016**). Research progress in phytochemistry and biology of *Aframomum* species. *Pharmaceutical Biology* 54(11), 2761–2770.
6. Lock, J. M., Hall, J. B., Abbiw, D. K. (**1977**). The Cultivation of Melegueta Pepper (*Aframomum melegueta*) in Ghana. Economic Botany 31 (3) pp. 321-330
7. Lock, A. J. M. (**2016**). Notes on *Aframomum* (Zingiberaceae) in West Africa, with a New Key to the Species Published by Springer on behalf of Royal Botanic Gardens, Kew Stable
8. Guarro, J., Gené, J., Stchigel, A. M. (**1999**). Developments in fungal taxonomy. *Clinical Microbiology Reviews* (Vol. 12)
9. Aguilar, C., Pujol I., Sala J., and Guarro J., (**1998**). Antifungal susceptibility of *Paecilomyces* species. *Antimicrobial Agents and Chemotherapy* 42:1601–1604
10. De Lucca, A. (**2007**). Harmful fungi in both Agriculture and Medicine. *Revista iberoamericana de micología* 24. 3-13.
11. World Checklist of Selected Plant Families: Royal Botanic Gardens, Kew. (n.d.). Retrieved July 31, 2018, from https://wcsp.science.kew.org/namedetail.do?name_id=218345
12. Tane, P., Tatsimo, S. D., Ayimele, G. A., & Connolly, J. D. (**2005**). Bioactive metabolites from *Aframomum* species. *11th NAPRECA Symposium Book of Proceedings, Antananarivo, Madagascar*, 214–223
13. Duke, J. A. (**2002**). Peppermint (*Mentha x piperita* L.). Handbook of Medicinal Herbs, Second Edition.
14. El-Halawany, A. M., Dine, El-Sayed, N. S., & Hattori, M. (**2014**). Protective effect of *Aframomum melegueta* phenolics against CCl_4 -induced rat hepatocytes damage; Role of apoptosis and pro-inflammatory cytokines inhibition. *Scientific Reports*, 4, 1–9.
15. Escoubas, P., Lajide, L., & Mizutani, J. (**1995**). Termite antifeedant activity in *Aframomum*

melegueta. *Phytochemistry* 40(4), 1097–1099.

16. Kuete, V., & Efferth, T. (**2010**). Cameroonian medicinal plants: Pharmacology and derived natural products. *Frontiers in Pharmacology* 1–19.
17. Wabo, H. K., Tane, P., Connolly J. D., (**2006**). Diterpenoids and sesquiterpenoids from *Aframomum arundinaceum*. *Biochemical Systematics and Ecology* 34:603–605
18. Smith, P. M., (**1976**). The chemotaxonomy of plants. London: Edward Arnold
19. Tane, P., Tatsimo, S. D., Ayimele, G. A., Connolly, J. D. (**2005**). Bioactive metabolites from *Aframomum* species. *11th NAPRECA Symposium Book of Proceedings, Antananarivo, Madagascar* 214–223
20. Wabo, H.K., Tane, P., Connolly, J. D., (**2006**). Diterpenoids and sesquiterpenoids from *Aframomum arundinaceum*. *Biochemical Systematics and Ecology* 34:603–605.
21. Ayimele, G. A., Tane, P., Connolly, J. D., (**2004**). Aulacocarpin A and B, nerolidol and β-sitosterol glucoside from *Aframomum escapum*. *Biochemical Systematics and Ecology* 32:1205–1207.
22. Mapitse, R., Kapche, G. D., Kuete, V., Mbaveng, A. T., Yeboah, S. O., Ngadjui, B. T., Efferth, T. (**2014**). Cytotoxicity of four *Aframomum* species (*A. arundinaceum, A. alboviolaceum, A. kayserianum and A. polyanthum*) towards multi-factorial drug resistant cancer cell lines. *BMC Complementary and Alternative Medicine*, *14*(1), 1–7.
23. Obike, H. I., Ezejindu, D. N., & Aj, A. (**2014**). The effects of *Aframomum Melegueta* aqueous extract on the adrenal gland of adult wistar rats. *International Journal of Research In Medical and Health Sciences* 1. *3*(6), 1–6
24. Ayafor, J., Tchuendem, M., Nyasse, B., Tillequin, F., Anke, H. (**1994a**). Aframodial and other bioactive diterpenoids from *Aframomum* species. *Pure Applied Chemistry* 66:2327–2330.
25. Okogbenin, O. B., Emoghene, A.O., Okogbenin, E. A.Airede C. E. (**2014**). Antifungal effect of polar and nonpolar extracts of *Aframomum sceptrum* on two isolates of oil palm. *Journal of Applied Science and Environmental Management* 18(2), 173.
26. Uzeh, R. E., Oguntosin, D. O. (**2013**). Efficacy of essential oils from some African spices against two strains of *Bacillus cereu*s isolated from vegetable salad. *Journal of Food Research* 2:48–54.
27. El-Halawany, A. M., & Hattori, M. (**2012**). Anti-oestrogenic diarylheptanoids from *Aframomum melegueta* with *in silico* oestrogen receptor alpha binding conformation similar to enterodiol and enterolactone. *Food Chemistr 134*(1), 219–226.

28. Kamtchouing, P., Mbongue, G., Dimo, T., Watcho, P., Jatsa, H., Sokeng, S. (**2002**). Effects of *Aframomum melegueta* and *Piper guineense* on sexual behavior of male rats. *Behavioural Pharmacology* 13:243–247

29. Mbongue, G., Kamtchouing, P., Dimo, T. (**2012**). Effects of the aqueous extract of dry seeds of *Aframomum melegueta* on some parameters of the reproductive function of mature male rats. *Andrologia* 44:53–58

30. Abo, K. A., Fred-Jaiyesimi, A. A., Jaiyesimi, A. E. A. (**2008**). Ethnobotanical studies of medicinal plants used in the management of *diabetes mellitus* in South-Western Nigeria. *Journal of Ethnopharmacology* 115(1), 67–71.

31. Gbolade, A., (**2009**). Inventory of antidiabetic plants in selected districts of Lagos State, Nigeria. *Journal of Ethnopharmacology* 121, 135–139.

32. Sugita, J., Yoneshiro, T., Hatano, T., Aita, S., Ikemoto, T., Uchiwa, H., Iwanaga, T., Kameya, T., Kawai, Y., Saito, M., (**2013**). Grains of paradise (*Aframomum melegueta*) extract activates brown adipose tissue and increases whole-body energy expenditure in men. *British Journal of Nutrition* 110, 733–738

33. Mohammed, A., Koorbanally, N. A., Islam, M. S., (**2016**). Antioxidative activity, phytochemistry, and inhibition of key enzymes linked to type 2 diabetes by various parts of *Aframomum melegueta in vitro*. *Acta Poloniae Pharmaceutica - Drug Research*, 73(2):403-417

34. Onoja, S. O., Omeh, Y. N., Ezeja, M.I., Chukwu, M.N., (**2014**). Evaluation of the in vitro and in vivo antioxidant potentials of *Aframomum melegueta* methanolic seed extract. *Journal of Tropical Medicine*, 1–7

35. Kazeem, M. I., Akanji, M. A., Hafizur Rahman, M., Choudhary, M.I., (**2012**). Antiglycation, antioxidant and toxicological potential of polyphenol extracts of alligator pepper, ginger and nutmeg from Nigeria. *Asian Pacific Journal of Tropical Medicine* 2,727–732

36. Adegoke, G. O., Evwiehurhoma, F. O., Afolabi, M. O., (**2015**). *African cardamom (Aframomum danielli) oils*. *Essential Oils in Food Preservation, Flavor and Safety*. Elsevier Inc.

37. Odukayo, O. A., Houghton, P. J., Raman, A., (**1996**). Lipoxygenase Inhibitors in the seed of *Aframomum danielli* K. Schum (Zingiberaceae). *Phytomedicine*, 6(4), 251-256

38. Nair, K. P. P., (**2006**). *The Agronomy and Economy of Cardamom (Elettaria cardamomum M.): The "Queen of Spices." Advances in Agronomy* (Vol. 91).

39. Adegoke, G.O., Skura, B.J., (**1994**). Nutritional profile and antimicrobial spectrum of the spice *Aframomum danielli*. *Plant Foods for Human Nutrition* 45, 175–182
40. Adegoke, G.O., Iwahashi, H., Komatsu, Y., Obuchi, K., Iwahashi Y., (**2000**). Inhibition of food spoilage yeasts and aflatoxigenic moulds by monoterpenes of the spice *Aframomum danielli*. *Flavour and Fragrance Journal* 15, 147–150
41. Titanji, V.P., Zofou, D., Ngemenya, M.N., (**2008**). The antimalarial potential of medicinal plants used for the treatment of malaria in Cameroonian folk medicine. *African Journal of Traditional Complementary and Alternative Medicines* 5 (3), 302–321
42. Tane, P., Tatsimo, S. D., Ayimele, G. A., & Connolly, J. D. (**2005**). Bioactive metabolites from *Aframomum* species 17- *Aframomum sulcatum* (Oliv . & Hanb) K . Schum 19- *Aframomum subsericeum* 20- *Aframomum zambesiacum* 21- *Aframomum limbatum* 22- *Aframomum leptolepis* 23- *Aframomum flavum*, 11th NAPRECA Symposium Book of Proceedings, Antananarivo, Madagascar 214–223.
43. Kuete, V., Krusche, B., Youns, M., Voukeng, I., Fankam, A.G., Tankeo, S., (**2011c**). Cytotoxicity of some Cameroonian spices and selected medicinal plant extracts. *Journal of Ethnopharmacology* 134 (3), 803–812.
44. Adegoke, G.O., Gopalakrishna, A.G., (**1998**). Extraction and identification of antioxidants from the spice *Aframomum danielli*. *Journal of the American Oil Chemists' Society* 75, 1047–1052.
45. Odukoya, O. A., Houghton, P. J., Raman, A. (**1999**). Lipoxygenase Inhibitors in the seeds of *Aframomum danielli* K. Schum (Zingiberaceae). *Phytomedicine*, *6*(4), 251–256.
46. Gbolade, A., (**2009**). Inventory of antidiabetic plants in selected districts of Lagos State, Nigeria. *Journal of Ethnopharmacology* 121, 135–139
47. Kimbu, S.F., Thomas, K.N., Sondergam, B.L., Akinniyi, J.A., Sonnolly, J.D., (**1979**). The structure of labdane dialdehyde from *Aframomum danielli* (Zingiberaceae). *Journal of Chemical Society, Perkin Transactions 1*, 1303–1304
48. Kindu, S. E, Ngadjui, B., Sondergam, B. L.: A new labdane diterpenoid from the seeds of *Aframomum danielli*. *Journal of Natural Products* 50 (2): 230-231,1987.
49. Pasdaran, A., & Hamedi, A. (**2017**). Natural Products as Source of New Antimicrobial Compounds for Skin Infections. Clinical Microbiology: Diagnosis, Treatments and Prophylaxis of Infections (1st ed., Vol. 2). Elsevier Inc.
50. Koechlin, J. (**1965**). Flore du Cameroun, Scistaminales. Museum National d'histoire Naturelle, Paris, **4**: 43.

51. Kuete, V., Krusche, B., Youns, M., Voukeng, I., Fankam, A. G., Tankeo, S., Lacmata, S., Efferth, T. (**2011**). Cytotoxicity of some Cameroonian spices and selected medicinal plant extracts. *Journal of Ethnopharmacology* 134(3):803–812.
52. Duker-Eshun, G., Jaroszewski, J. W., Asomaning, W. M., Oppong-Boachie, F., Olsen, C.E., Christensen, S. B. (**2002**). *Planta Medica* 68, 642
53. Kenmogne, M., Prost, E., Harakat, D., Jacquier, M. J., Frédérich, M., Sondengam, L. B., Waffo-Téguo, P. (**2005**). Five labdane diterpenoids from the seeds of *Aframomum zambesiacum. Phytochemistry*, 67(5), 433–438.
54. Hao, D., Gu, X., Xiao, P., Liang, Z., Xu, L., Peng, Y. (**2013**). Research progress in the phytochemistry and biology of Ilex pharmaceutical resources. *Acta Pharmaceutica Sinica B.* 3:8–19
55. Miyoshi, N., Nakamura, Y., Ueda, Y., Abe, M., Ozawa, Y., Uchida, K., Osawa, T. (**2003**). Dietary ginger constituents, galanals A and B, are potent apoptosis inducers in Human T lymphoma Jurkat cells. *Cancer Letters*. 199:113–119
56. Rice-Evans, C., Miller, N., Paganga, G. (**1997**). Antioxidant properties of phenolic compounds. *Trends in Plant Science*. 2:152–159.
57. Özçelik, B., Kartal M., Orhan, I. (**2011**). Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. *Pharmaceutical Biology* 49:396–402.
58. Middleton, E., Kandaswami, C., Theoharides, TC. (**2000**). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacology Revision* 52:673–751.
59. Ilic, N., Schmidt, B. M., Poulev, A., & Raskin, I. (**2010**). Toxicological evaluation of Grains of Paradise (*Aframomum melegueta*) [Roscoe] K. Schum. *Journal of Ethnopharmacology*, *127*(2), 352–356.
60. Adesokan, A. A., Akanji, M. A., Adewara, G. S., (**2010**). Evaluation of hypoglycaemic efficacy of aqueous seed extract of *Aframomum melegueta* in alloxan-induced diabetic rats. *Sierra Leone Journal of Biomedical Research* 2, 91–94.
61. Mojekwu, T. O., Yama, O. E., Ojokuku, S. A., Oyebadejo, S. A. (**2011**). Hypoglyceamic effects of aqueous extract of *Aframomum melegueta* leaf on alloxan-induced diabetic male albino rats. *Pacific Journal of Medical Science* 8, 28–36
62. Vidari, G., Finzi, P. V., de Bernardi, M. (**1971**). Flavonols and quinones in stems of *Aframomum giganteum*. *Phytochemistry* 10:3335–3339
63. Park, H-H., Lee, S., Son, H-Y., Park, S-B., Kim, M-S., Choi, E-J., Singh, T. S., Ha J-H., Lee M-G., Kim J-E. (**2008**). Flavonoids inhibit histamine release and expression of

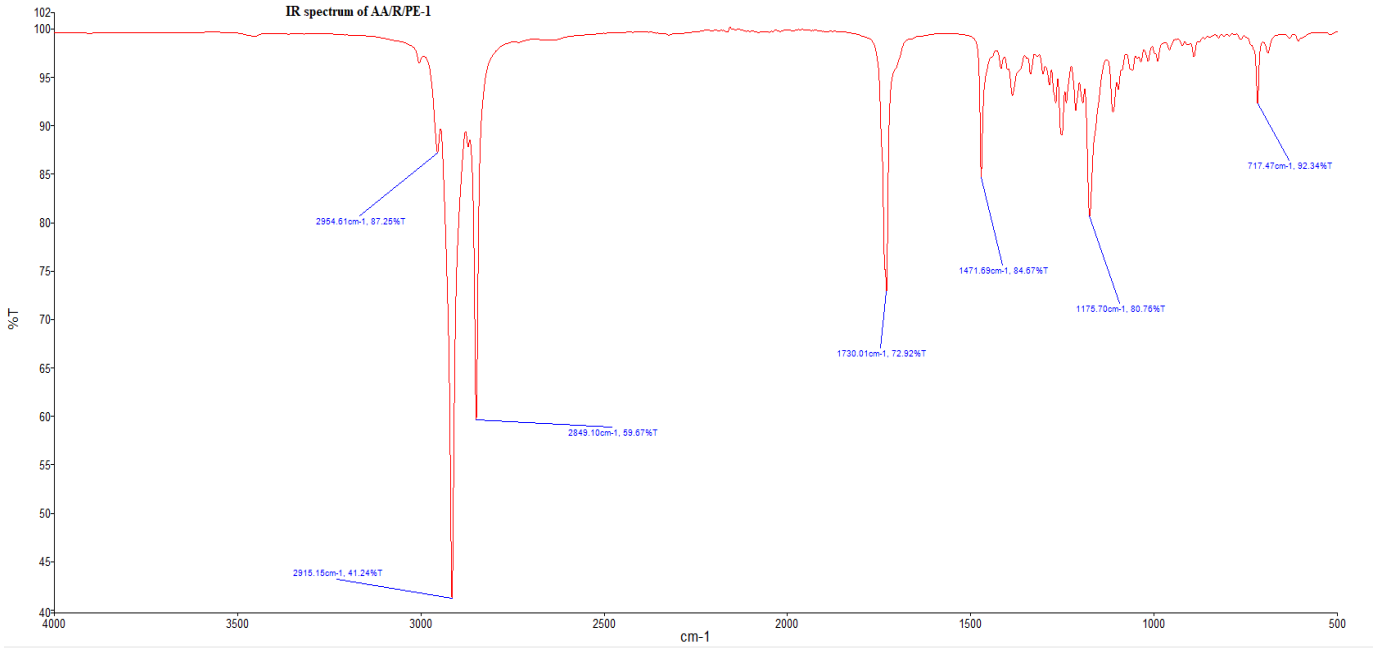
proinflammatory cytokines in mast cells. *Archives of Pharmacal Research* 31:1303–1311.

64. Kim, H. P., Son, K, H., Chang H. W., Kang S. S. (**2004**). Anti-inflammatory plant flavonoids and cellular action mechanisms. *Journal of Pharmacological Sciences* 96:229–245
65. Haining, L., She, G. (**2012**). Naturally occurring diarylheptanoids – a supplementary version. *Records of Natural Products*. 6:321–333.
66. Kim, H-J., Yeom, S-H., Kim, M-K., Shim, J-G., Paek, I-N., Lee, M-W. (**2005**). Nitric oxide and prostaglandin E2 synthesis inhibitory activities of diarylheptanoids from the barks of *Alnus japonica* Steudel. *Archives of Pharmacal Research*. 28:177–179.
67. Ocansey, B. K., Pesewu, G. A., Codjoe, F. S., Osei-djarbeng, S., Feglo, P. K., & Denning, D. W. (**2019**). Estimated Burden of Serious Fungal Infections in Ghana, *Journal of Fungi* 1–15.
68. Arif, T., Bhosale, J. D., Kumar, N., Mandal, T. K., Bendre, R. S., & Lavekar, G. S. (**2009**). Antifungal agents derived from plants, *Natural products* 11(7), 621–638.
69. Escalante, A. M., Santecchia, C. B., Lopez, S. N., Gattuso, M. A., Gutierrez Ravelo, A., Delle Monache, A., Gonzalez Sierra, M., and Zacchino, S. A. (**2002**). *Journal of Ethnopharmacology* 82, 29.
70. Svetaz, L., Aguero, M. B., Alvarez, S., Luna, L., Feresin, G., Derita, M., Tapia, A., Zacchino, S., (**2007**). *Planta Medica* 73 (10), 1074.
71. Varsha, K., Sharma, A., Kaur, A., Madan, J., Pandey, R. S., Jain, U. K., & Chandra, R. (**2017**). Natural plant-derived anticancer drugs nanotherapeutics: A review on preclinical to clinical success in '*Nanostructures for Cancer Therapy'* by Alexandru Grumezescu and Antoni Ficai (Eds), Elsevier, Amsterdam, Netherlands, pp. 775-809.
72. Hao, D. C., Gu, X.-J., & Xiao, P. G. (**2015**). Phytochemical and biological research of Cannabis pharmaceutical resources. Medicinal Plants: Chemistry, Biology and Omics 217-251 .
73. Oladunmoye, M. K. (**2019**). Characterization of Organic Compounds in *Aframomum melegueta* K. Schum Using GC-MS Medicinal and Aromatic Plants (Los Angeles), 8:1
74. Liu, X., Yang, X.-W., Chen, C.-Q., Wu, C.-Y., Zhang, J.-J., Ma, J.-Z., Xu, G. (**2013**). Hypercohones A–C, acylphloroglucinol derivatives with *homo*-adamantane cores from Hypericum cohaerens. *Natural Products and Bioprospecting*, 3(5), 233–237.
75. Diomandé, G. D., Koffi, A. M., Tonzibo, Z. F., Bedi, G., Figueredo, G. (**2012**). GC and GC/MS Analysis of Essential Oil of Five *Aframomum* Species from Côte D'Ivoire. *Middle-East Journal of Scientific Research* 11 (6): 808-813

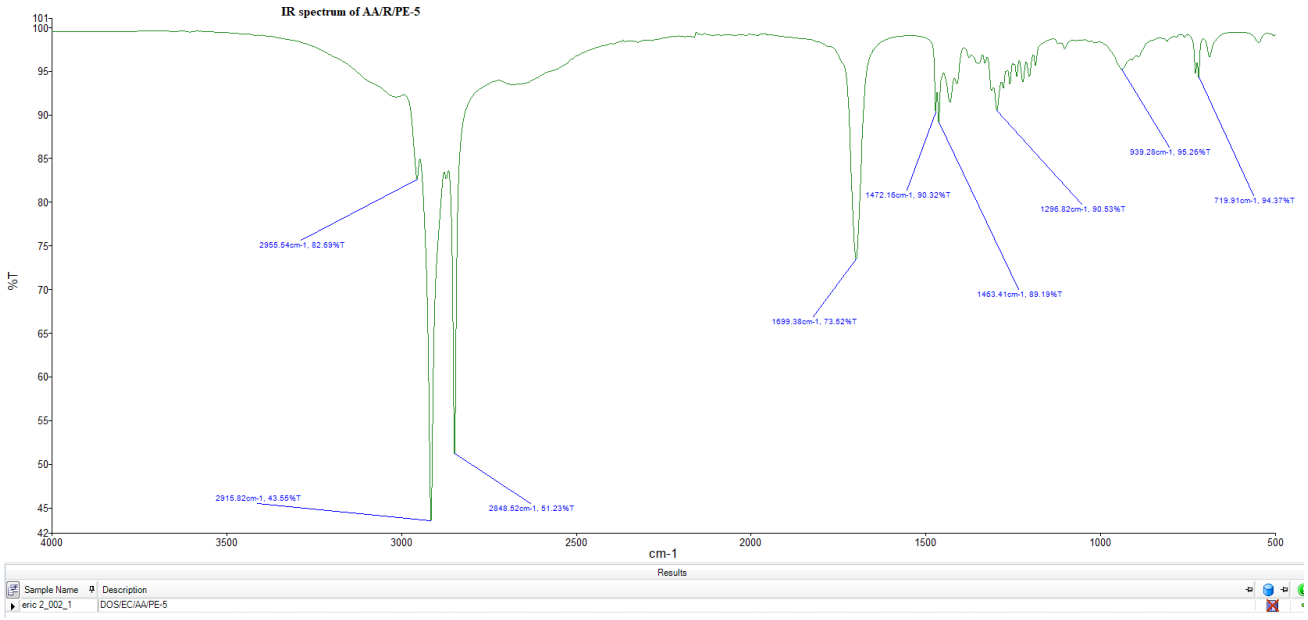
76. Essien, E. E., Thomas, P. S., Oriakhi, K., Mohammad, I. C (**2017**). Characterization and Antioxidant Activity of Volatile Constituents from Different Parts of *Aframomum danielli* (Hook) K. Schum, *Medicines* 4, 29.
77. Nguikwie, S. K., Nyegue, M. A., Ndoye-Foe Belinga, F., Ngono Ngane, R. A., Romestand, B., Kouzayha, A., Casabianca, H., Amvam Zolloa, P. H., Menute, C. (**2013**). The Chemical Composition and Antibacterial Activities of the Essential Oils from Three Aframomum Species from Cameroon, and Their Potential as Sources of (E)-(R)-Nerolidol *Natural Product Communications* Vol. 8 (6) 829 – 834
78. Amvam Zollo, P. H., Abondo, R., Biyiti, L., Menut, C., Bessière, J. M. (**2002**). Aromatic plants of Tropical Central Africa XXXVIII: Chemical composition of the essential oils from four *Aframomum* species collected in Cameroon (1). *Journal of Essential Oil Research* 14(2), 95–98.
79. Maruthupandian, A., Mohan, V. R. (**2011**). GC-MS analysis of some bioactive constituents of *Pterocarpus marsupium* roxb. *International Journal of ChemTech Research* 3(3), 1652–1657
80. Pondugula, S. R., Mani, S. (**2013**). Pregnane xenobiotic receptor in cancer pathogenesis and therapeutic response. *Cancer Letters*, *328*(1), 1–9.
81. Stanczyk, F. Z. (**2007**). Structure-function relationships, pharmacokinetics, and potency of orally and parenterally administered progestogens. Menopause (3rd Ed). Elsevier Inc.
82. Marrufo, T., Nazzaro, F., Mancini, E., Fratianni, F., Coppola, R., De Martino, L., De Feo, V. (**2013**). Chemical composition and biological activity of the essential oil from leaves of *Moringa oleifera* Lam. cultivated in Mozambique. *Molecules*, 18(9), 10989–11000.
83. Shin, S., Kang, C.A., (**2003**) Letter in Applied Microbiology 36, 111.
84. Rama Mohan, T., Alice Branton, T., & Gopal Nayak, D. T. (**2015**). Physical, Spectroscopic and Thermal Characterization of Biofield treated Myristic acid. *Journal of Fundamentals of Renewable Energy and Applications*, *05*(05).
85. Beare-Rogers, J. L, Dieffenbacher, A. Holm, J. V (**2001**). "Lexicon of lipid nutrition (IUPAC Technical Report)". *Pure and Applied Chemistry*. **73** (4): 685–744.
86. Gomez M., Saenz M., Garcia M., Farnandez M., (**1999**), Study on topical anti-inflammatory activity of *Achillea ageratum* on chronic and acute inflammation, *Z. Naturforsch* 55c, 937-941.
87. Sexton, A. C., Howlett, B. J. (**2006**). Parallels in fungal pathogenesis on plant and animal hosts. *Eukaryotic cell*, *America Society for Microbiology 5*(12), 1941–1949.

APPENDIX

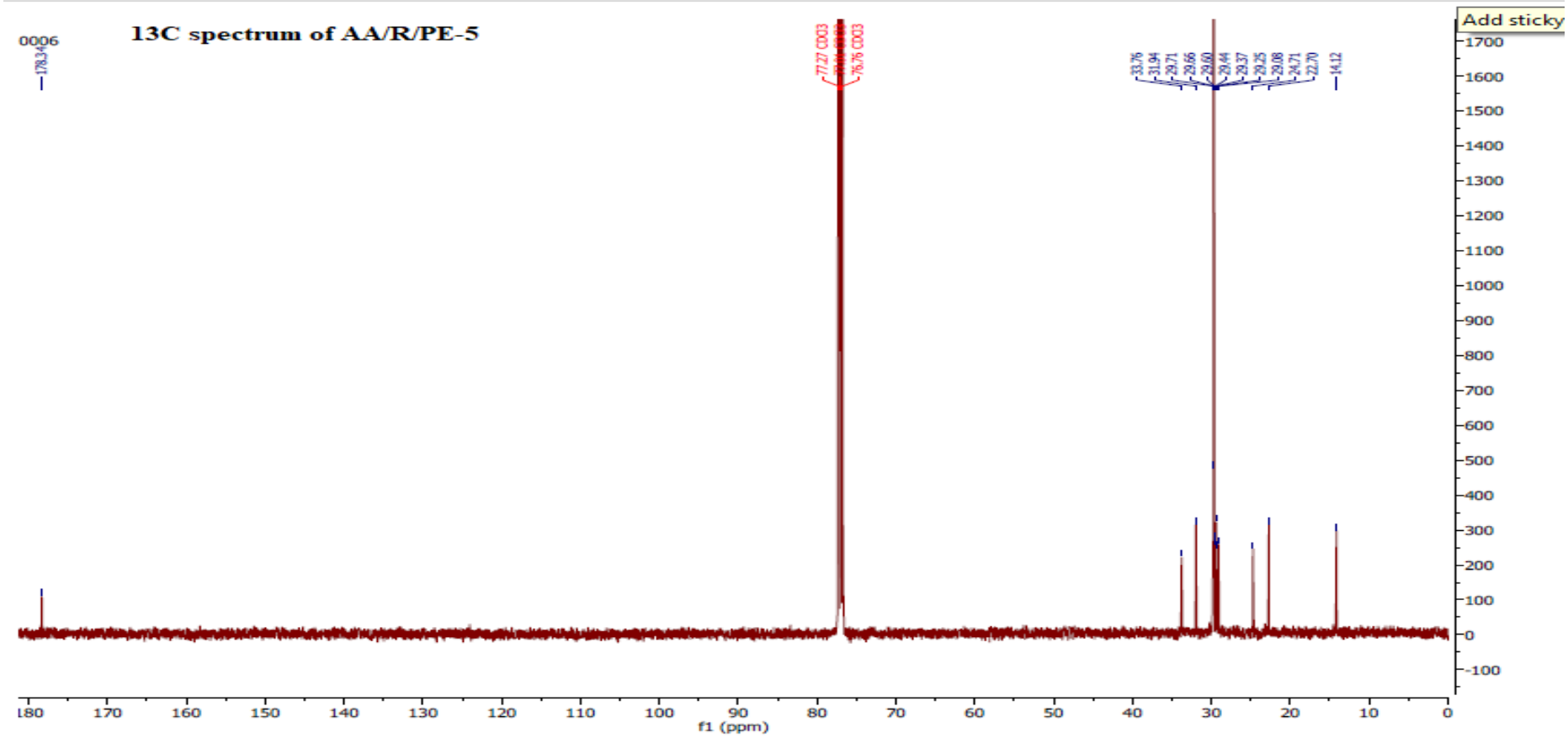
Appendix I



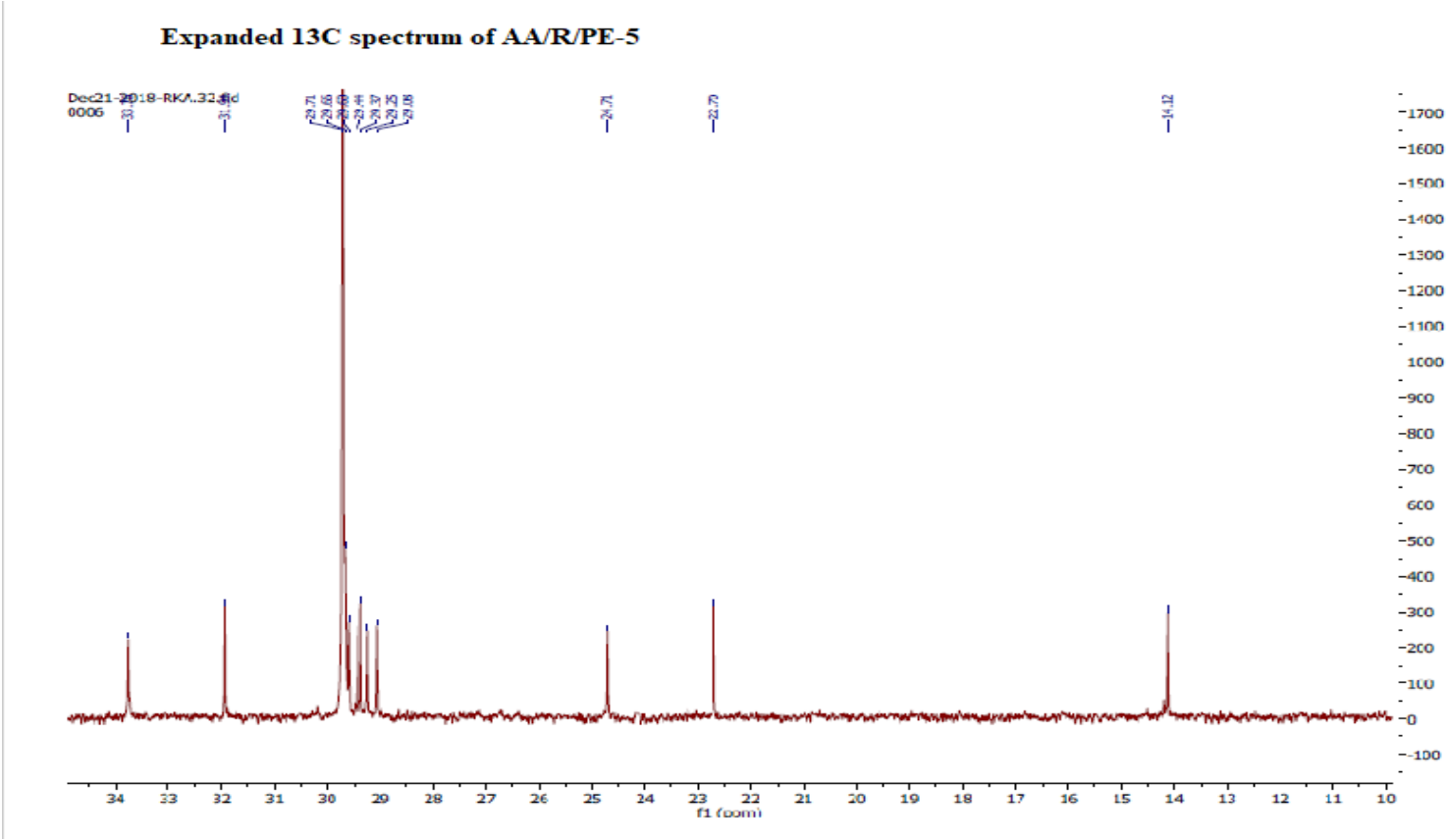
Appendix II



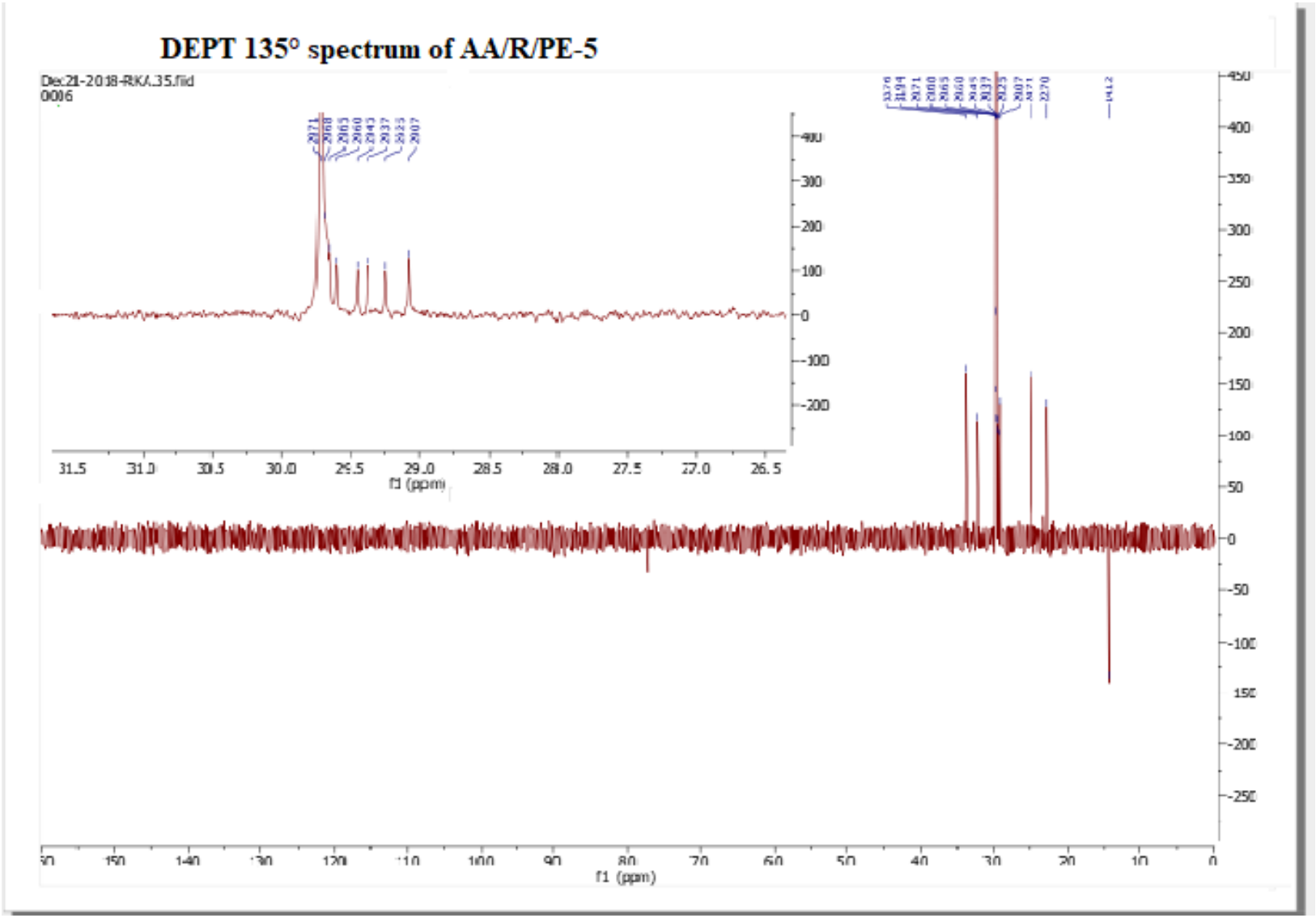
Appendix IIIA



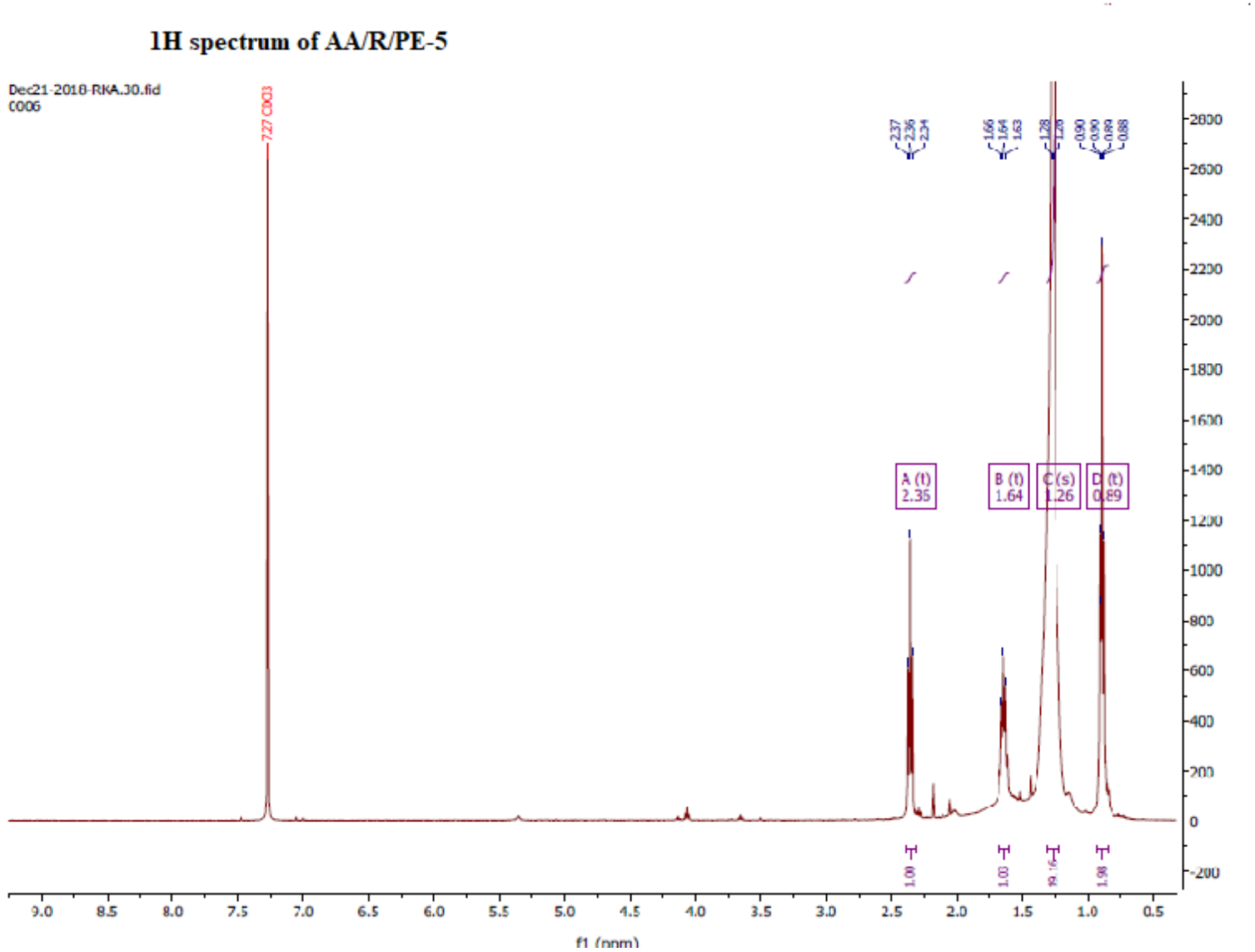
Appendix IIIB



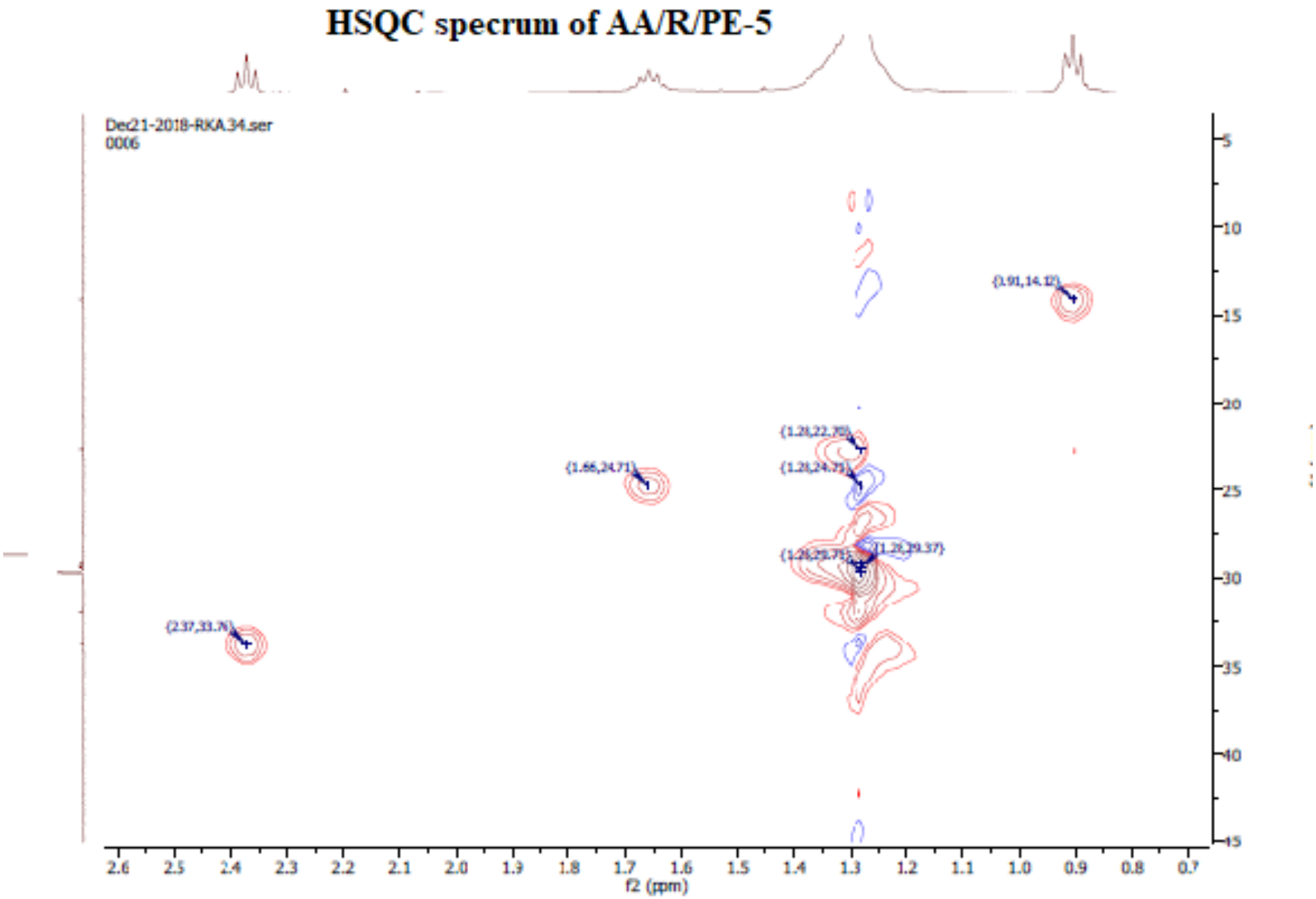
Appendix IV



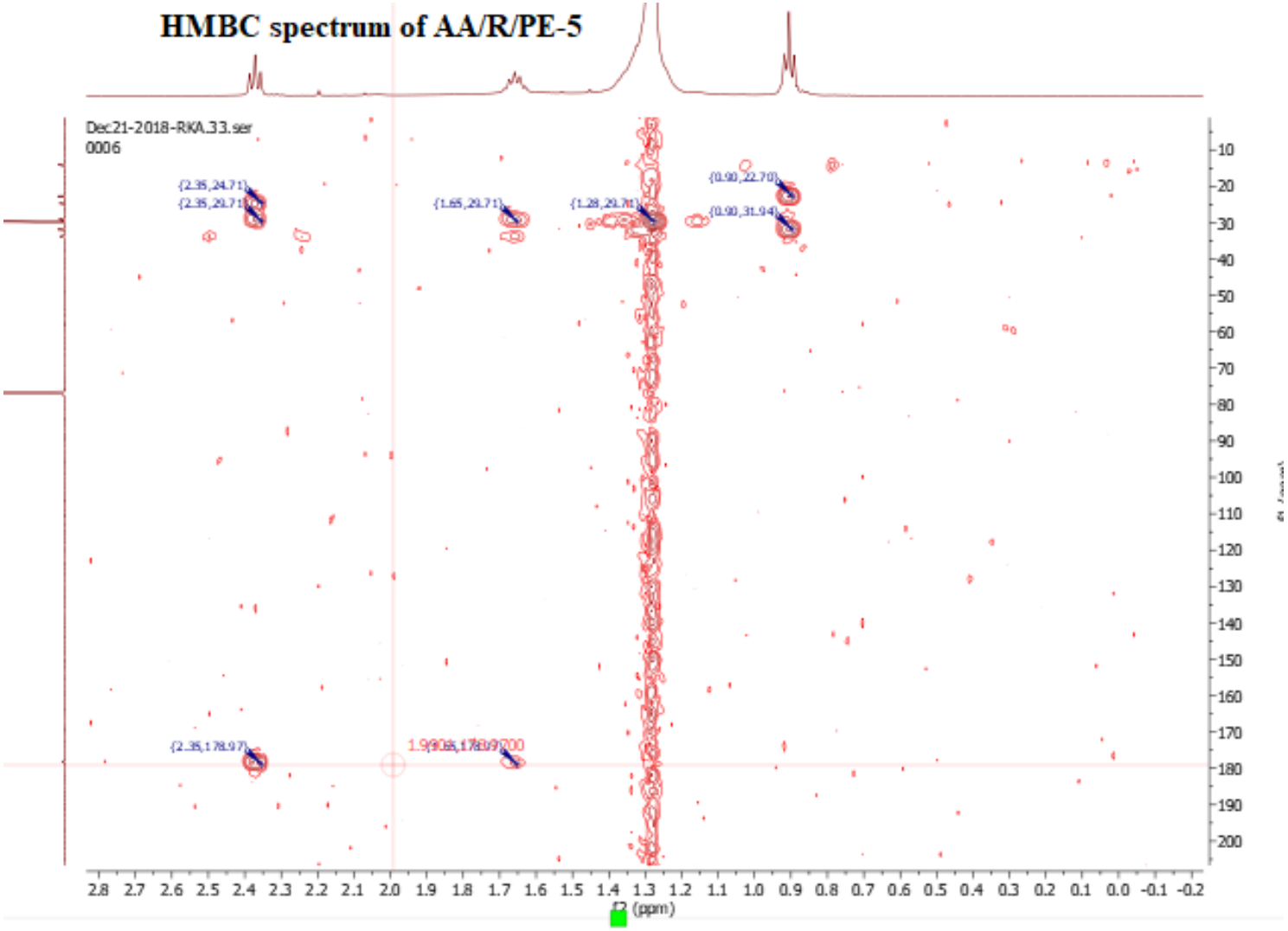
Appendix V



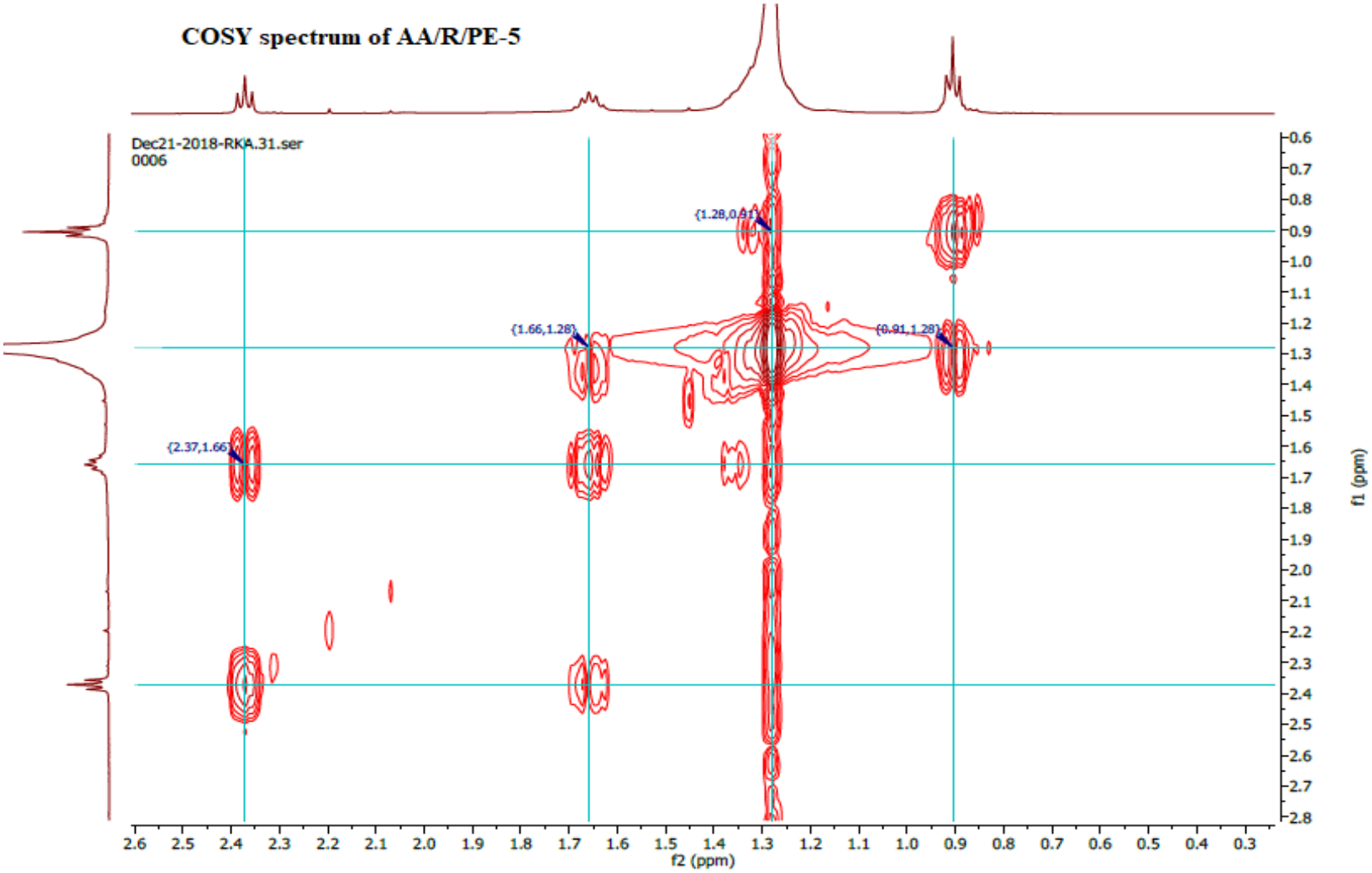
Appendix VI



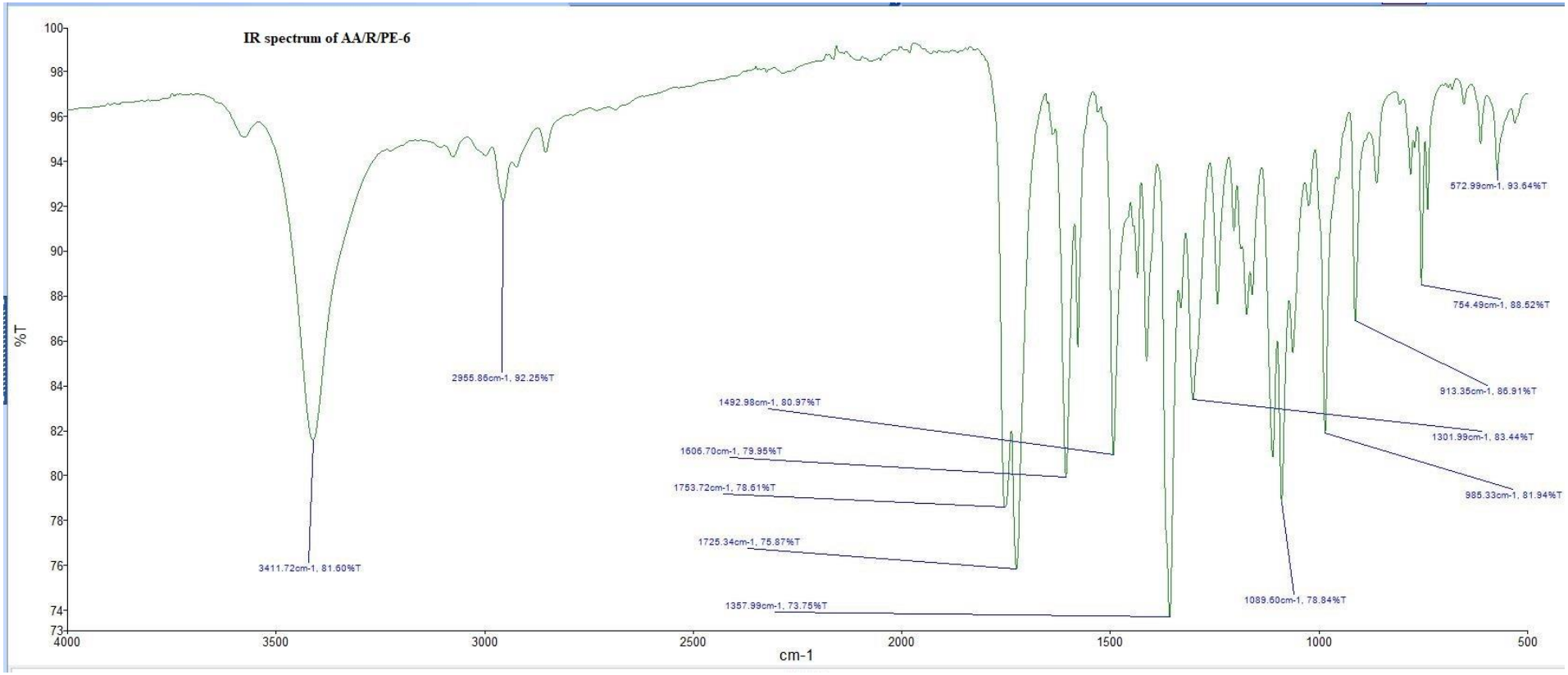
Appendix VII



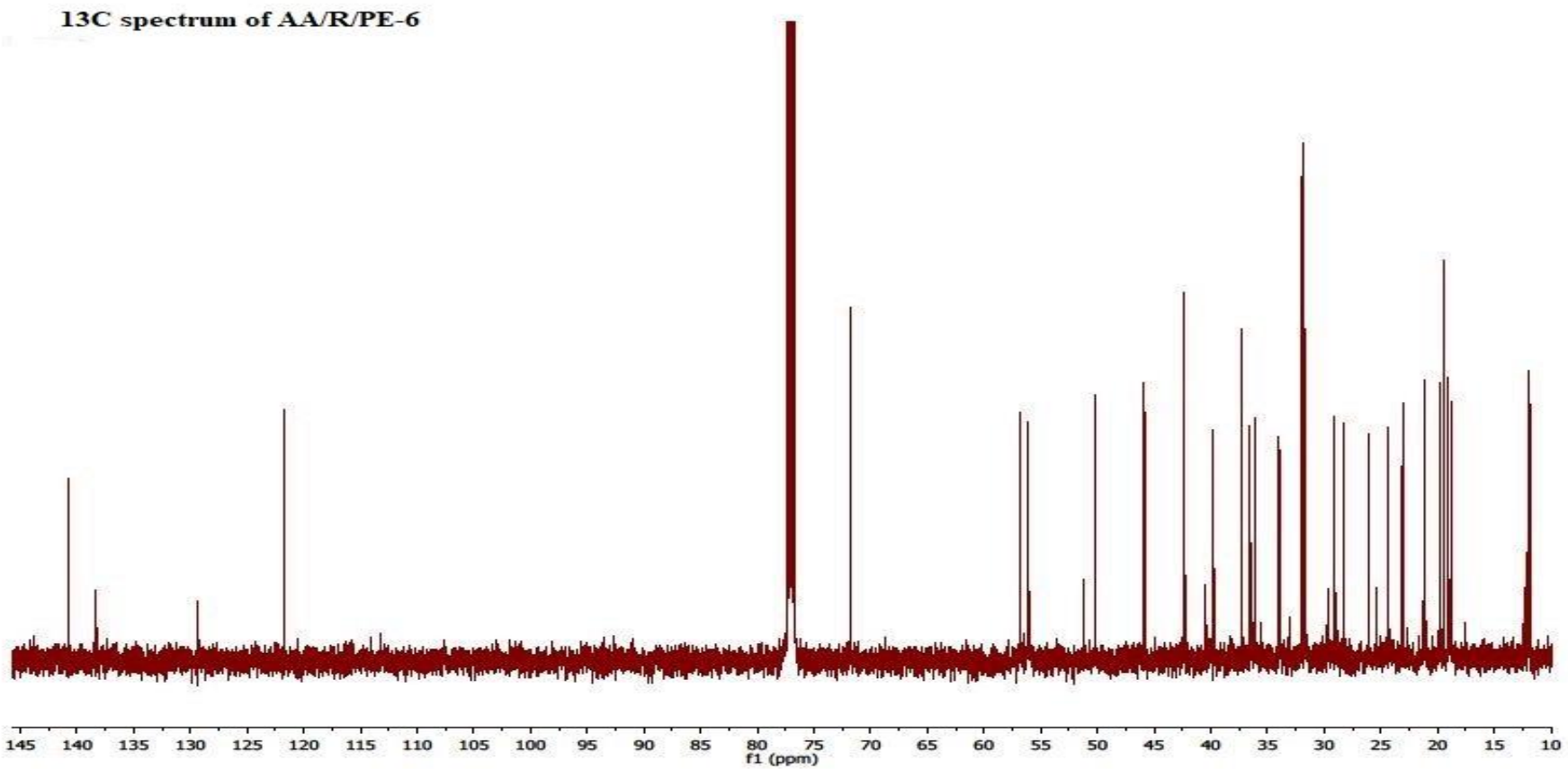
Appendix VIII



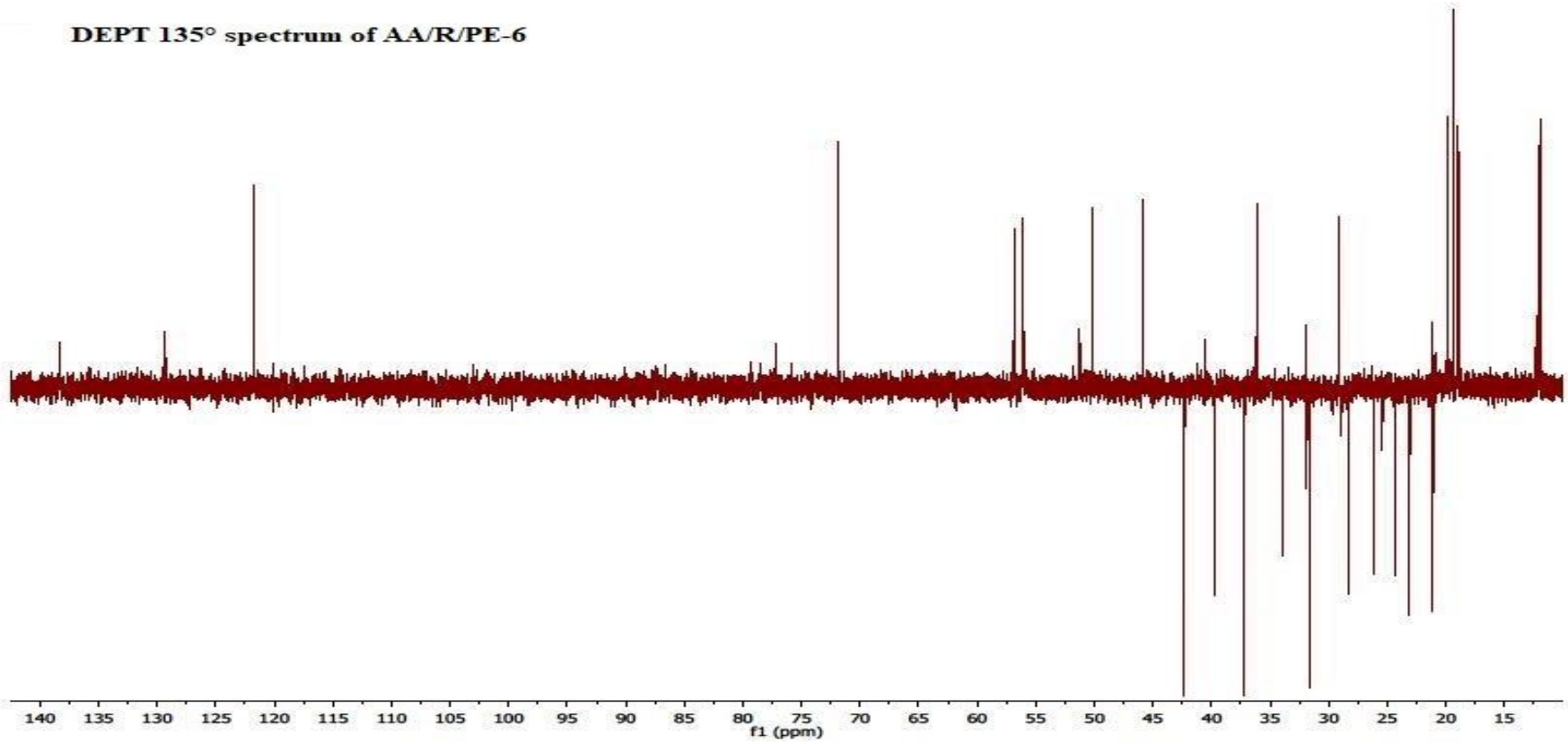
Appendix IX



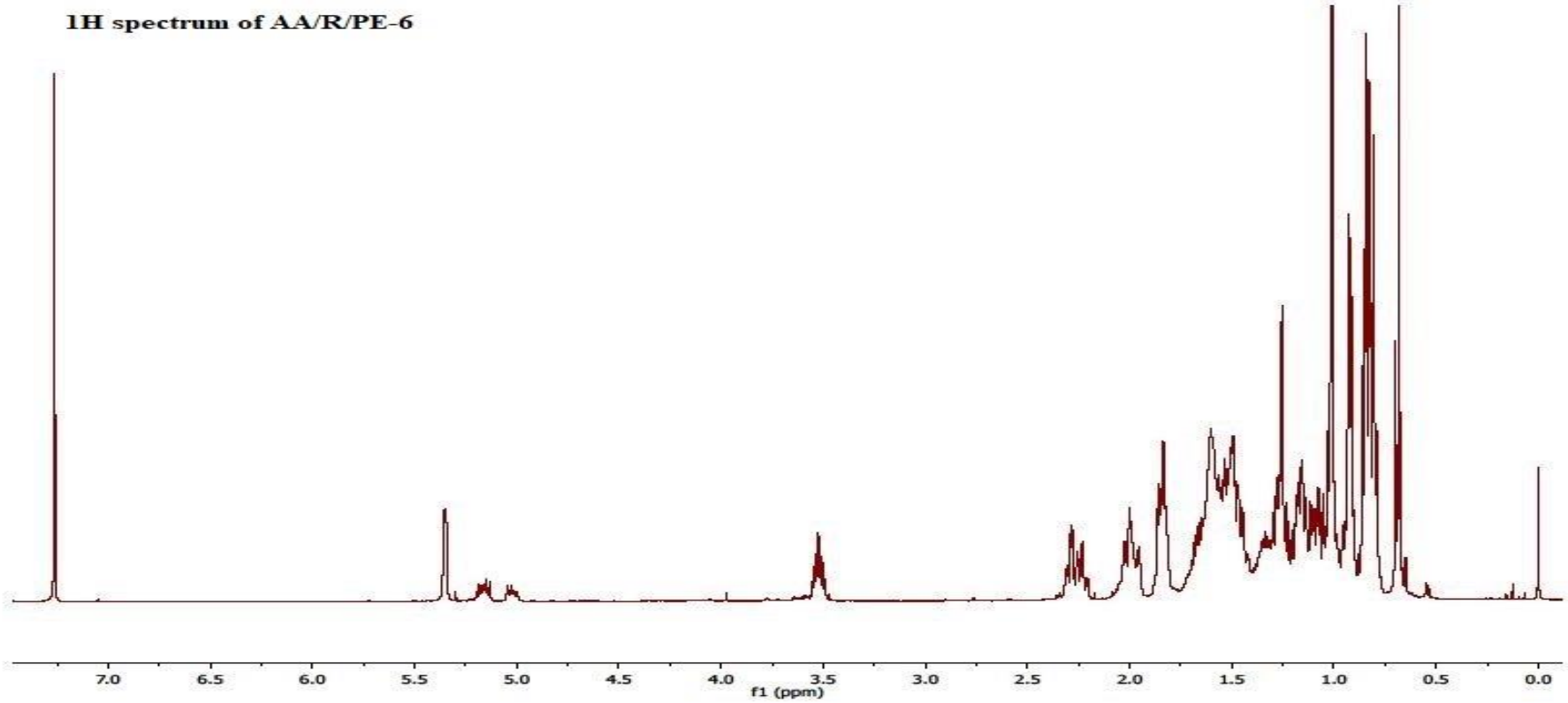
Appendix X



Appendix XI



Appendix XII



Appendix XIII