PHYTOCHEMICAL EVALUATION OF *Ocimum kilimandscharicum* GUERKE, *Gnidia subcordata* MEISN AND *Annona mucosa* JACQ LEAVES FOR SECONDARY METABOLITES AND THEIR INSECTICIDAL ACTIVITIES AGAINST *Sitophilus zeamais* AND *Prostephanus truncatus*.

BY

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DECLARATION

I declare this to be my own original work and as far as am aware, it has not been presented (submitted) for award of a degree in any institution.

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DEDICATION

This thesis is dedicated to my wife Betty and children Ivy, Laura and Precious for their encouragement and support they gave during the long and tedious period I undertook this study.

ABSTRACT

Global food security of which maize is a major component is threatened by food loss due to storage insect pests. *Sitophilus zeamais* and *Prostephanus truncatus* cause stored maize losses of 20% world wide and 45% in Africa. Synthetic insecticides though effective in control of insect pests, insect resistance and the adverse side effects associated with the insecticides calls for search of effective and safe botanical insecticides. *Ocimum kilimandscharicum, Gnidia subcordata* and *Annona mucosa* leaves have been used traditionally to control insect pests. Efficacy of their crude extracts and active principles occasioning their uses are however not documented. This work evaluated insecticidal activities of the plants' leaf extracts against *S. zeamais* and *P. truncatus*, isolated and characterised pure isolates and evaluated the pure isolates for their insecticidal activity against the two insects. Powdered plant leaves were separately sequentially soaked in n-hexane, ethyl acetate and methanol then filtered, concentrated and subjected to column chromatography fractionation with eluents of differing polarities and then thin layer chromatography. Structural elucidation of isolated compounds was done using physical and spectroscopic methods including NMR, IR, UV-VIS, mass spectrometry and comparison with literature data. Extracts and pure isolates were assayed for toxicity and antifeedant activities against *S. zeamais* and *P. truncatus*. Deltamethrine and azadirachtin were used as positive controls. *Gnidia subcordata* methanol extracts exhibited the highest contact toxicity and antifeedant activities against *S. zeamais*: LC_{50} = 27.03 µg/mL, AFI_{50} = 20.47 µg/mL and *P. truncatus:* LC50 =22.55 µg/mL, AFI_{50} = 20.99 µg/mL, which compared favourably with activities of the positive controls. *Gnidia subcordata* yielded β-amyrin acetate **(199)**, 3β-hydroxy-11-oxoolean-12-ene **(200),** dihydronitidine **(201),** dihydrochelerythrine **(202),** gedunin **(203),** obacunone **(204),** nagilactone **(205),** quercetin **(140),** kaempferol-3-*O*-β-galactoside **(206)** and 4', 5-dihydroxystilbene-3-*O*-β-glucoside **(207).** *n*-Eicosanol **(208),** friedelin **(209),** stigmasterol **(6),** lupeol **(49),** 2α-hydroxy-3-oxodammar-20, 24-diene **(210)**, 2α, 3β-dihydroxy dammar-20, 24-diene **(211)**, chrysin **(212)**, apigenin **(144),** fisetin **(213),** quercetin **(140)** and apigenin-7-*O*-neohesperidoside (**214**) were isolated from *Ocimum kilimandscharicum. Annona mucosa* yielded; α-amyrin acetate **(215),** β-sitosterol **(96),** 3α, 24-diacetoxy-12-oleanene **(216),**3-oxo-11β-hydroxyurs-12-ene **(217),** (3R,20S)-3-acetoxy-20-hydroxydammar-24-ene **(218),** 3β-acetoxy oleanolic acid **(219)**, 3β-acetoxytirucallic acid **(220)**, quercetin **(140),** oleanolic acid **(4),** quercetin 3-*O*-β-D-arabinoside **(221)** andquercetin-3-*O*-β-D-glucoside **(222).**Compounds **210**, **211** and **216** are new. Among the isolated compounds, **203** had the highest contact toxicity activities LC_{50} 15.68 and 16.99µg/mL while **222** had the highest antifeedant activities AFI_{50} 14.93and 16.84 µg/mL., against *S. zeamais* and *P. truncatus* respectively, which compared well with activities of the positive controls. This study has validated the traditional use of the plants in stored maize protection against insect pests. It has also identified the individual compounds responsible for the activities. These compounds can be developed into formulations individually or as mixtures to control *S. zeamais* and *P. truncatus* in maize.

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ABBREVIATIONS AND SYMBOLS

$[\alpha]^{25}_{D}$.....Specific rotation measured with sodium D-line light (589nm) at 25^{0}C

BAW…….Butanol-Acetic acid-Water

brs……....Broad singlet

CC ……Column chromatography

$CDCl_3$……Deuterated chloroform

CD_3OD…… Deuterated methanol

CI-MS……...Chemical ionization mass spectrometry

COSY……...Correlation spectroscopy

DEPT……Distortionless enhancement by polarization transfer

d……Doublet signal in NMR

DCM…….Dichloromethane

DMSO……..Dimethylsulphoxide

DMSO-d_6…….Deuterated dimethyl sulphoxide

ESI-MS**……**Electro- Spray Ionisation Mass Spectrometry

eV…….electron Volts

EI-MS……...Electron Ionization Mass Spectroscopy

EtOAc……. Ethyl acetate

HMBC……. Heteronuclear Multiple Bond Correlation

HMQC**.....**Heteronuclear Multiple Quantum Correlation

HSQC……. Heteronuclear Single Quantum Correlation

HR-EI-MS……...High Resolution Electron Spray Ionisation Mass Spectrometry

Hz……Hertz

IR……. Infra-red

J……...Coupling constant

LC_{50}……. Concentration of 50% response

m……. Multiplate

$[M]^+$ ……Molecular ion

MeOH-……. Methanol

M/z……Mass to Charge ratio

m.p.……Melting point

NMR.....Nuclear Magnetic Resonance

NOESY……. Nuclear Overhouser Effect Spectroscopy

OD…….Optical Dichroism

ORD …….Optical Rotary Dispersion

q……....Quartet

RDA……. Retro-Diels-Alder

R_F……. Retention factor

TLC……..Thin Layer Chromatography

V_{max} …….Maximum wave number of absorption

DEFINITION OF TERMS

Coleoptera	An order of insects consisting of beetles and weevils
Pest resurgence	Rapid reappearance of a pest after it has been controlled
Antifeedant	Natural or synthetic substance that stops or inhibits feeding by a pest especially an insect
Toxicity	The degree to which a chemical substance can damage an organism
Food security	The state of having reliable access to sufficient quantity of nutritious food
Botanical insecticides	Products used to kill or repel insects that consist of dried ground plant material, crude plant extracts or chemicals isolated from plants
Carcinogenic	Capable of causing cancer in living tissue
Anti-inflammatory	Substance that reduces the redness, swelling and pain in the body
Antinociceptive	The action or process of blocking the detection of a painful or injurious stimulus by sensory neurons
Antioxidant	A substance that protects cells from damage caused by free radicals
Biodegradable	Substance/ object capable of being decomposed by bacteria
Insect infestation	Invasion of an area, human, plant or animal by insects
Environmental degradation	Deterioration in environmental quality from through depletion of resources e.g. quality air, water and soil.
Terpenes and terpenoids	Terpenes are simple hydrocarbons while terpenoids are modified terpenes containing different functional groups and oxidized methyl groups. These two terms have however been used interchangeably in this study

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CHAPTER ONE

INTRODUCTION

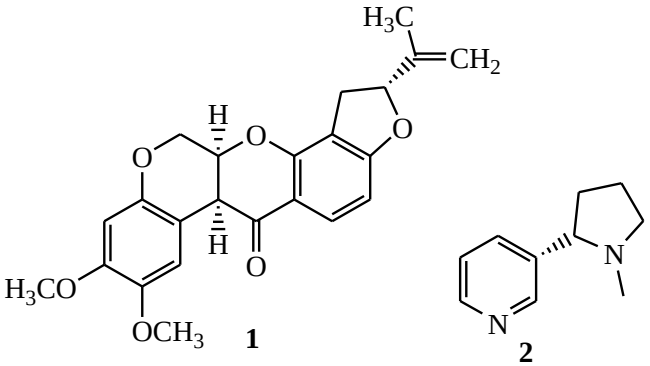
1.1 Background of the study

Post-harvest losses caused by insect pests accounts for 9% of stored food loss in developed countries and 20% or more in developing countries (Phillips *et al.,* 2010). In Africa pre- and post-harvest pests were responsible for about 40% of food losses (Mandava, 1985). Most of these losses have been caused by coleopteran insects which constitute almost 25% of all known types of animal life-forms (Rosenzweig, 1995). Maize (*Zea mays*), a staple food in many countries of Africa, Latin America and Asia (Mulungu *et al.*, 2011) is an essential component of global food security (Pingali & Pandey, 2000). Apart from being food for humans and animals, maize is a raw material for many industrial products including starches, sweeteners, oils and ethanol, (Maribet & Aurea, 2008). Despite the uses, maize grain losses as high as 80% due to insect pests infestation have been reported in developing countries (Pingali& Pandey, 2000; Tapondjou *et al.,* 2002).

Maize weevil, *Sitophilus zeamais* Motchulsky and larger grain borer, *Prostephanus truncatus* Horn are the most serious stored maize insect pests (Win *et al.,* 2013). These insects attack maize grains whose moisture content may be as low as 10.5% (Meikle *et al*., 1998) resulting in severe damage and weight loss during storage (Holst *et al.*, 2000). *S. zeamais* caused more than 20% grain loss for untreated maize worldwide (Giga & Mazarura, 1991; Markham *et al.*, 1994; Oduor *et al.*, 2000).*P. truncatus,* damaged well-dried maizeeven when stored on the cob (Dick, 1988) and a serious pest in sub-Saharan Africa (Boxah *et al.,* 1997), that caused yield losses of up to 45% in West Africa and about 62% in Mozambique (Muatinte *et al.,* 2014). Despite the great losses caused by these insects, effective, safe and sustainable methods of controlling them are lacking.

Synthetic insecticides are effective in insect pest control but their repeated use has led to residual effects that cause, environmental pollution, toxicity to non-target organisms and adversely affect food availability (Dubey *et al.,* 2007; Kumar *et al.,* 2007). Repeated use of synthetic pesticides has also led to development of resistant pest strains, pest resurgence and accumulation of toxic residues in food grains leading to health hazards (Sharma & Meshram, 2006). Several insecticides have therefore either been banned or restricted (Yallapa *et al.,* 2012).Though synthetic insecticides are effective in controlling insects, their numerous adverse effects on organisms and environment makes them unsuitable for pest control. In addition, in developing countries small-scale farmers may not afford these commercial products. This has led to the need to search for locally available plant products as alternative sources of controlling insects

Over the years botanical insecticides have provided effective control against insect pests which were resistant to synthetic insecticides (Weinzierl, 2000). In contrast with synthetic pesticides, most plant-derived pesticides are less toxic to mammals, have less persistence in the environment and are selective towards target pests (Rosenthal, 1986; Isman, 2006). Phytochemicals such as rotenone **(1)** and nicotine **(2)** were used as pesticides before the advent of synthetic insecticides (Ismam, 2006). Despite their effectiveness in the control of insect pests, rotenone **(1)** is toxic to aquatic life and mammals while nicotine **(2)** is toxic to mammals and can be absorbed through the eyes, skin and mucous membranes (Ismam, 2006)**.** Search for effective botanical insecticides with little side effects that can control insect pests of stored food is necessary.



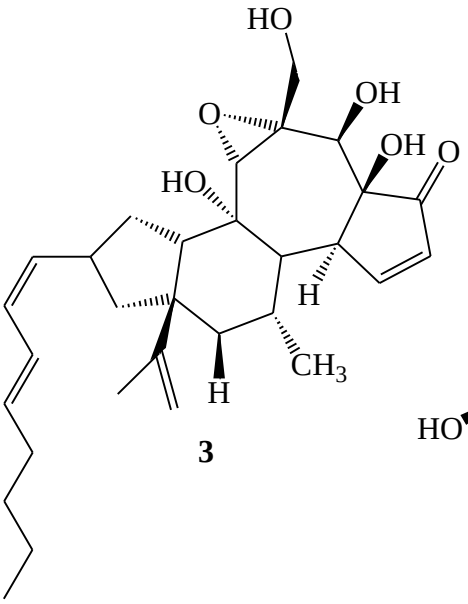
Some plants have been used in African folklore for the control of insect pests as botanical insecticides. For most of these plants, the scientific validation of their effectiveness which should be based on their biological activities and their phytochemistry is not documented. *Ocimum kilimandscharicum, Gnidia subcordata* and *Annona mucosa* are traditionally used for their insecticidal properties.

Leaves and roots of most *Gnidia* species are used in the traditional treatment of various conditions in humans and as insecticides (Kupchan *et al.,* 1976; Sohni *et al.*, 1994; Ferrari *et al.*, 2000; Munkombwe *et al.,* 2003). *Gnidia subcordata* leaves are used in treatment of skin diseases and in insect pest control (Bellakhdar, 1997). Extracts of some *Gnidia* species have exhibited a range of activities against various insects. Evaluation of hexane extracts of *Gnidia kraussiana* against *Callosobruchus maculatus* (F.) by contact toxicity assay demonstrated that, 1 ml/50 g grains dosage killed all the insects within two days of exposure Kosini & Nukunine, (2017). On the other hand, Conceição*et al.*, (2010) on testing methanol leaf extracts of *Daphne gnidium* at3.5% concentration against *Sitophilus zeamais* adults caused 66% mortality in a one-day exposure.

Extracts from other plants in the genus *Gnidia* showed activities against organisms such as Insects, bacteria, molluscs, viruses, fishes (Kareru *et al*., 2006; Teklehaymanot & Gidday, 2007; Roger, 2009; Berhan *et al.,* 2006).There is however no information on insecticidal activity of *Gnidia subcordata* crude leaf extracts against *S. zeamais* and *P. truncatus.* Documentation on whether

crude leaf extracts of *G. subcordata* have any insecticidal activity against *S. zeamais* and *P. truncatus* is therefore lacking.

Diterpenoids, triterpenoids, coumarins, benzophenone glycosides, flavonoids, lignans, phenolic glycosides, sterols and spiro-bis-γ-lactones isolated from the *Gnidia* species exhibited various biological activities including insecticidal activities (Sakata *et al.*, 1971; Mallavadhani *et al.*, 2003; Riaz *et al.*, 2018). Most of the activities were mainly against microorganisms. Reports on insecticidal activities of these compounds are scanty. Among the compounds however, exocariatoxin **(3)** from *Gnidia kulprantus* demonstrated piscicidal and insecticidal activities (Bala *et al.*, 1999) while oleanolic acid **(4)** from *Gnidia glauca* (Sannabommaji *et al.*, 2018) showed antifeedant activity (Mallavadhani*et al.*, 2003**).**



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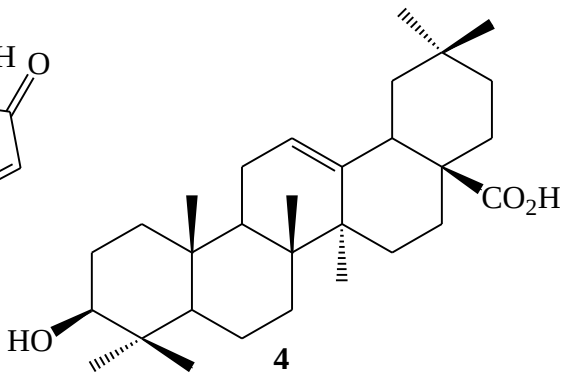

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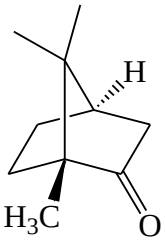
Despite the different biological activities of compounds isolated from various parts of the plants in the genus *Gnidia*, there are no reports on isolation of compounds from crude leaf extracts of *G. subcordata* and their biological activities. It is not known whether compounds with insecticidal activities against *S. zeamais* and *P. truncatus* would be isolated from crude leaf extracts of *G. subcordata*.

Traditionally stored food stuffs were mixed with dry leaves of *O. kilimandscharicum* for protection against insect damage during storage (Obeng-Ofori *et al.,* 1996). In the test of dried ground leaves and seed essential oils of *O. kilimandscharicum*, (Jembere *et al*., 1995) established that 25.0 g of the leaf powder and 0.3 g essential oil per 250 g of grain (maize and sorghum) killed 100 % of *S. zeamais and Rhyzopertha dominica* in 48 hours (Singh *et al.,* 2014). Exposure of *Helicoverpa armigera* larvae to *O. kilimandscharicum* whole leaves deterred larval feeding and mortality of the larvae increased (Lawal, 2014; Singh *et al*., 2014; Jembere *et al*., 1995). These tests were carried out using leaf powder, whole leaves and essential oils of *O. kilimandscharicum.* There are no reports on the insecticidal activities of *O. Kilimandscharicum* crude leaf extracts against *P. truncatus.* It has therefore not been documented as to whether *O. Kilimandscharicum* crude leaf extracts have any insecticidal activities against *P. truncatus*.

Compounds isolated from different species of *Ocimum*, exhibited various biological activities (Singh *et al.*, 2014; Fajriah & Darmawan, 2016; Marchese *et al.*, 2017). Camphor **(5)** isolated from flowers and seeds of *O. kilimandischaricum* exhibited insecticidal activities against *S. zeamais, P. truncatus, S. granarius* and *T. casterneum* (Jembere *et al.,* 1995; Obeng-Ofori *et al.,* 1998). Stigmasterol **(6),** a sterol from the ethanol stem extracts of the plant exhibited antifeedant activity against *Plutella xylostella* (Singh *et al.*, 2014; Huang *et al.*, 2008).



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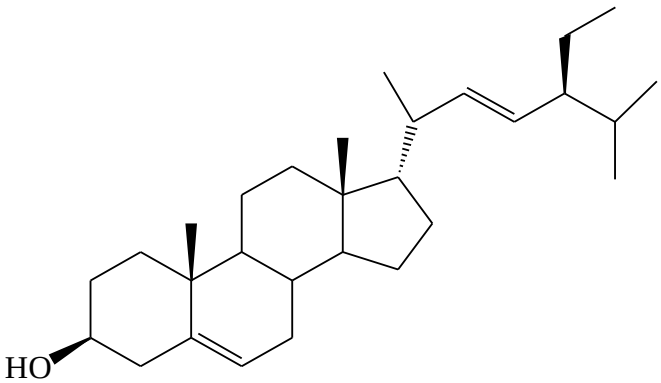

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Compounds from different parts of *O. kilimandscharicum* were subjected to various biological activities, but most compounds in crude leaf extracts of *O. kilimandscharicum* have not been subjected to the phytochemical analysis and insecticidal activities tests.

Though compounds isolated from flowers, seeds and stems of *O. kilimandscharicum* have promising activities against insect pests including *S. zeamais* and *P. truncatu*s, information on isolation and biological activities evaluation of compounds in crude leaf extracts of *O. kilimandscharicum* is scanty. It is not established whether new compounds with insecticidal activities against *S. zeamais* and *P. truncatus* would be isolated from crude leaf extracts of the plant.

The *Annona* species have traditional applications in treatment of various diseases and as insecticides (Ngamo & Hance, 2007; de Lima *et al.,* 2012; Akanksha *et al.*, 2015). Crude leaf extracts of *A. mucosa* have been used for elimination of fleas and other insects (de Lima *et al.,* 2012). There is however no scientific validation of the traditional use of *A. mucosa* leaves as an insecticide. In an experiment involving ethanol seed extracts of *A. mucosa*, Bernadi *et al.*, (2017) showed that a concentration of 2000 mg L^{-1} led to 85% mortality of *Drosophila suzukii*. Similarly, methanol seed extract of *A. mucosa* at 5 mg L^{-1} concentration led to 90% mortality of the *Euschistus heros* nymphs (Turchen *et al*., 2016). In another experiment, chloroform-methanol extracts of *A. mucosa* seeds at a concentration of 8.0% led to about 93.3% mortality of *Chrysodeixis inludens* (Massarolli *et al.,* 2016). All tests described, were carried out on seed extracts of *A. mucosa*, with no reports on the insecticidal activity of the crude leaf extracts of the plant. There has been no evaluation on whether crude leaf extracts of *A. mucosa* would have insecticidal activities against *S. zeamais* and *P. truncatus.*

Annona species elaborates terpenoids, flavonoids, alkaloids, megastamanes and acetogenins (Rieser *et al.,* 1993; Nawwar *et al.,* 2012; El-Azim *et al.,* 2015; Pino & Roncal, 2016). Most of these compounds were tested against various microorganisms and found to be active. Reports on their insecticidal activities are however scanty. There is limited documentation on isolation of compounds in leaves of *A. mucosa* and their insecticidal activities. It is thus not established whether fractionation of *A. mucosa* leaf extracts would lead to isolation of new compounds and whether such compounds would have insecticidal activities against *S. zeamais* and *P. truncatus.*

1.2 Statement of the problem

O. kilimandscharicum, G. subcordata and *A. mucosa* are known traditionally for their insecticidal properties yet basis of the insecticidal activities and effectiveness of their extracts and active principles have not been established. There have been no evaluation of insecticidal activities of *G. subcordata, O. Kilimandscharicum* and *A. mucosa* crude leaf extracts against *S. zeamais* and *P. truncatus*. Isolation and characterization of compounds from *G. subcordata, O. Kilimandscharicum* and *A. mucosa* crude leaf extracts have not been established. Information on insecticidal activities of compounds in *G. subcordata*, *O. Kilimandscharicum* and *A. mucosa* crude leaf extracts against *S. zeamais* and *P. truncatus* is lacking.

1.3 Research objectives

1.3.1 General objective

To evaluate the phytochemical composition and insecticidal potency of *Gnidia subcordata, Ocimum kilimandscharicum* and *Annona mucosa* leaves against insect pests of stored maize; *Sitophilus zeamais* and *Prostephanus truncatus*.

1.3.2 Specific objectives

1. To evaluate the contact toxicity and antifeedant activities of *G. subcordata, O. kilimandscharicum* and *A. mucosa* crude leaf extracts against *S. zeamais and P. truncatus*.

2. To characterize pure isolates from leaves of *G. subcordata, O. kilimandscharicum* and *A. mucosa.*

3. To evaluate the contact toxicity and antifeedant activities of the pure isolates from *G. subcordata, O. kilimandscharicum* and *A. mucosa* against *S. zeamais and P. truncatus*.

1.4 Null hypothesis

1. Extracts from *O. kilimandscharicum, G. subcordata* and *A. mucosa* leaves do not exhibit insecticidal activities against *S. zeamais* and *P. truncatus*.

2. Pure isolates from leaf extracts of *G. subcordata, O. kilimandscharicum* and *A. mucosa* have dissimilar structures to other previously characterised compounds from the plants.

3. Compounds from *O. kilimandscharicum, G. subcordata* and *A. mucosa* leaves do not exhibit insecticidal activities against *S. zeamais* and *P. truncatus.*

1.5 Justification of the research

Identification of isolates from *G. subcordata, O. kilimandscharicum* and *A. mucosa* that can effectively deter *S. zeamais* and *P. truncatus* from infesting stored maize will eliminate the losses caused by the pests, thus improve food security. Successful identification of the plant metabolites with insecticidal activities will provide effective, safe and biodegradable insecticides that will minimize environmental pollution.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Zeamays* (maize)

Zeamays L., commonly known as maize, belongs to the family Gramineae, is grown in many parts of the world (Maribet & Aurea, 2008). Maize is an essential component of global food security as a major diet for millions of people. The high demand of maize (Pingali & Pandey, 2000) and the high post harvest losses (Pimentel, 2007) presents an urgent challenge for most developing countries. Maize is a staple food in many countries of Africa, Latin America and Asia (Mulungu *et al.*, 2011). Apart from being food for humans and animals, maize is processed into starches, sweeteners, oils and ethanol. Items such as toothpaste, cosmetics, adhesives, shoe polish, ceramics, explosives, paints and textiles contain maize components (Maribet & Aurea, 2008; Soujanya *et al.*, 2016). Despite its many uses, maize grain losses due to postharvest storage insect pests are high due to lack of effective and eco-friendly insecticides.

2.2 Stored food losses to insect pests

Global food security requires world agricultural sector to achieve a production level that ensures sustainable and adequate food supply for the increasing population (Ngamo *et al.*, 2007).Insect pests constitute a major threat to food production, destroying approximately 14% of all potential food production, including maize (Pimentel, 2007).A serious threat to food security is loss due to insect pest infestation during post-harvest storage (Belmain & Stevenson, 2001). Food availability could be enhanced by increasing agricultural productivity through the use of sustainable good agricultural practices and by reducing post-harvest crop losses (Tscharntke *et al.*, 2012). The less developed countries where the losses are large may benefit most from eradication of post-harvest

food losses (Kosini & Nukunine, 2017). In the tropics, between 60-80% of all grain produced is stored at the farm level (Golob *et al.,* 1999) and can be subjected to post harvest losses especially caused by insect pests. In sub-Saharan Africa food security partially depends on reduction of postharvest losses caused by pests (Ogendo *et al.*, 2004). Post-harvest losses caused by stored insect pests are up to 9% in developed countries and can be 20% or more in developing countries (Phillips *et al.,* 2010), while pre and post-harvest pests are responsible for about 40% of Africa's food losses (Mandava, 1985). In the tropical region insect pest infestation inflict 20-30 % damage on stored maize grain(Haque *et al.*, 2000), while in developing countries maize grain losses as high as 80%have been reported (Pingali & Pandey, 2000; Tapondjou *et al.,* 2002). Due to insect pest infestation, the stored food products may have dead insect bodies, cast skins, faecal remnants, excretions and dusts (Win *et al.,* 2013), resulting in stored food of low quality and quantity (Yallapa *et al.,* 2012).Insect infestation-induced changes in the storage environment may cause warm moist "hotspots" that provide suitable conditions for storage fungi, to cause further food losses (Yallapa *et al.,* 2012). Despite the destruction caused by stored food insect pests on stored food, an effective insecticide with no side effects to control the insect pests is yet to be discovered. Effort to control insect pests by use of synthetic insecticides led to development of resistant pest strains, pest resurgence and accumulation of toxic residues on food grains (Sharma & Meshram, 2006).Some effective botanical insecticides were found to be toxic to aquatic life and to mammals (Carr *et al.*, 1991) and to persist on food crops after treatment (Isman, 2006)

2.3 Stored maize insect pests

Coleoptera is an order of insects which constitute almost 25% of all known types of animal life-forms (Rosenzweig, 1995). Most of these insects such as the maize weevil, *S. zeamais*

(Motschulsky) (Coleoptera: Curculionidae) and the larger grain borer, *P. truncatus* (Horn) (Coleoptera: Bostrichidae), are serious maize storage insect pests (Win *et al.*, 2013). Both insects can infest standing maize in the field once the crop has attained maturity and is drying, before harvesting (de Pury, 1968).

S. zeamais (Plate 1) is a serious, internal feeding pest of maize grain found in all warm and tropical parts of the world (Dobie *et al.*, 1984) and is considered a worldwide pest of stored products (Torres *et al.*, 2014).

Plate 1: Adult *Sitophilus zeamais*
(Source: Research Gate)

It infests maize in the field before harvest and extends the infestation throughout the storage period (Oliveira *et al.*, 2007). The larvae and adult feed on the endosperm and this damage allows the attack of secondary insect pests or fungi (Rees, 1996), causing more than 20% grain loss for untreated maize worldwide (Giga & Mazarura, 1991). In many tropical countries, *S. zeamais* cause an estimated 30 to 80% weight loss during storage (Agoda *et al.*, 2011). Voracious feeding on whole grains by this insect can completely destroys stored grain (Trematerra, 2009) and the damage it causes reduce the nutritive value, weight loss and contaminates the stored maize grain rendering it unfit for human consumption (Ouko *et al.*, 2017). The huge post-harvest losses and quality deterioration of maize grains caused *S. zeamais* is a major obstacle to achieving food security in developing countries (Rouanet, 1992).

P. truncatus (Plate 2) is a major storage pest of maize in Africa (Nboyine *et al.*, 2015) and is the most serious pest in parts of sub-Saharan Africa (Boxah *et al.,* 1997).

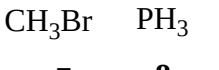
Plate 2: Adult *Prostephanus truncatus*
(Source: G. Goergen)

The pest has ability to damage well-dried maize, even when stored on the cob (Dick, 1988).The larvae and adults of *P. truncatus* produce lots of dust and frass(Shires, 1980) and causes yield losses of between 30% -90% (Nhamucho *et al.,* 2017). The destruction caused by these insect pests is a hindrance in achieving global food security and there is therefore need to search for effective and sustainable insecticides in control of *S. zeamais* and *P. truncatus*.

2.4 Conventional stored food insect pest control

Synthetic insecticides are effective in controlling insect pests but their uncontrolled use has led to serious problems (Yallapa *et al.,* 2012). These problems include development of resistant pest strains, pest resurgence and accumulation of toxic residues on food grains leading to health hazards (Sharma & Meshram, 2006). The repeated use of synthetic pesticides causes environmental pollution, toxicity to non-target organisms and adverse effects on food besides toxicity to humans (Dubey *et al.,* 2007*;* Kumar *et al.,* 2007*).* Indeed, toxicity of synthetic pesticides leads to destruction of beneficial fauna, domestic animal poisoning, contamination of livestock products, fish and wild life losses and contamination of underground and river waters (Adilakshmi *et al.,* 2008).Public awareness of environmental and food contamination from pesticides has led environmental

protection agencies to ban the use of some synthetic insecticides like chlorinated, organophosphorus and carbamate insecticides (Begum *et al.,* 2013). Synthetic insecticides such as methyl bromide **(7)** and phosphine **(8)** have been in use for control of stored food insect pests (Ayvaz *et al.,* 2010). Methyl bromide **(7)** has however been banned in many countries because of its ozone layer depleting properties (Hansen & Jensen, 2002) while pest resistance to phosphine **(8)** is high leading to control failures (Leelaja *et al.,* 2007; Rajeshakar *et al.,* 2006). Resistance by insect pests to the more recently developed synthetic insecticides such as chlorpyrifos-methyl **(9),** cynaphos **(10),** carbaryl **(11)**, dichlorophenyltrichloroethane (DDT) **(12),**cypermethrin **(13),**deltamethrin **(14),** diazinon **(15),** dichlorovos **(16),** ethylene bromide **(17),** ethyl formate **(18)**, permethrin **(19),** methyl diphenyl phosphine **(20)** and bioresmethrin **(21)** has been reported(Yallapa *et al.,* 2012). Carbaryl **(11)** is also toxic to human beings while DDT **(12)** accumulates in the body of animals and over a long period becomes toxic to the animals and human beings (Inge, 2004). Deltamethrin **(14)** and permethrin **(19)** are toxic to fish and other water organisms while dichlorovos **(16)** is highly toxic to human beings and warm blooded animals (Inge, 2004). Lindane **(22)** is toxic to animals and humans, is persistent on the grains while its residues build up in the food chain leading to chronic poisoning with its long term use (Inge, 2004). Some insect pests such as lesser grain borer have developed resistance to malathion **(23)** and pirimiphos-methyl **(24)** (Inge, 2004). The persistence of these chemicals in the environment and in treated foods is also a major constraint to their use as pesticides (Jovetic, 1994).



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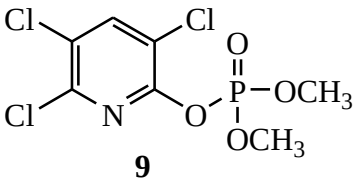

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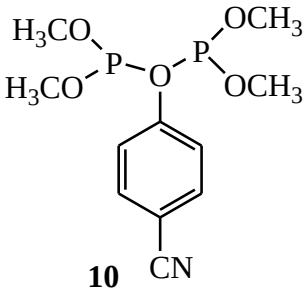

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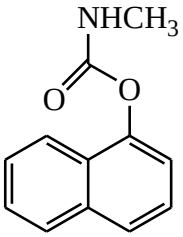

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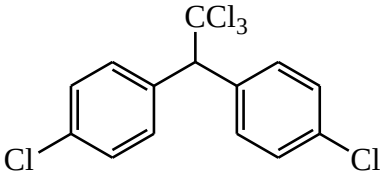

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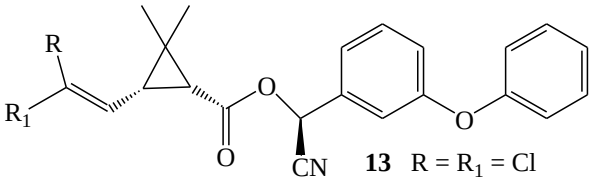
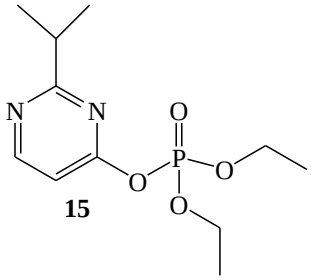


13 R = R_1 = Cl
14 R = R_1 = Br



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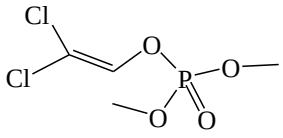

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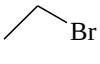

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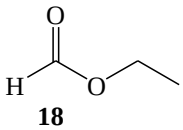

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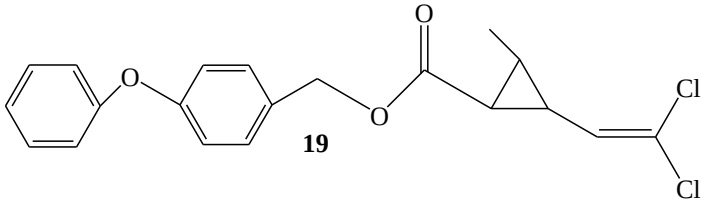

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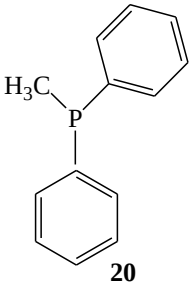

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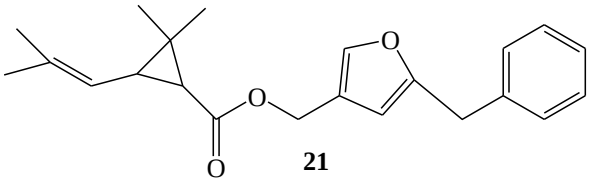

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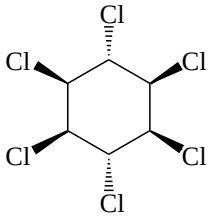

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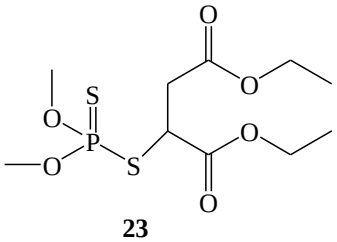

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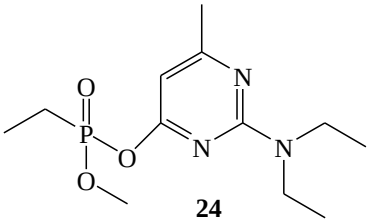

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Synthetic insecticides are effective in controlling insect pests, but the many adverse side effects of these insecticides calls for search and identification of effective alternative insecticides that have little or no environmental pollution and side effects on non-target organisms including human.

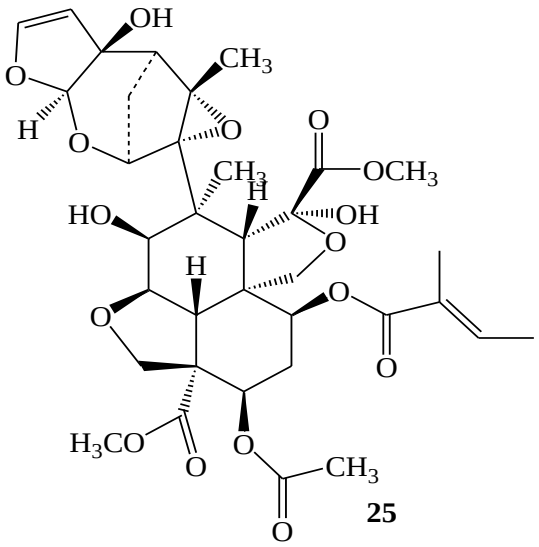
2.5 Use of botanical insecticides in pest control of stored food and food products

Knowledge on negative impacts of repeated use and problems associated with synthetic insecticides have prompted research into the development of new substances with insecticidal properties from plants as postharvest storage agents with lower environmental degradation characteristics (Souza *et al.,* 2010; Ladan *et al.,* 2013).The use of plant extracts in agriculture for pest control has been in practice for at least two millennia, when botanical insecticides were considered important products for pest management in ancient China (Long *et al.,* 2006), Egypt, Greece and India (Isman, 2006). Plants with insecticidal action have been used as protectants in form of powders, oils and extracts for control of major pests of stored products in many countries of Latin America, Africa and Asia (Gonçalves *et al.,* 2015).

Botanical insecticides provide effective control against insect pests that are resistant to synthetic insecticides (Weinzierl, 2000). These insecticides possess low mammalian toxicity, less persistence in the environment, selectivity towards target pests and non-phytotoxicity (Rosenthal, 1986; Isman, 2006). They also provide novel modes of action against insects that can reduce the risk of cross-resistance (Isman, 2008). Plants may contain compounds with activities against pest species of stored grains and could be good candidates of current stored grain integrated pest management programs (IPM) (Moreira *et al.,* 2007; Ribeiro *et al.,* 2016).Identification of compounds with modes/mechanisms of action which may be different from those found in the currently used insecticides is desirable for the management of resistant populations of insect pests in the storage

units (Ribeiro *et al.*, 2013). The identification could lead to the development of new class of safer insect control agents (Kim *et al.*, 2003). Plant-derived compounds show great potential for the management of populations of arthropod pests, both through homemade preparations for direct use in the field and in the development of botanical insecticides, as well as templates of molecules for the synthesis of new synthetic insecticides (Ribeiro *et al.*, 2017).

Due to these benefits, some plants have been used in African folklore for the control of insect pests, but the basis of their effectiveness has not been determined.Neem oil, obtained by cold-pressing of seeds of neem tree (*Azadirachta indica)* is effective against soft-bodied insects and mites while extracts of the neem seed residue after removal of the neem oil, contain azadirachtin **(25)** which is an effective insecticide (Isman, 2006). The roots or rhizomes of *Tephrosia spp* provide rotenone **(1)**, an effective insecticide comparable to synthetic insecticides (Hollingworth *et al.*, 1994). In addition rotenone **(1)** was found to be toxic to aquatic life and to mammals (Carr *et al.*, 1991) and persist on food crops after treatment (Isman, 2006). Leaves of the tobacco plant (*Nicotiana tabacum*) provided the insecticide, nicotine **(2)** (Isman, 2006) while leaves of wild tobacco (*Nicotiana gossei*) provided sugar esters which were insecticidal to certain soft-bodied insects and mites (Isman, 2006). Nicotine **(2)** though effective in pest control, was highly toxic to mammals and could readily be absorbed through the eyes, skin and mucous membranes (Carr *et al.*, 1991).



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With these limitations by the botanical insecticides, there is need for continued search of alternative control agents which are less toxic to human, more readily degradable, effective, economical, environmentally friendly and convenient to use for the control of insect pests of stored maize. Among these is the use of botanical insecticides with low mammalian toxicity which can effectively prevent and/or suppress insect pests especially in storage (Golob & Webly, 1980; Ayvaz *et al.*, 2008). An effective insecticide from plants that has fewer side effects, is biodegradable and is environmentally safe in the control of stored maize insect pests is yet to be identified**.**

2.6 Plants used in this study

The use of botanical pesticides which are indigenous, effective and with low mammalian toxicity can provide safe, environment-friendly and cheap sources of preventive measures for stored food product losses caused by pests (Maribet and Aurea, 2008). Folklore has information on use of several plants to control insect pests. These plants include *Gnidia subcordata*, *Ocimum kilimandscharicum* and *Annona mucosa*. Leaves of *Gnidia subcordata*, *Ocimum kilimandscharicum* and *Annona mucosa* are used traditionally in insect pest control (Bellakhdar,

1997; Obeng-Ofori *et al.*, 1996; Traore-Culibaly *et al.*, 2013). The basis of the insecticidal activities and effectiveness of their extracts and active principles are however not documented.

2.6.1 The genus *Gnidia*

2.6.1.1 Botanical information on the genus *Gnidia*

Gnidia is a genus of flowering plants in the family Thymelaeaceae, which is a small family consisting of about 1200 species distributed into 67 genera (Beaumont *et al.*, 2009; Borris *et al.*, 1988). Members of this family are widespread in the tropics and temperate climate, particularly in Africa (Borris *et al.*, 1988).Thymelaeaceae family consists of species that possess toxic, irritant or carcinogenic principles which affect animals and humans (Rajarajeshwari *et al.*, 2013).The toxic effect of the members of this family is due to polyfunctional diterpenoid esters of daphnane, tigliane and 1-alkyldaphnane type (Beaumont *et al.*, 2001).*Gnidia* is the largest genus in the Thymelaeaceae family with 140-160 species found growing in form of perennial herbs, shrubs, under-shrubs and small trees (Beaumont *et al.*, 2009; Levyns, 1950). The bark of the species in the genus *Gnidia* varies from smooth to rough texture with or without lenticels. Thegenus elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex (Rajarajeshwari *et al.*, 2013). Despite the many active compounds in the genus *Gnidia*, there is scanty information about their activities against stored insect pests.

2.6.1.2 Ethno-medicinal information on the genus *Gnidia*

The various species of *Gnidia* have been used in traditional medicine to manage various conditions in humans and as insecticides (Kupchan *et al.*, 1976; Sohni *et al.*, 1994; Ferrari *et al.*, 2000; Munkombwe *et al.*, 2003). Some of the uses are summarised in Table 2.1.

Table-2.1: Ethno-medicinal uses of some common *Gnidia* species

Species	Plant part	Uses	References
Gnidia buchananii	Roots	Bronchitis, abdominal pains	Kokwaro, 2009
Gnidia capitata,	Leaves	Relieves toothache, asthma, earache & constipation	Philander, 2011
Gnidia cuneata	Roots	Treats snake bite and relieves	Huchings & Staden, 1994
Gnidia glauca			
	Whole plant	Wound healing, viral infection	Kareru *et al.,* 2006:
		treatment.	Teklehaymanot & Gidday, 2007
		Molluscicide and pesticide in paddy fields	Vinayaka *et al.,* 2007
Gnidia gymostachya	leaves	Analgesic	Hutchings & Staden, 1994
Gnidia glabra	Roots	Laxative and emetic	Peterson, 1958
Gnidia involucrata	Leaves	Insecticide, insect repellent antimalarial	Berhan *et al*., 2006
Gnidia krausianna	Roots	Fish poison, insecticide	Roger, 2009
		Post harvest protection	Kosini & Nukunine, 2017
Gnidia latifolia	Roots	Purgative, antifeedant	Kiptoon *et al*., 1982
Gnidia polycephala	Roots	Insecticide, insect repellent & antimalarial,	Berhan *et al.,* 2006
		Treatment of tuberculosis, wound healing	Hedberg and Staugart, 1989
Gnidia stenophylla	Roots	Remedy for malaria, leprosy syphilis and gonorrhoea	Ashenafi *et al.,* 2007;
	Leaves	Treatment of malaria and rabies	Assefa *et al.,* 2009.
Gnidia subcordata	Leaves	Hypoglycaemic, skin disease treatment.	Ziyyat *et al*., 1997
		Insect pest control	Bellakhdar, 1997
Gnidia stenophylla	Roots	Treatment of syphilis and gonorrhoea	Tilahum *et al.,* 2017
			Mulungu *et al.,* 2011

Though some of the species in the *Gnidia* genus have been used traditionally as insecticides and as post-harvest grain protectants, the use of most of these species and more specifically *Gnidia subcordata* in insect pest control have not been scientifically validated.

2.6.1.3: Botanical description of *Gnidia subcordata*

Gnidia subcordata, a species in the genus *Gnidia*, is a shrub, up to 4 m tall, with slender branches and purple-grey outer bark that is smooth. The leaves are opposite, simple and entire and flowers

are greenish-white, white or cream with a cylindrical calyx tube. Its fruits are, small and enclosed by base of the calyx tube (Brinks, 2009). It grows at 1400–2400 m altitude above mean sea level, in dry evergreen forests, acacia woodlands and wooded grasslands (Brinks, 2009).

Plate 3: *Gnidia subcordata* Meisn. Aerial parts
(Source: Calvinsmit)

2.6.1.4 Biological activities of the species in genus *Gnidia*

Extracts and essential oils of different parts of the species in the genus *Gnidia* were shown to exhibit various biological activities. Hexane and chloroform extracts of dried bark of *Gnidia glauca* exhibited moderate mosquito parricidal activity, whereas hexane, chloroform and methanol extracts of fresh bark of the plant showed superior parricidal activity against second in-star larvae of *Aides aegyptus* (Ghosh *et al.*, 2015) and approximately 5 g/kg, of the extracts hindered adult emergence (Ghosh *et al.*, 2015). In another experiment, the hexane, acetone and methanol extracts from *Gnidia kraussiana* Meisn (Thymelaeaceae), each at dosages of 0.2 and 1 ml/50 g grains (1g/kg and 5g/kg grains), were evaluated for toxicity against *Callosobruchus maculatus* (F.). There was

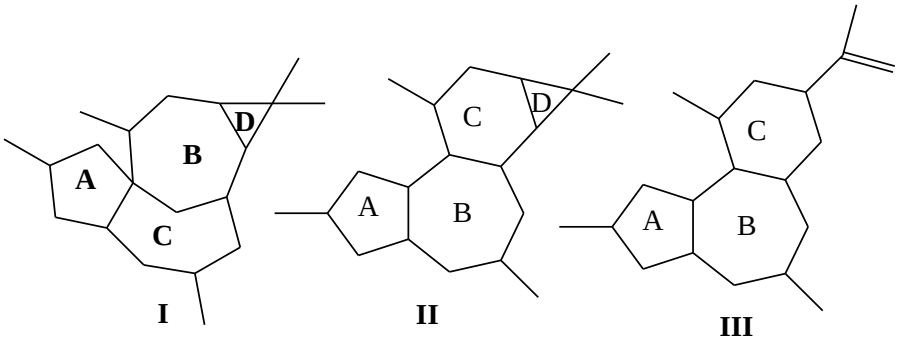
no adult survival recorded in grains treated with hexane extract at 5 g/kg dosage within 2 days' exposure (Kosini & Nukunine, 2017). In the insecticidal activity test for *Daphne gnidium* leaves against *Sitophilus zeamais*, a 3.5% methanol leaf extract caused 66% mortality. The methanol extract at 5% concentration, led to 72% mortality, while 6.5% methanol extract led to 81% mortality of adult *Sitophilus zeamais* (Conceição *et al.*, 2010).Conceição*et al*. (2010) in the experiment used *Daphne gnidium* leaf extracts against *S.zeamais* and *P. truncatus* and not *Gnidia subcordata* crude leaf extracts. It is therefore not established whether crude leaf extracts from *G. subcordata* would be active against *S. zeamais* and *P. truncatus* for information about insecticidal activities of *G. subcordata* is scanty.

2.6.1.5 Phytochemistry of the genus *Gnidia*

Phytochemical studies on some *Gnidia* species led to isolation of diterpenes, triterpenes, coumarins, phenyl glycosides, lignans and sterols. Some of these compounds exhibited diverse biological activities while some were not tested for their activities.

2.6.1.5.1 Diterpenes isolated from *Gnidia* species and their biological activities

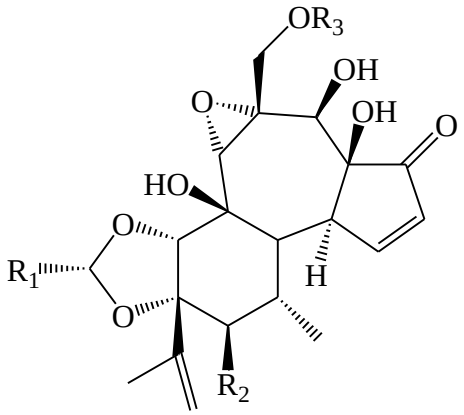
In previous phytochemical analysis of the *Gnidia* species, diterpenes of ingenane, tigliane and daphnane types were isolated (Figure 1). Ingenanes are highly oxygenated tetracyclic diterpene esters, whose skeleton is based on 5/7/7/3 tetracyclic system **(I)** (Apppendino, 2016). They possess potent tumour-promoting properties (Rigby *et al.*, 1986). Tiglianes are tetracyclic compounds found in plants in the form of polyhydroxyl diterpenes. The compounds have a skeleton based on 5/7/6/3 tetracyclic ring system **(II)**. Daphnanes are 5/7/6 tricyclic diterpenes which is the basis of their carbon skeleton **(III)** (Jin *et al.*, 2019)



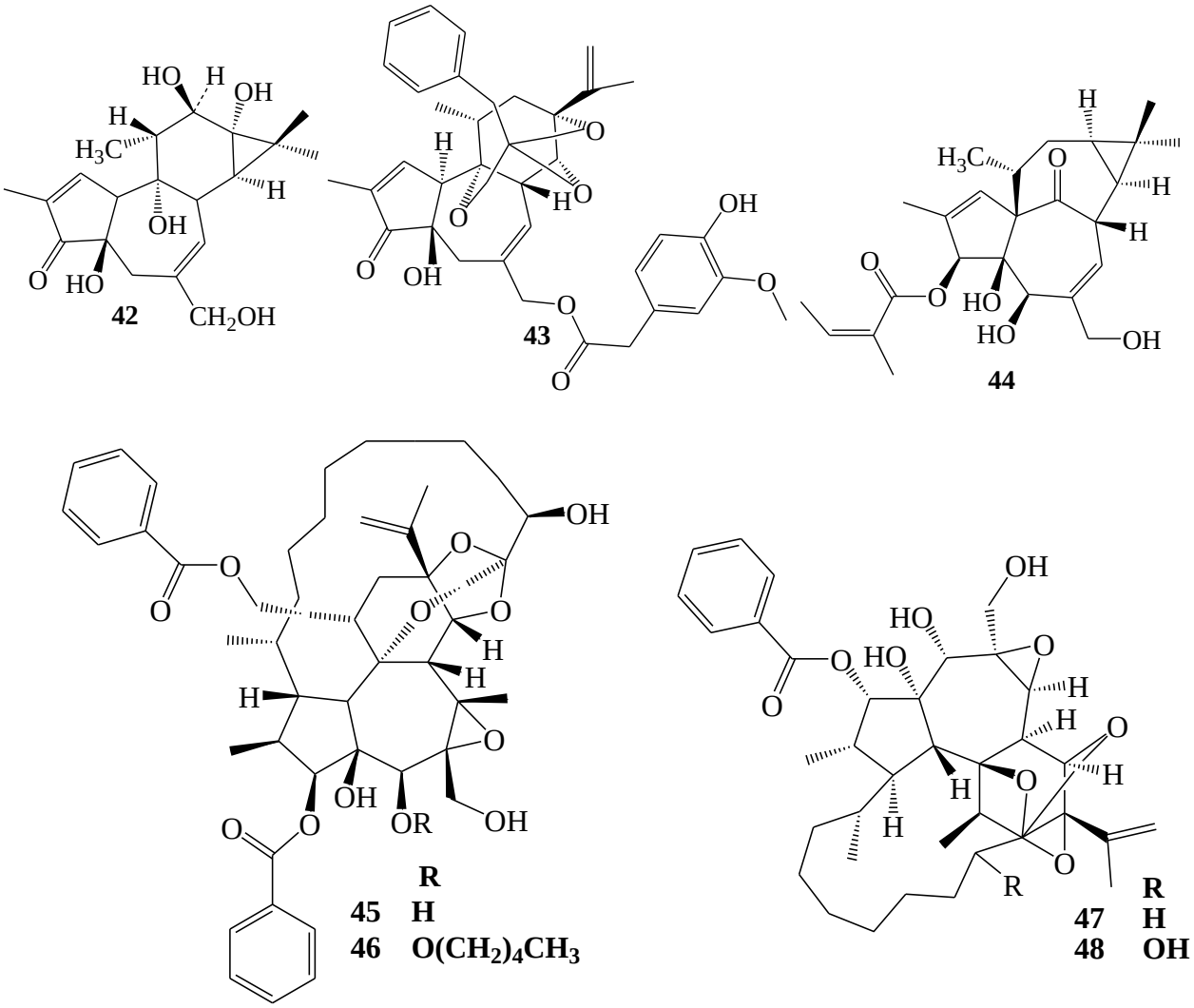
Figures 1: The ingenane (**I**), tigliane (**II**) and daphnane (**III**) ring systems

Among the daphnane type diterpenes previously isolated from the *Gnidia* species, daphnetoxin **(26)** isolated from *G. polystachya* (Fuller *et al.*, 1981) exhibited anticancer activity (Diogo *et al.*, 2009), mezerein **(27)** from *G. kraussiana* (Evans & Soper, 1978) showed piscicidal activity (Sakata *et al.*, 1971) while huratoxine **(28)** and12β-hydroxydaphnetoxine **(29)** from *G. polystachya* (Fuller *et al.*, 1981) showed inhibitory effects on interleukin 1 (Yesilada *et al.*, 2001). Acetoxyhuratoxine **(30)** and kirkinine **(31),** which are neurotrophic (He *et al.*, 2000); gnididin **(32),** gniditrin **(33)** and gnidicine **(34)** which are antileukemic were all isolated from *G. kraussiana* (Fujita & Nagao, 1977). Gnidilatin **(35)** and gnidilatidin **(36)** isolated from *G. kraussiana* (Borris & Cordell, 1984) exhibited piscicidal and antileukemic activities (Fujita & Nagao, 1977; Wang *et al.*, 1981). Gnidilatin-20-palmitate **(37)** and gnidilatidin-20-palmitate **(38)** isolated from *G. latifolia* showed antileukemic activity (Fujita & Nagao, 1977). Exoecariatoxin **(3)** obtained from *G. kulprantus* demonstrated piscicidal and insecticidal activities *(*Bala *et al.*, 1999). Gnidiglaucin **(39)** from *G. glauca* (Kupchan *et al.*, 1976 a), montanin **(40)** from *G. kraussiana* (Borris & Cordell, 1984) and genkwadaphnin **(41)** from *G. burchellii* (Pieterse, 1971) showed antileukemic activity (Adolf & Hecker, 1977). Tigliane type diterpenoids isolated from *G. kulprantus* **(**Bala *et al.*, 1999) were phorbol **(42)** and resiniferotoxin **(43)** which exhibited antileukemic, inflammatory and cytokines inhibitory activity (Fujita & Nagao, 1977; Yesilada *et al.*, 2001). Ingenane type diterpene was ingenol **(44)** isolated

from *G. kraussiana* (Borris & Cordell, 1984).Other daphnane diterpenoids were; gnidimacrin (**45)** and gnidimacrin-20-palmitate (**46)** isolated from *Gnidia subcordata* (Kupchan *et al***.,** 1976b**),** gnilamacrin (**47**) and kraussianin **(48)** isolated from *G. kraussiana* (Borris & Cordell, 1984). Compounds **43-48** showed antileukemic activity (Bala *et al.*, 2000; Kupchan *et al***.,** 1976a, b).



No.	R_1	R_2	R_3
26	C_6H_5	H	H
27	C_6H_5	$CO(CH=CH)_2C_6H_5$	H
28	$(CH=CH)_2(CH_2)_8CH_3$	H	H
29	C_6H_5	OH	H
30	$(CH=CH)_2CH)_8CH_3$	$OCOCH_3$	H
31	$(CH=CH)_3(CH_2)_2CH_3$	$OCOCH_3$	H
32	C_6H_5	$COCH=CHC_6H_5$	H
33	C_6H_5	$CO(CH=CH)_3(CH_2)_2CH_3$	H
34	C_6H_5	$COCH=CHC_6H_5$	H
35	$(CH_2)_8CH_3$	COC_6H_5	H
36	$(CH=CH)_2(CH_2)_4CH_3$	COC_6H_5	H
37	$(CH_2)_8CH_3$	COC_6H_5	H
38	$CH=CH)_2(CH_2)_4CH_3$	H	H
39	$(CH_2)_8CH_3$	H	H
40	$CH_{11}H_{23}$	H	H
41	C_6H_5	$COCH_3$	H



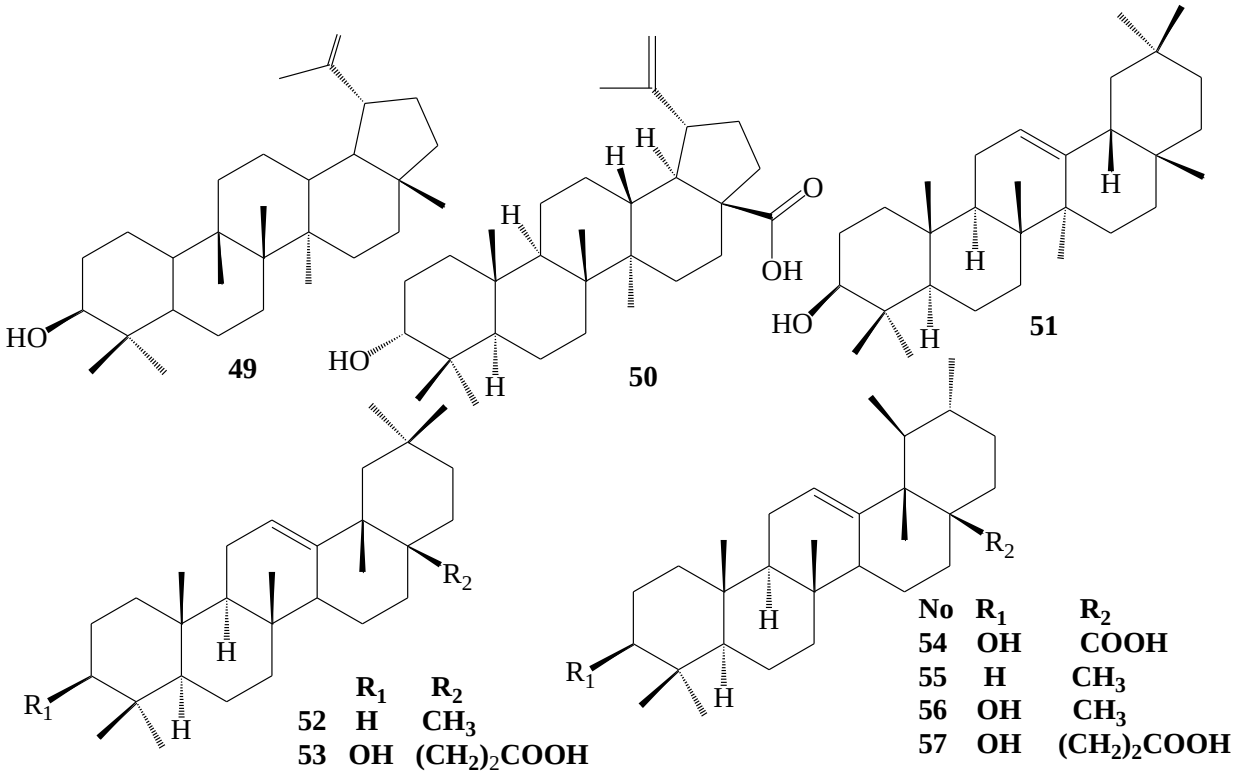
All the diterpenoids isolated from the various *Gnidia* species exhibited biological activities against different organisms including insect pests. Information on isolation of compounds from crude leaf extracts of *G. subcordata* is however lacking. It is not determined whether diterpenoids would be isolated from crude leaf extracts of *G. subcordata* and whether such diterpenoids would have insecticidal activities against *S. zeamais* and *p. truncatus.*

2.6.1.5.2 Triterpenoids isolated from *Gnidia* species and their biological activities

Triterpenoids are secondary metabolites with 30 carbon skeletons identified from terrestrial and marine living organisms (Mahato *et al*., 1992). Most of triterpenic skeletons are tetracycles

containing three six-membered and one five-membered rings and pentacycles, either with four six membered and one five-membered rings or five six-membered rings (Sandjo & Kuete, 2013).

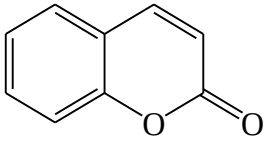
In the phytochemical analysis of the species in the genus *Gnidia*, lupane, ursane and oleanane type triterpenoids were isolated. The lupane type triterpenoids; lupeol **(49)** which showed anti-inflamatory, antiprotozoal and anticancer activities (Gallo & Sarachine, 2009) and betulinic acid **(50)** which showed anti-inflamatory activity (Xinxin *et al.,* 2017) were isolated from *Gnidia polycephala* (Munkombwe *et al*., 2003). β-amyrin **(51),** an anti-inflamatory (Okoye *et al.,* 2014), 12-oleanene **(52)** an antitumor (Sun *et al.*, 2006), oleanolic acid **(4)** which showed antifeedant activity (Malladhavani *et al.*, 2003**)** and 28-methylcorboxyolean-12-en-3β-oic acid **(53)** are oleanane type triterpenoids isolated from *G. glauca* (Sannabommaji *et al.,* 2018).Ursolic acid **(54)** an antifeedant (Mallavadhani*et al.*, 2003**),** 12-ursene **(55)** which demonstrated antitumor activity (Sun *et al.*, 2006)**,** α-amyrin **(56)** an anti-inflamatory (Okoye *et al.,* 2014) and 28-methylcorboxyurs -12-en-3α-oic acid **(57)** whose activity has not been determined are among the ursane type triterpenoids isolated from *G. glauca* (Sannabommaji *et al.*, 2018).



Among the triterpenoids isolated from the species in the genus *Gnidia*, Malladhavani *et al* (2003) demonstrated that oleanolic acid and ursolic acid isolated from *G. glauca* had antifeedant activities. The compounds were however not tested for their contact toxicity activities. For a plant such as *G. subcordata* that is traditionally used in insect pest control, there are no reports on isolation of triterpenoids from its crude leaf extracts. It is thus not established whether such isolation would yield triterpenoids with insecticidal activities against *S. zeamais* and *P. truncatus.*

2.6.1.5.3 Coumarins isolated from *Gnidia* species and their biological activities

Coumarins are a group of polyphenolic compounds belonging to the benzopyrone class, which consists of benzene ring joined to a pyrone ring (1-benzopyran-2-one or coumarin **(58**) **(**Lacy & O'kennedy, 2004)



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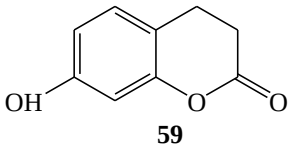
In the genus *Gnidia*, coumarins were found in form of simple coumarins, as dimmers and trimmers or as coumarin glycosides, flavone-coumarins and coumarinolignans. Umbeliferone **(59)** isolated from *G. polycephala* (Munkombwe, 2003) showed inhibitory activity against elongation of seed radicles (Morikawa *et al.*, 2011), lasiocephalin **(60)** from *G. polycephala* (Munkombwe, 2003) tested positive for the toxicity activity against insects (Khajja *et al.*, 2011). Coumarinolignin **(61),**dicumarol **(62),** gerberinol **(63),** daphnetin **(64)** and daphnetin-8-β-D-glucoside **(65),** isolated from *G. polycephala* (Zhang *et al.*, 2007; Shen & Bryan, 1985), exhibited immunosuppressive activities(Song *et al.*, 2014).Daphnoretin **(66)** which has antiviral activity (Ho *et al.*, 2010) and euphorbetin **(67**) an anticoagulant(Zhou *et al.*, 2009) were isolated from *G. involucrata* (Ferrari *et al.*, 2000)**.** Lasioerin **(68),** 7-acetoxydaphoretin **(69),** edgeworthin **(70)** and gnidicoumarin **(71)** were also isolated from *G. involucrata* (Ferrari *et al.*, 2003: Rajarajeshwari *et al.*, 2013) but their biological activities are not documented.



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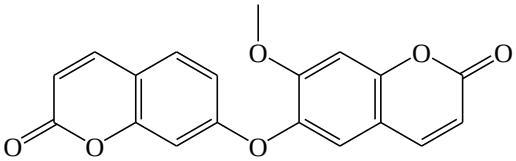

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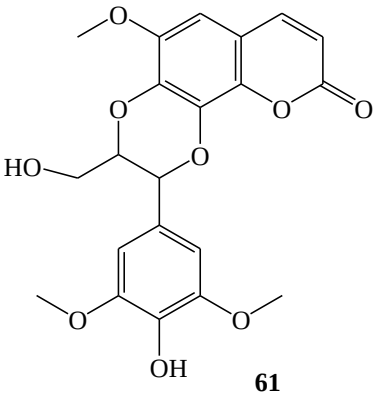

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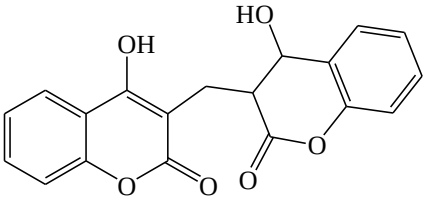

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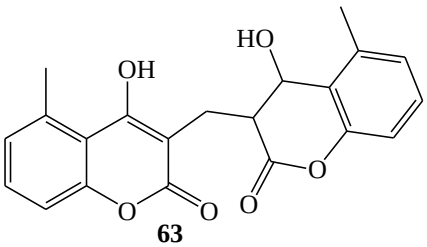

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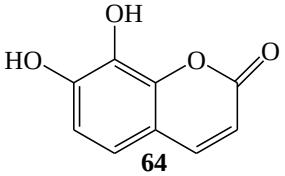

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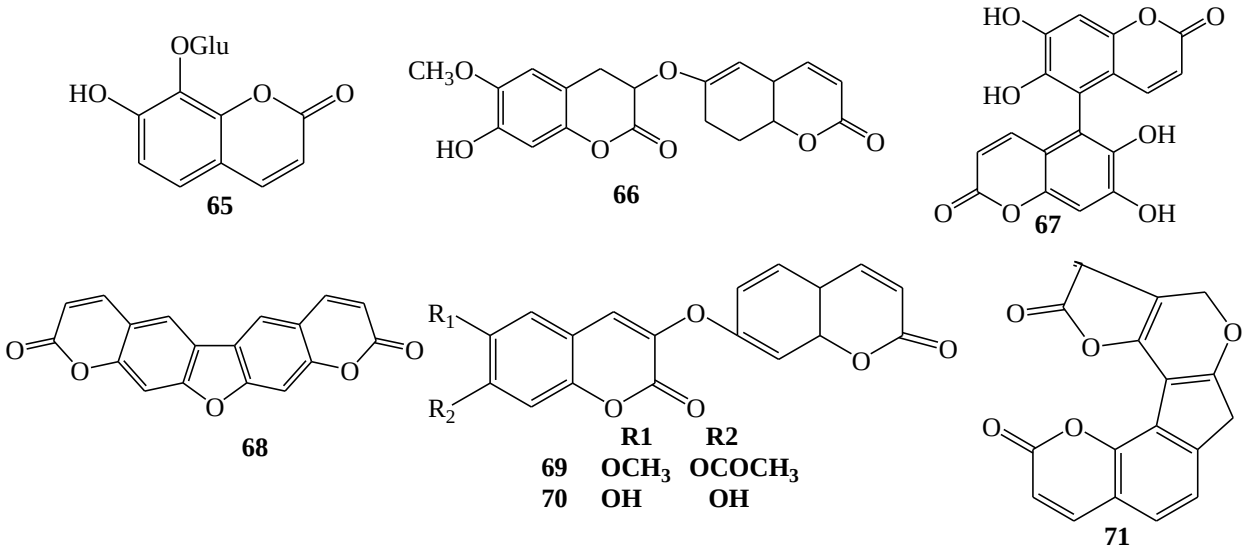
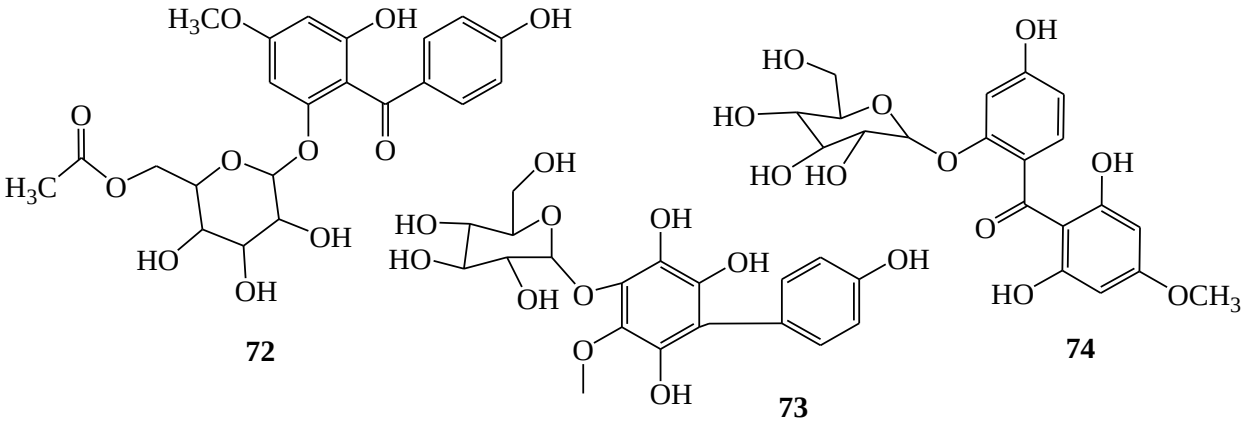


Some of the coumarins isolated from the *Gnidi*a species were shown to be biologically active. Biological activities of some of the coumarins are however not known. Among the coumarins, Khajja *et al*., (2011) found lasiocephalin from *G. polycephala* to have contact toxicity activity. The coumarin was however not tested for the antifeedant activity. Isolation of coumarins from *G. subcordata* is not documented. It is not known whether isolation of compounds from crude leaf extracts of the plant may yield coumarins with insecticidal activities against *S. zeamais* and *P. truncatus*.

2.6.1.5.4 Benzophenone glycosides isolated from *Gnidia* species and their biological activities

Benzophenone glycosides isolated from *Gnidia* species included; mahkoside **(72)** which is cytotoxic (Zhang *et al.*, 2007), 2, 3, 4', 5, 6-pentahydroxy benzophenone-4-C-glucoside **(73)** and2, 4', 6-trihydroxy-4-ethoxybenzophenone-2-O-glucoside **(74)** (Ferrari *et al.*, 2003: Rajarajeshwari *et al.*, 2013), whose activities against insect pests have not been reported. All the compounds were all isolated from *G. involucrata*



Some species in the genus *Gnidia*, elaborates benzophenone glycosides some of which are biologically active, having cytotoxic activity (Zhang *et al.*, 2007). There is no documentation on phenolic glycosides from *G. subcordata* and activities of some of the compounds are not documented. It is therefore not determined whether benzophenone glycosides can be isolated from crude leaf extracts of *G. subcordata* and whether the isolated compounds would have insecticidal activities against *S. zeamais* and *P. truncatus*.

2.6.1.5.5 Flavonoids isolated from *Gnidia* species and their biological activities

Flavonoids are a group of polyphenolic compounds with two substituted benzene rings connected by the chain of three carbon atoms and an oxygen bridge, (Figure 2**)** (Reynaud & Lussignol, 2005)

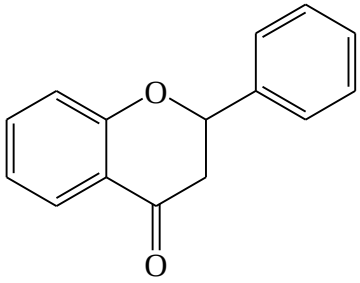
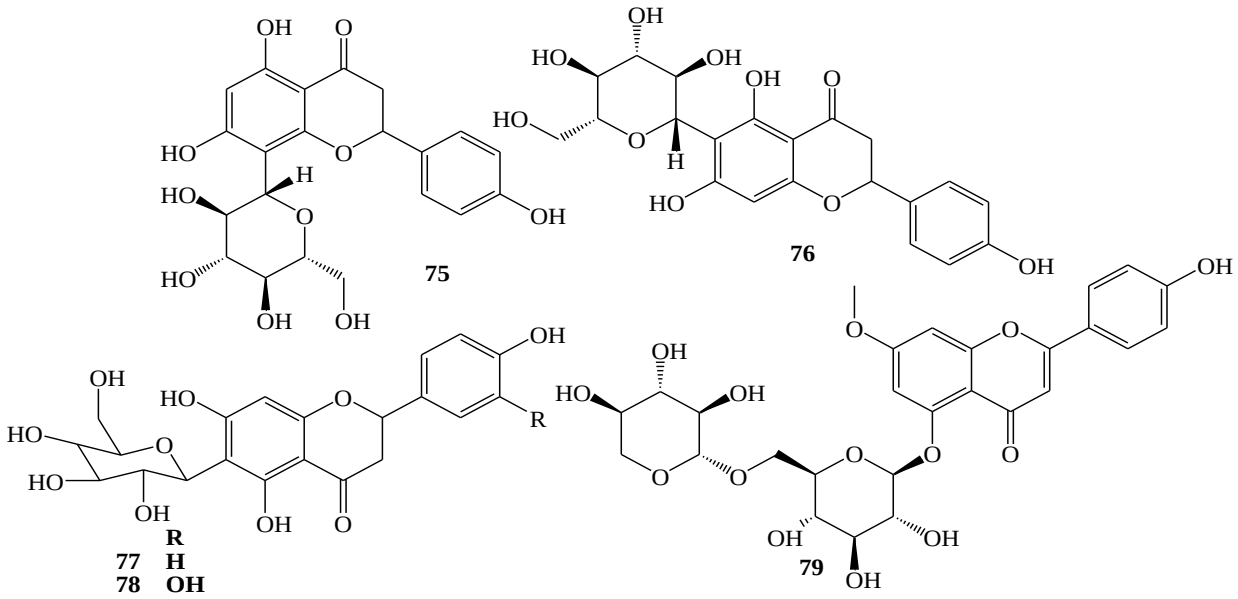
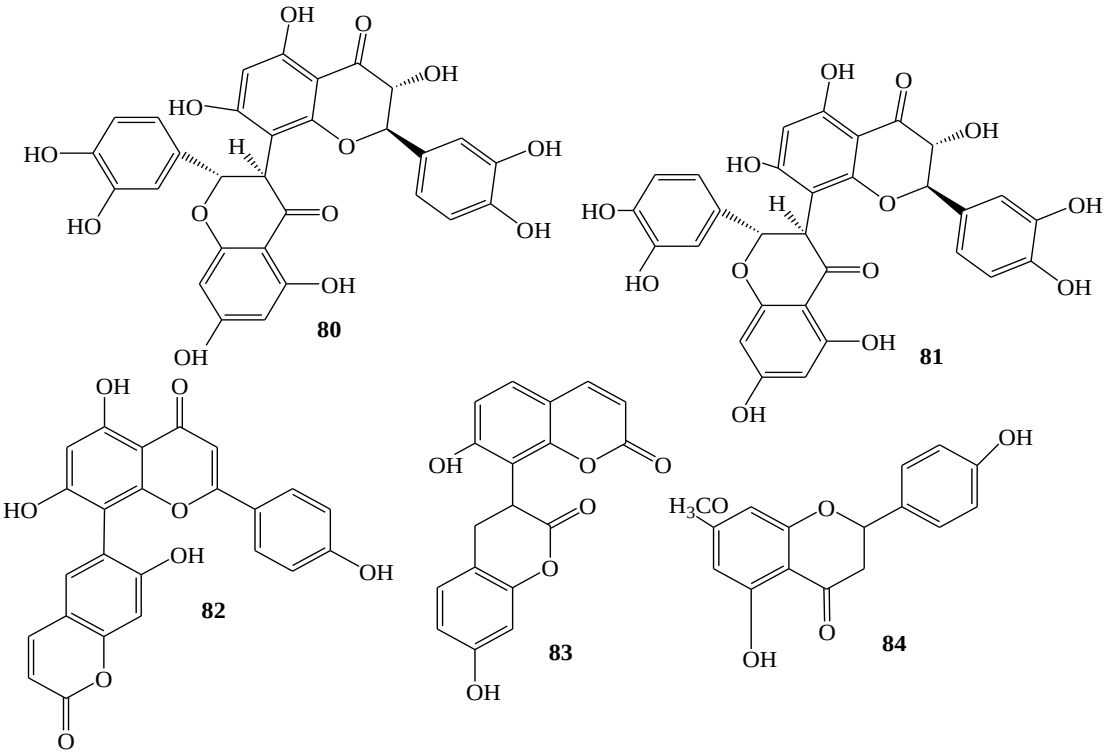


Figure 2: Basic structure of flavonoids

Some of the flavonoids previously isolated from the *Gnidia* species possess various biological activities. Vitexin **(75),** an α-glucosidase inhibitor (Yao *et al.*, 2011), isovitexin **(76)** an antioxidant (Delazae *et al.*, 2006), isoorientin **(77)** an antioxidant and antimicrobial (Ulubelen *et al.*, 1986) were

isolated from *G. involucrata* (Ferrari *et al.*, 2000)**.** Mangiferin **(78)** another *G. involucrata* isolate has antioxidant, antiplasmodial, anti-amoebic, antibacterial and antifungal activities (Singh *et al.*, 2009) while the activity of yuankanin **(79),** also isolated from the same plant is not documented. Manniflavanone**(80**) an antioxidant (Achaya *et al.*, 2017), 6-(8''-umbeliferyl) apigenin **(81)** and8-(6''-umbeliferyl) apigenin **(82)** which are compounds consisting of a flavone and a coumarin moiety connected by C-C linkage, together with 7,7'-dihydroxy-3,8'-biscoumarin **(83)** were isolated from *G. socotrana* (Franke *et al.*, 2002). The latter three compounds have anticancer activity (Fajriah & Darmawan, 2016). Genkwanin **(84)** an antiplasmodial, antibacterial (Martini *et al.*, 2014; Kraft *et al.*, 2003) and antimicrobial (Ulubelen *et al.*, 1986) and astragalin **(85)** an anticancer, anti-inflammatory, antioxidant, neuroprotective, antidiabetic, cardioprotective and antiulcer (Riaz *et al.*, 2018), were isolated from *G. involucrata* aerial part (Ferrari *et al.*, 2000).There is no documentation on the activities of compounds such as Gnidia biflavonoid 4a **(86)** also from aerial parts of *G. involucrata* (Ferrari *et al.*, 2000) and kaempferol-3-(p-coumaroyl)-*O*-β- glucopyranoside **(87)** from *G. kraussiana* (Borris *et al.*, 1988).





The species in genus *Gnidia* elaborates many flavonoids, most of which are biologically active. These flavonoids were however mainly tested against micro-organisms and none was tested against insects. There is no information on whether these compounds would have any insecticidal activities. It has not been determined whether isolation of compounds from *G. subcordata* crude leaf extracts would lead to flavonoids that have insecticidal activities against *S. zeamais* and *P. truncatus.*

2.6.1.5.6 Lignans isolated from *Gnidia* species and their biological activities

Lignans are polyphenolic substances derived from phenylalanine via dimerization of substituted cinnamic alcohols known as monolignols, to a dibenzyl butane skeleton (Figure 3) with oxidative enzymes as catalysts (Axelson *et al.*, 1982)

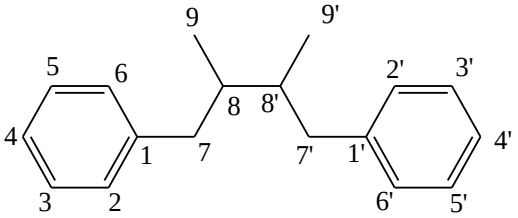
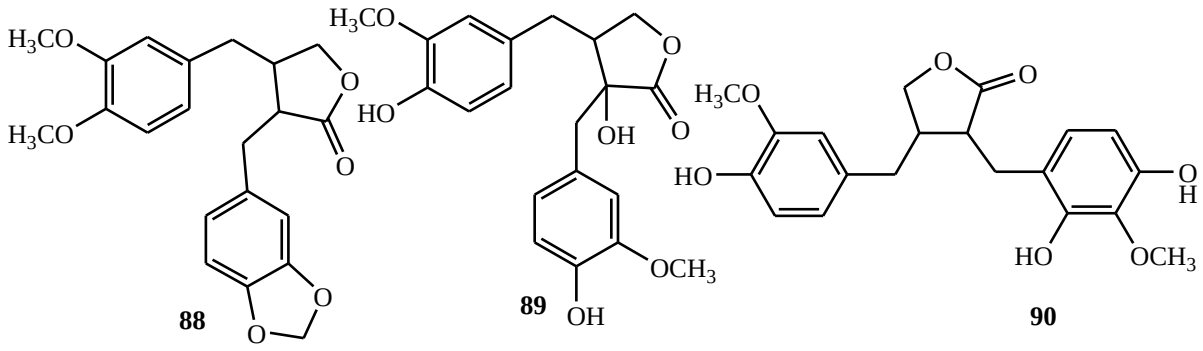


Figure 3: Dibenzyl butane skeleton

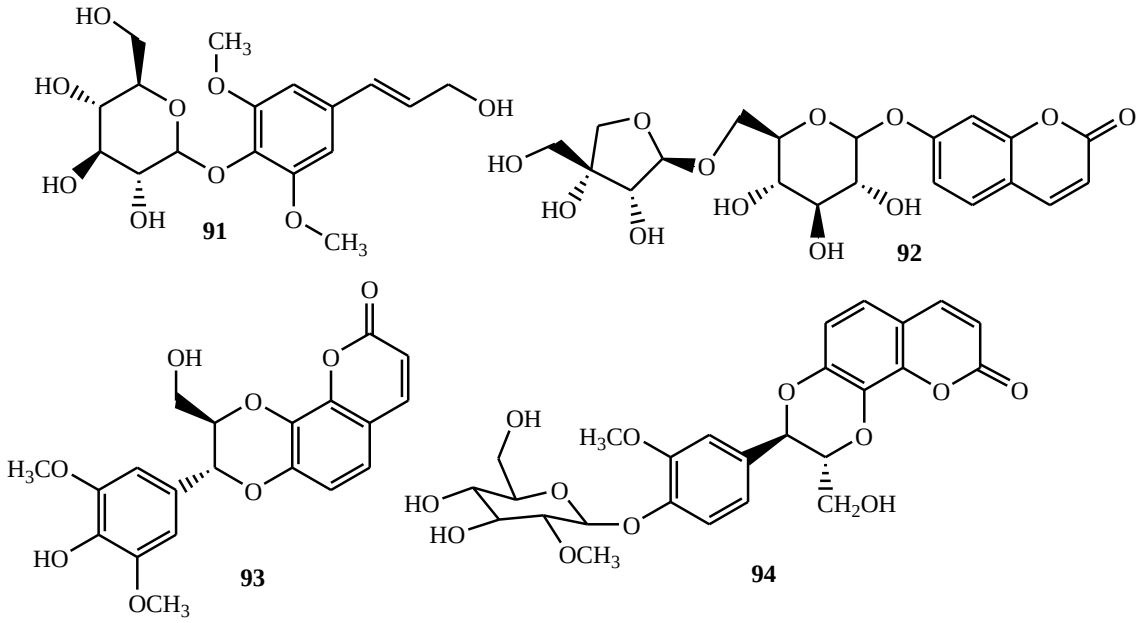
Lignans isolated from the *Gnidia* species were biologically active. Kusunokinin **(88)** and wikstromol **(89)** which showed anticancer activity (Rajachana *et al.*, 2013), were isolated from *G. latifolia* (Kupchan *et al.*, 1977). Gnidifolin **(90)** was isolated from *G. latifolia* (Bryan & Shen, 1978; Rajajeshwari *et al.*, 2013), but there are no reports about its biological activities.



Lignans isolated from the *Gnidia* species were biologically active (Rajachana *et al.*, 2013: Kupchan *et al.*, 1977). There are however no reports on lignans from *G. subcordata.* It is therefore not known whether isolation of compounds from *G. subcordata* crude leaf extracts would lead to lignans that have insecticidal activities against *S. zeamais* and *P. truncatus.*

2.6.1.5.7: Phenolic glycosides isolated from *Gnidia subcordata* and their biological activities

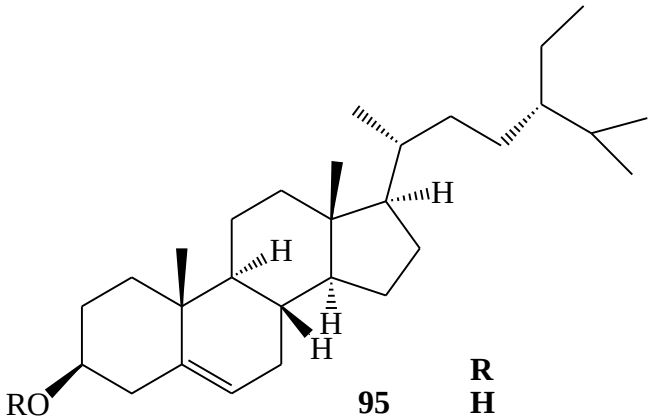
Syringin **(91),** an anti-inflamatory, antinociceptive, antiulcer and antioxidant (Rao *et al.*, 2015) and adicardin **(92)** that has an anti-chronic renal failure activity (Wei *et al.*, 2009) were reported in *G. Polycephala* **(**Munkombwe *et al.*, 2003**).** Daphneticin **(93)** and daphneticin-4-*O*-α-D-glucopyranoside **(94)** were isolated from *G. latifolia* (Bryan & Shen, 1978; Rajajeshwari *et al.*, 2013), but there are no reports about their biological activities



Some of the few phenolic glycosides isolated from the *Gnidia* species are biologically active with activities such as anti-inflamatory, antinociceptive, antiulcer and antioxidant (Rao *et al.*, 2015), however there are no reports on isolation of these compounds from *G. subcordata*. As to whether phenolic glycosides with insecticidal activities against *S. zeamais* and *P. truncatus* could be isolated from *G. subcordata* is yet to be established.

2.6.1.5.8 Sterols isolated from species in genus *Gnidia* and their biological activities

β- sitosterol **(95)** used for treatment of heart diseases, tuberculosis and rheumatoid arthritis and also exhibited anticancer and antidiabetic activities (Saeidnia *et al.*, 2014) and β-sitosterol 3-*O*-β-D-glucoside **(96),** that exhibited antigastro-ulcerative activity (Xiao *et al.*,1992), were isolated from *G. kraussiana* (Peterson, 1959).

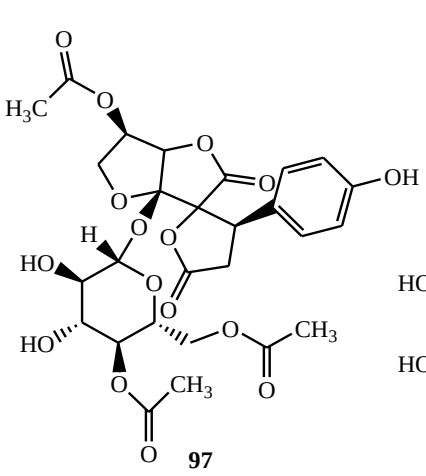


	R
95	**H**
96	**Glu**

Both sterols isolated from *G. kraussiana* are biologically active. It is therefore not known whether fractionation of *G. subcordata* would lead to isolation of biologically active sterols against *S. zeamais* and *P. truncatus.*

2.6.1.5.9 Spiro-bis-γ-lactones isolated from species in genus *Gnidia*

Compounds with the rare spiro-bis-γ-lactone structure were first reported in the family of Thymeleaeceae by Franke *et al* (2002), who isolated tetraacetoxy-viburnolide A **(97),** 4', 6'-diacetoxy-12-coumaroylviburnolide A **(98)** and4', 6'-diacetylviburnolide A **(99)** from *G. socotrana* leaves. There are however no reports on theirbiological activities.



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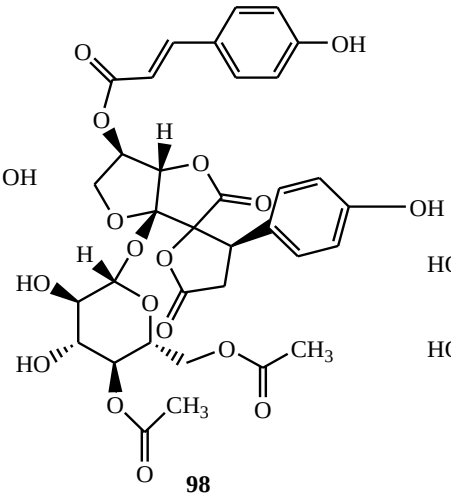

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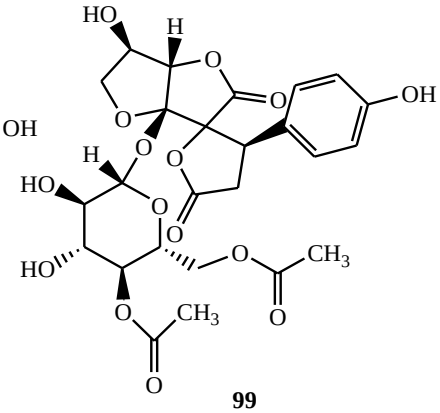

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Spiro-bis-γ-lactones though isolated from *G. socotrana*, have not been tested for their biological activities. There are no reports on isolation of these compounds from other *Gnidia* species. It is not known whether spiro-bis-γ-lactones can be isolated from leaf extracts of *G. subcordata* and whether the compounds would have insecticidal activities.

2.6.2 The genus *Ocimum*

The genus *Ocimum*, a member of Lamiaceae family is made of almost 200 species consisting of herbs and shrubs (Charles & Simon, 1990) which have tremendous medicinal potentials and are mostly native to the tropical and warm temperate regions including Asia, Africa and South America (Narwal *et al.*, 2011; Kashyap *et al.*, 2011).

2.6.2.1 Ethnomedicinal information on the genus *Ocimum*

Species in the genus *Ocimum* are used traditionally in control of various diseases and as insecticides (Bello, 2006; Sakkir *et al*., 2012; Caamal-Herrera *et al*., 2016; Upadhyay, 2017). Some of these uses are summarised in Table 2.2.

Table 2.2: Ethnomedicinal uses of some common species of *Ocimum* species

Species	Plant part	Uses	Reference
Ocimum forskolei	Leaves	Mosquito repellent, getting rid of fleas	Holme, 1999
Ocimum forskolei	Leaves	Treatment of headache, fever, ear ache, relief for insect bites	Dekker *et al.*, 2011
		Mosquito repellent	Sakkir *et al.*, 2012
Ocimum grattissimum	Leaves	Treatment of fever, coughs and diarrhoea	Elsaid *et al.*, 1969
		Treats stomach problems	Aiyeloja & Bello, 2006:
		Post-harvest protection	Ngamo *et al.*, 2007;
Ocimum	Whole plant	Mitigates coughs, measles, abdominal	(Kokwaro, 2009).
Kilimandscharicum		pains, diarrhoea, insect repellent against mosquitoes and storage pest control	
	Leaves	Treatment of colds, coughs, abdominal pains, measles and diarrhoea.	Agrawal, 2017
		Grain protectants	Jembere *et al.*, 1995
Ocimum micranthum	Leaves	Mosquito repellent and in grain storage	Caamal-Herrera *et al.*, 2016
Ocimum sanctum	Leaves	Treatment of bronchitis, influenza and asthma. Provides relief in cold, sneezing nose, cough, malaria, and dengue	Upadhyay, 2017
		Treatment of fevers, cough & cold	Joshi, 2017
Ocimum suave	Leaves	Stored grain protection and driving away insects at night	Githinji & Kokwaro, 1993

Though the species in genus *Ocimum* have many traditional applications, most of these applications have not been scientifically validated. There is therefore only scanty scientific knowledge to back the traditional use of *O. kilimandscharicum* in control of *S. zeamais* and *P. truncatus*

2.6.2.2 Botanical description of *Ocimum kilimandscharicum* Guerke

Ocimum kilimandscharicum commonly known as camphor basil is one of the species in the genus *Ocimum.* It is an economically important medicinal perennial herb that is widely distributed in East Africa (Soumen *et al.*, 2010). The plant is an aromatic shrub which is easily recognized by its shrubby habit, growing up to a height of eight feet (Agrawal, 2017). It has oblong or ovate green

coloured leaves, oppositely arranged with a pubescent leaf surface, about 3-7 cm in length including petioles which are 4 to 12 mm long, 1 to 2.5 cm wide, narrow at base and deeply serrated (Tanuj & Vijay, 2017; Gill *et al.,* 2012; Kashyap *et al.,* 2011). Stems are brownish green, much branched, woody with epidermis sometimes peeling off in strips below, arising from a large woody rootstock (Paton, 1992). It bears pale yellow, white or purple, hermaphrodite flowers in clusters with long flower stems up to 18 inches long (Kashyap *et al.,* 2011; Sonia *et al.,* 2012; Joshi, 2013). The fruits are in clusters with seeds that are very small, black, oval shaped and about 1mm in the middle and 2mm long (Narwal *et al.,* 2011; Kashyap *et al.,* 2011).

Plate 4: Aerial part of *Ocimum kilimandscharicum*
(Courtesy: Calvinsmit)

2.6.2.3 Biological activities of species in genus *Ocimum*

Previous studies showed that essential oils and extracts of *O. kilimandscharicum* were biologically active.The antimicrobial activities of flower and leaf oils of *O. kilimandscharicum* were assayed against two Gram-positive, seven Gram-negative and one standard bacterial strains using agar-disc diffusion and micro dilution-broth methods. The mean zones of inhibition (I Z) ranged between 7.3 ± 1.5 and 15.1 ± 1.5 mm for the flower oil and 9.3 ± 1.7 and 24.7 ± 1.0 mm for the leaf oil at a concentration of between 10mg/mL and 0.078 mg/mL (Lawal *et al.,* 2014). The minimum

inhibitory concentration (MIC) values varied between 2.5 mg/mL and 10 mg/mL flower oil which was weak to moderate activity and between 0.16 mg/mL and 5.0 mg/mL leaf oil which was better activity (Lawal *et al.,* 2014). In the choice assays conducted by exposing *Helicoverpa armigera* larvae to *O. kilimandscharicum* and tomato leaves, *O. kilimandscharicum* leaves deterred larval feeding and when the larvae were fed on *O. kilimandscharicum* leaves, the average body weight decreased and mortality of the larvae increased (Singh *et al.,* 2014). Dried ground leaves and essential oil of *O. kilimandscharicum* in doses of 25.0 g leaves and 0.3 g essential oil per 250 g grain (maize or sorghum) killed 100% of *S. zeamais and Rhyzopertha dominica* in 48 hours (Jembere *et al.,* 1995).

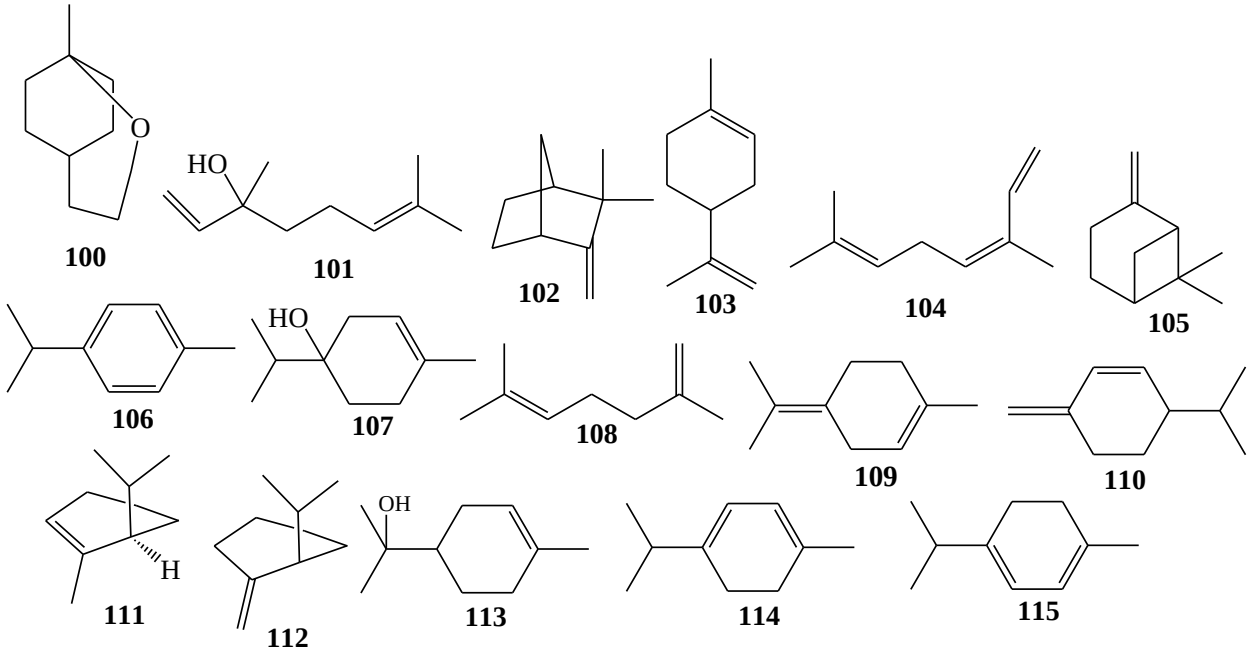
Lawal (2014), Singh *et al*., (2014) and Jembere *et al*., (1995) carried out their tests using leaf powder and essential oils of *O. kilimandscharicum* against the various insects. Crude leaf extracts of *O. kilimandscharicum* were however not used in these experiments. There are no reports on the insecticidal activities of *O. Kilimandscharicum* crude leaf extracts against *S. zeamais* and *P. truncatus.* It can therefore not determined whether *O. Kilimandscharicum* crude leaf extracts would have better insecticidal activities against *S. zeamais* and *P. truncatus.*

2.6.2.4Compounds from *Ocimum* species and their biological activities

Previous phytochemical investigation of *Ocimum* species yielded monoterpenes, sesquiterpenes, triterpenes, phenolic acids and flavonoids (Lawal *et al.,* 214; Hakkim *et al.,* 2008; Singh *et al.,* 2014). Some of these compounds were biologically active (Farre-Armengol *et al*., 2017; Sieniawska *et al.,* 2018; Chen, 2016).

2.6.2.4.1 Monoterpenoids from *Ocimum* species and their biological activities

Phytochemical analysis of seed and flower volatile oils of *O. kilimandscharicum* revealed the presence of1, 8-cineole **(100)** an antioxidant (Ciftci *et al.,* 2011) linalool **(101)** which checks the growth of *Rhizoctonia solani* (Thakur *et al.,* 1989), camphor **(5)**, an insect repellent against *S. Zeamais* and *P. truncatus* (Jembere *et al.,* 1995) and also against *S. granarius* and *T. casterneum* (Obeng-Ofori *et al.,* 1998). Camphene **(102)** a hypolipidemic (Valianou & Hadzopoulou-Cladaras, 2016) and limonene **(103)** an antimicrobial compound (Rancic *et al.,* 2003). Other compounds isolated were: β-ocimene **(104)** a flower pollinator attractant (Padalia & Verma, 2011; Farre-Armengol *et al.,* 2017), *β*-pinene (**105)** an antimicrobial (da Silva *et al.,* 2012), p-cymene **(106**) an antioxidant (de Oliveira *et al.,* 2015), 4-terpeneol **(107)** an anticancer, antibacterial and antioxidant (Zengin & Baysal, 2014; Shapira *et al.,* 2016)**.** Also isolated from the same plant were; β-myrcene **(108)** an antioxidant (Ciftci *et al.*, 2011). α-terpinolene **(109)** an antioxidant and anticancer (Aydin *et al.,* 2013**),** and β-phellandrene **(110)** an antifungal (Zhang *et al.,* 2017; Kumar *et al.,* 2011). All these compounds were isolated from leaves, inflorescences and succulent stem essential oils, seed and flower volatile oils of *O. kilimandscharicum* (Lawal *et al.,* 2014; Obeng-Ofori *et al.,* 1998; Ntezurubanza *et al.,* 1984) and from leaves and flower essential oils of *O. basilicum* (Zekovic *et al.,* 2015).α-thujene (**111**) an antimicrobial and antioxidant (Kelen & Tepe, 2008), sabinene **(112)** an antibiotic(Carvalho *et al.,* 2017) andα-terpeneol (**113**) which had gastro-protective activity (Souza *et al.,* 2011) were all isolated from *O. kilimandscharicum* volatile oils (Singh *et al.,* 2014). α-terpenine **(114)** and γ-terpinene **(115)** were isolated from *Ocimum gratissimum* (Nguemtchouin *et al.,* 2013) and have antioxidant activities (Rudback *et a*l., 2012)

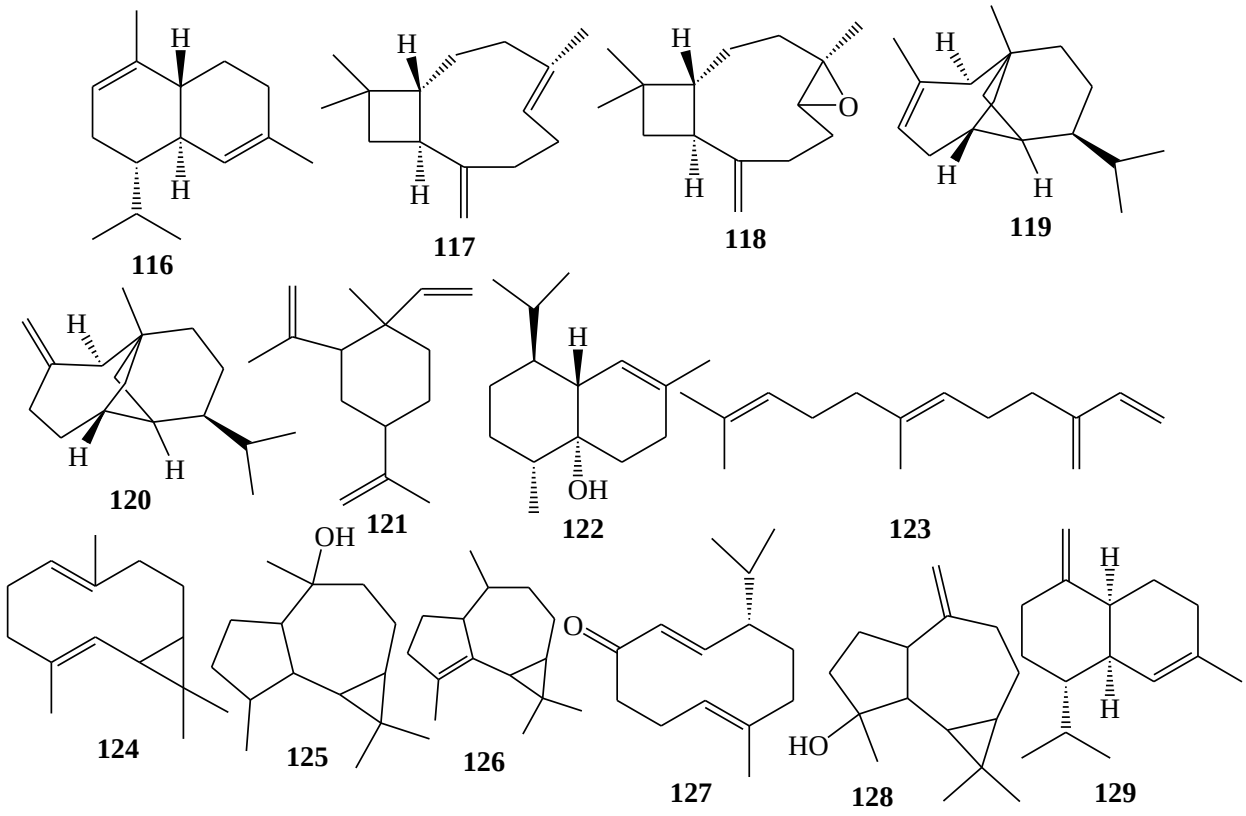


Essential oils and volatile oils from leaves, stems, seeds and flowers of *O. kilimandscharicum* contain monoterpenoids which are active against various microorganisms. Among these compounds, Jembere *et al*., (1995) found camphor to be active against *S. zeamais* and *P. truncatus*. It is not established whether new monoterpenenoids with insecticidal activities against *S. zeamais* and *P. truncatus* would be isolated from crude leaf extracts of *O. kilimandscharicum.*

2.6.2.4.2 Sesquiterpenoids from species in genus *Ocimum* and their biological activities

A variety of sesquiterpenoids were isolated from the species in genus *Ocimum* and some were biologically active. The isolated compounds include α-cadinene **(116),** β-caryophyllene **(117)** and β-caryophyllene oxide **(118)** with anticancer activity **(**Fidyt*et al.,* 2016**).** α-copaene **(119),** β-copaene **(120),** β-elemene **(121)** and cubenol **(122)** exhibited antimicrobial activity (Lang &Buchbauer, 2012; Martins *et al.,* 2015: Sieniawska *et al.,* 2018)**.** β-farnesene **(123)** had insect repellence and aphicidal activity (Zhang *et al.*, 2017) while bicyclogermacrene **(124)** and globulol

(125) showed antimicrobial activity (Tan *et al.*, 2008; Tabanca *et al.*, 2001). The biological activities of α-gurjunene **(126)** and germacrene D **(127)**are not documented although they were found as major components of essential oils that showed antimicrobial and antioxidant activities (Ei-Kalamouni *et al.*, 2017)**.** Spathulenol **(128)** and γ-muurolene **(129)** have immunomodulatory activity (Ziaei *et al.*, 2010; Singh *et al.*, 2014). Compounds **116-129** were isolated from *Ocimum basilicum* leaves and flower essential oils (Filip *et al.*, 2014)



The biological activities of sesquiterpenes in leaf essential oils of *Ocimum* species against microorganisms were established. Among these compounds Zhang *et al* (2017) found β-farnesene **(123)** from *O. basilicam* essential oils to have repellence and aphicidal activities. There are however no reports on insecticidal activities of sesquiterpenes in leaf extracts of *O. kilimandscharicum* against *S. zeamais* and *P. truncatus.* It is not been determined whether sesquiterpenes isolated in

leaf extracts of *O. kilimandscharicum* would have insecticidal activities against *S. zeamais* and *P. truncatus.*

2.6.2.4.3 Triterpenoids from *Ocimum* species and their biological activities

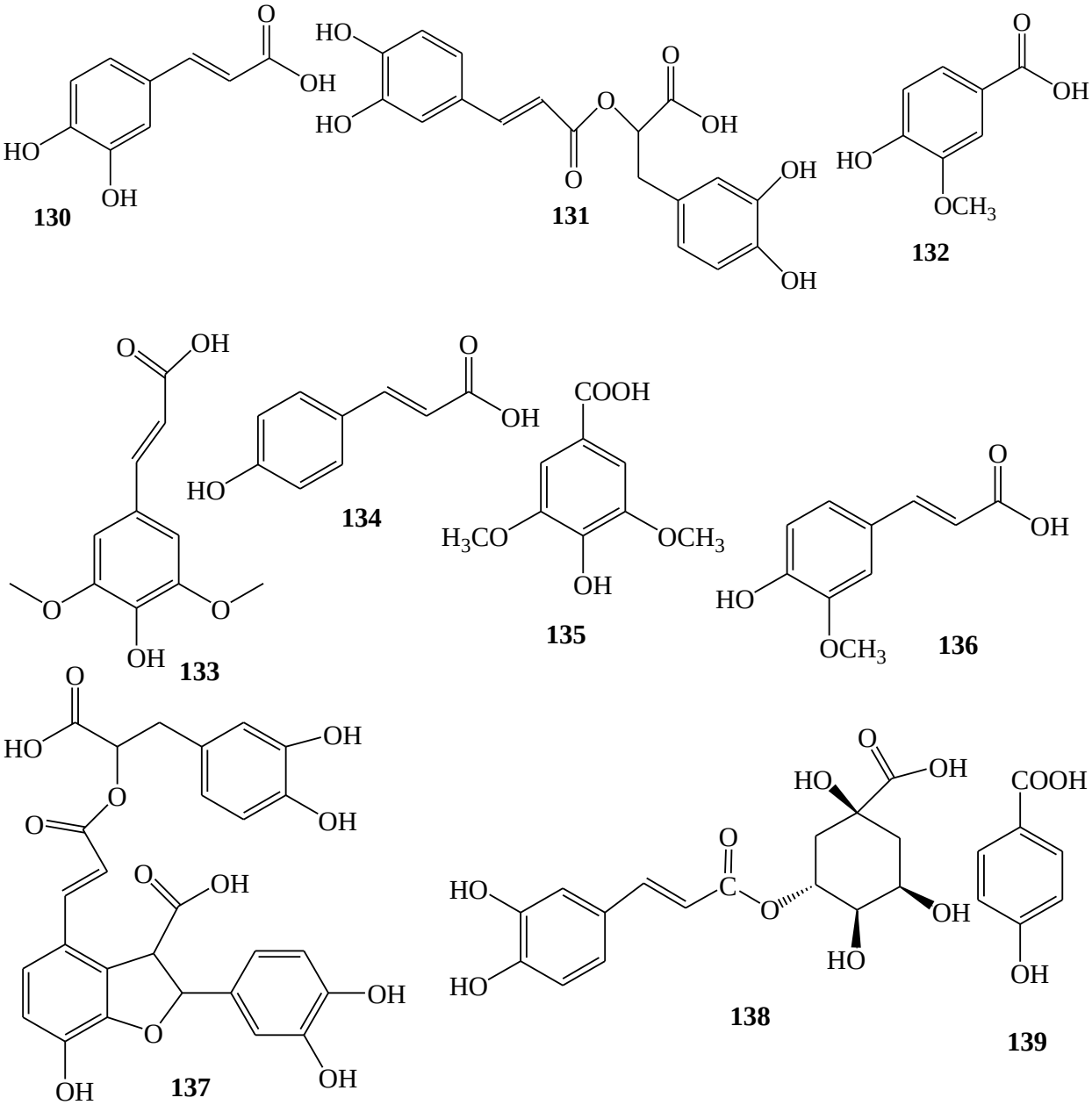
Triterpenoids isolated from species in the genus *Ocimum* included: Oleonolic acid **(4)** which is cytotoxic (Prabhu *et al.*, 2009; Woo *et al.,* 2014) and ursolic acid **(54),** an anti-inflamatory, antioxidant and antitumor which were isolated from *O. kilimandscharicum* stem (Tewari *et al.*, 2012)

The two triterpenoids isolated from stem extracts of *O. kilimandscharicum* were active against microorganisms (Prabhu *et al.*, 2009; Woo *et al.*, 2014). Their activity against insects is however not known. There is no information on isolation of triterpenoids in leaf extracts of *O. kilimandscharicum.* It has therefore not been established whether fractionation of leaf extracts of *O. kilimandscharicum* would lead to isolation of triterpenoids with insecticidal activities against *S. zeamais and P. truncatus*

2.6.2.4.4 Phenolic acids from *Ocimum* species and their biological activities

A number of phenolic acids were reported from *Ocimum* species and were known to have various biological activities. Caffeic acid **(130)** and rosmarinic acid **(131)** showed antibacterial, cytotoxic and genotoxic activities (Matejczyk *et al.*, 2018; Fernandez *et al.*, 1996). Vanilic acid **(132)** showed antioxidant and hypertensive activities (Kumar *et al.*, 2011),sinapic acid **(133),** an antioxidant, anticancer, antibacterial and anti-inflammatory (Chen, 2016),p-coumaric acid **(134)** had antioxidant activity (Masek *et al.*, 2016: Hakkim *et al.*, 2008),syringic acid **(135**) and ferulic acid**(136)**had antibacterial activities (Fernandez *et al.*, 1996), while lithospermic acid **(137)** had antioxidative and hepatoprotective activities (Chan & Ho, 2015). Compounds **130-137** were isolated from *Ocimum*

grattisimum (Hakkim *et al*, 2008). Chlorogenic acid **(138)** an antioxidant (Morishta & Ohnishi, 2001) and *p*-hydroxy benzoic acid **(139)** which showed antimicrobial activity (Cho *et al.*, 1998**),** were isolated from *Ocimum basilicum* (El-Azim *et al.*, 2015)

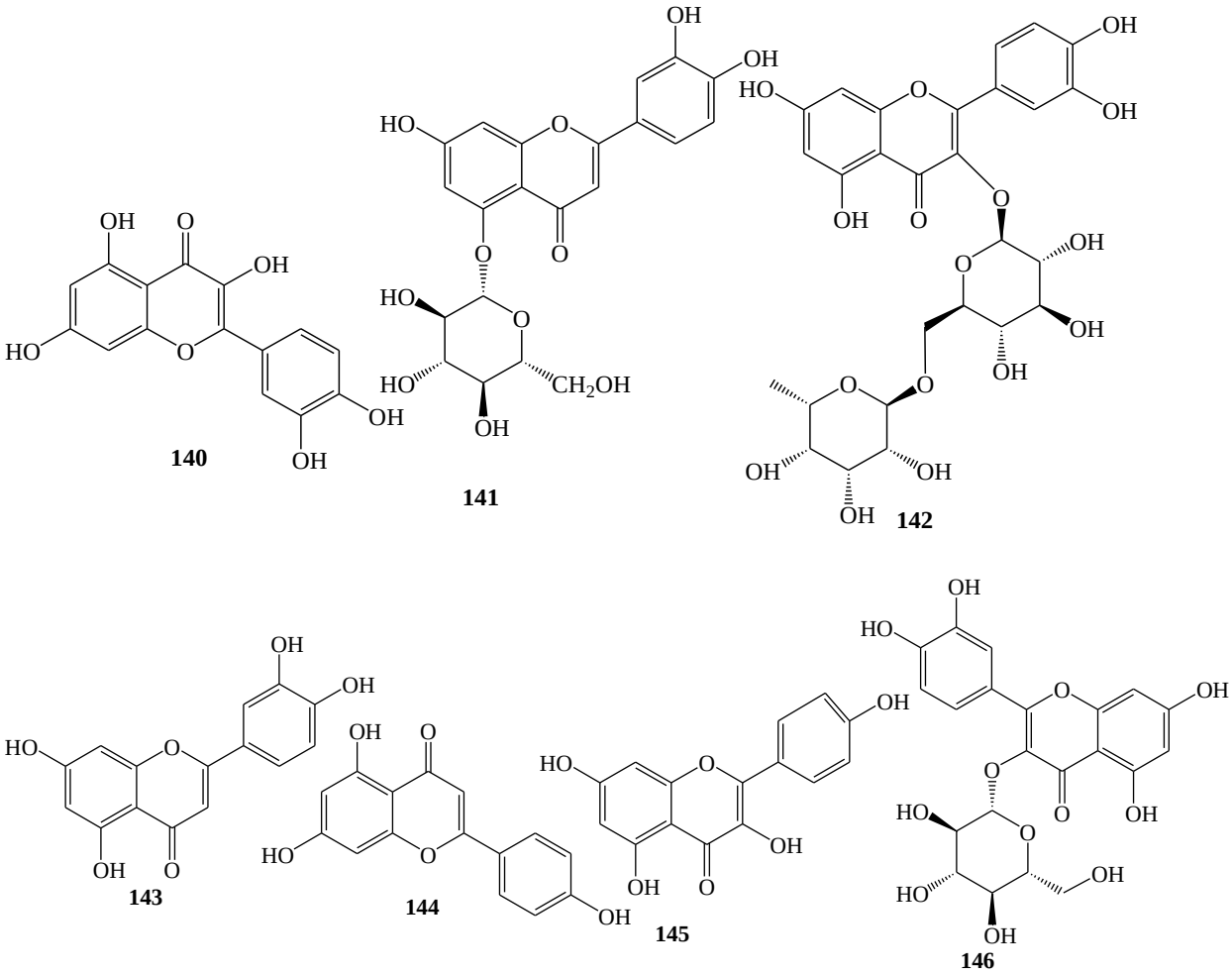


Phenolic acids obtained from the species in the genus *Ocimum* were active against bacteria (Matejczyk *et al.*, 2018; Fernandez *et al.*, 1996). There is no information on the activities of the compounds against insect pests. Information on isolation of phenolic acids from crude leaf extracts

of *O. kilimandscharicum i*s also lacking. It is not established whether phenolic acids would be isolated from crudes leaf extracts of *O. kilimandscharicum* and whether the isolated compounds would have insecticidal activities against *S. zeamais and P. truncatus*.

2.6.2.4.5 Flavonoids from *Ocimum* species and their biological activities.

Astragalin **(85)** an anticancer, anti-inflammatory, antioxidant, neuroprotective, antidiabetic, Cardioprotective and antiulcer agent (Riaz *et al.*, 2018) was isolated from *Ocimum basilicum* (Marwat *et al.*, 2011). Quercetin **(140)** an anti-inflamatory, anticancer and antioxidant, galuteolin **(141)** and rutin **(142),** which demonstrated antibacterial, antifungal and anti-mycobacterial activities (Johann *et al.*, 2011; da Cruz *et al.*, 2012; Dubey *et al.*, 2013) were isolated from *O. kilimandscharicum* stems and leaves (Joshi *et al.*, 2011; Singh *et al.*, 2014, Grayer *et al*., 2002). Luteolin **(143)** an antioxidant, anti-inflammatory, antimicrobial and anticancer activities (Lopez-Lazaro, 2009) and apigenin **(144)** an anticancer (Fajriah & Darmawan, 2016) were isolated from *O. sanctum* (Kaewnarin & Rakariyatham, 2017) whereaskaempferol **(145)** an antioxidant, anti-inflamatory, anticancer and antimicrobial (Calderon *et al.*, 2011) and isoquercetin **(146)** an antimicrobial and antioxidant (Razavi *et al.*, 2009) and were isolated from *Ocimum basilicum* (Marwat *et al.*, 2011).



Flavonoids obtained from the species in the genus *Ocimum* including those from *O. kilimandscharicum* stem extracts had antibacterial, antifungal anticancer and antioxidant activities (Johann *et al*., 2011; da Cruz *et al*., 2012; Dubey *et al*., 2013). There is however no information on their activities against insect pests. Though some compounds from stem extracts of *O. kilimandscharicum* had antibacterial, antifungal and antimycobacterial activities (Johann *et al.*, 2011; da Cruz *et al.*, 2012; Dubey *et al.*, 2013), information on isolation of compounds from crude leaf extracts of the plant is lacking. It is therefore not determined whether flavonoids would be isolated from crude leaf extracts of *O. kilimandscharicum* and whether the flavonoids would have insecticidal activities against *S. zeamais and P. truncatus.*

2.6.2.4.6Sterols from *Ocimum* species and their biological activities

β-sitosterol **(95)** which has antidiabetic and anticancer activities and treatment of heart disease, tuberculosis and rheumatoid anthritis (Saeidnia *et al.,* 2014; Zeb *et al.,* 2017) and stigmasterol **(6)** which is antimicrobial and antifeedant (Huang *et al*., 2008; Yusuf *et al.*, 2018) were isolated from *O. kilimandscharicum* stems (Singh *et al.,* 2014). Sterols isolated from stem extracts of *O. kilimandscharicum* were active against microorganisms and insect pests (Huang *et al*., 2008; Yusuf *et al.,* 2018). There is however no information on isolation of sterols from leaf extracts of the plant. It has not determined whether fractionation of the crude leaf extracts would lead to isolation of sterols with insecticidal activities against *S. zeamais* and *P. truncatus.*

2.6.3 The genus *Annona*

Annonaceae is the largest plant family in the order Magnoliales (Westra & Maas, 2012).It is a family of angiosperms with 135 genera and approximately 2500 species (Chatrou *et al.,* 2004). The genus exhibits a pan tropical distribution with 40 genera and 900 species in the Neotropical region (Ribeiro *et al.,* 2016). Despite the great diversity, it is one of the lesser phytochemically studied tropical plant families (Ribeiro *et al.,* 2013). Isoquinoline alkaloids are characteristic compounds of the Annonaceae family commonly associated with plant defence against herbivorous insects (Cordell *et al.*, 2001; Bermojo *et al.*, 2005). Extracts from Annonaceae were tested for control of lepidoptera, hymenoptera, coleoptera and diptera, especially against *Spodoptera frugiperda, Plutella xylostella, Aedes aegypti*, and stored grain insect pests (Isman &Seffrin, 2016). Plants from the family such as *Annona mucosa* and *Annona sylvatica* have shown promising biopesticides among tropical plants (kumar, 2017).

2.6.3.1 Ethno-medicinal information on species in the genus *Annona*

The *Annona* species have various traditional applications as shown in Table 2.3.

Table 2.3: Ethnobotanical uses of some common species in the genus *Annona*

Species	Plant part	Uses	References
Annona cherimola	Whole plant	An emetic, skin disease treatment and insecticide	Bories *et al.*, 1991
Annona mucosa	Leaves	Elimination of fleas and other insects	de Lima *et al.*, 2012
Annona muricata	Leaves	Treatment of anthritis, diarrhea	Moghamtousi *et al.*, 2015
Annona reticulata	Whole plant	Control fever, treatment of ulcers and diarrhea	Pathak & Zaman, 2013
Annona senegalensis	Whole plant	Stored grain protection	Ngamo & Hance, 2007
Annona squamosa	Leaves, fruits, and roots	Overcoming hysteria, fainting and dysentery, Insecticide, vomiting relief ıtive to heart, expectorant and ıal disease treatment	Galajakshmi *et a*l., 2011 Akanksha *et al.*, 2015

Species in the genus *Annona* have many traditional applications including insecticidal activities against stored food insect pests (Bories *et al.*, 1991; Ngamo & Hance, 2007). Among these plants, *A. mucosa* leaves are used in elimination of fleas and other insects (de Lima *et al*., 2012). The traditional use of *A. mucosa* leaves as an insecticide has not been scientifically validated.

2.6.3.2: Botanical description of *Annona mucosa* (Jacq)

Annona mucosa [synonym *Rollinia mucosa*] (Jacq) Baill] is a native fruit tree of the Amazon and the Atlantic forest that grows well in different habitats (Ferreira *et al.*, 2010). The plant is a fast-growing tree up to 4 m in height; has brown, hairy twigs. The flowers are 1 to 3 formed together in the leaf axils and the fruit is conical or heart-shaped 15 cm in diameter (Morton, 1987).

Plate 5: *Annona mucosa* Jacq. Trees
(source: National parks flora & fauna)

2.6.3.3 Biological activities of *Annona mucosa*

In a previous insecticidal assay of *A. mucosa, n*-hexane and dichloromethane seed extracts were tested against *Sitophilus zeamais*. The hexane extract was the most promising treatment with LC_{90} values of 259.31 mg kg^{-1} followed by dichloromethane seed extract with LC_{90} of 425.15 mg kg^{-1}(Ribeiro *et al.*, 2013).In other experiments, ethanolic seed extracts from *A. mucosa* were active against looper *Trichoplusiani hübner* (Lepidoptera: Noctuidae) through oral and topical administration, with greater than 98% mortality against third instar *T. ni* larvae (Ribeiro *et al.*, 2013). In the test for the nyphacidal effect, the chloroform-methanol seed extracts of *A. Mucosa* controlled more than 75% of *T. limbativentris* nymphs at the concentration of 1.0% after the first 24 hours and reached 88% at same concentration after five days from the application of the extract (Krinski & Massaroli, 2014). In the ingestion and topical application tests of the chloroform-methanol (2:1) seed extracts of *A. mucosa* on *Chrysodeixis includens* (Walker), mortality of first

instars was high after 24 hr treatment for both ingestion and topical application tests with the topical application resulting in mortality of 93.3% at the 8% concentration (Massarolli *et al.*, 2016). The methanol seed extract of *A. mucosa* showed promising results for control of nymphs and adults of brown stink bug, *Euschistus heros* (F.) with mortality higher than 90% of nymphs at concentrations of 5mg/mL.

All these tests were carried out on seed extracts of *A. mucosa,* with no reports on the insecticidal activity of the crude leaf extracts of the plant against *S. zeamais* and *P. truncatus.* It has therefore not been determined whether leaf extracts of *A. mucosa* may have insecticidal activity against *S. zeamais* and *P. truncatus.*

2.6.3.4 Phytochemistry of the species in the genus *Annona*

2.6.3.4.1 Monoterpenoids from *Annona* species and their biological activities

Previous phytochemical analysis of *Annona cherimola* essential oils (Pino & Roncal, 2016), revealed the presence of 1, 8-cineole **(100)** an antioxidant (Ciftci *et al.*, 2011), linalool **(101)** which checks the growth of *Rhizoctonia solani* (Thakur *et al.,* 1989), camphene **(102)** a hypolipidemic (Valianou & Hadzopoulou-Cladaras, 2016) and limonene **(103)** an antimicrobial (Rancic *et al.*, 2003). Other compounds isolated from *Annona cherimola* fruit essential oils were: *β*-pinene (**105)** an antimicrobial (da Silva *et al.*, 2012)**,** p-cymene **(106**) an antinociceptive and antioxidant (Quintans *et al.*, 2013)**,** 4-terpinenol **(107)** an anticancer (Shapira *et al*., 2016) and β-myrcene **(108)** an antioxidant (Ciftci *et al.*, 2011). Other compounds isolated were: α-terpinolene **(109)** an antioxidant and anticancer (Aydin *et al.*, 2013**),** β-phellandrene **(110)** an antifungal (Zhang *et al.*, 2017; Kumar *et al.*, 2011), α-thujene (**113**) an antimicrobial and antioxidant (Kelen & Tepe, 2008),

sabinene **(114)** an antibiotic (Carvalho *et al.*, 2017) andα-terpeneol (**115**) which has gastro protective activity (Souza *et al.*, 2011).

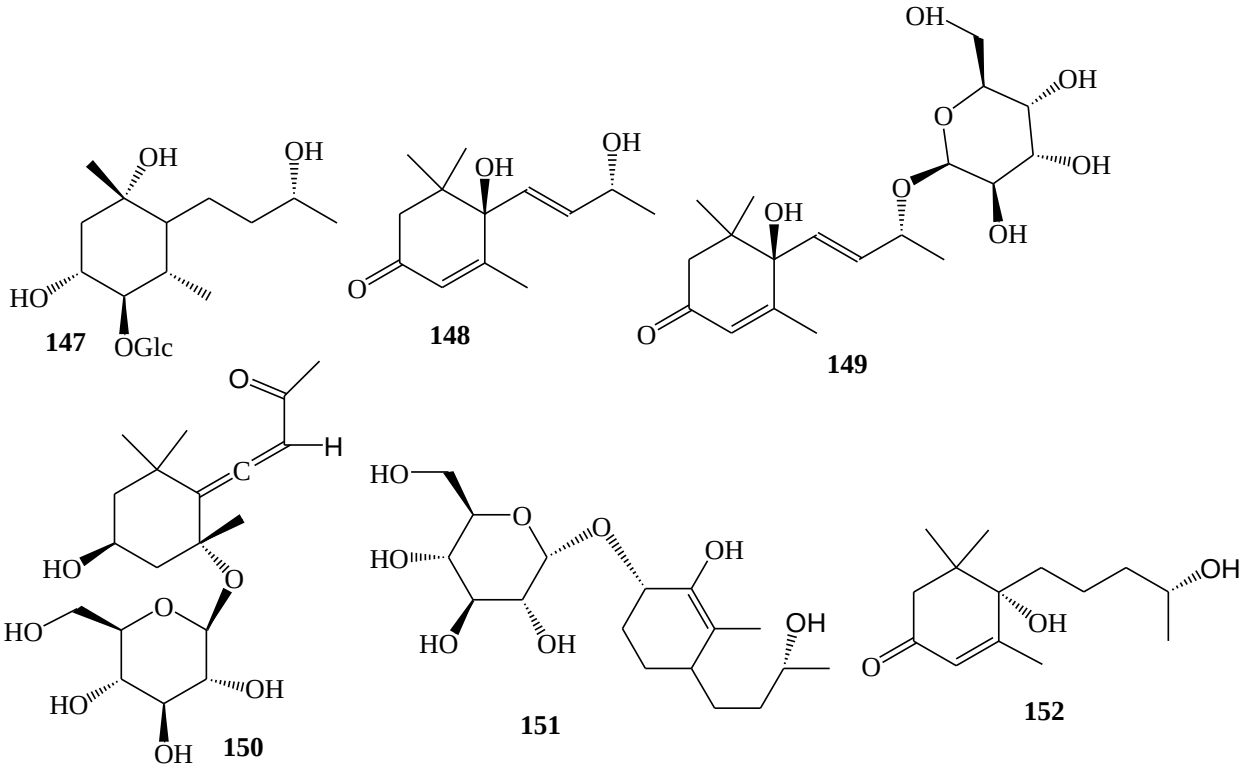
Monoterpenoids from essential oils of the species in the genus *Annona* have antimicrobial, (Rancic *et al.*, 2003; da Silva *et al.*, 2012; Kelen & Tepe, 2008) and antifungal (Zhang *et al.*, 2017; Kumar *et al.*, 2011). Information on insecticidal activities of the monoterpenes is however lacking. There are no reports on insecticidal activities in crude leaf extracts of *A. mucosa* against *S. zeamais* and *P. truncatus.* It is therefore not established whether monoterpenoids in leaf extracts of *A. mucosa* would have insecticidal activities against *S. zeamais* and *P. truncatus.*

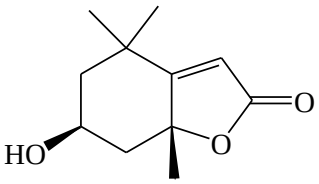
2.6.3.4.2 Sesquiterpenoids from the *Annona* species and their biological properties

Previous phytochemical analysis of fruit essential oils of *Annona cherimola* (Pino and Roncal, 2016), revealed the presence of α-cadinene **(116),** an antioxidant (Kundu *et al.*, 2013), β-caryophyllene **(117)** an antibiotic, antioxidant and anti-carcinogenic (Leqault & Pichette, 2007) and-caryophyllene oxide **(118)** that has anticancer activity **(**Fidyt*et al.*, 2016**).** Additionally, α-copaene **(119),** β-Copaene **(120),** β-elemene **(121)** and globulol **(125)** were also isolated from the plant**.** Compounds **119-121**and **125** had antimicrobial activity (Tan *et al.*, 2008; Lang & Buchbauer 2012; Martins *et al.*, 2015; Sieniawska *et al.*, 2018). Germacreme-D **(127),** an isolate from the same plant had immunomodulatory, antimicrobial and antioxidant activities (Ziaei *et al.*, 2010; Ei-Kalamouni *et al.*, 2017).Sesquiterpenoids from essential oils of the species in the genus *Annona* were active against microorganisms. Reports on their insecticidal activities are scanty and there are no reports on these compounds in crude leaf extracts of *A. mucosa.* It has therefore not been evaluated whether sesquiterpenoids in leaf extracts of *A. mucosa* would have insecticidal activities against *S. zeamais* and *P. truncatus.*

2.6.3.4:5 Megastigmanes from *Annona* species and their biological activities

Megastigmanes isolated from the *Annona* species included; annoinoside **(147),** vomifoliol **(148),** roseoside **(149)** and citroside A **(150)** which had antitumour activity (Ito *et al.*, 2002). Turrpinionoside (A) **(151),** an antioxidant and antibacterial (Voravuthikunchai *et al.*, 2010), blumenol A **(152)** and (+)-epiloliolide **(153)** were cytotoxic (Ren *et al.*, 2009) while annoionol A **(154)** had α-glucosidase inhibitory activity (Raynil *et al*., 2016). The activity of Z-3-hexeneyl-D-glucopyranoside **(155)** is not known. Compounds **147-155** were isolated from *Annona muricata* (Matsushige *et al.*, 2012**;** Nawwar *et al.*, 2012)





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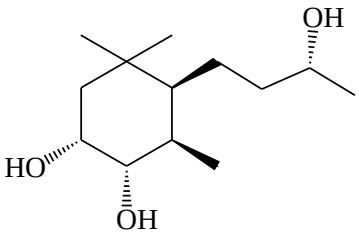

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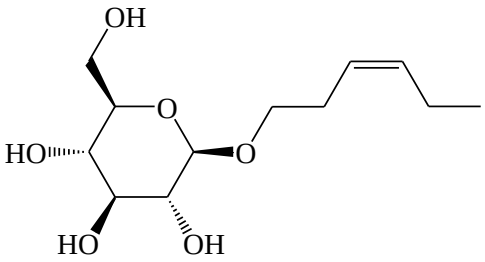

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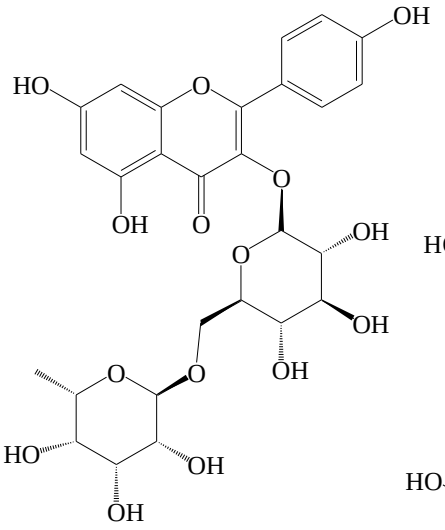
Megastamanes isolated from *A. muricata* are biologically active. Reports on isolation of megatamanes from other *Annona* species are lacking. There is therefore no evaluation on whether fractionation of the *A. mucosa* leaf extracts would lead to isolation of megastamanes and whether the compounds would have insecticidal activity against *S. zeamais* and *P. truncatus.*

2.6.3.4.3 Flavonoids from *Annona* species and their biological activities

In the previous phytochemical screening of the *Annona* species, flavonoids and flavonol glycosides were isolated. The compounds isolated included astragalin **(85)** which is cytotoxic, phytotoxic and hasanti-inflammatory, antioxidant, neuroprotective, cardioprotective, antiobesity, anticancer, antiulcer and antidiabetic properties (Riaz *et al.*, 2018; Razavi *et al.*, 2009). Quercetin **(140)** an anti-inflamatory, anticancer and antioxidant (Singh *et al.*, 2014; Joshi *et al.*, 2011), rutin **(142)** an antibacterial and antifungal (Dubey *et al.*, 2013; da Cruz *et al.*, 2012; Johann *et al.*, 2011)**,** isoquercetin **(145)** an antimicrobial and antioxidant(Razavi *et al.*, 2009; Veras *et al.*, 2011), kaempferol **(146)** an antitumor, antioxidant and anti-inflammatory (Wang *et al*., 2018) were isolated from *Annona muricata* leaves (Nawwar *et al.*, 2012). Other compounds isolated from *A. muricata* leaves werekaempferol-3-*O*-rutinoside **(156),** isoquercetin hyperin **(157)** which were anti hyperglycemic (Verma *et al.*, 2013), catechin **(158)** and epicatechin **(159)** which were neurotoxic and antibacterial (Nawwar *et al.*, 2012; Moghadamtousi *et al.*, 2015) and kaempferol-3-*O*-robinobioside **(160)** a human lymphocyte proliferation inhibitor (Brochado *et al.*, 2003).



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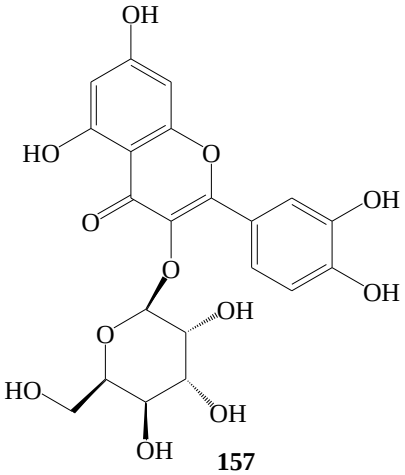

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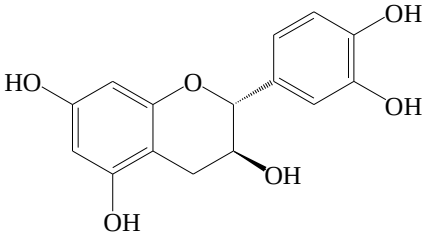

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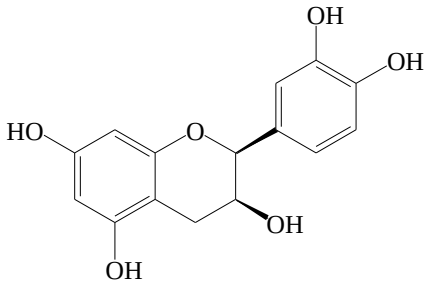

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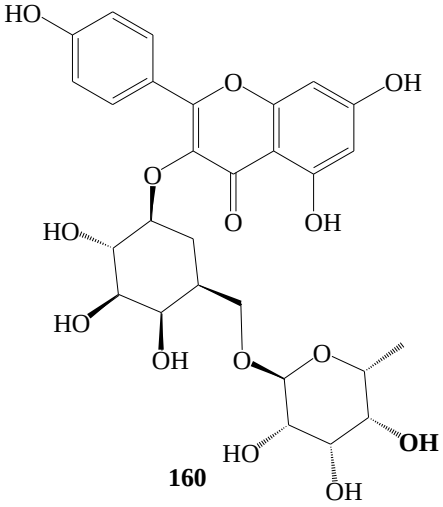

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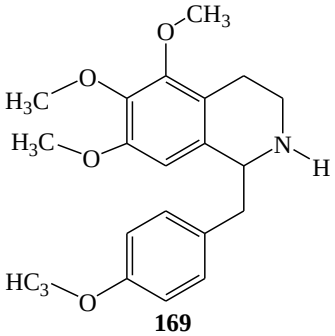
Flavonoids isolated from leaf extracts of *A. muricata* are biologically active against several microorganisms. There are no reports on their activities against insect pests and therefore no documentation on whether flavonoids isolated from leaf extracts of *A. mucosa* would have insecticidal activities against *S. zeamais* and *P. truncatus*.

2.6.3.4.4 Alkaloids from *Annona* species and their biological activities

Previous phytochemical investigation of *Annona species* extracts led to isolation of the alkaloids; atherospermidine **(161)** and liriodenine **(162)** obtained from *Annona mucosa* (de Lima *et al*., 2012)

and *Annona pickelii* (Dutra *et al.*, 2012) and annomontine **(163)** isolated from *Annona foetida* (Rejo'n-Orantes *et al.*, 2011). The compounds were reported to possess antileishmanial activity (de Lima *et al*, 2012) and tripanocidal activity (Costa *et al.*, 2011) while *O*-methyl moschatoline **(164)** from *Annona foetida* had tripanocidal activity (Costa *et al.*, 2011). Other alkaloids isolated from the *Annona* species were; reticuline **(165),** coclaurine **(166),** coreximine **(167),** atherosperminine **(168),** anomurine **(169),** anomuricine **(170)** and annonaine **(171)**.All these compounds showed anti-depressive activity (Hasrat *et al.*, 1997). The compounds were isolated from *Annona muricata* (Laboeuf *et al.*, 1981). Nomuciferine **(172)** and asimilobine **(173)** isolated from *Annona pickelii* (Dutra *et al.*, 2012) were anti-depressive (Hasrat *et al.*, 1997). Isolaureline **(174)** and xylopine **(175)** which had cytotoxic activity (Santos *et al.*, 2017), were isolated from *Annona muricata* **(**Moghadamtousi *et al*., 2015: Fofana *et al.*, 2011). Oxopurpureine **(176)** an antioxidant (de Lima *et al.*, 2012: Puvanendran *et al.*, 2008) and nornantenine **(177),** an antimicrobial (Baskar *et al.*, 2007) were obtained from *Annona rugulosa* (Dutra *et al.*, 2012).Hydroxynornantenine **(178)** isolated from *Annona glabra* (Garcia *et al.*, 2012) had antimicrobial activity (Baskar *et al.*, 2007) while lysicamine **(179)** and orientaline **180)** showed anti proliferative effect (Nakano *et al.*, 2013), were isolated from *Annona pickelii* (Dutra *et al.*, 2012)**.** Romucosine **(181)** which had an anti-platelet activity (Kuo *et al.*, 2014), stepharine **(182),** glaucine **(183)** and oxoglaucine **(184)** which were anti-inflammatory (Cortijo *et al.*, 1999**)** were isolated from *Annona mucosa* unripe fruits (Chen *et al.*, 1996). *N*-Nitrosoxylopine **(185),** duguevaline **(186),** roemerolidine **(187)** which were isolated from *Annona squamosa,* had antimalarial activities (Johns *et al.*, 2011).



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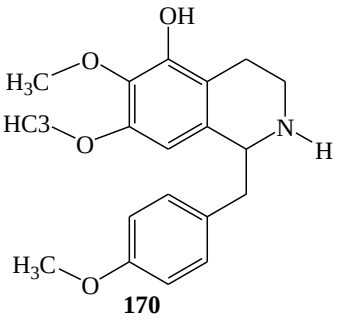

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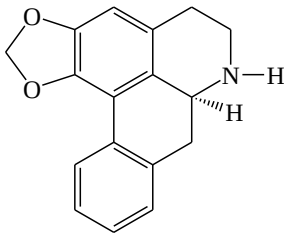

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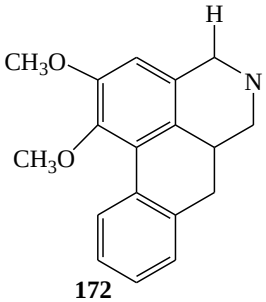

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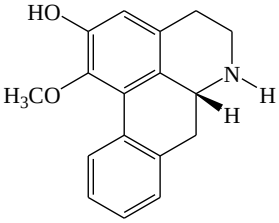

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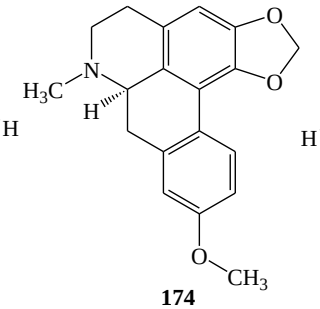

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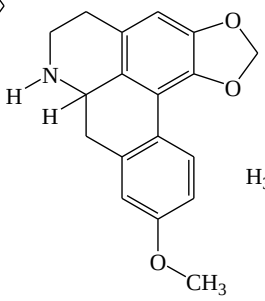

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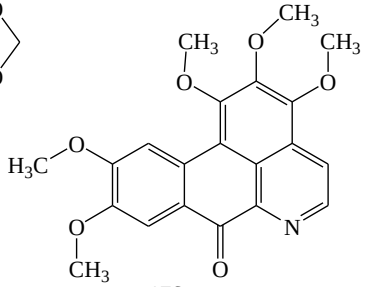

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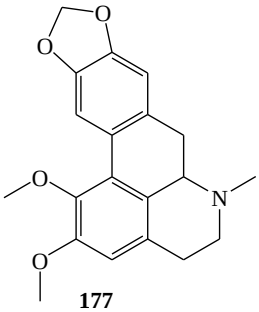

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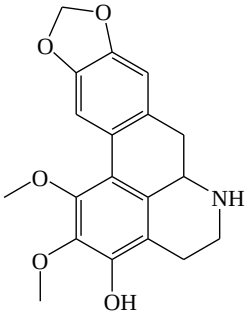

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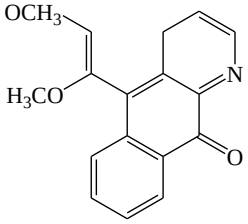

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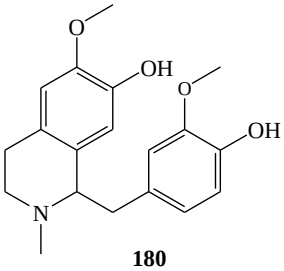

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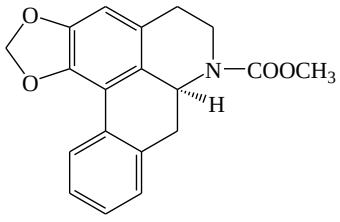

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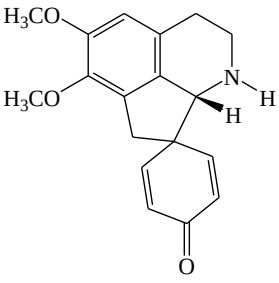

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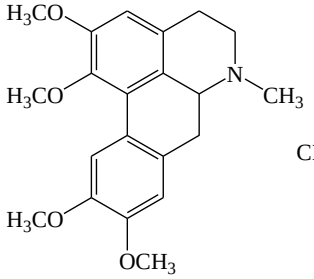

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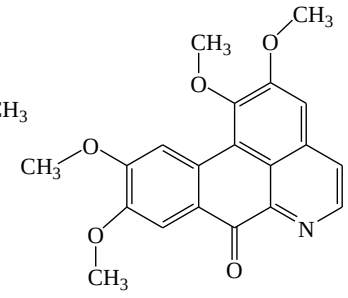

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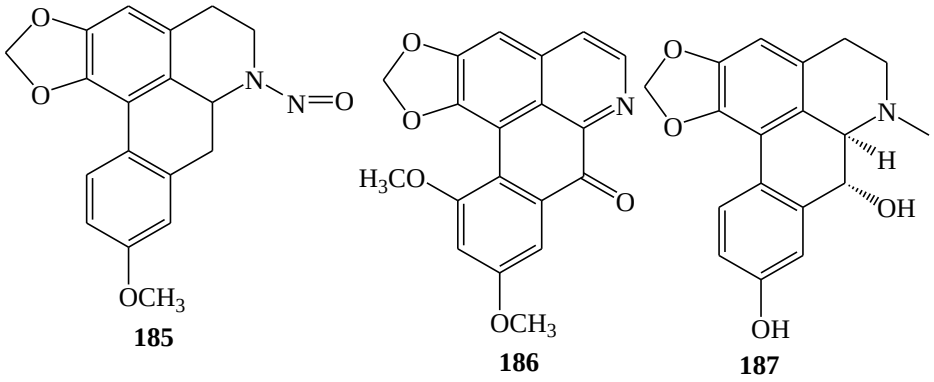


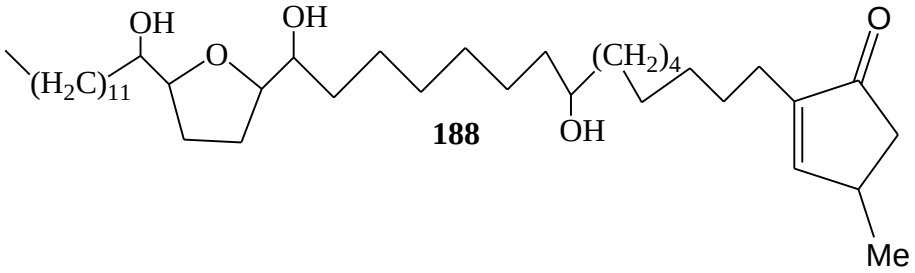
185 **186** **187**

Romucosine **(181),** stepharine **(182),** glaucine **(183)** and oxoglaucine **(184)** isolated from *Annona mucosa* unripe fruits (Chen *et al.,* 1996) had anti-platelet and anti-inflammatoryactivities (Cortijo *et al.,* 1999**;** Kuo *et al.,* 2014) while *N*-Nitrosoxylopine **(185),** duguevaline **(186),** roemerolidine **(187)** had antimalarial activities (Johns *et al.,* 2011).The compounds were however not tested for their insecticidal activities against *S. zeamais* and *P. truncatus.* It is therefore not known whether fractionation of *A. mucosa n*-hexane, ethyl acetate and methanol leaf extracts would lead to isolation of new alkaloids and whether the compounds would have insecticidal activities against *S. zeamais* and *P. truncatus.*

2.6.3.4:6 Acetogenins from *Annona* species and their biological activities

Acetogenins are a family of naturally occurring polyketides (C-35/C-37) derived from long-chain fatty acids (C-32/C-34) combined with a 2-propanol unit at C-2 (Alali *et al*, 1999). Some acetogenins were previously isolated from the *Annona* species and their biological activities were evaluated. The isolated compounds included; annonamutacin **(188**) which had toxicity against lung A549 cancer cells (Wu *et al.,* 1995) and annohexocin **(189)**that hadtoxicity against brine shrimp and different cancer cells. Other compounds were; muricapentocin **(190)** (Zeng *et al.,* 1995) that hadtoxicity against pancreatic MIA PaCa-2and colon HT-29 cancer cells (Kim *et al.,* 1998), sabadelin**(191)** and muricoreacin **(192)** that had toxicity against different cancer cells (Kim *et al*.,

1998). Gigantetronenin **(193)**, isonnonacin **(194)**, muricatacin **(195)** and annonacin **(196)** were neurotoxic and molluscicidal (Luna *et al.*, 2006; Jaramillo *et al.*, 2000). Other compounds were corosolone **(197),** a gall bladder tumor inhibitor and had toxicity against brine shrimp (Liaw *et al.*, 2002) and solamin **(198)** that had toxicity against oral KB cancer cells (Cortes *et al.*, 1991: Gleye *et al.*, 1998). Compounds **188-198** were isolated from *Annona muricata* seeds (Rieser *et al.*, 1993).



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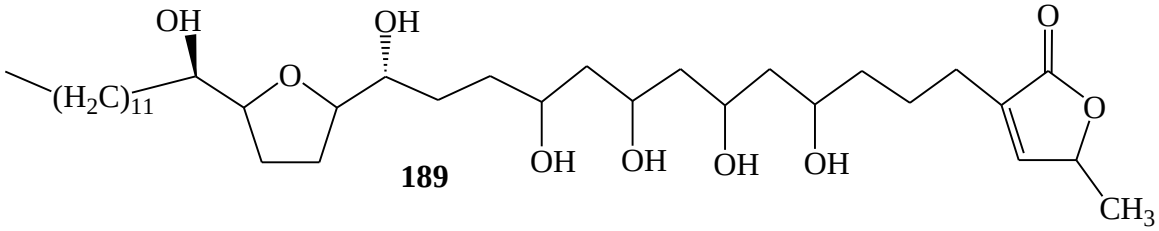

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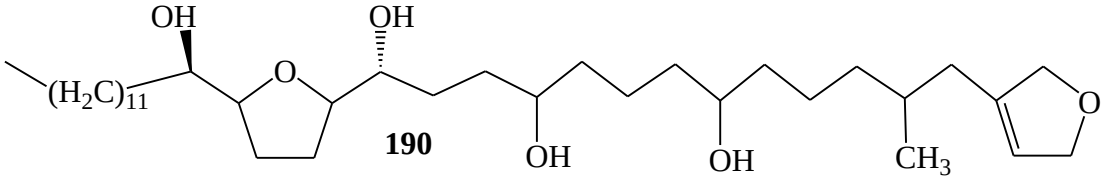

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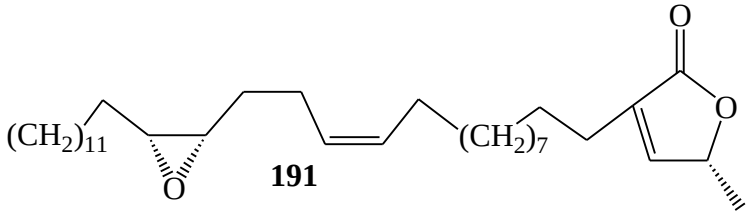

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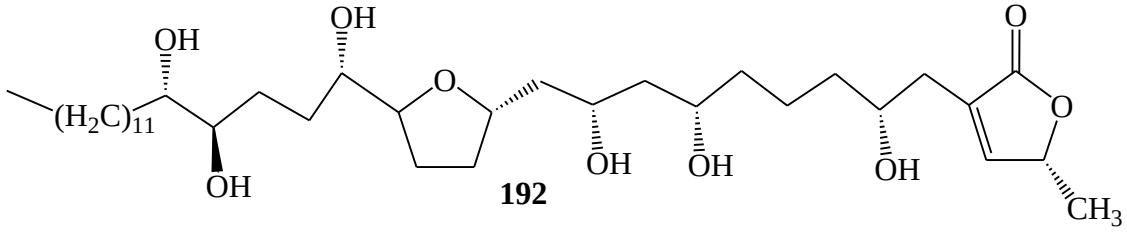

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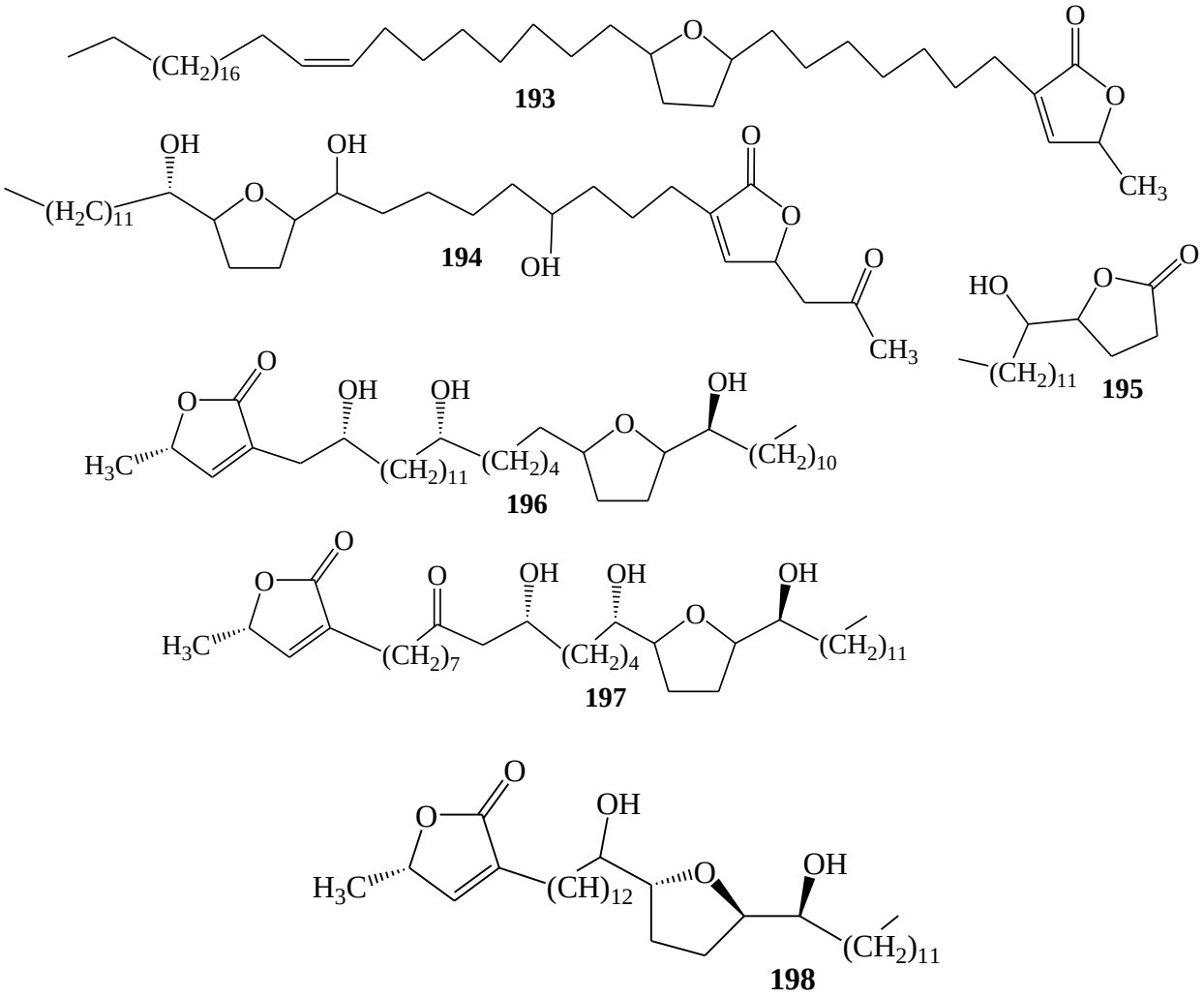

Acetogenins isolated from *Annona* species had activities such as toxicity against different cancer cells (Kim *et al.*, 1998; Wu *et al.*, 1995; Cortes *et al.*, 1991: Gleye *et al.*, 1998). There is however no report on insecticidal activity of acetogenins from *A. mucosa* leaf extracts against *S. zeamais* and *P. truncatus*. There is therefore no documentation on whether isolation of compounds from leaf extracts of *A. mucosa* would lead to isolation of acetogenins that have insecticidal activity against *S. zeamais* and *P. truncatus*.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant material collection and identification

Leaves and twigs of *G. subcordata* (Meisn) were collected from the wild in the outskirts of Kitui town, Kitui County (Lat: 1° 22' 30.2916" S: Long. 37° 59' 42.7668" E), Kenya, in April 2014 while those of *Ocimum kilimandscharicum* (Guerke) were collected from Kakamega Forest, Kakamega county (Lat: 0° 17' 3.19" N, Long: 34° 45' 8.24" E) in February 2014. On the other hand, leaves and twigs of *Annona mucosa* (Jacq) were collected from Kitale, Trans Nzoia County (Lat: 1° 00' 56.59" N, Long: 35° 00' 22.39" E), in January 2015.The plant specimens for *G. subcordata* and *Ocimum kilimandscharicum* were identified and authenticated at the herbarium section, Department of Botany, University of Nairobi by a taxonomist, Mr. Mutiso after comparison with authentic specimens and where voucher specimens are deposited(Reference No.GS/MU/2014) and (Reference No.OK/MU/2014). The plant specimens for *Annona mucosa* were identified by the same taxonomist. Authentication of the plant was done at the herbarium of the National Museums of Kenya where voucher specimens are deposited (Reference No.AM/MU/EA/2015).

3.2 General experimental procedure, solvents and fine consumables

Melting points were determined using Gallenkamp melting point apparatus (Manchester, UK). Optical rotation was measured on a Jasco P-1020 Polarimeter (Jasco Corporation, Tokyo, Japan). UV spectra were analysed using a Shimadzu UV-2401 A Spectrophotometer (Shimadzu Corporation, Kyoto, Japan). IR data were recorded on a Bruker Tensor 27 FTIR spectrophotometer (Bruker Corporation, Bremen, Germany) as KBr pellets. NMR data were measured in $CDCl_3$, CD_3OD and DMSO-d_6 on a JOEL NMR instrument operating 600 and 150MHz, respectively. Some

NMR data were done using Brucker AM 300 spectrometer operating at 360 and 90 MHz, respectively. Tetramethylsilane (TMS) was used as reference in NMR analyses. The mass spectral data were obtained using a Varian MAT 8200 A instrument. Electron-impact (EI) mass spectra (70 eV) were measured on a Hewlett-Packard 5989B mass spectrometer. Silica gel 60 (63-200 μm, Merck, Darmstadt, Germany) was used for gravity column chromatography (CC). TLC was performed on pre-coated DC Alufolien 60 F254 sheets (Merck, Darmstadt, Germany) and detected by spraying with anisaldehyde spraying agent. UV light and iodine were also used for detecting spots. Paper chromatography was done on standard Whatman No 1 chromatography paper. All solvents used were of analytical grade

3.3 Phytochemistry of *Gnidia subcordata* leaves

The sample materials were separated from the stems. The leaves obtained were then air dried under a shade for four weeks and finally ground into powder in a model 4E grinding mill

3.3.1 Solvent extraction of *G. subcordata* powdered leaves

Ground leaves (2.0 kg) were cold extracted sequentially using *n*-hexane, ethyl acetate and methanol (4 L x 3) each with occasional shaking using an orbital shaker set at 150 revolutions per minute lasting 24 hours. The extracts were filtered then concentrated under *vacuo,* to give green (18.0 g), greenish-yellow (39.0 g) and greenish-brown (100.0 g) of *n*-hexane, ethyl acetate and methanol extracts, respectively. Analysis of the *n*-hexane extract on TLC using *n*-hexane-EtOAc (4:1) revealed three spots of R_f values 0.71, 0.60, and 0.45 which were stained purple with acidified anisaldehyde. On the other hand, TLC analysis of the EtOAc extract using *n*-hexane-EtOAc (4:1, 3:2, 1:1, 1:3) and EtOAc-MeOH (99:1, 97:3, 95:5 and 9:1) gave a total of seven spots of R_f values

0.71, 0.60, 0.45, 0.40, 0.36, 0.30 and 0.27. All spots on spraying with acidified anisaldehyde followed by heating showed purple to pale yellow colour range.

3.3.2 Fractionation of the *n*-hexane and ethyl acetate extracts

TLC profiles of both the extracts revealed that the compounds extracted by the *n*-hexane solvent were also present in the ethyl acetate extract. For column chromatography 15.0 g of *n*-hexane extract was combined with 30.0 g of ethyl acetate extract. The whole extract (45.0 g) was made into slurry with silica gel (20 g) in 200 ml EtOAc after which the solvent was removed under *vacuo* and the solid charged onto silica gel column (5.0 x 40 cm, SiO_2, 300 g, pressure ≈1 bar). Fractionation of the column using solvent gradient; *n*-hexane-ethyl acetate mixture with increasing polarity of the latter, ethyl acetate and ethyl acetate-methanol (increment 1% of more polar solvent) afforded 255 fractions (each 20 mL). Their compositions were monitored by TLC, using solvent systems; *n*-hexane-ethyl acetate (9:1, 4:1, 2:1, 1:1) and ethyl acetate-methanol (99:1 and 97:3). Fractions showing similar TLC profiles were pooled together resulting into five pools **(I-V).** Pool **1** (fractions 5-46, 7.0 g) eluted using *n*-hexane-EtOAc (9:1) gave a yellow-orange pigment which faded with time and was not considered for further analysis. This is because the pigment may have contained compounds that underwent structural changes. Fractions 47-76 (pool **II**, 3.40g) showed two spots on TLC (R_f values 0.71 and 0.60; solvent system; *n*-hexane: EtOAc, 4:1) which upon repeated fractionation over silica gel using *n*-hexane-EtOAc (9:1) afforded **GS1** (R_f = 0.71, 70 mg) and **GS2 (**R_f = 0.60, 55.5 g).Fractions 78-130 constituted pool **III** (5.0 g) exhibited a single spot contaminated with chlorophyll. Pool **IV** (fractions 133-200, 4.5 g), was similarly resolved into individual component as described for the above case using *n*-hexane-EtOAc (4:1) followed by same solvents in the ratio 3:1 to give **GS3** (R_f=0.45, 53.3 mg), **GS4** (R_f=0.40, 65.0 g) and **GS**5

(33.5 mg, R_f = 0.36). Fractions 203-255 which constituted pool **V** (3.5 g) upon repeated chromatographic elution over silica gel using CH_2Cl_2-MeOH (99:1) followed by the same solvents in the ratio 49:1 gave two white solids which were further purified by crystallization in *n*-hexane-CH_2Cl_2 (4:1) to give **GS6** (R_f = 0.30, 25.7 mg) and **GS7** (R_f = 0.27, 35.2 mg).

3.3.3 Physical and spectral data of compounds isolated from *n*-hexane and ethyl acetate extract of *G. subcordata* leaves

3.3.3.1 Isolate GS1: Isolated as white amorphous powder, m.p. 239-241^oC, R_f = 0.71 (silica gel, TLC, *n*-hexane-EtOAc, 4:1); IR ν_{max} (KBr) cm^{-1}: 2947.5, 1734.6, 1654.0, 1455.8, 1366.2, 1244.4, 1146.3, 1095.7, 1024.5, 984.6, 877.4; ^1H and ^{13}C NMR δppm: see Table 4.1; EI-MS: *m/z* (*rel. int*.) 468.2 $[M]^+$ (10), 453.2 (5), 249.1 (8), 218.1 (100), 203.1 (15), 189.1 (18), 161.1(8), 135.1 (12), 109.1 (11), 95.1 (12), 69.1 (10), 55.1 (6), 43.1 (28).

3.3.3.2. Isolate GS2: White amorphous powder, m.p. 184-186^oC, R_f = 0.60 (silica gel, TLC, *n*-hexane-EtOAc, 4:1); ^1H and ^{13}C NMR δppm see Table 4.2; EI-MS: *m/z* (rel. int.) 440.1 $[M]^+$ (100), 422.1 (100), 407.1 (20), 371.1 (10), 355.1 (11), 341.1 (8), 311.1 (4), 287.0 (10), 255.1 (10), 234.1 (100), 205.1 (11), 191.1 (85), 163.0 (20), 135.0 (42), 123.0 (53), 95.0 (75), 69.0 (75), 55.0 (38), 43.1 (27).

3.3.3.3. Isolate GS3: Isolated as white crystals; m.p. 174-175^oC and R_f = 0.45 (silica gel, TLC, *n*-hexane-EtOAc, 4:1); IR ν_{max} (KBr) cm^{-1}: 2950.5, 1463.7, 1334.5, 1216.8, 1039.4, 881.3, 736.6, 553.5; ^1H and ^{13}C NMR ($CDCl_3$) δppm: see Table 4.3; EI-MS: *m/z* 350.0 $[M+H]^+$

3.3.3.4. Isolate GS4: Isolated as UV active white crystals, m.p. 166-168^oC; R_f = 0.40 (*n*-hexane-EtOAc, 4:1);^1H and ^{13}C NMR ($CDCl_3$) δppm: see Table 4.4; EI-MS: *m/z* 348 $[M-H]^+$ (100), 333 $[M-H-Me]^+$ (26), 318 $[M-OMe]^+$(4), 304 (4), 290 (4), 275 (2), 260 (2), 247 (2), 232 (2), 217 (2), 204 (1), 174 (3), 145 (5), 123 (2), 102 (2), 95 (2), 81 (2), 69 (3), 57 (3), 55 (4).

3.3.3.5. Isolate GS5: White amorphous powder, R_f = 0.36 (silica gel, TLC, *n*-hexane-EtOAc, 4:1); ^1H and ^{13}C NMRδppm: see Table 4.5; ESI-MS (positive ion mode) *m/z* 482.6$[M]^+$; HR TOF-MS peak at *m/z* 505.5654 $[M+Na]^+$ (calcd for $C_{28}H_{34}O_7$ 482.5653).

3.3.3.6. Isolate GS6: Was obtained as white powder with R_f = 0.30 and m.p 215-216^{o}C; IR ν_{max} (KBr) cm^{-1}: 3431.9, 2937.3, 1774.5, 1744.3, 1664, 1458.1, 1375.3, 1254.2, 1054.0, 959.6, 886.6; ^1H and ^{13}C NMR δppm: see Table 4.6; EI-MS (*re lint*.): *m/z* 455 $[M]^+$ (5),454 (10), 453 (50), 424 (5), 407 (20), 406 (4), 343 (3), 313 (3), 297 (15), 245 (18), 207(4), 206 (20), 205(70), 189 (85), 161 (38), 147 (40), 135 (78), 109 (100), 93 (77), 69 (100); HRESI-MS 454.23819 (calcd for $C_{26}H_{30}O_7$ 454.2382)

3.3.3.7. Isolate GS7: White amorphous powder, m.p. 298-301^{o}C; R_f=0.27 (silica gel, TLC, *n*-hexane-EtOAc, 4:1); $[\alpha]^{25}_D$ + 15^o (c0.5, MeOH);^1H NMR and ^{13}C NMR, see Table 4.7;EI-MS: *m/z*365 $[M+3H]^+$ (10),364 $[M+2H]^+$ (64), 362 $[M]^+$ (45), 355 (10), 348 (14), 347 (20), 346 (13), 326 (17), 310 (20), 309 (7), 295 (18), 294 (90), 293 (100), 278 (5)

3.3.4: Fractionation of methanol extract of *G. subcordata* leaves

MeOH extract (56.0 g) was made into slurry with silica gel (25 g) in 200 mL MeOH. It was then flash chromatographed (5.0 x 30 cm column, SiO_2 300 g, pressure ≈1 bar), eluting with CH_2Cl_2 followed by CH_2Cl_2-MeOH gradient with increasing concentration of the more polar solvent (increment 3%) and elution concluded with MeOH. A total of 300 fractions each 20 mL were sampled and their homogeneity determined by TLC (eluent: CH_2Cl_2-MeOH, 98:2, 97:3, 19:1, 9:1 and 4:1; *n*-BuOH-HOAc-H_2O,(BAW): 4:5:1. Those exhibiting similar profiles were combined into four major pools (**I-IV)**. Pool **I** (fractions 30-130, 3 g) mainly eluted using CH_2Cl_2 afforded more of compounds **GS6** and **GS7** in 25 mg and 36 mg, respectively. Pool **II** (Fractions 134-183, 8.0 g) showed a major spot R_f = 0.46 (solvent system: BAW, 4:1:5) accompanied by minor impurities.

This was further purified by crystallization in 5% aqueous MeOH-H_2O to give **GS8** (R_f= 0.46, 80 mg). Fractions 187-230 (10.5 g) which constituted pool **III** afforded two spots of R_f values 0.46 and 0.33 (BAW, 4:1:5). The spots turned yellow on exposure to concentrated ammonia vapour, signifying the presence flavonoid derivatives. Repeated fractionation of this pool (8.3 g, 3.5 x 60 cm, SiO_2 150 g, pressure ≈ 1.0 bar) using BAW (4:1:5, 2.0 L) and collecting 10 mL each gave further **GS8** (43.0 mg) and **GS9 (**R_f = 0.33, 76.3 mg). Fractions 232-300 constituted pool **IV** (5.5 g) mainly from CH_2Cl_2-MeOH (1:1) elution. On repeated flash chromatography using CH_2Cl_2-MeOH (2:1), collecting 10 mL each afforded further **GS9** in 52.5 g and **GS10 (**R_f = 0. 25**)** in 50.7 mg.

3.3.4.1: Acid hydrolysis of GS9 and GS10.

Compounds **GS9** and **GS10** (each 10 mg) in a mixture of 2% HCl (2 mL) and MeOH (20 mL) were separately heated under reflux for 2 hrs. The reaction mixtures were heated to dryness, dissolved in H_2O (2 mL) and neutralized with NaOH. The neutralized products were then subjected to TLC analysis (solvent system: EtOAc-MeOH-H_2O-HOAc, 6:2:1:1) and paper chromatography (PC) (eluent: *n*-BuOH-HOAc-H_2O, 4:1:5). The chromatograms were sprayed with aniline hydrogen phthalate followed by heating at 100°C and the compounds were then identified after comparison with authentic samples. On the other hand, the aglycones were identified by TLC co-chromatography with authentic samples and ^{1}H NMR spectra.

3.3.5 Physical and spectral data of compounds isolated from methanol extracts of *G. subcordata* leaves

3.3.5.1. IsolateGS8: Amorphous yellow powder**,** R_f = 0.46 (silica gel, TLC, BAW; 4:1:5), 79.0 mg, m.p 315-317^oC; UV $\lambda_{max,}$ (MeOH) nm: 354 (band I), 304, 258 (band II), $AlCl_3$: 436 (band I), 316, 270 (band II), $AlCl_3$ + HCl: 402 (band I) 308, 272 (band II), NaOMe: 394 (band I), 322, 270 (band II), NaOAc: 366 (band I), 314, 272 (band II), NaOAc + H_3BO_3: 374 (band I), 300, 260 (band II); ^1H (600 MHz) and ^{13}C NMR data δppm: (See Table 4.8)*;* ESI-MS: *m/z* (%): 302 $[M]^+$ (45), 301 $[M-H]^+$ (100), 284 (2), 272 (10), 256 (5), 228 (15), 200 (1), 153 (23), 137 (30), 128 (14), 81 (10), 69 (23).

3.3.5.2. IsolateGS9: Amorphous yellow powder, m.p. ≈ 250C; R_f = 0.33 (silica gel, TLC, BAW, 4:1:5); UV λ_{max} (MeOH) nm: 354 (band I), 302, 262 (band II), $AlCl_3$: 406 (band I), 272 (band II), $AlCl_3$ + HCl: 400 (band I), 352, 300, 272 (band II), NaOMe: 396 (band I), 324, 272 (band II), NaOAc: 362 (band I), 264 (band II), NaOAc + H_3BO_3: 378 (band I), 262 (band II): IR ν_{max} (KBr) cm^{-1}, 3888.4, 2946.8, 1720.9, 1630.9, 1567.0, 1457.7, 1376.3, 1255.2, 1215.9, 1181.7, 1033.5, 986.9, 910.1; ^1H and ^{13}C NMR δppm: see Table 4.9; EI-MS: *m/z* (*rel. int*.) 287.6 $[M+H\text{-galactose}]^+$ (100), 244.8 (20), 228.0 (24), 135.0 (15), 55.0 (12), 43.3 (20).

3.3.5.3. IsolateGS10: Pale yellow powder R_f = 0.25 (solvent system: BAW, 4:1:5), m.p. 220-222^oC;^1H and ^{13}C NMR (CD_3OD) data δppm: see Table 4.10; EI-MS: *m/z* 390.0 (4), 260.9 (2), 229.8 (1), 218.0 (2), 177.0 (1), 166.8 (50), 148.8 (100), 121.8 (4), 113.0 (25), 97 (5), 70.9 (25), 56.9 (30), 42.9 (27).

3.4 Phytochemistry of *Ocimum kilimandscharicum* leaf extracts

The leaves of *Ocimum kilimandscharicum* were spread under shade for one week to dry and thereafter pulverized using model 4E grinding mill

3.4.1 Plant material preparation and solvent extraction.

The powdered leaf (2.0 kg) was extracted with MeOH at room temperature (3 x 6 L for 3 days). The extracts were combined, filtered and evaporated under reduced pressure to give a dark green MeOH extract (364.0 g). The extract was separately partitioned between H_2O and *n*-hexane and EtOAc to give the soluble layers: *n*-hexane extract (22.0 g, green material) and EtOAc extract (41.5 g, green material). The aqueous layer was freeze dried to give 300 g brownish-green extract.

3.4.2 Fractionation of *n*-hexane extract of *O. kilimandscharicum* leaves

Part of the *n*-hexane-soluble fraction (15.0 g) was mixed with silica gel (≈10.0 g) in 30 mL dichloromethane and solvent removed under *vacuo* to obtain a green solid. This was fractionated over silica gel column (5.0 x 30 cm, SiO_2 300 g, pressure ≈ 1 bar) using *n*-hexane and *n*-hexane-EtOAc (95:5, 9:1, 6:1, 4:1 and 3:1) to give 190 fractions, each 20 mL. Their homogeneity was monitored by TLC (solvent systems: *n*-hexane EtOAc, 9:1 and 4:1). Those that exhibited similar TLC profiles were combined resulting into five major pools (**OK1**-**OK5**). Pool **OK1** (*n*-hexane elution) was made up of fractions 1-20 which showed no spot on TLC and was set aside. Fractions 21-75 constituted pool **OK2** (*n*-hexane-EtOAc, 95:5 elution) produced yellow oil which lost colour with time and was set aside for it may have contained compounds that underwent structural changes. Pool **OK3** (*n*-hexane-EtOAc, 9:1 elution, fractions 77-100) gave a single spot R_f= 0.75 that was further purified by crystallization in CH_2Cl_2-MeOH mixture to give **OL1** (R_f = 0.75, 120.0 mg). Fractions 102-130 (pool **OK4** from *n*-hexane-EtOAc elution, 6:1, 1.0 g) gave two spots on TLC

with R_f values 0.75 and 0.65 and were separated by medium pressure chromatography to give further **OL1** (20.5 mg) and **OL2** (R_f = 0.65, 100.0 mg). Pool **OK5** (*n*-hexane-EtOAc, 6:1 and 4:1 elution, fractions 132-190, 2.5 g) afforded two spots of R_f values 0.65 and 0.53. These were resolved into individual components using medium pressure chromatography (column 2.0 x 24 cm, SiO_2 90 g; solvent system: *n*-hexane-EtOAc, 4:1) to give further **OL2** (90.0 mg) and **OL3** (R_f = 0.53, 70.5 mg).

3.4.3 Physical and spectroscopic data of compounds isolated from *n*-hexane extract of *O. kilimandscharicum* leaves

3.4.3.1 Isolate OL1: White amorphous powder with m.p 112-114^oC; R_f= 0.75 (silica gel, TLC, *n*-hexane-EtOAc, 4:1);^1H NMR δppm &^{13}C NMR δppm: see Table 4.11; EI-MS (rel. int): (%) 300.2 $[M]^+$ (30), 285.2 (26), 257.2 (17), 217.1 (10), 189.1 (50), 175.1 (11), 164.1 (20), 135.1 (25), 108.1 (30), 94.1 (100), 81.1 (25), (69.1 (24), 55.1 (15), 41.1 (15).

3.4.3.2. Isolate OL2: White crystals with m.p. 254-256^{o}C; R_f = 0.65 (silica gel, TLC, *n*-hexane-EtOAc, 4:1);^1H and ^{13}CNMR δppm: see Table 4.12; EI-MS (rel. int): *m/z* (%) 426.2 $[M]^+$ (24), 408.2 (9), 343.2 (12), 313.2 (27), 316.2 (12), 205.0 (6), 154.2 (9), 127.1 (21), 97.2 (32), 73.2 (100)

3.4.3.3. Isolate OL3: White crystals, m.p. 163-165^{o}C; R_f = 0.53 (silica gel, TLC, *n*-hexane-EtOAc, 4:1);^1H-NMR and ^{13}C-NMR δppm: see Table 4.13; EI-MS (rel. int): *m/z* 412.4 (16), 397.4 (10), 369.4 (7), 327.2 (8), 300.4 (10), 281.2 (28), 271.3 (65), 255.3 (23), 207.1 (50), 191.0 (9), 161.2 (15), 147.1 (25), 95.2 (30), 81.1 (60), 55.1 (100), 43.1 (89).

3.4.4 Fractionation of ethyl acetate extract of *O. kilimandscharicum* leaves

The ethyl acetate extract approximately 35.0 g was subjected to pass over silica gel column (5.0 x 40.0 cm, silica gel 300.0 g, pressure ≈1 bar) using *n*-hexane-ethyl acetate (10% increment of ethyl acetate), ethyl acetate neat and finally with CH_2Cl_2-MeOH (0.5 and 1% increment of MeOH). Three hundred and sixty (360) fractions (each 20 mL) were sampled and their composition similarly monitored by TLC; solvent system: *n*-hexane-ethyl acetate (4:1, 3:2 and1:1) and CH_2Cl_2-MeOH (99:1, 98:2 and 97:3). Those that exhibited similar TLC profiles were combined resulting into four major pools (**OK6-OK9**). Pool **OK6** (fractions 20–80, 3.5 g), upon removal of solvent, afforded a yellow oily paste which lost colour with time and was set aside. This is because the oil may have contained compounds that decomposed. Pool **OK 7** (fractions 83–120, 6.0 g) was similarly subjected to silica gel column and elution with *n*-hexane-ethyl acetate (4:1) followed by (3:2) afforded 70 fractions of 20 ml each, which were combined into two major fractions (**Oki** and **OKii**) depending on their TLC profiles. Fraction **OKi** contained two spots which upon further purification gave **OL3** (R_f = 0.53**,** 55.4 mg) and **OL4** (R_f = 0.48, 89.2 mg). Fraction **OKii** (1.5 g) on evaporation of the solvent crystallized out to give white powder, which upon re-crystallization (*n*-hexane-EtOAc, 3:2) gave **OL5 (**R_f = 0.43**,** 75.4 mg). Fractions 125–250 constituted pool **OK 8** (7.0 g), which showed three spots of R_f values 0.48, 0.43 and 0.35 (eluent: *n*-hexane-EtOAc, 3:2). Repeated medium pressure chromatography using *n*-hexane-EtOAc (4:1 followed by 3:2) afforded a further **OL4** (70.6 mg), **OL5 (**15.1 mg) and **OL6** (R_f= 0.35, 43.3 mg). Fractions 253-357 (Pool **OK9** (8.5 g) showed three spots with R_f values 0.51, 0.44 and 0.37 on TLC using CH_2Cl_2-MeOH (99:1). The constituents of this pool were separated using medium pressure chromatography [solvent system: *n*-hexane-EtOAc (1:1) followed by CH_2Cl_2-MeOH (99:1)] to give **OL7 (**R_f = 0.51, 35.0 mg), **OL8** (R_f = 0.44, 27.0 mg) and **OL9** (R_f = 0.37, 24 mg).

3.4.5 Physical and spectroscopic data of compounds isolated from ethyl acetate extract of *O. kilimandscharicum* leaves

3.4.5.1. IsolateOL4: White crystals with R_f = 0.48(solvent system: *n*-hexane-EtOAc, 3:2), m.p 216-218^{o}C; ^{1}H and ^{13}C NMR spectral data δppm: see Table 4.14; ESI-MS: *m/z* (rel. int) 426 $[M]^+$(20), 411 (10), 393 (2), 370 (1), 315 (4), 299 (2), 272 (3), 257 (3), 234 (5), 218 (45), 207 (51), 189 (50), 175 (20), 147 (25), 135 (55), 109 (60), 95 (75), 81 (47), 68 (95), 43 (100)

3.4.5.2 Isolate OL5: White powder from CH_2Cl_2-MeOH (9:1); m.p 191–193^{o}C; R_f=0.43, (solvent system: *n*-hexane-EtOAc, 3:2); $[\alpha]^{25}_D$ + 19^o(c 0.05, MeOH); IR ν_{max} (KBr) cm^{-1}: 3357.3, 2930.1, 1764.1, 1589.0, 1454.0, 1121.1; ^{1}H and ^{13}C NMR spectral data δppm: see Table 4.15; EI-MS(rel. int.) *m/z* 440.3 $[M]^+$ (2), 391.2 (4), 390.2 (12), 364.2 (15), 362.2 (36), 336.2 (12), 308.2 (2), 249.1 (16), 219.1 (100), 163.0 (88), 134.0 (40), 105.0 (16), 91.0 (9); HRESI-MS 463.3430 [M + Na]+ (calcd for $C_{30}H_{48}O_2$+Na, 463.3424).

3.4.5.3 Isolate OL6: White crystals from CH_2Cl_2-MeOH with m.p. 198-199^{o}C; R_f = 0.35, (solvent system: *n*-hexane-EtOAc, 3:2); $[\alpha]^{25}_D$ + 59^o (c 0.1, MeOH);^{1}H and ^{13}C NMR spectral data δppm: see Tables 4.16; EI-MS (rel. int.) *m/z* 442.1 $[M]^+$ (12), 424.0 (13), 409.0 (10), 371.0 (100), 353.0 (5), 329.0 (3), 313.0 (4),287.0 (3), 274 0 (5), 247.0 (7), 217.9 (25), 204.9 (42), 189.0 (23), 164.9 (34), 150.9 (42), 108.9 (45), 94.9 (42), 80.9 (30), 54.9 (20), 42.9 (27); ESI-MS m/z 465.4 $[M+Na]^+$; HRESI-MS 465.3430 $[M+Na]^+$ (calcd for $C_{30}H_{48}O_2$+Na, 465.3424)

3.4.5.4. Isolate OL7: Yellow powder, m.p. 285-287^{o}C; R_f =0.51 (solvent system: CH_2CL_2 9:1); ^{1}Hand ^{13}CNMR data δppm: See Table 4.17; ESI-MS (%): *m/z* 255 $[M+H]^+$ (70), 210 (47), 186 (32), 153 (70), 143 (75), 110 (52), 104 (100).

3.4.5.5 Isolate OL8: Pale yellow amorphous powder, (R_f = 0.44, solvent system: CH_2Cl_2-MeOH, 9:1)**;** m.p. 346 -348^{o}C; ^{1}H and ^{13}C NMR data, δppm: See Table 4.18; EI-MS: *m/z* (%): 270.2 $[M]^+$

(12), 201.1 (1), 175.1 (5), 159.1 (18), 151.1 (20), 134.1 (25), 109.1 (60), 93.0 (40), 81.1 (65), 43.1 (100)

3.4.5.6 Isolate OL9: Yellowish amorphous powder, R_f = 0.37 (solvent system: CH_2Cl_2-MeOH, 99:1) and m.p. 299-300^oC; UV (MeOH) λ_{max} nm 252 and 354; IR ν_{max} (KBr) cm^{-1}: 3366.6 (OH), 1631.1 (C=O), 1565.4, 1430.5 (-C=C-), 1311.9, 1215.3(-C-O-bend), 1114.5, 1037.5 (-C-O-stretch), 928.6 (-C-H out of plane bending); ^1H and ^{13} C NMR δppm. See Table 4.19; EI-MS *m/z* (rel. int): 286.2 $[M]^+$ (15), 218.1 (10), 206.1 (5), 175.1 (4), 161.1(6), 150.1 (10), 137.2 (21), 135.1 (22), 121. 1 (45), 93.1 (50), 82.1 (100), 67.1 (40), 41.1 (40)

3.4.6 Fractionation of aqueous MeOH extract of *O. kilimandscharicum* leaves

Approximately 100 g of the freeze dried (MeOH-H_2O soluble fraction) extract was chromatographed over silica gel column (5.0 x 40 cm, SiO_2 500g, pressure ≈ 1.5 bar) using CH_2Cl_2-MeOH (10% increment of MeOH) and MeOH neat to give a total of 200 fractions (each 50 ml). Their homogeneity was monitored by TLC using CH_2Cl_2-MeOH (98:2, 97:3, 95:5, 9:1, and 4:1) and the fractions were grouped into two pools (**OK10** and **OK11**) depending on TLC profile. Pools **OK10** and **OK11** (fractions 20–150, 9 g) were combined and on repeated medium pressure chromatography separation using CH_2Cl_2-MeOH (98:2) followed by the same solvents in the ratio (97:3) gave **OL10** (R_f = 0.46, 79 mg) and **OL11** (R_f = 0.19, 41 mg).

3.4.7 Acid hydrolysis of compound OL11.

Compound **OL11** (10 mg) in a mixture of 2% HCl (2 mL) and MeOH (20 mL) was heated under reflux for 2 hrs. The reaction mixture was reduced under pressure to dryness, dissolved in H_2O (2 mL) and neutralized with NaOH. The neutralized product was then subjected to TLC analysis (solvent system: EtOAc-MeOH-H_2O-HOAc, 6:2:1:1) and paper chromatography (PC) (eluent: *n*-

BuOH-HOAc-H_2O, 4:1:5). The chromatograms were sprayed with aniline hydrogen phthalate followed by heating at 100^oC and then identified after comparison with authentic samples.

3.4.8 Physical and spectroscopic data of compounds isolated from aqueous MeOH extract

3.4.8.1 Isolate OL10: Amorphous yellow powder**,** R_f = 0.46, m.p 314-316^oC (Lit. 316-318^oC; Esra *et al*., 2015); UV $\lambda_{max,}$ (MeOH) nm: 354 (band I), 304, 258 (band II), $AlCl_3$: 436 (band I), 316, 270 (band II), $AlCl_3$ + HCl: 402 (band I) 308, 272 (band II), NaOMe: 394 (band I), 322, 270 (band II), NaOAc: 366 (band I), 314, 272 (band II), NaOAc + H_3BO_3: 374 (band I), 300, 260 (band II); IR ν_{max} (KBr) cm^{-1}: 3500-2500 (OH), 1610 (conjugated C=O), 1450, 1340, 1250, 930, ^1H (600 MHz) and ^{13}C NMR data see table 4.8, δppm: 12.60 (s, 5-OH), 7.59 (d, *J*= Hz, H), 7.53 (d, *J*= Hz, H-), 6.40 (d, *J*=2.1 Hz, H-8), 6.20 (d, *J*=2.0 Hz, H-6); ESI-MS: *m/z* (%): 303.4 $[M+3H]^+$ (100), 272.3 (8), 228.5 (10), 153.2 (11), 137.1 (20), 69.5 (10).

3.4.8.2. Isolate OL11: Amorphous yellow powder R_f = 0.19 (solvent system; CH_2Cl_2-MeOH, 97:3), m.p. 346-348^oC; ^1H and ^{13}C NMR data δppm: (See Table 4.20**)***;* ESI-MS: *m/z* (%): 519.4 $[M+Na]^+$

3.5 Phytochemical analysis of *Annona mucosa* leaves

The leaves of the *Annona mucosa* were air dried under a shade for four weeks and then ground into powder in a model 4E grinding mill.

3.5.1 Solvent extraction of *A. mucosa* leaf powder

Air dried, pulverized leaves (2.5 kg) were sequentially soaked in *n*-hexane (3 x 4.5 L), EtOAc (3 x 4.5 L) and MeOH (3 x 4.5 L), each lasting three days at room temperature, with occasional shaking. The extracts were separately filtered and evaporated under vacuum using a rotary evaporator to

afford green (23 g), dark green (45 g) and brownish-green (126 g) extracts of *n*-hexane, EtOAc and MeOH, respectively.

3.5.2 Thin layer chromatography (TLC) analysis of *n*-hexane and ethyl acetate leaf extracts of *A. mucosa*.

TLC analysis of *n*-hexane extract showed three major spots of R_f values 0.82, 0.71 and 0.54 (solvent system: *n*-hexane-EtOAc, 4:1), of which the latter two spots turned greenish-purple on spraying with anisaldehyde-sulphuric mixture followed by heating on hot plate at 100^{o}C. On the other hand, TLC analysis of EtOAc extract using *n*-hexane-EtOAc (4:1) afforded six spots of R_f values 0.82, 0.71, 0.54, 0.45, 0.32 and 0.20 of which the latter five spots also turned greenish-purple on spraying with anisaldehyde-sulphuric reagent. Change of developing solvent system to *n*-hexane-EtOAc (3:2) gave four additional spots of R_f values 0.57, 0.47 and 0.30. Upon spraying the spots with anisaldehyde-sulphuric acid mixture followed by heating the spots turned greenish-purple. Ultra-violet light and iodine vapour were also used to confirm the number of spots on TLC.

3.5.3 Isolation of phytoconstituents in the *n*-hexane leaf extract of *A. mucosa*

The*n*-Hexane extract (20 g) was mixed with 5 g of silica gel in 100 mL of dichloromethane. The solvent evaporated under vacuum and the free flowing solid mixture chromatographed over silica gel column (4.0 x 40 cm, SiO_2, 300 g; pressure ≈1 bar) using *n*-hexane with increasing amount of EtOAc up to 100%. A total of 190 fractions, each 20 mL were collected and their homogeneity monitored by TLC (solvent systems: *n*-hexane EtOAc, 9:1 and 4:1). The fractions were grouped into four major pools (**1-IV**) depending on TLC profiles. Fractions 1-60 constituted pool **I**, which upon evaporation of solvent afforded a yellow oily substance (R_f= 0.82; solvent system: *n*-hexane-

EtOAc, 4:1) that lost colour with time and was set aside for the compound may have undergone structural changes with time. Pool **II** (fractions 62-95, 2.7 g) showed a single spot of R_f = 0.71 (solvent system: *n*-hexane-EtOAc, 4:1) which on crystallization (CH_2Cl_2-MeOH, 9:1) gave **AM1 (**R_f = 0.71, 100 mg) as white solid compound. Pool **III** (fractions 97-141, 1.5 g) also gave a single spot of R_f value 0.54 (eluent: *n*-hexane-EtOAc, 4:1), which on evaporation of solvent followed by crystallization (CH_2Cl_2-MeOH, 9:1) afforded **AM2 (**200 mg). Fractions 144-183 (3.0 g) constituted pool **IV**, which showed a major spot of R_f = 0.54 contaminated with chlorophyll and was further purified by crystallization to give a further 70 mg of **AM2**

3.5.4 Physical and spectroscopic data of compounds from *n*-hexane extract of *A. mucosa* leaves

3.5.4.1 Isolate AM1: White powder with a melting point of 192-194^oC; R_f = 0.71(silica gel TLC, *n*-hexane-EtOAc, 4:1); IR V_{max} (KBr) cm^{-1}: 3444.3, 2945.0, 2361.2, 1733.9, 1649.6, 1457.5, 1370.2, 1246.1, 1026.1, 901.7;^1H and ^{13}C NMR ($CDCl_3$) δppm: see Table 4.21; EI-MS *m/z* (rel. int.): 468.0 $[M]^+$ ($C_{32}H_{52}O_2$); 453.1 (2), 393.0 (4), 368.1 (1), 325.0 (1), 293.0 (1), 272.0 (3), 249.0 (10), 283.0 (1), 218.0 (100), 203.0 (15), 189.0 (20), 161.0 (8), 134.9 (11), 121.9 (10), 94.9 (15), 68.9 (14), 54.9 (8), 42.9 (9)

3.5.4.2Isolate AM2: White amorphous powder, m.p 137-138^oC; R_f = 0.54 (silica gel TLC, *n*-hexane-EtOAc, 4: 1).IR ν_{max} (KBr) cm^{-1}: 3444.3, 2945.0, 1733.9; 1649.3, 1144.5, 1025.7, 901.7, 823.6, 666.4, 607.4; ^1H and ^{13}C NMR ($CDCl_3$) *δ*ppm see Table 4.22. Formula $C_{29}H_{50}O$ m/z =414. EI-MS (70ev); m/z (rel. int.): 414 (100), 396 (70), 381 (40), 329 (50), 303 (50), 288 (5), 273(25), 255(35), 213 (40), 199 (14), 173 (15), 159 (35), 145 (50), 95 (36), 81 (35), 55 (27).

3.5.5 Fractionation of EtOAc extract of *A. mucosa* leaves

Approximately 40 g of the extract was adsorbed onto 10.0 g silica gel immersed in 100 mL of EtOAc. The solvent was removed and the mixture subjected to column chromatography (column 4.0 x 60 cm, SiO_2 300 g, and pressure ≈1 bar) using *n*-hexane-EtOAc mixture with increment of 10% of the more polar solvent up to 100% EtOAc and elution concluded with CH_2Cl_2-MeOH (99:1 and 95:5). A total of 323 fractions each 20 mL were collected, leading to pools **V-XI** as determined by TLC profiles using *n*-hexane-EtOAc (4:1, 3:2 and 1:2) and CH_2Cl_2-MeOH (99:1 and 95:5) solvent systems. Pool **V** (fractions 1-60) was a yellow oily substance which lost colour with time and was set aside due to the likely structural change. Pool **VI** (fractions 64-90, 2.90 g) showed a single spot which upon concentration and crystallization in CH_2Cl_2-MeOH (9:1) afforded a further **AM2** (25 mg). Pools V**II** and **VIII** (fractions 93-170, 4.5 g) which were combined due to similarity, showed two spots of R_f values 0.54 and 0.45 (contaminated with chlorophyll) on TLC using *n*-hexane-EtOAc (4:1) solvent system. Repeated column chromatography using *n*-hexane-EtOAc (4:1) led to the isolation of more **AM2** (20 mg) and **AM3 (**R_f= 0.45, 65 mg). Pool **IX** (fractions 172-210, 3.4 g) gave two spots of R_f values 0.32 and 0.20 (solvent system: n-hexane-EtOAc 4:1). Both spots turned purple on TLC after spraying with *p*-anisaldehyde-sulphuric acid mixture followed by heating. The compounds were separated using medium pressure chromatography with solvent system; *n*-hexane-EtOAc (4:1) to give **AM4** (R_f= 0.32, 46.5 mg) and **AM5 (**R_f = 0.20, 71.0 mg). Pool **X** (fractions 213-270, 3.5 g) on TLC analysis using *n*-hexane-EtOAc (3:2) gave two spots of R_f values 0.57 and 0.47. Upon repeated chromatographic separation (column 2.4 x 60 cm, SiO_2 = 200 g, pressure ≈ 1 bar) using solvent system *n*-hexane-EtOAc (3:2) followed by the same solvent system in the ratio 2:3 gave **AM6 (**R_f = 0.57, 76.4 mg) and **AM7 (**R_f = 0.47, 40 mg). Fractions 272-320 which constituted pool **XI** (4.1 g) showed two major spots R_f = 0.47 and 0.30 (eluent: *n*-hexane-

EtOAc, 2:3) which were contaminated with chlorophyll. On repeated chromatographic separation (column 2.5 x 50 cm, SiO_2 150 g, pressure≈1 bar) using the same solvent mixture, followed by crystallization (CH_2Cl_2-MeOH, 4:1) gave a further **AM7** (24.2 mg) and **AM8** (R_f = 0.30, 55.5 mg)

3.5.6 Physical and spectroscopic data of compounds from EtOAc extract of *A. mucosa* leaves

3.5.6.1. Isolate AM3: White amorphous powder, m.p. 176-178ºC; $[\alpha]_D^{25}$+ 14º ($CHCl_3$, c 0.50); R_f = 0.45 (silica gel TLC, *n*-hexane-EtOAc, 4:1). IR ν_{max} (KBr) cm^{-1}: 2848.9, 2814.1, 1737.3, 1457.0, 1376.3, 1248.3, 1191.2, 1028.0, 986.2, 914.5, 823.4, 657.1, 606.0; ^1H and ^{13}C ($CDCl_3$) NMR δppm: see Table 4.23; EI-MS (70 ev): *mz* (rel. Int.) 526.3 $[M]^+$ (13), 511.2 (3), 468.3 (2), 466.3 (4), 391.2 (3), 307.0 (5), 272.1 (4), 247.1 (6), 218.1 (100), 203.0 (38), 161.0 (10), 135.0 (12), 109.0 (15), 95.0 (17), 68.9 (12), 42.9 (22).HRESI-MS *m/z* 526.79016 **(**cald 526.78943 for $C_{34}H_{54}O_4$).

3.5.6.2 Isolate AM4: The compound was isolated as white amorphous powder, m.p.170-174ºC; R_f = 0.32 (silica gel TLC, *n*-hexane-EtOAc, 4:1); IR ν_{max} (KBr) cm^{-1}; 3400.3, 2954.7, 1702.4, 1454.5, 1378.4, 1112.2 and 835.4; ^1H and ^{13}C NMR ($CDCl_3$) δ_{ppm}: see Table 4.24**;** EI-MS (70 eV):*m/z* (rel. Int.) 440.1 $[M]^+$ (96), 422.1 (100), 407.1 (18), 371.1 (5), 355.1 (6), 341.1 (4), 311.1 (3), 287.0 (14), 273.0 (46), 234.1 (100), 255.1 (15), 205.1 (25), 191.1 (91), 163.0 (19), 135.0 (43), 95.0 (76), 69.0 (58), 55.0 (40), 43.1 (26)

3.5.6.3 Isolate AM5: White amorphous solid, m.p. 61-62ºC; R_f = 0.20 (silica gel TLC, *n*-hexane-EtOAc, 4:1); IR ν_{max} (KBr) cm^{-1} 3387.7, 1729.8, 1631.3, 1242.1; ^1H and ^{13}C NMR δppm: see Table 4.25; EI-MS (70 ev): *m/z* (rel. Int.) 468.1 $[M\text{-}H_2O]^+$(1), 418.0 (2), 408.1 $[M\text{-}H_2O\text{-}CH_3COOH]^+$(1), 306.9 (5), 292.9 (18), 278.9 (13), 218.0 (10), 203.0 (3), 189.0 (4), 166.8 (37), 148.8 (100), 113.0 (13), 97.0 (5), 71.0 (35), 56.9 (38), 42.9 (25).

3.5.6.4 Isolate AM6: white amorphous powder, m.p. 218-220ºC; R_f = 0.57 (silica gel TLC, *n*-hexane-EtOAc, 2:3) IR ν_{max} (KBr) cm^{-1}: 2940.1, 2861.1, 2361.2, 1731.1, 1456.3, 1379.9, 1278.2, 1237.8, 1188.4, 1111.7, 1028.5, 988.6, 895.0, 825.4, 795.3, 754.2, 728.7, 667.6, 627.5, 546.7; ^{I}H and ^{13}C NMR ($CDCl_3$) *δ*ppm: see Table 4.26; EI-MS: m/z (rel. int.) 498.0 (13), 483.0 (5), 423.0 (6), 279.9 (5), 255.0 (1), 218.0 (100), 203.0 (38), 161.0 (12), 134.9 (18), 94.9 (22), 68.9 (15), 42.9 (13).

3.5.6.5 Isolate AM7. white amorphous powder, m.p. 212-214ºC; R_f = 0.47 (silica gel TLC, *n*-hexane-EtOAc, 3:2);^{1}H and ^{13}C NMR δppm: see Table 4.27; EI-MS: *m/z* (rel. int.) 498.1 (11), 423.1 (100), 405.1 (2), 377.1 (10), 341.0 (1), 327.0 (2), 301.0 (3), 281.1 (10), 187.0 (13), 159.1 (6), 135.0 (8), 119.0 (10), 69.0 (13), 40.0 (9).

3.5.6.6 Isolate AM8: White amorphous powder, m.p. 302-305ºC; R_f = 0.30 (silica gel TLC, *n*-hexane-EtOAc, 3:2); ^{I}H and ^{13}C NMR δppm: see Table 4.28; ESI-MS (negative mode) 456.5 $[M-H]^{+}$(65), 438.4 (20), 423.4 (10), 411.4 (8), 369.4 (6), 328.3 (2), 316.3 (5), 320.3 (10), 259.2 (5), 248.2 (70), 220.2 (30), 202.2 (65), 189.2 (100), 175.2 (30), 147.2 (22), 135.1 (38), 107.1 (30), 95.1 (37), 81.1 (37), 69.1 (40), 43.1 (35), 41.0 (25)

3.5.7: Thin layer chromatography (TLC) analysis of methanol leaf extract of *A. mucosa*.

TLC analysis of MeOH leaf extract on non-deactivated silica gel (solvent system: CH_2Cl_2-MeOH, 99:1, 98:2, 97:3, 95:5, 4:1 and 1:1; *n*-BuOH-HOAc-H_2O, 4:1:5) revealed two distinct spots of R_f values 0.30 and 0.46. The former spot gave a purple colour upon spraying with *p*-anisaldehyde-sulphuric mixture, while the latter showed an intense yellow colour when exposed to conc. ammonia vapour suggesting the presence of flavonoid derivatives. However, when deactivated silica gel TLC plates (2 % oxalic acid solution) were used with solvent system CH_2Cl_2-MeOH (4:1),

two additional yellow spots with R_f values 0.34 and 0.21 were observed. The yellow colour of the spots intensified on exposure to concentrated ammonia vapour suggesting the presence of flavonoid derivatives.

3.5.8 Fractionation of the methanol extract of *A. mucosa* leaves

MeOH extract (40.0 g) was mixed with 20.0 g of silica gel, dried and subjected to column chromatography (5.0 x 48.0 cm; SiO_2 300.0 g; pressure ≈1 bar), eluting with CH_2Cl_2 followed by CH_2Cl_2-MeOH mixture with increasing concentration of the more polar solvent (increment 10%) and finally with 100% MeOH. A total of 150 fractions, each 50 mL were sampled and their homogeneity determined by TLC (eluent: CH_2Cl_2-MeOH, 99:1, 98:2, 97:3, 95:5, 4:1 and 1:1; *n*-BuOH-HOAc-H_2O, 4:1:5) and those exhibiting similar profiles were combined into two major pools (**XII** and **XIII**). Pool **XII** (fractions 20-35, 4.0 g) eluted with CH_2Cl_2-MeOH (99:1, 98:2) afforded a mixture of compounds which were separated using medium pressure column chromatography (2.5 x 24.0 cm, SiO_2 150.0 g, pressure ≈ 1 bar) to give **AM8 (**R_f = 0.30, 89.5 mg) and **AM9 (**R_f = 0.44, 20.3 mg). Fractions 36-120 (8.0 g) eluted using CH_2Cl_2-MeOH (95:5, 9:1 and 4:1)constituted pool **XIII** which was further repeatedly fractionated over 2% oxalic acid deactivated silica gel with CH_2Cl_2-MeOH (4:1) to give **AM10 (**R_f = 0.34, 30.1 mg) and **AM11** (R_f = 0.21, 24.4 mg)

3.5.8.1 Acid hydrolysis of compounds AM10 and AM11.

A 2% HCl solution containing 10 mg each of compounds **AM10** and **AM11**were separately heated under reflux for 2 hours and there after solvent removed under *vacuo*. The residues were dissolved in MeOH (10 ml) and neutralized with a drop of NaOH solution. The resulting solution was extracted with *n*-hexane and solvent removed under vacuum. The residues were crystallized in 5% aqueous MeOH (2 mg). A comparison analysis on 2% oxalic acid deactivated silica gel TLC (solvent system: CH_2Cl_2-MeOH, 4:1) of the aglycone with authentic quercetin afforded R_f value of 0.46. Similarly, the aqueous residues when compared with authentic samples of glucose, arabinose and galactose on TLC using solvent system (EtOAc-MeOH-H_2O-HOAc, 6:2:1:1) gave R_f values of 0.50 and 0.60 respectively, after spraying with aniline hydrogen phthalate followed by heating on hot plate for 1 minute.

3.5.9 Physical and spectral data of compounds from the methanol extract of *A. mucosa* leaves

3.5.9.1. IsolateAM9: Amorphous yellow powder(R_f = 0.46, 79.0 mg), m.p. 314-316^oC; UV $\lambda_{max,}$ (MeOH) nm: 354 (band I), 304, 258 (band II), $AlCl_3$: 436 (band I), 316, 270 (band II), $AlCl_3$ + HCl: 402 (band I) 308, 272 (band II), NaOMe: 394 (band I), 322, 270 (band II), NaOAc: 366 (band I), 314, 272 (band II), NaOAc + H_3BO_3: 374 (band I), 300, 260 (band II); IR ν_{max} (KBr) cm^{-1}: 3500-2500 (OH), 1610 (conjugated C=O), 1450, 1340, 1250, 930, ^1H (600 MHz) and ^{13}C NMR data δppm: (See Table 4.8)*;* ESI-MS: *m/z* (%): 302 $[M]^+$ (100), 272 (8), 228 (10), 153 (11), 137 (20), 69 (10).

3.5.9.2. IsolateAM10: Amorphous yellow powder, m.p = 250^oC, R_f = 0.34 (silica gel TLC, CH_2Cl_2-MeOH, 4:1);UV λ_{max} (MeOH) nm: 358 (band I), 302, 258 (band II), $AlCl_3$: 434 (band I), 274 (band II), $AlCl_3$ + HCl: 400 (band I) 360, 300, 270 (band II), NaOMe: 410 (band I), 328, 272(band II),

NaOAc: 382 (band I), 322, 274 (band II), NaOAc + H_3BO_3: 378 (band I), 262 (band II):^{1}H and ^{13}C NMR δppm; see Table 4.29, EI-MS (70 ev): *m/z* 301 (rel. Int): (100), 289 (11), 245(2), 216 (8) 153(10), 136 (15), 108 (3).

3.5.9.3: Isolate AM11: A greenish-yellow amorphous powder, m.p.= 250^{o}C, R_f = 0.21 (solvent system: CH_2Cl_2-MeOH, 4:1); UV λ_{max} (MeOH): 366 (band I), 304, 258 (band II), MeOH + $AlCl_3$: 446 (band I), 312, 272 (band II), MeOH +$AlCl_3$/HCl: 414, 310, 272, MeOH +NaOMe: 414, 324, 270, MeOH + NaOAc/H_3BO_3:386, 300, 260, ^{1}H and ^{13}C, δppm: see Table 4.30, EI-MS (*rel. int*)): 463.2 (25), 440.0 (5), 423.0 (8), 386.0 (2), 357.0 (1), 343.1 (5), 326.6 (3), 302 (80), 275.0 (3), 271.2 (4), 255.2 (4), 228.1 (2), 216.8 (2), 173.1 (3).

3.6: The bioassays

3.6.1 Test insects.

Stock cultures of *Sitophilus zeamais* and *Prostephanus truncatus* were obtained from a colony maintained at National Agricultural Research Laboratories (NARL), Nairobi, Kenya. The experiments were conducted in the Kenya Agricultural and Livestock Research Organization (KALRO) laboratories, Kitale. Adult insects were kept in glass jars at 28^{o}C and 75% relative humidity and reared on maize cobs (Kossou *et al*., 1992).Adults were removed from the jars after two weeks, after egg oviposition. The insects were then cultured in the laboratory at 27 ± 2^{o}C, 60 - 65% relative humidity and 12 hr: 12 hr light-dark regime. The jars were left undisturbed and emerging adults were collected. The freshly emerged adults were then used for the experiments (Rugumamu, 2005).

3.6.2. Contact toxicity assay

Contact toxicity assay was done following the method of Obeng-Ofori &Reichmuth (1997). The experiments were carried out in the laboratory at 27 ± 2^{o}C, 65-70% relative humidity and L12: D12 regime. The temperature and humidity were controlled in thermo-regulators. Different solutions of the extracts in concentrations of 30, 60, 150 and 300 µg in 1 mL of acetone and pure isolates in concentrations of 10, 30, 50 and 100 µg in 1 mL of acetone were prepared. Three to seven day old insects of mixed sex were first transferred into the Petri dishes lined with moist filter paper and chilled for three minutes to reduce their mobility and enable topical treatment to be carried out. The immobilized insects were picked individually for treatment and 0.5 µL of the solution was applied to the dorsal surface of the thorax of each insect using Hamilton's syringe (700 series, Microliter TM Hamilton Company, USA). Thirty insects in three replicates of 10 insects each were treated with each dose. The same numbers of insects were each treated with acetone only and deltamethrin a commercial insecticide, as the negative and positive control respectively. After treatment, the insects were transferred into 11.0 cm diameter glass Petri dishes (10 insects per Petri dish), containing maize seeds. The treatments were laid out in a completely randomized design.

The insects were examined daily for two days and those that did not move or respond to three probings with a blunt probe, were considered dead. Insect mortalities were recorded at 48 hours after treatment. This procedure was followed for hexane, ethyl acetate, methanol/aqueous extracts and pure isolates, on both *S. zeamais* and *P. truncatus.*

Corrected percent mortality was calculated using Abbot's formula (Abbot, 1925) as follows:

$$\% \text{ Mortality (adjusted)} = \frac{(\% \text{ DT} - \% \text{ DC})\text{X}100)}{(100 - \%DC)}$$

Where: DT = dead insects in test; DC = dead insects in control. Probit analysis was performed to calculate the lethal concentration for 50% (LC_{50}) insect mortality (Finney, 1971). The Microsoft excel, was used in determination of the LSD values (Fatunbi, 2009).

3.6.3 Antifeedant assay

Antifeedant activities of the plant extracts and pure isolates were determined using leaf disc no choice bioassay method of Arivoli & Tennyson, (2013) with some modification. Flour disks were prepared by mixing 10g of maize flour with 50 mL of water until the flour was completely suspended. The maize flour suspension was pipetted (200 µL) onto a plastic sheet, held for 24 hours at room temperature and then dried in an oven at 60 ^{o}C for one hour. Fresh maize flour leaf discs (1350sq.mm) were separately dipped in solutions of each plant extract and pure isolates of concentrations; 30, 60, 150 and 300 µg/mL for extracts and 10, 30, 50 and 100µg/mL for pure isolates, respectively with acetone as the solvent. After solvent evaporation at room temperature, the flour leaf discs were kept in individual Petri dishes (9 cm diameter). In each Petri dish a single 2 hours pre-starved adult *S. zeamais* or *P. truncatus* was introduced. The insects were pre-starved to make them have an urge to feed. The insects were allowed to feed on treated discs for twenty-four hours. The leaf discs sprayed with acetone and azadirachtin, a commercial antifeedant served as negative and positive controls respectively. At the end of the experiment, unconsumed area of leaf disc was measured with the aid of a leaf area meter. Each experiment was repeated three times. The antifeedant index was calculated based on the formula of (Zhang *et al*., 2018). Antifeedant index (AI) (%) = [(C - T)/C] x100

Where C is the leaf disk consumed in the blank control and T is the leaf disc consumed in the treated groups. The AFI_{50}, which is the effective concentration for 50% antifeedant activity of a substance

relative to the control (Huang *et al*., 2008) and the confidence upper and lower limits, were calculated by subjecting the data to probit analysis (Finney 1971).The Microsoft excel, was used in determination of the LSD values (Fatunbi, 2009).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Biological activities of the leaf extracts of *G. subcordata*, *O. kilimandscharicum* and *A. mucosa*

The crude leaf extracts of the three plants were investigated for their bioactivity against *Sitophilus zeamais* and *Prostephanus truncatus* by use of contact toxicity and antifeedant bioassays. The reports given about the activities of the extracts are presented herein.

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4.1.1 Contact toxicity activities

4.1.1.1 Contact toxicity activities of the crude extracts against *S. zeamais*

In the investigation for the contact toxicity activities of *n*-hexane, ethyl acetate and methanol extracts of the leaves from the three plants, the method of Obeng-Ofori &Reichmuth, (1997) was applied. Results from the tests revealed that all the plant extracts were active against *S. zeamais* and the activities exhibited a concentration-dependent response. Methanol extracts of the three plants had the highest contact toxicity activities among the extracts (Figure 4 a, b and c). The activities of the methanol extracts of *G. subcordata* were not significantly different from those of the positive control ($P \geq 0.05$).The higher activities of the methanol extracts could be due to the presence of polar compounds which are known to be important sources of potent insecticides (Obeng-Ofori *et al*, 1997; Obeng-Ofori & Riechmuth, 1997). It may also be due to the synergistic effects of the compounds present in the extracts. The ethyl acetate extracts showed moderately high activities that were significantly different ($P \leq 0.05$) from those of the positive control. The moderate activities of ethyl acetate extracts could be due to the presence of less polar compounds, fewer polar compounds or reduced synergistic effects among the compounds. The *n*-hexane extracts had the lowest

activities against the insect (Figure 4a, b, and c). This may be due to the presence of less polar compounds. Among the plants, *G. subcordata* extracts had the highest relative contact toxicities (Figure 4a). All these activities were however lower than those of the positive control. The activities of the methanol extract of *G. subcordata* had no significant difference from activities of the positive control ($p \geq 0.05$). The higher contact toxicity of *G. subcordata* extracts may be attributed to the kind of compounds that are present in the extracts that might be having high contact toxicities. The activities of *O. kilimandischaricum* (Figure 4b) tallies well with the results of Kaguchia *et al*., (2018), in which there was 100% mortality of *S. zeamais* at 105µl/mL concentration of *O. kilimandscharicum* leaf essential oils. In their assay, essential oils of the plant were used in the place of crude leaf extracts as for the current study. .*A. mucosa* extracts had the lowest activities against the insect (Figure 4c). The low insecticidal activity of *A. mucosa* leaf extracts compares well with the previously reported low contact toxicity activity of the plant's stem extracts against *Euchistus heros* nymphs (Turchen *et al.*, 2016). The seed extracts of *A. mucosa* were however shown to have high insecticidal activities (LC_{50}; 0.184 µg/mL) (Rivera & Alvarez, 2018) when tested against *Corythucha gossypii*. The low activities of the leaf extracts of *A. mucosa* as compared to activities of the seed extracts was possibly due to low concentration of bioactive compounds such as acetogenins that are highly concentrated in seed extracts, which could have led to the high insecticidal activities (Rivera & Alvarez, 2018).

The current results have revealed that plant extracts exhibit different levels of mortalities against *S. zeamais* at different concentrations which could be attributed to the different chemical constituents in the extracts. These extracts would therefore be good candidates for isolation of compounds with insecticidal activities against *S. zeamais*. The favourable contact toxicity activities of these extracts validate the traditional use of the plants in stored food insect pest control.

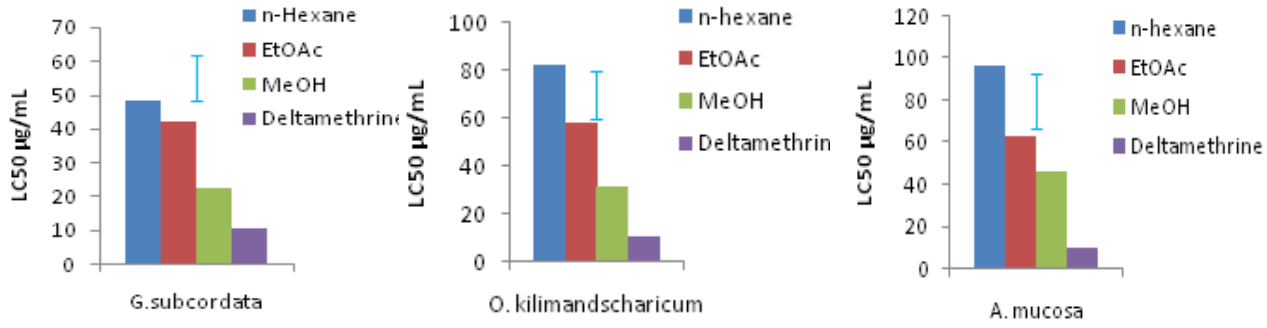


Figure 4: Contact toxicity activities of (a) *G. subcordata* (b). *O. kilimandscharicum* (c). *Annona mucosa* extracts (LC_{50} µg/mL) against*S. Zeamais*.

4.1.1.2 Contact toxicity activities of the crude extracts against *P. truncatus*

The contact toxicity results for the three plant extracts against *P. truncatus* followed the same trend as for *S. zeamais*, with activities depending on concentration of the extracts. Methanol extracts of the three plants had relatively high contact toxicities (Figure 5a, b, and c). The contact toxicity activity of the methanol extract of *G. subcordata* was lower and significantly different from that of the positive control ($P \leq 0.5$). The *n*-hexane extracts showed the lowest activities (Figure 5a, b, and c). This may have been due to the lower contact toxicity activities of the less polar compounds present in these extracts. *G. subcordata* extracts had the highest activities against the insect (Figure 5a). The higher activities suggested that active compounds were either more concentrated in *G. Subcordata* extracts or *P. truncatus* adults were more sensitive to active compounds from the plant (Kosini & Nukunine, 2017).*O. kilimandscharicum* showed moderate antifeedant activities (Figure 5b). *A. mucosa* leaf extracts had the lowest activities (Figure 5c), which agreed well with the results of (Krinski & Massaroli, 2014) in which the stem extracts showed low activities when tested against *Tirana limbativentris* even though the insects used in this case were coleopterans belonging to a different genus. All the extracts showed contact toxicity activities against *P. truncatus* thus validating the traditional use of the plants in stored insect pest control.

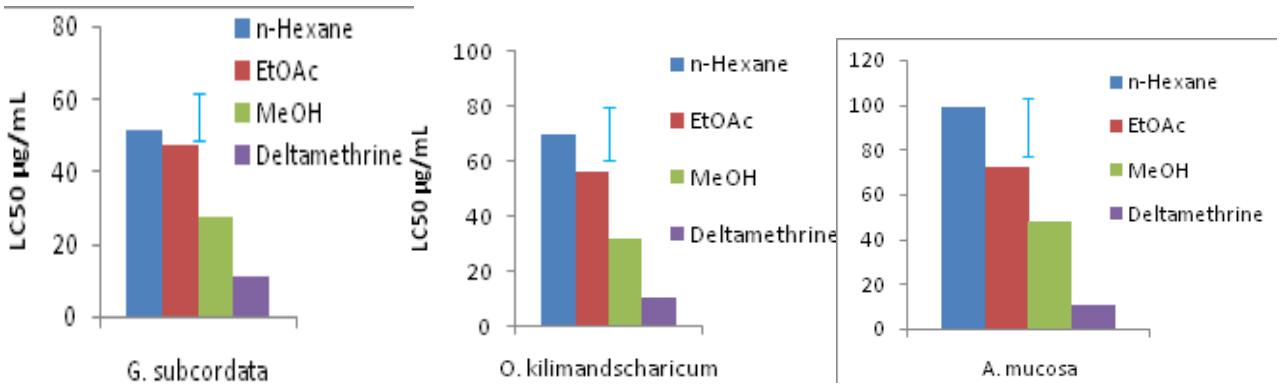


Figure 5: Contact toxicity activities of (a) *G. subcordata* (b). *O. kilimandscharicum* (c). *Annona mucosa* extracts (LC_{50} µg/mL) against*P. truncatus*.

4.1.2 Antifeedant activities

The crude extracts of *G. subcordata*, *O. kilimandscharicum* and *A. mucosa* were subjected to antifeedant assays against *S. zeamais* and *P. truncatus*. Insect antifeedant also known as feeding deterents are chemicals that inhibit feeding or disrupt insect feeding by rendering the treated materials unattractive or unpalatable (Munakata, 1997; Saxena *et al*, 1988).

4.1.2.1 Antifeedant activities of crude extracts of *G. subcordata, O. kilimandscharicum* and *A. mucosa* against *S. zeamais*

In the antifeedant activity assay of the crude extracts for the three plants against *S. zeamais*, the extracts showed varied degrees of activities. The activities however depended on concentration of the extracts, increasing with increase in concentration. The antifeedant activities of the plant extracts also depended on polarity of the solvents used for extraction. Among the plants *G. subcordata* extracts had relatively high activities which were however lower than the activity of the positive control (Figure 6a). *A. mucosa* had the lowest antifeedant activities against the insect (Figure 6c) which was also reported by (Ribeiro & Vendramin, 2017) on testing extracts against *S. zeamais* even though (Ribeiro &

Vendramin, 2017) used the tree branches of *A. mucosa* while for the current results leaves of the plant were used. Methanol extracts of the three plants exhibited highest antifeedant activities against the insect (Figure 6a, b and c). The antifeedant activities of *G. subcordata* and *O. kilimandscharicum* methanol/ aqueous extracts were not significantly different from those of the positive control ($P \geq 0.05$).Methanol extract activity of *A. mucosa* was significantly different from that of the positive control ($P \leq 0.05$)(Figure 6c). The *n*-hexane extracts of the three plants exhibited the lowest activities. The difference in activities of different solvent extracts showed the diversity of substances in extracts obtained from the same plant structure and species (Ribeiro & Vendramin, 2017). These were likely to cause different behavioural effects to *S. zeamais*, depending on the solvent used that led to significant changes in the chemical profile of the derivatives obtained and consequently, changes in their bioactivity (Ribeiro &Vendramin, 2017). The high antifeedant activities of the methanolic extracts may be attributed to the presence of polar compounds which were known to have antifeedant activities (Ribeiro &Vendramin, 2017). The activities of the methanol extracts of *G. sucordata* and *O. kilimandscharicum* were in good comparison with the activities of the positive control. The antifeedant activities of the plant extracts validated traditional use of the plant in post-harvest insect pest control. The methanol extracts from *G. sucordata* and *O. kilimandscharicum* may therefore be considered for use in integrated pest management programs.

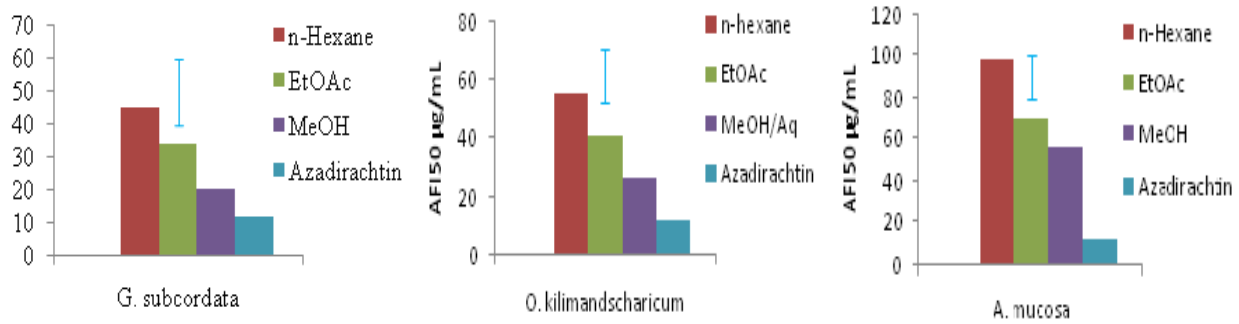


Figure 6: Antifeedant activity (AFI_{50} µg/ml) of (a).*G. subcordata* (b) *O. kilimandscharicum* (c). *A. mucosa* extracts against *S. zeamais*.

4.1.2.2 Antifeedant activities of crude extracts of *G. subcordata, O. kilimandscharicum* and *A. mucosa* leaves against *P. truncatus*

In the antifeedant activity assay of the crude extracts for the three plants against *P. truncatus,* all the extracts showed varying activities that were concentration dependent of the extracts. The activities followed the same trend as for *S. zeamais*. The antifeedant activities also depended on polarity of the solvents used for extraction. Among the plants, *G. subcordata* extracts exhibited comparatively higher activities relative to the other two plants. The activities were however lower than those of the positive control (Figure 7a). Methanol extracts of the three plants showed the highest antifeedant activities against the insect (Figure 7a, b, c) as compared to the activities of the ethyl acetate and *n*-hexane extracts.The methanol extracts of *G. subcordata* and *O. kilimandscharicum* had activities that were lower but not significantly different from the activity of the positive control ($P \geq 0.05$). The high activity of *O. kilimandscharicum* is reflected in the results of (Karakas, 2016) which showed leaf extracts of *Ocimum basilicum,* a species in the same genus to have moderately high antifeedant activities when tested against *Sitophilus granarius.* The plant species used in the two tests were however different species in the same genus. *A. mucosa* had the lowest activities, (Figure 7c). This is likely due to the presence of the less polar and less bioactive compounds in the *n*-hexane leaf extracts.

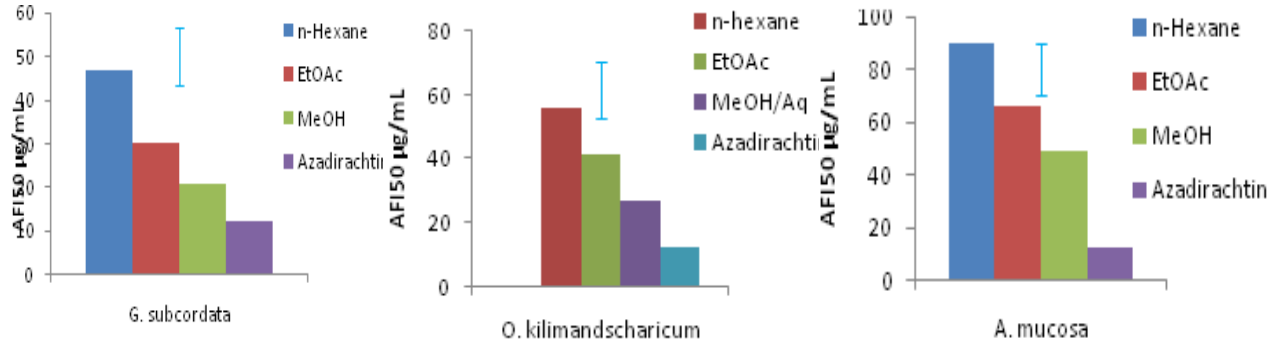


Figure 7: Antifeedant activity (AFI_{50} µg/ml) of (a).*G. subcordata (*b). *O. kilimandscharicum* (c). *A. mucosa* extracts against *P. truncatus*

4.2 STRUCTURALELUCIDATION OF THE ISOLATED COMPOUNDS

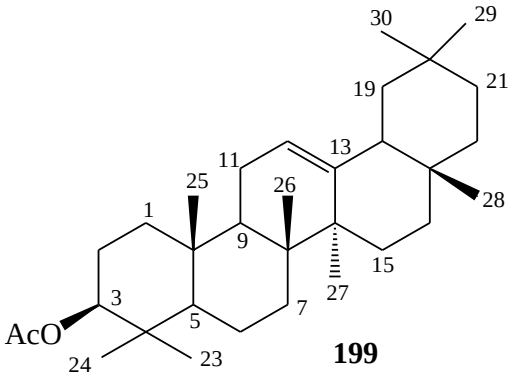
4.2.1 Structural elucidation of compounds from *G. subcordata* leaves

Ten compounds were isolated from the leaf extracts of *G. subcordata*. The structures of these compounds were established based on their physical and spectroscopic data as well as on comparison with those in the respective literature.

4.2.1.1 Structural elucidation of compounds from combined *n*-hexane and ethyl acetate leaf extracts of *G. subcordata*

4.2.1.1.1 IsolateGS1: The compound was isolated from the combined *n*-hexane and EtOAc extract of *G. subcordata* leaves as white amorphous powder with m.p. 239-241 ^{o}C and R_f value of 0.71 (solvent system: *n*-hexane-EtOAc 4:1). It showed a purple colour with acidified anisaldehyde after heating on hot plate at 100^{o}C suggesting the presence of a sterol or a terpenoid derivative. The compound responded positively to Liebermann-Buchard and ceric sulphate tests which further supported the presence of a terpenoid derivative (Sukumar *et al.*, 1995). It showed significant IR absorption bands at 1734.6 and 1244.4representing acetoxy group (Appendix 1a) and 1654.0 cm^{-1} (trisubstituted carbon-carbon double bond) (Sukumar *et al*., 1995). The ^{I}H NMR spectrum **(**Table 4.1; Appendix 1b**)** exhibited an outstanding triplet peak at δ_H 5.15 (J = 3.6 Hz) assignable to H-12 whereas the relatively downfield peak appearing at δ_H 4.64 (dd, J = 11.0, 3.0 Hz) was assignable to H-3. This together with nine methyl groups on quaternary carbons including the acetoxy moiety centred at δ_H 2.08, 1.12, 0.98, 0.92, 0.88, 0.84, 0.83, 0.81 and 0.80 were suggestive of oleanane type triterpenes (Okoye *et al.*, 2014). The ^{13}C NMR spectrum **(**Table 4.1; Appendix 1c**)** showed a total of 32 carbon signals which were sorted out by DEPT-135 (Appendix 1d) into nine methyls, ten methylenes, five methines including one olefinic and one oxygen bearing carbons as well as eight quaternary carbons. The olefinic carbons C-12 and C-13 appeared at δ_C 121.6 and 145.2, respectively. The up field value of C-13 further supported the compound to be an oleanane

derivative rather than an ursane derivative (Doddrell *et al.*, 1974; Sukumar *et al.*, 1995). Comparison of the ^{1}H and ^{13}C NMR spectra of **GS1** with those of β-amyrin acetate (Feleke & Brehane, 2005) revealed similarities as substantiated by EI-MS data *m/z* 249.1 for $C_{16}H_{25}O_2$, (Appendix 1e; Figure 8**).** The position of an acetoxy group was confirmed to be at C-3 and was equatorially oriented based on the coupling constants (Sukumar *et al.*, 1995; Okoye *et al.*, 2014). Thus, on the basis of accrued spectroscopic and mass spectrometric data as well as comparison with literature data (Feleke & Brehane, 2005), compound **GS1** was concluded to be 3β-acetoxyolean-12-ene (β-amyrin acetate) **(199).**



199

Table 4.1: ^{1}H NMR (360 MHz) and ^{13}C NMR (90 MHz) ($CDCl_3$) spectral data of**199**

C	^{1}H NMR (*J* in Hz)	^{13}CNMR	^{1}H NMR*(*J* in Hz)	^{13}C NMR*	DEPT
1		37.1		39.6	CH_2
2		26.9		27.9	CH_2
3	4.64 dd (*J* = 11.0, 3.0 Hz)	80.9	4.50 m	80.8	CH
4		34.7		39.5	C
5	0.83 m	55.2		55.1	CH
6		18.3		18.1	CH_2
7		31.1		33.6	CH_2
8		36.8		38.3	C
9		47.5		47.4	CH
10		32.5		35.0	C
11		23.5		23.5	CH_2
12	5.15 (t, *J* = 3.6 Hz)	121.6	5.15 (dt, *J* = 8.0, 4.0 Hz)	121.5	CH
13		145.2		145.1	C
14		41.7		42.0	C
15		26.1		28.2	CH_2
16		28.0		27.9	CH_2
17		32.6		32.5	C
18		55.2		55.0	CH
19		38.2		40.2	CH_2
20		39.8		41.4	C
21		28.4		31.0	CH_2
22		41.7		42.0	CH_2
23	0.88 s	28.5	0.88 s	29.5	CH_3
24	0.84 s	15.6	0.83 s	15.8	CH_3
25	0.92 s	15.6	0.92s	15.8	CH_3
26	0.98 s	16.7	0.98 s	16.8	CH_3
27	1.12 s	23.5	1.00 s	23.5	CH_3
28	0.81 s	26.9	0.80 s	28.8	CH_3
29	0.80 s	16.8	0.77 (d, *J* = 7.0 Hz)	17.6	CH_3
30	0.83 s	21.3	0.83 (d, *J* = 6.1 Hz)	21.2	CH_3
CH_3CO_{2-}	2.08 s	21.3	2.05 s	21.2	CH_3
CH_3CO_{2-}		171.1		170.8,	C

*Feleke & Brehane, 2005

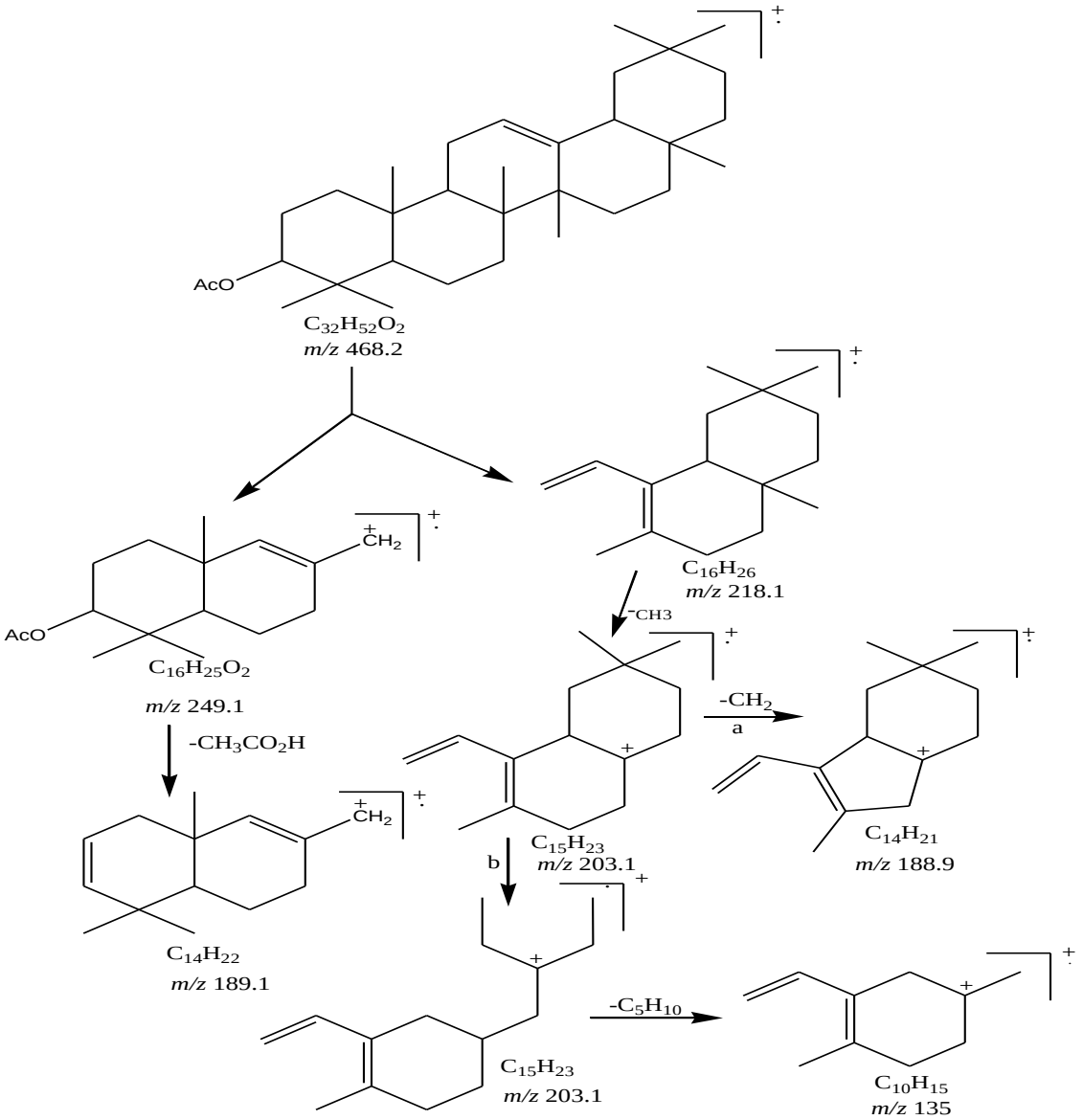
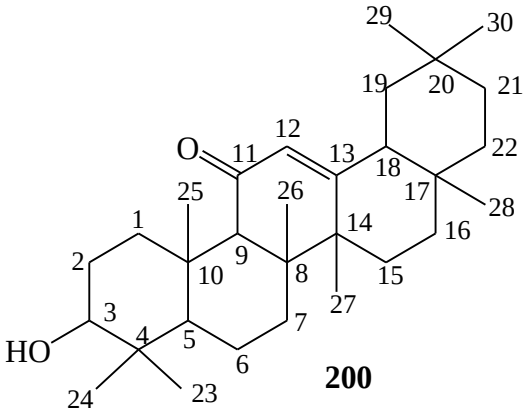


Figure 8: Proposed fragmentation pattern of compound **199**in EI-MS (70 eV)

4.2.1.1.2Isolate GS2

Compound **GS2** was obtained as white amorphous powder with m.p. 184-186ºC; R_f= 0.60 (silica gel TLC, solvent system: *n*-hexane-EtOAc, 4:1). It responded both to Liebermann-Buchard and ceric sulphate tests suggesting that it is a terpenoid compound. The ^{I}H NMR spectrum (Table 4.2; Appendix 2a), exhibited the presence of an oxygenated methine proton at δ_H 2.57 (dd, *J*= 12.0, 4.3 Hz, H-3) and eight tertiary methyl groups at δ_H 1.20, 1.17, 1.11, 1.08, 0.93, 0.87, 0.85 and 0.81 (all

singlets) indicating that compound GS**2** is an oleanane-type triterpenes(Sukumar *et al*., 1995). The unshielded nature of the signal at δ_H 2.57 is indicative of the unsubstituted hydroxyl group, which was suggested to be β-oriented based on the axial-axial and axial-equatorial coupling observed between H-3 and H-2 protons. On the other hand, the presence of eight tertiary methyls together with vinylic singlet at δ_H 5.20 further confirmed that the compound is a 12-oleanene derivative (Kuo & Chiang, 2000). The compound afforded 30 distinct carbon resonances in the ^{13}C NMR **(**Table 4.2; Appendix 2b**)** which were sorted out by DEPT-135into eight methyls, 9 methylenes, five methines including the oxymethine and eight quaternary carbons including the keto group. In the EI-MS spectrum, the compound exhibited a molecular ion peak at *m/z* 440.1 $[M]^+$ which corresponded to $C_{30}H_{48}O_2$ formula. This together with daughter fragment peaks at *m/z* 422.1 $[M-H_2O]^+$, 407.0 $[M-H_2O-CH_3]^+$ and 234.1 $[C_{16}H_{26}O]$ (Appendix 2c; Figure 9) indicated that the keto group is on ring C possibly at C-11. Thus on the basis of spectroscopic and mass spectrometric evidences as well as comparison with literature data of 3β-hydroxy-11-oxo-12-oleanene (Amgad *et al.*, 2013), compound **GS2** was concluded to be 3β-hydroxy-11-oxo-12-oleanene (**200)**, reported in this species for the first time.



200

Table 4.2: ^{1}H NMR (360 MHz) and ^{13}C NMR (90 MHz) ($CDCl_3$) of compound **200**

C$^{\#}$	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C MR*
1		39.3		37.3
2		27.9		27.2
3	4.28 (dd, *J* = 12.0, 4.3Hz)	76.7	3.0(dd, *J* = 12.9, 4.7Hz)	71.9
4		39.4		37.0
5		55.5		55.1
6		17.9		17.5
7		33.7		32.8
8		43.0		45.2
9	2.54 s	58.3	2.34 s	61.5
10		39.3		36.7
11		213.4		213.1
12	7.29s	128.8	5.54 s	130.5
13		142.9		143.1
14		42.4		43.7
15		26.5		27.2
16		26.8		27.3
17		34.4		33.9
18		56.3		59.1
19		39.4		39.2
20		41.2		39.3
21		33.2		30.9
22		37.6		36.9
23	0.93 s	28.7	0.98 s	28.1
24	1.11 s	17.5	1.00 s	16.7
25	1.20 s	16.3	1.13 s	16.5
26	1.08s	18.0	1.02 s	18.6
27	1.17s	19.7	1.07 s	20.5
28	0.81 s	31.1	0.79 s	28.9
29	0.85 s	34.4	0.88 s	33.5
30	0.87 s	23.0	0.88 s	23.6

*Amgad *et al*., 2013

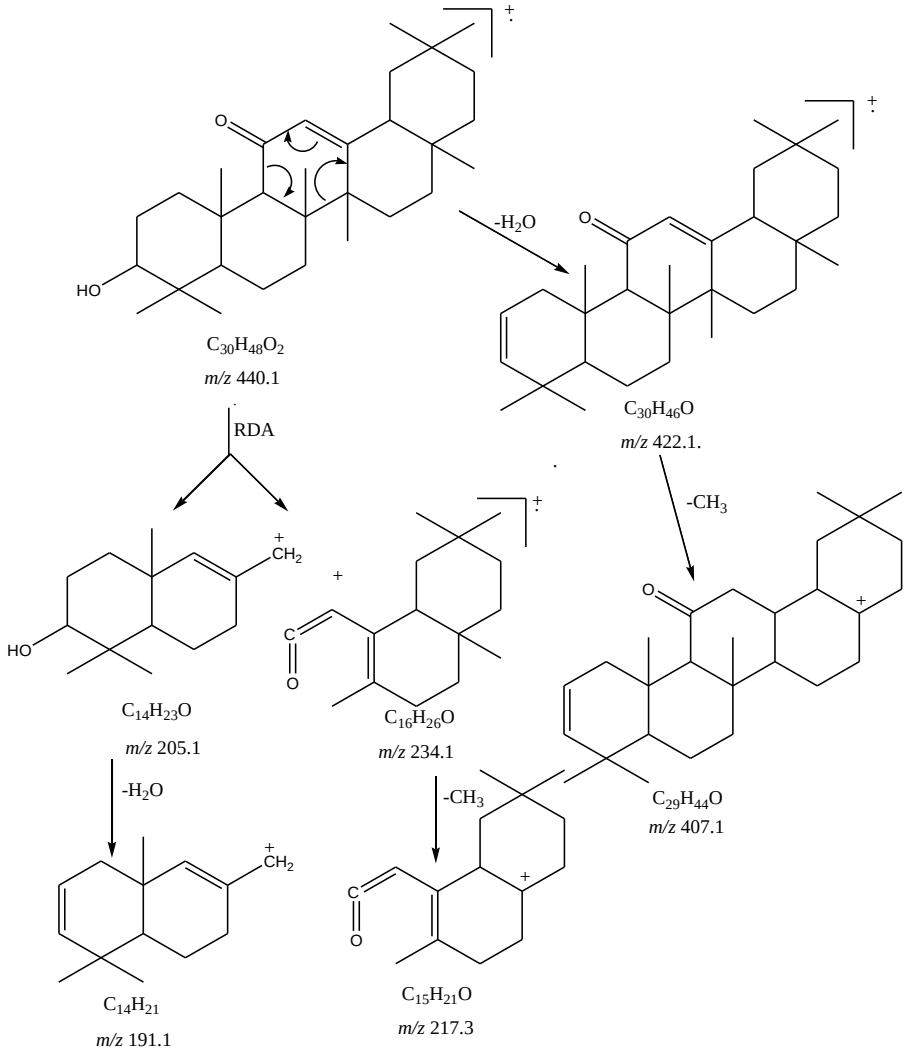
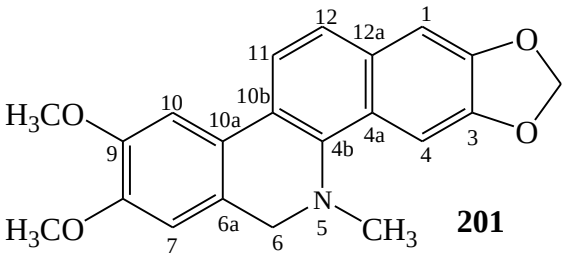


Figure 9: Proposed fragmentation pattern of compound **200** in EI-MS (70 eV)

4.2.1.1.3 Isolate GS3

The compound was isolated as white crystals with m.p 174-175ºC (solvent system: 25% EtOAc in *n*-hexane) and R_f = 0.45 (silica gel TLC, *n*-hexane-EtOAc, 4:1). It responded positively to Dragendorff's spray reagent by giving an orange colouration suggesting it is an alkaloid. Its spot on TLC on exposure to UV light gave a blue flouresence which on exposure to air and/ or light turned yellow, a characteristic feature of alkaloids containing benzophenanthridine skeleton (Nyahanga *et al.*, 2013; Moussavi *et al.*, 2015). The IR spectrum (Appendix 3a) gave a significant

absorption peak centered peak at 1463.71 cm^{-1} suggesting N-C stretch in the molecule. The ^{1}H NMR spectrum **(**Table 4.3; Appendix 3b) showed six signals in the aromatic region including the *ortho*-coupled proton doublets at δ_H 7.69 and 7.09 (each J = 8.4 Hz) assigned to H-11 and H-12, respectively. A set of singlets which appeared at δ_H 7.24 and 6.93 were assigned to H-10 and H-7, respectively, while the remaining singlet signals integrating into one proton each which resonated at δ_H 7.48 and 7.50 were attributable to H-4 and H-1, respectively. These together with peaks for two methoxy groups at δ_H 3.91 and 3.86, methylenedioxy moiety signal at δ_H 6.03 and *N*-methyl group peak at δ_H 2.58, all appearing as singlets suggested oxygenation pattern of C-2, C-3, C-8 and C-9 in the proposed structure. The ^{13}C NMR spectrum **(**Table 4.3; Appendix 3c**)** of compound **GS3** displayed a total of 21 carbon resonances out of which three were methyls including the $N\text{-}CH_3$, two methylenes, six methines and ten quaternary carbons as evidenced by the DEPT-135 spectrum (Appendix 3d), which is in agreement with a benzophenanthridine type alkaloid (Nissanka *et al.*, 2001; Iwasaki *et al*., 2006; Nyahanga *et al.*, 2013). In fact, considering the aromatic pattern displayed by both the ^{1}H and ^{13}C NMR data, compound **GS3** was suggested to be dihydronitidine (Nyahanga *et al.*, 2013) rather than dihydrochelerythrine **(**Scheuer*et al*., 1962). Comparison of the ^{1}H and ^{13}C NMR data of compound **GS3** with dihydronitidine (de Moura *et al*., 1997; Moussavi *et al*, 2015) revealed close similarity with no noticeable difference as substantiated by the EI-MS molecular ion peak at *m/z* 350.0 $[M+H]^+$ (Appendix 3e). Thus, on the basis of spectroscopic data as well comparison of these values with literature data, compound **GS3** was concluded to be dihydronitidine (**201**)**,** an alkaloid that has been previously reported from *Todalia asiatica* (de Moura *et al*., 1997) but being reported in this species for the first time.



201

Table 4.3: ^{1}H (600MHz) and ^{13}C NMR (150 MHz) ($CDCl_3$) of compound **201**

C#	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C NMR*	DEPT
1	7.50 s	100.7	7.54 s	99.8	CH
2		152.2		149.2	C
3		148.0		149.2	C
4	7.48 s	101.0	7.11 s	104.6	CH
4a		126.3		127.0	C
4b		124.2		124.4	C
5					
6	4.28 s	76.7	4.03 s	69.9	CH_2
6a		123.7		123.5	C
7	6.93 s	110.9	6.79 s	110.8	CH
8		147.4		148.5	C
9		146.1		147.9	C
10	7.24 s	104.3	7.25 s	106.6	CH
10a		126.2		124.4	C
10b		142.7		137.9	C
11	7.69 (d, *J* = 8.4 Hz)	118.6	7.64 s	119.6	CH
12	7.09 (d, *J* = 8.4 Hz	120.1	7.33 d	124.3	CH
12a		130.8		129.1	C
8-OMe	3.91 s	55.8	3.94 s	56.1	CH_3
9-OMe	3.86 s	61.0	3.99 s	65.4	CH_3
N-Me	2.58 s	41.3	2.73 s	42.7	CH_3
OCH_2O	6.03 s	101.0	6.04 s	101.1	CH_2

*****de Moura *et al*., 1997

4.2.1.1.4 Isolate GS4

GS4 was isolated as a UV active white crystalline substance with m.p. 166-168^{o}C and R_f = 0.40 (*n*-hexane-EtOAc, 4:1). Like compound **201**, it showed an orange colouration when sprayed with Dragendorff's reagent suggesting that it could be an alkaloid (Ahsan*et al*., 2014). Its spot on TLC on exposure to UV light gave a blue fluorescence further suggesting the presence of benzophenanthridine type of alkaloid (Iwasaki *et al*., 2006; Nissanka *et al*., 2001). The ^{1}H NMR

spectrum (Table 4.4; Appendix 4a) suggested that the compound could be a benzophenanthridine type of alkaloid (Ahsan *et al*., 2014) by the following characteristic signals: (a) methylenedioxy protons at δ_H 6.04(singlet); (b) two methoxy groups at δ_H 3.92 and 3.87 (each singlet); (c) an N-methyl group at δ_H 2.59 (singlet); (d) a pair of *ortho* coupled aromatic protons at δ_H 6.93 and 7.51 (each doublet, J = 8.5 Hz); (e) another pair of ortho coupled aromatic doublets at δ_H 7.70 and 7.50 (each J = 8.5 Hz); (f) two aromatic proton singlets at δ_H 7.10 and 7.68; and (g) a benzylic proton as a singlet at δ_H 4.29. In fact, the foregoing evidences suggested oxygenation at C-2, C-3, C-7 and C-8 as substantiated by ^{1}H-^{1}H COSY spectrum (Appendix 4b) which showed correlations between H-10 (δ_H 7.51) and H-9 (δ_H 6.93) and between H-11 (δ_H 7.70) and H-12 (δ_H 7.50). The ^{13}C NMR spectrum **(**Table 4.4; Appendix 4c**)** of compound **GS4** displayed a total of 21 carbon resonances including six methines, two methylenes, three methyl including the N-CH_3 and ten sp^2 hybridized quaternary carbons as evidenced by DEPT-135 (Appendix 4d), which is in agreement with a benzophenanthridine type alkaloid (Nissanka *et al.*, 2001; Ahsan*et al*., 2014). In fact, both the ^{1}H and ^{13}C NMR data were in agreement with published data of dihydrochelerythrine (Scheuer*et al*., 1962; Ahsan*et al*., 2014; Feng *et al*., 2012) which was further evidenced by EI-MS which exhibited a molecular ion peak at *m/z* 350 $[M+H]^+$ (Appendix 4e) corresponding to $C_{21}H_{19}O_4N$ formula. The accrued data were further supported by HSQC (Appendix 4f) and HMBC (Appendix 4g) data. Therefore, on the basis of physical and spectroscopic data as well comparison with those values in literature, compound **GS4** was structurally elucidated as dihydrochelerythrine **(202),** an alkaloid that has been previously reported from *Bocconia intengrifolia* (Oechslin *et al*., 1991) and from *Macleaya cordata* (Feng *et al*., 2012). This is the first time the compound is reported in this species.

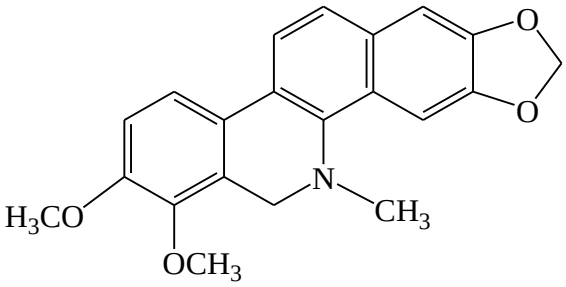


Table 4.4: ^{1}H (600MHz) and ^{13}C NMR (150 MHz) ($CDCl_3$) of compound **202**

C#	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C NMR*	DEPT
1	7.10 s	104.3	7.11 s	104.3	CH
2		148.1		148.0	C
3		147.5		147.4	C
4	7.68 s	100.7	7.67 s	100.7	CH
4a		126.4		126.3	C
4b		142.7		142.7	C
5					
6	4.29s	48.7	4.29 s	48.7	CH_2
6a		126.3		126.2	C
7		146.1		146.1	C
8		152.3		152.2	C
9	6.93 (d, *J* = 8.5 Hz)	111.0	6.94 (d, *J* = 8.5 Hz)	111.0	C
10	7.51 (d, *J* = 8.5 Hz)	118.7	7.51 (d, *J* = 8.5 Hz)	118.6	CH
10a		126.3		126.2	C
10b		124.3		124.2	C
11	7.70 (d, *J* = 8.4 Hz)	120.1	7.70 (d, *J* = 8.6 Hz)	120.0	CH
12	7.50 (d, *J* = 8.4 Hz	123.8	7.48 (d, *J* = 8.6 Hz)	123.7	CH
12a		130.8		130.8	C
7-OMe	3.92 s	61.1	3.87 s	61.0	CH_3
8-OMe	3.87 s	56.8	3.92 s	55.8	CH_3
N-Me	2.59s	41.3		41.2	CH_3
OCH_2O	6.04 s	100.9	6.04 s	101.0	CH_2

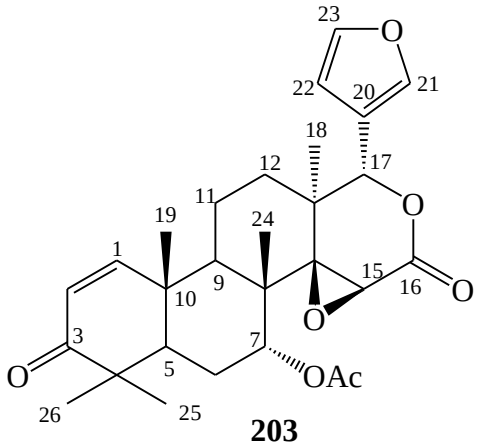
*Oechslin *et al*., 1991

4.2.1.1.5 Isolate GS5

The compound was isolated as white amorphous powder, m.p 214-218^{o}C and R_f= 0.36, solvent system; *n*-hexane-EtOAc, 4:1). It responded positively to both Liebermann-Burchard and ceric sulphate tests suggesting that it could be a terpenoid derivative (Manguro & Wagai, 2006). It also gave Ehrlich positive reaction on TLC which is typical of limonoids (Bennet *et al*, 1989). Analysis

of the ^{1}H NMR spectrum (Table 4.5; Appendix 5a) revealed the presence of six methyl groups on quaternary carbons including the acetoxy group (δ_H 2.10, 1.24, 1.11, 1.00, 1.02 and 0.94). The spectrum also showed a pair of vinylic protons appearing as doublets at δ_H 7.39 (d, J = 9.0 Hz) and 5.61 (d, J =10.2 Hz) which were ascribable to H-1 and H-2. Another characteristic feature of the ^{1}H NMR spectrum was a set of vinylic protons at δ_H 6.32 (H-22) and 7.40 (H-23), which together with a singlet at δ_H 7.41 (H-21) suggested the presence of β-furyl ring in the compound (Chianese, 2011), a fact supported by the ^{13}C NMR spectrum (Table 4.5; Appendix 5b) peaks at δ_C120.7 (C-20), 143.0 (C-21), 110.0 (C-22) and143.1 (C-23) (Chianese *et al*., 2010). The relatively low oxymethine proton appearing at δ_H 4.54 (brt, J = 3.6 Hz) suggested that the acetoxy group was connected to the carbon and its presence was substantiated by a peak appearing at δ_C 170.1 in the ^{13}C NMR spectrum. The ^{13}C NMR spectrum showed the presence of 28 carbon signals; multiplicity assignments from DEPT-135 **(**Appendix 5c**)** experiments revealed the presence of ten methines, three methylenes, six methyls and nine quaternary carbons. The combined interpretation of the ^{1}H and ^{13}C NMR which was aided by HSQC spectrum (Appendix 5d) allowed the association of all the protons with relevant carbon signals and in this way compound **GS5** was proposed to be gedunin, a limonoid-type terpenoid previously isolated from *Azadirachta indica* (Chianese *et al*., 2010), a fact evidenced by HRTOF-MS peak (Appendix 5e) at *m/z* 505.2864 [M +Na] $^{+}$corresponding to $C_{28}H_{34}O_7$ formula. In fact, the ^{1}H and ^{13}C NMR data as well as TOF-MS m/z 505.2202 (Appendix 5f), were in complete agreement with the structure of gedunin (Haldar *et al*., 2013). The position of an acetoxy group was confirmed to be at C-7 where it was oriented axially as evidenced by the narrow half-height with $w^{1/2}$ (3.2 Hz) of equatorially-positioned oxymethine proton which appeared down field at δ_H 4.54 (Haldar *et al*., 2013). Thus, on the basis of physical

and spectroscopic data as well as comparison with literature data (Khalid *et al*., 1989), compound **GS5** was concluded to be gedunin (**203**).



203

Table 4.5 ^{1}H (150 MHz) and ^{13}C NMR (600 MHz) ($CDCl_3$) spectral data of compound **203**

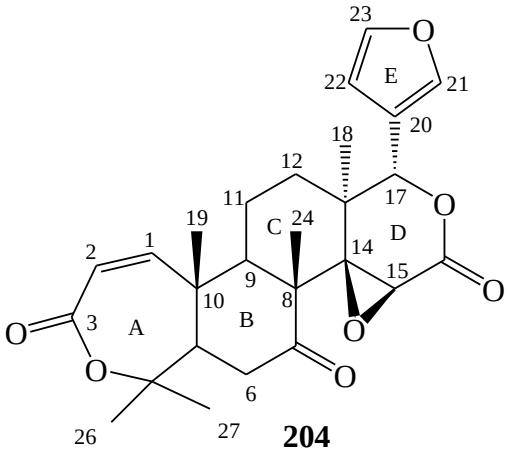
C#	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C NMR*	DEPT
1	7.39 (d, *J* = 9.0 Hz)	157.0	7.07 (d, *J* =10.2 Hz)	157.0	CH
2	5.61 (d, *J* = 10.2 Hz)	126.0	5.84 (d, *J* = 10.2 Hz)	125.9	CH
3		204.1		204.0	C
4		44.1		44.0	C
5	2.30 (dd, *J* = 12.0, 6.0 Hz)	46.1	2.12 (dd, *J* = 13.2, 2.3 Hz)	46.0	CH
6	1.92 m, 1.84 m	23.3	1.92 m, 1.79 m	23.2	CH_2
7	4.54 (br t, *J* = 3.6 Hz)	72.7	4.52 brs	73.2	CH
8		42.8		42.6	C
9	2.45 (dd, *J* = 10.2, 7.8 Hz)	39.6	2.46 (dd, *J* = 12.7, 6.2 Hz)	39.5	CH
10		40.2		40.0	C
11	1.92-1.61 m	14.1	2.00m, 1.81m	14.9	CH_2
12	2.10 m	25.3	1.70 m	25.9	CH_2
13		38.9		38.7	C
14		69.4		69.7	C
15	3.51 s	57.0	3.50 s	56.8	CH
16		165.1		167.4	C
17	5.60 s	77.9	5.59 s	78.2	CH
18	1.24 s	15.2	1.22 s	17.7	CH_3
19	1.00 s	18.9	1.19 s	19.7	CH_3
20		120.4		120.4	C
21	7.41 br s	143.1	7.38 (d, *J* = 1.3 Hz)	143.1	CH
22	6.32 (d, *J* = 1.4Hz)	109.9	6.31 (d, *J* = 1.3 Hz	109.8	CH
23	7.40 (d, *J* = 1.6Hz)	141.2	7.50 (d, *J* = 1.3 Hz	141.2	CH
24	1.02 s	27.3	1.03 s	27.1	CH_3
25	0.94 s	21.3	1.04 s	21.2	CH_3
26	1.11 s	17.8	1.08 s	18.3	CH_3
O-CO-Me		166.9		169.9	C
O-CO-Me	2.10 s	19.9	2.07 s	21.0	CH_3

*Khalid *et al.*, 1989)

4.2.1.1.6Isolate GS6

GS6 was isolated from ethyl acetate extract of the leaves of *G. subcordata* as a white powder with m.p 215-216^{o}C; R_f = 0.30 (solvent system: *n*-hexane-EtOAc, 4:1). It afforded a positive Liebermann-Burchard and ceric sulphate tests suggesting that it could be a terpene derivative. The compound also gave Ehrlich positive reaction on TLC which is typical of limonoids (Bennet *et al.*, 1989). In the IR spectrum (Appendix 6a), significant absorption peaks were observed at 1774.5, 1744.3 and 1664.1 cm^{-1} representing lactone, keto and C=C double bond functionalities, respectively. The ^{1}HNMR spectrum (Table 4.6; Appendix 6b) exhibited two pairs of ortho-coupled vinylic protons appearing as doublets at δ_C 6.52 (d, *J*=11.4 Hz, H-1) and 5.96 (*J*=12.0 Hz, H-2) which were observed to correlate with carbon resonances at δ_C 156.9 (C-1) and 123.1 (C-2), respectively in the HSQC spectrum (Appendix 6c). In addition, the ^{1}H NMR spectral peaks appearing at δ_H 7.41 (s, H-21), 6.36 (s, H-22) and 7.39 (d, *J* = 1.8 Hz, H-23) were consistent with a furan ring in the compound (Khalil *et al*., 2003). These together with oxymethine proton centred at δ_H 3.65 (s, H-15) and five methyls on quaternary carbons strongly suggested that the compound **GS6** is a limonoid (Dreyer *et al.*, 1976). The ^{13}C NMR spectrum (Table 4.6; Appendix 6d) gave 26 distinct carbon signals accounted for by five methyls, three methylenes, nine methines including five olefinic and oxygenated carbons and nine quaternary carbon atoms as evidenced by DEPT-135 (Appendix 6e). The proton attached to each carbon signal observed in the ^{13}C NMR spectrum were deduced by analysis of DEPT-135 and HSQC spectra and in this way it was established that oxygenated methyls at δ_H 3.65 signified an oxirane ring and its position was deduced to be at C-15 on the basis of HMBC correlation (Appendix 6f) between H-15 and C-13 (δ_C 37.6) and in turn with C-8 (δ_C 53.1). Comparison of both the ^{1}H and ^{13}C of compound **GS6** with those of compound **203** revealed close similarities with notable differences being the nature of ring A and substitution pattern in ring B as substantiated by the ^{13}C NMR and EI-MS (Appendix 6g). The acetoxy

group was apparently replaced by a keto group with corresponding ^{13}C NMR peak at δ_C207.6 in compound **GS6**, confirmed by HMBC contour between the keto carbon C-7 and H-5 (δ_H 2.61, dd, *J*= 13.8, 4.8 Hz) and in turn with H-9 (δ_H 1.89, dd, *J*=9.0, 5.4 Hz). The EI-MS afforded a molecular ion peak at *m/z* 454.5 corresponding to $C_{26}H_{30}O_7$ formula. This suggested 12 double bond equivalents, two of which were assigned to two lactone moieties, one attributable to the furyl ring, one to the oxirane ring, two to lactone rings, two to the hexacyclic rings and another assignable to the keto group. The remaining three represented six sp^2 hybridized carbons of the compound. The foregoing evidence led to the confirmation that ring A had additional oxygen atom which generated the second lactone moiety. The two olefinic methines signals at δ_C 156.9 and 123.1 spectrum were assigned H-1 and H-2, respectively. On the other hand, H-2 doublet showed HMBC correlation with C-10 (δ_C 43.2). In fact, these data were in complete agreement with those reported for obacunone (Khalil *et al*., 2003). Therefore, on the basis of accrued data as well as comparison with literature data, compound **GS6** was concluded to be obacunone (**204**), a compound reported in this species for the first time.



204

Table 4.6:^{1}H (600 MHz) and ^{13}C NMR (125MHz) ($CDCl_3$) spectral data of compound **204**

C#	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C NMR*	DEPT
1	6.52 (d, *J* = 11.4 Hz)	156.9	6.70 (d, *J* = 11.8 Hz)	158.8	CH
2	5.96 (d, *J* = 12.0 Hz)	123.1	5.81 (d, *J* = 11.8 Hz)	122.3	CH
3		167.1		167.6	C
4		84.1		84.7	C
5	2.61(dd, *J* = 13.8, 4.8 Hz)	57.5	2.66 (dd, *J* = 13.5, 5.0 Hz)	56.6	CH
6	3.00 (t, *J* = 14.4 Hz), 2.30 (dd, *J* = 14.4, 5.4 Hz),	40.0	2.20 (dd, *J* = 13.8, 4.8 Hz), 3.02 (*t*, *J* = 14.0 Hz)	40.2	CH_2
7		207.6		209.1	C
8		53.1		53.0	C
9	1.89 (dd, *J* = 9.0, 5.4 Hz)	49.4	2.10 (dd, *J* = 10.0, 2.0 Hz)	48.9	CH
10		43.2		43.5	C
11	1.86 m	19.5	1.70 m, 1.77 m	19.6	CH_2
12	1.88 m	32.9	1.77 m, 1.80 m	32.5	CH_2
13		37.6		37.7	C
14		65.2		66.2	C
15	3.65 s	53.4	3.75 s	53.8	CH
16		166.8		167.9	C
17	5.45 s	78.1	5.41s	78.3	CH
18	1.11 s	21.2	0.99 s	21.3	CH_3
19	1.50 s	17.1	1.34 s	17.3	CH_3
20		120.2		120.9	C
21	7.41 s	143.3	7.63 br s	144.2	CH
22	6.36 brs	109.9	6.48 br s	111.1	CH
23	7.39 (d, *J* = 1.8 Hz)	141.2	7.70 s	142.5	CH
24	1.23 s	16.6	1.14 s	17.2	CH_3
25	1.45 s	32.0	1.30 s	33.5	CH_3
26	1.15 s	26.8	1.38 s	27.4	CH_3

*Khalil *et al*., 2000

4.2.1.1.7 Isolate GS7

The compound was isolated as a white powder, m.p. 298-301^{o}C from ethyl acetate extract of *G. subcordata*, with R_f= 0.27 (*n*-hexane-EtOAc 4:1). It gave positive test with Lieberman-Buchard reagent and also responded positively to ceric sulphate test suggesting that it could be a terpenoid compound. Its IR spectrum determined as KBr pellet showed significant absorption peaks at 3431.9, 1774.3 and 1666.1 which are indicative of hydroxy, lactone and carbon-carbon double bond moieties, respectively (Kuo *et al*., 2008). The ^{1}H NMR spectrum **(**Table 4.7; Appendix 7a**)** showed the presence of two methyl singlets centred at δ_H1.27 and 1.36 attributable toMe-19 and Me-20, respectively, a single olefinic proton appearing at δ_H 7.26 attributable to H-11 and a doublet observable at δ_H 3.46 (d, *J* = 10.2 Hz) and a multiplet at 3.45 which were assigned to H-1 and H-2, respectively. Besides these ^{1}H NMR data,

the spectrum also exhibited two methyl doublets at δ_H 1.21 (J = 6.4 Hz, H-16) and 1.10 (J = 6.4 Hz, H-17) attributable to isopropyl methyls. The isopropyl methine proton which showed a peak at δ_H 3.44 (sept, J = 6.6 Hz, H-15) together with two oxymethine protons at δ_H 3.64 (d, J = dd, J = 10.0, 4.6 Hz, H-3) and 4.98 (d, J = 3.6 Hz, H-7), suggested that compound **GS7** is a nagilactone derivative (Kuo *et al.*, 2008). The foregoing evidence was substantiated by ^{13}C NMR spectrum (Table 4.7; Appendix 7b) which displayed 19 carbon signals with multiplicity assignment revealing the presence of four methyls, eight methines and seven quaternary carbons. This in turn was confirmed by the EI-MS spectrum (Appendix 7c) which gave a peak at *m/z* 365 $[M+3H]^+$ corresponding to molecular formula$C_{19}H_{22}O_7$indicative of nine degrees of unsaturation including the two carbonyls of δ-lactone (δ_C 163.4, C-12) and γ-lactone (δ_C 179.4, C-18), two lactone rings, two hexacyclic rings, an oxirane ring and two conjugated double bonds on a lactone ring. In the HSQC spectrum (Appendix 7d) peaks at δ_H 3.45 (H-1) and 3.32 (H-2) correlated with carbons at δ_C 58.6 and 53.5, respectively. This allowed placement of the epoxy ring at C-1/C-2, a fact that was further supported by HMBC (Appendix 7e) cross peaks between H-1 and C-3 (δ_C 63.3) and H-2 with C-10 (δ_C 36.3). Similarly, the oxymethine proton observed at δ_H 4.60 (d, J = 5.4 Hz, H-6) correlated with carbon signals at δ_C 73.0 (C-6) in the HSQC spectrum. The three bond long range correlation (HMBC) was applied to establish their positions in the molecule. The H-6 proton was observed to correlate with C-4 (δ_C 44.1) and in turn with C-8 (δ_C 116.7) and C-10 (δ_C 36.3). On the other hand, H-7 showed cross peaks with C-5 (δ_C 45.3), C-9 (δ_C 163.4) and C-14 (δ_C 163.4). Similarly, the three bond long range correlations between H-3 and C-5 and in turn with C-1 (δ_C 53.5) in the HMBC spectrum further supported the presence of a hydroxyl group at C-3 position. Their configurations as α- was established on the basis of coupling constants (Kuo *et al*., 2008) a fact confirmed by the ^{1}H-^{1}H proximity NOESY, spectrum (Appendix 7f) which showed correlation between H-7 and H-6; H-6 and H-5; H-5 and Me-20 (δ_H 1.36); H-3 and H-2 and in

turn with Me-20. On the other hand, the methine protons at δ_H 3.46 and 3.45 showed NOESY correlation indicative of their *syn* nature. This was further confirmed by ^1H-^1H proximity between H-3 and H-2. Thus on the basis of physical and spectroscopic data as well as comparison with relevant literature (Davila *et al*., 2014), compound **GS7** was deduced to be nagilactone C (**205**) which is reported in this species for the first time.

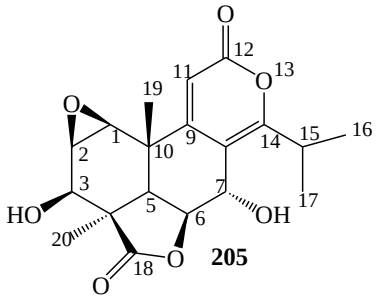


Table 4.7: ^{1}HNMR (600 MHz) and ^{13}C NMR 150 MHz, ($CDCl_3$) spectral data of compound **205**

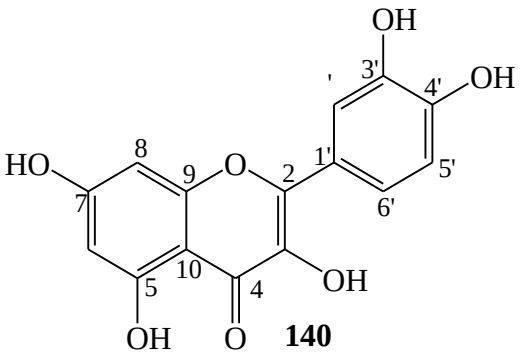
C$^{\#}$	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C NMR*
1	3.46 (d, *J* = 5.2 Hz)	58.6	3.56 (d, *J* = 4.5 Hz)	56.9
2	3.45 m	53.5	3.32 m	50.6
3	3.64(d, *J* = 10.0 Hz)	63.3	4.23 (dd, *J* = 4.5, 5.0 Hz)	66.4
4		44.1		49.0
5	1.86 (d, *J* = 5.4 Hz)	45.3	2.05(d, *J* = 6.8Hz)	49.8
6	4.60 (d, *J* = 5.4 Hz)	73.0	4.88 (dd, *J* = 8.5, 6.8 Hz)	72.7
7	4.98 (d, *J* = 3.6 Hz)	63.2	5.21 (dd, *J* = 8.5, 4.5 Hz)	58.7
8		116.7		111.3
9		163.4		165.0
10		36.3		36.9
11	7.26 s	82.9	6.27 s	85.8
12		158.1		161.2
13				
14		163.4		169.4
15	3.44 (sept, *J* =.6.4 Hz)	28.3	3.27 (sept, (*J* = 6.8 Hz)	28.5
16	1.21 (d, *J* = 6.4 Hz)	21.6	1.20 (d, *J* = 6.8 Hz)	20.1
17	1.10 (d, *J* = 6.4 Hz)	21.3	1.17 (d, *J*= 6.8 Hz)	20.3
18		179.4		176.8
19	1.27 s	26.7	1.31 s	25.5
20	1.36 s	16.4	1.33.s	18.3
3-OH	5.95 (d, *J* = 5.4 Hz)		5.29 (d, *J* =.5.0 Hz)	
7-OH	6.01 (d, *J* = 4.8 Hz)		5.78 (d, *J* = 4.5 Hz)	

*****Davila *et al*., 2014

4.2.1.2 Structural elucidation of compounds from methanol extract of *G. subcordata* leaves

4.2.1.2.1. Isolate GS8

GS8 was isolated as pale yellow amorphous powder R_f value of 0.46 (solvent system: BAW, 4.1.5). It gave a melting point of 315-317^{o}C (Lit. 316-318^{o}C, Esra *et al.*, 2015).The yellow colour intensified on exposure to conc. ammonia solution vapour and also turned dark brown when sprayed with ferric chloride solution on silica gel TLC suggesting that it could be a flavonol flavonoid derivative (Mabry *et al.*, 1970). The ^{1}H NMR spectrum (Table 4.8; Appendix 8a) exhibited two sets of aromatic systems: AX system at δ_H 6.19 (d, J = 1.8 Hz) for H-6 and δ_H 6.41 (d, J = 1.8 Hz) for H-8 and ABX system at δ_H 7.67 (d, J = 2.4 Hz) for H-2', 7.53 (dd, J = 8.4, 2.4 Hz) for H-6' and 6.88 (d, J = 8.4 Hz) for H-5', which suggested a flavonol pattern similar to quercetin (Sathyadevi &Subramanian, 2015). In addition, there was a signal at δ_H 12.48 representing a strongly hydrogen bonded C-5 hydroxyl group (Batterham & Highet, 1963). The ^{13}C NMR spectral data (Table 4.8, Appendix 8b) of compound **GS8**, showed a total of fifteen carbon signals including five aromatic CH and ten non-protonated carbons (one carbonyl and six C-O bearing carbons) as evidenced by DEPT-135 spectrum (Appendix 8c). This further suggested that the compound is 3, 5, 7, 3', 4' pentahydroxy flavone, commonly known as quercetin (Sathyadevi & Subramanian, 2015). Its mass spectrum showed a molecular ion peak at *m/z* 302 (Appendix 8d) which is consistent with $C_{15}H_{10}O_7$ formula. This together with daughter ions at *m/z* 153 and 137 **(**Figure 10**)** confirmed the compound is quercetin. Thus on the basis of spectroscopic and physical data, and on comparison with the literature values (Aisya *et al*., 2017), compound **GS8** was identified as quercetin (**140)**.



140

Table 4.8: ^{1}H (600 MHz) and ^{13}C NMR (125 MHz) (DMSO-d_6) spectral data of compound **140**

C#	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C NMR*	DEPT
2		147.7		147.7	C
3		135.7		135.7	C
4		175.8		176.8	C
5		160.7		160.7	C
6	6.19 (d, *J* = 1.8 Hz)	98.1	6.20 (d, *J* = 2.0 Hz)	98.2	CH
7		163.8		163.9	C
8	6.41 (d, *J* = 1.8 Hz)	93.3	6.40 (d, *J* = 2.0 Hz)	94.5	CH
9		156.1		156.1	C
10		103.0		103.0	C
1'		120.0		121.9	C
2'	7.67 (d, *J* = 2.4 Hz)	115.0	7.65(d, *J* = 2.1 Hz)	115.0	CH
3'		145.0		145.0	C
4'		146.8		145.8	C
5'	7.53(d, *J* = 8.4 Hz)	115.6	6.85 (d, *J* = 8.4 Hz)	115.6	CH
6'	6.88 (d, *J* = 8.4 Hz)	121.9	7.50 (dd, *J* = 8.4, 2.1	124.5	CH
3-OH	9.60 s				
5-OH	12.48 s		12.50 s		
3'-OH	9.30 s				
4'-OH	9.34 s				

*Aisya *et al*., 2017

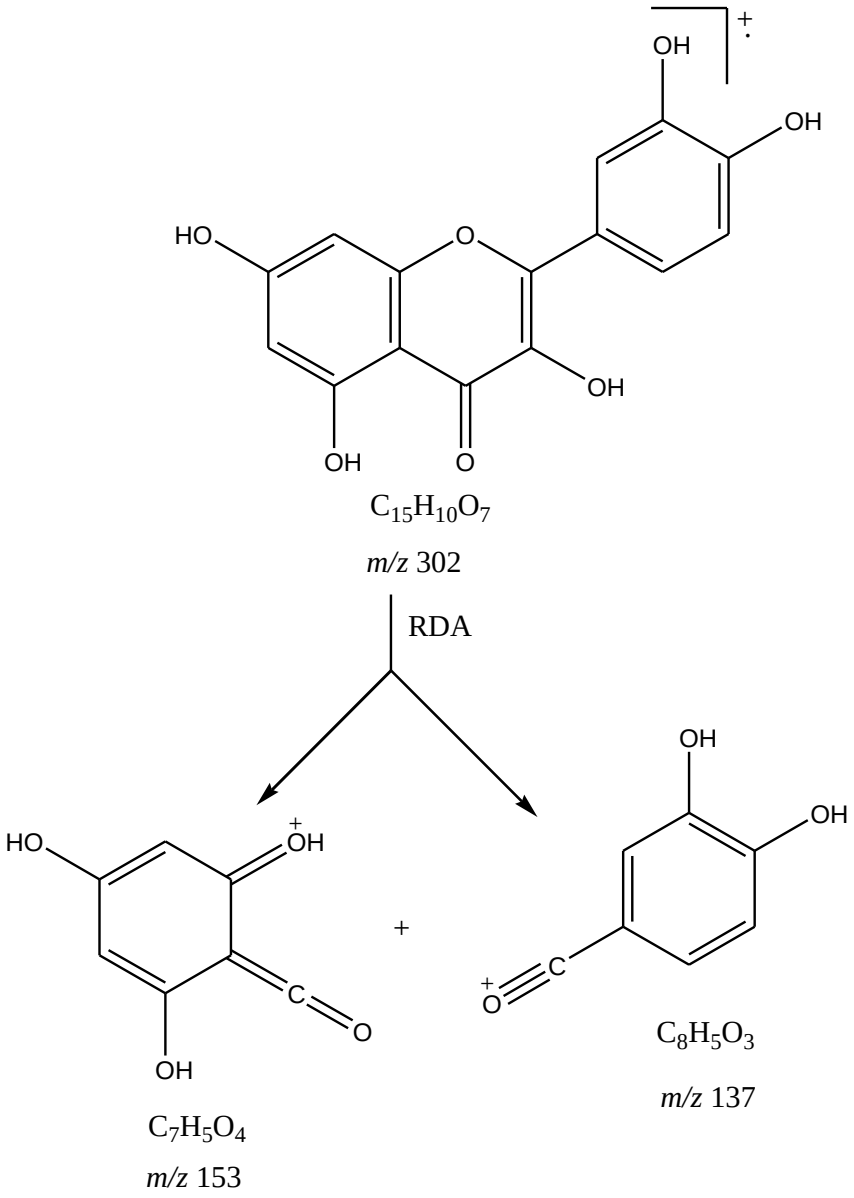


Figure 10: Proposed fragmentation pattern of compound **140** in EI-MS (70 ev)

4.2.1.2.2Isolate GS9

Compound **GS9** was isolated as a yellow amorphous powder with R_f = 0.33 (solvent system: BAW, 4.1.5). The UV spectrum of the compound in MeOH showed absorption maxima at 354 (band I) and 262nm (band I) (Appendix 9a) suggesting substituted hydroxyl group at C-3 of compound **140**. The bathochromic shift of band I with $AlCl_3$/HCl (46 nm) [Appendix 9b (i)] is a typical feature of non-transformed hydroxyl group at C-5, whereas the bathochromic shift of band II (10 nm) observed with

NaOAc [Appendix 9b (ii)] indicated the presence of unsubstituted hydroxyl group at C-7 (Mabry *et al*., 1970; Manguro, 1996). The foregoing evidences were supported by the ^{1}H NMR spectrum (Table 4.9; Appendix 9c) which exhibited two meta-coupled protons at δ_H 6.41 (d, J = 2.1Hz) and 6.20 (d, J = 1.8 Hz) assignable to H-8 and H-6, respectively. On the other hand, the non-degeneration of the UV NaOMe spectrum (Appendix 9d) with time suggested the absence of the 3'-OH group in the molecule (Mabry *et al*., 1970), a fact substantiated by the ^{1}H NMR two doublet A_2B_2 peaks at δ_H 7.57 (J = 8.5 Hz) and 6.86 (J = 9.0 Hz) attributable to C-2', C-6' and C-3', C-5', respectively (Zhang *et al*., 2014; Omar *et al*, 2015). On hydrolysis (2% HCl acid), of the compound, it yielded kaempferol and galactose. The identity of kaempferol as aglycone was confirmed by comparison of its TLC, UV,^{1}H NMR with authentic sample and EI-MS spectrum aglycone peak at *m/z* 287.6 [M+H-galactose] $^{+}$(Appendix 9e). Galactose was identified by comparison on TLC (solvent system: EtOAc-MeOH-H_2O-HOAc, 6:2:1:1) with authentic galactose, confirmed by ESI-MS [M+H] $^{+}$ 449.4 (Appendix 9f) representing $C_{21}H_{20}O_{11}$. In the sugar region, the ^{1}H NMR spectrum displayed only one resolved doublet at δ_H 5.48 (J = 7.2 Hz) assigned to anomeric proton H-1". Thus on the basis of physical, chemical and spectroscopic data, as well as comparison with literature data (Liu *et al.,* 2009) **GS9** was confirmed to be kaempferol -3-*O*-β-galactoside (**206**), reported in this species for the first time.

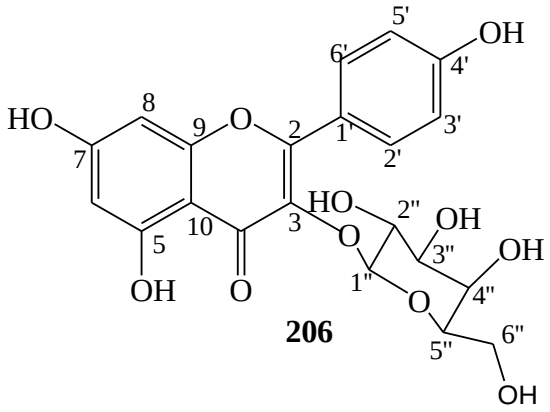


Table 4.9:[1]H (600 MHz) and [13]C NMR (150 MHz) ($CDCl_3$) data of compound **206**

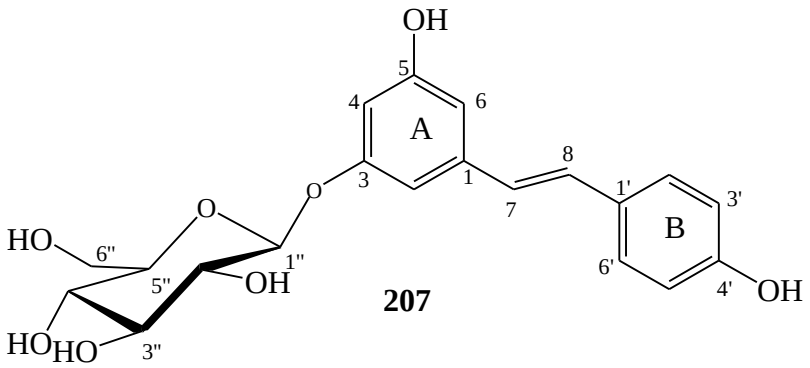
C#	[1]H NMR	[1]H NMR*
2		
3		
4		
5		
6	6.20 (d, *J* = 1.8 Hz)	6.21 (d, *J* = 2.0 Hz)
7		
8	6.41 (d, *J* = 2.1 Hz)	6.43 (d, *J* = 2.0 Hz)
9		
10		
1'		
2'	7.57 (d, *J* = 8.5 Hz)	8.07 (d, *J* = 9.0 Hz)
3'	6.86 (d, *J* = 9.0 Hz)	6.88 (d, *J* = 9.0 Hz)
4'		
5'	6.86 (d, *J* = 9.0 Hz)	6.88 (d, *J* = 9.0 Hz)
6'	7.57 (d, *J* = 8.5 Hz)	8.07 (d, *J* = 9.0 Hz)
1"	5.48 (d, *J* = 7.2 Hz)	5.25 (d, *J* = 7.0 Hz)
2"	4.97-3.09m	
3"	4.97-3.09 m	
4"	4.97-3.09 m	
5"	4.97-3.09 m	
6"	4.97-3.09 m	

*Da Liu *et al*., 2009)

4.2.1.2.3 Isolate GS10

The compound was obtained as pale yellow powder; m.p. 220-222^{o}C. The[1]HNMR spectrum (Table 4.10, Appendix 10a) of the compound showed characteristic aglycone pattern of resveratol derivative with three *meta*-coupled aromatic protons of ring A appearing at δ_H 6.62 (H-6), 6.77

(H-4) and 6.45 (H-2) (Ana *et al.*, 2009). The other set of aromatic protons signifying AA'XX' were observed at δ_H 7.36 (d, *J* = 9.0 Hz, H-2'/H-6') and 6.79 (d, *J* = 10.2, 6.6 Hz, H-3'/H-5'), respectively. These were supported by ^{13}C NMR spectrum **(**Table 4.10; Appendix 10b) which exhibited signals at δ_C 125.8 (C-2'), 128.6 (C-3'), 128.9 (C-5') and 128.0 (C-6'). These together with isolated olefinic protons observed at δ_H 7.00 (d, *J* = 16.8 Hz, H-8) and 6.83 (d, *J* = 16.8 Hz, H-7) suggested that the aglycone is resveratol (Fulvia *et al.*, 1997; Xiao-Hua *et al.*, 2013). The anomeric proton signal in the ^{1}HNMR spectrum appeared at δ_H 4.83 (H-1", d, *J* = 7.2 Hz) was in accordance with the axial-axial coupling between protons on C-1" and C-2" in a β-linked hexose (Markham, 1982). The other signals in the relatively up field region of the spectrum at δ_H 3.47, 3.38, 3.31, 3.39 and 3.55, 3.70 integrated to one proton each accounting for H-2", H-3", H-4", H-5" and H-6", respectively. The ^{13}C NMR spectrum displayed twenty signals with multiplicity assignment revealing the presence of one methylene, fourteen methines and five quaternary carbons atoms as evidenced by DEPT-135 (Appendix 10c).In the EI-MS spectrum (Appendix 10d), molecular ion peak at *m/z* 390 corresponded to $C_{20}H_{22}O_8$ formula. Acid hydrolysis of isolate **GS10** gave resveratol and glucose confirmed by the TLC and paper chromatography with authentic samples. Thus, the above data for **GS10** and in comparison with literature data (Feng *et al.*, 2005) are consistent with the structure of 4', 5-dihydroxystilbene-3-*O*-β-glucoside (**207**), reported in this species for the first time,



207

Table 4.10: ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) (CD_3OD)) spectral data of **207**

C#	^{1}H NMR (*J* in Hz)	^{13}C NMR	^{1}H NMR*(*J* in Hz)	^{13}C* NMR	DEPT-135
1		141.6		140.3	C
2	6.45, s	105.6	6.46, s	106.3	CH
3		160.5		160.1	C
4	6.77(dd, *J* = 4.2,1.2)	104.1	6.46 (d, *J* = 2.3, 1.7)	104.1	CH
5		159.6		159.4	C
6	6.62, s	107.1	6.59, s	108.8	CH
7	6.83(d, *J* = 16.8)	130.4	6.45 (d, *J* = 16.6)	129.2	CH
8	7.00(d, *J* = 16.8)	126.7	6.62 (d, *J* = 16.6)	129.7	CH
1'		141.4		138.2	C
2'	7.36 (d, *J* = 9.0)	126.8	7.35 (d, *J* = 8.6)	127.0	CH
3'	6.79 (dd, *J* = 10.2, 6.6)	128.6	6.75 (dd, *J* = 10.1, 8.6)	129.4	CH
4'		130.0		128.3	C
5'	6.79 (d, *J* = 9.0)	128.9	6.75 (d, *J* = 10.1)	129.4	CH
6'	7.36 (d, *J* = 9.0)	128.0	7.35 (d, *J* = 8.6)	127.0	CH
1"	4.84 (d, *J* = 7.2)	102.4	3.91 (dd, *J* = 7.1, 1.7)	100.4	CH
2"	3.47 (dd, *J* = 9.4, 6.8)	75.0	3.41, s	77.2	CH
3"	3.38 (dd, *J* = 9.0, 4.2)	78.1	3.36, s	77.6	CH
4"	3.31 (d, *J* = 9.8)	71.5	3.35, s	71.4	CH
5"	3.39 (dd, *J* = 9.8, 6.0)	78.3	3.38, s	79.0	CH
6_b"	3.55 (dd, *J* = 13.2, 6.6)	62.6	3.44 (dd, *J* = 12.4, 6.3)	62.4	CH_2
6_a"	3.70 (dd, *J* = 10.1, 5.7)				

*Feng *et al.*, 2005

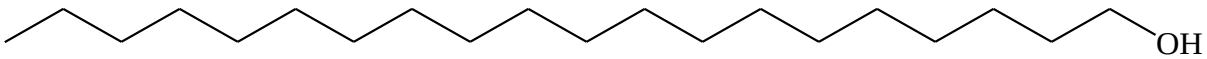
4.2.2 Structural elucidation of compounds from *O. kilimandscharicum* leaves

Eleven compounds were isolated from the leaf extracts of *O. kilimandscharicum*. The structures of these compounds were established based on their physical and spectroscopic data as well as on comparison with the data in the respective literature.

4.2.2.1 Structural elucidation of compounds from *n*-hexane extracts of *O. kilimandscharicum* leaves

4.2.2.1.1 Isolate OL1

Compound **OL1** was isolated as white amorphous powder with m.p 112-114^{o}C and R_f = 0.75 (solvent system, *n*-hexane-EtOAc, 4:1). The ^{1}H NMR spectrum (Table 4.11, Appendix 11a) showed a methyl triplet at δ_H 0.90 (J = 7.2 Hz) representing a terminal methyl group. A triplet peak which appeared at δ_H 3.65 could be assigned to a methylene proton next to hydroxyl group while the multiplet appearing at δ_H 1.59 was assigned to the methylene protons on the carbon atom adjacent to the carbon bearing the hydroxyl group. The multiplet appearing at δ_H 1.25 was due to the remaining seventeen methylenes forming the long chain. The EI-MS (Appendix 11b) corroborated the ^{1}H NMR information by showing a molecular ion peak at *m/z* 300.2 $[M + 2]^{+}$and base peak of *m/z* 94.1 (100). The ^{13}C NMR spectrum of compound **OL1** (Appendix 11c) showed the presence of a long chain terminal methyl group at δ_C 14.1 while the methylene next to the hydroxyl group appeared at δ_C 63.1. The remaining peaks (δ_C 32.8, 31.9, 29.7, 29.7, 29.6, 29.6, 29.6, 29.3, 25.6 and 22.7) (Table 4.11) represented the long chain methylene groups. Thus on the basis of physical, spectroscopic data and on comparison with the literature data (Mamun *et al*., 2021), compound **OL1** was concluded to be *n*-eicosanol **(208)**



208

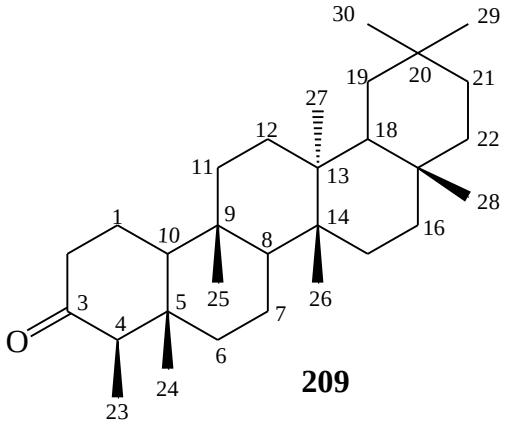
Table 4.11: ^{1}H (400 MHz) and ^{13}C NMR (150 MHz) ($CDCl_3$) spectral data of 208

C	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C NMR*
1	3.65 t (J = 3.2 Hz)	63.1	3.66	63.1
2	1.59 m	32.8	1.59	32.8
3-19	1.25 m	31.9-22.7	1.28	31.9-22.7
20	0.89 t (J = 7.2 Hz)	14.1	0.90	14.1

*Mamun *et al.*, 2021

4.2.2.1.2Isolate OL2

OL2 was isolated as white powder, m.p; 254-256^{o}C. It gave a positive Liebermann-Burchard test suggesting that it could be a terpene or sterol (Attarde *et al.*, 2010). The presence of keto functionality was confirmed by a signal at δ_C 213.4 typical of a ketonic carbon (Majidul *et al.*, 2015). In the ^{1}H NMR spectrum (Table 4.12, Appendix 12a), a doublet signal at δ_H 1.20 (J = 6.8 Hz, 23-Me), integrating into three protons together with resonances for seven quaternary methyl groups observed at δ_H 0.74 (25-Me), 0.88 (29-Me), 0.89 (30-Me), 0.91 (26-Me), 1.02 (24-Me), 1.03 (27-Me) and 1.07 (28-Me) with corresponding ^{13}C NMR signals at 18.9 (C-25), 32.4 (C-29), 32.7 (C-30), 20.5 (C-26), 14.9 (C-24), 18.5 (C-27) and 32.1 (C-28) (Table 4.12, Appendix 12b), suggested that the compound was a triterpene of friedelin-type (Jong *et al.*, 2012). The fore-going evidence was further supported by a quartet signal integrating into one proton at δ_H 2.27 which was assigned to H-4. The ^{13}C NMR spectral data (Table 4.12**,** Appendix 12b) showed the presence of 30 carbon signals resolved into eight methyls, eleven methylenes, four methines and seven quaternary carbons as evidenced by DEPT 135 experiments (Appendix 12c).On the other hand, the EI-MS (70 eV) (Appendix 12d) afforded a molecular ion peak at *m/z* 426.2 which suggested the molecular formula $C_{30}H_{50}O$. In fact, the presence of a signal due to one tertiary and seven quaternary methyl groups in the ^{13}C NMR spectrum together with cross correlation between a doublet methyl signal at δ_H1.20 and a quartet of methine at δ_H 2.27 strongly supported the structure of the compound as friedelin. Thus on the basis of spectroscopic evidence and on comparison with the literature data (Ragasa *et al*., 2015), **OL2** was confirmed to be friedelin **(209).**



209

Table 4.12: ^{1}H NMR (600 MHz) and ^{13}C (150 MHz) ($CDCl_3$) spectral data of compound **209**

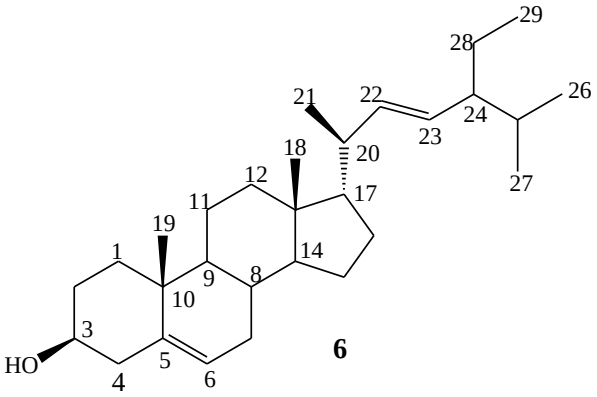
C#	^{1}H NMR	^{13} CNMR	^{1}H NMR*	^{13}C NMR*	DEPT
1	1.76 (dd, *J* = 13.0, 7.5 Hz)	22.5	1.78 (dd, J = 13.0, 7.4 Hz)	22.3	CH_2
2	2.25 (d, *J* = 6.8 Hz)	41.6	2.32 m	41.5	CH_2
3		213.4		213.2	C
4	2.27 (q, *J* = 7.3 Hz)	58.5		58.2	CH
5		42.4		42.1	C
6		41.5		41.3	CH_2
7	1.31 m	18.5	1.31 m	18.2	CH_2
8	1.39 m	53.4	1.39 m	53.1	CH
9		37.7		37.4	C
10	1.39 m	59.8	1.30 m	59.4	CH
11	1.56 m	33.0	1.56 m	35.6	CH_2
12	1.56 m	30.8	1.56 m	30.5	CH_2
13		41.8		39.7	C
14		36.3		36.3	C
15	1.31 m	30.3	1.31 m	32.4	CH_2
16	1.31 m	35.6	1.31 m	36.0	CH_2
17		29.9		30.0	C
18	1.39 m	43.1	1.39 m	42.8	CH
19	1.45 m	35.3	1.45 m	35.3	CH_2
20		29.9		28.2	C
21	1.31 m	33.1	1.21 m	32.8	CH_2
22	1.31 m	40.0	1.31 m	39.2	CH_2
23	1.20 (d, *J* = 6.8 Hz)	7.1	0.88 (d, *J* = 6.5 Hz)	6.8	CH_3
24	1.02 s	14.9	1.04 s	14.6	CH_3
25	0.74 s	18.9	0.72 s	17.9	CH_3
26	0.91 s	20.5	0.87 s	20.2	CH_3
27	1.03 s	18.3	1.00 s	18.7	CH_3
28	1.07 s	32.1	1.05 s	32.1	CH_3
29	0.88 s	32.4	0.95 s	35.0	CH_3
30	0.89 s	32.7	1.18 s	31.8	CH_3

*Ragasa *et al*., 2015

4. 2.2.2 Structural elucidation of compounds from ethyl acetate extract of *O. kilimandscharicum* leaves

4. 2.2.2.1 Isolate OL3

OL3 was isolated as a white amorphous powder R_f = 0.53 (solvent system: n-hexane-EtOAc, 4:1) and a melting point of 163-166^{o}C. It was obtained from the leaves of *O. kilimandscharicum* using medium pressure column chromatography with solvent system CH_2Cl_2-EtOAc (4:1). The compound gave a bluish-purple colour on TLC (silica gel) after spraying with acidified vanillin suggesting presence of a sterol or a terpenoid derivative, a fact that was supported by a positive Liebermann-Buchard test characteristic of sterols or terpenoids. The compound however failed the specific ceric sulphate test for terpenoids suggesting it could be a sterol derivative. The ^{1}HNMR spectrum (Table 4.13; Appendix 13a) showed the presence of three vinylic protons at δ_H 5.36 (1H, m, H-6), 5.13 (1H, m, H-23) and 5.07 (1H, m, H-22), and an oxymethine peak at δ_H 3.56 (1H, m, H-3).. The ^{13}C NMR spectrum **(**Table 4.13**;** Appendix 13a) of compound **OL3** displayed a total of 29 carbon resonances. The EI-MS (70 eV) mass spectrum (Appendix 13b) showed a molecular ion peak at *m/z* 412.4 which is 2 a.m.u less than that of β-sitosterol**(95)** possibly due to the presence of an additional isolated double bond in the side chain besides the endocyclic double bond in the molecule. The foregoing evidence was manifested by the ^{13}C NMR spectrum two sets of olefinic carbon signals at δ_C 140.71 (C-5) and 121.30 (C-6) due to trisubstituted double bond functionality, whereas the peaks at δ_C 138.32 and 129.30 were attributed to isolated double bond between C-22 and C-23, respectively. Furthermore, the methine carbon signal at δ_C 71.21 was assigned to C-3 further suggesting that the compound is a sterol derivative (Habib *et al.*, 2007; Rao *et al.*, 2012). Thus, from the physical and spectral data and on comparison with the literature data (Cyme & Ragasa, 2004) compound **OL3** was confirmed to be stigmasterol **(6)**



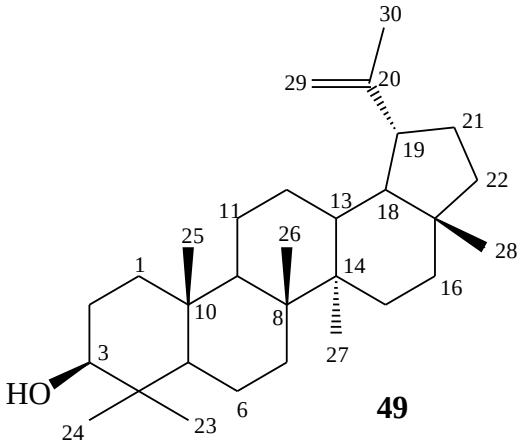
6

Table 4.13: ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) ($CDCl_3$) spectral data for compound **(6)**

C	^{1}H NMR (*J* in Hz)	^{13}CNMR	*^{1}H NMR (*J* in Hz)	*^{13}C NMR	DEPT
1		36.4		37.2	CH_2
2		31.1		31.6	CH_2
3	3.56 m	71.7	3.53 m	71.8	CH
4		42.2		42.3	CH_2
5		140.7		140.7	C
6	5.36 m	121.7	5.35 (t, *J* = 6.1 Hz)	121.7	CH
7		31.8		31.9	CH_2
8		28.8		31.9	CH
9		50.1		50.1	CH
10		33;9		36.5	C
11		21.0		21.1	CH_2
12		39.7		39.7	CH_2
13		40.4		42.2	C
14		56.8		56.8	CH
15		24.5		24.4	CH_2
16		28.3		28.9	CH_2
17		56.0		55.9	CH
18	0.81 s	12.1	0.70 s, me	12.0	CH_3
19	1.51s	18.9	1.01 s	19.4	CH_3
20		39.6		40.5	CH
21	1.49(d, *J* =5.9 Hz)	19.3	1.02 (d, *J* = 6.8 Hz)	21.1	CH_3
22	5.07 m	138.2	5.15dd (*J* = 8.4,15.1Hz)	138.3	CH
23	5.13 m	129.3	5.02dd (*J* = 8.4, 15.1Hz)	129.2	CH
24	0.83 (t, *J* = 7.0 Hz)	51.2	0.84 (t, *J* = 6.4 Hz)	51.2	CH
25		31.6		31.9	CH
26		21.2		21.2	CH_3
27	0.94(d, *J* = 6.3 Hz)	20.0	0.97 (d, *J* = 6.6 Hz)	19.0	CH_3
28		24.4		25.4	CH_2
29		11.8		12.3	CH_3

*Cyme *& Ragasa*, 2004

4.2.2.2.2Isolate OL4: The compound was isolated as white crystals with R_f = 0.48, (solvent system: *n*-hexane –EtoAc, 4:1) and m.p 216-218ºC. It gave a positive Liebermann-Buchard test suggesting terpenoid or a sterol skeleton (Attarde *et al.,* 2010). The ^{1}H NMR spectrum (Table 4.14, Appendix 14a) had signals appearing at δ_H 4.69 brs and 4.57 brs representing double bond protons typical of lupane- type triterpenes (Gallo & Sarachine, 2009). The presence of the exocyclic double bond was supported by presence of a peak at δ_C 108.7 attributable to the olefinic carbon at C-29 in the ^{13}C NMR spectrum (Table 4.14,Appendix 14b), an evidence further confirmed by the down field olefinic carbon at δ_C 149.7 (C-20) typical of quaternary sp^2 hybridized carbon (Gallo & Sarachine, 2009). In addition, the ^{1}H NMR spectrum showed a signal suggesting the presence of a hydroxy methine proton (H-3) at δ_H3.18 (dd, *J*=11.4, 3.0 Hz) (Gallo & Sarachine, 2009) with the corresponding carbon peak at δ_C 76.6 (C-3). The hydroxyl group was deduced to be in equatorial orientation based on axial-axial and axial-equatorial couplings between H-3 and H-2. Furthermore seven singlets for quaternary methyl protons observed at 0.94 (Me-23), 0.74 (Me-24), 0.80 (Me-25), 1.01 (Me-26), δ_H 0.92 (Me-27), 0.76 (Me-28) and 1.66 (Me-30), (Integrating into 3H each) with corresponding ^{13}C NMR signals at 28.4 (C-23), 15.1 (C-24), 15.3 (C-25), 17.3 (C-26), δ_C 13.9 (C-27), 18.6 (C-28) and 20.6 (C-30) respectively so deduced from HSQC (Appendix 14c) were in agreement with the structure of lupeol previously isolated from *Lonchocarpus sericens* and *Bowdichia virgilioides*(Abdullahi *et al*., 2013; Beserra *et al*., 2018). Confirmation of lupeol structure was further supported by ESI-MS (70 ev.) molecular ion peak at m/z 426.0 $[M]^+$ (Appendix 14d) corresponding to the molecular formula $C_{30}H_{50}O$. Therefore, on the basis of physical and spectroscopic data as well as comparison with literature data (Beserra *et al*., 2018) compound **OL4** was confirmed to be lupeol (**49)**.



49

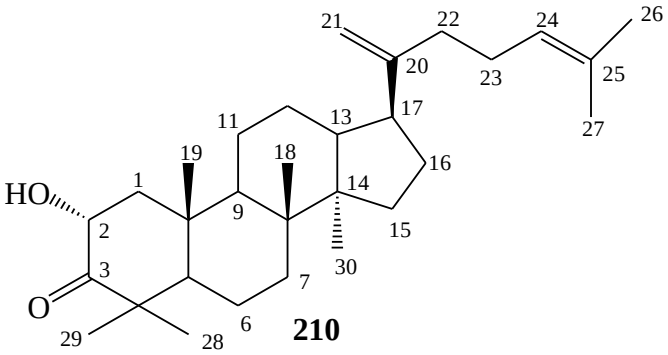
Table 4.14: ^{1}H (600 MHz) and ^{13}C NMR (125 MHz) $(CD_3)_2SO$) spectral data for **49**

C#	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C NMR*
1	1.68 m, 1.38 m	39.0		38.8
2	1.66 m, 1.64 m	27.6	1.89 m	27.5
3	3.00 (dd, *J* = 11.4, 3.0 Hz)	76.6	3.16 m	79.0
4		39.2		38.9
5		54.7		55.3
6	1.68 m, 1.26 m	18.6	0.66 m	18.9
7	1.48 m, 1.32 m	34.8	1.36 m	34.3
8		40.2		40.8
9		49.7		50.4
10		37.4		37.2
11		18.6		21.0
12		24.6		25.1
13		38.1		38.1
14		42.1		42.1
15	1.46 m, 1.26 m	26.8		27.4
16	1.64 m, 1.32 m	36.4		35.6
17		42.1		43.1
18		47.7		48.3
19		47.0		48.0
20		149.7		150.9
21		29.1		29.9
22		40.0		40.1
23	0.94 s	28.4	0.94 s	28.1
24	0.74 s	15.1	0.74 s	15.5
25	0.83 s	15.3	0.80 s	16.0
26	1.04 s	17.3	1.01 s	16.2
27	0.92 s	13.9	0.92 s	14.6
28	0.80 s	17.6	0.77s	18.1
29	4.57 brs, 4.69 brs	108.7	4.55s, 4.67s,	109.5
30	1.66 s	20.2	1.66s	19.4

*Beserra *et al*., 2018

4.2.2.2.3 Isolate OL5. The compound was isolated as an amorphous white powder with m.p. 191-193^{o}C. Its IR spectrum (Appendix 15a) showed absorption peaks for hydroxyl (3357.3 cm^{-1}), keto (1764.1 cm^{-1}) and double bond (1589.0 cm^{-1}) functional groups. The high resolution electron mass spectrum exhibited a molecular ion peak at *m/z* 440.36550 $[M]^{+}$ suggesting that **OL5** had the formula $C_{30}H_{48}O_2$. This molecular formula corresponded to seven degrees of unsaturation, of which four were assigned to the tetracyclic rings, two to the two exocyclic double bonds and one to the keto group. The EI-MS (Appendix 15b) on the other hand gave significant peaks at *m/z* 440.3 $[M]^{+}$, 391.2 $[M\text{-}Me\text{-}2H_2O]^{+}$, 362.2 [391-2Me] $^{+}$which are characteristic of tetracyclic triterpene alcohols with di-unsaturated side chain and also a saturated skeleton (Leong &Harrison, 1999). The ^{1}H NMR data of compound **OL5** [Table 4.15, Appendix 15c(i & ii)], which was compared with those of known compounds dammar-20, 24-dien-3β-ol (Leong & Harrison 1999), dammaradienone (Teles *et al.*, 2014) and 2-oxo-3β,19α-dihydroxyolean-12-en-28-oic (Kuang *et al.*, 2011) showed the presence of a vinylic proton on a tri-substituted double bond at δ_H 5.12 (d, *J*=6.4 Hz), two proton doublets resonating at δ_H 4.70 (d, *J* = 1.4 Hz), and 4.60 (d, *J* = 1.4 Hz), assignable to C-21 methylene protons and two vinylic methyl protons at δ_H 1.68 (Me-26) and 1.64 Me-27). The vinylic proton at δ_H 5.12 was shown to allylically coupled with the vinylic methyls by homonuclear decoupling experiments, thus authenticating the presence of a terminal $–CH_2\text{-}CH=C(CH_3)_2$ group (Teles *et al.*, 2014). Furthermore, the spectrum exhibited five quaternary methyls at δ_H 0.81, 0.91, 1.14, 1.42, 1.43, 1.64 and 1.68 which were ascribable to C-19, C-18, C-29, C-28, C-30, C-27, C-28 and C-26 methyl protons, respectively. An up field double of doublets in the aliphatic region which resonated at δ_H 2.84 was assigned to C-2 proton and its orientation was suggested to be α-on the basis of the coupling constant, *J* = 9.6, 3.8 Hz (Nick *et al.*, 1995; Duan *et al.*, 2000). Thus, this inferred that the hydroxyl functionality in this position was in β-

orientation. The ^{13}C NMR spectrum [Table 4.15, Appendix 15d (i & ii)] displayed a total of 30 carbon resonances, their multiplicities assigned using DEPT experiments, including DEPT-135 (Appendix 15e) established the presence of seven methyls, 10 methylenes (including one olefinic carbon), six methines (including an oxymethine at δ_C 69.7 and one olefinic methine at δ_C 124.4) and seven quaternary carbons including a keto group at δ_C 216.7. In fact, the ^{13}C NMR of **OL5** was similar to those of dammara-20, 24-dien-3β-ol (Leong & Harrison, 1999) with notable differences being the presence of an oxo group on ring A. In **OL5**, the C-3 hydroxyl group was replaced by a keto functionality and the positions of the hydroxyl and the keto groups on ring A were ascertained from HMBC experiments, which showed one-bond H, C- and three bond H, C-correlations, respectively (Figure 13). In this way it was proved that the hydroxyl moiety was at C-2 and the keto group was at C-3, a fact cemented by NOESY correlations as outlined in (Figure 11). Thus, on the basis of spectroscopic data, isolate **OL5** was elucidated as2α-hydroxy-3-oxodammar-20, 24-diene (**210**), which is a new compound.



210

Table 4.15: ^{1}H (600 MHz) and ^{13}C (150 MHz) ($CDCl_3$) spectral data of **210**.

C#	^{1}H NMR	^{13}C NMR	DEPT	HMBC NMR	NOESY NMR
1	2.24 m, 1.07 m	43.1	CH_2	H-1, C-3	
2	2.84 (dd, *J* = 9.6, 3.8 Hz)	69.7	CH	H-2, C-4, C-10	H-2, Me-24
3		216.7	C		
4		37.2	C		
5	0.79(dd, *J* = 11.5, 2.4 Hz)	55.9	CH	H-5, C-3	H-5, Me-23
6	1.25 m	21.4	CH_2		
7	1.51 m	35.5	CH_2		
8		42.1	C		
9	1.43 m	54.7	CH		H-9, H-5
10		39.7	C		
11	1.90 m, 1.42 m	23.3	CH_2		
12	2.17 m, 1.14m	25.0	CH_2		
13	2.21 m	42.2	CH		
14		50.7	C		
15	1.41 m, 1.00 m	28.7	CH_2		
16	1.42 m, 1.25 m	27.3	CH_2		
17	2.38 m	47.7	CH	H-17, C-21	H-17, H-5
18	0.91 s	17.4	CH_3		Me-18, Me-19
19	0.81 s	16.9	CH_3		
20		150.8	C		
21	4.70 (d, *J* = 1.4 Hz), 4.60, (d, *J* = 1.4 Hz),	109.4	CH_2		
22	1.51 m, 1.43 m	39.9	CH_2		
23	2.24 m, 1.25 m	27.5	CH_2		
24	5.12 (t, *J* = 6.4 Hz)	124.4	CH	Me-24, C-26	
25		133.6	C		
26	1.68 s	25.2	CH_3		
27	1.64 s	18.8	CH_3	Me-24, C-27	
28	1.42 s	29.8	CH_3		
29	1.14 s	23.7	CH_3		
30	1.43 s	17.5	CH_3		

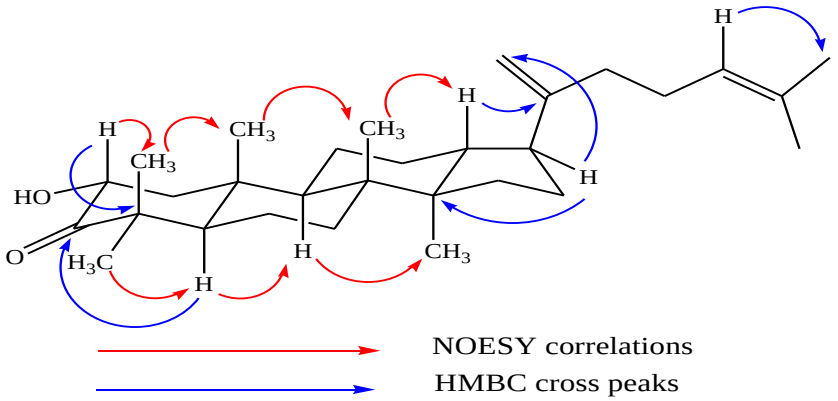
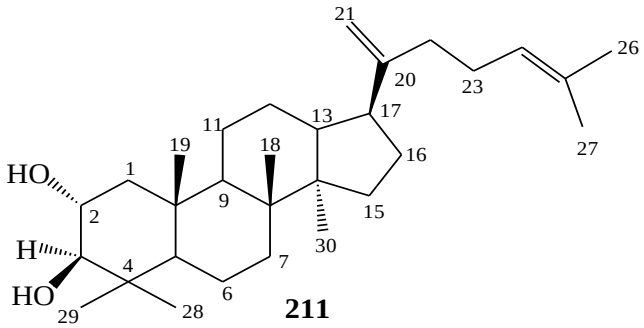


Figure 11: Selected HMBC and NOESY correlations of compound **210**

4.2.2.2.4 Isolate OL6

The compound was obtained as white crystals from CH_2Cl_2-MeOH mixture. The compound exhibited a HRESI-MS molecular ion peak at *m/z* 465.3430 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_2$+Na, 465.3424), ESI-MS m/z 465.4 $[M+Na]^+$ (Appendix 16a)and EI-MS (rel. Int.) m/z 442.1 (12) (Appendix 16b). The ^{13}C NMR spectrum [Table 4.16**,** Appendix 16c (i &ii)] revealed the presence of 30 carbon signals including CH_3 x 7, >CH-OH x 2, >CH- x 4, >C=CH_2 x 1, >CH_2- x 9, >C< x 4, -CH=C< x 1 as evidenced by DEPT-135 spectrum (Appendix 16d). Comparison of the ^1H and ^{13}C NMR spectral data of compound **OL6** with those of **210** revealed close similarities with notable difference being the replacement of the oxo group by hydroxyl functionality in compound **OL6** as substantiated by the molecular ion peak which is 2 a.m.u more than that of compound **210.** Furthermore, from the ^1H NMR spectrum [Table 4.16**;** Appendix 16e (I & ii)] the methine bearing hydroxyl group exhibited a doublet at δ_H3.23 (d, J = 9.0 Hz) assignable to H-3α on the basis of coupling constant characteristic of H-3α and H-2β axial-equatorial interaction (Liu *et al.*, 2005). Again comparing both the ^1H and ^{13}C NMR data of compound **OL6** with those of dammara-20, 24-dien-3β-ol revealed a shift of a C-1 methylene peak δ_C 43.1 with corresponding ^1H NMR peaks at δ_H 2.20 m and 1.01 m, suggesting that the second hydroxyl group was possibly at C-2. Additional

evidence from comparative studies between the two structures revealed the presence of an exomethylene with ^{1}H NMR peaks appearing at δ_H 4.70 (br s) and 4.58 (brs). Thus, on the basis of spectroscopic evidences and comparison with the literature, compound **OL6** was established to be 2α, 3β-dihydroxy-3-dammar-20, 24-diene **(211**) which is a new compound.



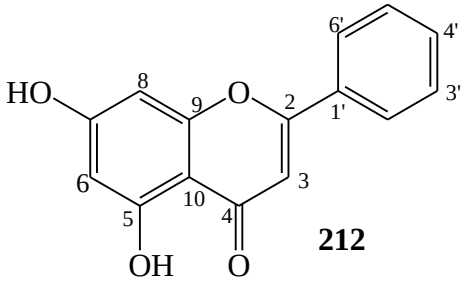
211

Table 4.16: ^{1}HNMR (600MHz) and ^{13}C NMR (150 MHz) ($CDCl_3$) of **211**

C#	^{1}H NMR	^{13}C NMR	DEPT	HMBC NMR	NOESY NMR
1	2.20 m, 1.01 m	42.9	CH_2	H-1, C-3	
2	2.80 m	69.7	CH	H-2, C-10	H-2, Me-24
3	3.23 (d, *J* = 9.0 Hz)	79.1	CH		
4		38.8	C	C-4, H-2	H-5, Me-23
5	0.87 m	56.6	CH	H-3, C--5	
6	1.64 m, 1.51 m	21.3	CH_2		
7	1.64 m, 1.25 m	35.5	CH_2		
8		42.1	C		
9	1.51 m	55.2	CH		H-9, Me-18
10		39.6	C		
11		22.3	CH_2		
12	1.68 m, 1.64 m	23.0	CH_2		
13	1.93 m	41.5	CH	H-13, C-20	H-13, Me-18
14		49.0	C		
15	1.51 m, 1.07 m	29.8	CH_2		
16	1.93 m, 1.25 m	27.3	CH_2		
17	1.68 m	47.9	CH	H-17, C-21	
18	0.91 s	16.7	CH_3		Me-18, Me-19
19	0.79 s	17.9	CH_3		
20		150.8	C		
21	4.70 (br s), 4.58 (br s)	109.4	CH_2		
22	1.64 m, 1.43 m	40.1	CH_2		
23	2.20 m, 1.90 m	27.5	CH_2		
24	5.12 (t, *J* = 6.6 Hz)	124.4	CH	H-24, C-26	
25		132.6	C		
26	1.68 s	26.6	CH_3		
27	1.64 s	18.4	CH_3	C-27, H-24	
28	1.43 s	28.2	CH_3		
29	1.41 s	21.4	CH_3		
30	1.42 s	17.5	CH_3		

4.2.2.2.5 Isolate OL7

Compound **OL7** was isolated as yellow powder, R_f = 0.51 (solvent system: CH_2Cl_2-MeOH, 99:1) and m.p. 285-287^oC. Its ^1H NMR spectrum (Table 4.17**,** Appendix 17a**)** showed the presence of two aromatic protons of AX spin system which are *meta*-coupled typical of ring A of a flavone derivative at δ_H 6.48 (d, *J* = 1.5 Hz, H-8) and 6.23 (d, *J* = 1.5 Hz, H-6). Another characteristic singlet resonance at δ_H 6.73 was assigned to H-3. Furthermore, another set of five aromatic signals of ring B involving ortho-coupled doublets at δ_H 7.99 (d, *J* = 6.5 Hz) for H-2' and H-6' and a multiplet at δ_H 7.58 (3H) assigned to H-3', H-4' and H-5' were observed suggesting that **OL7** is a flavone derivative without substitution in ring B (Vijay *et al*., 2011; Chaturvedula & Prakash, 2012). On the other hand, the ^{13}C NMR spectrum (Table 4.17**,** Appendix 17b)revealed the presence of 15 carbon signals out of which eight were methine and seven were quaternary carbons including the carbonyl carbon at δ_C183.9 (C-4) as evidenced by DEPT-135 spectrum **(**Appendix 17c). The EI-MS (Appendix 17d) showed a molecular ion peak at *m/z* 255 $[M+H]^+$ which was consistent with the formula $C_{15}H_{10}O_4$ (Liu *et al*., 2010), confirming that **OL7** was chrysin (**212).**



212

Table 4.17: ^{1}H (600 MHz) and ^{13}CNMR (150 MHz) (MeOD) spectral data for **212**.

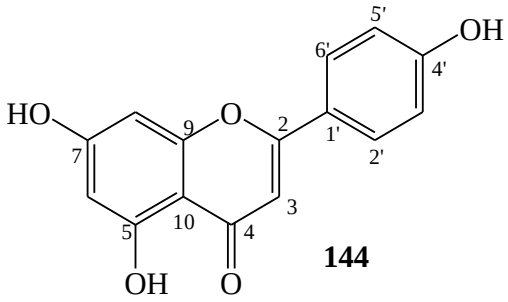
C#	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C NMR*	DEPT-135
2		163.3		163.1	C
3	6.73 s	106.1	6.96 s	105.2	CH
4		183.9		181.8	C
5		159.6	12.81 s	161.4	C
6	6.23 (d, *J* = 1.5 Hz)	100.4	6.21 (d, *J* = 2.0 Hz)	99.0	CH
7		165.7	10.71 s	164.4	C
8	6.48 (d, *J* = 1.5 Hz)	95.2	6.52 (d, *J* = 2.0 Hz)	94.1	CH
9		157.4		157.4	C
10		105.6		104.0	C
1'		132.6		130.7	C
2'	7.99 (d, *J* = 6.5 Hz)	127.5	8.06 (brd, *J* = 8.0 Hz)	126.4	CH
3'	7.58 m	130.3	7.59 m	129.1	CH
4'	7.58 m	133.1	7.59 m	132.0	CH
5'	7.58 m	130.3	7.59 m	129.1	CH
6'	7.99 (d, *J* = 6.5 Hz)	127.5	8.06 (brd, *J* = 7.0Hz)	126.4	CH

*Liu *et al.*, 2010

4.2.2.2.6 Isolate OL8

OL8 was isolated as a pale-yellow amorphous powder m.p. 346-348^{o}C and R_f = 0.44(solvent system, CH_2Cl_2-MeOH, 99:1).The yellow colour on TLC intensified on exposure to conc. ammonia vapour. It also turned dark brown upon spraying with ferric chloride solution suggesting that it could be a flavonoid (Batterham & Highet, 1963). The molecular ion peak at *m/z* 270.2 (Appendix 18a) which is 16 a.m.u less than that of kaempferol **(146)** corresponded to the formula $C_{15}H_{10}O_5$. This together with typical fragmentation ions (Figure 13) appearing at *m/z* 153.1 and 121.1 confirmed the presence of 5, 7, 4'-trihydroxyflavone. The^{1}H NMR spectral data (Table 4.18**,**

Appendix 18b) of compound **OL8** exhibited the presence of two *meta*-coupled aromatic doublets at δ_H 6.21 (d, J = 2.2 Hz) and δ_H 6.45 (d, J = 2.2 Hz) corresponding to H-6 and H-8 respectively. On the other hand, the two doublets at 6.94 (d, J = 9.0 Hz) and δ_H 7.86 (d, J = 9.0 Hz) were assigned to H-3'/H-5' and H-2'/H-6' respectively while a singlet at δ_H 6.59 represented H-3 proton. The ^{1}H NMR spectral data suggested the presence of a 5, 7, 4'-trisubstituted flavone (Batterham & Highet, 1963). The ^{13}C NMR data (Table 4.18, Appendix 18c) showed the presence of fifteen carbon signals sorted out into seven aromatic CH of the double bond and eight quaternary carbons including a conjugated carbonyl carbon at δ_C 181.7. Thus, basing on physical and spectroscopic data as well as comparison with data already reported in literature (Wang *et al.*, 2011), **OL8** was identified as apigenin **(144).**



144

Table 4.18: ^{1}H (600 MHz) and ^{13}C NMR [150 MHz, $(CD_3)_2SO$] data for compound **144**

C#	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C NMR*
2		161.4		162.5
3	6.59 s	103.6	6.66 s	104.2
4		181.7		183.2
5		163.7		163.5
6	6.21(d, J = 2.2 Hz)	98.8	6.26 (d, J = 2.4 Hz)	99.8
7		164.5		165.0
8	6.45 (d, J = 2.2 Hz)	94.0	6.55 (d, J = 2.4 Hz)	94.8
9		157.3		158.9
10		102.8		105.5
1'		123.2		123.5
2'	7.86 (d, J = 9.0 Hz)	128.4	7.94 (d, J = 9.0 Hz)	129.3
3'	6.94 (d, J = 9.0 Hz)	115.9	7.04 (d, J = 9.0 Hz)	116.9
4'		161.2		162.0
5'	6.94 (d, J = 9.0 Hz)	115.9	7.04 (d, J = 9.0 Hz)	116.9
6'	7.86 (d, J = 9.0 Hz)	128.4	7.94 (d, J = 9.0 Hz)	129.3

*Wang *et al.*, 2011

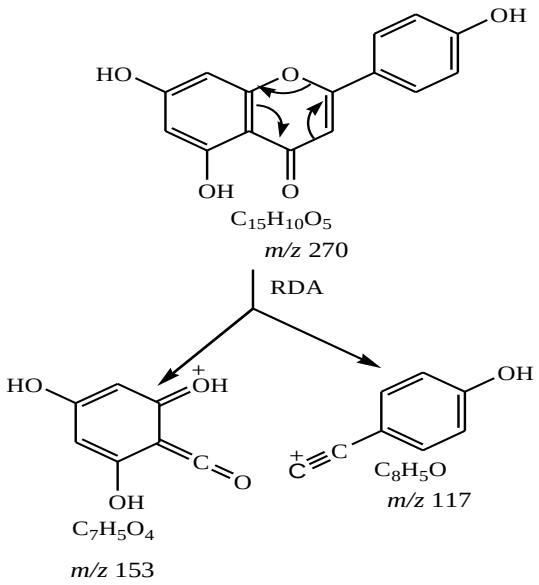
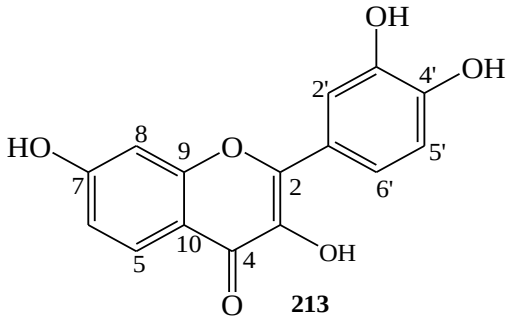


Figure 12: Fragmentation pattern of compound **144** in EI-MS (70 eV)

4.2.2.2.7 Isolate OL9

OL9 was isolated as yellow amorphous powder with m.p. 299-300^{o}C and R_f= 0.37(solvent system, CH_2Cl_2-MeOH, 99:1). The compound turned yellow upon exposure to conc. ammonia vapour or upon spraying with 5% aluminium chloride reagent indicating that it could be a flavonoid derivative (Manguro *et al.*, 2007; Ramalingam *et al.*, 2016). The IR spectrum (KBr) (Appendix 19a) showed significant absorption peaks at 3366.6 (OH) and 1631.1 (C=O) cm^{-1} representing hydroxyl and conjugated carbonyl functionalities. The ^{1}H NMR spectrum (Table 4.19,Appendix 19b) of compound **OL9** revealed the presence of a meta-coupled proton appearing at δ_H 7.80 (d, J = 2.1 Hz) and an ortho-coupled proton positioned at δ_H 7.99 (d, J = 8.7 Hz) representing H-8 and H-5, respectively. Similarly, a one doublet of doublet of *ortho-meta*-coupled aromatic proton at δ_H 7.79 (dd, J = 8.7, 2.1 Hz) was assignable to H-6. These evidences along with 3H ABX aromatic system appearing at δ_H 6.90 (d, J = 2.1 Hz, H-2'), δ_H 7.80 (dd, J = 8.1, 2.1 Hz, H-6') and δ_H 6.90 (d, J = 8.1 Hz, H-5') suggested a flavonol pattern typical of fisetin moiety (Oladimeji *et al.*, 2015; Shefaghat & Salimi, 2008). This suggestion was further supported by ^{13}C NMR (Table 4.19; Appendix 19c) peak at δ_C138.6 which is characteristic of C-3 hydroxylated flavonol derivatives (Manguro *et al.*,

2006). The HMBC spectrum (Appendix 19d) allowed the assignment of H-5, H-6 and H-8 in ring A as well as H-2', H-5' and H-6" in ring B. In fact, the absence of C-5 hydroxyl group in the molecule is responsible for the up field shift of C-5 to δ_C 127.6 in the ^{13}C NMR spectrum due to absence of hydrogen bonding between 5-OH and C=O. Thus, on the basis of accrued spectroscopic data supported by EI-MS molecular ion peak at *m/z* 286.2 and the fragmentation pattern (Figure 13; Appendix 19e) observed (*m/z* 137) and on comparison with literature data (Boukhary *et al*., 2017), **OL9** was confirmed to be 3, 7, 3′, 4′-tetrahydroxyflavone (Fisetin) (**213**). This is the first time the compound is reported in this species.



213

Table 4.19: ^{1}H (600 MHz) and ^{13}C NMR (125 MHz, CD_3OD) data for **213**

C#	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C NMR*
2		146.3		145.4
3		138.6		137.6
4		174.4		172.4
5	7.99 (d, *J* = 8.7 Hz)	124.4	7.93(d, *J* = 7.2 Hz)	126.9
6	7.67 (dd, *J* = 8.7, 2.1Hz)	116.0	7.58 (dd, *J* = 8.4, 2.1 Hz)	115.0
7		164.3		162.2
8	7.78(d, *J* = 2.1 Hz)	103.0	7.7 (d, *J* = 2.0 Hz)	102.3
9		158.5		156.9
10		116.3		120.0
1'		121.7		122.9
2'	6.90 (d, *J* = 2.1 Hz)	127.5	6.92 (d, *J* = 2.0 Hz)	128.9
3'		115.5		115.4
4'		147.5		147.7
5'	6.90 (d, *J* = 8.1 Hz)	116.3	6.92 (d, *J* = 7.4 Hz)	116.0
6'	7.79 (dd, *J* = 8.1, 2.1 Hz)	116.0	7.90 (dd, *J* = 8.5, 2.0 Hz)	115.1

*Boukhary *et al.*, 2017

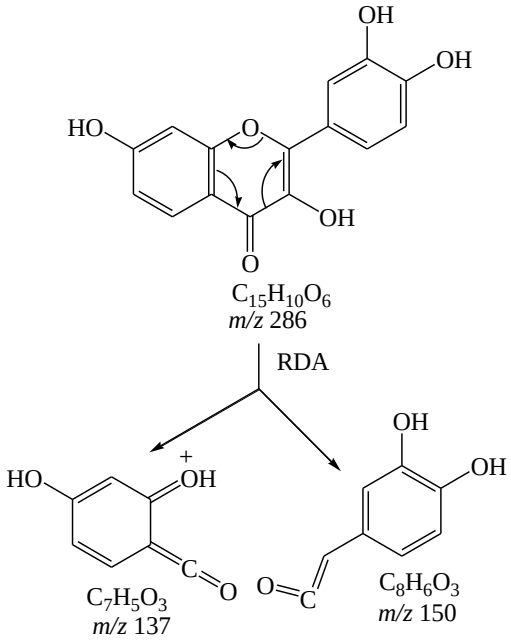


Figure 13: Proposed fragmentation pattern of compound **213** in EI-MS (70 eV)

4.2.2.3 Structural elucidation of compounds from aqueous methanol extracts of *O. kilimandscharicum* leaves

4.2.2.3.1 Isolate OL10

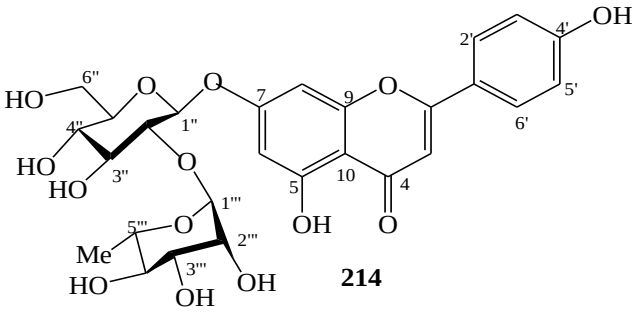
OL10 was elucidated as quercetin **(140),** a compound that was also isolated from methanol leaf extract of *Gnidia subcordata.* See pages 105-107.

4.2.2.3.2Isolate OL11

OL11 was obtained as yellow amorphous powder with R_f = 0.19 (solvent system: CH_2Cl_2-MeOH, 3:2; 2% oxalic deactivated silica gel TLC plate) and melting point 346-348^{o}C. The yellow colour intensified on exposure to concentrated ammonia vapour. It also turned dark-brown upon spraying with ferric chloride solution suggesting that it was a flavonoid derivative (Batterham & Highet, 1963, Mabry *et al*., 1970; Harborne & Mabry, 1982; Manguro *et al*., 2007). The ^{1}HNMR spectral data (Table 4.20; Appendix 20a) of the aglycone exhibited two *meta*-coupled aromatic doublets at

δ_H 6.80 (d, *J* = 2.1 Hz) and 6.39 (d, *J* = 2.1 Hz) corresponding to H-8 and H-6 respectively. In ring B, an AA'XX' system at δ_H 7.82 (d, *J* = 1.8 Hz) and 6.90 (d, *J* = 8.4 Hz) were assigned to H-2'/H-6' and H-3′/H-5', respectively, while a characteristic singlet at δ_H 6.70 represented H-3 proton, thus suggesting the presence of a flavone moiety (Fajriah *et al*., 2016). In fact, the ^{1}H NMR spectral data were in agreement with a 5,7,4'-trisubstituted flavone apeginin **(144)** (Batterham & Highet, 1963) except for the two sugar unit residues evidenced by ^{1}H NMR anomeric peaks at δ_H 5.30 (d, *J* = 7.4 Hz) and 5.16 (d, *J* = 1.3 Hz), with corresponding ^{13}C NMR peaks at δ_C 105.5 and 99.8, respectively. Acid hydrolysis gave glucose and rhamnose as the sugar residues confirmed by TLC (solvent system: EtOAc-MeOH-H_2O-HOAc, 6:2:1:1) and PC (solvent system: *n*-BuOH-HOAc-H_2O-pyridine, 1:5:3:3) co-chromatography with authentic samples. The large coupling constant of the anomeric proton (*J* = 7.4 Hz) indicated that the glucose moiety was present in the β-configuration while the small coupling constant of the anomeric proton *J* = 1.03 Hz evident of α configuration of rhamnose sugar (Manguro *et al*., 2011). The presence of 15 carbons due to aglycone moiety was also evident in the ^{13}C NMR spectrum (Table 4.20; Appendix 20b) further supporting the close structural relationship between compound **OL11** and apeginin (**144**). The attachment of glucose to the aglycone was assigned to C-7 where it is in equatorial configuration as evidenced by HMBC correlation between H-1'' (δ_H 5.30) and C-7 (δ_C 164.3) (Appendix 20c). Similarly, HSQC experiment (Appendix 20d) was used to correlate the protons with corresponding carbons and this allowed the assignment of the inter-glycosidic linkage. In the ^{13}C NMR, glycosylation shift was observed for C-2''at δ_C 79.1, thus suggesting the terminal rhamnose was linked to primary glucose through 1'''and 2'' as in neohesperidoside (Sathyadevi & Subramanian, 2015). The foregoing evidence was substantiated by HMBC correlation between rhamnosyl H-1''' (δ_H 5.16) and glucosyl C-2'' (δ_C 79.1) which was further supported by ESI-MS (Appendix 20e) molecular ion at *m/z* 601.4

corresponding to $C_{27}H_{30}O_{14}Na$.Therefore, from the spectroscopic data and on comparison with literature data (Reefat *et al*., 2015), **OL11** was deduced as apigenin 7-*O*-neohesperidoside (**214**) a compound reported in this species for the first time.



214

Table 4.20: ^{1}H (600 MHz) and ^{13}C NMR (125 MHz) (MeOD) data for **(214)**

C#	^{1}H NMR	^{13}C NMR	^{1}H* NMR	^{13}C* NMR
2		166.6		164.4
3	6.70 s	104.2	6.80 (s)	103.2
4		184.0		182.1
5		162.7		161.1
6	6.39 (d, *J* = 2.2 Hz)	101.0	6.33 (d, *J* = 2.0)	99.4
7		164.3		162.6
8	6.80 (d, *J* = 2.1 Hz)	95.9	6.84 (d, *J* = 2.0)	94.6
9		158.9		157.1
10		107.0		105.5
1'		123.0		120.9
2'	7.82(d, *J* = 7.8 Hz)	129.6	7.91 (d, *J* = 8.8)	128.7
3'	6.90 (d, *J* = 8.4 Hz)	117.1	6.92 (d, *J* = 8.8)	116.2
4'		162.9		161.7
5'	6.90 (d, *J* = 8.4 Hz)	117.1	6.92 (d, *J* = 8.8)	116.2
6'	7.82 (d, *J* = 7.8 Hz)	129.6	7.91 (dd, *J* = 8.8)	128.7
1"	5.30 (d, *J* = 7.4 Hz)	102.6	5.20 (d, *J* = 7.3)	100.5
2"		79.1		77.6
3"		79.0		77.4
4"		72.3		71.1
5"		78.3		76.8
6"		62.5		60.9
1'''	5.16 (d, *J* = 1.3 Hz)	99.8	5.08 s	98.2
2'''		72.2		71.0
3'''		71.4		70.8
4'''		74.0		72.3
5'''		70.0		68.8
6'''	1.32 (d, *J* = 6.4 Hz)	18.3	1.16(d, *J* = 6.3)	18.5

*Refaat *et al*., 2015

4.2.3. Structural elucidation of compounds from *A. mucosa* leaf extracts

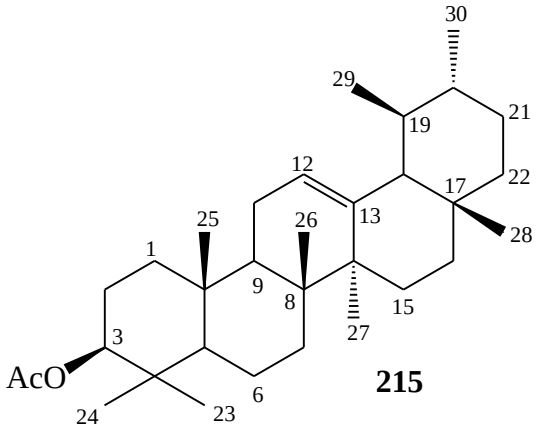
Eleven compounds were isolated from the leaf extracts of *A. mucosa*. The structures of these compounds were established based on their physical and spectroscopic data as well as on comparison with the data in the respective literature

4.2.3. 1Structural elucidation of compounds from *n*-hexane extracts of *A. mucosa* leaves

4.2.3.1.1 Isolate AM1

AM1 was obtained as an amorphous white powder with m.p 192-194^{o}C and Rf = 0.71 (solvent system *n*-hexane-EtOAc, 4:1) in a yield of 0.008% of the starting plant material. It gave positive tests to both Liebermann-Buchard and ceric sulphate tests, indicating that it is a terpenoid derivative. It showed significant IR peaks (Appendix 21 a) 1733.9 cm^{-1} (acetyl-carbonyl) 1649.6 and 901.7 cm^{-1}(trisubstituted double bond) (Sukumar *et al*., 1995. The ^{1}H NMR spectrum (Table 4.21**;** Appendix 21a) exhibited the presence of seven tertiary methyl singlets including the acetoxy (δ_H 2.01, 1.06, 0.98, 0.94, 0.88, 0.87 and 0.80) and two methyl doublets at δ_H 0.79 (d, J = 7.1 Hz) and 0.84(d, J = 6.7 Hz). The ^{I}H NMR also showed olefinic proton at δ_H 5.12 (t, J = 3.3 Hz) and oxygenated proton at δ_H 4.50 (dd, J = 10.9, 4.8 Hz) assigned to H-12 and H-3, respectively. In fact, the presence of two methyl doublets together with the downfield methyl singlet at δ_H 2.01 suggested that the compound is an acylated ursane type triterpene derivative (Okoye *et al*, 2014; Abdullahi *et al*., 2017). The downfield shift of the oxygenated proton suggested the attachment of the acetate unit at position C-3. The ^{13}C NMR spectrum (Table 4.21, Appendix 21b) showed a total of 32 carbon signals which were sorted out by DEPT experiments including DEPT-135 spectrum (Appendix 21c) into nine methyls, nine methylenes, eight methines including one olefinic and one oxygen bearing carbon as well as five quaternary carbons. In the ^{13}C NMR spectrum of compound

AM1, the olefinic carbons C-12 and C-13 appeared at δ_C 124.3 and 139.6 respectively. The up field value of C-13 clearly suggested the compound to be an ursane derivative rather than an oleonane derivative (Doddrell *et al.*, 1974). The mass spectrum of **AM1** revealed characteristic Retro-Diels-Alder fragments at m/z 249.0 [$C_{16}H_{25}O_2$] and 218.0 [$C_{16}H_{26}$] (Figure 14) due to cleavage of ring C, which indicated that the acetoxy group is in ring A/B. The ready loss of the methyl group at C-17 from fragment [$C_{16}H_{26}$] to give m/z 202.9 confirmed further that the acetoxy group is at C-3. This was further supported by HMBC correlation between H-3 and the carbonyl carbon at δ_C171.0, based on spectral data, the acetoxy group was attached to C-3 and the orientation being β from the ^{1}H NMR coupling constant (Okoye *et al*., 2014). The EI-MS spectrum (Appendix 21e) gave a molecular ion peak at m/z 468.0, corresponding to $C_{32}H_{52}O_2$ formula. The formula exhibited seven double bond equivalents, five of which are in a pentacyclic carbon frame work and the remainder two in C=C and C=O double bonds. Thus on the basis of physical and spectroscopic data together with comparison with literature data (Okoye *et al.,* 2014), compound **AM1** was identified as α-amyrin acetate (**215)**.



215

Table 4.21: ^{1}H (360 MHz) and ^{13}C NMR (90 MHz) ($CDCl_3$) spectral data of compound **215**.

C#	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C NMR*	DEPT
1	1.55m, 1.52 m	38.4		38.6	CH_2
2	1.64m, 1.55m	23.6	1.61	23.8	CH_2
3	4.50 (dd, *J* = 10.9, 4.8 Hz)	80.92	4.48 dd	81.2	CH
4		37.7		37.9	C
5	0.82 m	55.2	0.81 m	55.5	CH
6	1.52 m, 1.32 m	18.2	1.51 m, 1.34 m	18.5	CH_2
7		33.7		33.1	CH_2
8		40.0		40.2	C
9	1.57 m	47.6	1.54 m	47.8	CH
10		36.8		37.0	C
11	1.91 m	23.6	1.89 m	23.6	CH_2
12	5.12 (t, *J* = 3.3 Hz)	124.3	5.10 m	124.5	CH
13		139.6		139.8	C
14		42.0		42.4	C
15		28.1		28.3	CH_2
16		26.6		26.8	CH_2
17		32.8		34.0	C
18	1.28 m	59.0	1.29 m	59.3	CH
19	1.37 m	39.6	1.38 m	39.8	CH_2
20	1.99 m	31.2	1.98 m	39.8	CH
21		36.6		31.5	CH_2
22		41.5		41.7	CH_2
23	0.88 s	28.4	0.85 s	28.3	CH_3
24	0.87 s	16.7	0.84 s	17.0	CH_3
25	0.94 s	15.7	0.96 s	16.0	CH_3
26	0.98 s	17.5	0.98 s	17.7	CH_3
27	1.01 s	23.3	1.04 s	23.4	CH_3
28	0.80 s	28.7	0.78 s	29.1	CH_3
29	0.79(d, *J* =7.1 Hz)	16.8	0.77 s	17.0	CH_3
30	0.84 (d, *J* = 6.7 Hz)	23.6	0.83 s	21.6	CH_3
$\underline{CH_3}CO$	2.01 s	21.3	2.02 s	21.0	CH_3
$CH_3\underline{C}O$		171.0		171.5	C

*Okoye *et al*., 2014

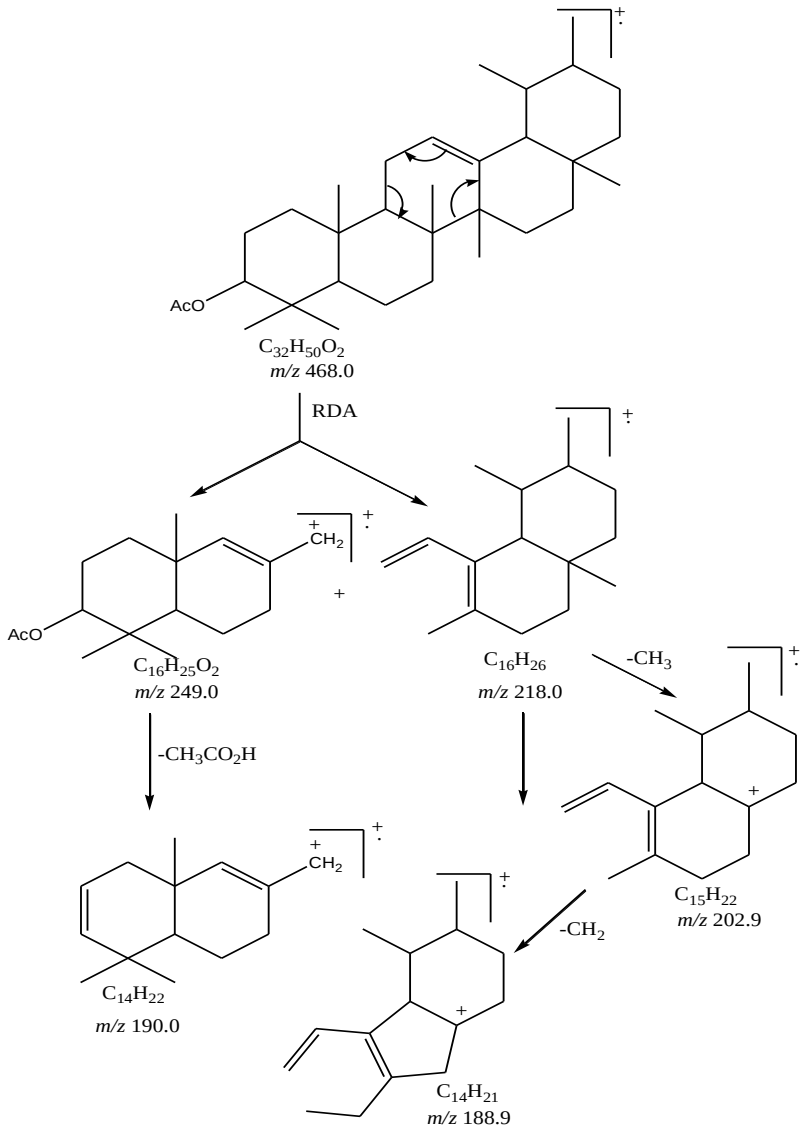
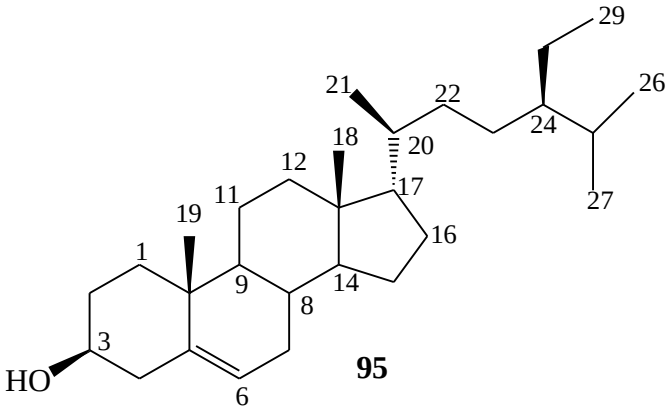


Figure 14: Proposed fragmentation pattern of compound **215** (EI-MS 70 eV**.**)

4.2.3.1.2 Isolate AM2

Compound **AM2** was isolated as white powder R_f = 0.54 (solvent system: *n*-hexane-EtOAc, 4:1) and m.p 137-138ºC, Lit 134-135ºC. It afforded a positive colour with Liebermann-Buchard reagent but failed the ceric sulphate test suggesting it could be a sterol derivative (Nurwidayati, 2012). The compound exhibited an IR spectrum (Appendix 22a) data at 3357.3 and 1569.0 cm^{-1} respectively, indicating the presence of hydroxyl and carbon-carbon double bond functionalities. The ^{1}H and ^{13}C

NMR spectral data (Table 4.22) of the compound were in good agreement with the cholesterol skeleton (Johnson & Jankowski, 1972). In fact, the ^{1}H NMR spectrum (Table 4.22**,** Appendix22b) showed the presence of six methyl groups of which two were observed as singlets at δ_H 0.68 and 1.01 attributed to C-19 and C-18 methyls respectively. Other three methyl doublets at δ_H 0.81, 0.83 and 0.93 represented methyl groups at Me-27, Me-26, and Me-21, respectively while a methyl triplet at δ_H 0.85 was assigned to terminal Me-29. The ^{1}H NMR spectrum of compound **AM2** also exhibited an endocyclic olefinic double bond proton at δ_H5.35 (t, *J*= 6.4 Hz, H-6) which was attributable to H-6. The ^{13}C NMR spectrum (Appendix 22c) revealed the presence of 29 distinct carbon resonances attributable to six methyls, eleven methylenes, nine methines and three non-protonated carbon atoms from DEPT-135 experiments (Appendix 22d)which accounted for the 49 protons of the molecule. The electron impact mass spectrum (70eV gave a parent ion peak at *m/z* 414 consistent with the formula $C_{29}H_{50}O$ corresponding to five degrees of unsaturation. Thus the remaining proton to ascertain 50 protons was part of the hydroxyl functionality as already suggested by IR spectrum, a fact corroborated by the ^{1}H NMR peak at δ_H 3.52multiplet. Further support for the cholestane moiety was accrued from other prominent peaks in EI-MS besides the molecular ion 414 $[M]^+$ (Appendix 22e) which appeared at *m/z* 396 $[M\text{-}H_2O]^+$, there was 329 [M-part of the side chain $(C_6H_{13})]^+$ which is consistent with already reported data for **AM2**. The identification of **AM2** as sitosterol was further accomplished by comparing its physical properties, co-spotting on TLC with authentic sample and by comparison of its spectroscopic data with those of corresponding data reported in the literature (Chaturvedula & Prakash, 2012) for β- sitosterol **(95)**



95

Table 4.22: ^{1}H (600 MHz) and ^{13}C NMR (125 MHz) ($CDCl_3$) spectral data of compound **95**

C#	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C NMR*	DEPT
1		37.3		37.5	CH_2
2		31.9		31.9	CH_2
3	3.52 m	71.8	3.53 (dd, *J* = 4.5, 4.2. 3.8 Hz)	72.0	CH
4		42.3		42.5	CH_2
5		140.8		140.9	C
6	5.35 (t, *J* = 6.44 Hz)	121.7	5.36 (t, *J* = 6.4 Hz)	121.9	CH
7		31.7		32.1	CH_2
8		32.4		32.9	CH
9		50.2		50.3	CH
10		36.5		36.7	C
11		21.1		21.3	CH_2
12		39.8		39.9	CH_2
13		42.9		42.6	C
14		56.8		56.9	CH
15		26.1		26.3	CH_2
16		28.2		28.5	CH_2
17		56.1		56.3	CH
18	1.01 s	12.0	1.01 s	12. 2	CH_3
19	0.68 s	18.8	0.68 s	18.9	CH_3
20		36.2		36.3	CH
21	0.93 (d, *J* = 5.9 Hz)	19.4	0.93 (d, *J* = 6.5 Hz)	19.2	CH_3
22		34.0		34.2	CH_2
23		23.1		23.3	CH_2
24		45.9		46.1	CH
25		29.2		29.4	CH
26	0.83 (d, *J* = 6.3 Hz)	20.2	0.83 (d, *J* = 6.4 Hz)	20.2	CH_3
27	0.81 (d, *J* = 6.2 Hz)	19.8	0.81 (d, *J* = 6.4 Hz)	19.6	CH_3
28		23.1		23.3	CH_2
29	0.85 (t, *J* = 7.3 Hz)	11.9	0.83 (t, *J* = 7.2 Hz)	12.0	CH_3

*Chaturvedula & Prakash, 2012

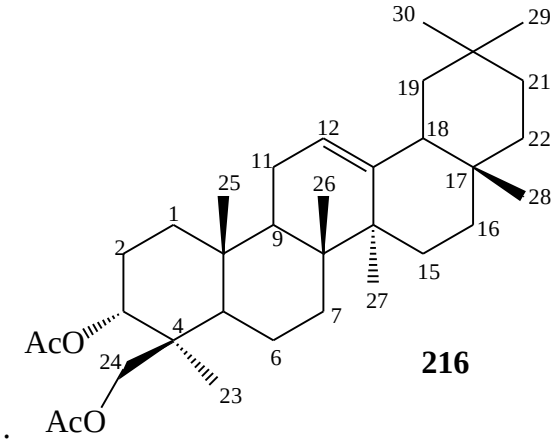
4.2.3.2Structural elucidation of compounds from EtOAc extracts of *A. mucosa* leaves

4.2.3.2.1 IsolateAM3

AM3 was isolated from the EtOAc leaf extract of *A. mucosa* by repeated medium pressure chromatography on silica gel using solvent system *n*-hexane-EtOAc 4:1 as a white solid compound, m.p. 176-178^{o}C and R_f = 0.45 (Solvent system: *n*-hexane-EtOAc, 4:1). It exhibited the characteristic of a triterpene derivative by giving positive Liebermann-Burchard test and also responding to ceric sulphate test (Firdous *et al.*, 1999). Its IR spectrum (Appendix 23a) determined as KBr pellet showed significant absorption bands at 1737.3 and 1248.3 cm^{-1} representing an acetoxy group. It was assigned the molecular formula of $C_{34}H_{54}O_4$ as evidenced by HRESI-MS molecular ion peak at *m/z* 526.79016**.** The EI-MS molecular ion peak m/z 526.3 suggested eight double bond equivalents, six of which were assigned to the ring of the pentacyclic triterpene skeleton and the remaining two were attributable to a primary and a secondary acetoxy groups in the molecule. The fragment *m/z* 307.0 suggested that both the acetoxy groups are on ring A/B portion of the molecule (Sukumar *et al.*, 1995). In the ^{1}H NMR spectrum (Table 4.23; Appendix 23b), the vinylic proton δ_H 5.20 (t, *J* = 3.7 Hz, H-12), an oxymethine proton δ_H 4.95 (t, *J*= 4.8Hz) and two geminal methylene protons δ_H 4.22 [(d, J_{gem} = 11.0 Hz) and 3.99 (d, J_{gem} = 11.5 Hz)] together with nine methyl groups located on quaternary carbons including two acetoxy groups (2.09, 2.06, 1.24, 1.11, 1.00, 0.96, 0.87, 0.83 and 0.80, all singlets) unambiguously confirmed the presence of Δ^{12}-oleanene skeleton (Manguro *et al.*, 2018). An equivalent assignable to the double bond consistent with Δ^{12}-oleanane skeleton exhibited ^{13}C NMR (Table 4.23, Appendix 23c) resonance peak at δc 121.5 (C-12) and 145.0 (C-13). This together with significant fragment peaks at *m/z* 307.0 $[C_{18}H_{27}O_4]^+$and *m/z* 218.1 $[C_{16}H_{26}]^+$ (100%), (Figure 15; Appendix 23d) suggested typical Retro-Diels-Alder fission diagnostic of 12-oleonene or 12-ursene type triterpene derivatives (Noel & Dayrit, 2005). In fact,

the down field value of C-13 clearly suggested the compound to be an oleanane derivative rather than an ursane (Okoye *et al*., 2014). The ^{13}C NMR spectrum showed the presence of 34 carbon signals; their multiplicity assignments using DEPT experiments including DEPT-135 experiments (Appendix 23e) showed the presence of five methines, eleven methylenes, nine methyls and nine quaternary carbon atoms. Comparison of the ^{1}H and ^{13}C NMR and EI-MS spectral data with that of 3β-acetoxy-12-oleanene **(199)**(Okoye *et al.,* 2014) revealed close similarity with notable difference between the two compounds being substitution in ring A in compound **AM3** as evidenced by replacement of the Me-24 with CH_2OAc group. The methylene group attached to acetoxy moiety showed ^{13}C NMR peak at δ_C 66.7 while the acetoxy group at C-24 exhibited peaks at δ_C 170.5 and 21.3. HMBC correlations (Figure 17; Appendix 23f i & ii) between the oxymethine carbon δ_C 73.4 (C-3) and H-5 (δ_H 1.00-0.96, m) and in turn with the exo-methylene doublets δ_H 4.22 (J = 11.0 Hz) and δ_H 3.99 (d, J = 11.5 Hz) suggested that acetylations were at C-3 and C-24 positions. Also comparing the data for compound **AM3** with those of synthetically derived urs-12-ene-3α, 24-diol (Tapandjou *et al.,* 2005) revealed a shift of the oxymethylene peaks relatively downfield by approximately 1.40 δppm, which further suggested that the exomethylene was acetylated. The foregoing evidence was substantiated by ROESY cross peaks (Figure 16; Appendix 23g) observed between the acetylated primary alcohols with CH_3-25 group (Mahajan *et al.,* 1995). The acetoxy group at C-3 was axially oriented as substantiated by the narrow peak height $w^{½}$ (J = 4.80 Hz) of equatorially positioned geminal proton which appeared relatively downfield at δ_H 4.95 (Dekebo *et al.*, 2002). This was confirmed by HMBC correlation (Figure 16; Appendix 23f) between H-5 (δ_H 1.00-0.96 m) and C-3 (δ_C 73.4). Similarly, the three bond long-range correlation from H-3 to carbons ascribable to C-5 (δ_C 50.6) and C-24 (δ_C 66.7) in the HMBC spectrum suggested the presence of acetylated hydroxyl group at C-24, possibly with β-configuration, a fact substantiated

by ^{1}H-^{1}H proximity (ROESY) correlation between CH_3-23 and H-5. On the other hand, a triplet at δ_H5.20 (H-12) correlated with C-8 (δ_C 40.3) and in turn with C-13 (δ_C 145.0) and C-14 (δ_C 41.7). On this basis, the position of the double bond was concluded to be between C-12 and C-13, a fact that was corroborated by EI-MS daughter ion at *m/z* 218.1 [$C_{16}H_{26}$] originating from *Retro*-Diels-Alder cleavage (Figure 15). Therefore, on the basis of spectroscopic data, and in comparison with literature data, **AM3** was deduced to be 3α, 24-diacetoxy-12-oleanene (**216**) which is a new compound.



216

Table 4.23: ^{1}HNMR (600 MHz) and ^{13}C NMR (125 MHz) $CDCl_3$data of **216**

C#	^{1}H NMR	^{13}C NMR	DEPT
1	1.87-1.68 m, 1.11-1.12 m	37.1	CH_2
2	1.68-1.43 m, 1.19-1.12 m	26.9	CH_2
3	4.95 (t, *J* = 4.8 Hz)	73.4	CH
4		39.8	C
5	1.00-0.96 mm	50.6	CH
6	1.43 -1.38 m	18.3	CH_2
7	1.43 -1.38 m, 1.24-1.19 m	32.7	CH_2
8		40.3	C
9	2.06-1.89 m	47.2	CH
10		36.6	C
11	1.90-1.88 m	23.5	CH_2
12	5.20 (t, *J* = 3.7 Hz)	121.5	CH
13		145.0	C
14		41.7	C
15	1.43-1.38 m, 1.24-1.19 m	26.0	CH_2
16	1.68-1.43m	22.4	CH_2
17		32.4	C
18	2.09-2.06m	47.5	CH
19	1.68-1.42 m	46.9	CH_2
20		31.0	C
21	1.68-1.42m	34.7	CH_2
22	2.10-1.89 m, 1.12-1.00 m	33.5	CH_2
23	1.11 s	15.7	CH_3
24	4.22 (d, J_{gem} = 11.0), 3.99 (d, J_{gem} = 11.5 Hz)	66.7	CH_2
25	0.87 s	15.7	CH_3
26	0.80 s	16.7	CH_3
27	1.24 s	26.1	CH_3
28	0.83 s	28.3	CH_3
29	0.96 s	33.3	CH_3
30	1.00 s	23.6	CH_3
3-CH_3C(O)O-	2.06 s	20.9	CH_3
3-CH_3C(O)O-		171.2	C
24-CH3C(O)O-	2.09 s	21.3	CH_3
24-CH_3C(O)O-		170.5	C

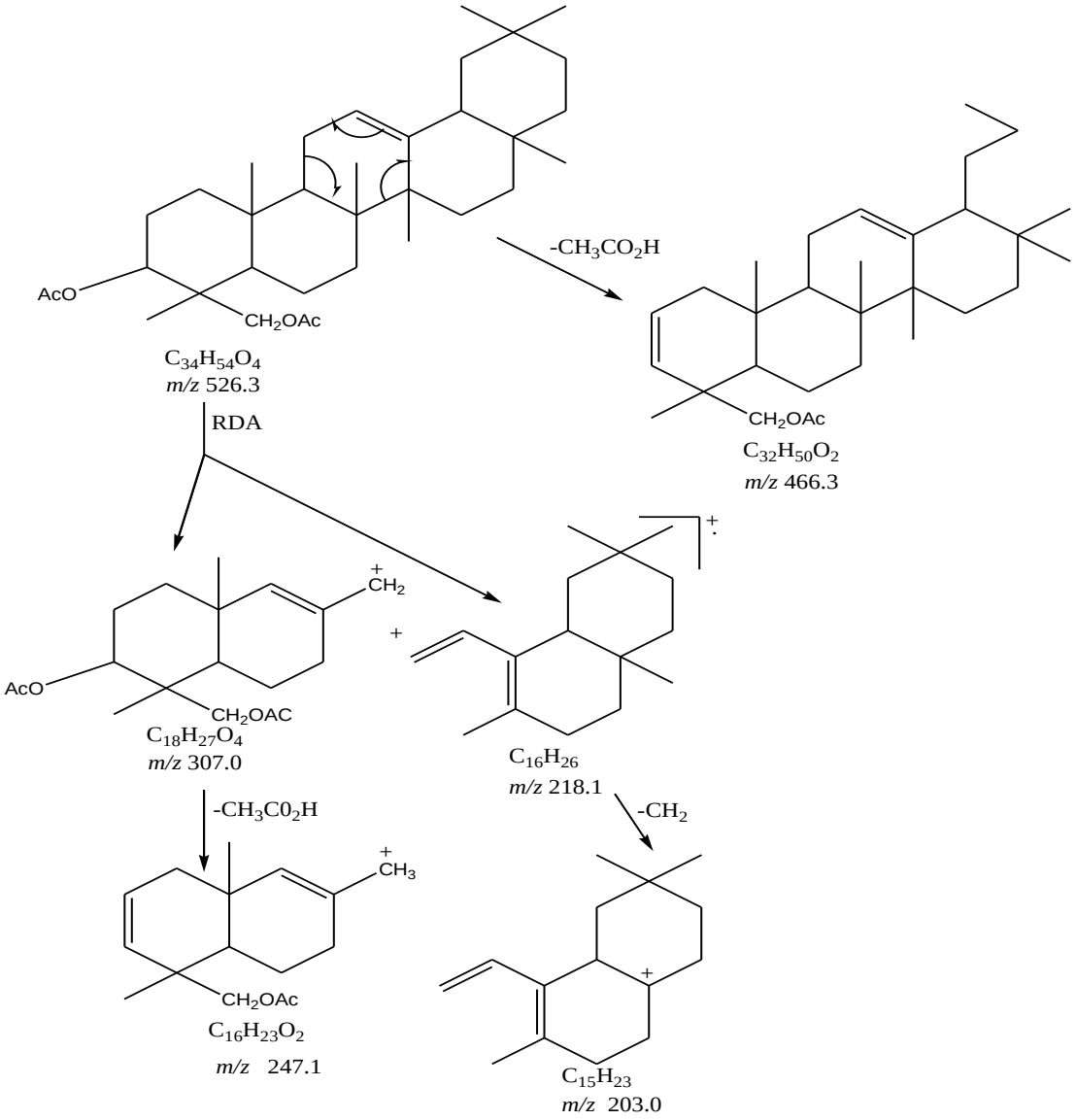


Figure 15: Proposed fragmentation pattern of compound **216**in EI-MS (70 eV)

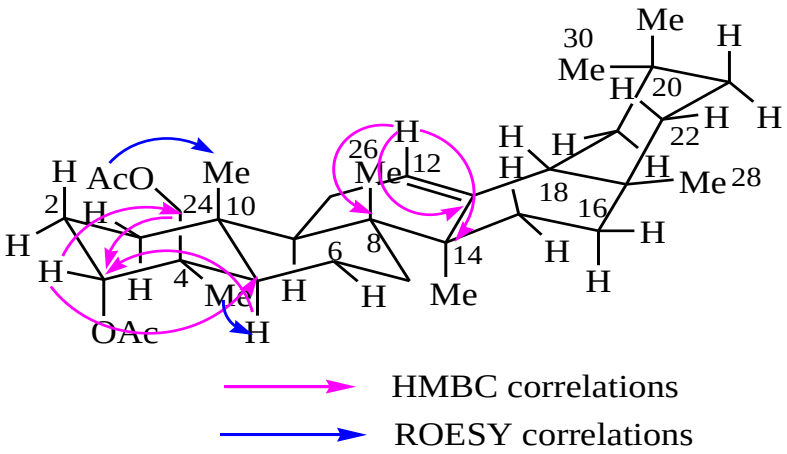
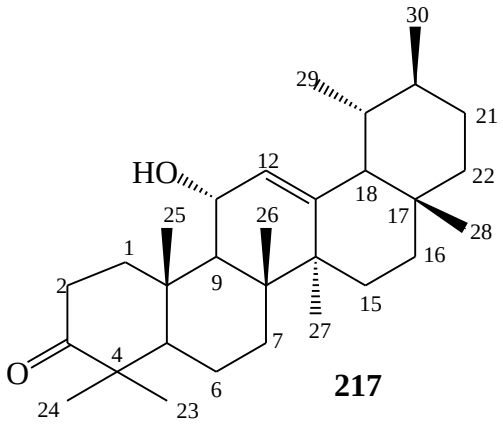


Figure 16 HMBC and ROESY cross peaks of compound **216**

4.2.3.2.2 Isolate AM4

AM4 was isolated using conventional medium pressure column chromatography on fractionating ethyl acetate portion of *A. mucosa* leaf extract. The process led to the isolation of a white amorphous powder $R_{f\,=}$ 0.32 (solvent system: *n*-hexane-EtOAc) and m.p. 170-174^{o}C. The compound showed the characteristics of a triterpene by positively responding to both Liebermann-Buchard and ceric sulphate tests. The IR spectrum (KBr pellets) (Appendix 24a) exhibited significant absorption peaks at 3400.3, 1702.4 and 1454.5 representing hydroxyl, ketone and carbon-carbon double bonds functionalities, respectively. In the ^{1}H NMR spectrum (Table 4.24; Appendix 24b), of compound **AM4**, the presence of vinylic proton (δ_H 5.20, t, *J*= 3.0 Hz, H-12), an oxymethine proton δ_H 4.32 (dd, *J*= 11.8, 2.5 Hz, H-11), six tertiary methyls (δ_H 1.20, 1.17, 1.11, 1.08, 0.93 and 0.81 all singlets), and two secondary methyls (δ_H 0.87 (d, *J*=7.1 Hz, H-29), 0.85 (d, *J*=6.7 Hz, H-30)] were observed unambiguously confirming the presence of Δ^{12}-ursane skeleton (Lima *et al*., 2005). The ^{13}C, NMR spectrum (Table 4.24; Appendix 24c), exhibited a total of 30 carbon signals. In the ^{13}C NMR spectrum, *inter alia* signals for saturated carbonyl C-3 (δ_C 217.0), oxymethine carbon C-11 (δ_C 77.4) and a tri-substituted carbon-carbon double bond C-12 and C-13 (δ_C 128.8 and 142.9) were evident, thus cumulatively this data suggested an ursane-type triterpenoid with a carbonyl and hydroxyl substituents. The foregoing evidences were further confirmed by the EI-MS molecular ion peak at *m/z* 440.1 corresponding to $C_{30}H_{48}O_2$ formula. This together with the significant peaks at *m/z* 234.1 [$C_{16}H_{26}O$] and 205.1 [$C_{14}H_{21}O$], suggested the Retro-Diels-Alder cleavage of ring C resulting in fragmentations (Figure 17; Appendix 24d) commonly encountered in the spectra of urs-12-ene derivatives possessing the keto group in ring A and hydroxyl group in ring C (Lima *et al*., 2005). Comparison of ring A signal with those of 3-oxours-12-ene (Lima *et al*., 2005) permitted the assignment of the carbonyl at position 3, a fact supported by the EI-MS peak at *m/z* 191.1 [$C_{14}H_{22}$]**.**

Similarly, the decoupling experiment established position C-11 as the site for the hydroxy methine [δ_C 77.4; δ_H4.32 (dd, J = 11.80, 2.50 Hz)] with stereochemistry of the hydroxyl group being β- from coupling constant values. Therefore, from the spectroscopic data and on comparison with the literature data (Lima *et al.*, 2005), compound **AM4** was deduced to be 3-oxo-11β-hydroxyurs-12-ene **(217).**



217

Table 4.24: ^{1}H (600 MHz) and ^{13}C NMR (125 MHz) ($CDCl_3$) spectral data of **217**

C	^{1}H NMR	^{13}CNMR	^{1}H NMR*	^{13}CNMR*
1		39.3		40.1
2		33.7		34.2
3		217.0		218.0
4		43.0		47.6
5		55.5		55.3
6		19.7		19.7
7		31.1		32.4
8		42.4		43.0
9	2.38 (d, *J* = 8.4)	47.7	2.00 (d, *J* = 9.4 Hz)	47.8
10		34.4		37.4
11	4.32, dd (*J* = 11.80, 2.50 Hz)	77.4	4.56 (dd, *J* = 9.4, 3.0 Hz)	81.8
12	5.20 d (*J* = 2.95 Hz)	128.8	5.38 (d, *J* = 3.0 Hz)	124.8
13		142.9		146.1
14		41.4		42.0
15		26.8		27.9
16		26.5		26.3
17		33.2		33.8
18		58.3		58.7
19		41.2		39.4
20		37.6		39.3
21		31.1		31.1
22		39.4		41.3
23	0.93 s	26.5		26.5
24	1.08 s	21.3		21.4
25	1.11 s	17.5		18.1
26	1.17 s	16.3		16.2
27	1.20 s	23.0		22.0
28	0.81 s	27.5		28.5
29	0.87 (d, *J* = 7.1 Hz)	17.9		17.5
30	0.85 (d, *J* = 6.7 Hz)	21.5		21.3

*Lima *et al.*, 2005

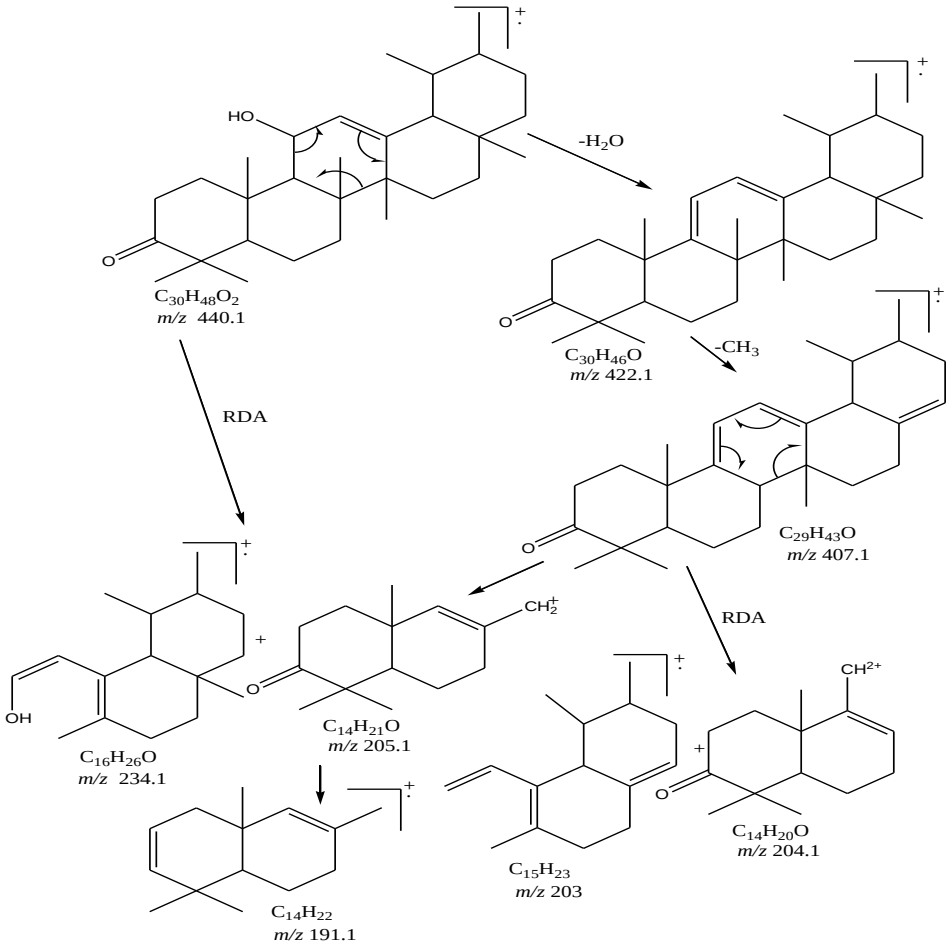
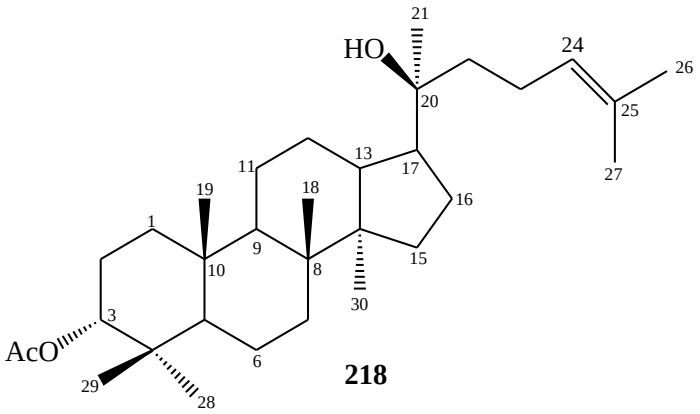


Figure 17: proposed fragmentation pattern of compound **217** in EIMS (70 eV)

4.2.3.2.4. Isolate AM5

AM5 was isolated as a white amorphous powder, M.P. 61-62^{o}C and R_f = 0.20 (solvent system: *n*-Hexane-EtOAc, 3:2). It was obtained after repeated column chromatography using gradient of ethyl acetate- *n*-hexane to yield 45 mg of the compound. The compound responded positively both to Liebermann-Buchard and ceric sulphate tests indicating that it is a terpenoid derivative (Sukumar *et al.,* 1995).It showed significant IR spectrum (Appendix 25a) peaks at 3387.7 (OH), 1729.8 and 1242.1 (acetoxy group) and 1631.3 cm^{-1} (trisubstituted double bond) (Sukumar *et al.,* 1995). The ^{1}H NMR spectrum (Table 4.25; Appendix 25b) showed signals due to vinyl proton at δ_H 5.13 (t, *J* = 7.2, 3.6 Hz) and an oxymethine proton at δ_H 4.53 (dd, *J* = 12.1, 6.0 Hz) as well as nine tertiary

methyls including a methyl attached to a carbonyl carbon (δ_H 2.05, 1.68, 1.63, 1.13, 1.03, 1.01, 0.93, 0.91 and 0.87, all singlets). The methyl singlets appearing at δ_H 1.68 and 1.63 appeared deshielded possibly due to their attachment to a double bond. The ^{13}C NMR (Table 4.25; Appendix 25c) and DEPT-135 spectrum (Appendix 25d) indicated six methines, ten methylenes, nine methyls and seven quaternary carbons, including carbonyl carbon at δ_C 170.8, two oxygen bearing carbons at δ_C 78.4 and 75.4 and two olefinic carbons at δ_C 124.7 and 131.5, respectively. In the EI-MS spectrum (Appendix 25e), a peak at *m/z* 468.1 represented $[M\text{-}H_2O]^+$. A three proton singlet at δ_H 2.05 and IR absorption at 1729.8 cm^{-1} revealed the presence of acetoxy group while the absorption at 3387.7 cm^{-1} and the ^{13}C NMR resonance at δ_C 75.4 indicated the presence of a tertiary alcohol (Dekebo *et al*., 2002). Spin decoupling experiments showed that the acetoxy group was linked in a $–CH_2\text{-}CH_{ax}\text{-}(OAC)_{eq}\text{-}C(CH_3)_2$ system requiring it to be at C-3 as in(3R, 20S)-3-acetoxy-20-hydroxydammar-24-ene (Dekebo *et al*., 2002). The presence of the acetoxy group created appreciable shielding of the Me_{ax}-4 compared to an equivalent (Dekebo *et al*., 2002). Previous findings have shown that in 3β-and 3α-oxygenated dammarane derivatives, the ^{13}C NMR signals of C-5 appear at approximately δ_C 55.0 and δ_C 50.0, respectively (Dekebo *et al*., 2002; Asakawa *et al*., 1977). In the case of compound **AM5,** the C-5 carbon resonated at δ_C 50.7, a fact further supported by the ^{13}C NMR resonance peak at δ_C 78.4 rather than at δ_C 81.0 expected for the corresponding 3β- isomer (Dekebo *et al*., 2002; Rouf *et al*., 2001).Similarly, the double bond was possibly between C-24 and C-25 as evidenced by an EI-MS peak at *m/z* 418.0 $[C_{27}H_{46}O_3]^+$ which is due to methyl butenyl fragment from C-17 side chain. From the accrued physical and spectroscopic data as well as comparison with literature (Dekebo *et al*., 2002), compound **AM5** was concluded to be (3R, 20S)-3α-acetoxy-20-hydroxydammar-24-ene (**218)**.



218

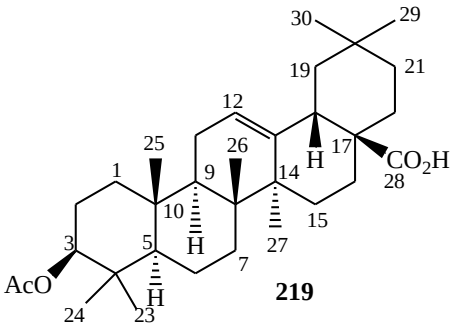
Table 4.25: ^{1}H (600 MHz) and ^{13}C NMR (125 MHz) ($CDCl_3$) spectral data of **218**

C#	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C NMR*	DEPT
1		31.2		34.6	CH_2
2		25.4		23.2	CH_2
3	4.53 (dd, *J* = 12.1, 6.0 Hz)	78.4	4.59 (t, *J* = 3.0 Hz)	78.7	CH
4		36.7		37.5	C
5		50.7		50.7	CH
6		18.1		18.4	CH_2
7		34.2		35.4	CH_2
8		37.1		40.8	C
9		49.8		51.1	CH
10		35.0		37.1	C
11		20.8		21.6	CH_2
12		22.9		25.1	CH_2
13		40.6		42.5	CH
14		50.4		49.8	C
15		27.8		31.5	CH_2
16		25.7		27.9	CH_2
17		42.2		50.2	CH
18	1.01 s	15.5	0.89 s	15.8	CH_3
19	1.03 s	16.0	0.94 s	16.3	CH_3
20		75.4		75.8	C
21	1.13 s	24.7	1.12 s	25.7	CH_3
22		40.5		40.9	CH_2
23	2.04 m	22.5		22.9	CH_2
24	5.13 (t, *J* = 7.2, 3.6 Hz)	124.7	5.09 m	125.1	CH
25		131.5		131.9	C
26	1.63 s	27.5	1.59 s	26.1	CH_3
27	1.68 s	17.7	1.66 s	18.1	CH_3
28	0.93 s	27.5	0.86 s	28.2	CH_3
29	0.87 s	21.4	0.81 s	22.1	CH_3
30	0.91 s	16.6	0.84 s	16.9	CH_3
CH_3CO		170.8		171.2	C
CH_3CO	2.05 s	21.7	2.06 s	21.7	CH_3
OH-20	3.48 br s				

* Dekebo *et al*., 2002

4.2.3.2.5 Isolate AM6

AM6was isolated as an amorphous powder with R_f value 0.57 (solvent system: *n*-hexane -EtoAc, 2:3) and m.p. 218-220^{o}C. Like compound **218**, it gave a positive Lieberman-Buchard test suggesting that it could be a terpenoid or a sterol derivative (Attarde *et al.*, 2010). Its IR spectrum (Appendix 26a) showed significant absorption bands for carboxylic acid (2940.1 and 1278.2 cm^{-1}) and an ester (1731.7cm^{-1}) suggesting the presence of these moieties in the compound (Attarde *et al.*, 2010). Both the ^{1}H and ^{13}C NMR (Table 4.26; Appendices 26b and 26c) of **AM6** were in agreement with those of oleanolic acid **(6)** with notable difference being the presence of an acetoxy group which appeared at δ_H 5.30 (dd, J = 11.0, 4.4 Hz, H-3) in the former compound. Apparently in compound **6,** the hydroxyl group was replaced by an acetoxy group as evidenced by peaks at δ_C 170.4 and 21.4 in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum displayed eight tertiary methyls at δ_H 2.10, 1.05, 0.84, 1.12, 0.81, 1.22, 0.84 and 0.92 with corresponding ^{13}C NMR signals at δ_C 21.4 (OAc), 16.9 (C-23), 28.8 (C-24), 13.2 (C-25), 17.5 (C-26), 23.6 (C-27), 21.3 (C-29) and 17.5 (C-30) respectively. This indicated that compound **AM6** is an acetylated derivative of oleanolic acid **(6)** in which C-3, has an acetoxy group. This was confirmed with ESI-MS spectrum (Appendix 26d) which exhibited molecular ion peak at *m/z* 498.0 $[M]^{+}$ which is 42 a.m.u more than that of compound **6** corresponding to $C_{32}H_{50}O_4$ formula. Thus on the basis spectroscopic data accrued in combination with those obtained from literature search (Endo *et al*., 2019) compound **AM6** was concluded to be 3β- acetoxy oleanolic acid **(219)**



219

Table 4.26: ^{I}H (600 MHz) and ^{13}C NMR (125 MHz) ($CDCl_3$) spectral data of **219**

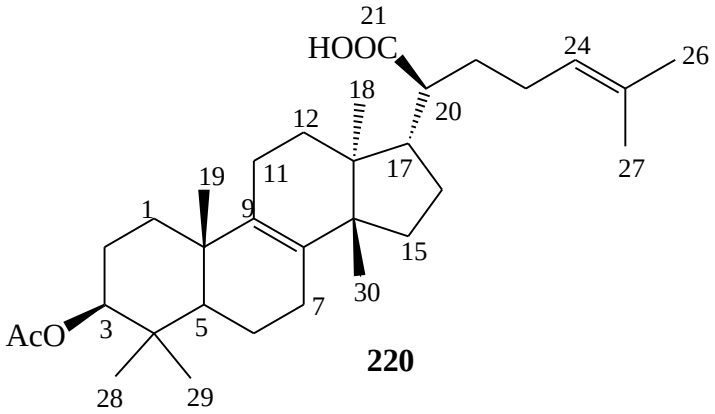
C#	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C NMR*	DEPT-135
1		37.4		38.1	CH_2
2	2.09 m	23.4	1.87-1.90 m	23.5	CH_2
3	5.30 (dd, *J* = 11.0, 4.4Hz)	77.4	4.49 m	80.9	CH
4		37.4		37.7	C
5	0.81 (t, *J* = 0.35)	50.6	0.83-0.88 m	55.3	CH
6		19.6		18.2	CH_2
7		31.0		32.6CH_2	
8		39.6		39.3	C
9		46.8		47.6	CH
10		37.4		37.0	C
11		23.6		23.4CH_2	
12	5.20 (brt, *J* = 3.8 Hz)	124.5	5.28 (t, *J* = 3.5 Hz)	122.6	CH
13		139.5		143.6	C
14		41.5		41.6	C
15		28.1		27.7	CH_2
16		23.2		22.9	CH_2
17		46.8		46.5	C
18	1.62 m	40.0	2.82 (dd, *J* = 13.6, 4.1 Hz)	41.0	CH
19	1.34 m	45.7		45.9	CH_2
20		28,8		30.7	C
21		33.7		33.8	CH_2
22		31.2		32.4CH_2	
23	1.05 s	16.9	0.85 s	16.7	CH_3
24	0.84 s	28.7	0.87 s	28.0	CH_3
25	1.12 s	15.3	0.94 s	15.4	CH_3
26	0.81 s	17.6	0.76 s	17.1CH_3	
27	1.22 s	26.5	1.13 s	25.9CH_3	
28		183.0		182.7CH_3	
29	0.84	23.9	0.93 s	23.9	CH_3
30	0.92 s	33.0	0.91 s	33.1	CH_3
MeCO	2.10 s	21.3	2.05 s	21.3CH_3	
MeCO		170.4		171.0	C

*Endo *et al*., 2019

4.2.3.2.6 Isolate AM7

Compound **AM7** was isolated as a white amorphous solid with m.p. 212-214^{o}C and Rf = 0.47 (solvent system *n*-hexane-EtOAc 3:2) from the ethyl acetate extract of *A. mucosa* by repeated medium pressure chromatography using *n*-hexane-EtOAc (3:2). It showed a positive test with Liebermann-Buchard reagent and also responded positively to ceric sulphate test suggesting that it may be a terpenoid compound (Baser, 2003). The ^{1}H NMR spectrum (Table 4.27; Appendix 27a) displayed resonances for vinylic proton at δ_H 5.12 (t, *J*=3.6 Hz), an oxymethine proton at δ_H 4.67 (d, *J*=11.7 Hz) and eight methyl groups including the acetyl group situated on quaternary carbon (δ_H 2.05, 1.68, 1.59, 0.98, 0.93, 0.88, 0.87, 0.84 and 0.75 all singlets). The two methyls appearing at δ_H 1.68 and 1.59 are deshielded and by homonuclear decoupling experiments, they coupled allylically with the vinyl proton (δ_H 5.12), thus suggesting the presence of a $–CH_2$-CH=C $(CH_3)_2$ group in the C-17 side chain (Manguro *et al*., 2003). The oxymethine proton appearing at δ_H 4.67 was possibly at C-3 on biogenetic grounds and its α-configuration was evident from the coupling constant *J*=11.7, 4.6 Hz (Manguro *et al*., 2004). The ^{13}C NMR spectrum (Table 4.27; Appendix 27b) showed the presence of 32 carbon atoms and their multiplicity assignments using DEPT-135 experiment (Appendix 27c) revealed the presence of nine methyls including the acetoxy group, ten methylenes, five methines and nine quaternary carbons. The ^{13}C NMR spectrum also showed a peak at δ_C 778.3 representing C-3 while non-protonated olefinic carbons peak at δ_C 134.4, 133.0 and 132.2 represented C-8, C-9 and C-25 respectively and were characteristic of tirucallic acid derivative (Badria *et al*., 2003). The EI-MS (70eV) molecular ion peak at *m/z* 498.1 (Appendix 27d) suggested a molecular formula of $C_{32}H_{50}O_4$ corresponding to eight double bond equivalents, which further support that the compound is a tirucallic acid derivative (Badria *et al.,* 2003). Comparison of the ^{I}H and ^{13}C NMR spectra of compound **AM7** with those of 3β-hydroxytirucallic acid (Badria

et al., 2003) revealed close similarities with notable differences between the compounds being the presence of acetoxy group in **AM7** as substantiated by the ^{1}H and ^{13}C NMR and EI-MS 498.1 $[M]^{+}$-. The foregoing evidences along with significant fragments in the EI-MS (70eV) *m/z* 483.1 [$C_{31}H_{47}O_4$], 423.1 [$C_{29}H_{43}O_2$] and 377.1[$C_{28}H_{41}$] (Figure 18; Appendix 27d) demonstrated that the compound is 3β-acetoxy tirucallic acid. Thus on the basis of physical and spectroscopic data as well, comparison with literature data (Badria *et al*., 2003), compound **AM7** was structurally elucidated as 3β-acetoxytirucallic acid **(220).**



220

Table 4.27: ^{I}H (600 MHz) and ^{13}C NMR (125 MHz) ($CDCl_3$) spectral data of **220**

C#	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C NMR*	DEPT
1	2.00 (dd, *J* = 7.6, 2.7)	36.5	1.2 (dd, *J* = 9.9, 3.1 Hz)	35.7	CH_2
2		27.4		27.9	CH_2
3	4.67 (d, *J* = 11.7, 4.6 Hz)	78.3	3.23 (dd, *J* = 11.5, 4.44Hz	79.4	CH
4		37.1		39.3	C
5		51.0		51.3	CH
6		19.7		19.3	CH_2
7	1.49 (dd, *J* = 12.1, 6.5 Hz)	32.4	1.53 (dd, *J* = 14.4, 7.3 Hz)	29.2	CH_2
8		134.4		134.4	C
9		133.0		132.6	C
10		36.8		37.7	C
11		26.9		26.3	CH_2
12	1.68 (dd, *J* = 11.6, 2.8)	32.4	1.75 (dd, *J* = 12.1, 3.5 Hz)	32.8	CH_2
13		45.9		44.3	C
14		49.7		50.0	C
15		29.3		29.7	CH_2
16	2.05 m	28.8	1.37(dd, *J* = 12.2, 6.8 Hz)	28.2	CH_2
17	2.30 (d, d, *J* = 11.4, 3.5)	47.0	2.27 (dd, *J* = 12.1, 3.5 Hz)	47.3	CH
18	0.93 s	19.8	0.93 s	20.4	CH_3
19	0.75 s,	15.9	0.74 s	15.9	CH_3
20		47.6		48.1	CH
21		183.2		183.1	C
22		21.9		21.9	CH_2
23		27.2		27.3	CH_2
24	5.12 (t, *J* = 3.6 Hz)	123.9		124.0	CH
25		132.2		133.7	C
26	1.68 s	24.5	1.67 s	24.8	CH_3
27	1.59 s	18.5	1.58 s	18.1	CH_3
28	0.84 s	28.8	0.82 s	28.4	CH_3
29	0.88 (d, *J* = 6.0Hz)	17.6	0.99 s	16.2	CH_3
30	0.87 (d, J = 12.0 Hz)	26.0	0.87 s	26.1	CH_3
MeCO		170.9		168.1	CH_3
MeCO	2.07 s	21.4			CH_3

* Badria *et al.*, 2000

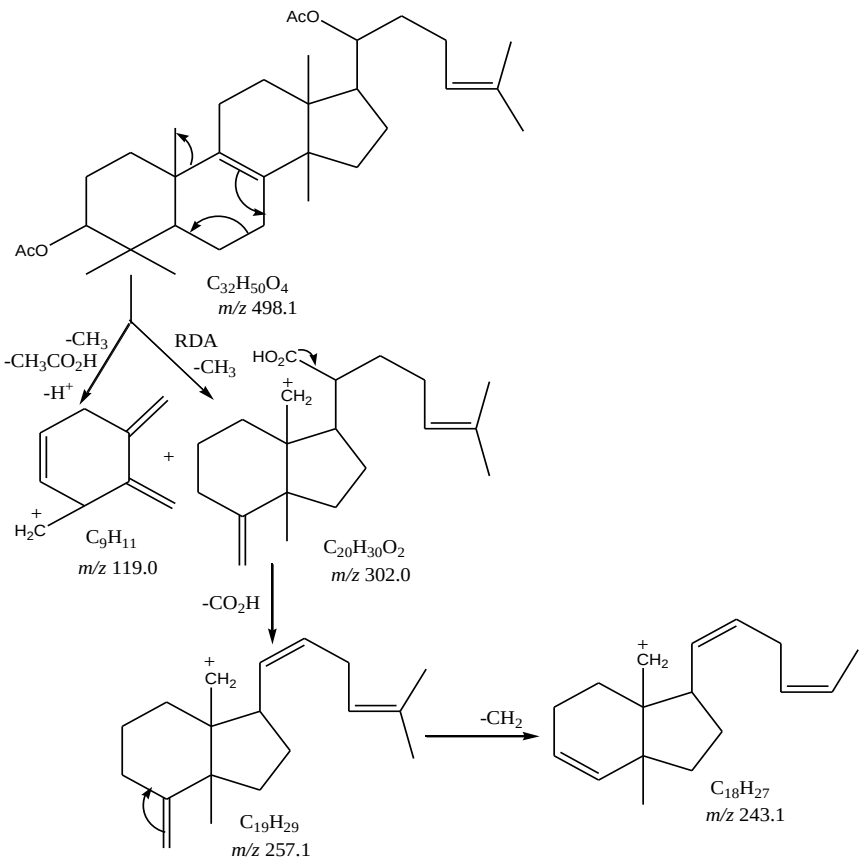


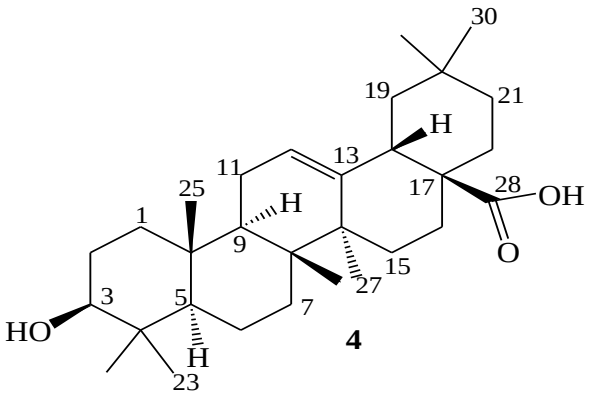
Figure 18: Proposed fragmentation pattern of compound **220** in EI-MS (70 eV)

4.2.3.3 Structural elucidation of compounds from methanol extracts of *A. mucosa* leaves

4.2.3.3.1 Isolate AM-8

AM8 was isolated as white powder with m.p. 302-305ºC. It showed a positive Liebermann-Burchard test suggesting that it could be a terpenoid or a sterol (Attarde *et al.,* 2010). In the ^{1}H NMR spectrum (Table 4.28; Appendix 28a) two singlets at δ_H 0.93 and 0.89, together with resonance for five tertiary methyl groups observed at δ_H1.10 (27-Me), 0.77 (26-Me),0.89 (25-Me), 0.74 (24-Me),0.96 (23-Me) and suggested that the compound is oleanane type triterpene (Ragasa *et al.,* 2014). The foregoing evidence was further supported by doublet at δ_H2.82 (d, *J*=11.3 Hz), typical of an oleanane skeleton in which C-9 is unsubstituted (Ragasa *et al*., 2014). The large

coupling constant indicated that H-18 and H-19 are *trans* to each other (Babalola & Shode, 2013). The EI-MS (Appendix 28c) showed a molecular ion peak at *m/z* 456.5 $[M]^+$corresponding to a $C_{30}H_{48}O_3$formula. Furthermore, in the ^1H NMR spectrum, compound **AM8** exhibited a vinyl proton (δ_H 5.27, t, *J* = 3.5 Hz) and an oxygenated methine proton (δ_H 3.30, dd, *J* = 11.0, 4.8 HZ, H-3) which corresponded to carbon resonances at δ_C 121.4 (C-12) and 77.6 (C-3) (Table 4.28; Appendix 28b) respectively**.** This further suggested the compound was an oleanane type triterpene with a hydroxyl group at C-3 and double bond between C-12 and C-13 (Ragasa *et al.*, 2014). The hydroxyl group at (C-3) was deduced to be in equatorial position based on axial-axial and axial-equatorial couplings between H-3 and H-2 protons as previously observed in 3β-hydroxy-olean-12-ene (Babalola & Shode, 2013). On the other hand, a comparative analysis of both ^1H and ^{13}C NMR spectra of compound **AM8**, with those of ursolic acid (Woo *et al*. 2014, Babalola & Shode, 2013) showed similar results, a fact that was supported by the ^{13}C NMR at δ_C 179.8 representing the CO_2H functionality at C-17 (Babalola and Shode, 2013). Based on spectroscopic evidence as well as comparison with literature data (Ragasa *et al*., 2014), compound (**AM8)** was concluded to be oleanolic acid **(4)**



4

Table 4.28: ^{I}H (600 MHz) and ^{13}C NMR (125 MHz) ($CDCl_3$) spectral data of compound **4**

C#	^{1}H NMR	^{13}CNMR	^{1}H NMR*	^{13}C NMR*	DEPT
1		37.9		37.7	CH_2
2		26.7		27.2	CH_2
3	3.26 (dd, *J* = 11.0, 4.8 Hz)	77.6	3.20 (dd, *J* = 4.2, 11.4 Hz)	77.0	CH
4		38.3		38.7	C
5		54.5		55.2	CH
6		22.1		18.3	CH_2
7		31.8		32.6	CH_2
8		40.5		39.3	C
9		48.0		47.6	CH
10		37.7		37.1	C
11		24.5		23.4	CH_2
12	5.27 (t, *J* = 3.5 Hz)	121.4	5.28 (t, *J* = 3.6 Hz)	122.6	CH
13		142.9		143.6	C
14		40.7		41.6	C
15		26.7		27.7	CH_2
16		22.0		22.9	CH_2
17		46.3		46.5	C
18	2.82 (d, *J* = 11.3Hz)	45.0	2.81(dd, *J* = 4.2, 13.8 Hz)	41.0	CH
19		45.4		45.9	CH_2
20		29.5		30.7	C
21		31.6		33.8	CH_2
22		31.8		32.4	CH_2
23	0.96, s	29.5	0.96 s	28.1	CH_3
24	0.74 s	15.7	0.73 s	15.5	CH_3
25	0.89 s	14.3	0.89 s	15.3	CH_3
26	0.77 s	17.4	0.75 s	17.1	CH_3
27	1.10 s	25.7	1.11 s	25.9	CH_3
28		179.8		183.3	C
29	0.93 s	36.0	0.91 s	33.1	CH_3
30	0.89 s	22.4	0.88 s	23.6	CH_3

*Ragasa *et al*., 2014

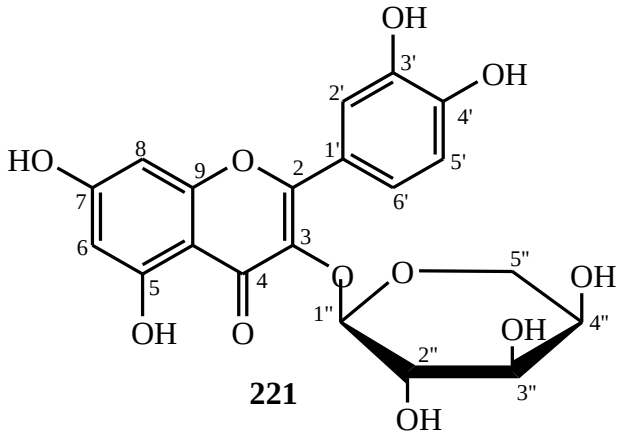
4.2.3.3.2. Isolate AM9

Compound**AM9** was elucidated as quercetin **(140),** a compound also isolated from the methanol leaf extract of *Gnidia subcordata.* See pages 105-107.

4.2.3.3.3Isolate AM10

AM10 was obtained from the methanol leaf extract of *Annona mucosa***,** and gave R_f value of 0.34 in 2% oxalic acid deactivated silica gel TLC [CH_2Cl_2-MeOH, 4:1]. It was isolated as a yellow amorphous powder with melting point 250^{o}C. The compound on exposure to conc. ammonia vapour appeared on TLC (both cellulose and silica gel) as dark UV absorbing spots suggesting a flavonol with substituted C-3-hydroxyl group and free C-5-hydroxyl group (Markham, 1982). The compound exhibited two major MeOH UV absorption bands**:** band I at 358 nm and band II at 258 nm (Appendix 29a), which suggested a flavonol nucleus with substituted C-3-hydroxy group (Mabry *et al*., 1970). Addition of shift reagent NaOAc/H_3BO_3 (Appendix 29b) caused a bathochromic shift of band 1 (20 nm) relative to the spectrum in methanol, which indicated the presence of *ortho*-dihydroxyl groups in the B-ring. This was in turn supported by the absorption spectrum in NaOMe/MeOH (Appendix 29c) which gave a bathochromic shift of (42 nm) which rapidly decreased in intensity confirming the presence of free 3', 4'-dihydroxyl groups. Similarly, a Bathochromic shift of 14 nm band II in the presence of NaOAc/MeOH (Appendix 29d) relative to MeOH indicated flavonols with free C-7 hydroxyl group (Markham, 1982), whereas the presence of free C-5-OH group was ascertained by a Bathochromic shift of 42 nm in band I obtained with $AlCl_3$/HCl (Appendix 29e) relative to MeOH (Mabry *et al.*, 1970). Thus, the UV data of compound **AM10** suggested that the compound is a flavonol with free hydroxyl groups at C-7, C-5, C-4' and C-3'. The presence of free C-5 OH group was confirmed by a singlet at δ_H 12.64. The ^{1}H and ^{13}C

NMR data (Table 4.29; Appendixes 29f and 29g**)** of the aglycone were similar with those of quercetin **(140)**, implying that the compound is quercetin derivative (Shahat*et al*., 2004). This was confirmed by acid hydrolysis of the compound (2% HCl, reflux for 2 hours) which released the aglycone quercetin and a sugar moiety identified as α-arabinose after comparison with standard samples of arabinose, glucose and galactose using silica gel TLC (solvent system: EtOAc-MeOH-H_2O-HOAc, 6:2:1:1). The ^{13}C NMR spectrum corroborated the acid hydrolysis results by exhibiting the presence of 20 carbons in the molecule of which five carbon signals in the glycosidic region corresponded to a pentose moiety and the remaining 15 carbons were due to the aglycone. The assignment of all the carbon signals due to the aglycone was done by comparison with reported data for quercetin 3-*O*-α-arabinoside. The anomeric proton signal of the sugar moiety appeared as doublet at δ_H 5.46 (J=5.1 Hz) with corresponding ^{13}C NMR peak at δ_C 102.05. The coupling constant of J=5.1 Hz signified axial-equatorial interaction which is characteristic of α-sugar conformers (Arima & Danno, 2002). The position of attachment of arabinose on the aglycone was suggested to be at C-3 due to a chemical shift value of the aglycone at δ_C 134.1. In the EI-mass spectrum (70 eV) (Appendix 29 h), a peak at *m/z* 302 is consistent with the quercetin aglycone $C_{15}H_{10}O_7$ indicating the loss of arabinose unit from the molecule. Thus on the basis of accumulated evidences as well as comparison with literature data (Ahmadu *et al*., 2007), compound**AM10** was established as quercetin 3-*O*-β-D-arabinoside **(221)**



221

Table 4.29: ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) $(CD_3)_2SO$ spectral data of **221**

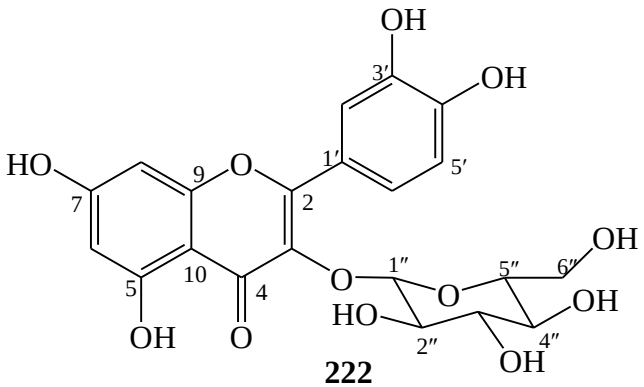
C#	^{1}H NMR	^{13}C NMR	^{1}H NMR*	^{13}C NMR*	DEPT
2		156.7		158.7	C
3		134.1		135.7	C
4		177.8		179.5	C
5		161.6		163.2	C
6	6.20 (d, *J* = 1.8 Hz)	99.0	6.20 (d, *J* = 1.6Hz)	100.1	CH
7		164.4		166.8	C
8	6.40 (d, *J* = 2.1 Hz)	93.8	6.39 (d, *J* = 1.2Hz)	94.9	CH
9		156.7		158.6	C
10		104.5		105.7	C
1'		122.0		123.0	C
2'	7.59 (d, *J* = 3.3 Hz)	116.3	7.74 (d, *J* = 1.5Hz)	117.6	CH
3'		145.1		146.1	C
4'		148.8		150.1	C
5'	6.80(d, *J* = 9.2 Hz)	115.6	6.88 (d, *J* = 8.5Hz)	116.3	CH
6'	7.59 (dd, *J* = 9.0, 3.3 Hz)	121.4	7.58 (dd, *J* =2.0, 8.0 Hz)	123.2	CH
1"	5.46 (d, *J* = 5.1 Hz)	102.1	5.17 (d, *J* = 3.5Hz)	104.8	CH
2"	4.28-3.32 m, overlapping	71.1		73.0	CH
3"	4.28-3.32 m, overlapping	66.7		69.3	CH
4"	4.28-3.32 m, overlapping	72.3	3.65-3.62(d, *J* = 7.0 Hz)	74.3	CH
5"	4.28-3.32 m, overlapping	65.0	3.46-3.48(d, *J* = 7.0 Hz)	67.1	CH_2
5-OH	12.64 s				

*Ahmadu *et al*., 2007

4.2.3.3.4 Isolate AM11: Compound **AM11** was isolated as greenish-yellow amorphous powder with m.p = 250^{o}C.It also appeared deep purple on paper chromatography under UV light and turned

yellow with conc. ammonia solution vapour suggesting that the compound is a flavonoid derivative (Mabry *et al*., 1970). The compound showed a green colour when reacted with aqueous ferric chloride indicating the presence of 5-hydroxyl group (Wolbis & Krolikowa, 1988) and this was supported by its UV spectrum which exhibited a bathochromic shift of 48 nm in band I with shift reagent $AlCl_3$/HCl relative MeOH (Appendix 30a] (Mabry *et al.*, 1970). The compound dissolved in aqueous NaOAc (Appendix 30b) suggesting the presence of free hydroxyl groups at C-7 and C-4' positions (Wolbis & Krolikowa, 1988). The presence of C-7-hydroxyl group was further supported by a bathochromic shift of 14 nm in band II with NaOAc relative to the methanol spectrum, while the presence of 4'-OH group was substantiated by bathochromic shifts of 44 nm and 20 nm in band I with NaOMe (Appendix 30c) and NaOAc/H_3BO_3 (Appendix 30d) respectively (Mabry *et al*., 1970). The rapid decomposition of the compound in NaOMe (Appendix 30e) could be attributable to the alkali sensitive 3', 4', 5-hydroxylation pattern in the compound (Howard & Mabry, 1970). In fact, the foregoing evidences suggested glycosylation at C-3 position. Acid hydrolysis (2% HCl) yielded quercetin and glucose confirmed by TLC co-chromatography with authentic samples. Quercetin structure was further confirmed by mass spectrum (70 eV) (Appendix 30f) which showed a peak at *m/z* 463.2 $[C_{21}H_{20}O_{12}\text{-}H]^+$. The downfield part of the ^{1}H NMR spectrum (Table 4.30; Appendix 30g) obtained in CD_3OD showed the characteristic aglycone pattern of quercetin derivative (Mabry *et a*l., 1970). Also in the spectrum, a 3H ABX systems was observed with peaks being exhibited at7.68 (d,d, J = 8.5 Hz, 2.0 Hz) and6.81 (d, J= 9.0) attributable to H-2', H-6' and H-5', respectively. Together with these were *meta*-coupled doublets at δ_H6.34 (d, J = 2.0 Hz) and 6.18 (d, J = 1.8 Hz) which represented H-8 and H-6, respectively. The anomeric proton signal appeared at δ_H 5.23 as a doublet with coupling constant J = 7.1 Hz which is in accord with *diaxial* coupling between the proton on C-1" and C-2" in a β-linked D-glucopyranoside

(Anderson *et al*., 1991; Markham, 1982). From physical, chemical and spectroscopic data and in comparison with literature data (Shahat *et al*., 2004), isolate **AM11** was concluded to be quercetin 3-*O*-β-D-glucoside **(222).**



222

Table 4.30:^{1}H (600 MHz)(CD_3OD)spectral data of (**222**).

C#	^{1}H NMR	^{1}H NMR*
2		
3		
4		
5		
6	6.18 (d, *J* = 1.8 Hz)	6.36 (d, *J* = 2.1)
7		
8	6.34 (d, *J* = 2.0 Hz)	6.80 (d, *J* = 2.3)
9		
10		
1'		
2'	7.71 (d, *J* = 2.0 Hz)	7.70 (d, *J* = 2-0)
3'		
4'		
5'	6.81 (d, *J* = 9.0)	6.90 (d *J* = 2.0)
6'	7.68 (d,d, *J* = 8.5 Hz, 2.0 Hz)	7.56 (dd, *J* = 8.4, 2.1)
1"	5.23 (d, *J* = 7.1 Hz)	5.12 (d, *J* = 7.5)
2"	3.49 m	3.70-3.20 m
3"	3.44 m	3.70-3.20 m
4"	3.33 m	3.70-3.20 m
5"	3.22 m	3.70-3.20 m
6"	3.73 m, 3.63 m	3.70-3.20 m

*Shahat *et al*., 2004

4.3 BIOLOGICAL ACTIVITIES OF COMPOUNDS ISOLATED FROM *GNIDIA SUBCORDATA, OCIMUM. KILIMANDSCHARICUM* AND *ANNONA MUCOSA* LEAVES

The pure isolates of leaves from the three plants were investigated for their bioactivities against *Sitophilus zeamais* and *Prostephanus truncatus* by use of contact toxicity and antifeedant bioassays. The reports given about the activities of the pure isolates are presented herein.

4.3.1 Contact toxicity of the isolated compounds

4.3.1.1 Contact toxicity of *G. subcordata* compounds against *S. zeamais* and *P. truncatus*

S. zeamais and *P. truncatus* when separately treated with pure isolates from *G. subcordata* leaf extracts, all the compounds exhibited contact toxicity activities against the two insects. The activities depended on the concentration of the compounds. Among the compounds, gedunin **(203)** had the highest contact toxicity activities (Figures 19&20) against *S. zeamais* and *P. truncatus* respectively. These activities were lower than but not significantly different from the activities of the positive control ($P \geq 0.05$).Quercetin **(140)** andobacunone **(204)** exhibited moderately high contact toxicity activities(Figure 19 & 20) which were lower and significantly different from the activities of the positive control ($P \leq 0.05$). On the other hand,*β*-amyrin acetate **(199),** nagilactone **(205)** and 4′, 5-dihydroxystilbene-3-O-β-glucoside **(207)** also exhibited moderately high contact toxicity activities against the two insects (Figure 19 and 20). The results of Yasui, (2001) however showed that nagilactone **(205)** had high activity when tested against *Eocanthecona furcellata*, which may be due to the ingestion toxicity assay used as opposed to contact toxicity assay used currently and the difference in the species of insects used. Among the isolated compounds, 3-hydroxy-11-oxo-olean-12-ene **(200)** had the lowest activities (Figures 19 & 20) against *S. zeamais*

and *P. truncatus*.Compounds that had activities that were not significantly different from those of the positive controls would be suitable for use in stored insect pest control.

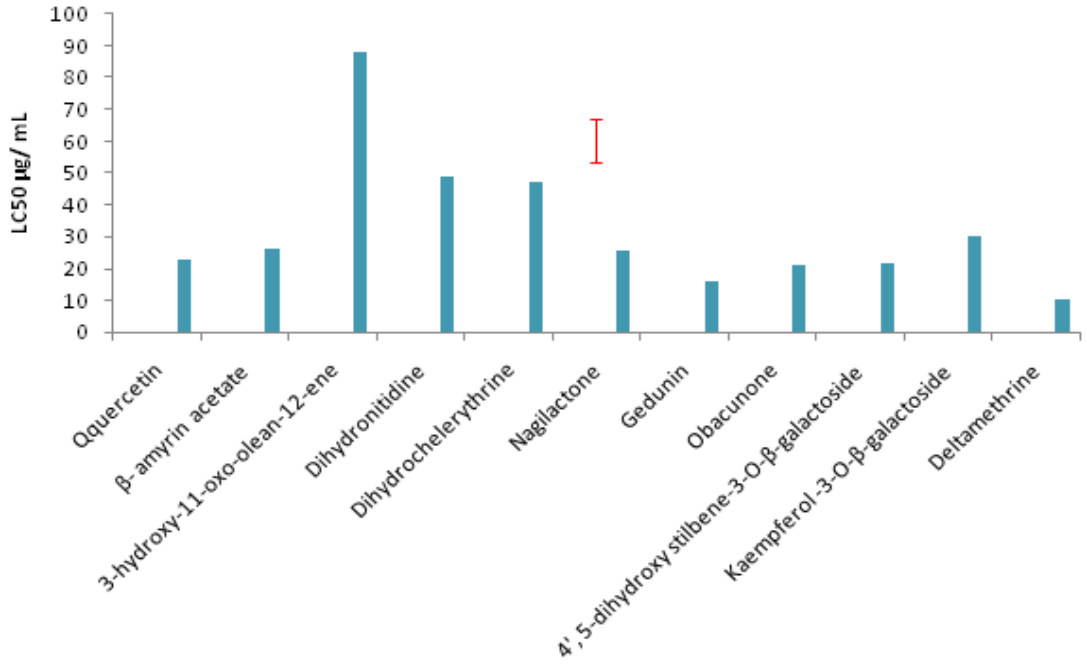


Figure 19: **Contact toxicity activities (LC_{50} µg/ml) of *G. subcordata* compounds against *S. zeamais*

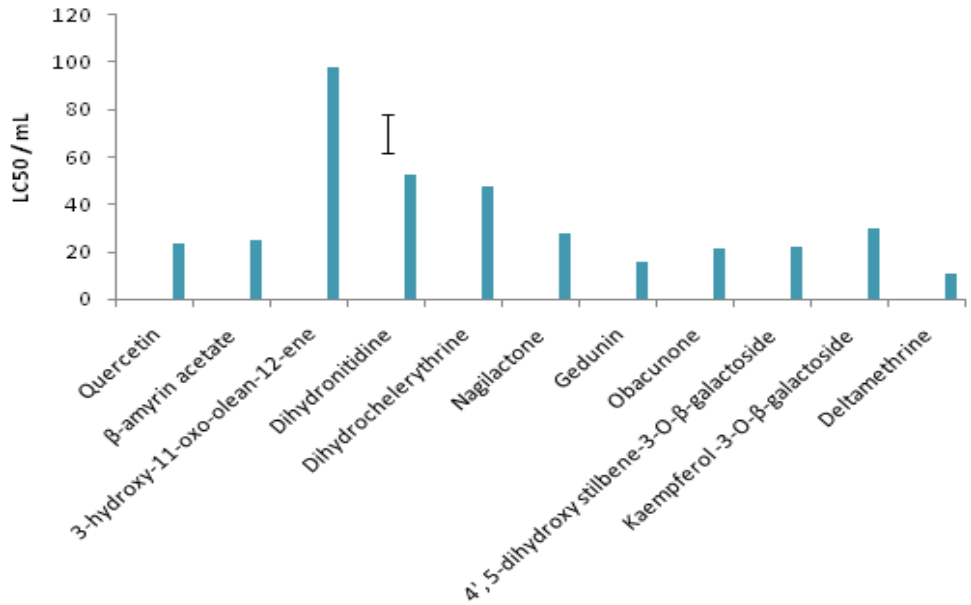


Figure 20: Contact toxicity activities (LC_{50} µg/ml) of *G. subcordata* compounds against *P. truncatus*

4.3.1.2 Contact toxicity activities of *Ocimum kilimandscharicum* compounds against *S. zeamais* and *P. truncatus*

Allpure isolates of *O. Kilimandscharicum* had activities against *S. zeamais* and *P. truncatus*. The activities increased with increase in concentration. All the isolates however showed activities that were lower than the activities of the positive control, deltamethrine. Quercetin **(140)** had the highest activities against both *S. zeamais and P. truncatus* (Figures 21 & 22). These activities were however lower and significantly different from the activities of deltamethrine ($P \leq 0.05$) against *P. truncatus* and *S. zeamais* (Figures 21 & 22). The high activity of quercetin agrees well with the results of (Golawska *et al*., 2014) which showed high activity of the compound when tested against *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae). .Apigenin -7-*O*-neohesperidoside **(214)** exhibited relatively high contact toxicity activities against *S. zeamais* and *P. truncatus* respectively (Figures 21 & 22). The activities of this compound were lower and significantly different from the activities of the positive control ($P \leq 0.05$) for *S. zeamais* and *P. truncatus*. Other flavonoids including apigenin **(144),** friedelin **(209)** and fisetin **(213)** showed moderately high contact toxicity activities (Figure 21 and 22) against the two insects. Among the triterpenoids, the dammarane type triterpenoids, 2α-hydroxy-3-oxo-dammar-20, 24-diene **(210)** and 2α, 3β-dihydroxy dammar-20, 24-diene **(211)** exhibited moderately high activities (Figure 21 and 22) against *S. zeamais* and *P. truncatus* respectively. Lupeol **(49**) and chrysin **(212)** had moderate activities against the two insects.Stigmasterol **(6)** exhibited low activities against the two insects. The compounds that had activities comparable to the activities of the positive control may be considered for application as insecticides in post-harvest insect pest control.

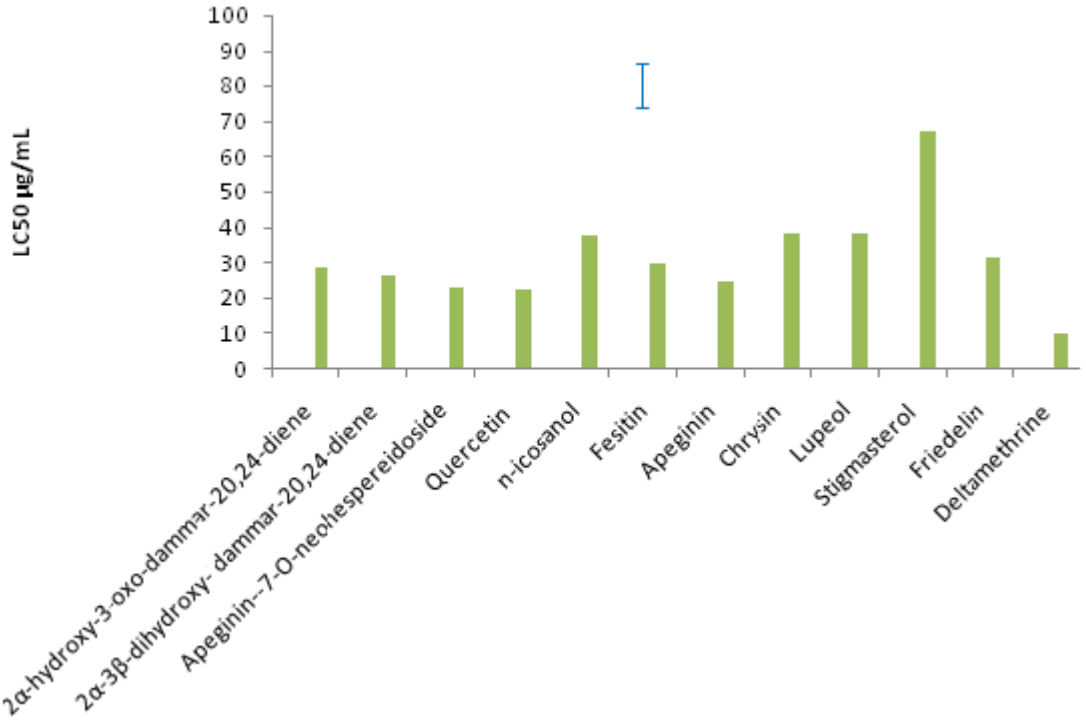


Figure 21: Contact toxicity activities (LC_{50}) of *O. kilimandscharicum* compounds against *S. zeamais*

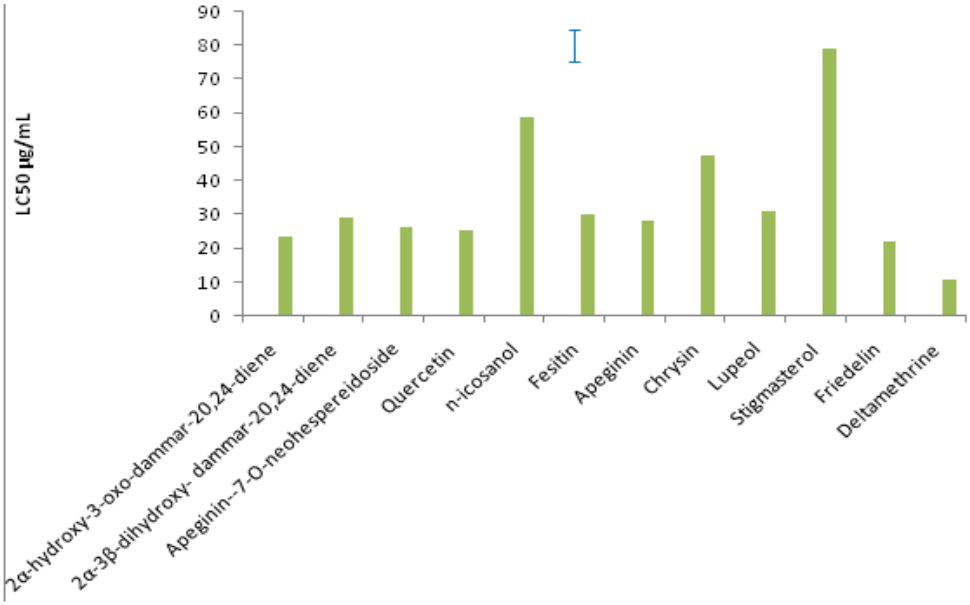


Figure 22: Contact toxicity activities (LC_{50}) of *O. kilimandscharicum* compounds against *P. truncatus*

4.3.1.3: Contact toxicity of *Annona mucosa* compounds against *S. zeamais* and *P. truncatus*

Compounds isolated from *A. mucosa* were tested against *S. zeamais* and *P. truncatus* and all of them exhibited contact toxicity activities against the two insects. The two insects had similar susceptibilities on exposure to the *A. mucosa* compounds. Among the compounds, the flavonoid, quercetin-3-*O*-β-D-glucoside **(222)** from the methanol leaf extract had the highest contact toxicity activities against *S. zeamais* and *P. truncatus* (Figure 23 and 24). These activities were lower than but not significantly different from those of the positive control, deltamethrine ($P \geq 0.05$) against *S. zeamais* and *P. truncatus* respectively. Another flavonoid, quercetin- 3-*O*-β-D-arabinoside **(221)** also from the methanol extract had relatively high activities among the compounds when tested against *S. zeamais* and *P. truncatus* respectively, which were again lower than but not significantly different from the activities of deltamethrine ($P \geq 0.05$). Quercetin **(140)** exhibited promising contact toxicity activities against the two insects (Figures 23 & 24) and the activities were also significantly different from those of the positive control ($P \leq 0.05$). The triterpenoids; 3α, 24-diacetoxy-12-oleonene **(216)**, 3-oxo-11α-hydroxy-12-ursene **(217),** (3R, 20S)-3-acetoxy-20-hydroxy dammar-24-ene **(218)**, 3β-acetoxy oleanolic acid **(219)** and 3β-acetoxy tirucallic acid **(220),** had moderate activities against the two insects (Figures 23 & 24). The activities of β-sitosterol **(95),** a sterol were the lowest against both *S. zeamais* and *P. truncatus* (Figures 23 & 24). Since the activities of some the compounds are not different from those of the positive controls, such compounds are suitable candidates for use in stored insect pest control after field testing.

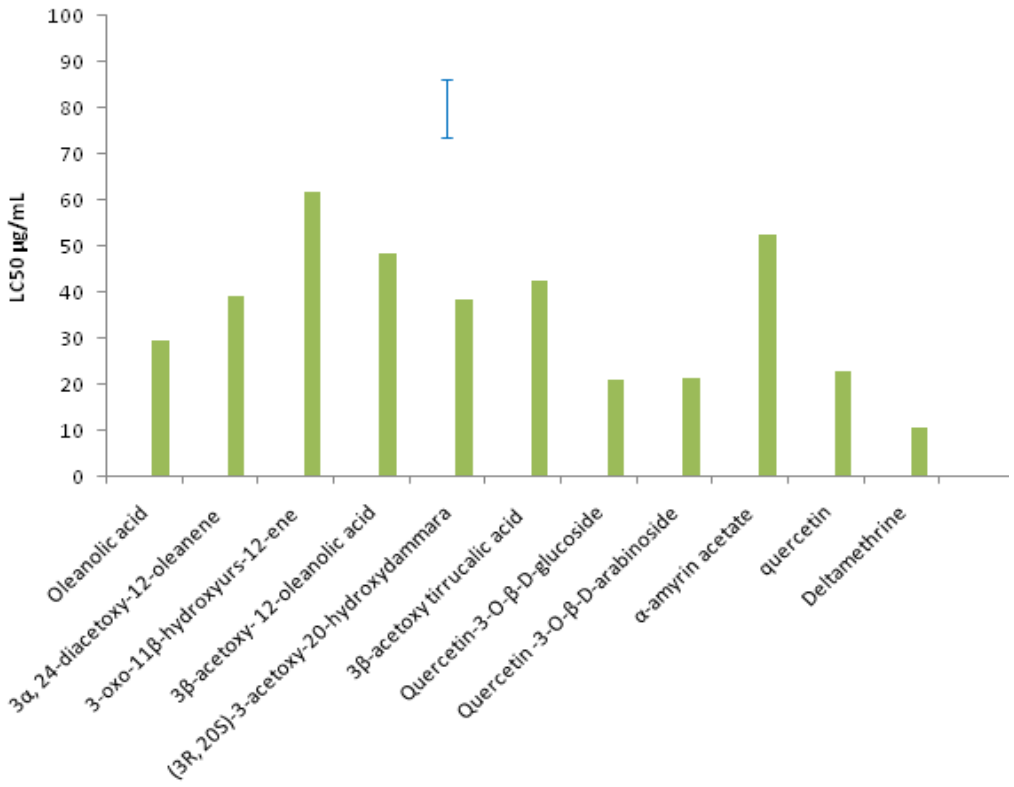


Figure 23: Contact toxicity activity (LC_{50}) of *A. mucosa* compounds against *S. zeamais*

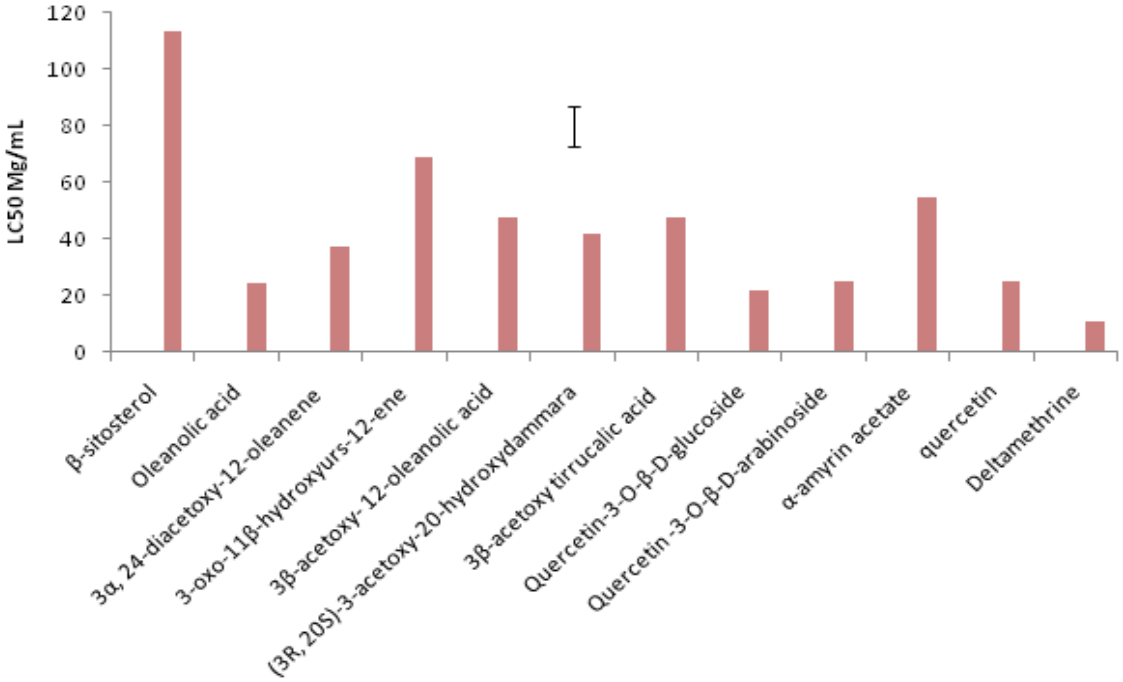


Figure 24: Contact toxicity activity (LC_{50}) of *A. mucosa* compounds against *P. truncatus* ($p \leq 0.05$)

4.3.2 Antifeedant activities of *Gnidia subcordata, Ocimum kilimandscharicum* and *Annona mucosa* isolated compounds

Compounds of *G. subcordata, O. kilimandscharicum* and *A. mucosa* were subjected to antifeedant assays against *S. zeamais* and *P. truncatus*.

4.3.2.1 Antifeedant activities of *Gnidia subcordata* compounds against*S. zeamais* and *P. truncatus*

The compounds exhibited various levels of antifeedant activities against the two insects. Gedunin **(203)** displayed high antifeedant activities among the compounds (Figures25 & 26). These were however lower than the activities of the positive control, azadrachtin, when tested against *S. zeamais* and *p. truncatus* respectively. There were no significant differences in the activities of the compound and the positive control ($P \geq 0.05$) for *S. zeamais* and *P. truncatus*. Similar results were obtained when the compound was tested against *Sitophilus oryzae* at 0.50% w/w (Omar *et al*., 2007). Quercetin **(140)** was found to exhibit high activities which were lower but not significantly different ($p \geq 0.05$) from the activities of the positive control when tested against the two insects. High activity of compound **140** was also reported by (Adeyemi *et al*., 2010) on testing the compound against *Tribolium castaneum*. Obacunone **(204)**, nagilactone **(205)** and kaempferol-3-O-β-galactoside (**206**) exhibited moderately high activities which were lower than the activities of the positive control, while 4′, 5-dihydroxystilbene-3-*O*-β-glucoside **(207**) had moderate activities (Figure 25 and 26). The moderately high activity of obacunone may have been due to the presence of the carbonyl group at C-7 and the presence of the furan ring in the structure of the compound (Ruberto *et al.*, 2002). 3-hydroxy-3-oxo-oleanane **(200)** exhibited the lowest activities among the compounds (Figure 25 and 26). Some of the compounds isolated had activities which compared

well with the activities of the positive control. Such compounds maybe considered in application as part of compounds to be used in post-harvest insect pest control after field testing.

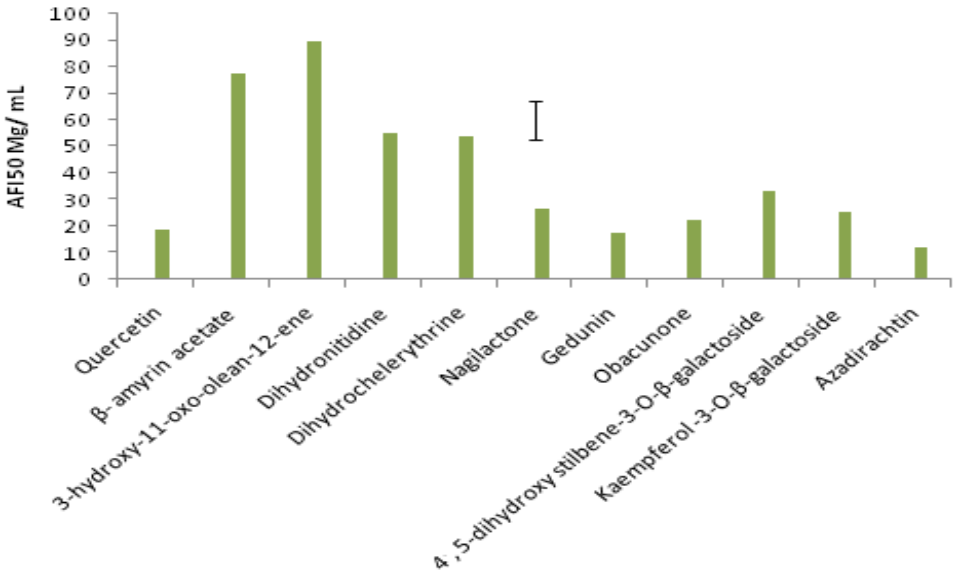


Figure 25: Antifeedant activity (AFI_{50} µg/ml) of *G. subcordata* compounds Against *S. zeamais* ($P \leq 0.05$)

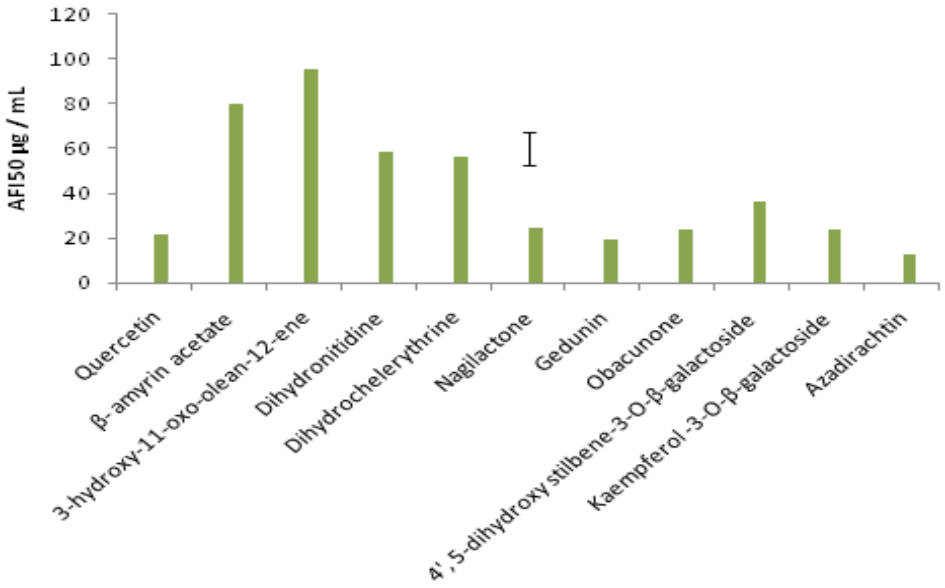


Figure 26: Antifeedant activity (AFI_{50} µg/ml) of *G. subcordata* against *P. truncatus* ($p \leq 0.05$)

4.3.2.2 Antifeedant activities of *Ocimum kilimandscharicum* compounds against *S. zeamais* and *P. truncatus*

When tested against *S. zeamais* and *P. truncatus* all the compounds isolated from *O. kilimandscharicum* exhibited concentration dependent activities. The antifeedant activities of the compounds showed similar trends against the two insects. Among the compounds, quercetin **(140)** exhibited the highest activities against *S. zeamais* and *P. truncatus* (Figures 27 & 28). These activities were lower but significantly not different from the activities of the positive control ($P \geq 0.05$) when tested against *S. zeamais* and *P. truncatus* respectively. Similar results were reported by (Adeyemi *et al.,* 2010) on testing the compound against *Tribolium casteneum*. Lupeol **(49)** exhibited high antifeedant activities against the two insects. These activities were lower but not significantly different ($P \geq 0.05$) from those of the positive control against *S. zeamais* and *P. truncatus* (figures 27 & 28). Friedelin **(209),** fisetin **(213)** and apeginin -7-O-neohesperidoside **(216)** had moderately high antifeedant activities (Figures 27& 28) which were lower than the activities of the positive control. Apigenin **(144),** 2α-hydroxy-3-oxodammar-20, 24-diene **(210)** and 2α, 3β-dihydroxydammar-20, 24-diene **(211)** had moderate activities against the two insects (Figure 27 and 28). Low antifeedant activities were exhibited by stigmasterol **(6).**For the flavonoids; quercetin **(140),** apigenin **(144),** chrysin **(212),** fisetin **(213)** and apeginin 7-O-neohesperidoside **(214),** the presence of a keto group at C-4 of the flavonoid may be considered to be important for the antifeedant activity (Medeiros *et al.,* 1994). Chrysin **(212)** had lower activity most probably due to the presence of fewer hydroxyl groups in its structure (Medeiros *et al*., 1994). Some of the compounds exhibited superior activities that compared well with the activities of the positive control. These compounds may be considered for use in stored insect pest control after field testing.

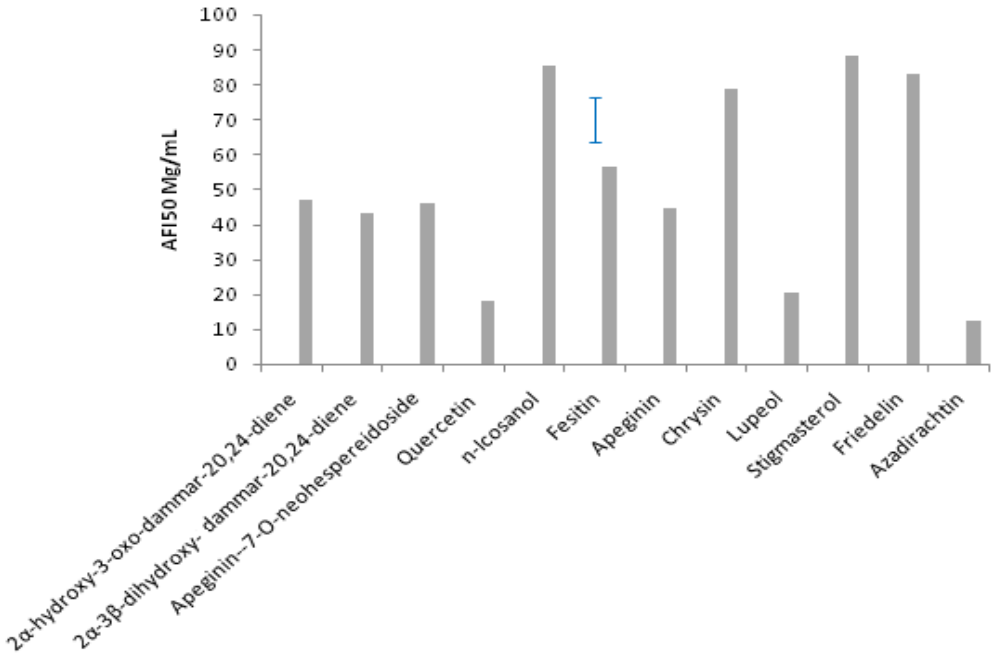


Figure 27: Antifeedant activity (AFC_{50} µg/ml) of *O. kilimandscharicum* compounds against *S. zeamais* ($p \leq 0.05$**)**

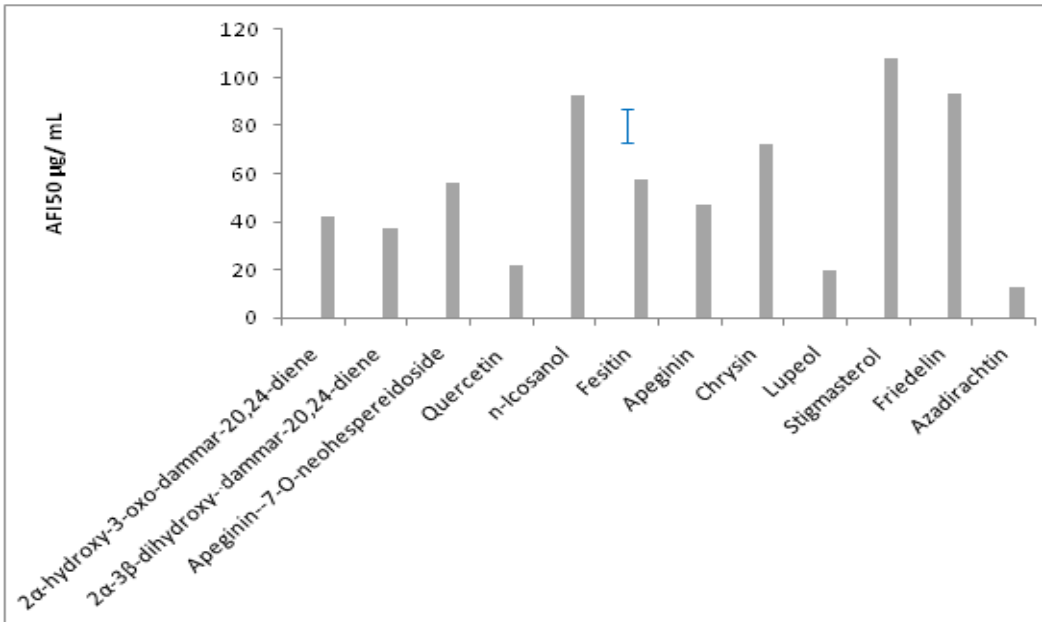


Figure 28: Antifeedant activity (AFC_{50} µg/ml) of *O. kilimandscharicum* compounds against. *P. truncatus* ($P \leq 0.05$**)**

4.3.2.3: Antifeedant activities of *Annona mucosa* compounds against *S. zeamais* and *P. truncatus*

When *S. zeamais* and *P. truncatus* were both separately treated with pure isolates of *A. mucosa,* all the compounds showed antifeedant activities on the two insects. The antifeedant activities of *A.*

mucosa compounds against the two insects increased with increase in concentration. The flavonoid, quercetin-3-*O*-β-D-glucoside **(222)** had the highest antifeedant activities when tested against *S. zeamais* and *P. truncatus*. Though lower than the activity of the positive control, they were not significantly different from activities of the positive control ($P \geq 0.05$), *zeamais* and *P. truncatus.*Quercetin-3-*O*-β-D-arabinoside **(221)**and quercetin **(140)**had relatively high antifeedant activities, against *S. zeamais* and *P. truncatus* respectively which had no significant differences from the activities of the positive control, ($P \geq 0.05$) (Figures 29 & 30).Flavonoids such as quercetin **(140),** quercetin-3-*O*-β-D-arabinoside **(221)** and quercetin-3-*O*-β-D-glucoside **(222)** are known to have high antifeedant activities (Medeiros *et al.*, 1994). This may be due to the presence of a keto group at C-4 in the pyran ring, a hydroxyl group at C-5, C-7 and a large number of hydroxyl groups in its structure (Nascimento *et al.*, 2013; Medeiros *et al.*, 1994). Other compounds, 3α, 24-diacetoxy-12-oleonene **(216),** 3-oxo-11β-hydroxyurs-12-ene **(217),** (3R, 20S)-3-acetoxy-20-dammar-24-ene **(220)** and 3β-acetoxy oleanolic acid **(219)** showed moderate activities against the two insects (Figure 29 and 30).β-sitosterol **(95)** had the lowest activities against the two insects which was also expressed in the results of (Santana *et al.*, 2012), when tested against *L. decemlineata*. Oleanane type pentacyclic triterpene acids such as 3β-acetoxy oleanolic acid **(219)** are known to be potent antifeedants due to the presence of the acid moeity at C-7 and ester functionality at C-3 (Mallavadhani *et al.*, 2003; Kashiwada *et al.*, 2000). The low antifeedant activity of the phytosterol, β-sitosterol may be due to the absence of O-bearing substituent at C-7 such as –Ome or –OH which gives sterols their antifeedant activity (Santana *et al.*, 2012). In addition, herbivorous insects do not have the capacity to synthesize cholesterol and they therefore depend on the ingestion of phytosterols which are then metabolized to cholesterol (Behmer & Elias, 1999) hence they may not serve as potent antifeedants. Some of the compounds exhibited superior

activities that compared well with the activities of the positive control. These compounds may thus be considered for use in stored insect pest control after field testing.

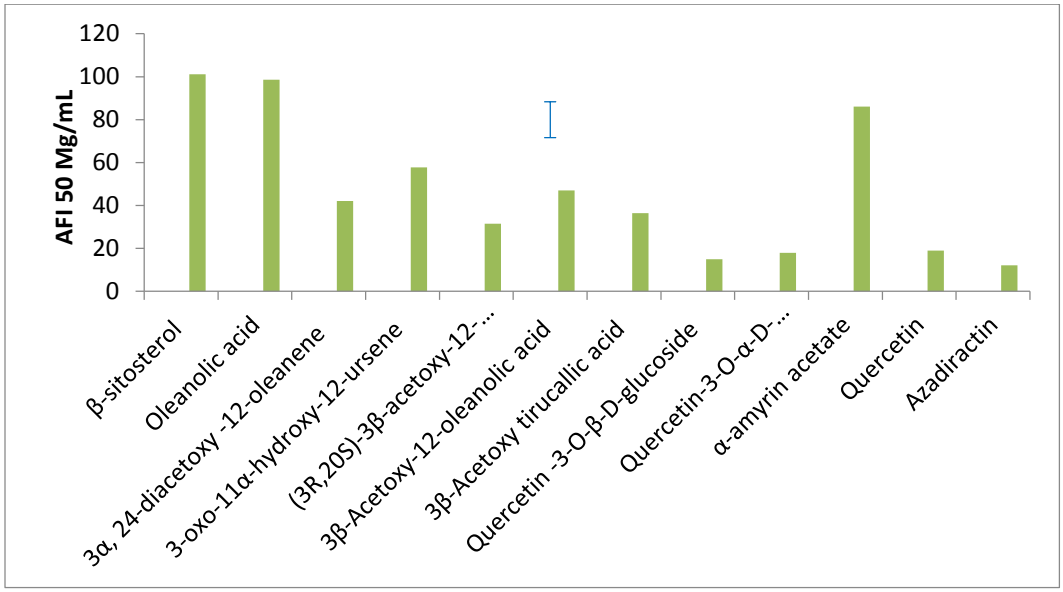


Figure 29: Antifeedant activity (AFI_{50} µg/ml) of *A. mucosa* compounds against *S. Zeamais* ($p \leq 0.05$)

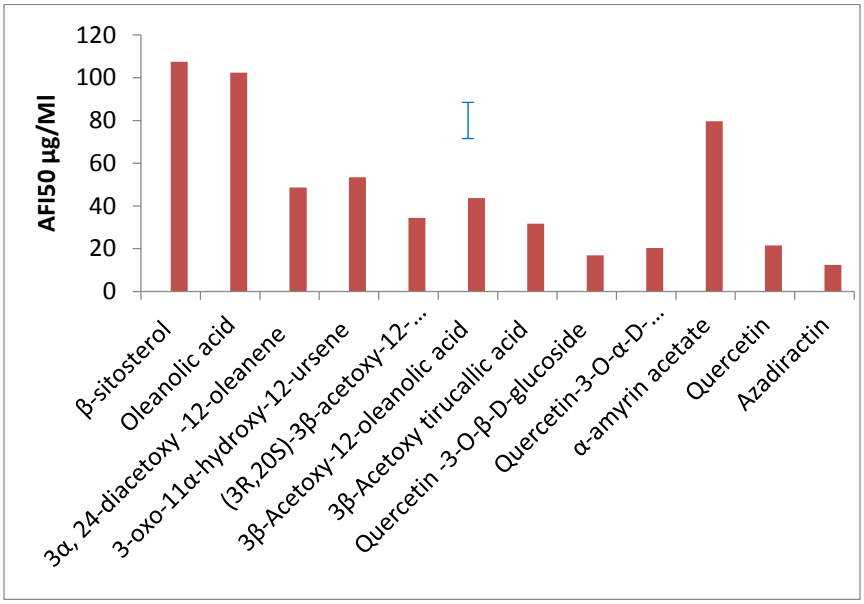


Figure 30: Antifeedant activity (AFI_{50} µg/ml) of *A. mucosa* compounds Against *S. zeamais* ($p \leq 0.05$)

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The study was intended to establish the compounds present and determine the insecticidal efficacy of *G. subcordata*, *O. kilimandscharicum* and *A. mucosa* crude leaf extracts and chemical isolates against *S. zeamais* and *P. truncatus*, insect pests of stored maize. In the investigation the summary and conclusions that follow, were generated and recommendations arising from the study listed

.

5.1 Summary

- *G. subcordata* leaf extracts exhibited higher contact toxicity and antifeedant activities as compared to the activities of *O. kilimandscharicum* and *A. mucosa*. The activities were lower than the activities of the positive control though some were not significantly different ($p \geq 0.05$) from activities of the positive control.

- For each of the plants, methanol extracts had higher activities than the ethyl acetate and the *n*-hexane extracts. All the activities were lower than the activities of the positive control, though some were not significantly different from those of the positive control.
- Phytochemical evaluation of *G. subcordata* gave rise to ten compounds; *β*-amyrin acetate **(199),** 3β-hydroxy-11-oxoolean-12-ene **(200),** dihydronitidine **(201),** dihydrochelerythrine **(202),** gedunin **(203),** obacunone **(204),** nagilactone **(205),** quercetin **(140),** kaempferol-3-*O*-β-galactoside **(206)** and 4', 5-dihydroxystilbene-3-*O*-β-glucoside **(207).** Compounds;**200, 201, 202, 204, 205 206** and **207** are being reported in this plant for the first time

- *O. kilimandscharicum* afforded eleven compounds; *n*-eicosanol **(208),** friedelin **(209),** stigmasterol **(6),** Lupeol **(49),** 2α-hydroxy-3-oxodammar-20, 24-diene **(210)**, 2α, 3β-dihydroxy dammar-20, 24-diene **(211)**, chrysin **(212)**, apigenin **(144),** fisetin **(213),** quercetin **(140)** and apigenin-7-*O*-neohesperidoside (**214**). Among these compounds, 2α-hydroxy-3-oxodammar-20, 24-diene **(210)** and 2α, 3β-dihydroxy dammar-20, 24-diene **(211)** are new while compounds, **213** and **214** are being reported in this plant for the first time.
- *Annona mucosa* yielded eleven compounds; β-sitosterol **(95),** quercetin **(140),** α-amyrin acetate **(215),** 3α, 24-diacetoxy-12-oleanene **(216),** 3-oxo-11β-hydroxyurs-12-ene **(217),** (3R,20S)-acetoxy-20-hydroxydammar-24-ene **(218),** 3β-acetoxy oleanolic acid **(219)**, 3β-acetoxytirucallic acid **(220)**, oleanolic acid **(4),** quercetin 3-*O*-β-D-arabinoside **(221)** andquercetin-3-*O*-β-D-glucoside **(222).** Among them 3α, 24-diacetoxy-12-oleanene **(216),** a compound isolated from the ethyl acetate leaf extract of the plant is new, while compounds **221** and **222** are being reported for the first time from this plant.
- Gedunin **(203)** and quercetin-3-O-β-D-glucoside **(222)** are among the compounds that had promising contact toxicity activities against the two insects. The activities compared favourably with the activities of deltamethrin, the positive control.
- In the antifeedant activity compounds that had promising activities were quercetin **(140),** gedunin **(203),** quercetin-3-*O*-β-D-arabinoside **(221)** and quercetin-3-O-β-D-glucoside **(222).** These activities compared well with the activities of azadirachtin, the positive control

5.2 Conclusions

- The *n*-hexane, ethyl acetate and methanol/aqueous extracts of the three plants exhibited contact toxicity and antifeedant activities against *S.zeamais* and *P. truncatus.* This validates the traditional use of these plants in post-harvest insect pest control.

- Among the extracts from the three plants, methanol extracts had the highest activities. *G. subcordata* methanol extract exhibited activities that were not significantly different from those of the positive controls.
- Thirty compounds were isolated from the three plants and were characterized. *G. subcordata* yielded ten compounds; *O. kilimandscharicum* gave forth to eleven compounds while *A. mucosa* also yielded eleven compounds. Quercetin was isolated from each of the three plants.
- .Three of the isolated compounds were new; 3α, 24-diacetoxy-12-oleanene **(216),** 2α-hydroxy-3-oxodammar-20, 24-diene **(210)** and 2α, 3β-dihydroxy dammar-20, 24-diene **(211)**.
- All the compounds isolated from the three plants exhibited various contact and antifeedant activities against *S. zeamais* and *P. truncatus*.
- Some of the compounds had activities that were comparable to activities of the positive control. Gedunin **(203),** quercetin **(140),** quercetin-3-*O*-α-D-arabinoside **(221**) and quercetin-3-O-β-D-glucoside **(222)** had promising contact toxicity and antifeedant activities that were comparable to those of the positive controls.

5.3 Recommendations

- Extracts from the three plants were active against the two insects. Extracts from these plants may therefore be used as a cheaper option for post-harvest protection against the two insects.
- The methanol extracts of *G. subcordata* had the highest activities that were comparable to those of the positive control. These extracts may be used in the integrated pest management (IPM) programs.
- Compounds that showed high contact toxicity and antifeedant activities; quercetin **(140)** isolated from the methanol extracts of the three plants, gedunin **(203**) from the ethyl acetate extracts of *Gnidia subcordata*, quercetin 3-*O*-β-D-glucoside **(221)**, and quercetin 3-*O*-β-D-glucoside **(222)** from

methanol extracts of *Annona mucosa* may be developed into formulations to be used as insecticides in control of *S. zeamais* and *P. truncatus* after field testing.

5.4 Suggestions for further study

- Essential oils extracted from leaves of the three plants may be screened to determine their phytochemical composition and insecticidal activities.
- The synergistic effects of the most active compounds may be investigated against the two insects.
- The extracts and isolated compounds may be tested against the two insects using other bioassay methods such as repellence
- The newly isolated compounds; 2α-hydroxy-3-oxodammar-20, 24-diene **(210)**, 2α, 3β-dihydroxy dammar-20, 24-diene **(211)** from *O. kilimandscharicum* and 3α, 24-diacetoxy-12-oleanene **(216)** from *A. mucosa* may be subjected to bioassay tests against other insects to determine their activities.

5.5 Significance of the study

- The results validate the folklore use of these plants in control of stored maize insect pests and thus offering options to improving food security.
- Positive results from this study have provided insecticides from locally available plants that can effectively control insect pests of stored maize. This will minimize the threat to global food security
- Use of the plants as insecticides will reduce environmental pollution and toxicity to humans.

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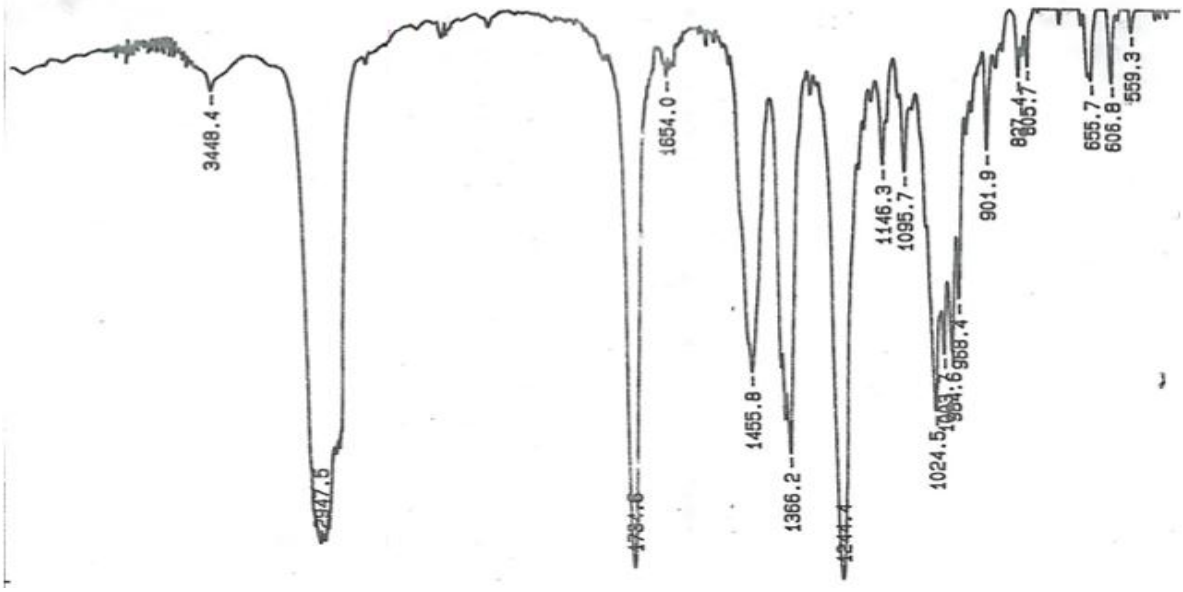
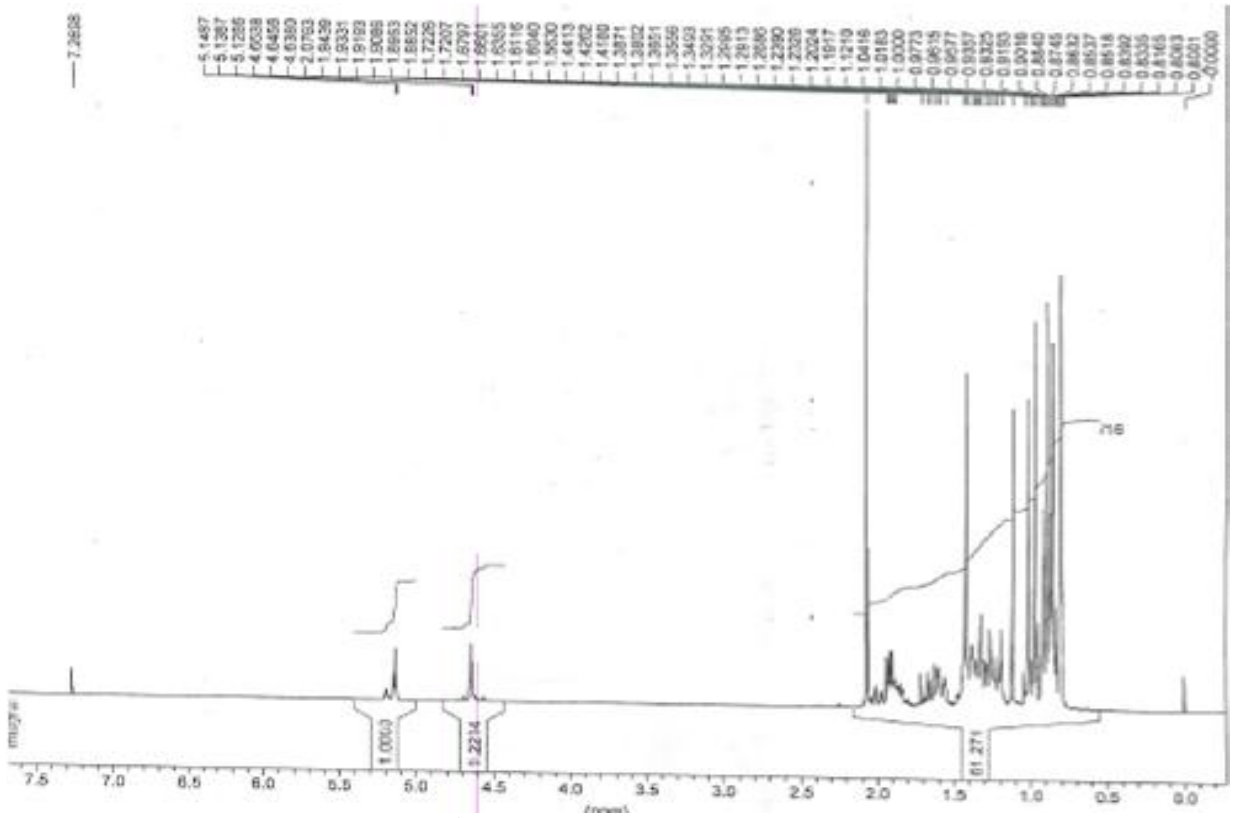
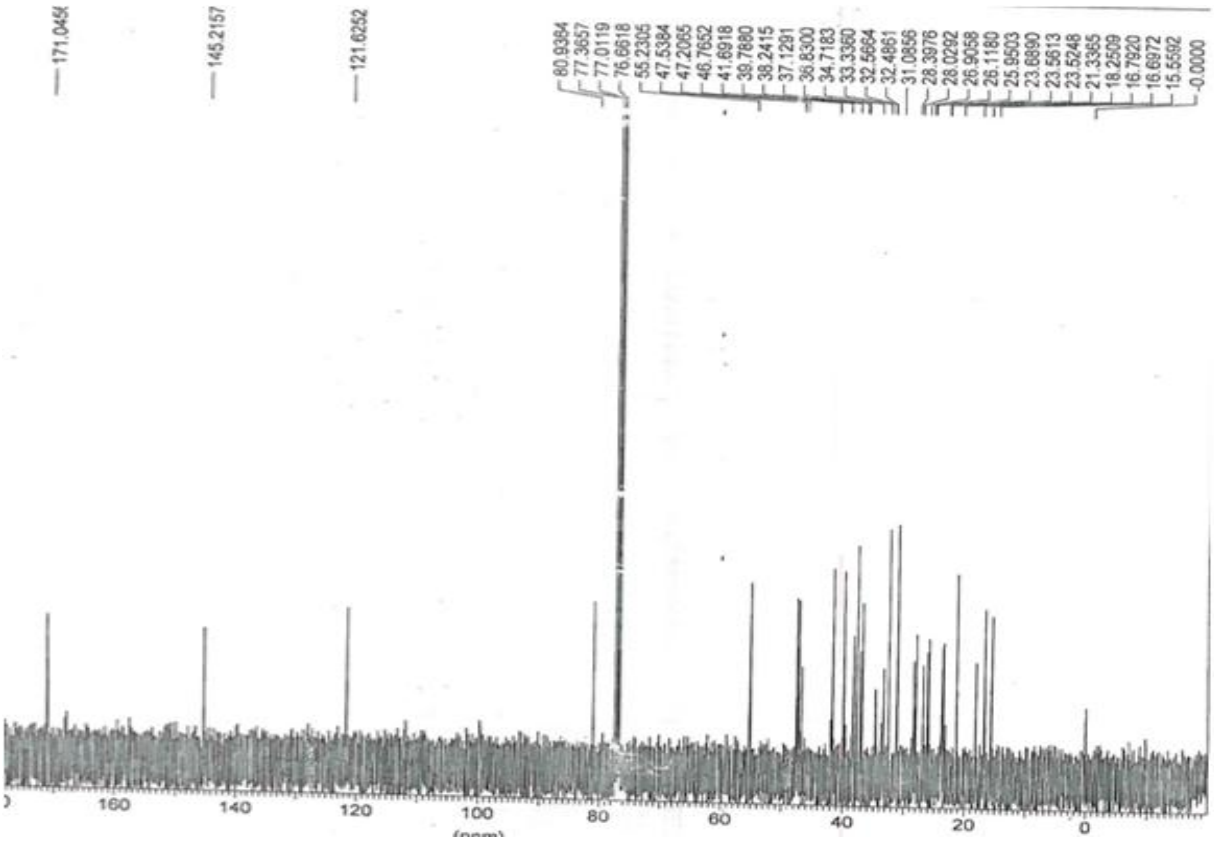
THE APPENDICES



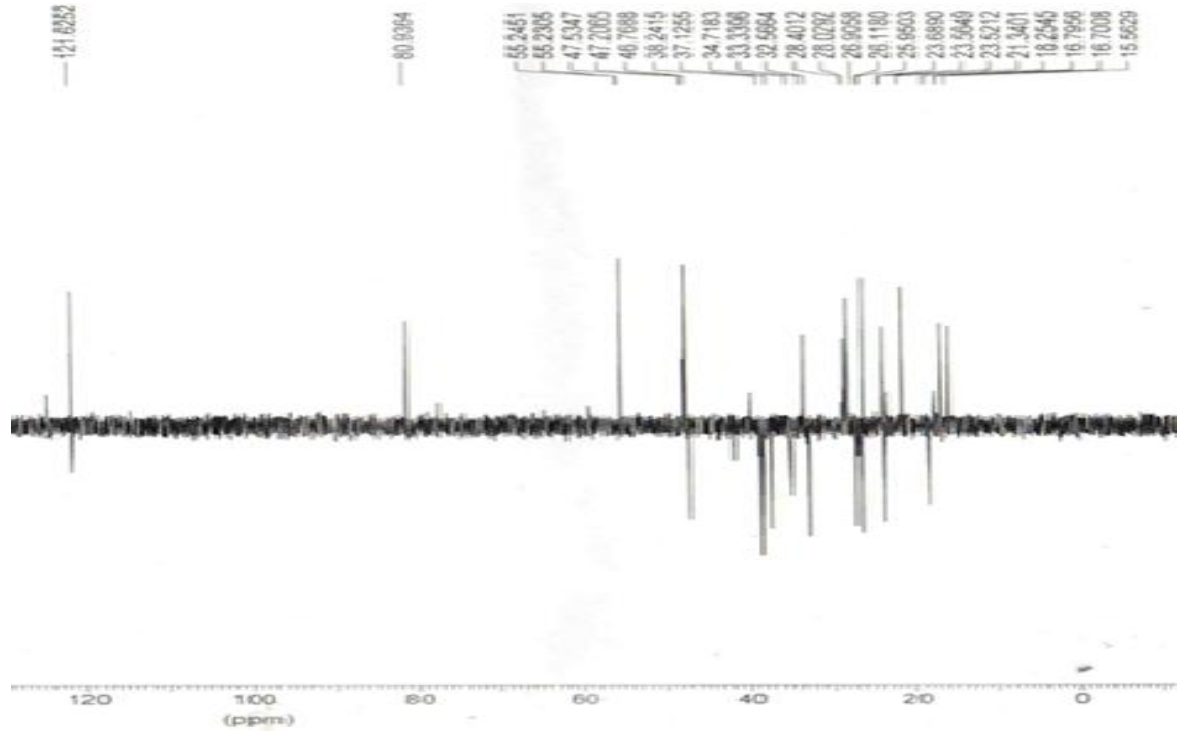
Appendix 1a: IR spectrum for compound 199



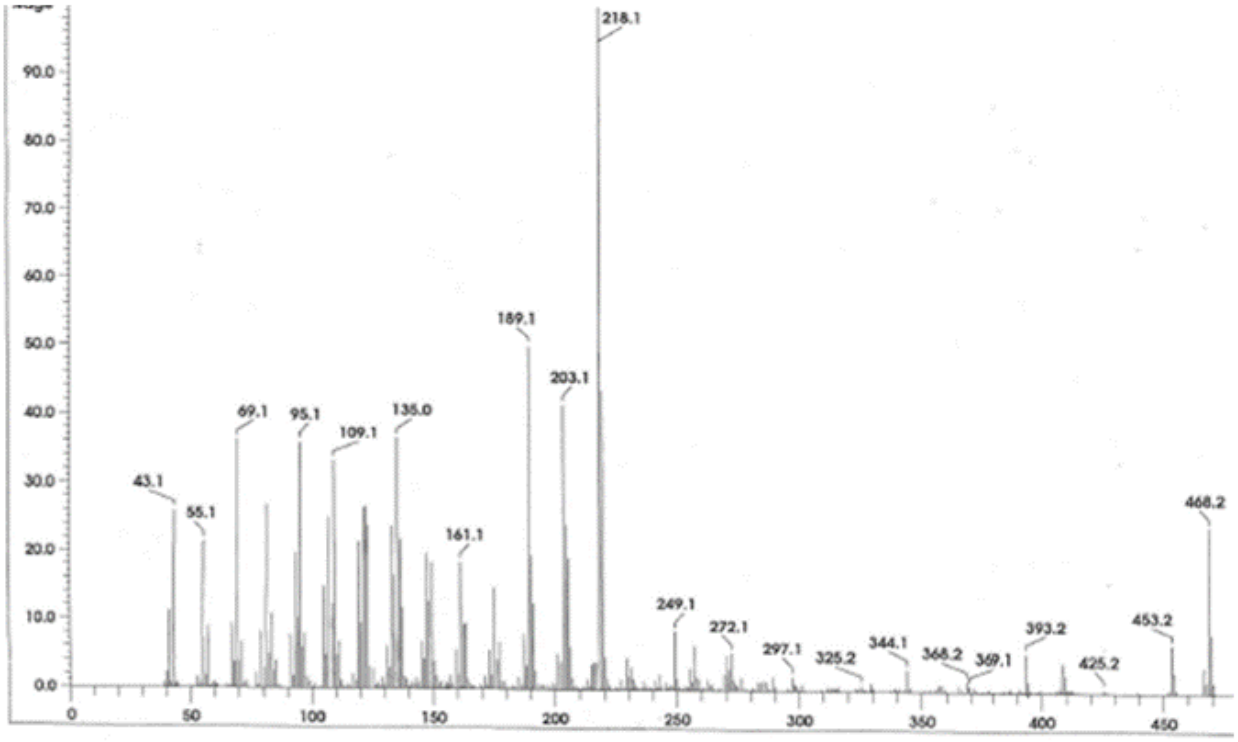
Appendix 1b:^{1}H NMR spectrum for compound 199



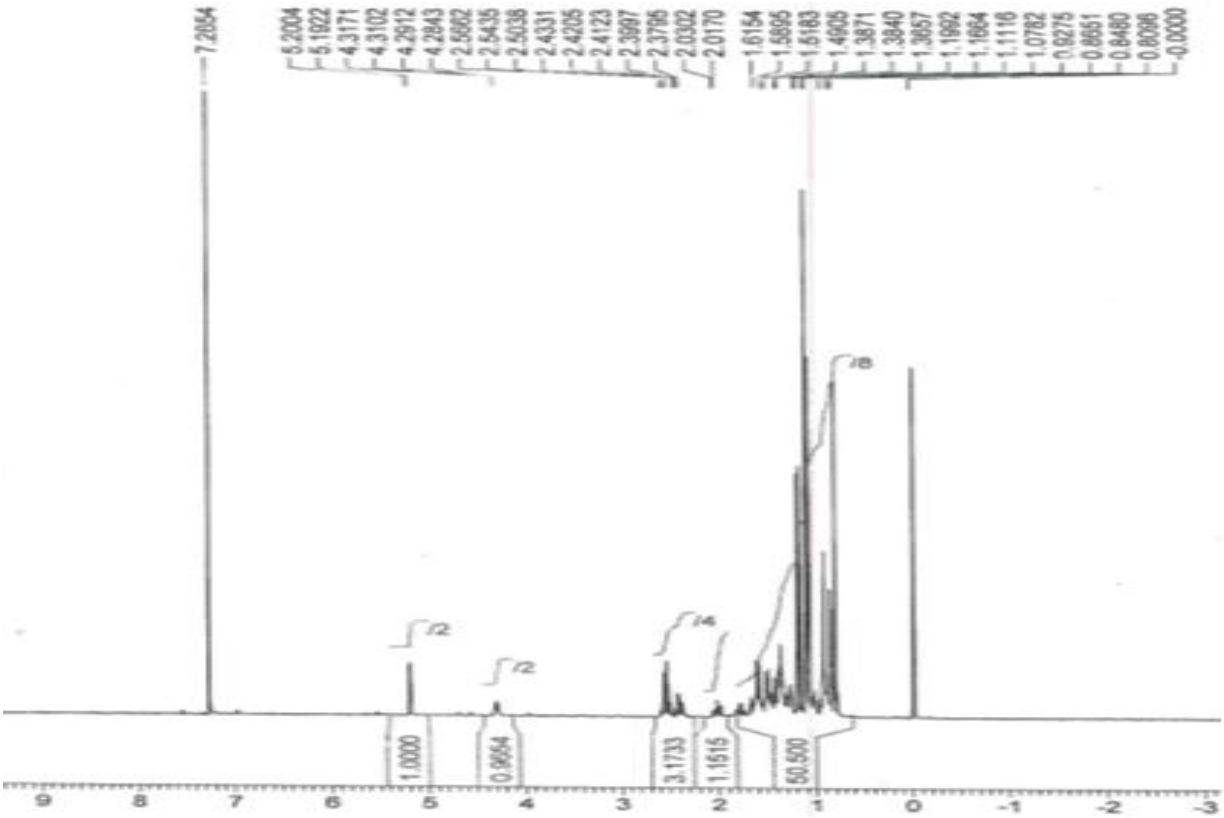
Appendix 1c: ^{13}C NMR spectrum for compound 199



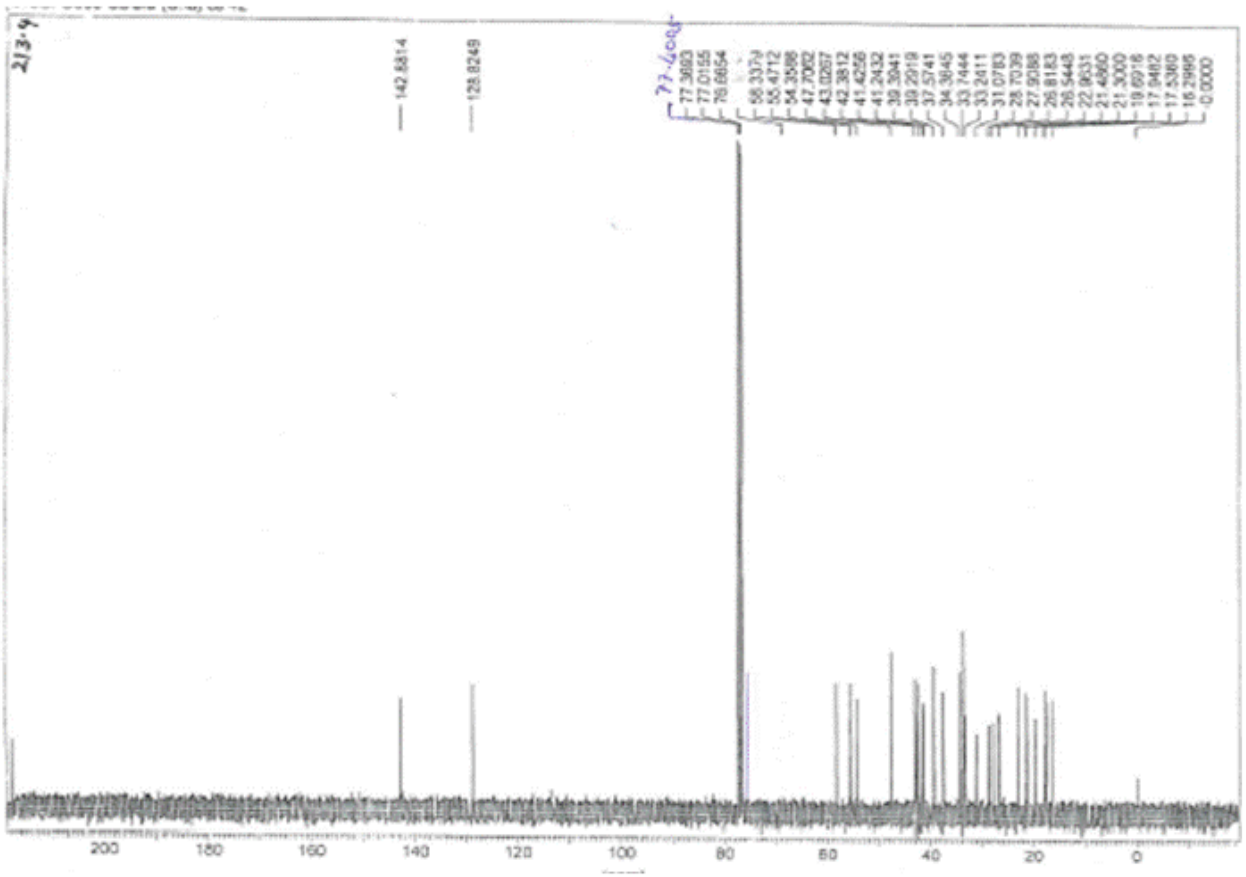
Appendix 1d: DEPT 135 spectrum for compound 199



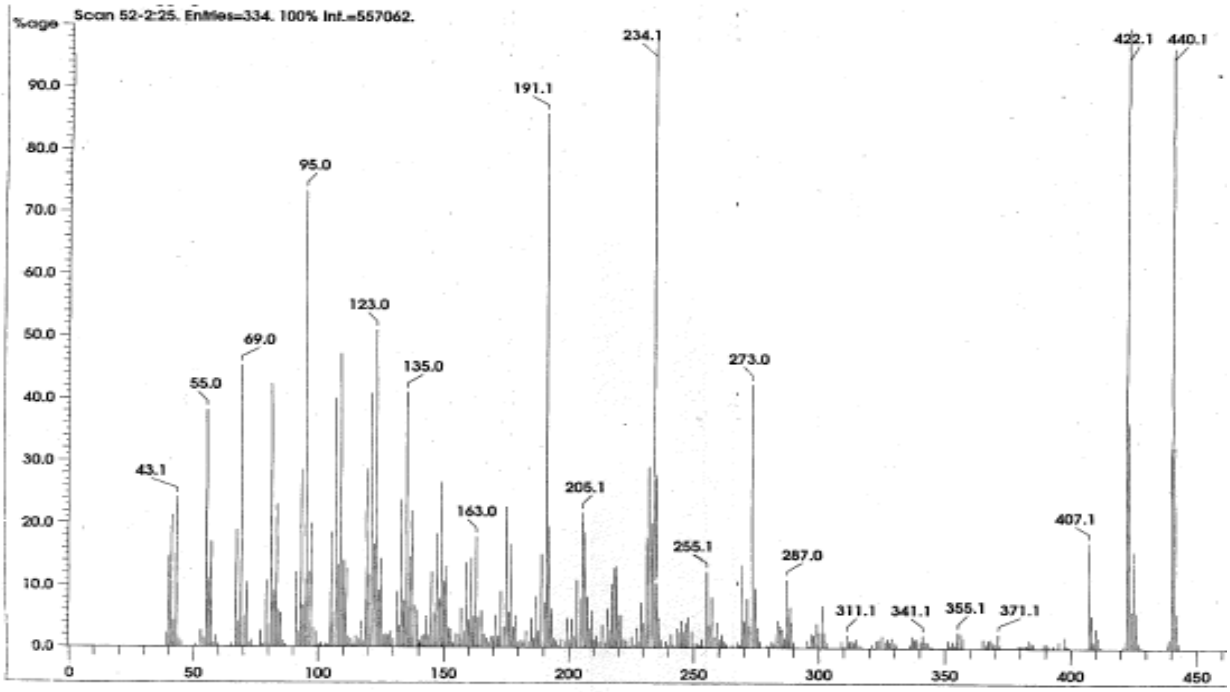
Appendix 1e: EI-MS spectrum for compound 199



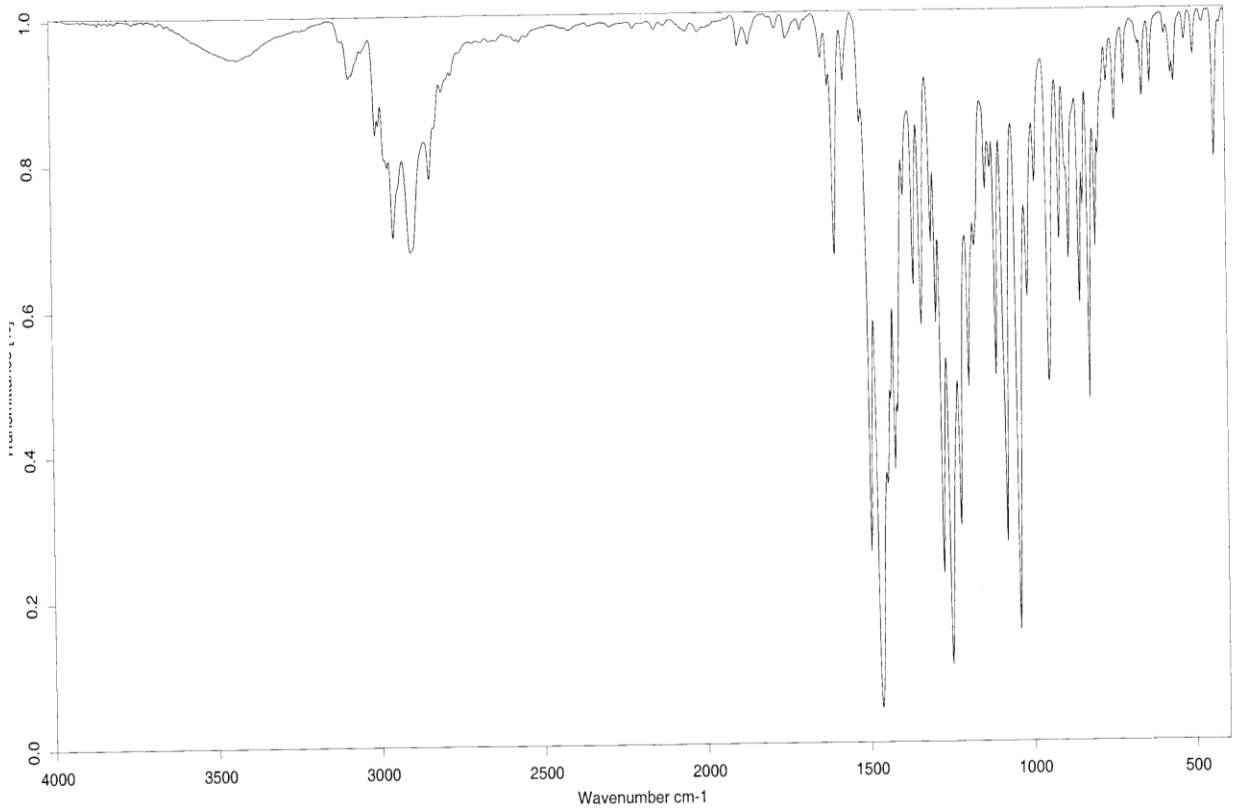
Appendix 2a: ^{1}H NMR spectrum for compound 200



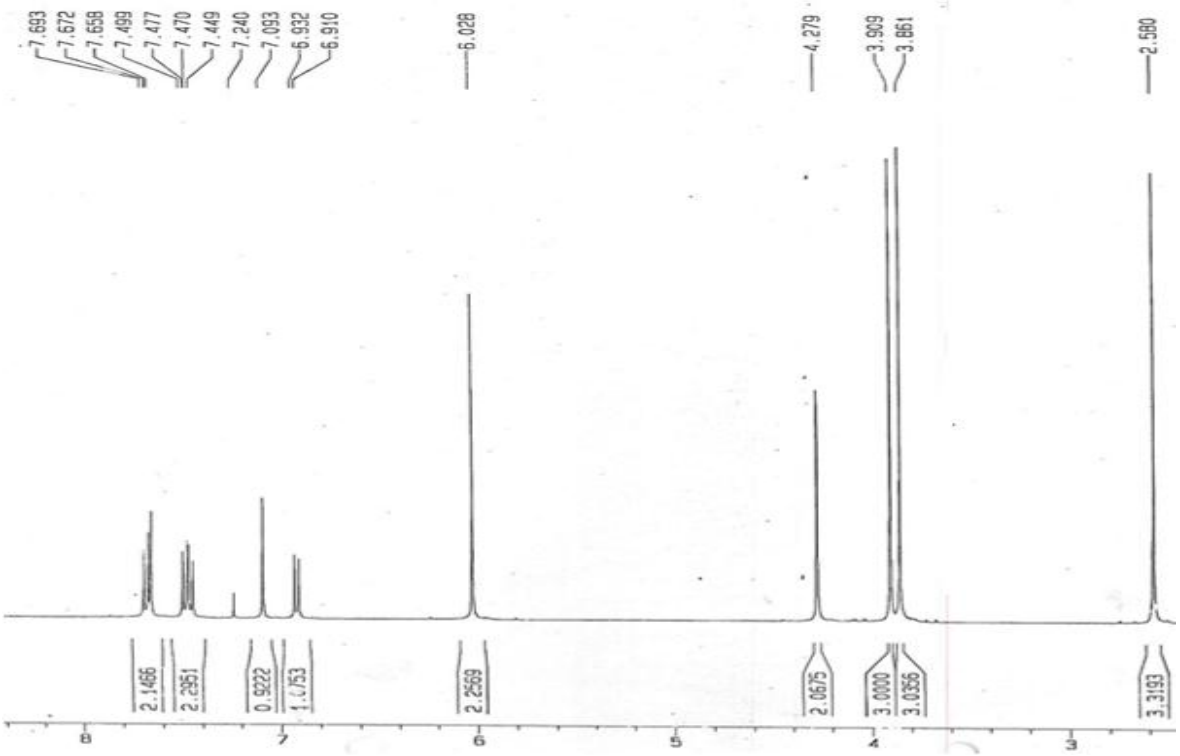
Appendix 2b: ^{13}C NMR spectrum for compound 200



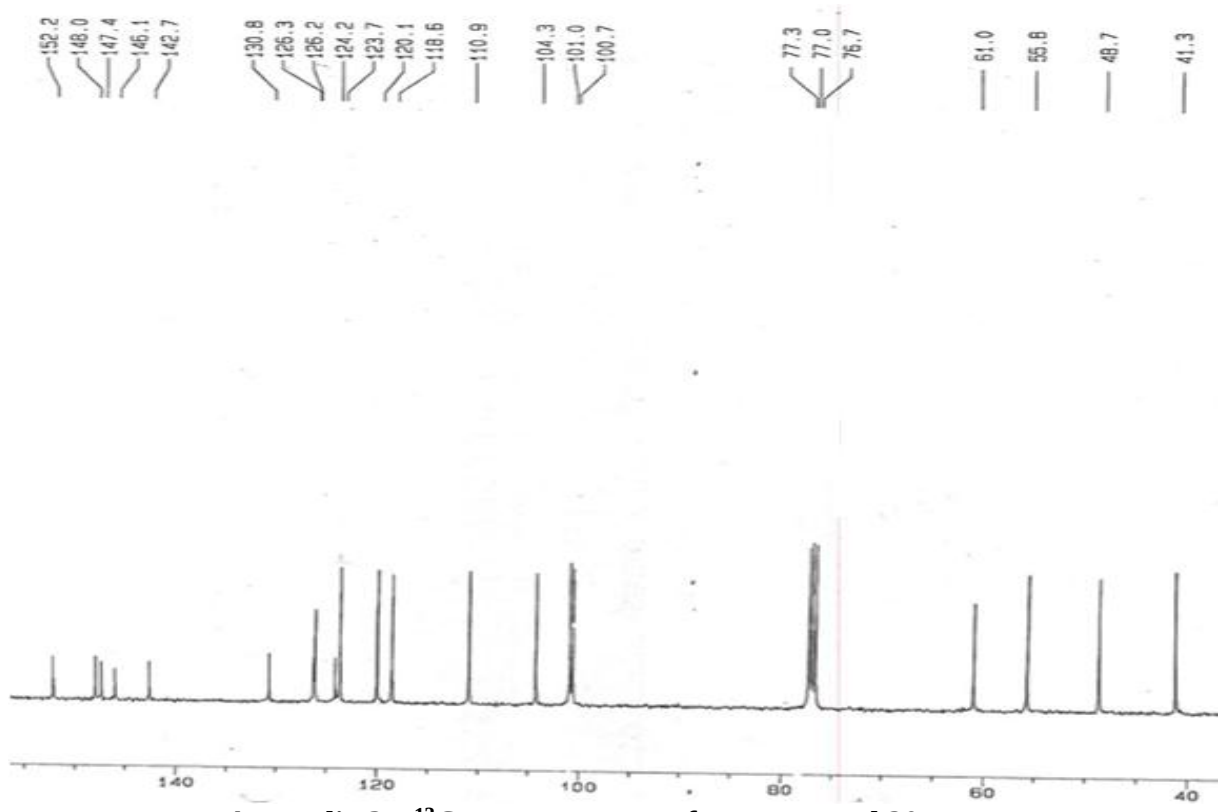
Appendix 2c: EI-MS spectrum for compound 200



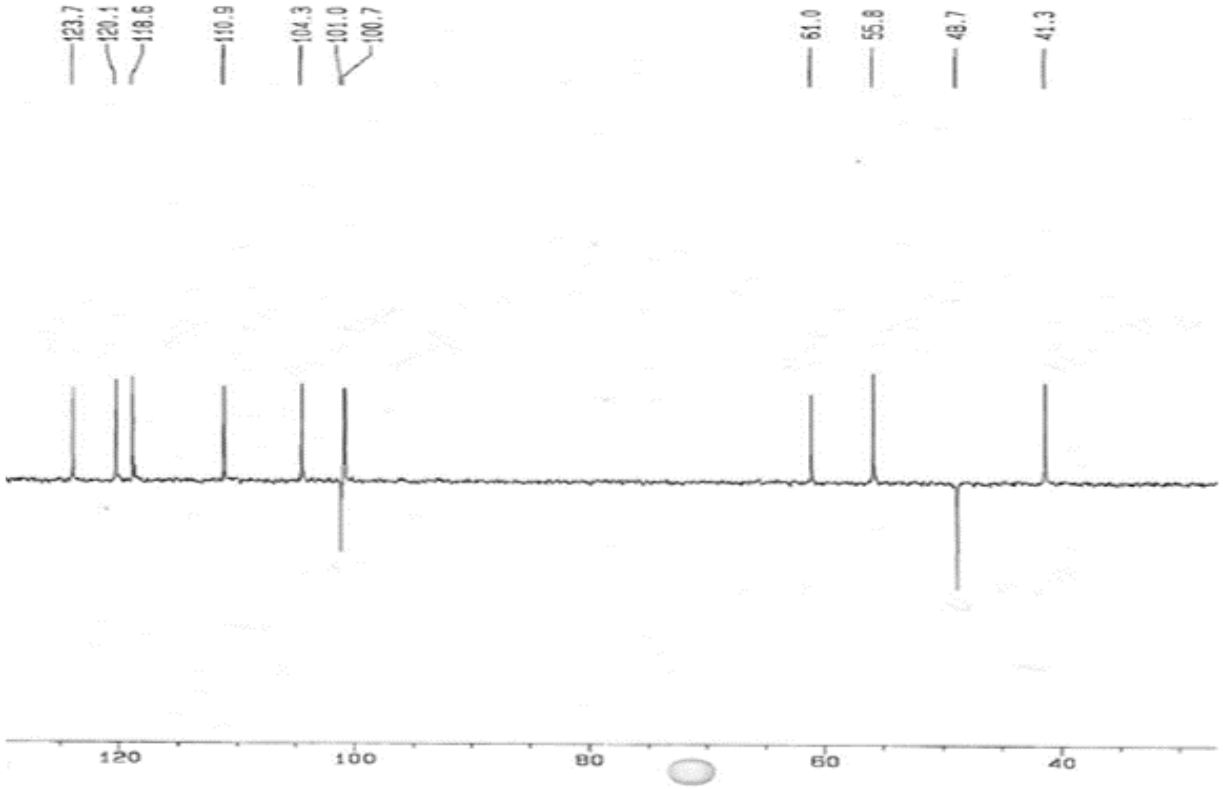
Appendix 3a: IR spectrum for compound 201



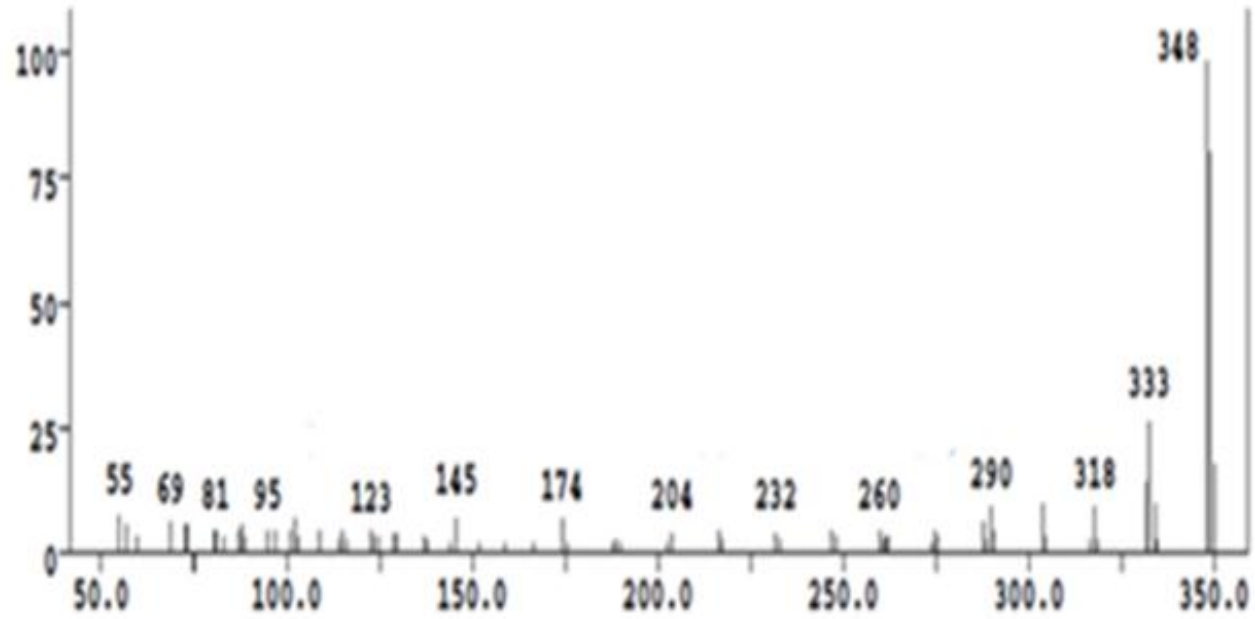
Appendix 3b: ^{1}H NMR spectrum for compound 201



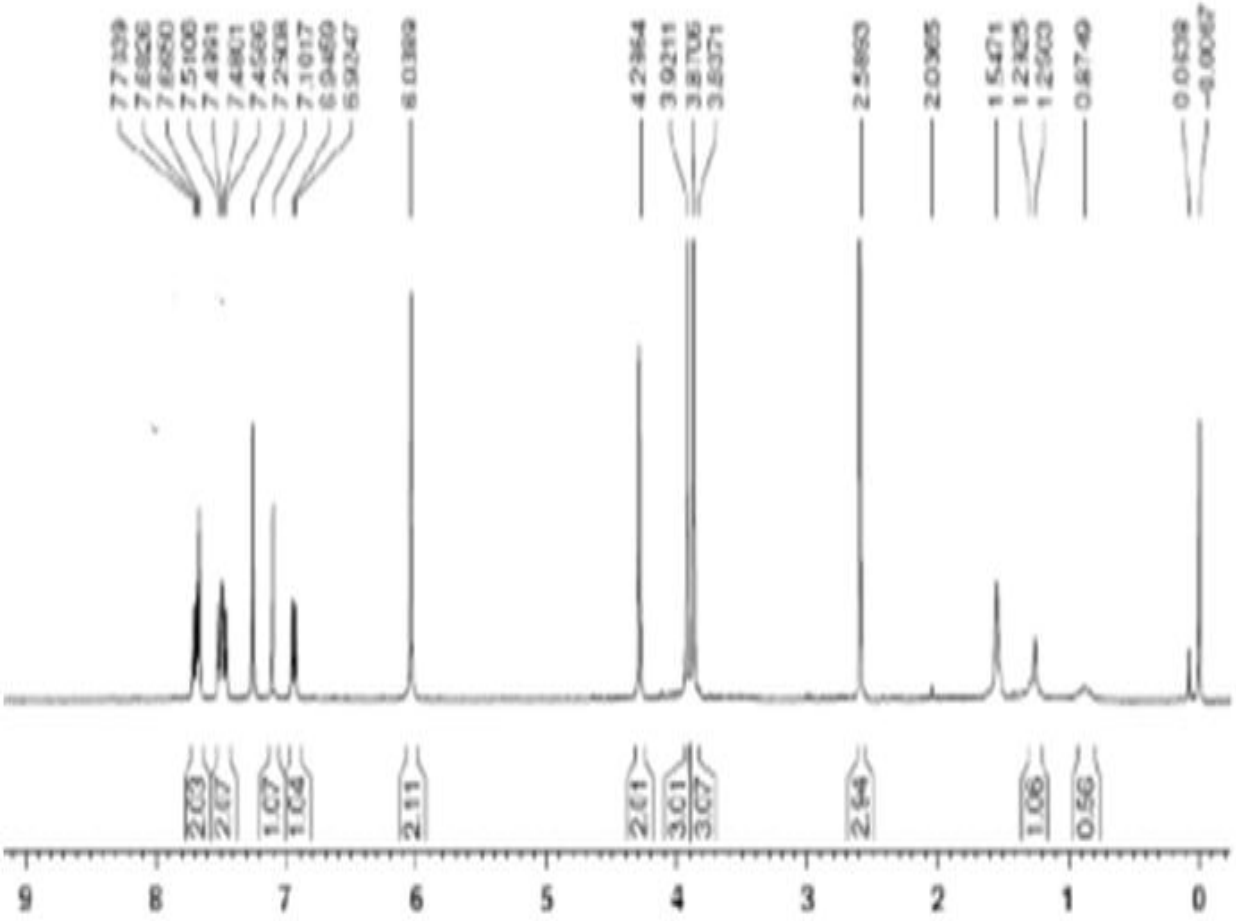
Appendix 3c: ^{13}C NMR spectrum for compound 201



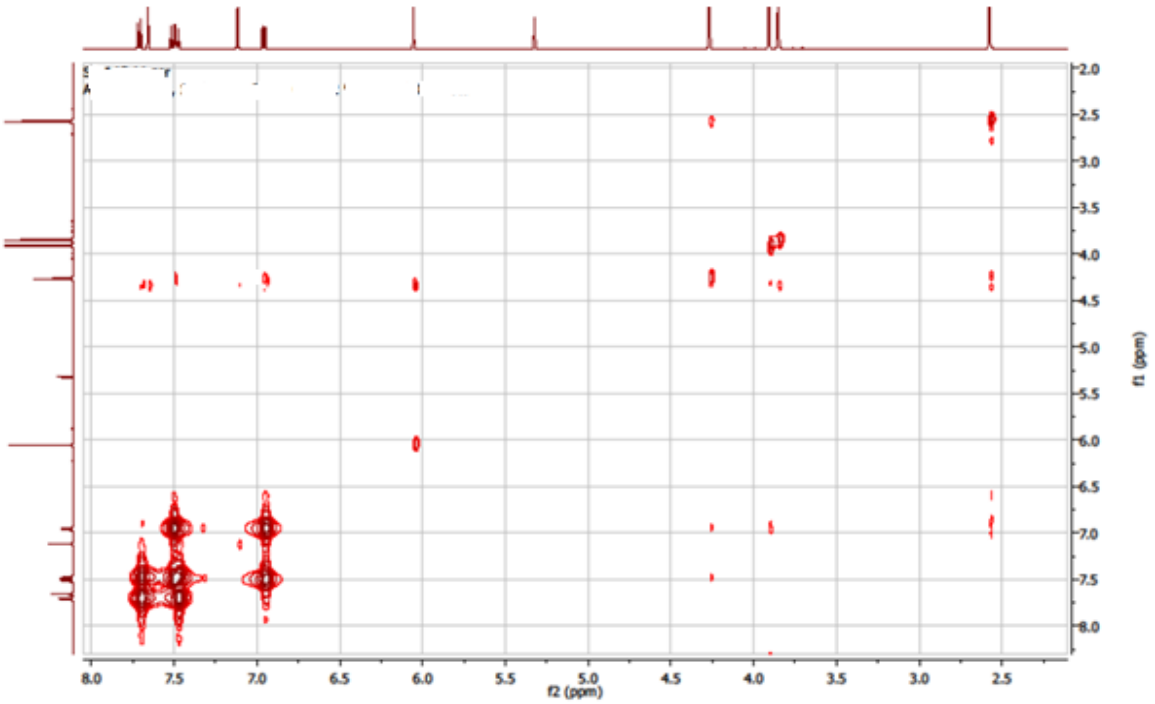
Appendix 3d: DEPT-135 spectrum for compound 201



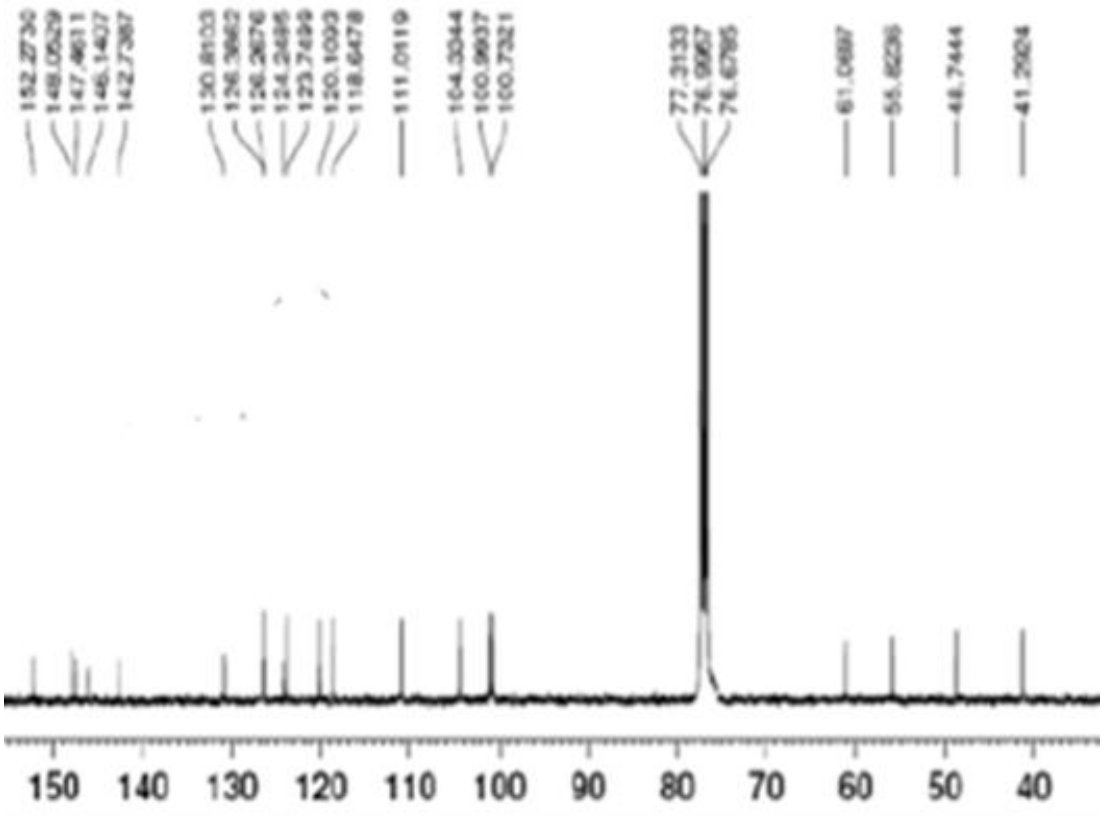
Appendix 3e: EI-MS spectrum for compound 201



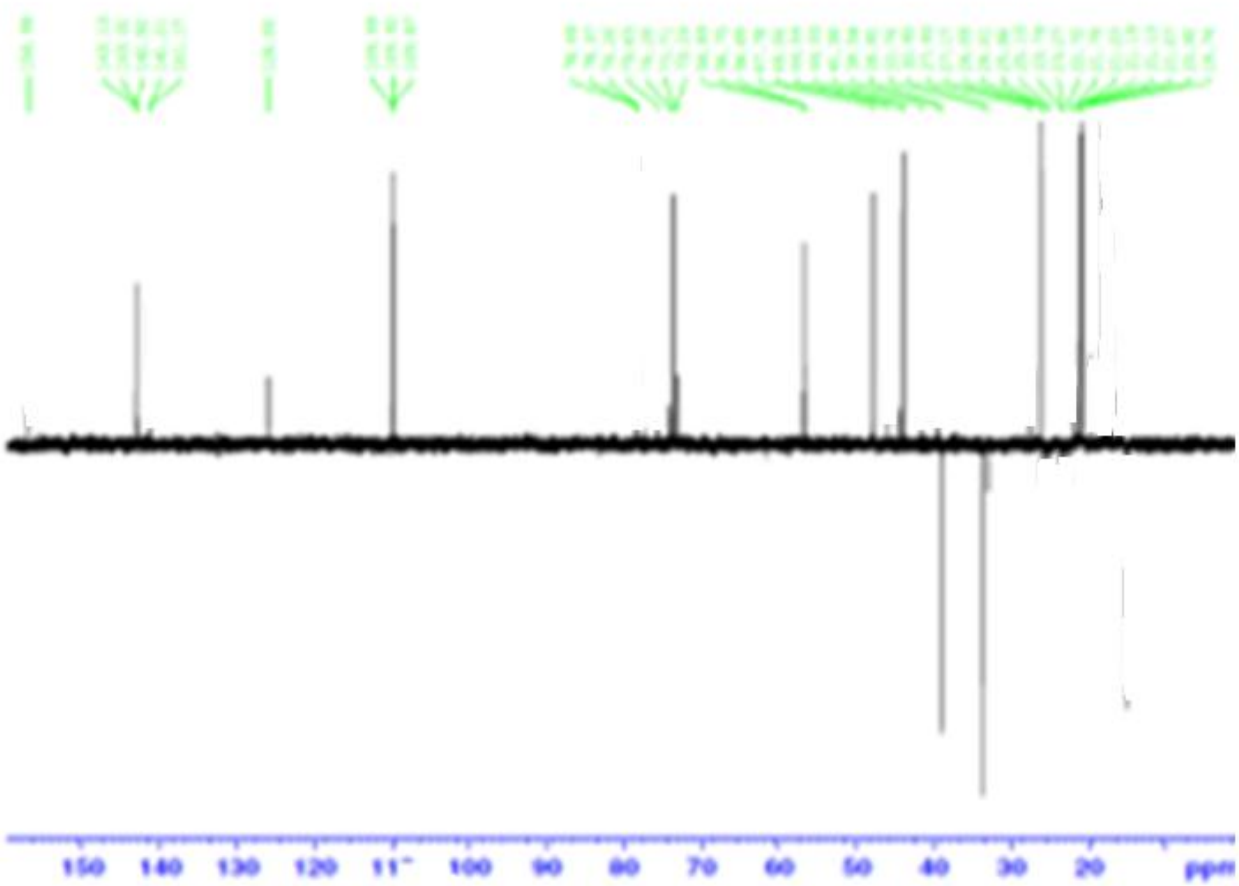
Appendix 4a: ^{1}H NMR spectrum for compound 202



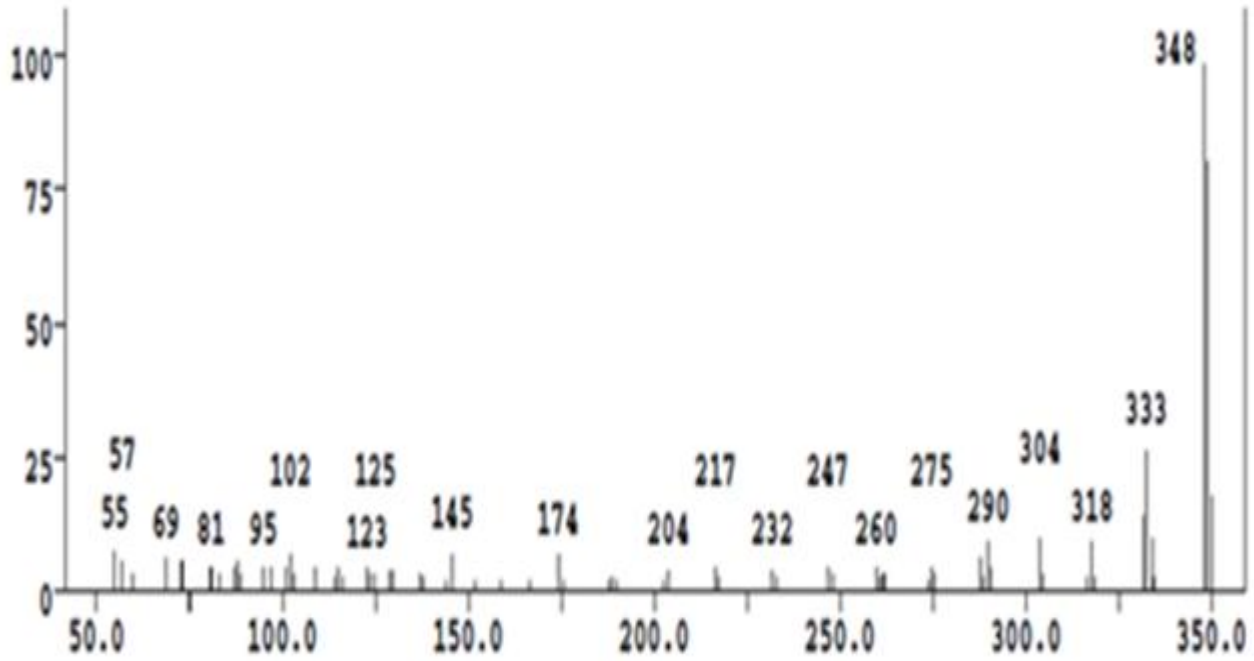
Appendix 4b: ^{1}H-^{1}H COSY spectrum for compound 202



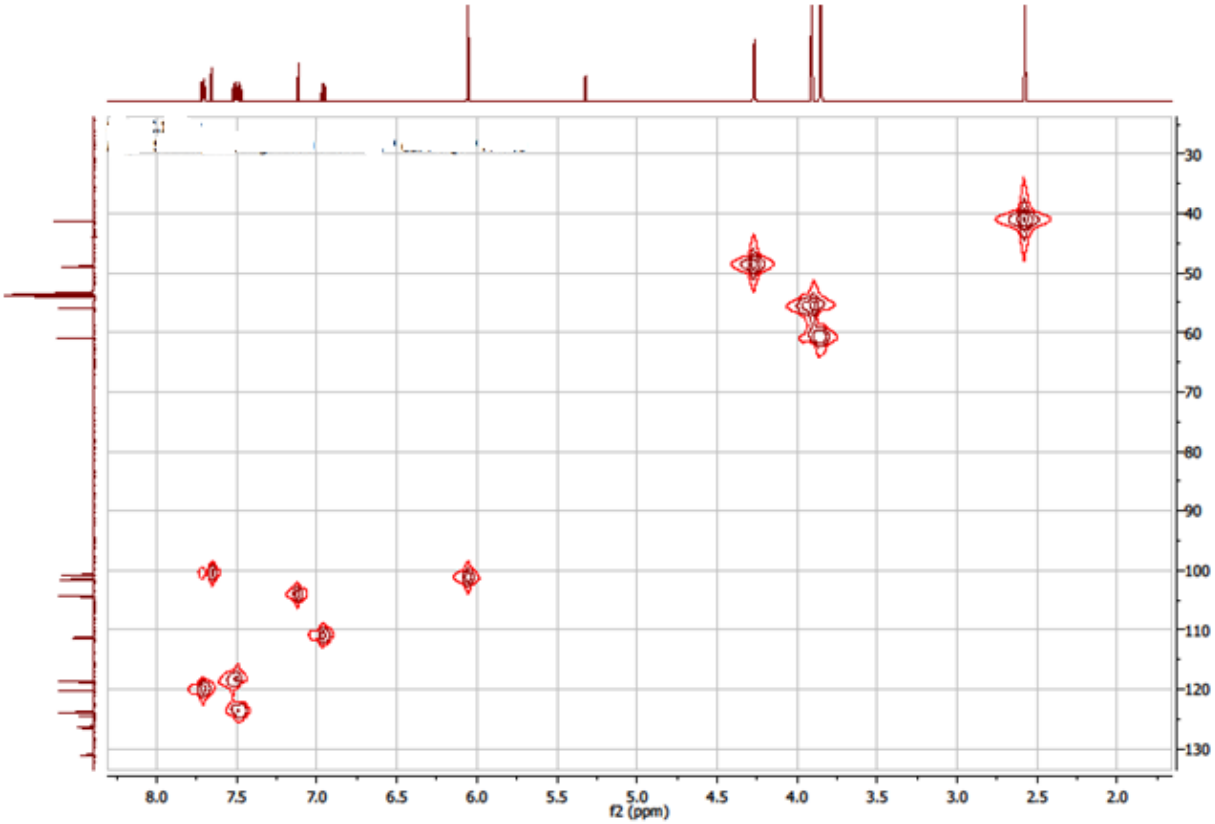
Appendix 4c: ^{13}C NMR spectrum for compound 202



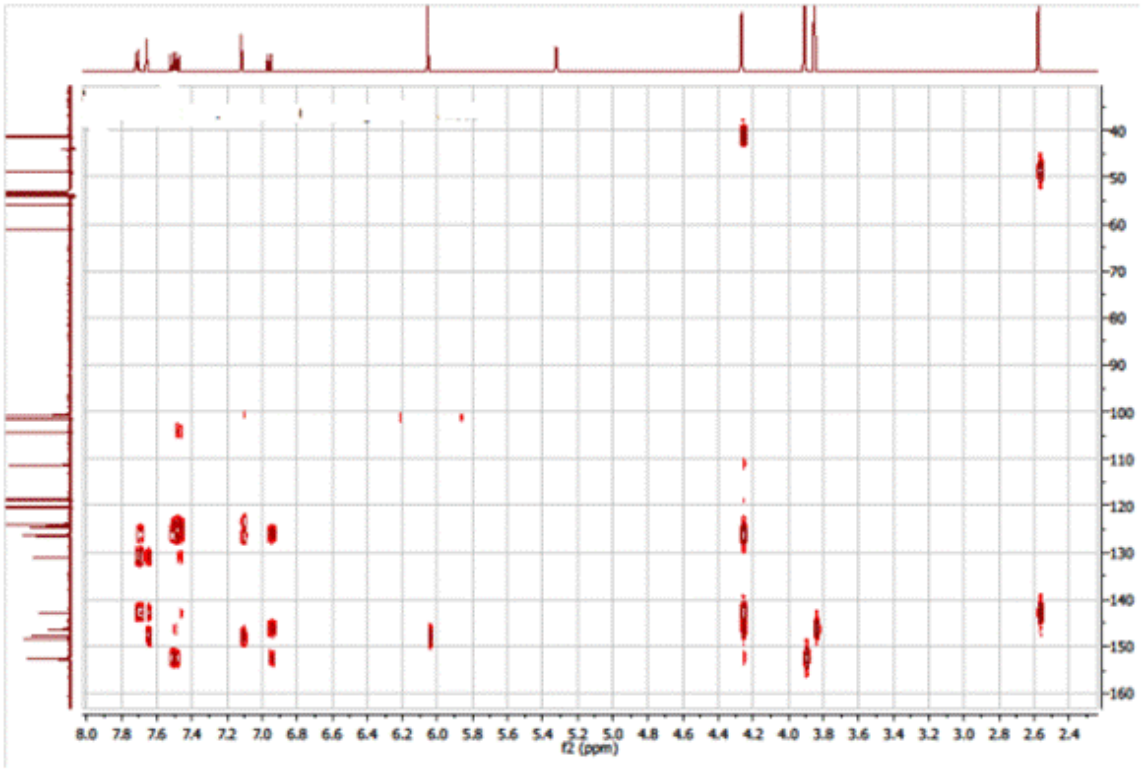
Appendix 4d: DEPT-135 spectrum for compound 202



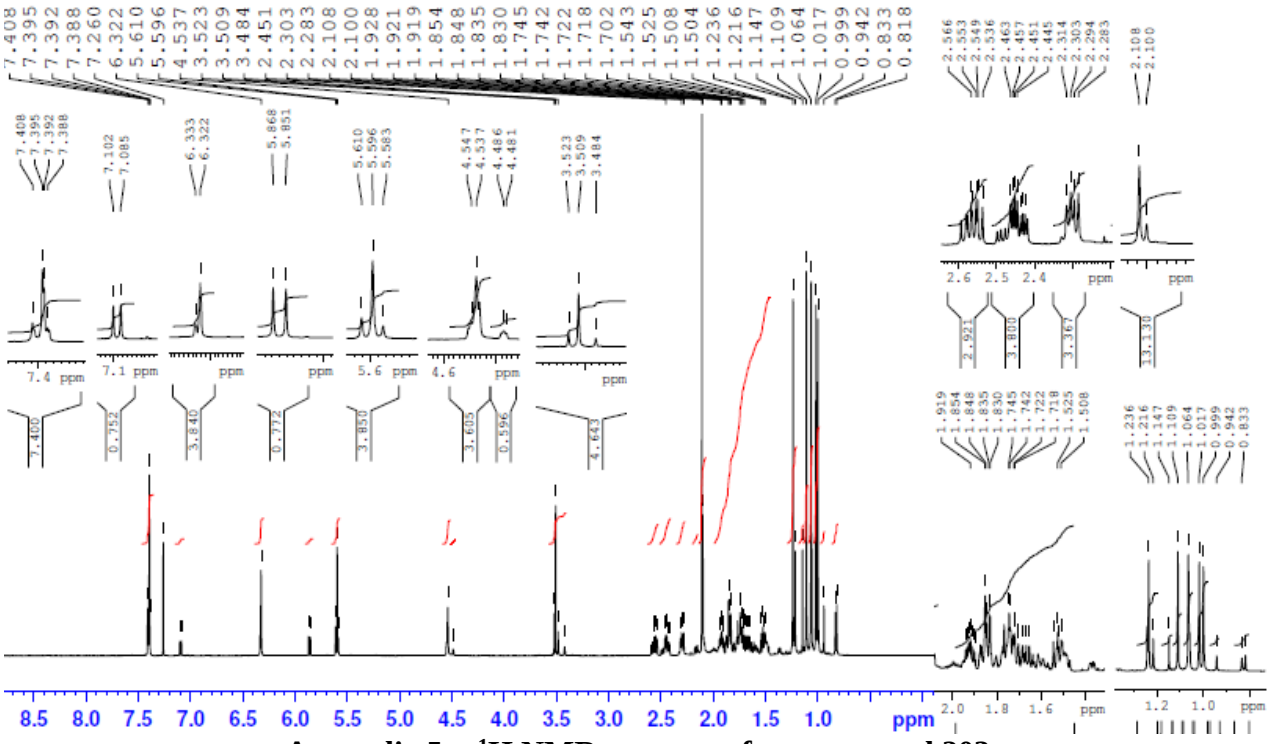
Appendix 4e: EI-MS spectrum for compound 202



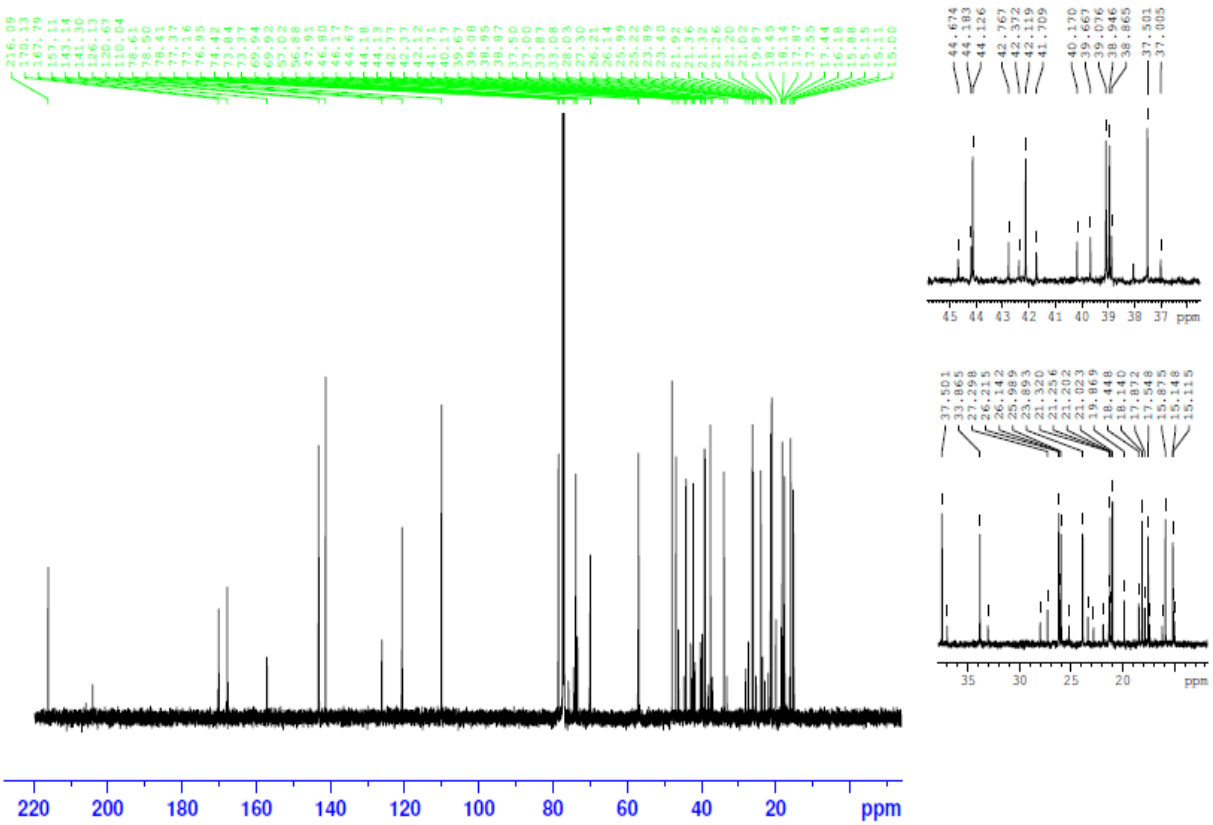
Appendix 4f: HSQC spectrum for compound 202



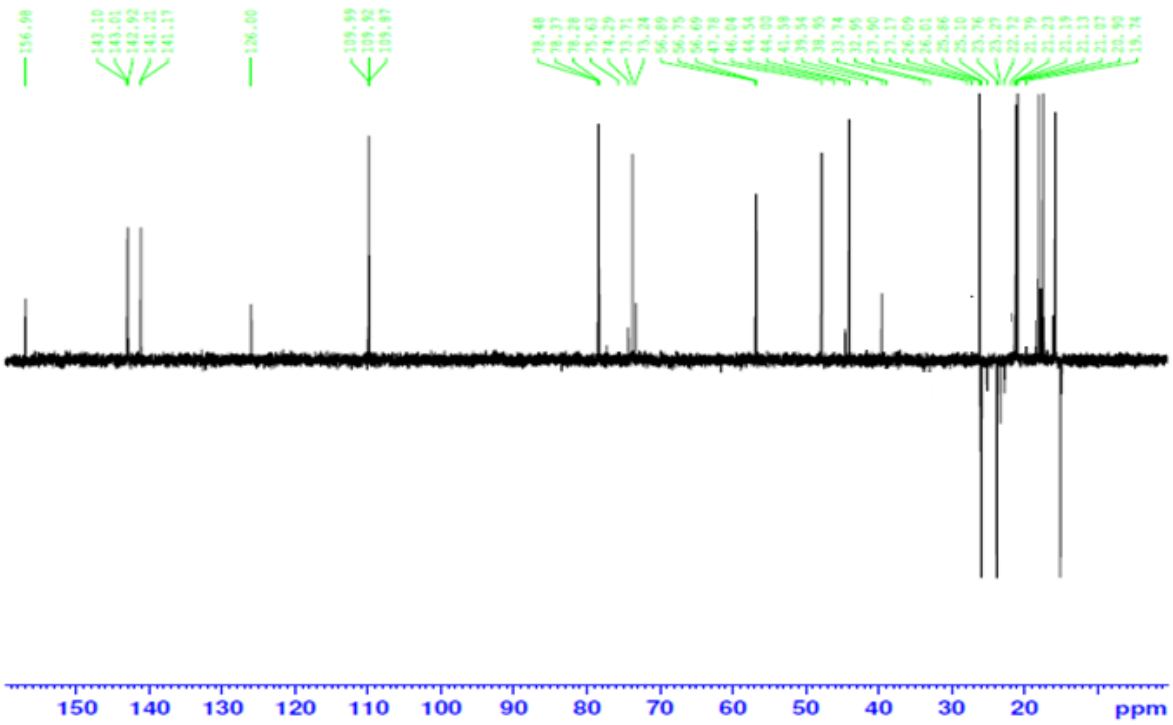
Appendix 4g: HMBC spectrum for compound 202



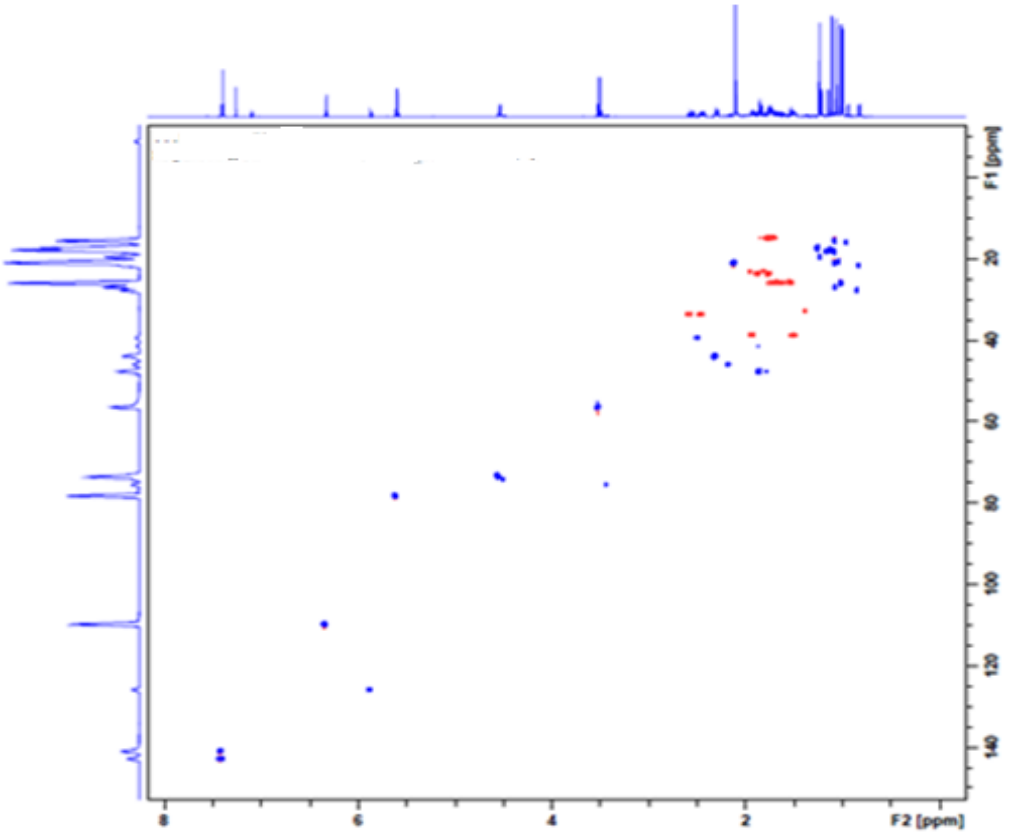
Appendix 5a: ^{1}H NMR spectrum for compound 203



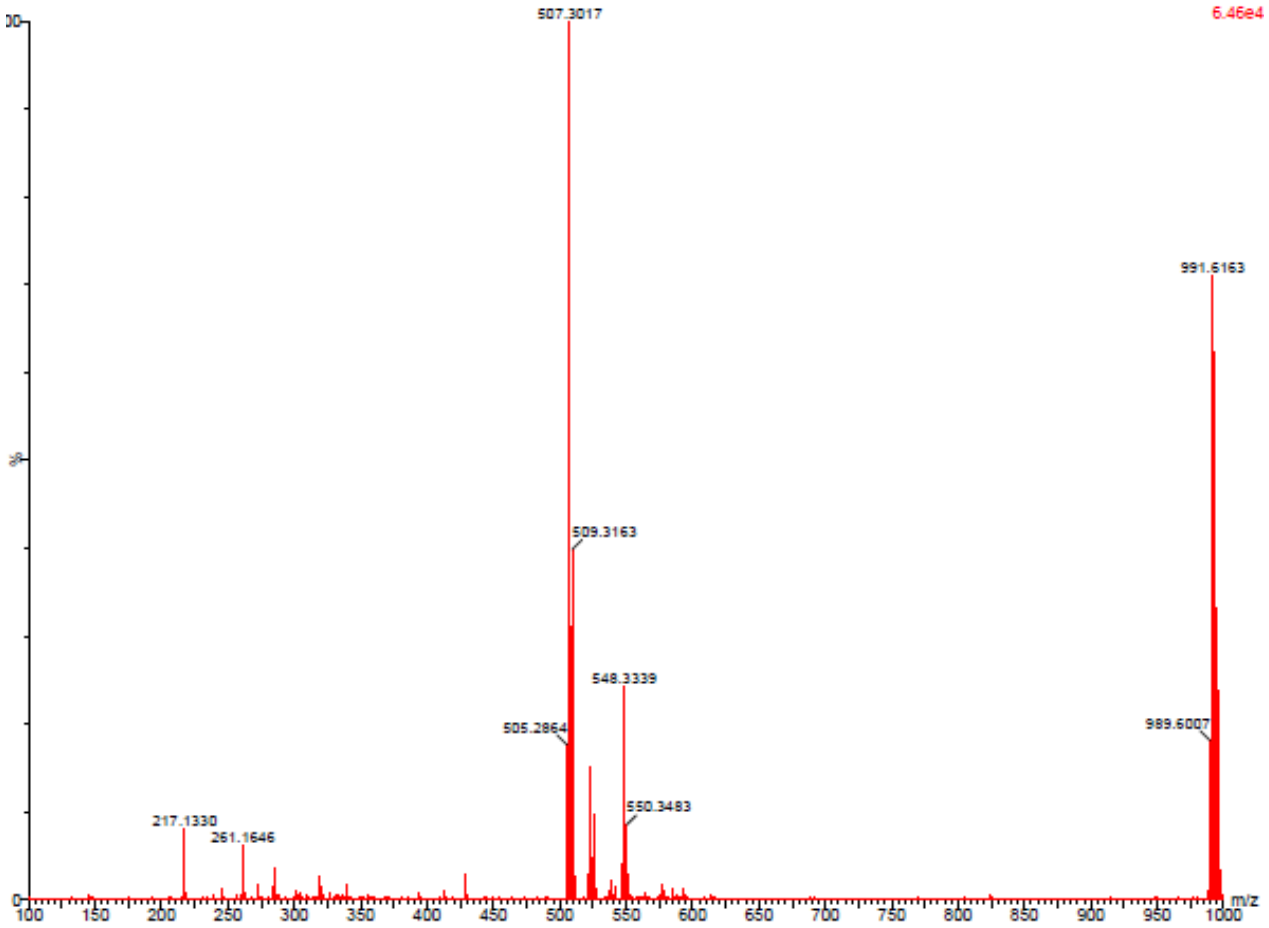
Appendix 5b: ^{13}C NMR spectrum for compound 203



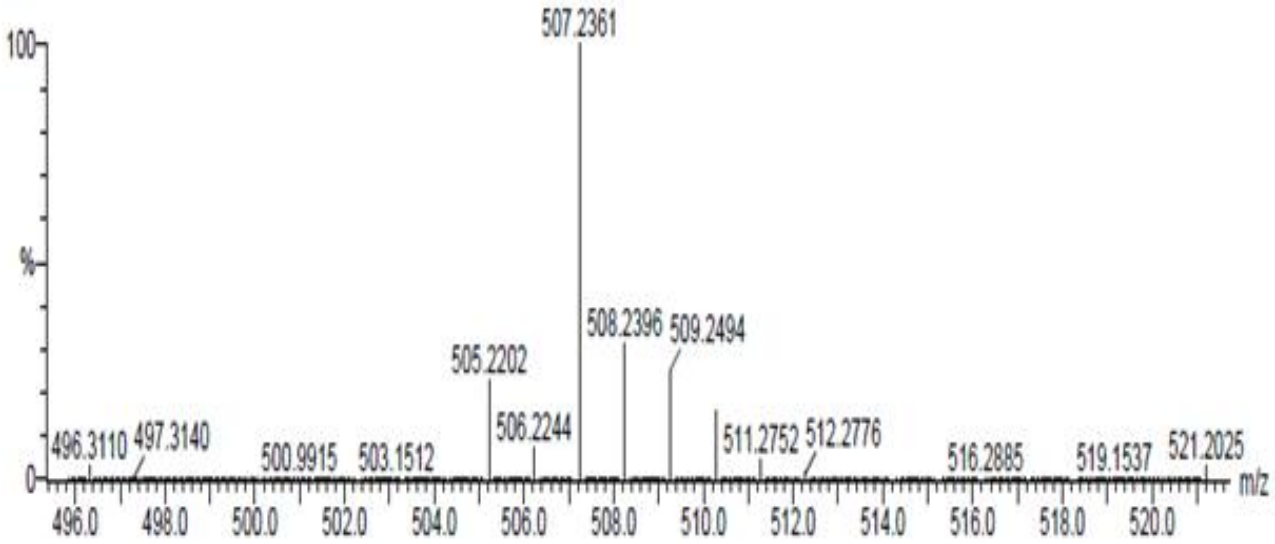
Appendix 5c: DEPT-135 NMR spectrum for compound 203



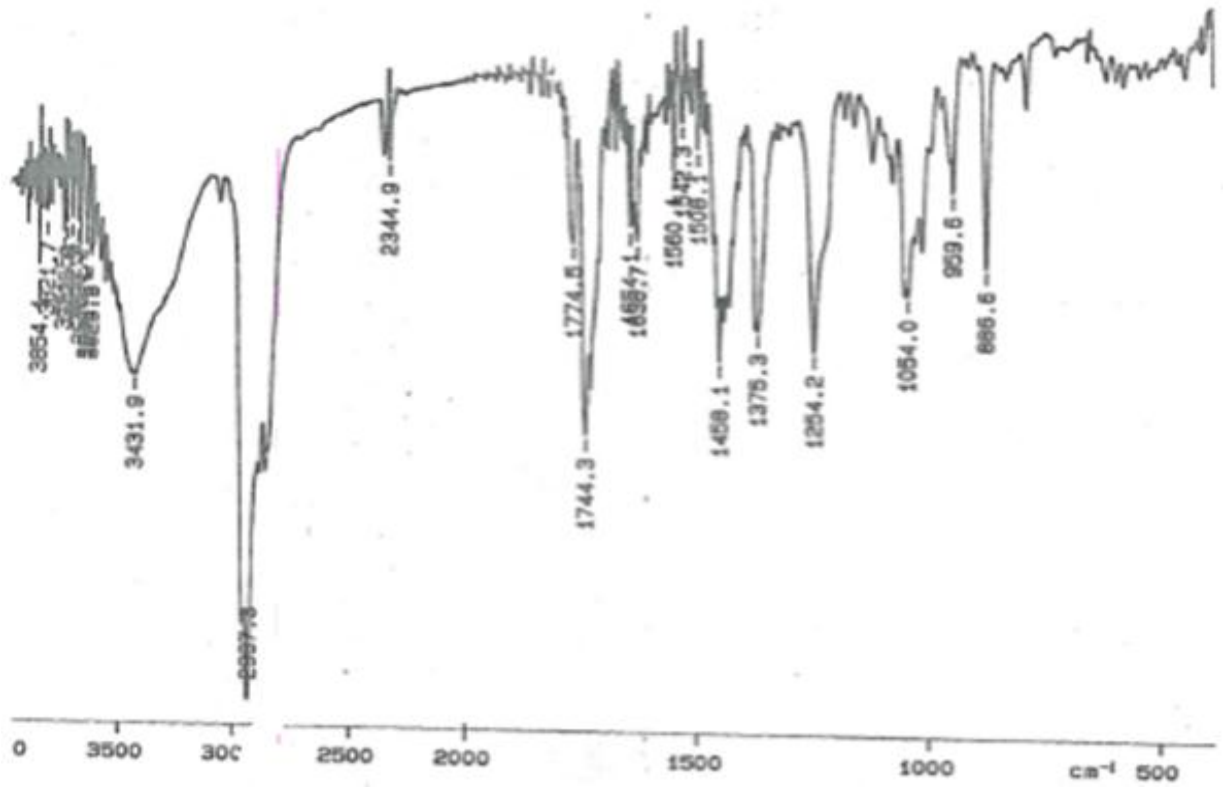
Appendix 5d: HSQC spectrum for compound 203



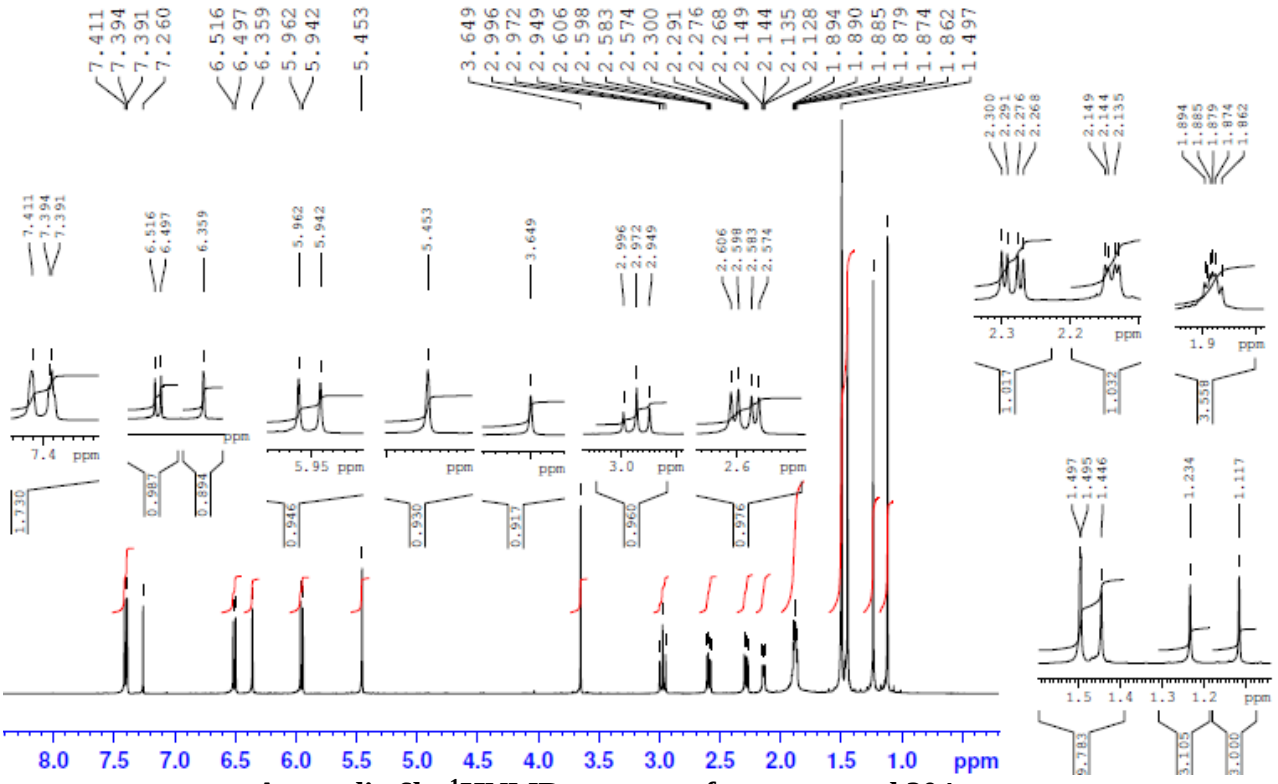
Appendix 5e; HRTOF-MS spectrum for compound 203



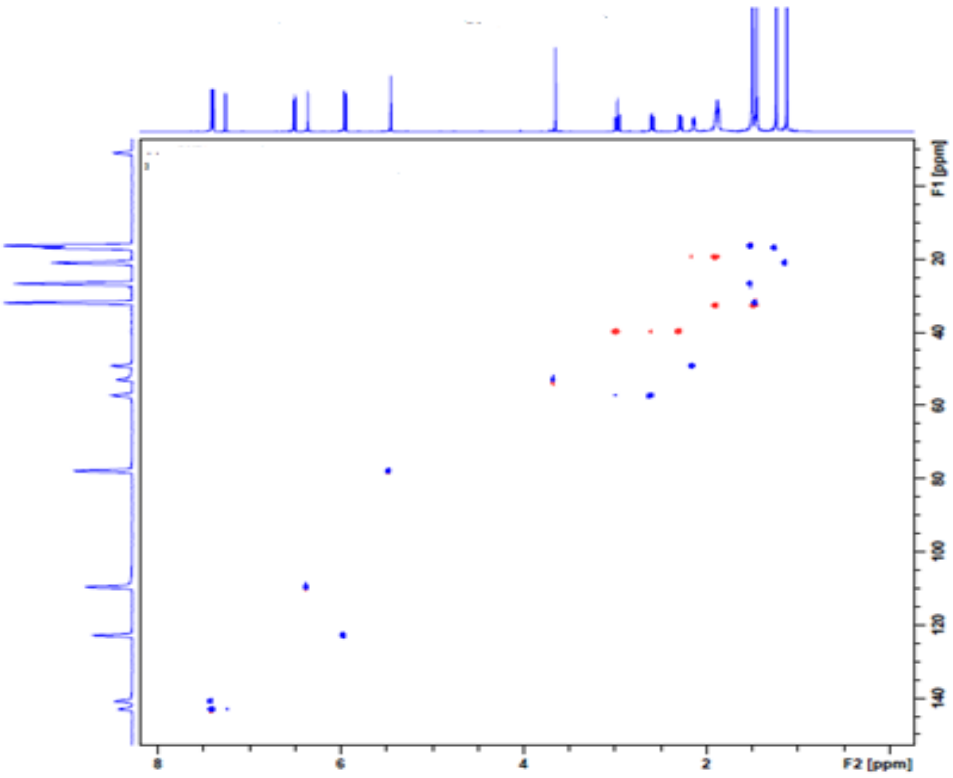
Appendix 5f: TOF-MS spectrum for compound 203



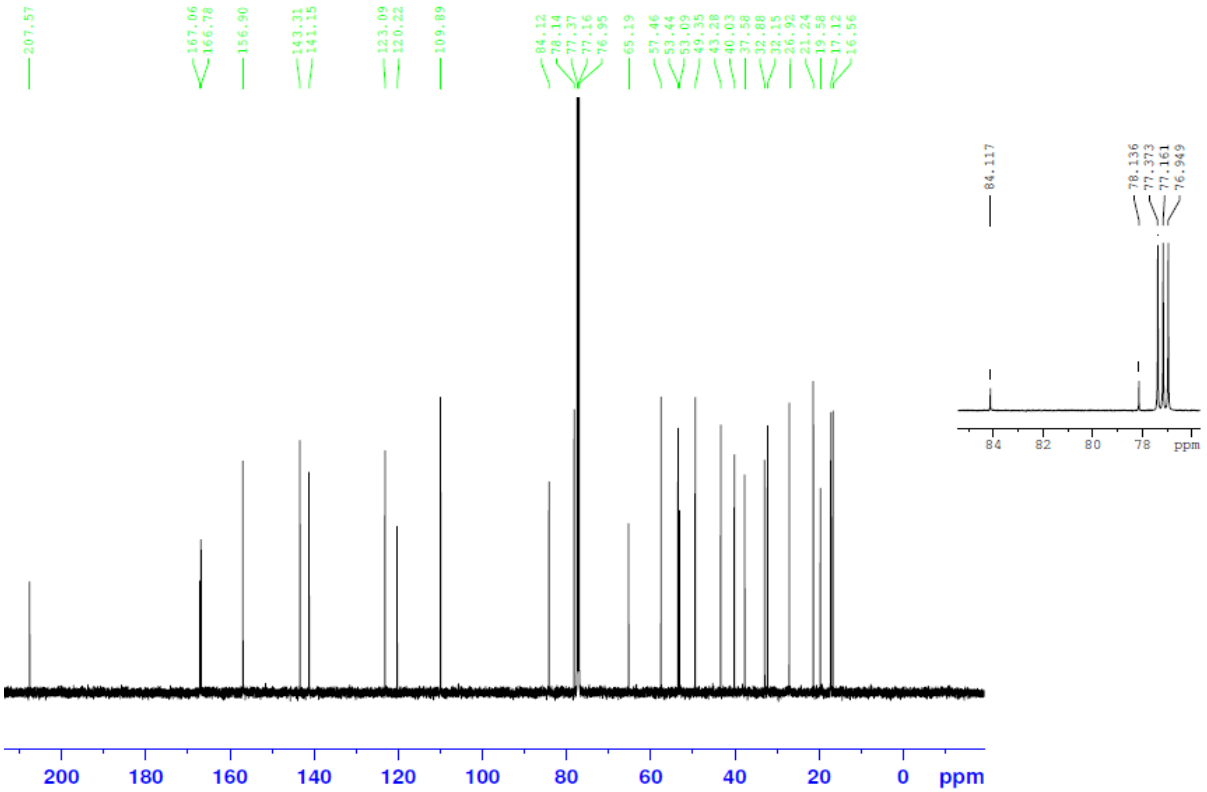
Appendix 6a: IR spectrum for compound 204



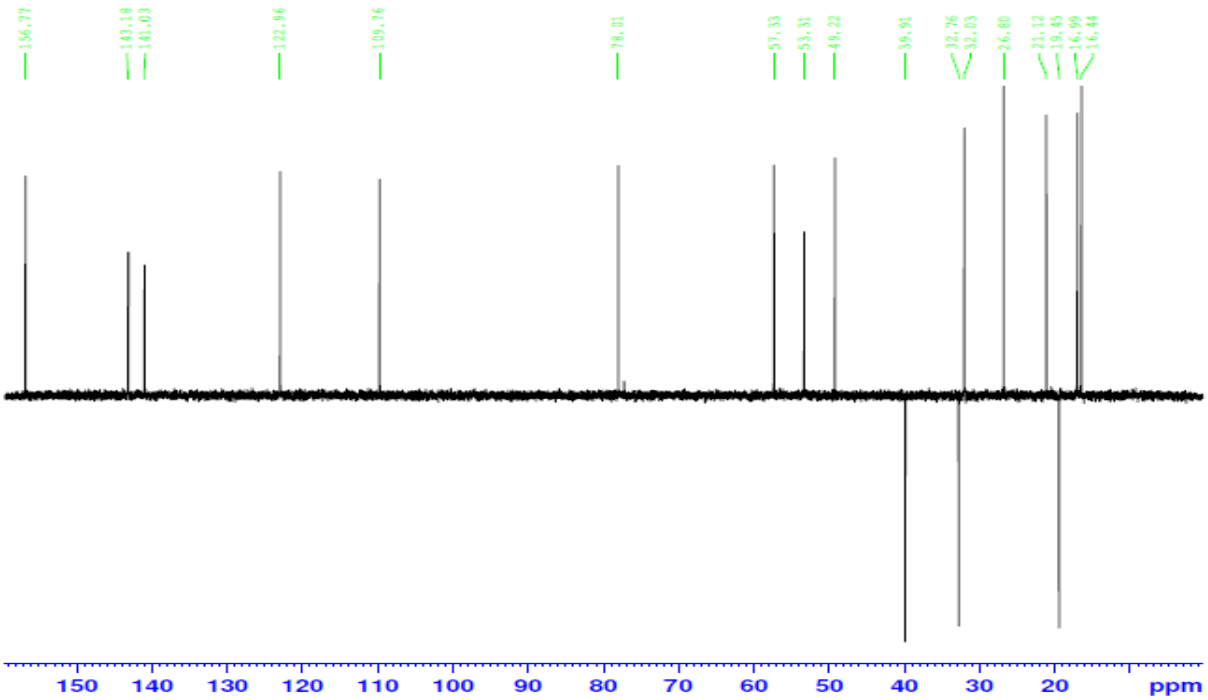
Appendix 6b: ^{1}HNMR spectrum for compound 204



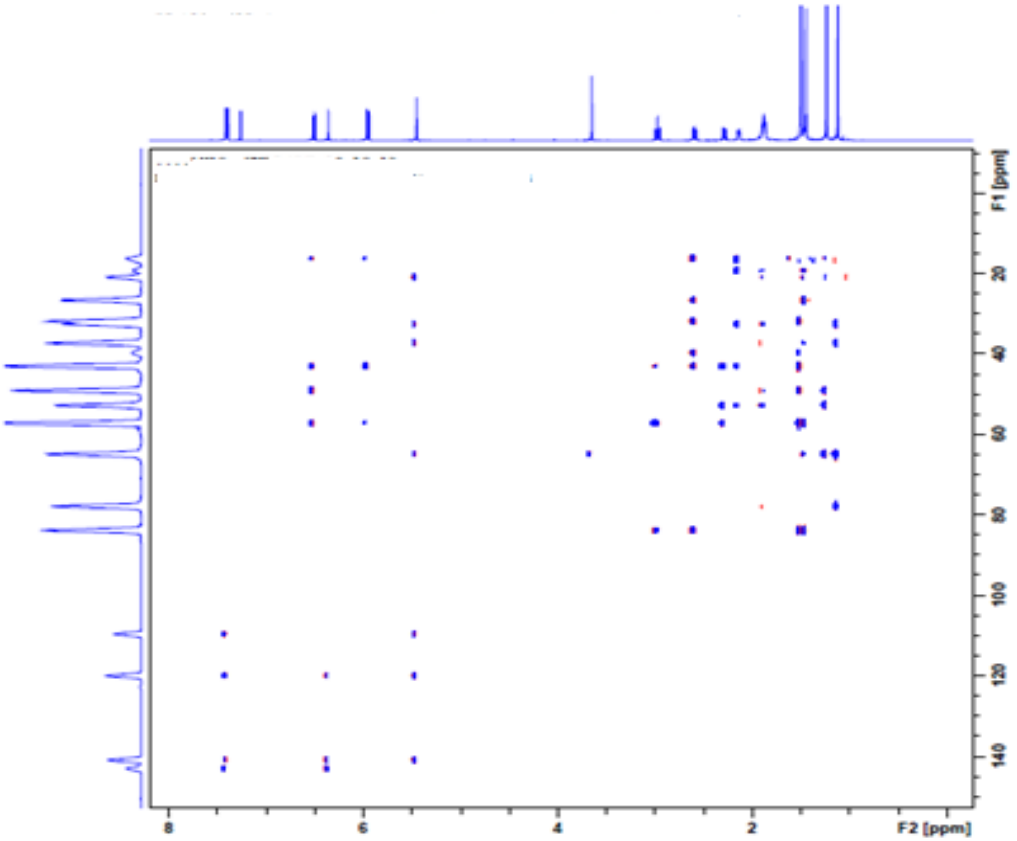
Appendix 6c: HSQC spectrum for compound 204



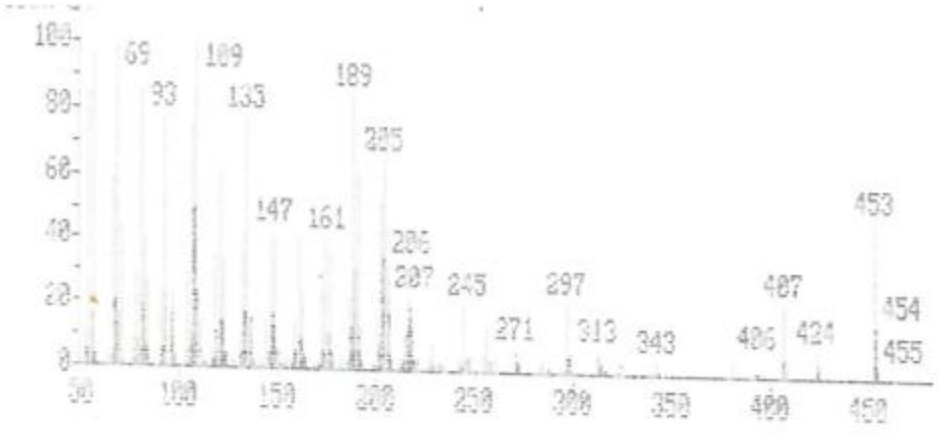
Appendix 6d: ^{13}C NMR spectrum for compound 204



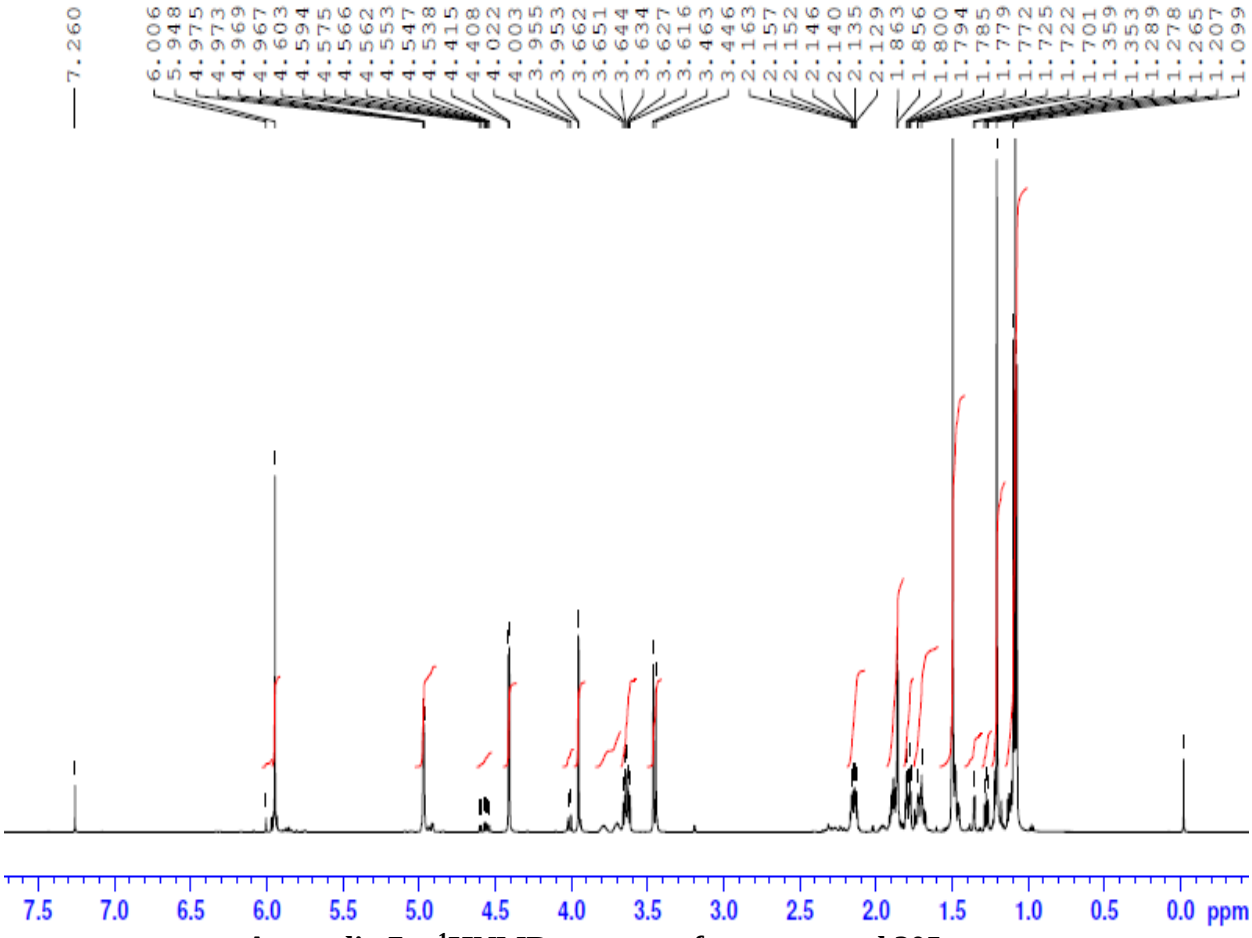
Appendix 6e: DEPT-135 spectrum for compound 204



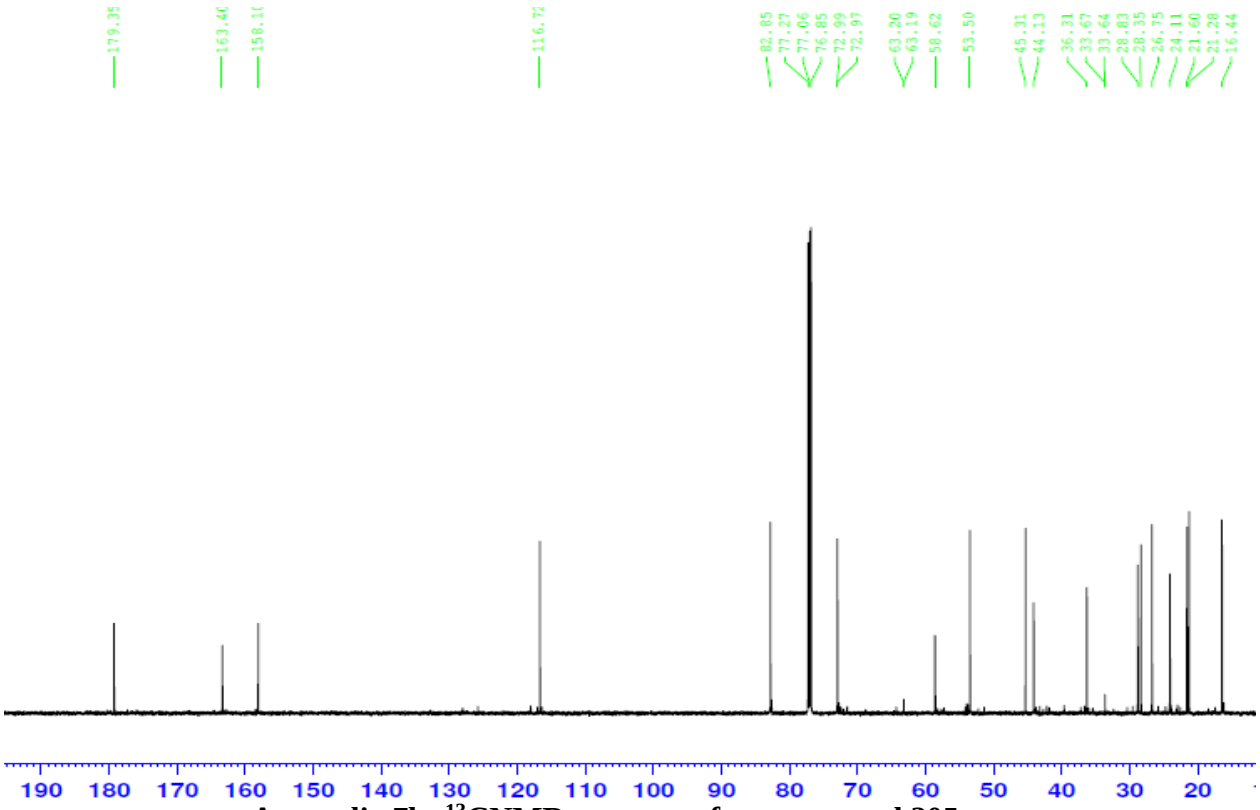
Appendix 6f: ^{1}HMBC spectrum for compound 204



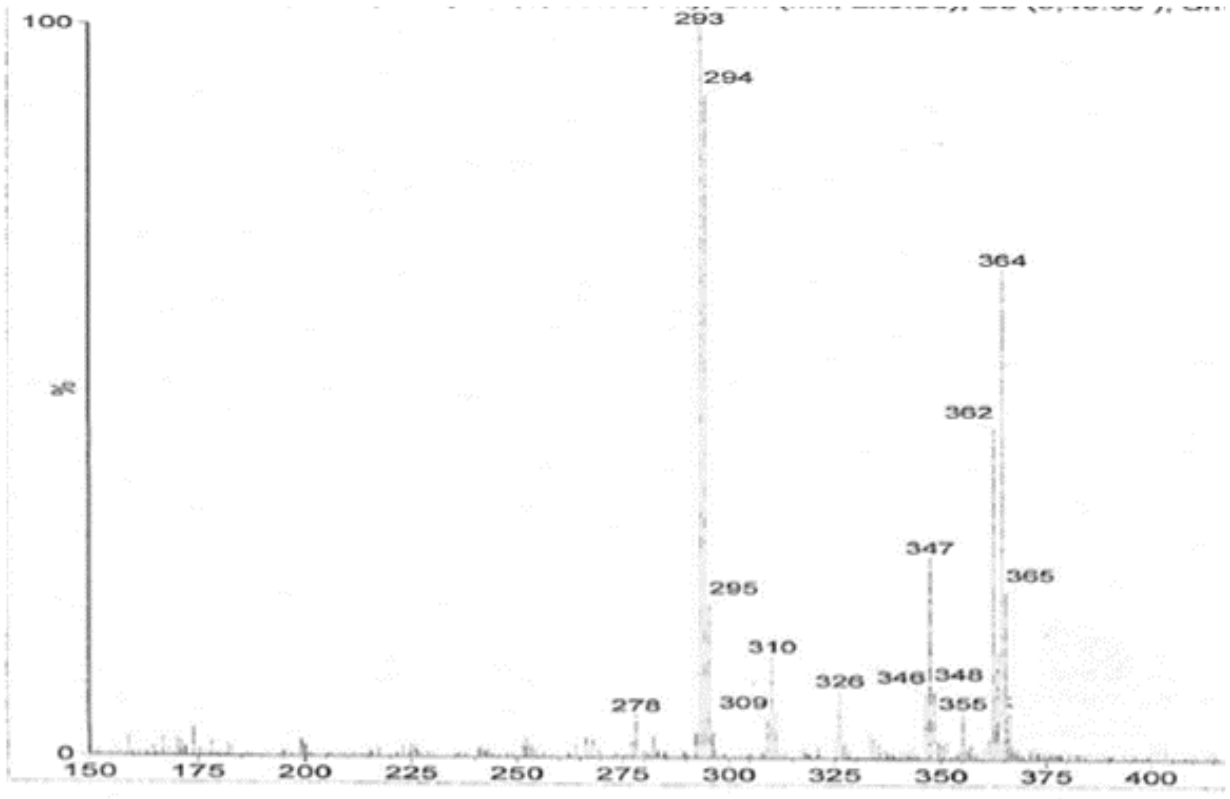
Appendix 6g: EI-MS spectrum for compound 204



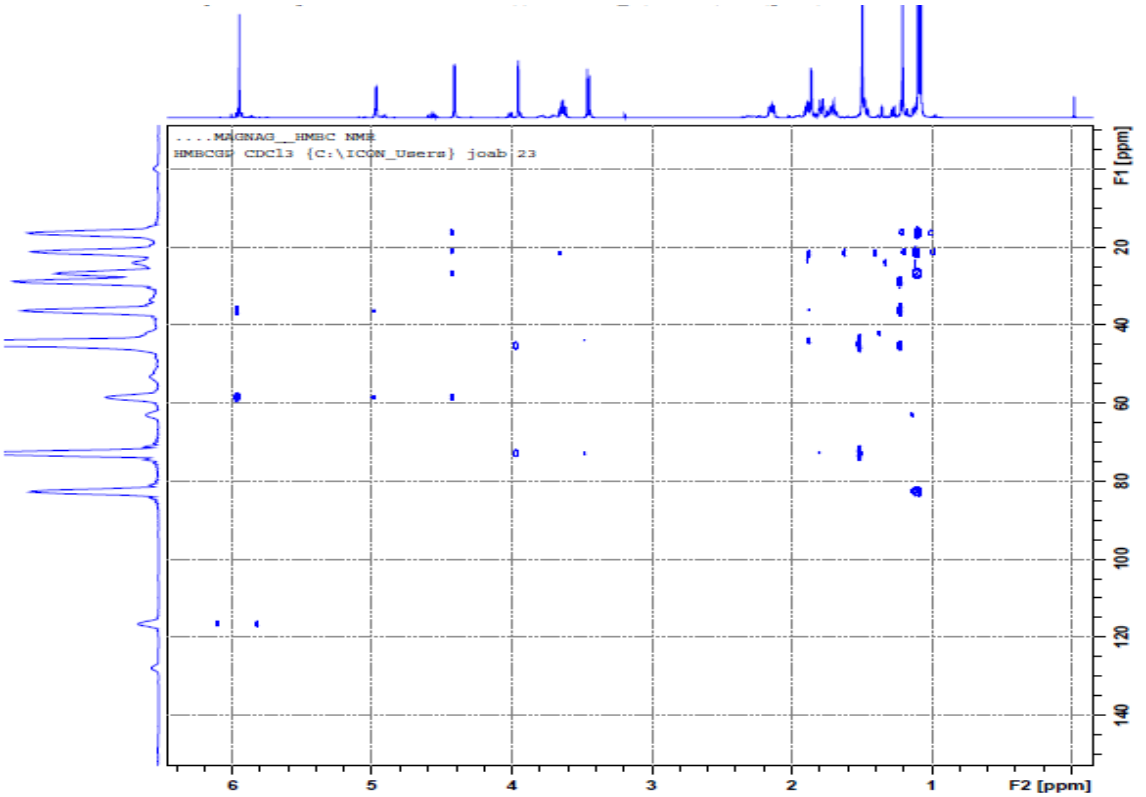
Appendix 7a: ^{1}HNMR spectrum for compound 205



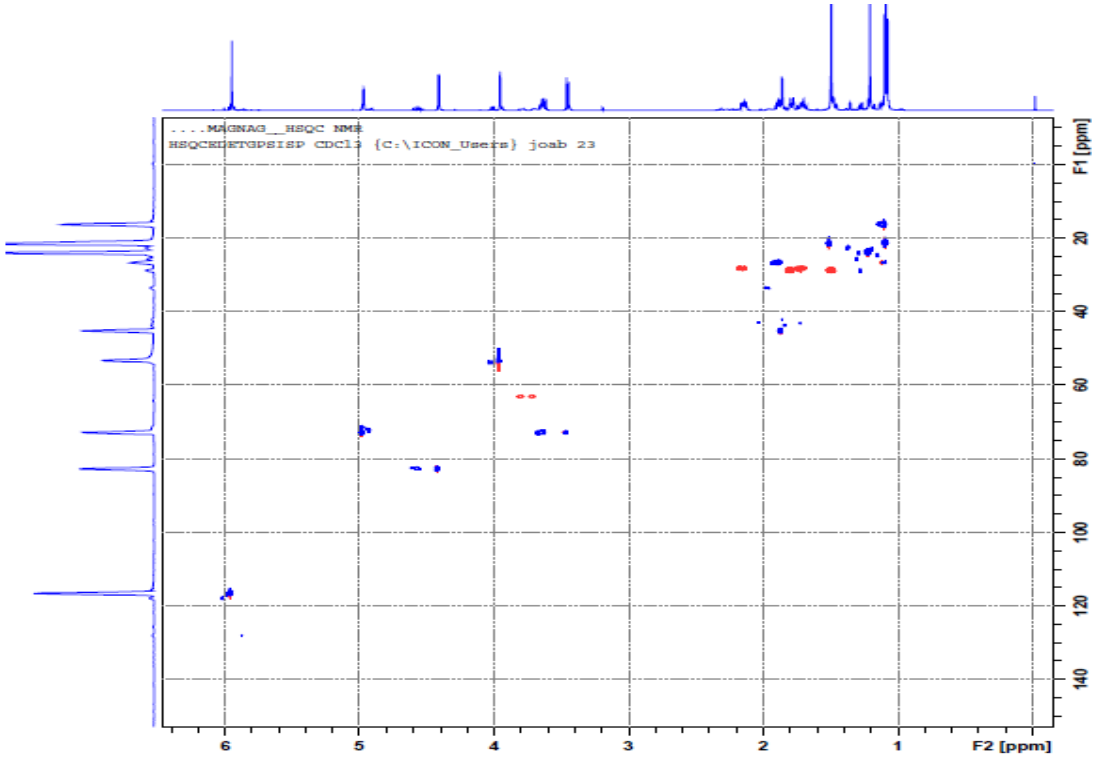
Appendix 7b: ^{13}CNMR spectrum for compound 205



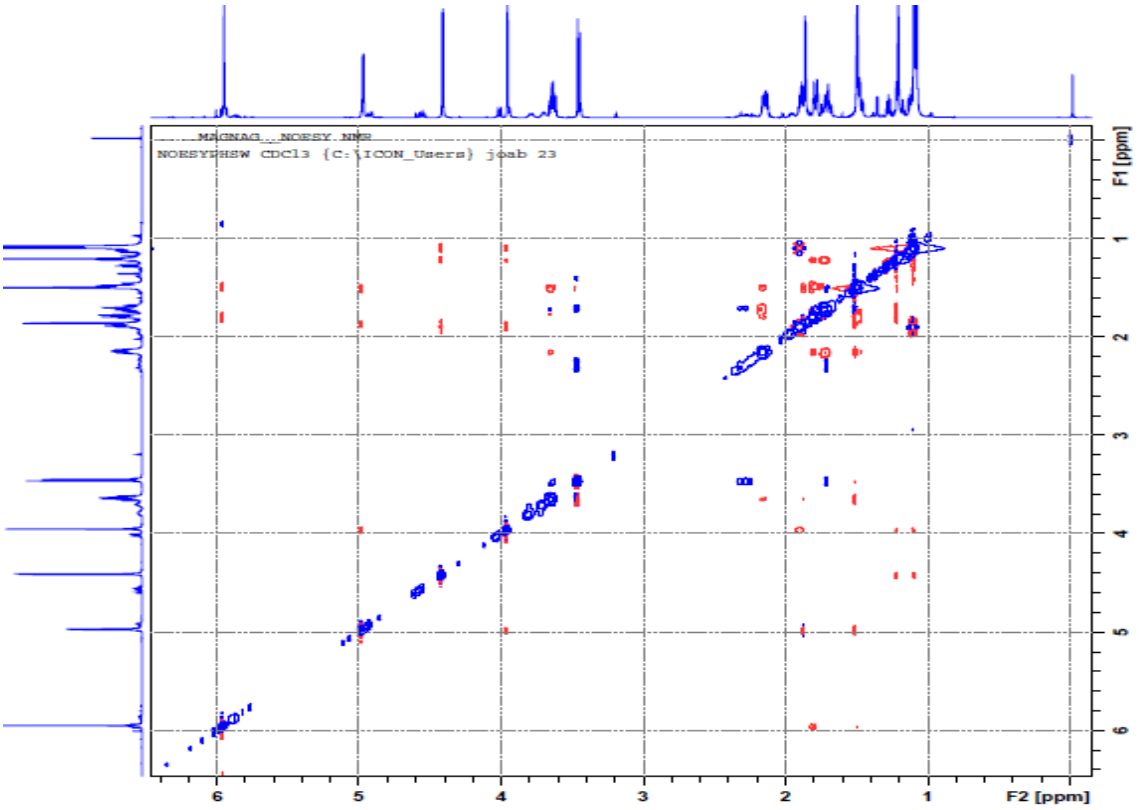
Appendix 7c: EI-MS spectrum for compound 205



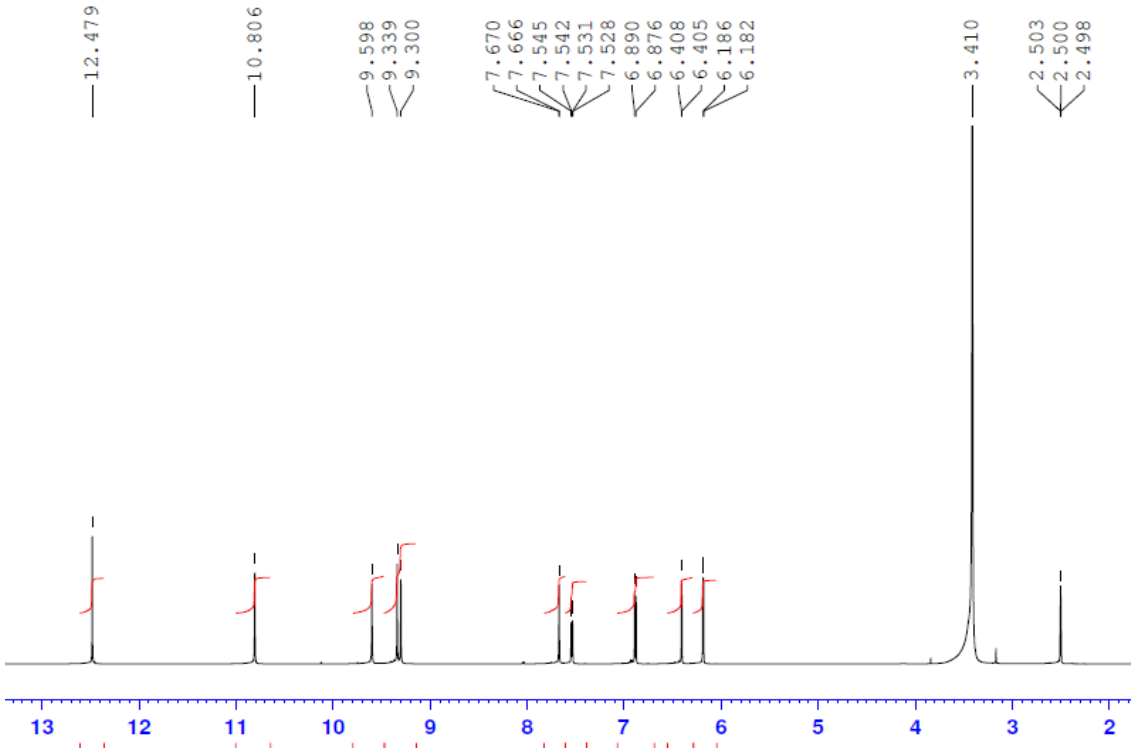
Appendix 7d: HMBC spectrum for compound 205



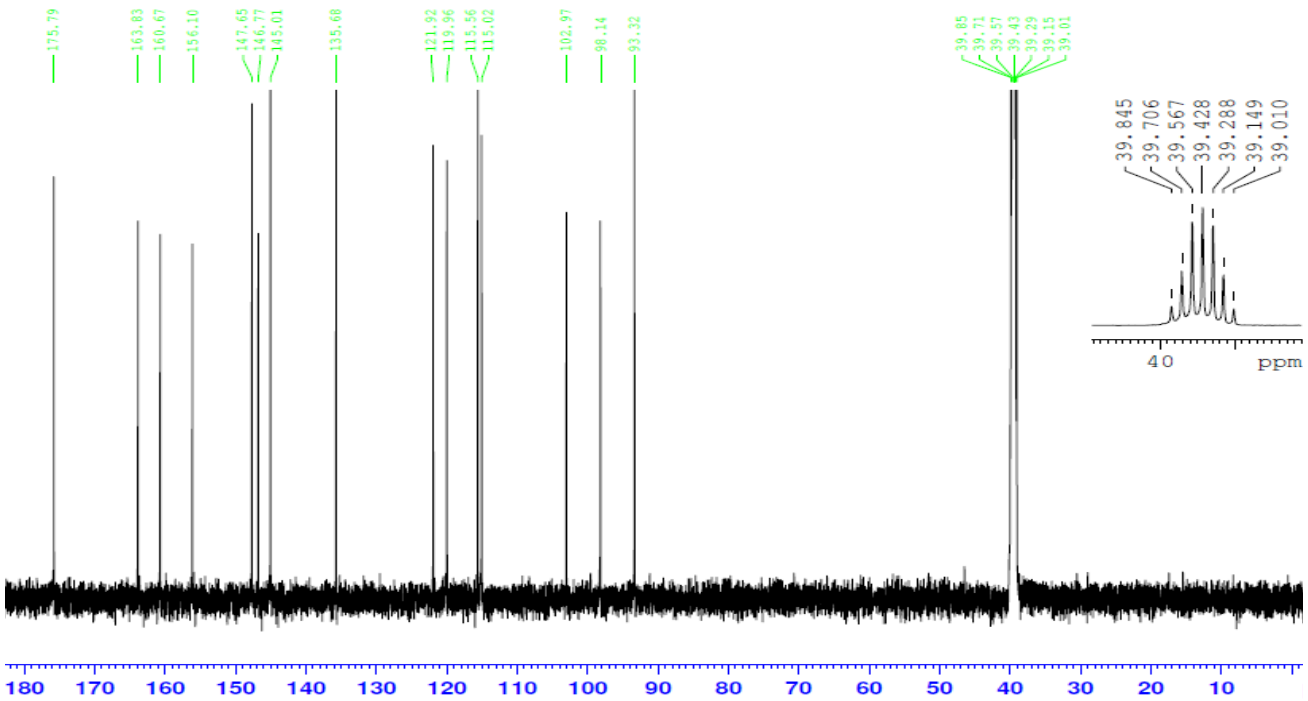
Appendix 7e: HSQC spectrum for compound 205



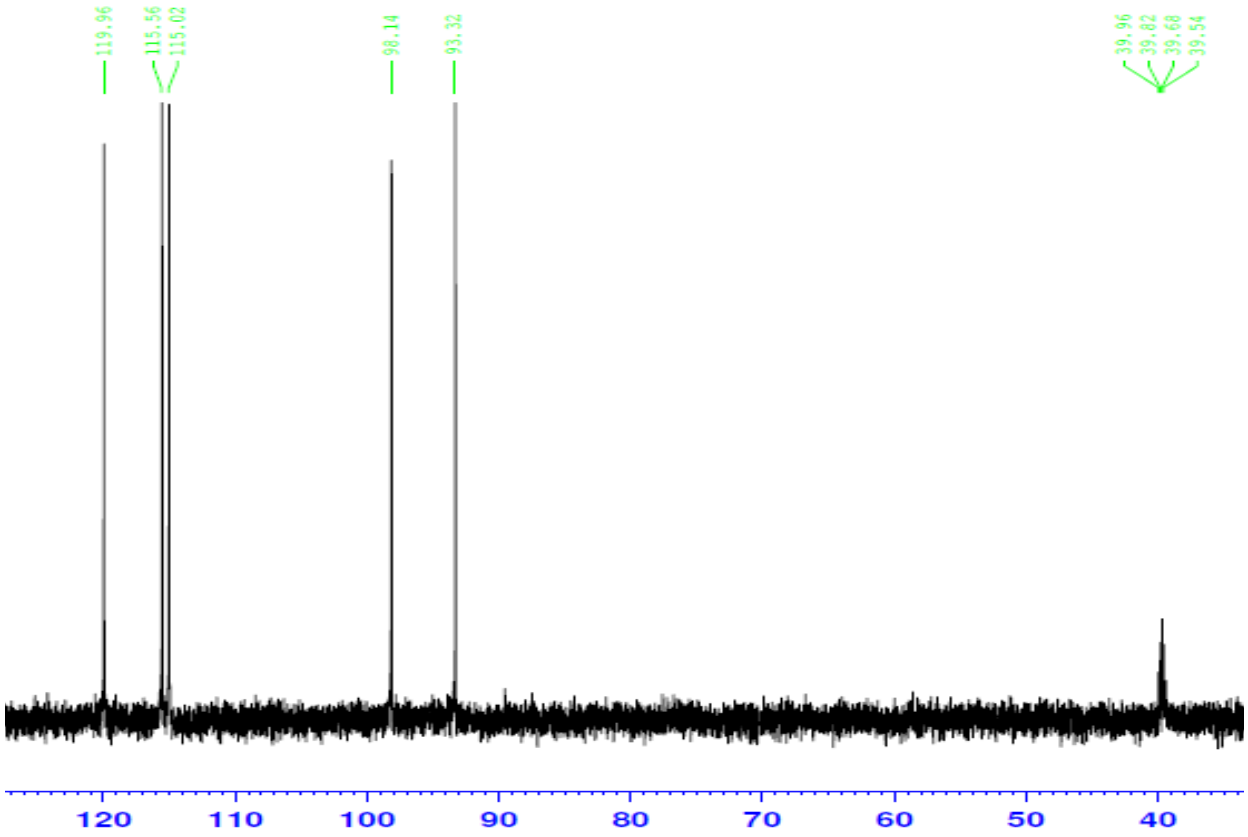
Appendix 7f: NOESY spectrum for compound 205



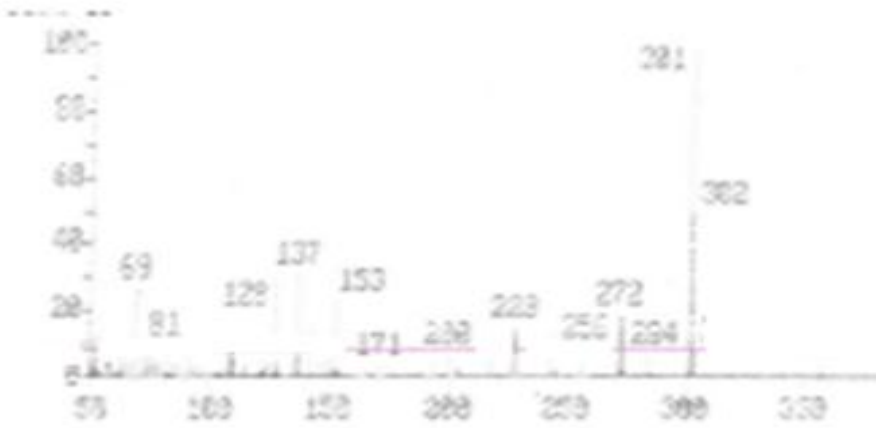
Appendix 8a:^{1}H NMR spectrum for compound 140



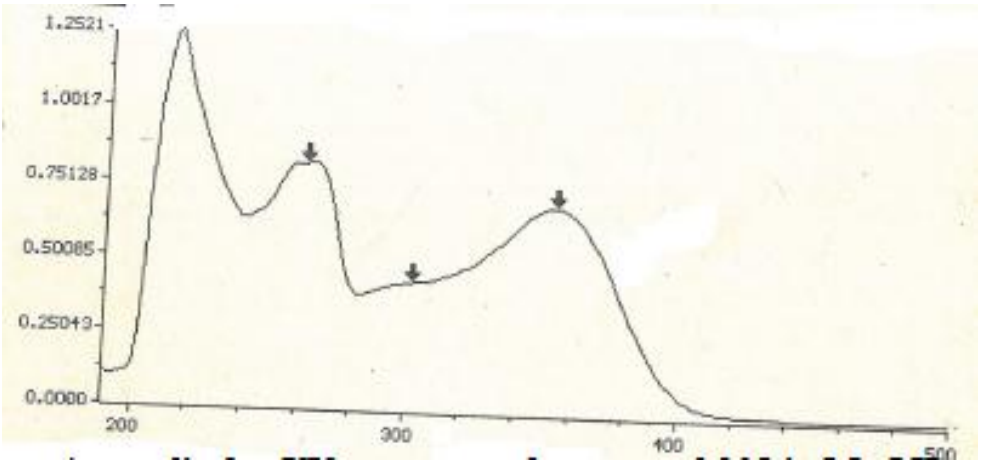
Appendix 8b:^{13}C NMR spectrum for compound 140



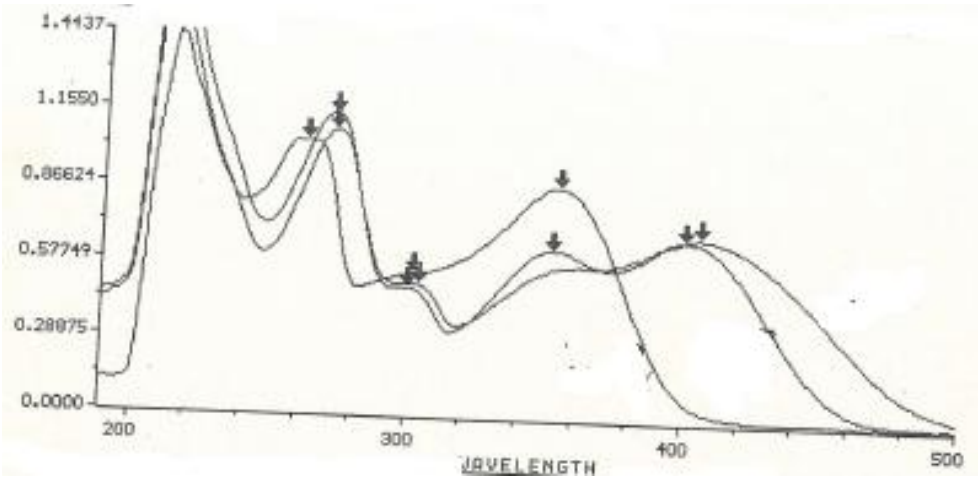
Appendix 8c; DEPT -135 NMR spectrum for compound140



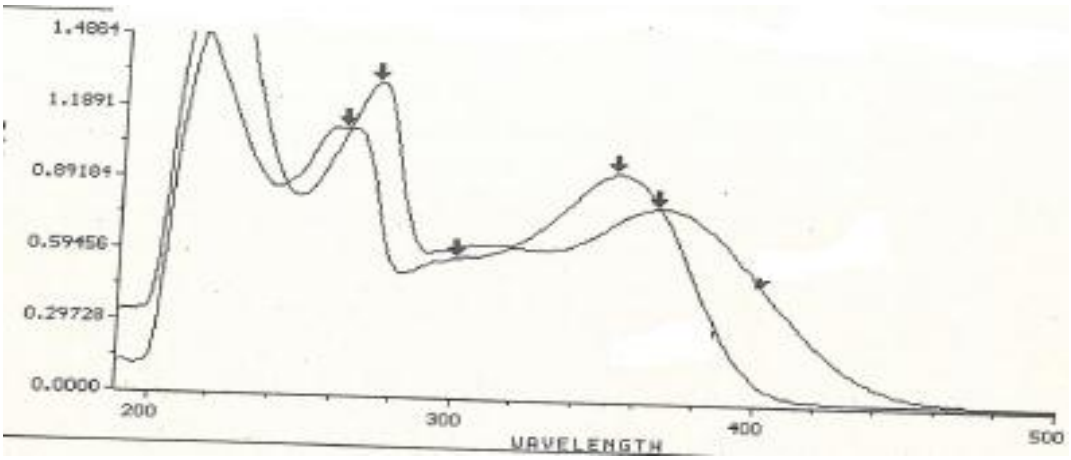
Appendix 8d: EI-MS spectrum for compound140



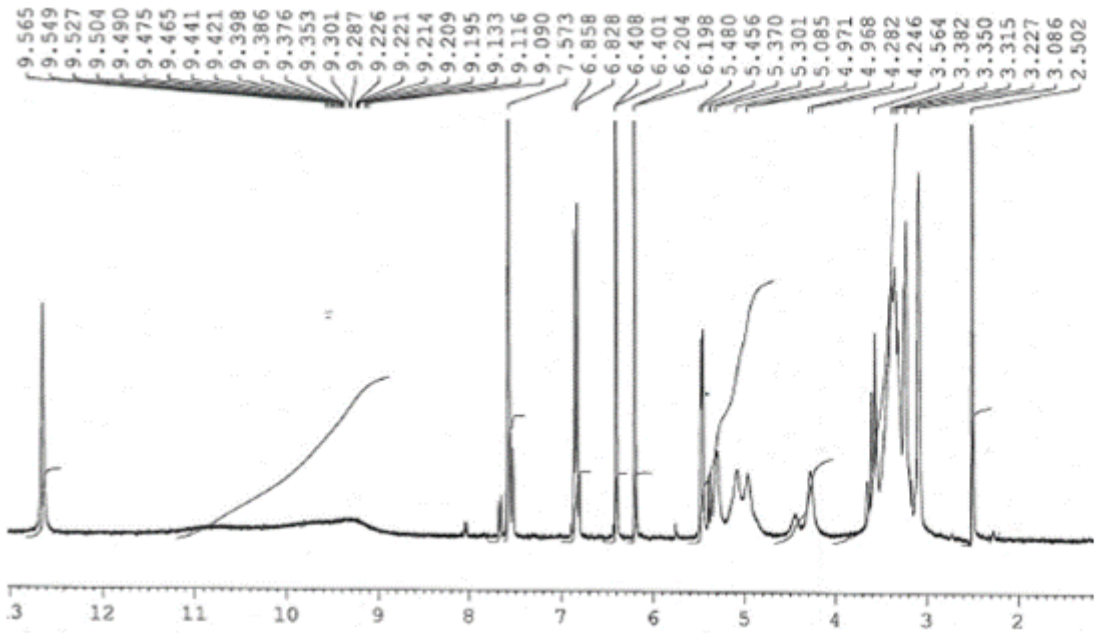
Appendix 9a: UV spectrum for compound 206 in MeOH



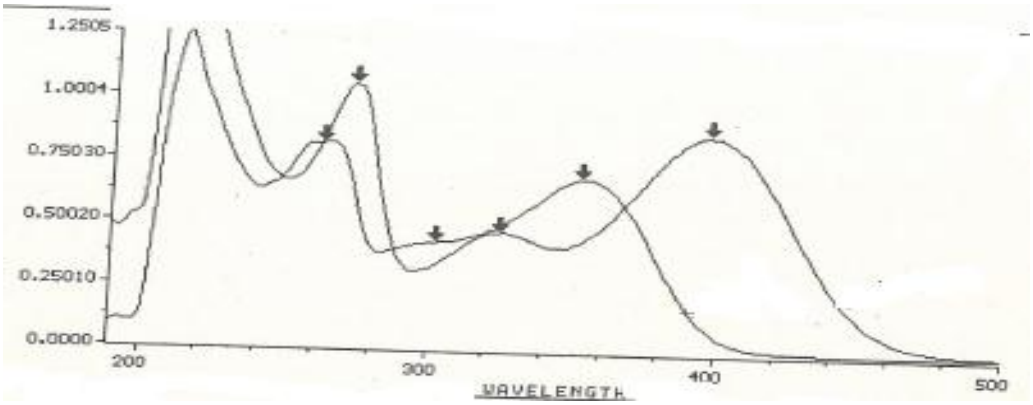
Appendix 9b (i): UV spectrum for compound 206 showing Bathochromic shift of band I in $AlCl_3$/ HCl



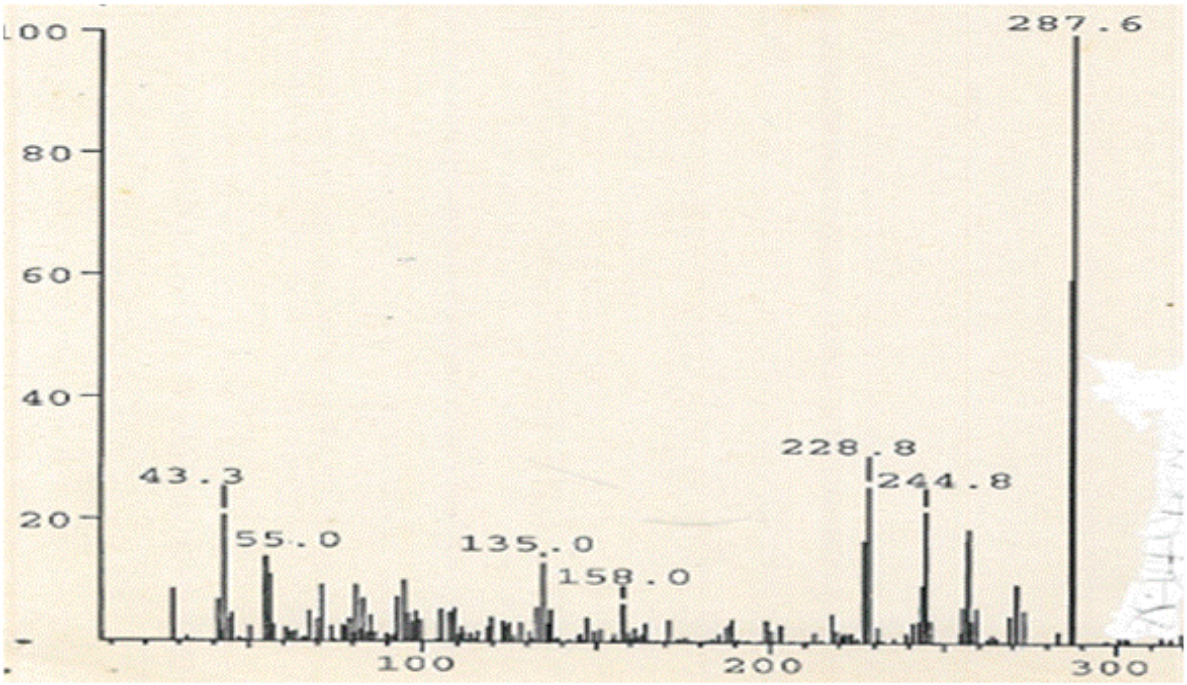
Appendix 9b (ii): UV spectrum for compound 206 showing bathochromic shift of band II in NaOAc



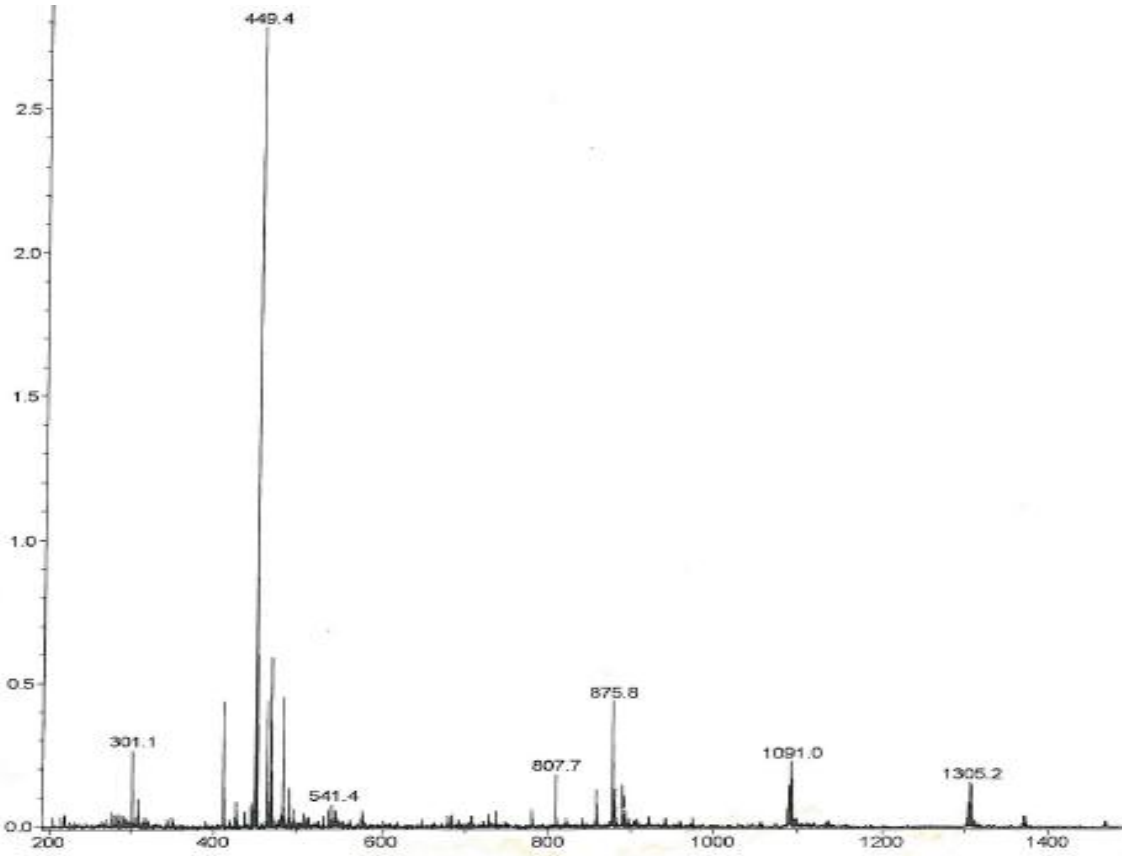
Appendix 9c: ^{I}H NMR spectrum for compound 206



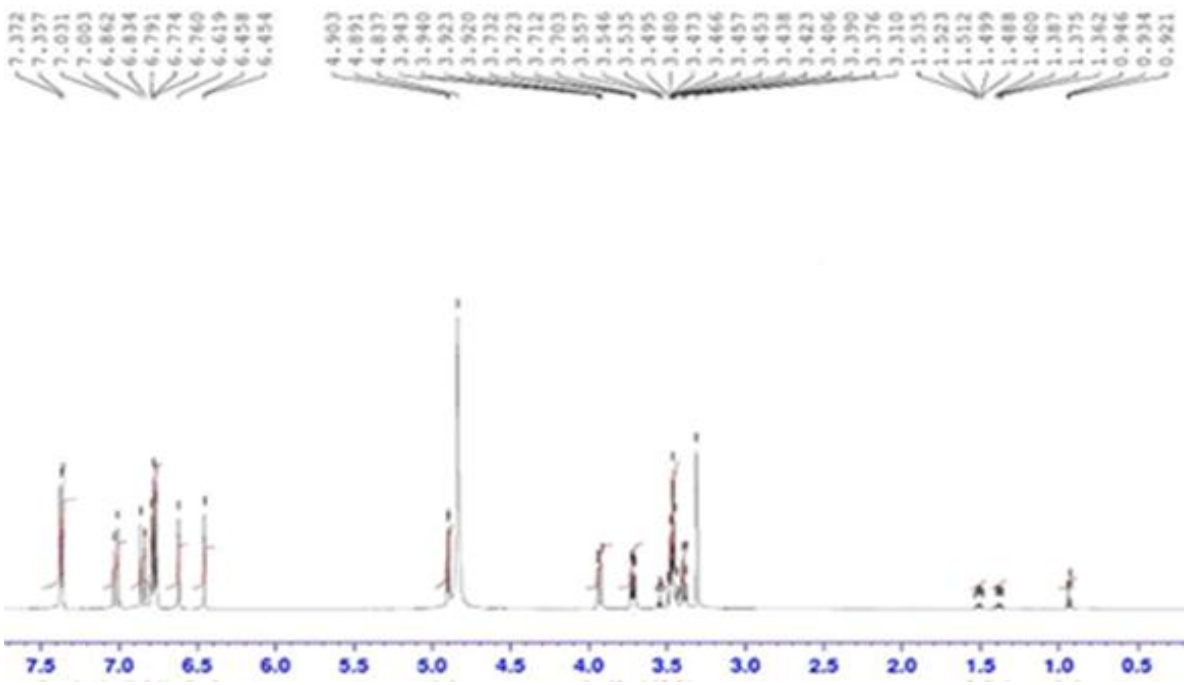
Appendix 9d: UV spectrum for compound 206 in NaOMe



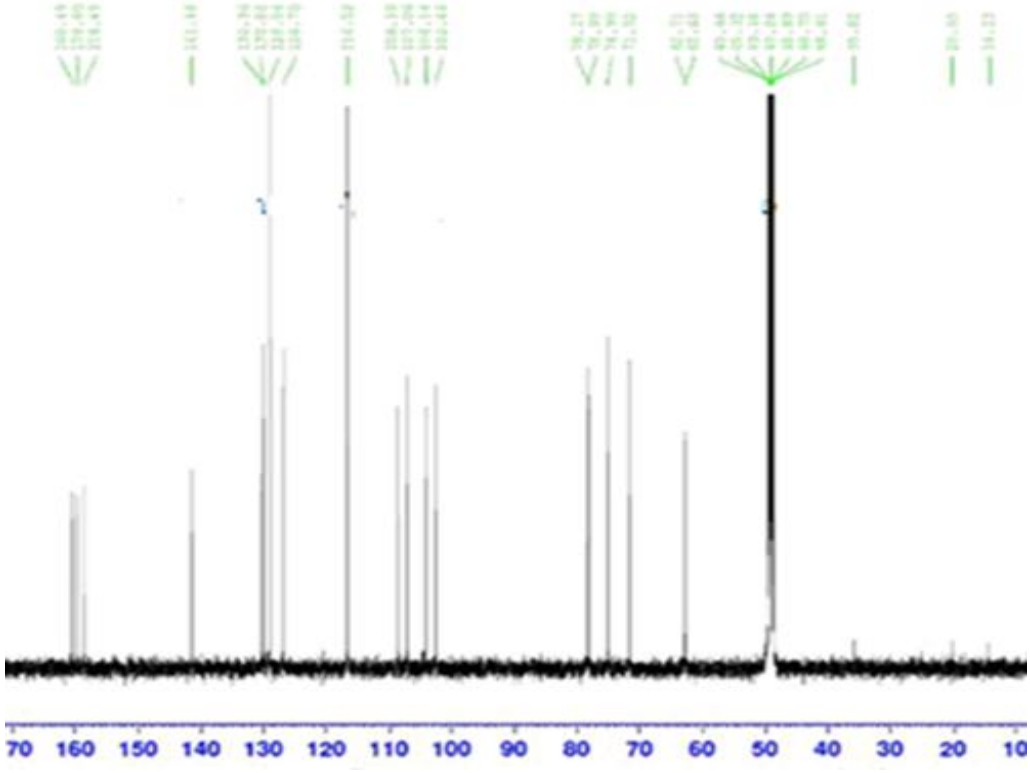
Appendix 9e: EI-MS spectrum for the aglycone of compound 206



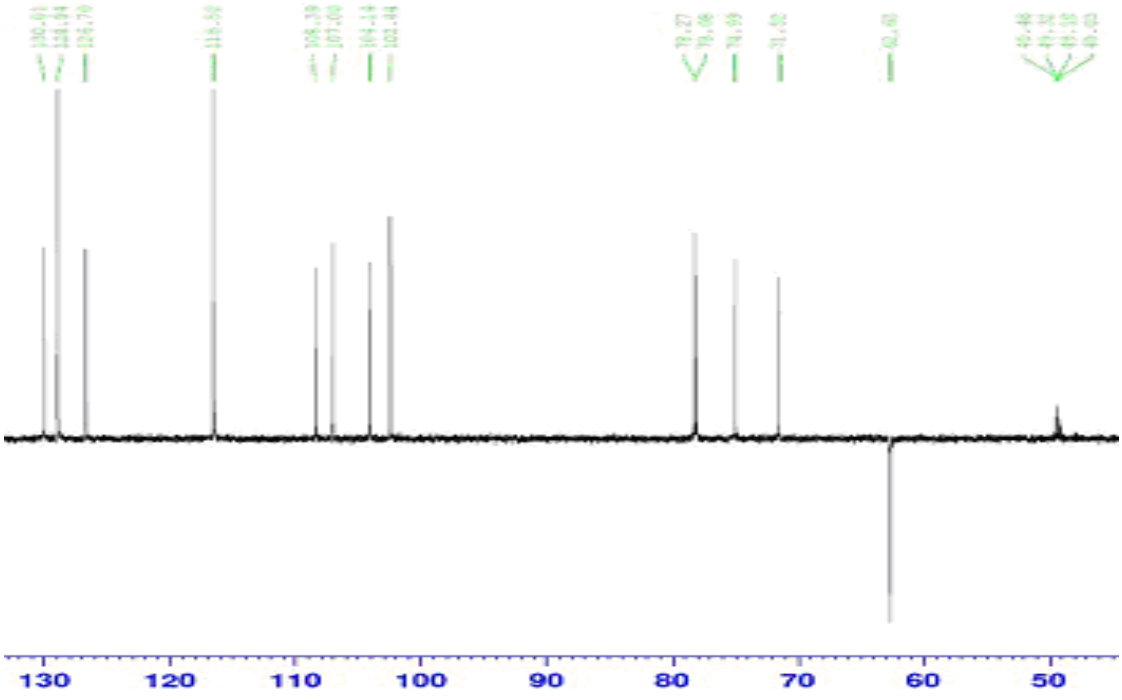
Appendix 9f: ESI-MS spectrum for compound 206



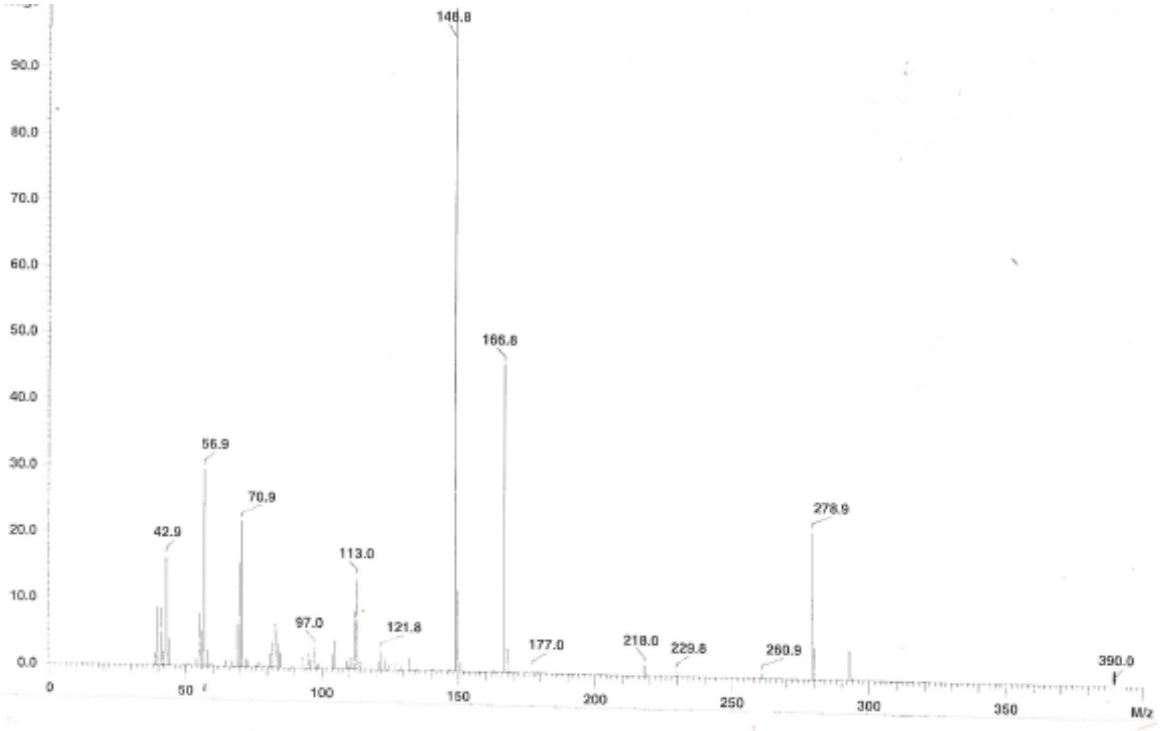
Appendix 10a: ^{1}H NMR spectrum for compound 207



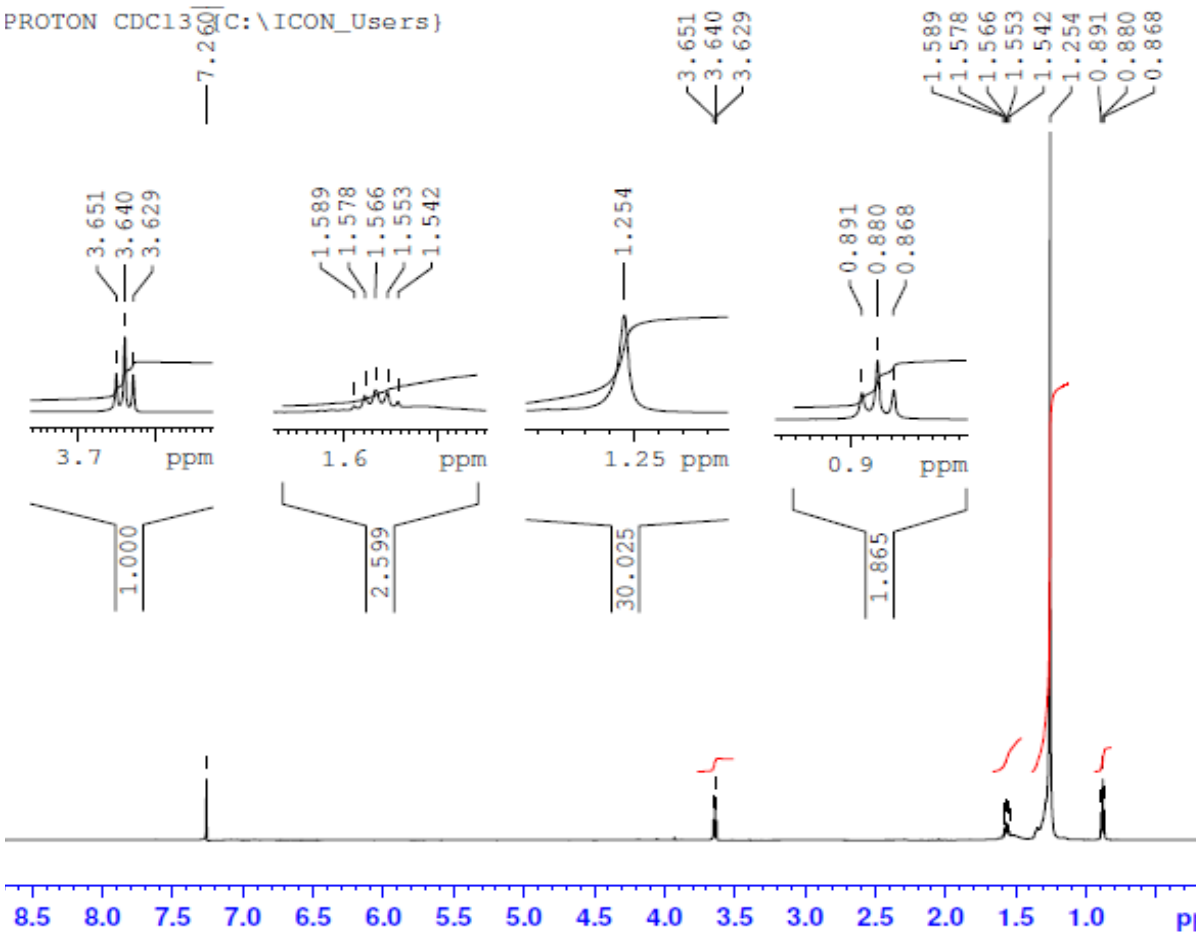
Appendix 10b: ^{13}C NMR spectrum for compound 207



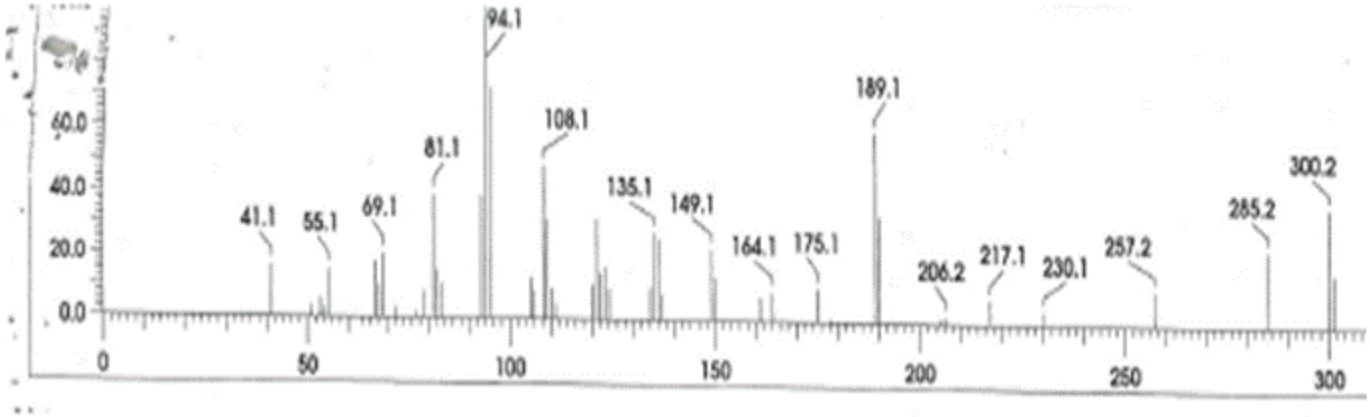
Appendix 10c: DEPT -135 spectrum for compound 207



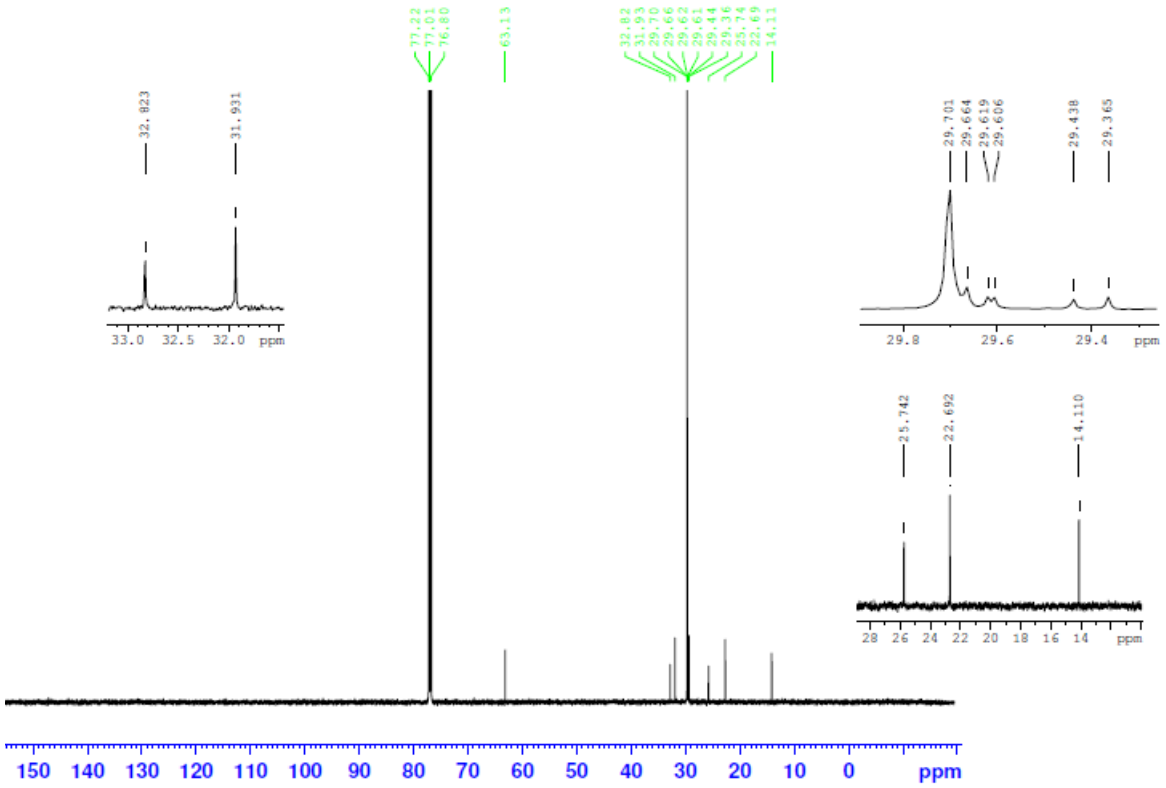
Appendix 10d: EI-MS spectrum for compound 207



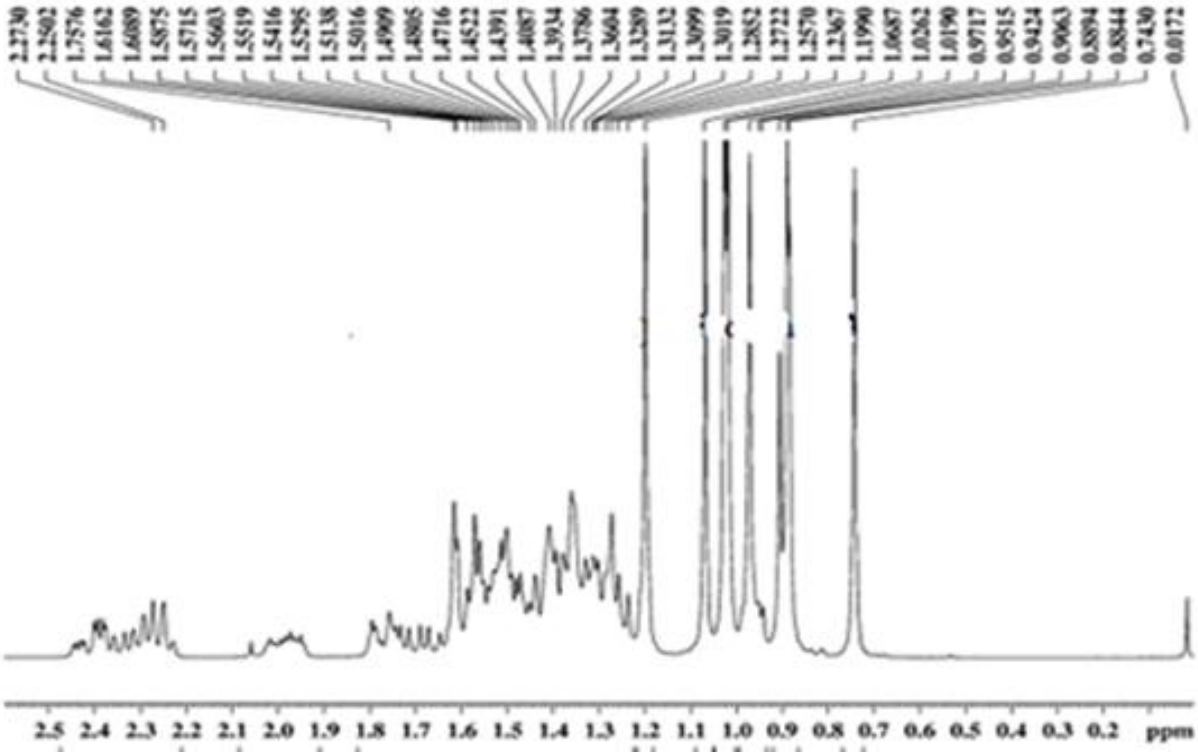
Appendix 11a: ^{13}C NMR spectrum for compound 208



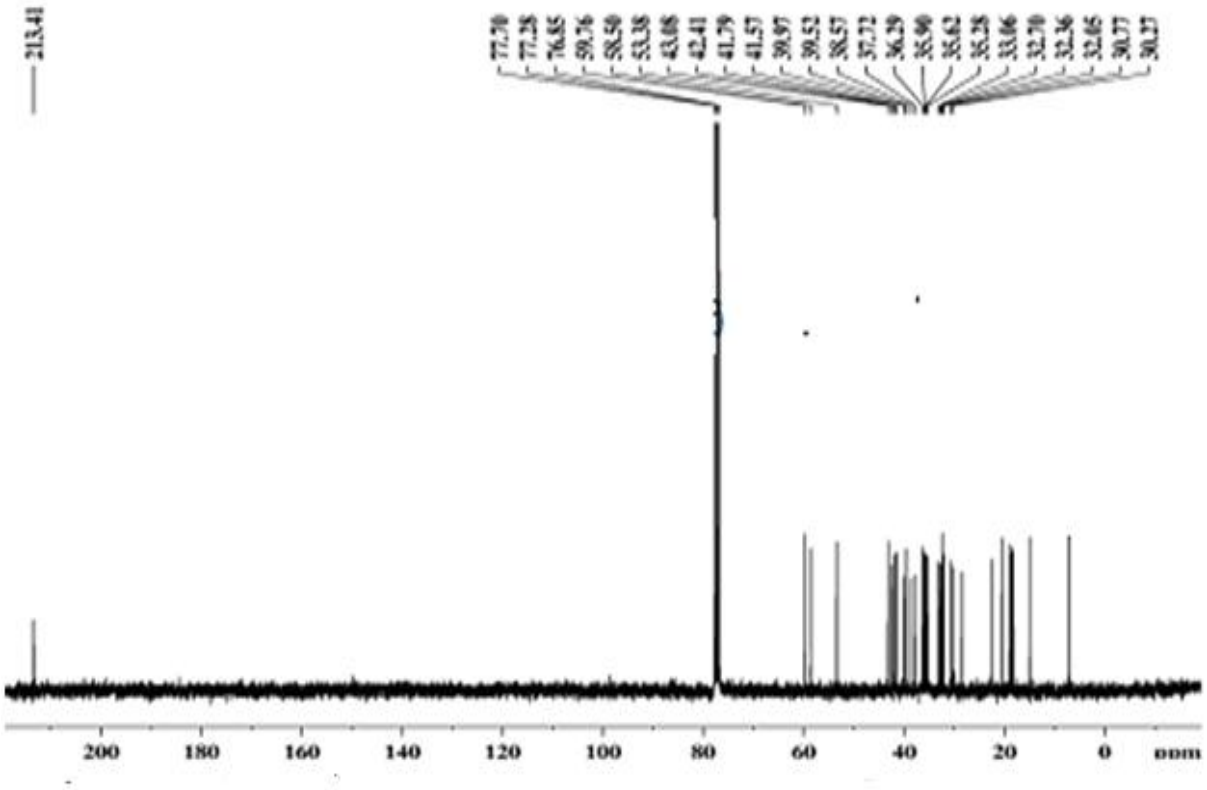
Appendix 11b: EI-MS spectrum for compound 208



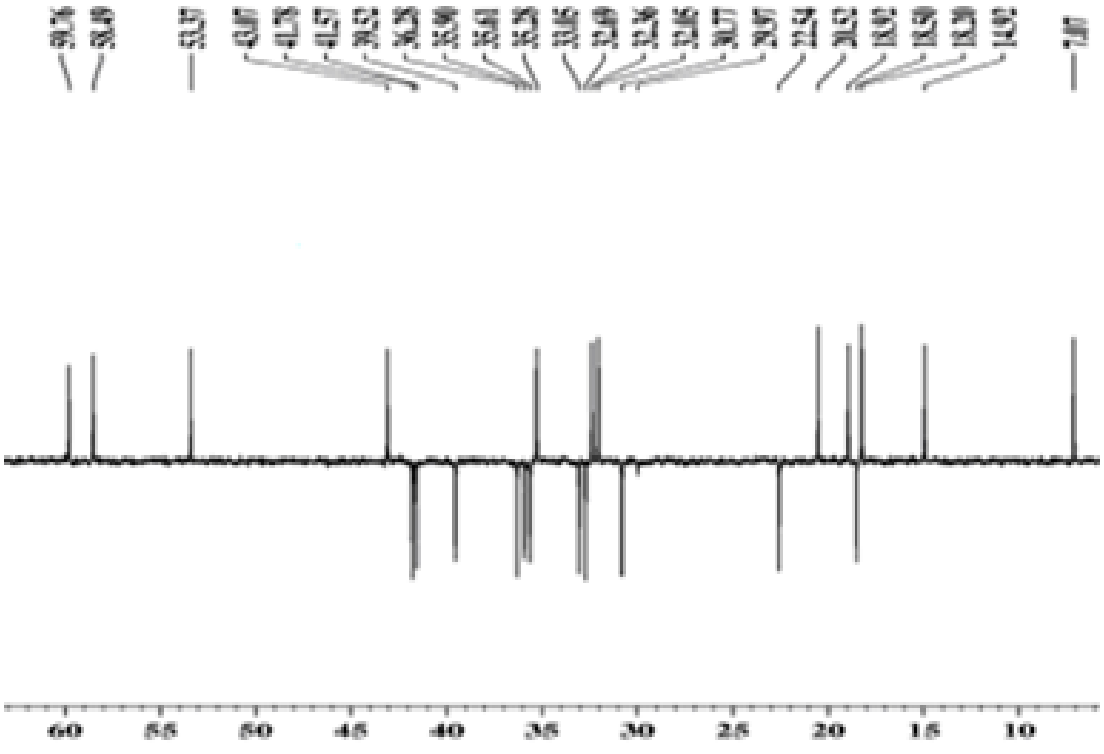
Appendix 11c: ^{13}C NMR spectrum for compound 208



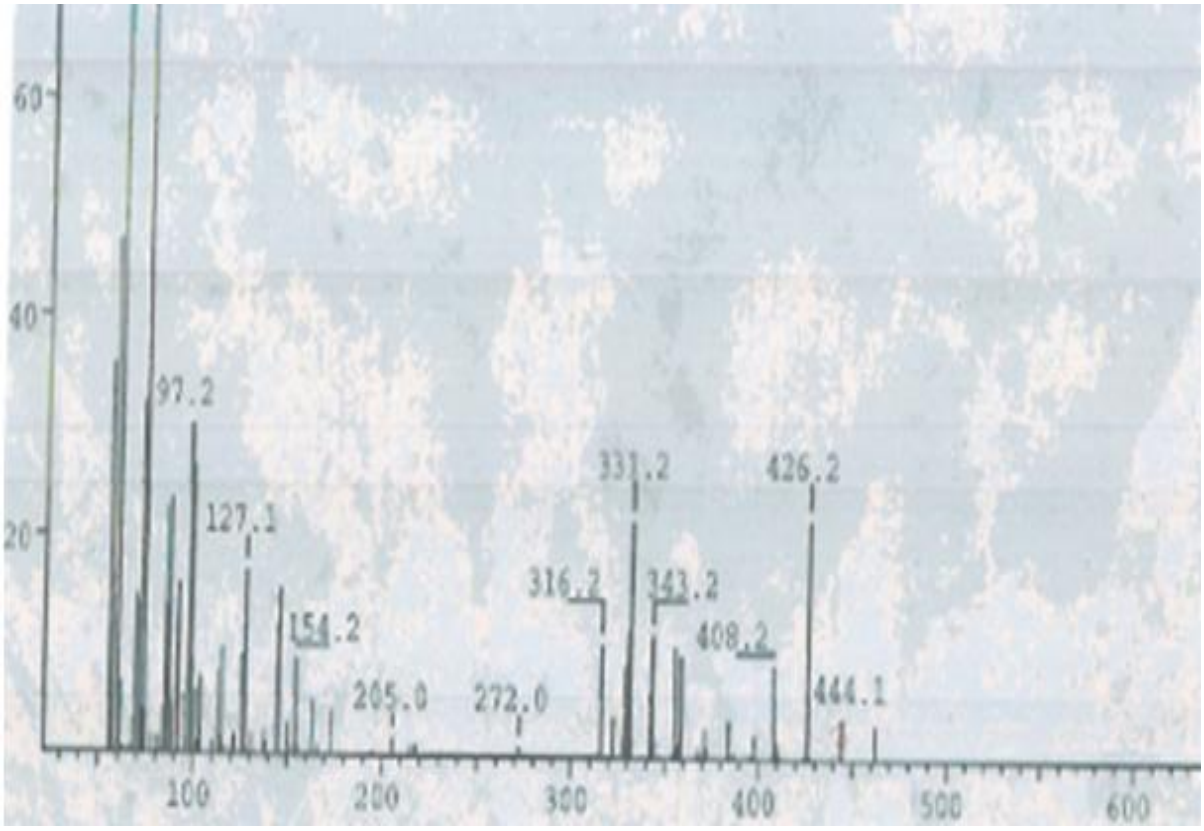
Appendix 12a: ^{1}H NMR spectrum for compound 209



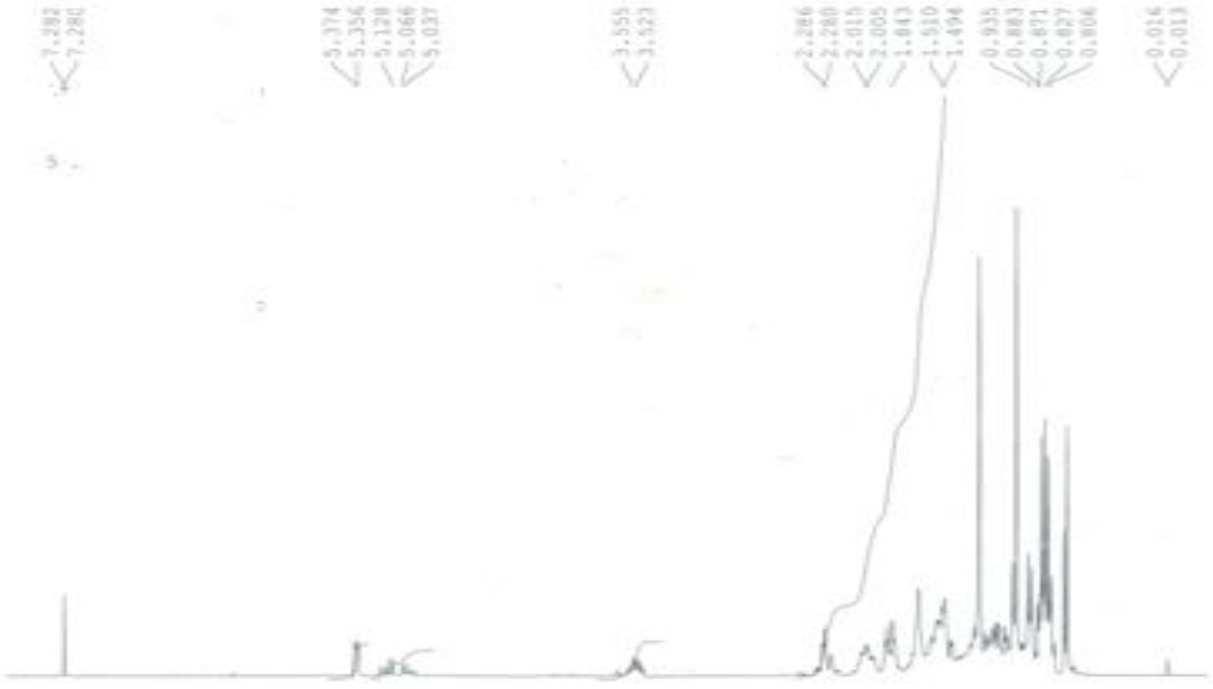
Appendix 12b: ^{13}C NMR spectrum for compound 209



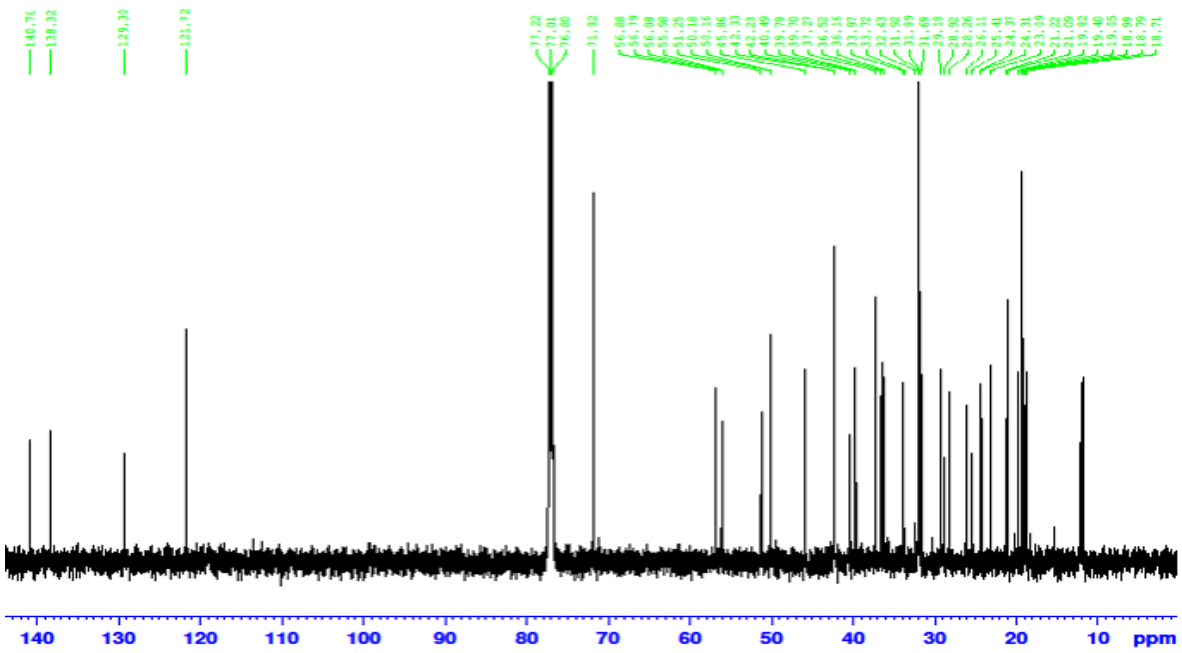
Appendix 12c: DEPT-135 spectrum for compound 209



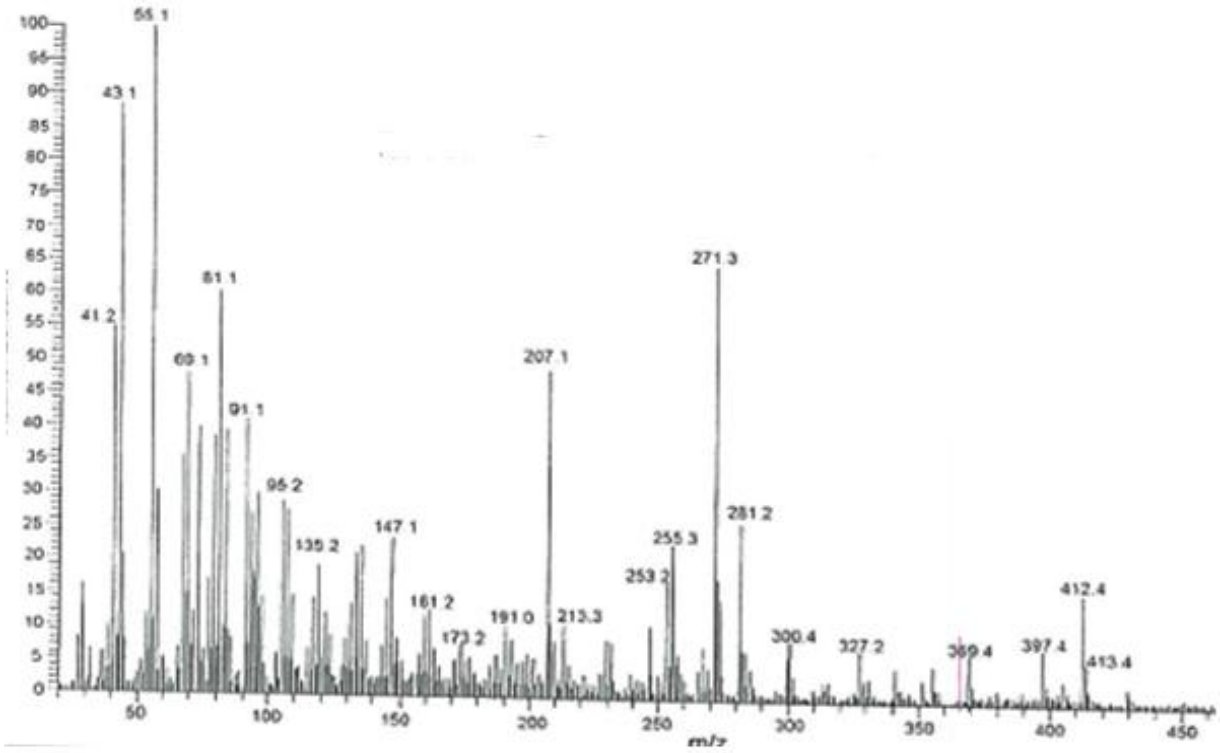
Appendix 12d: EI-MS spectrum for compound 209



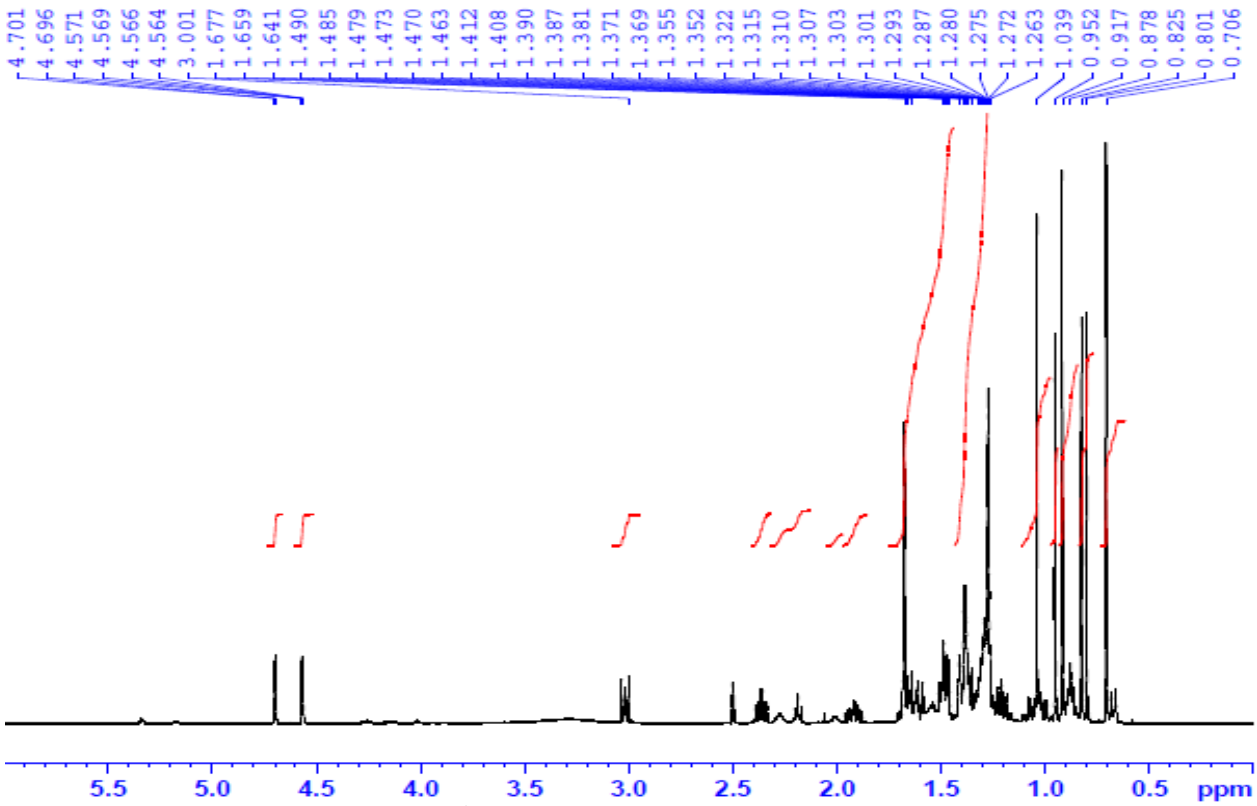
Appendix 13a: ^{1}H NMR spectrum for compound 6



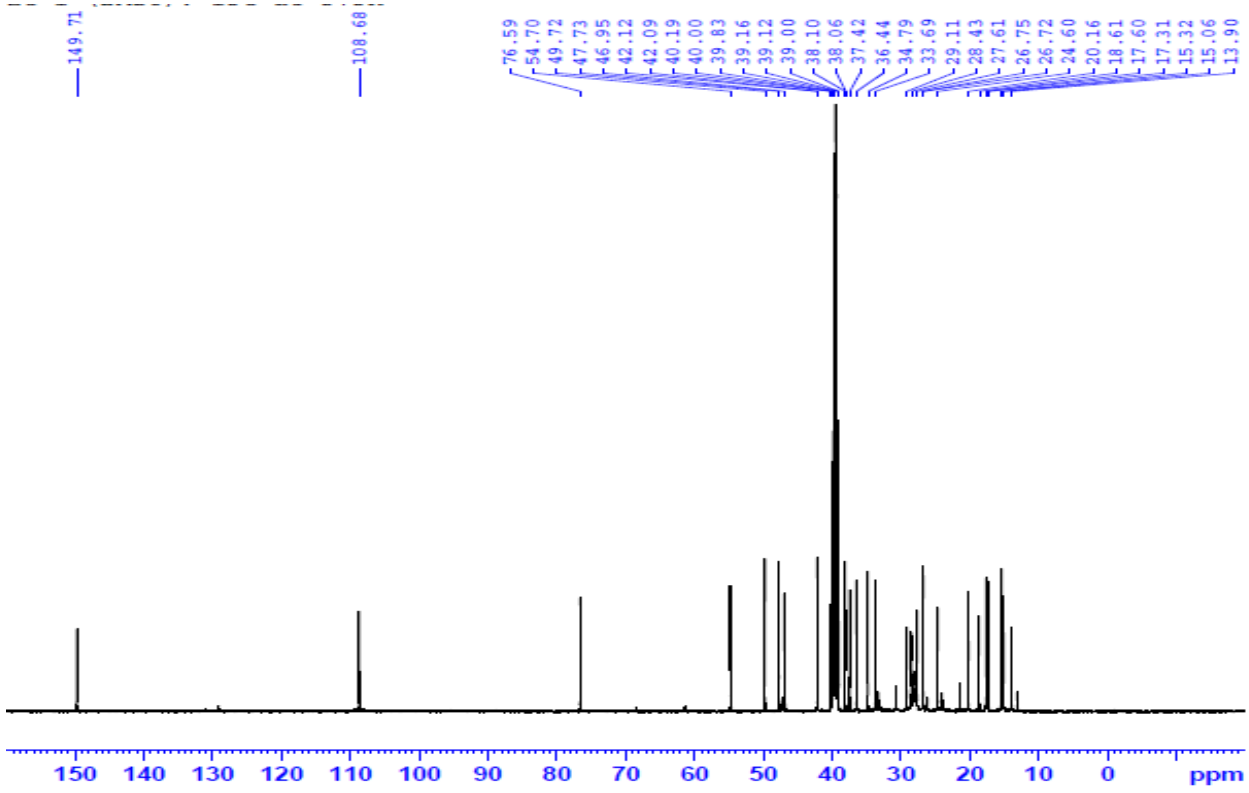
Appendix 13b: ^{13}C NMR spectrum for compound 6



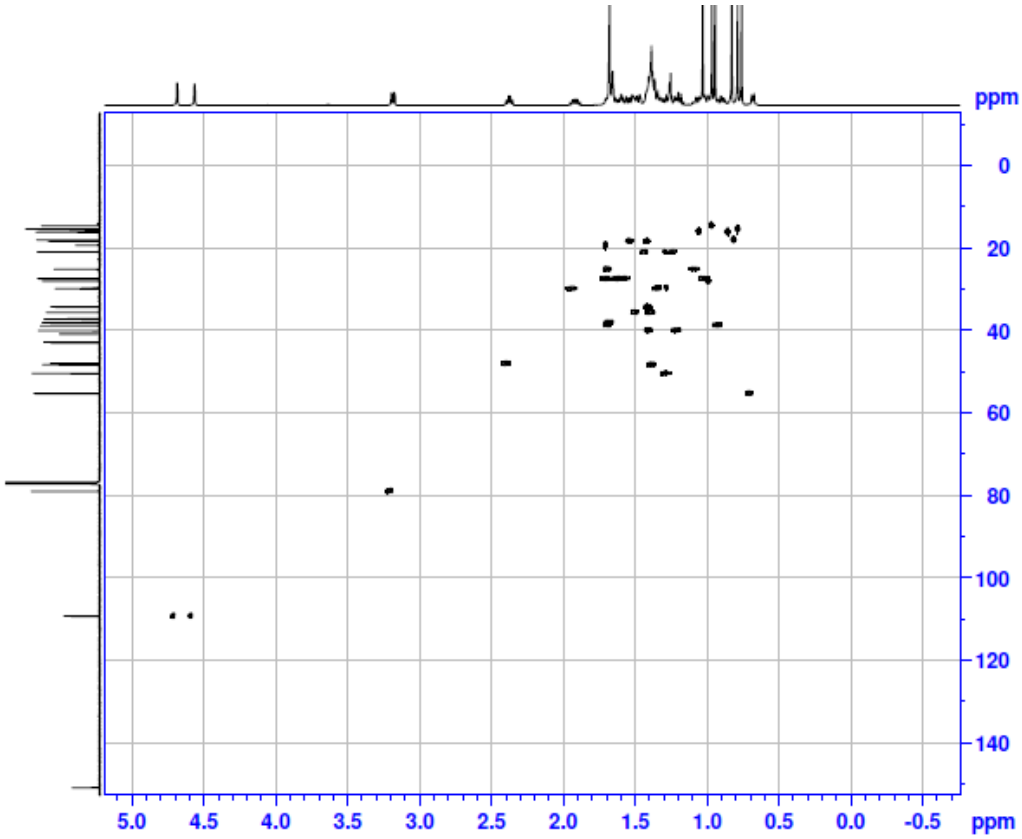
Appendix 13c: EI-MS spectrum for compound 6



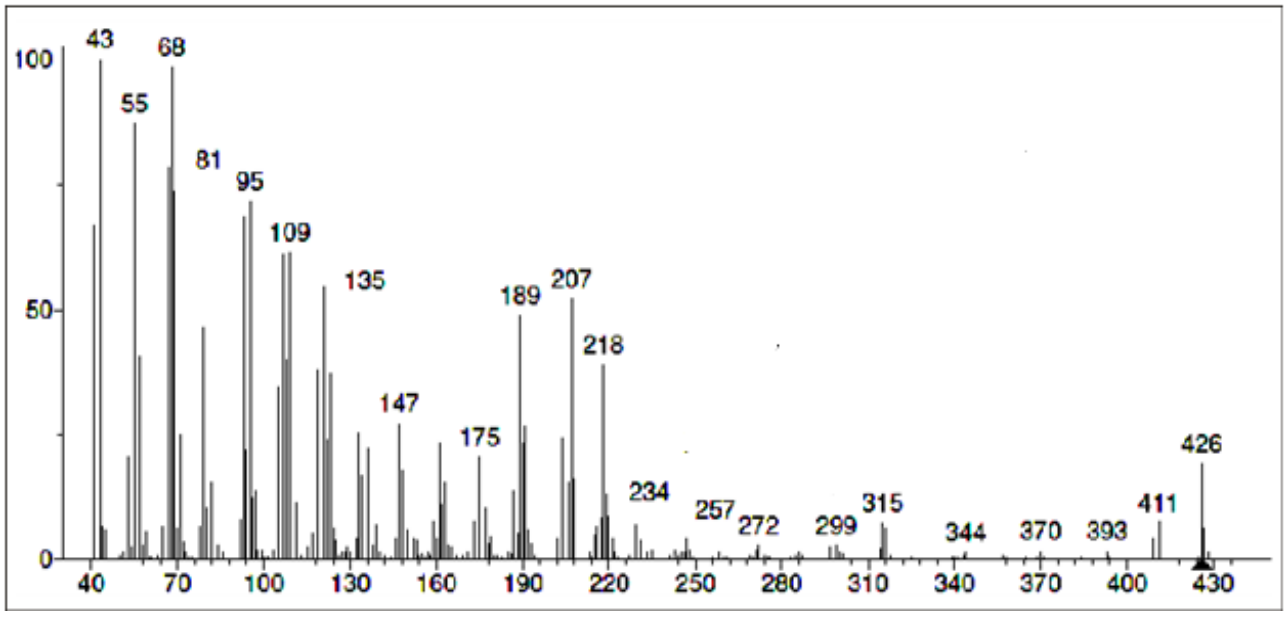
Appendix 14a: ^{1}H NMR spectrum for compound 49



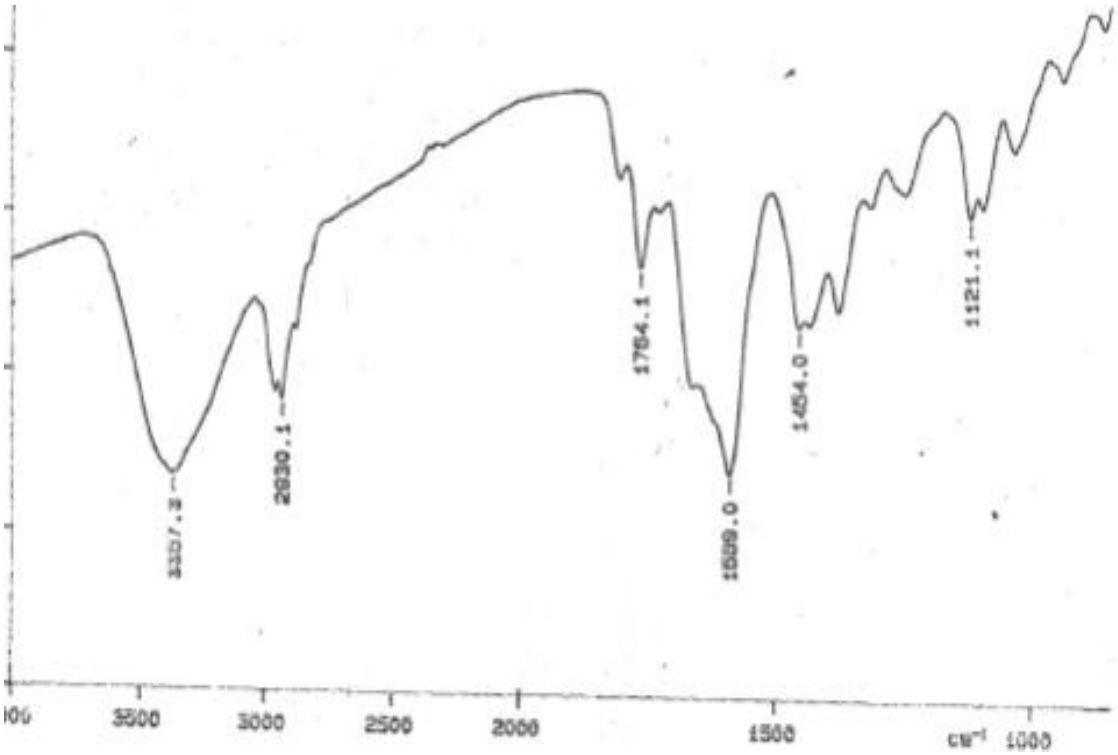
Appendix 14b: ^{13}C NMR spectrum for compound 49



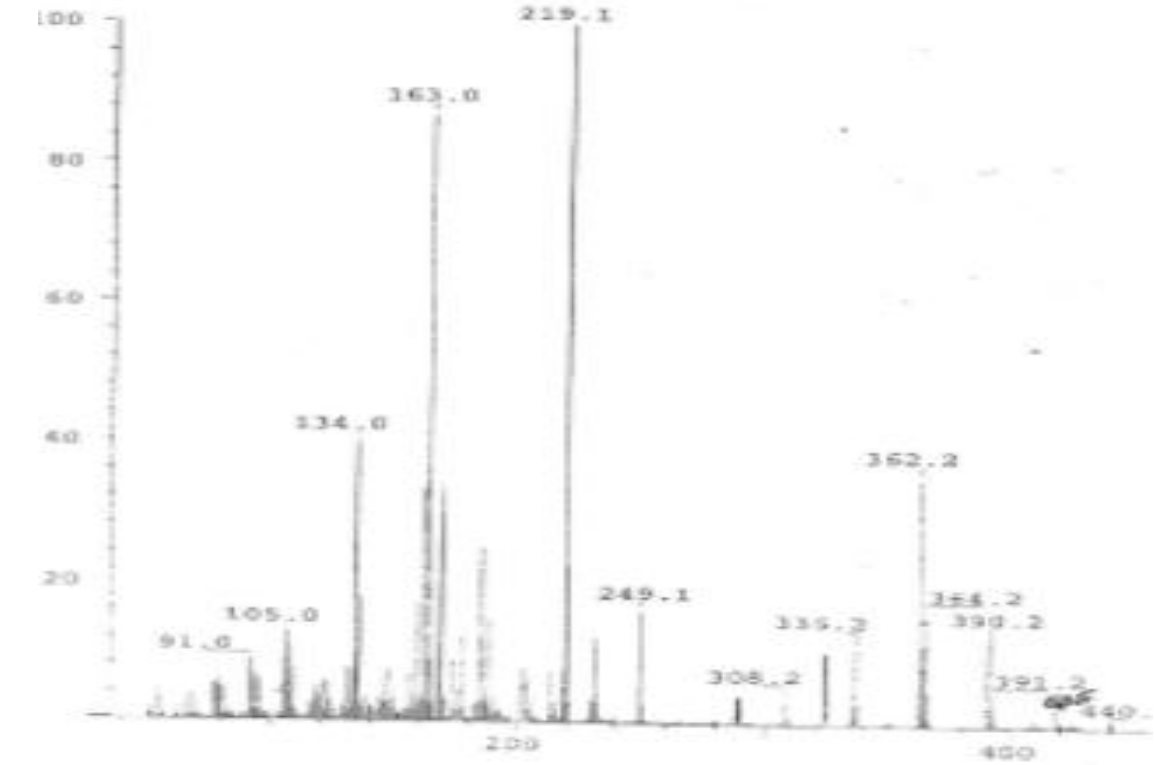
Appendix 14c: HSQC spectrum for compound 49



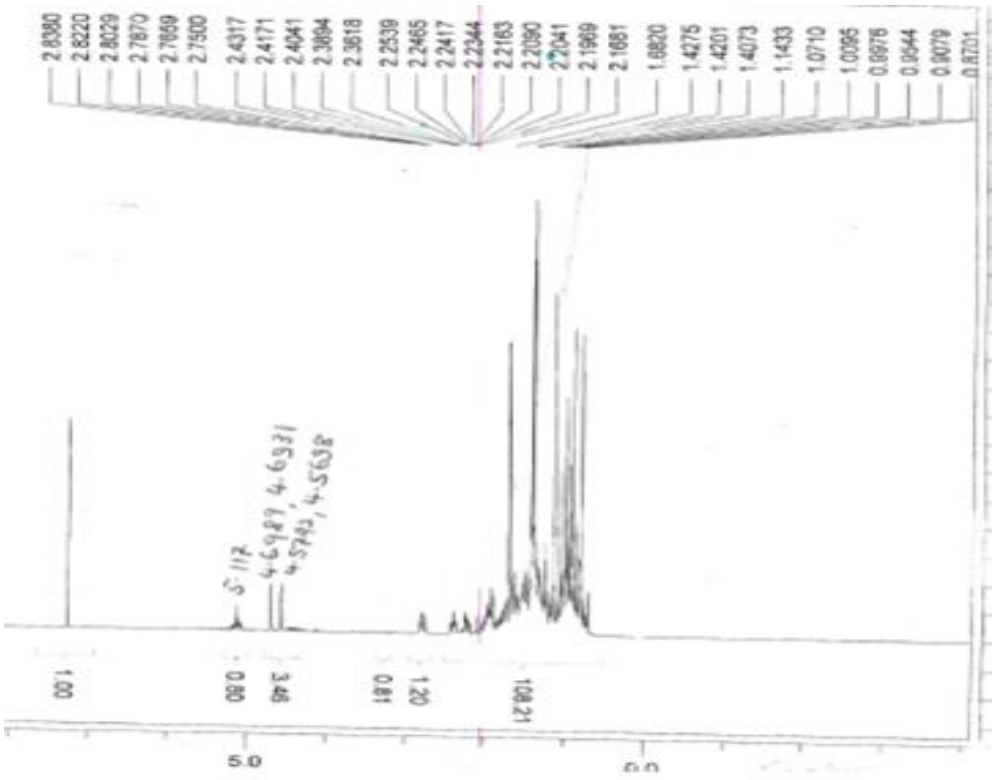
Appendix 14d: ESI-MS spectrum for compound 49



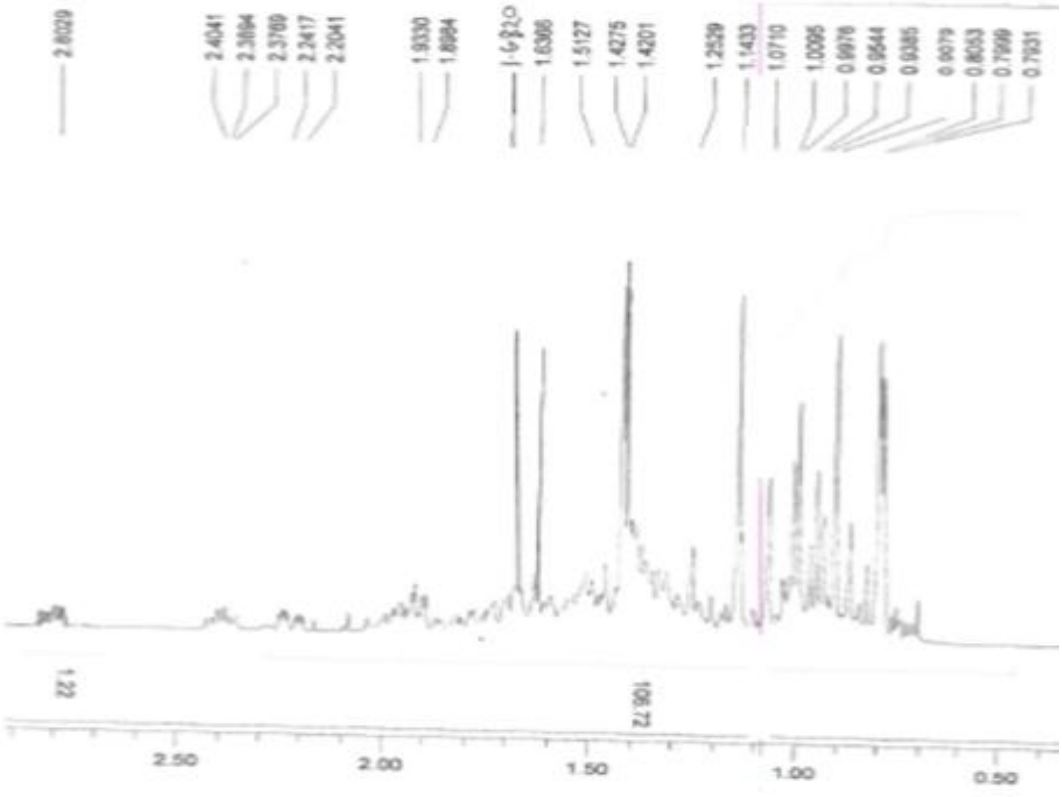
Appendix 15a: IR spectrum for compound 210



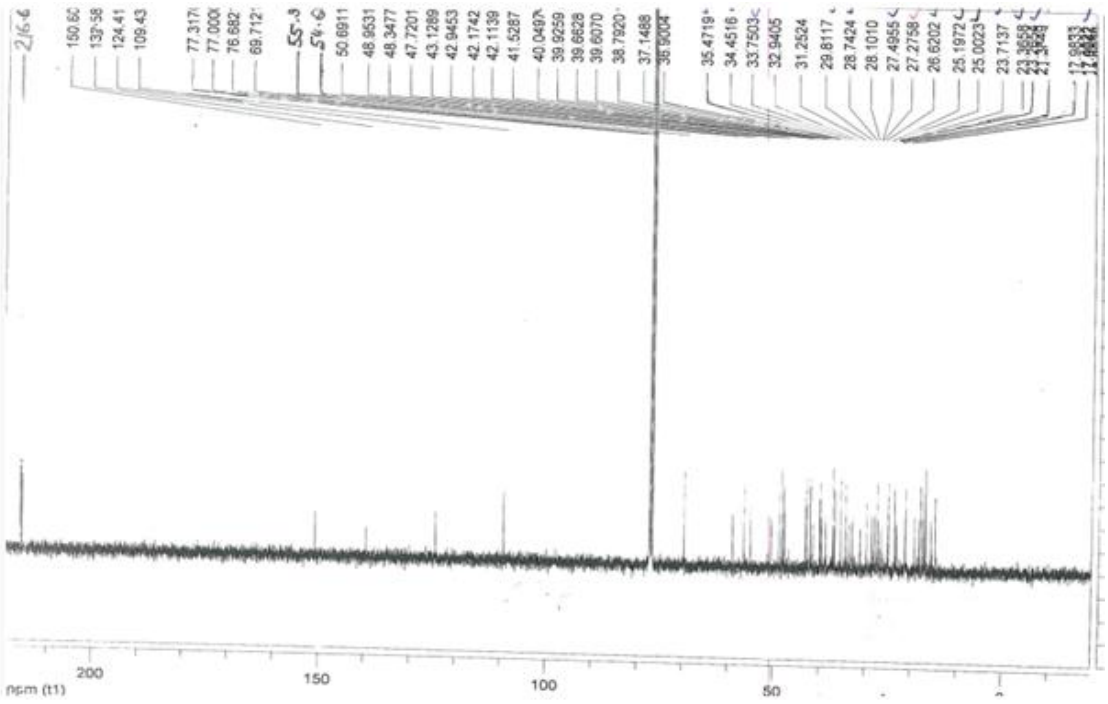
Appendix 15b: EI-MS spectrum for compound 210



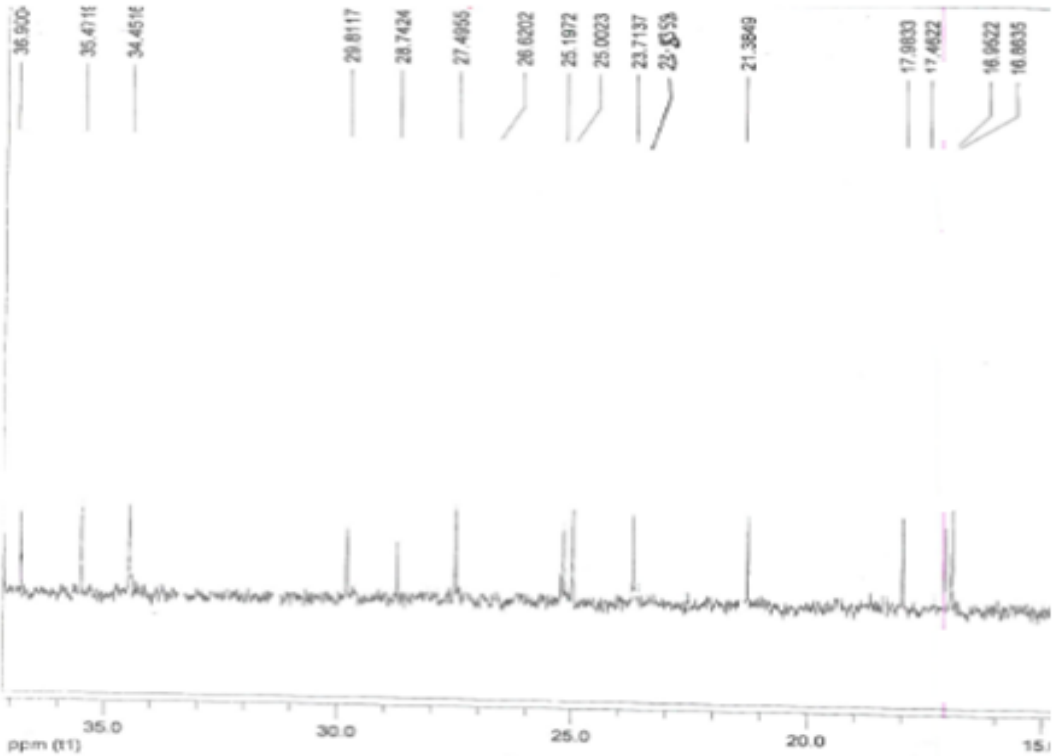
Appendix 15c (i): ^{1}H NMR spectrum for compound 210



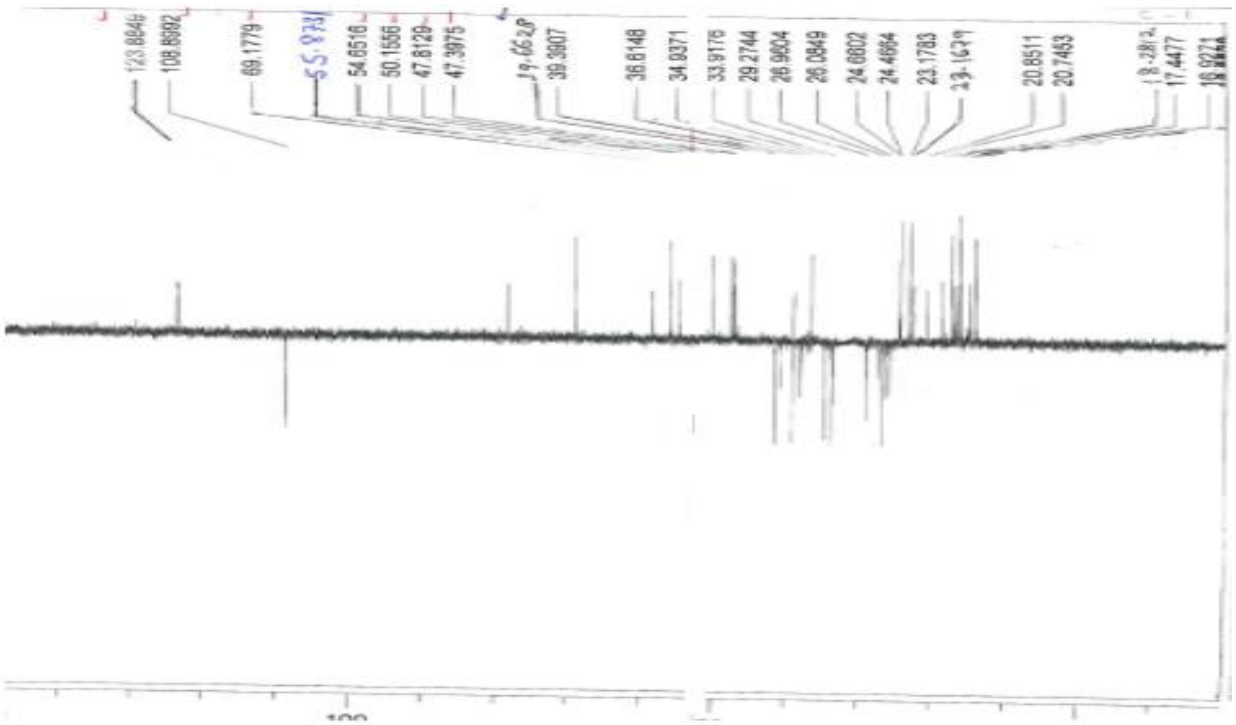
Appendix 15c (ii): ^{1}HNMR (expanded) for compound 210



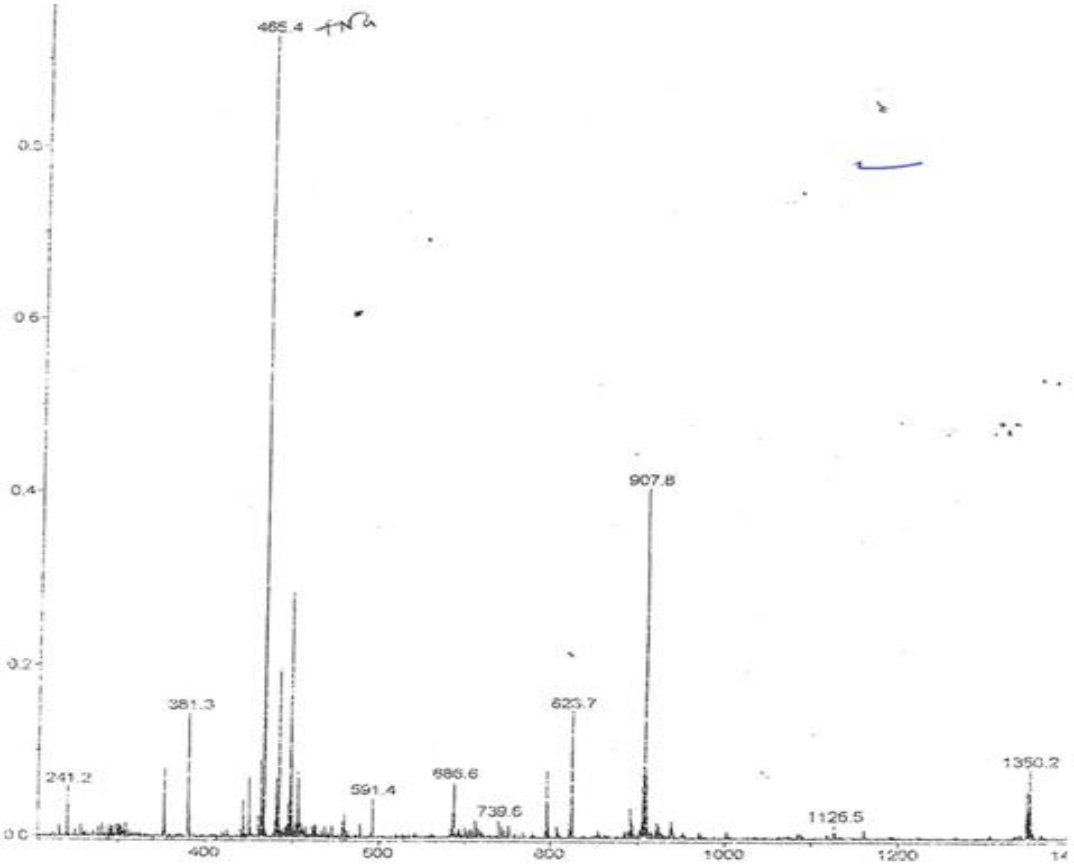
Appendix 15d (i): ^{13}CNMR spectrum for compound 210



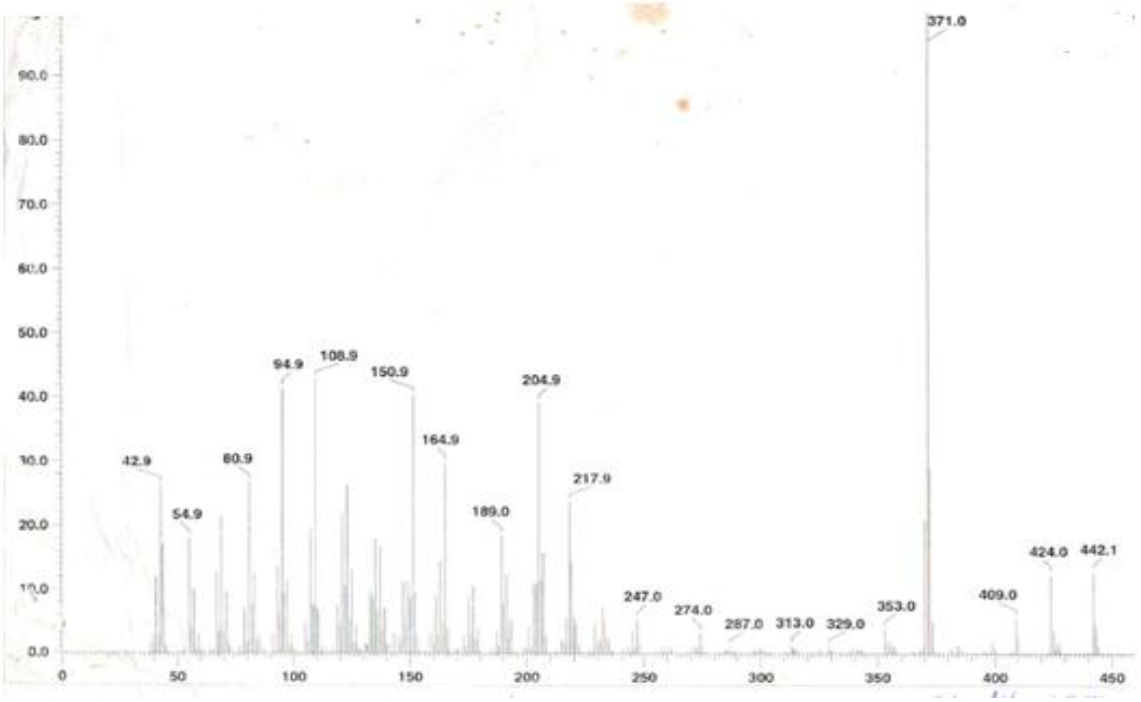
Appendix 15d(ii): ^{13}C NMR spectrum (expanded) for compound 210



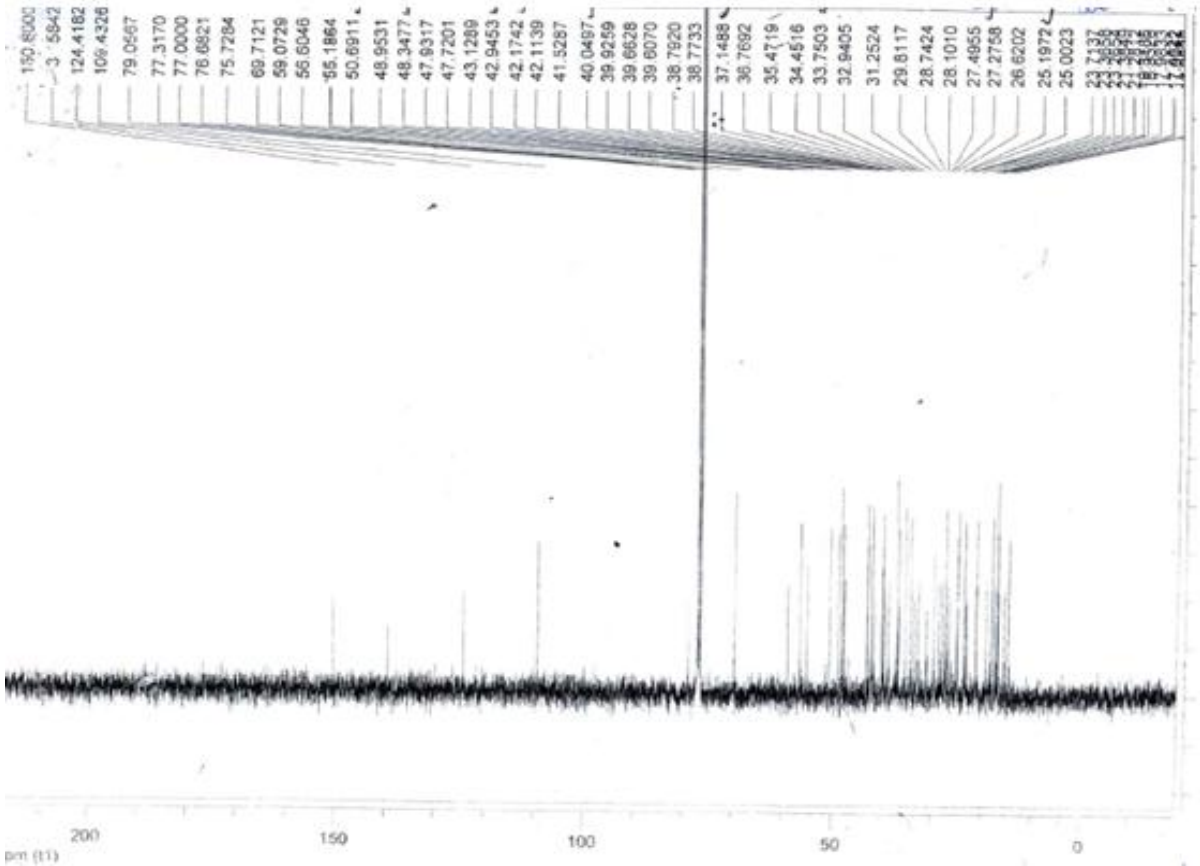
Appendix 15e: DEPT-135 spectrum for compound 210



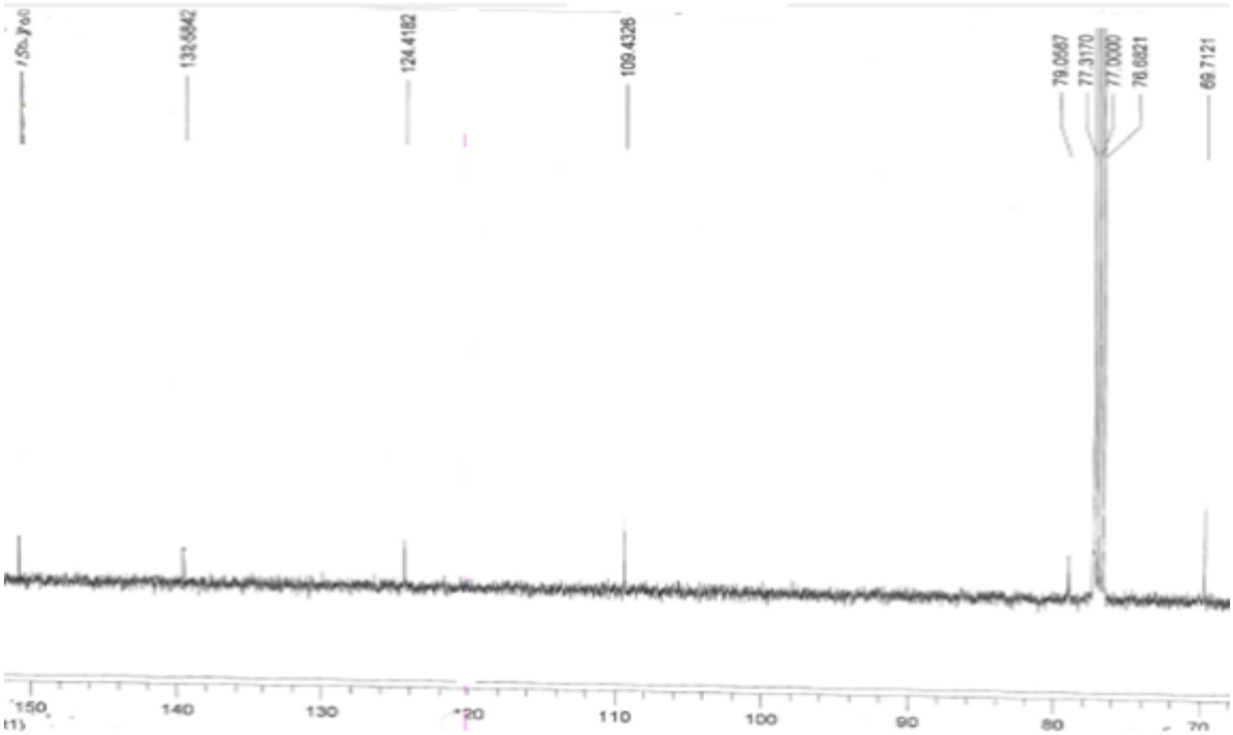
Appendix 16a; ESI-MS spectrum for compound 211



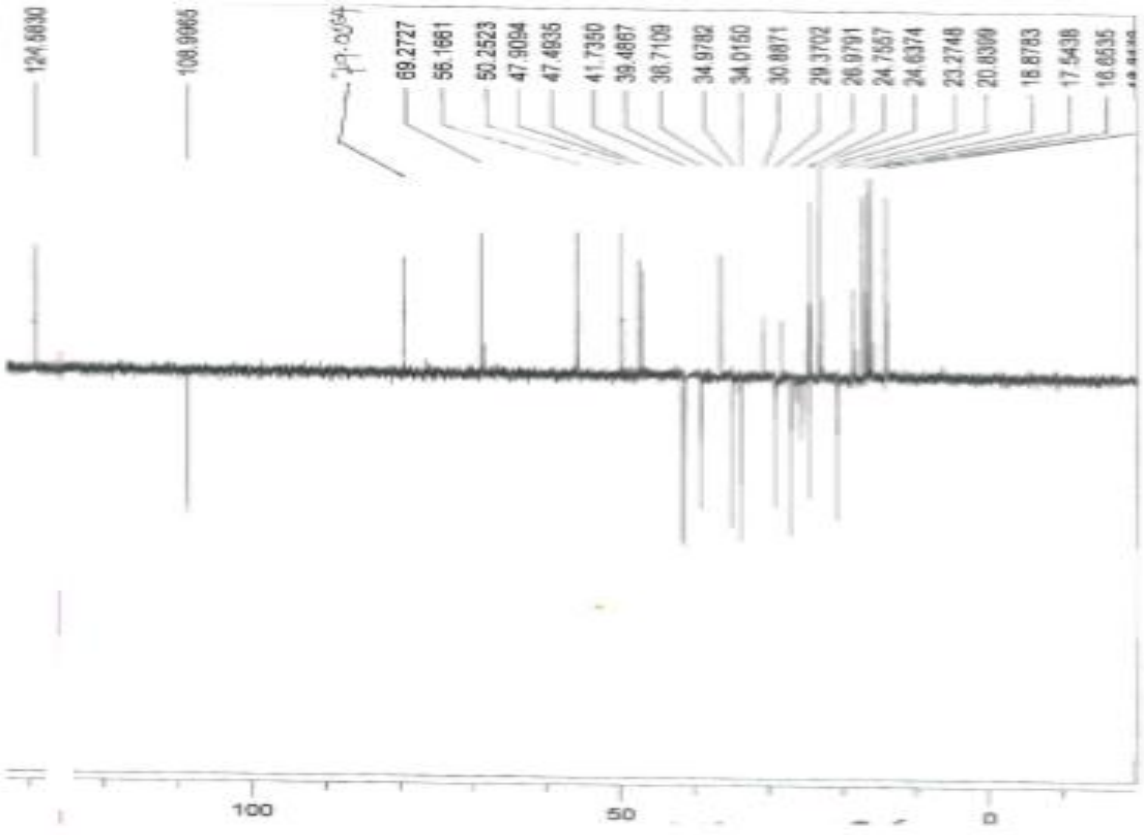
Appendix 16b; EI-MS spectrum for compound 211



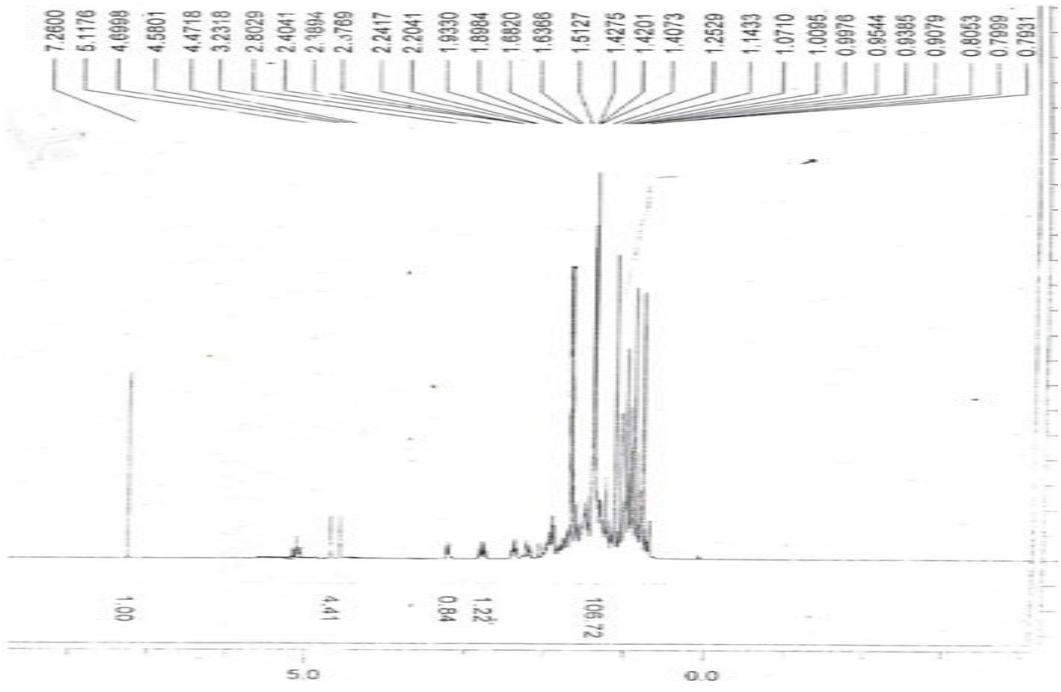
Appendix 16c (i): ^{13}C NMR spectrum for compound 211



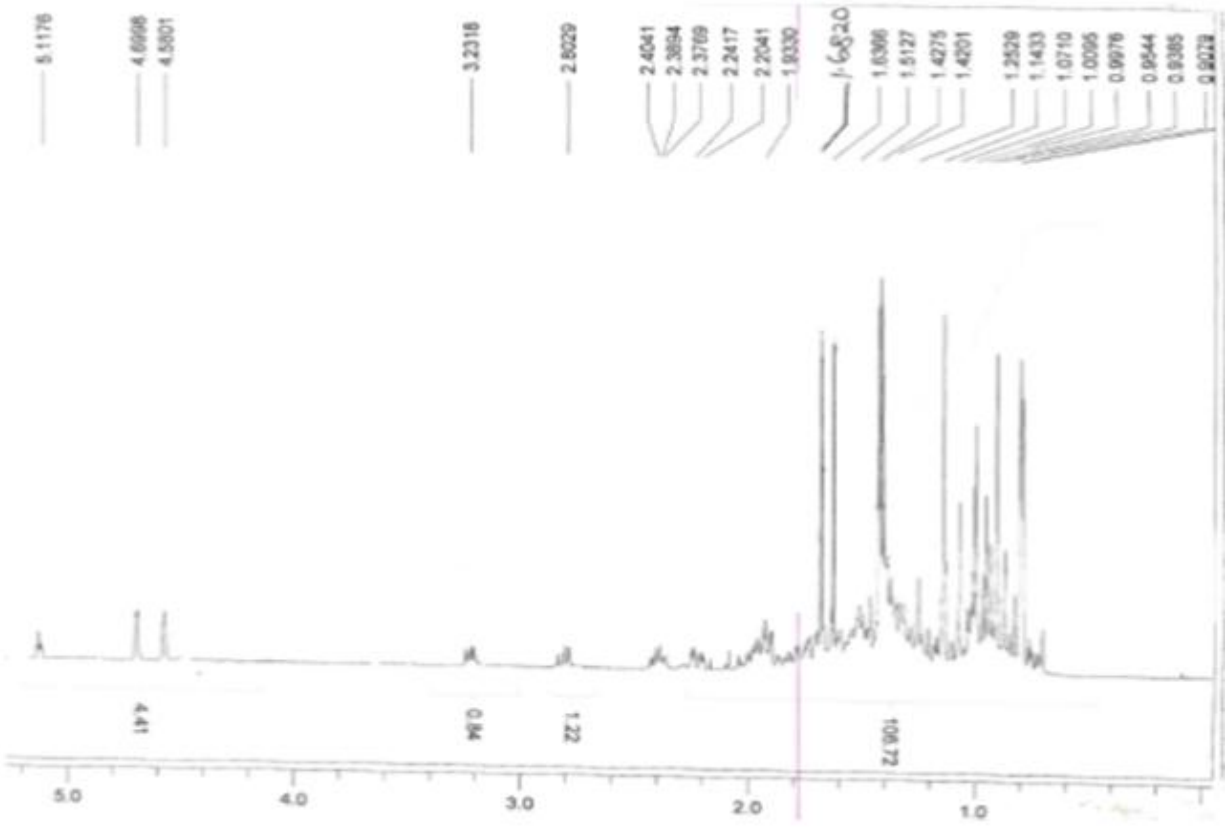
Appendix 16c (ii): ^{13}C NMR spectrum (expanded) for compound 211



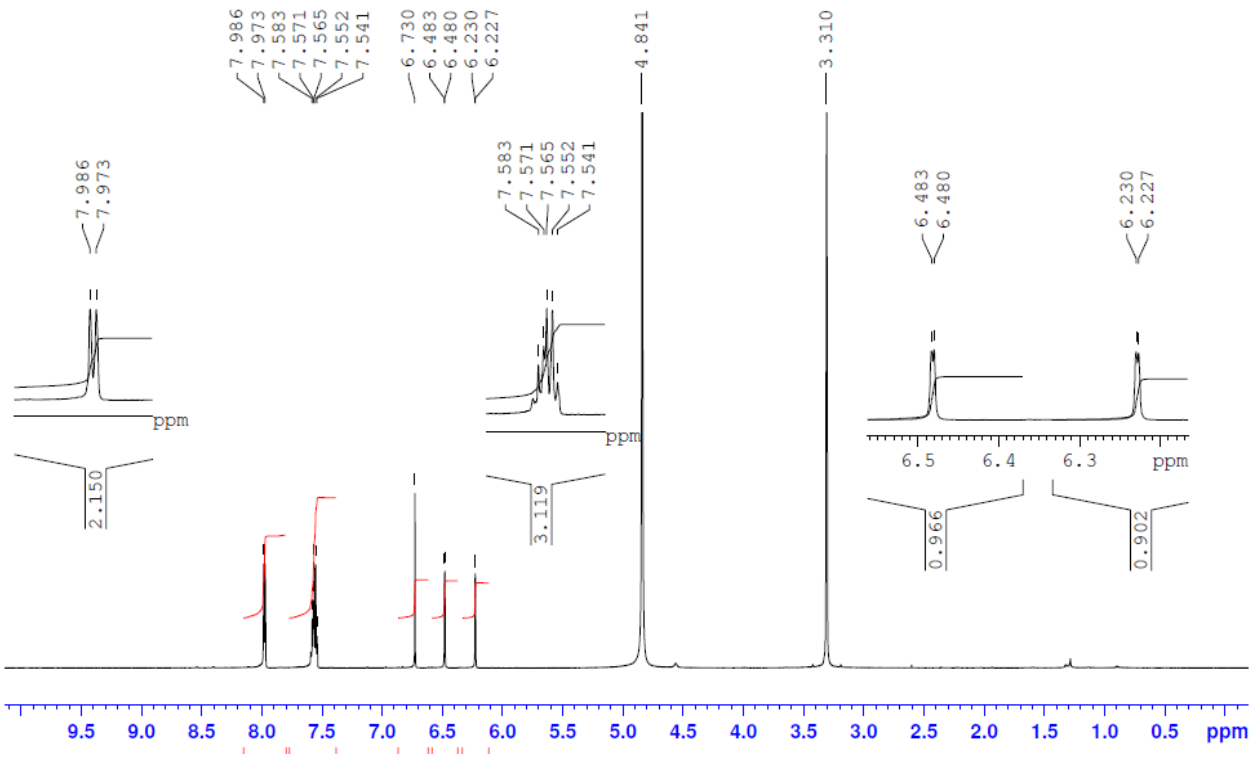
Appendix 16d: DEPT -135 spectrum for compound 211



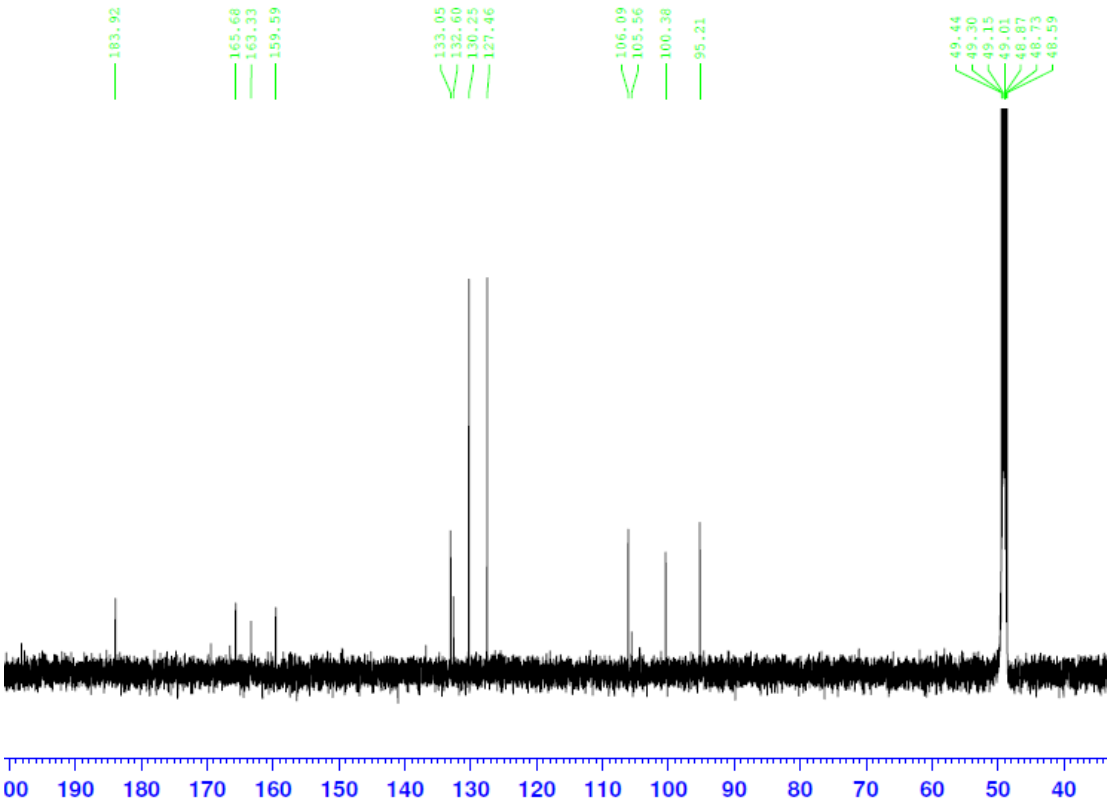
Appendix 16e (i):[1]HNMR spectrum for compound 211



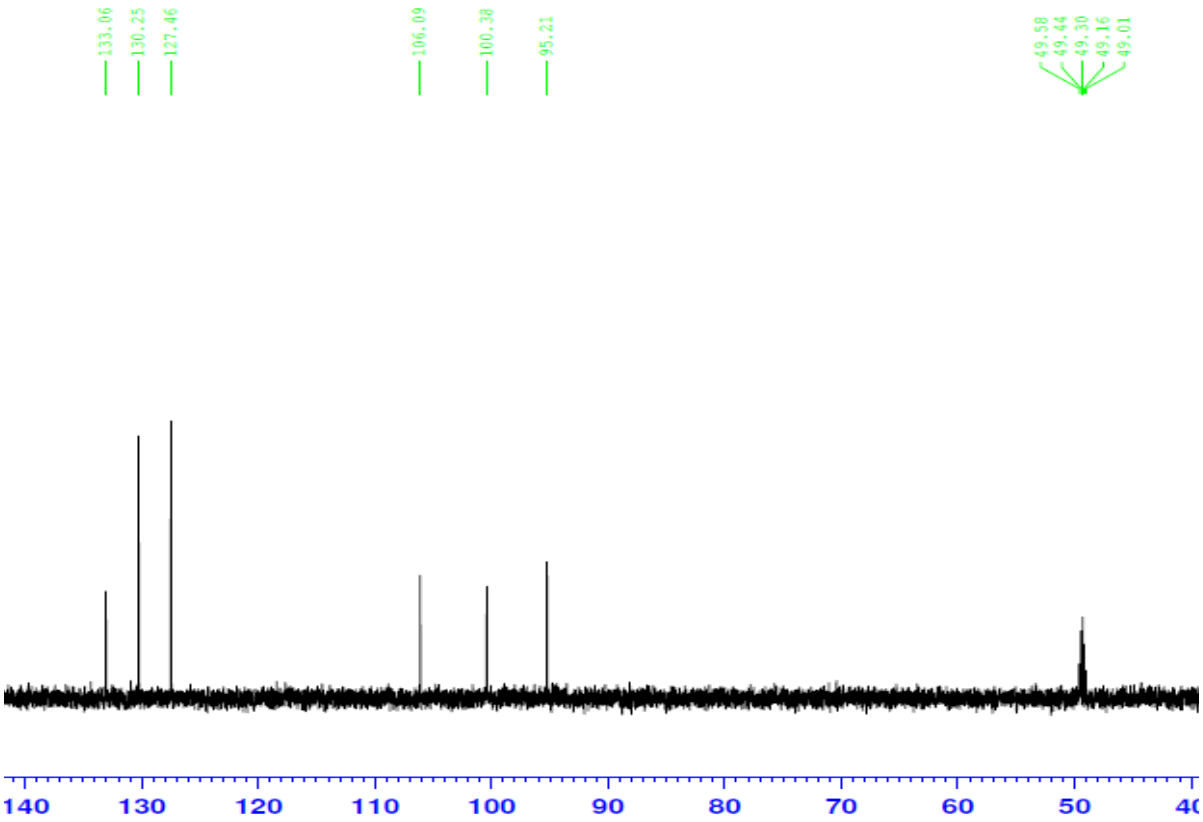
Appendix 16e (ii); [1]HNMR spectrum (expanded) for compound 211



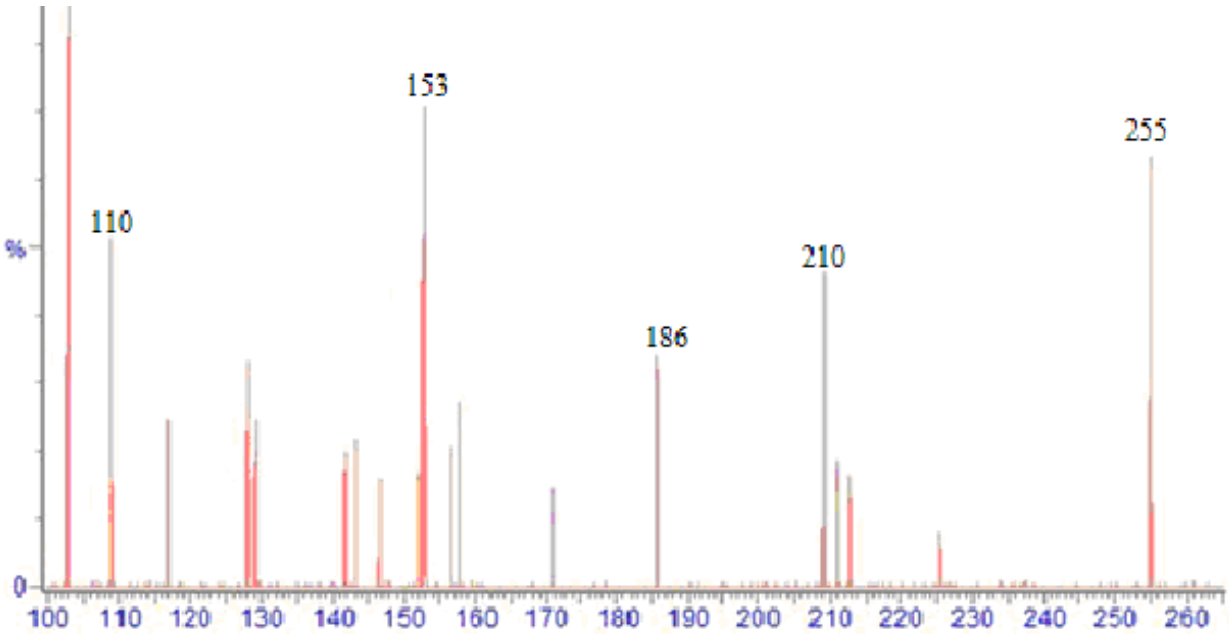
Appendix 17a: ^{1}HNMR spectrum for compound 212



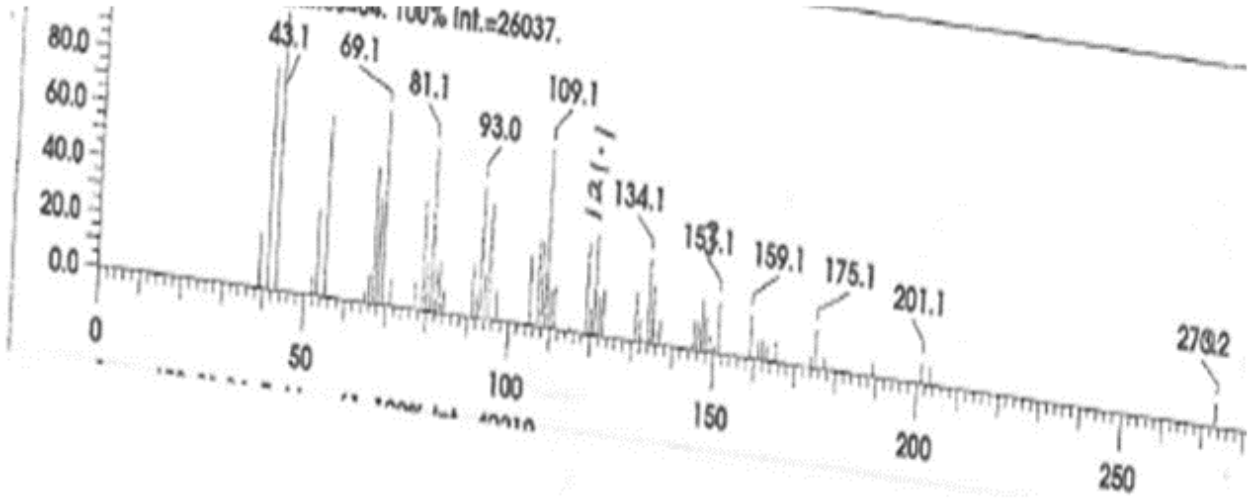
Appendix 17b: ^{13}C NMR spectrum for compound 212



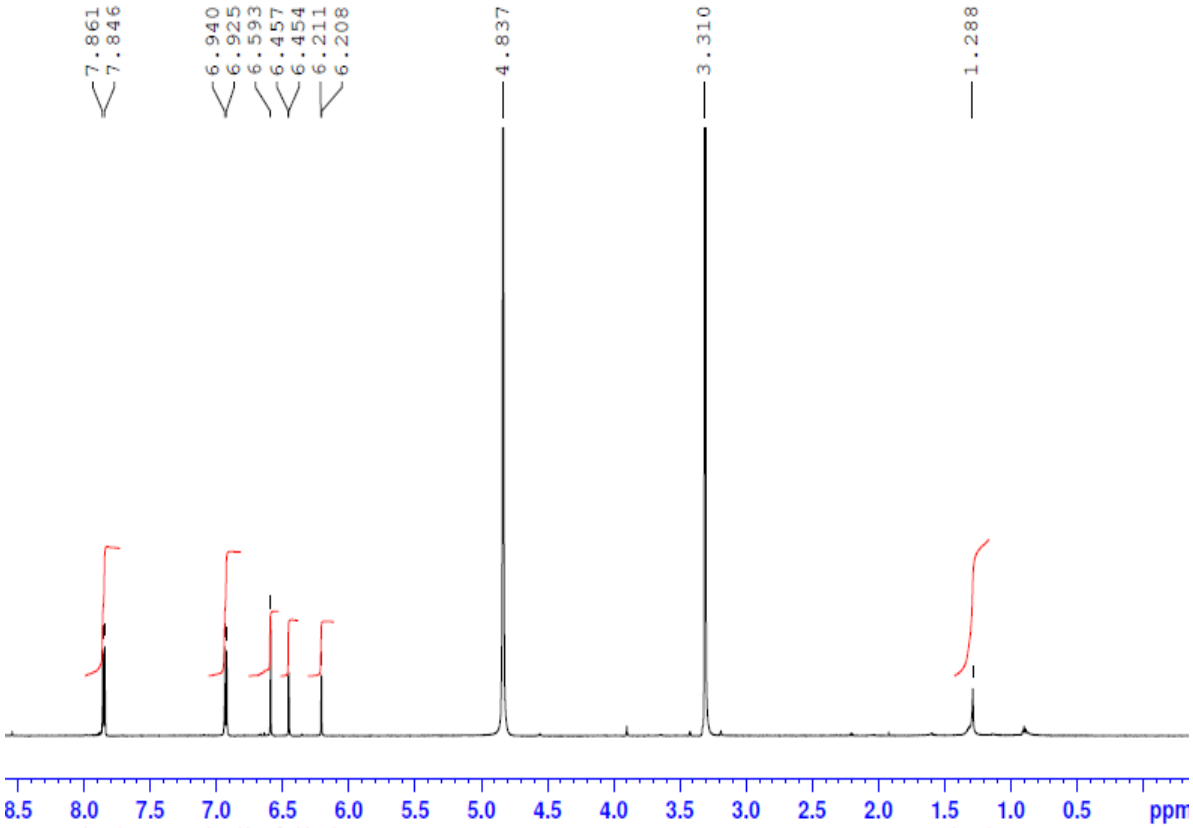
Appendix 17c: DEPT-135 spectrum for compound 212



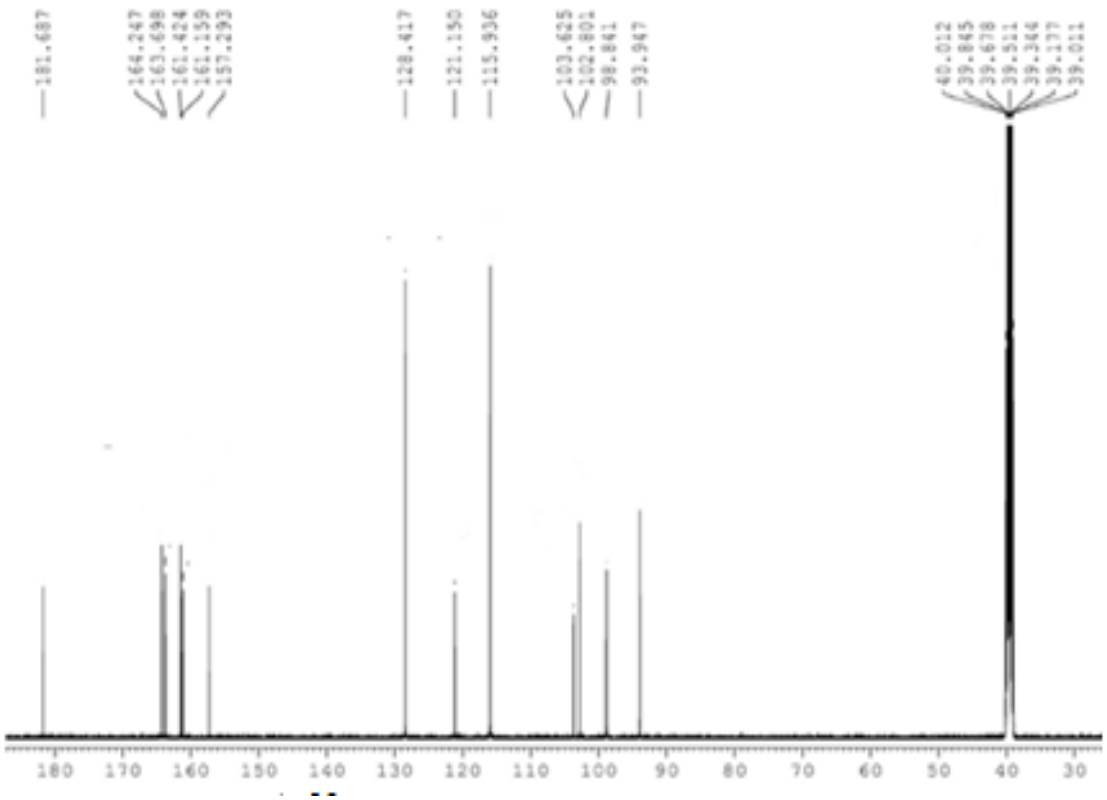
Appendix 17d: EI-MS spectrum for compound 212



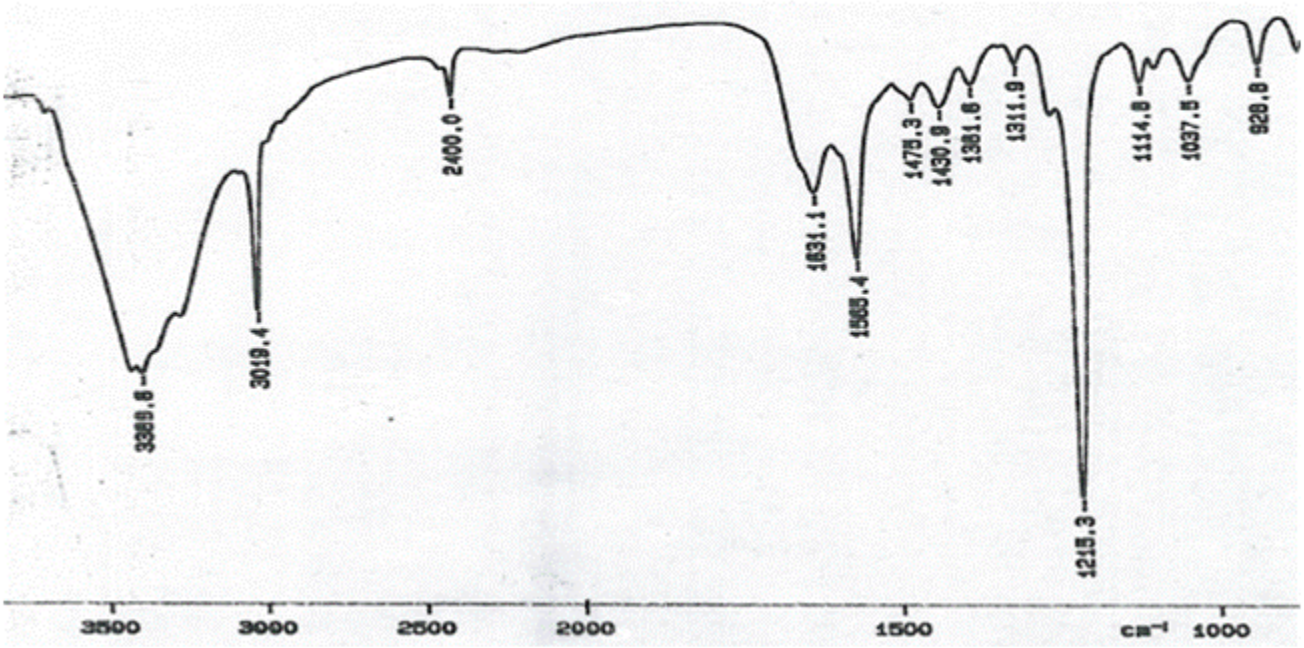
Appendix 18a: EI-MS spectrum for compound 144



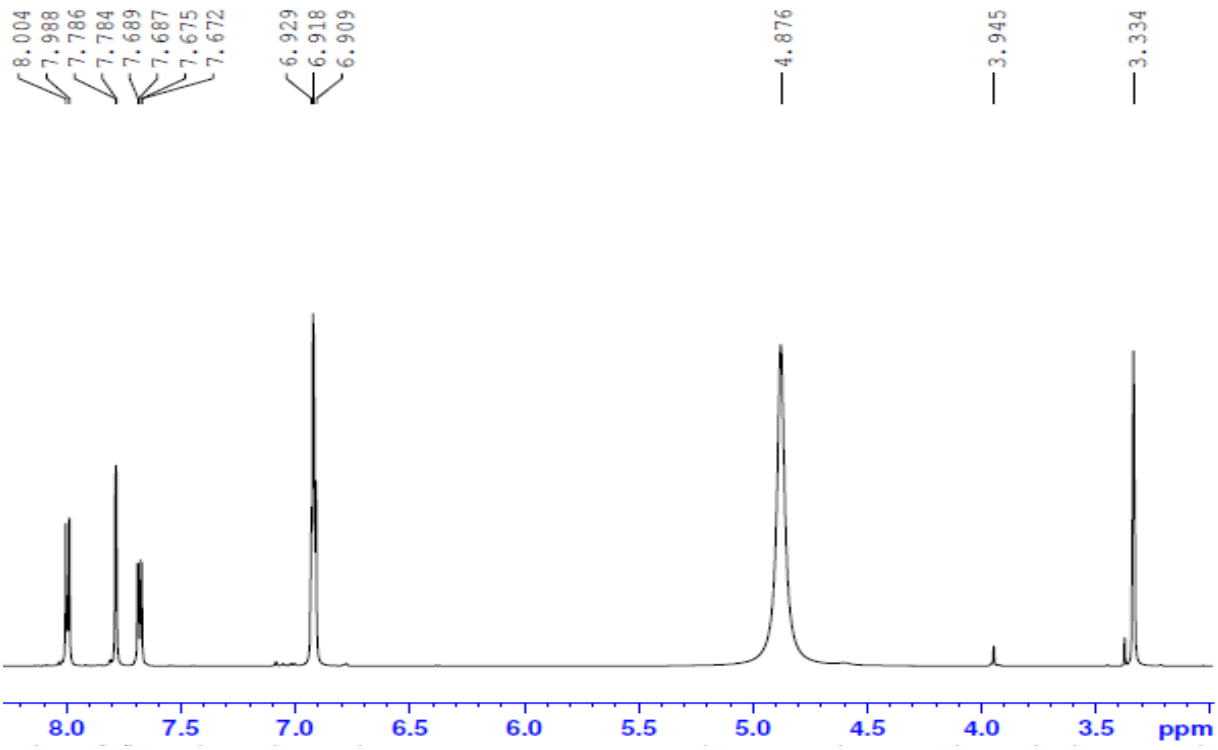
Appendix 18b: ^{1}H NMR spectrum for compound 144



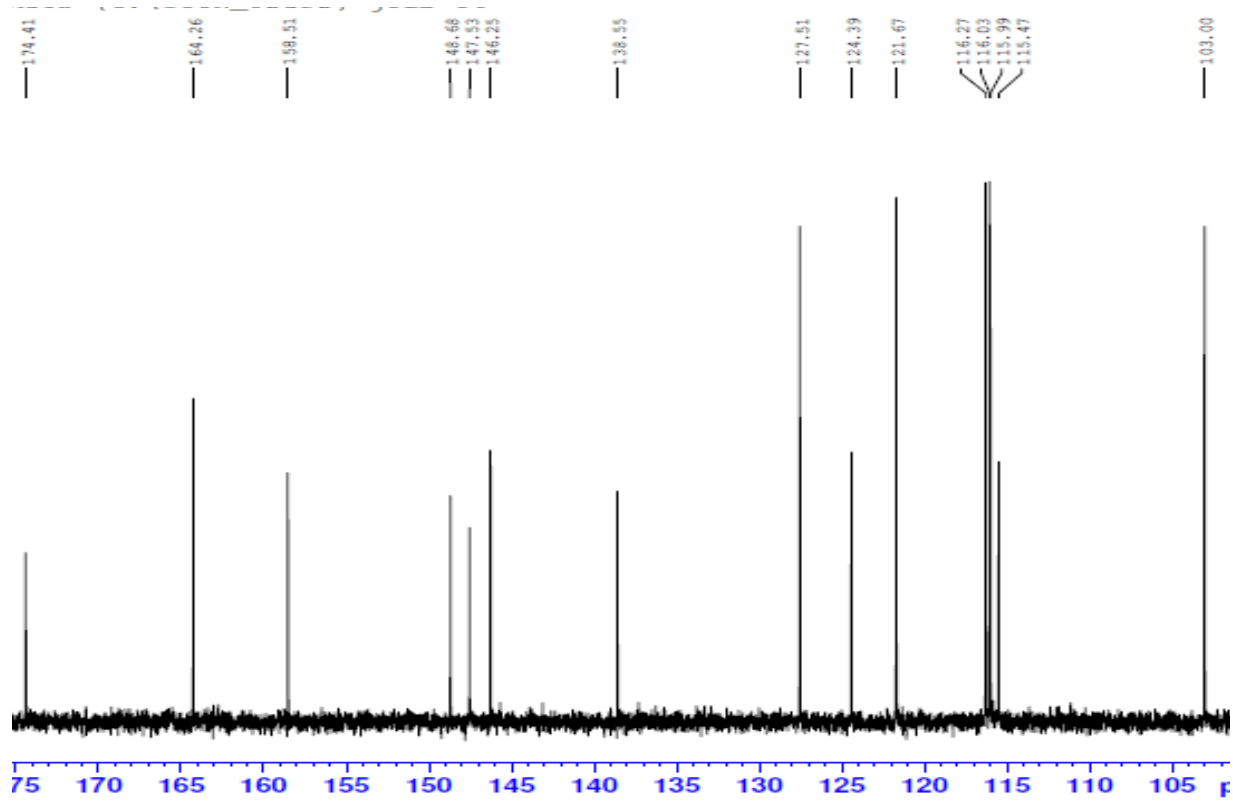
Appendix 18c: ^{13}C NMR spectrum for compound 144



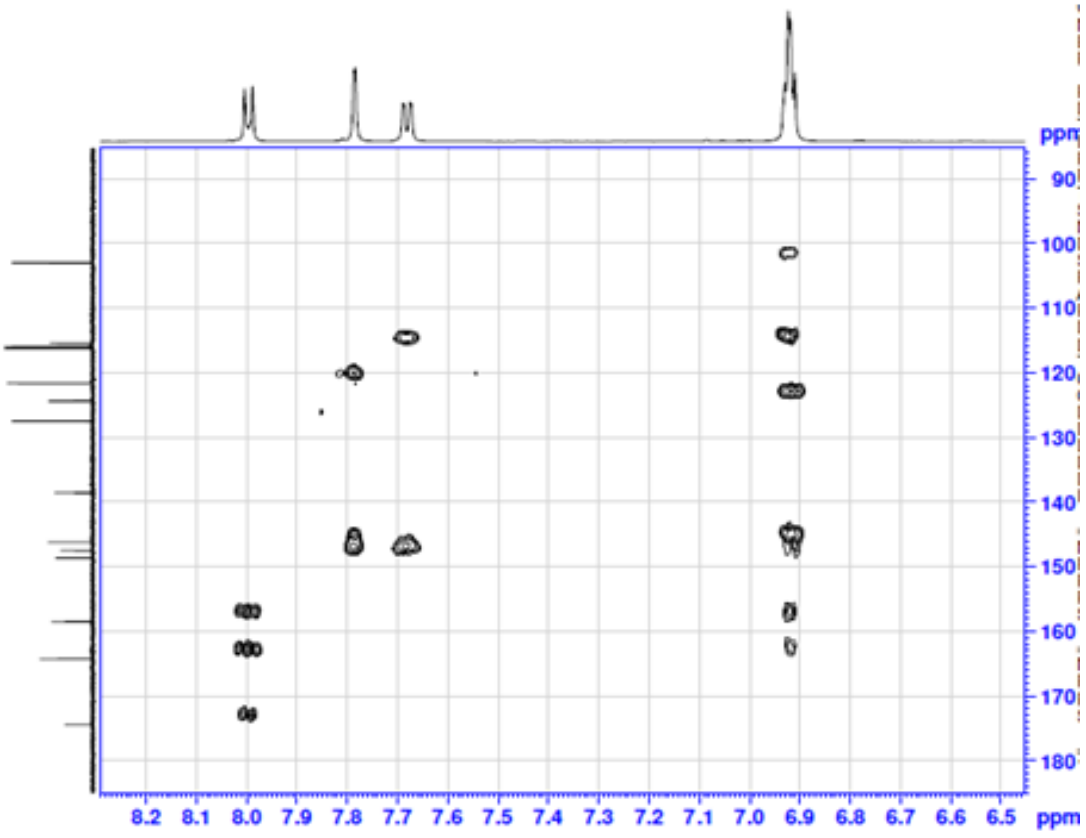
Appendix 19a: IR spectrum for compound 213



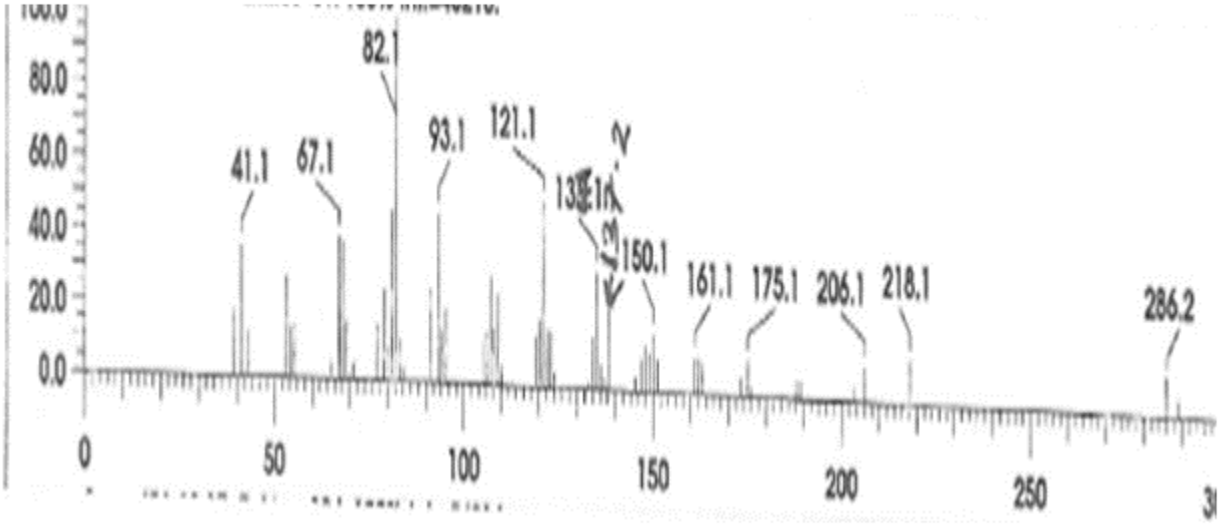
Appndix 19b: ^{1}H NMR spectrum for compound 213



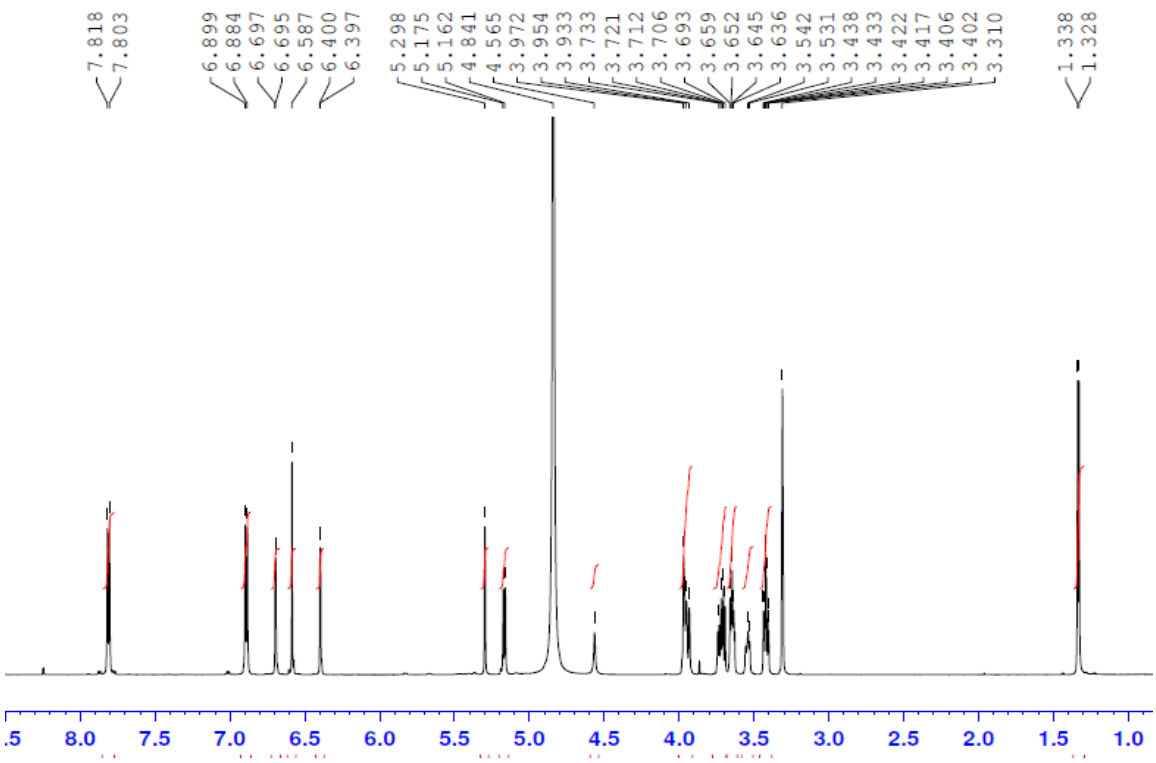
Appndix 19c: ^{13}C NMR spectrum for compound 213



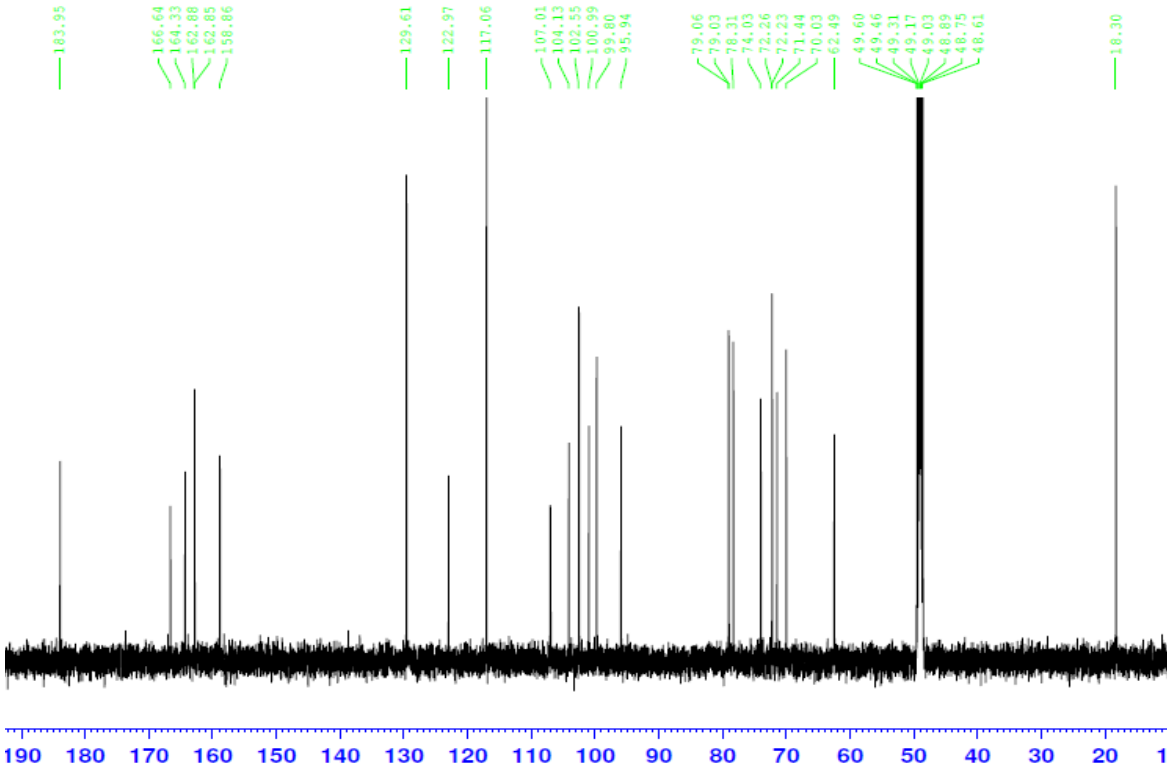
Appendix 19d: HMBC spectrum for compound 213



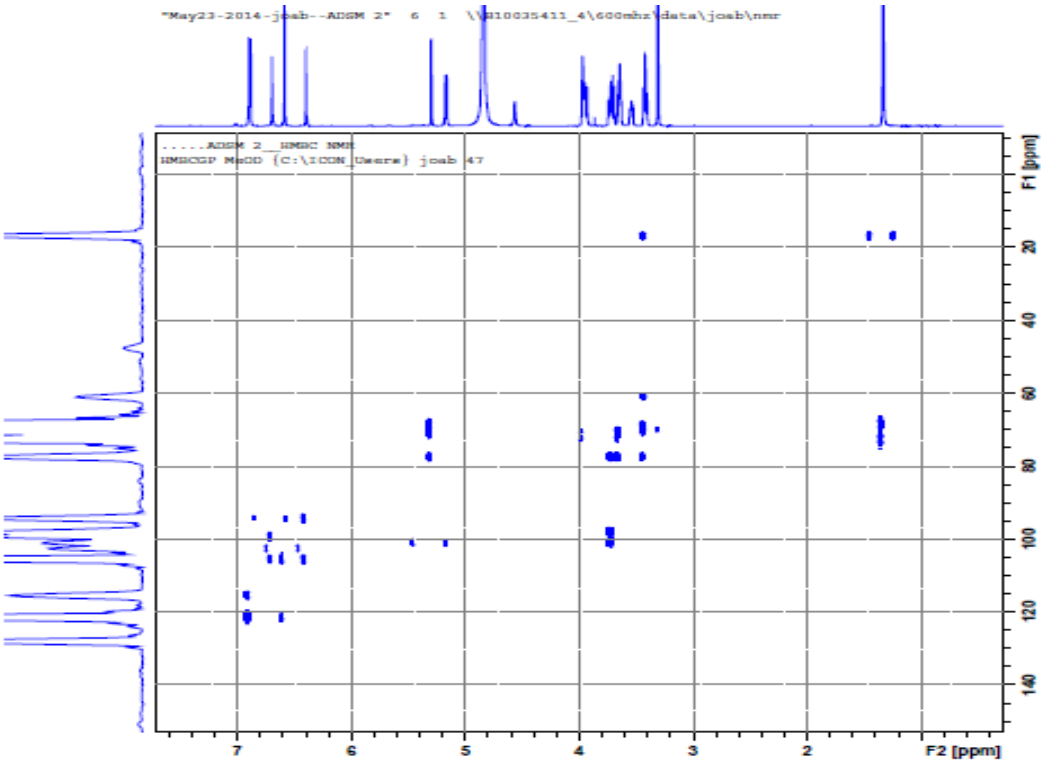
Appendix 19e: EI-MS spectrum for compound 213



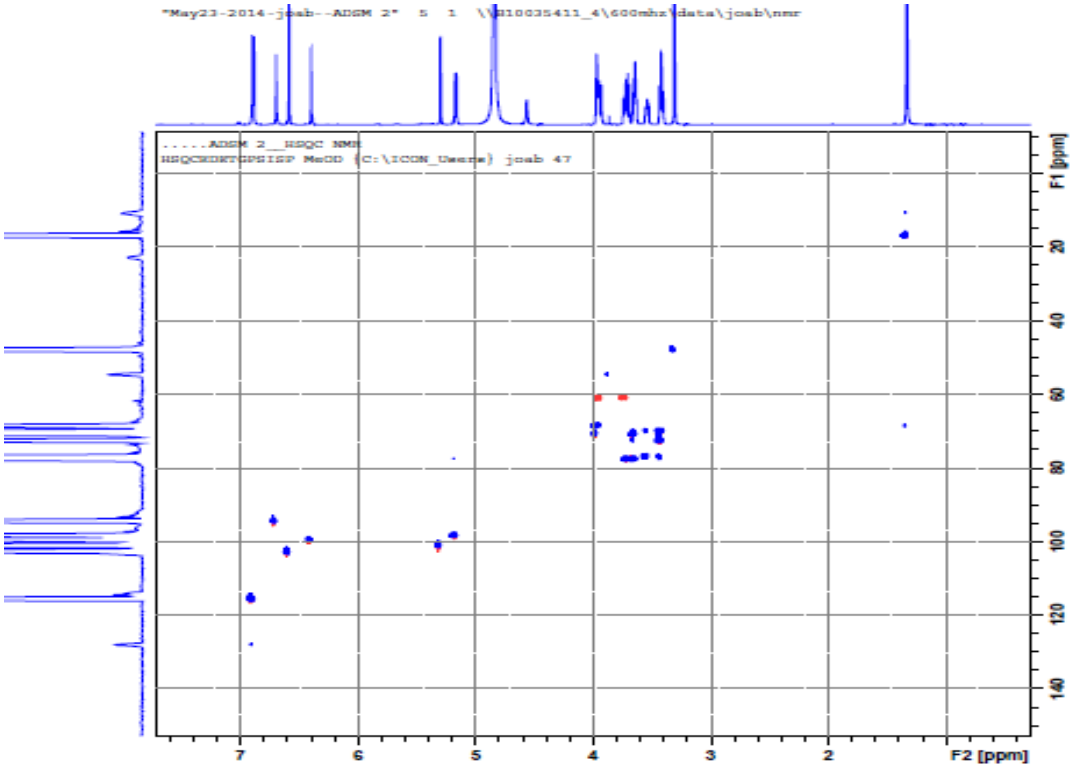
Appendix 20a: ^{1}H NMR spectrum for compound 214



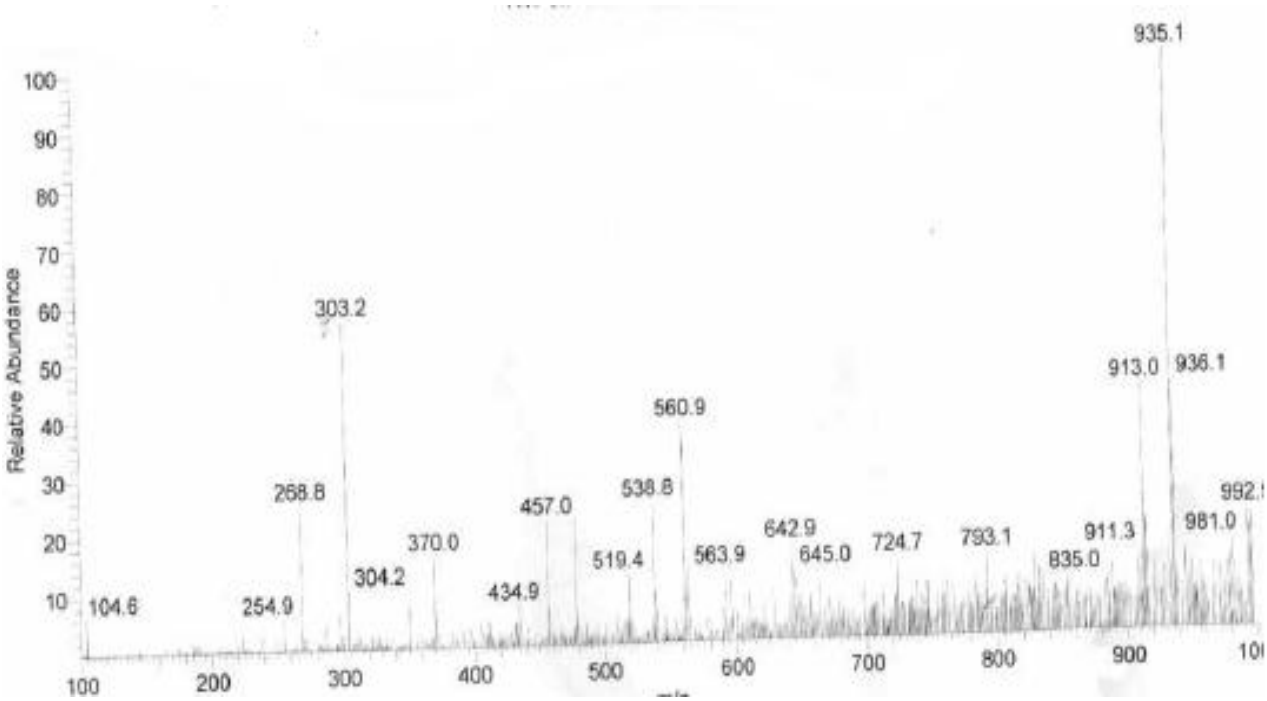
Appendix 20b: ^{13}CNMR spectrum for compound 214



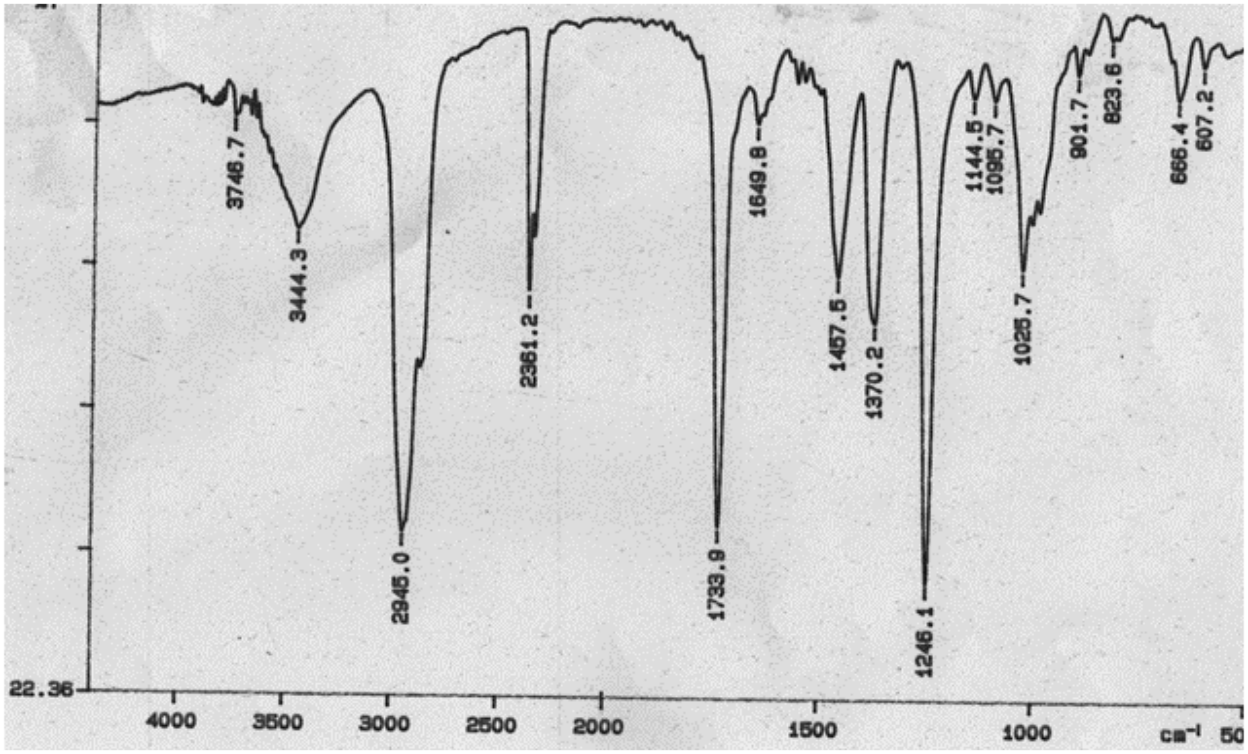
Appendix 20c: HMBC spectrum for compound 214



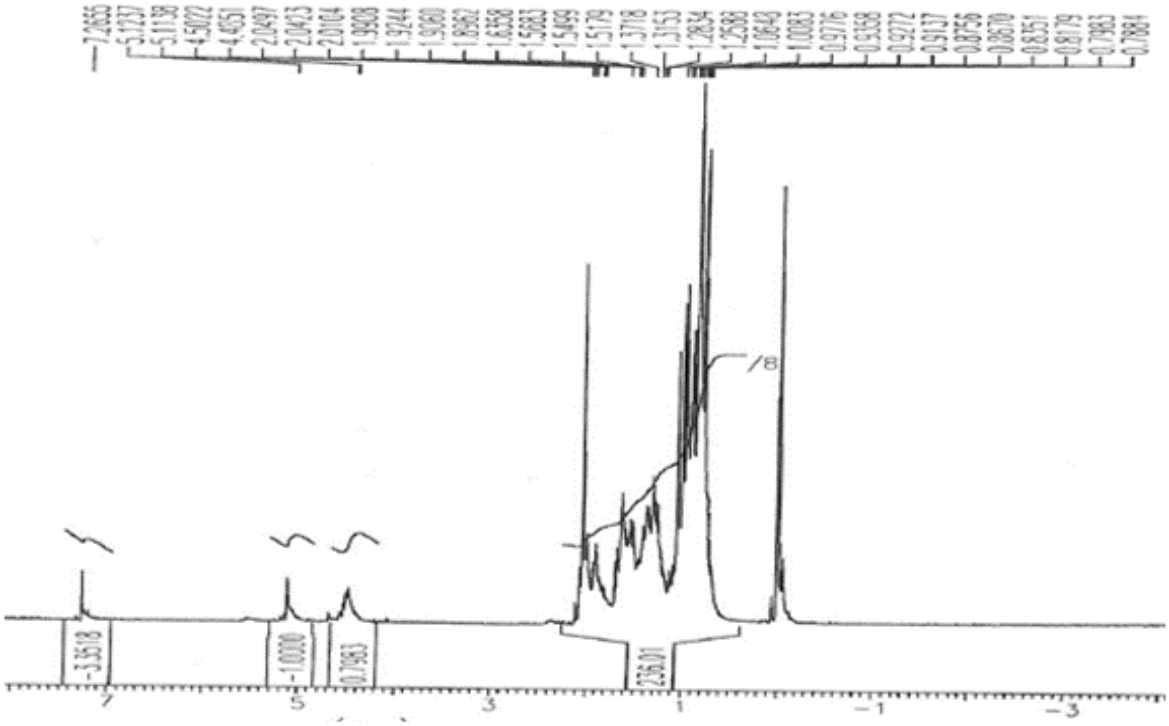
Appendix 20d: HSQC spectrum for compound 214



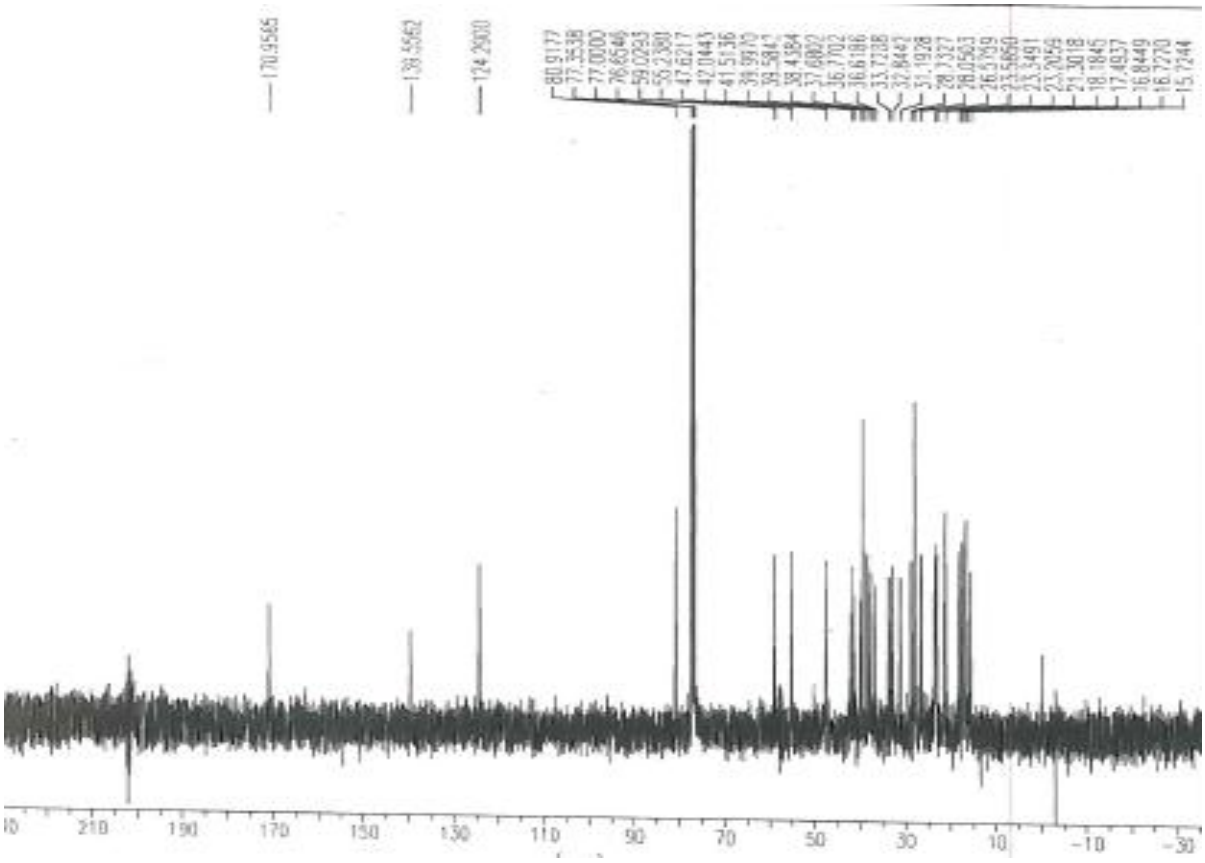
Appendix 20e: ESI-MS spectrum for compound 214



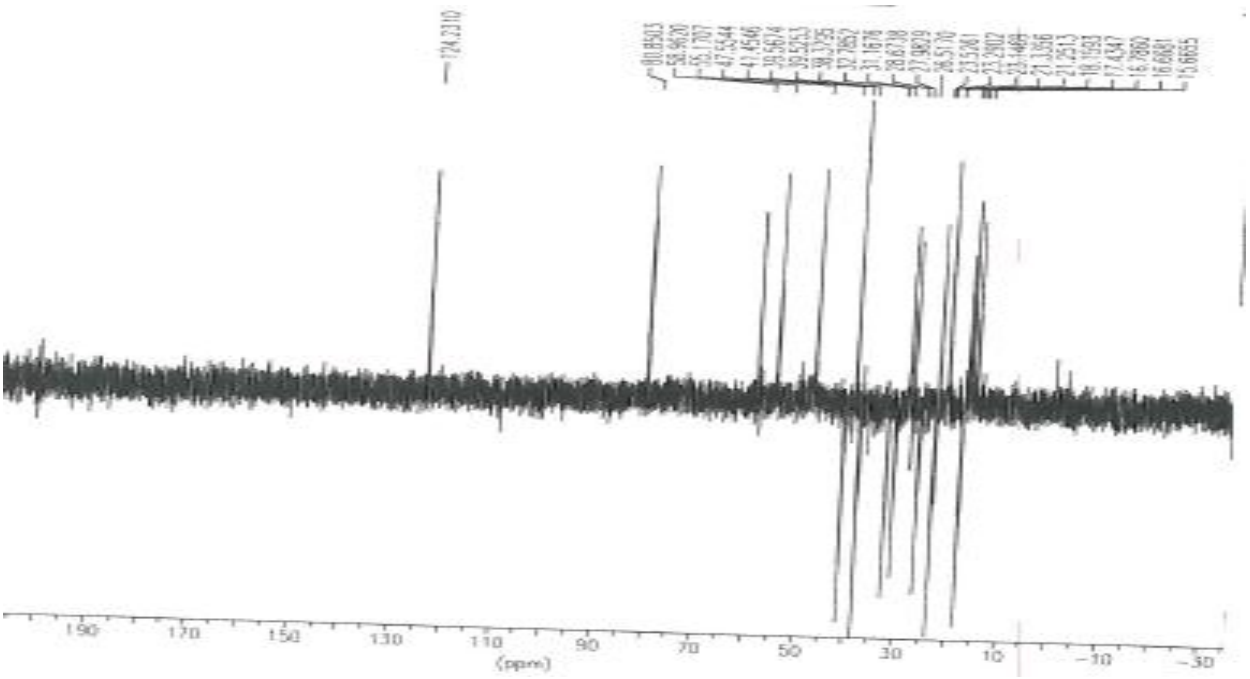
Appendix 21a: IR spectrum for compound 215



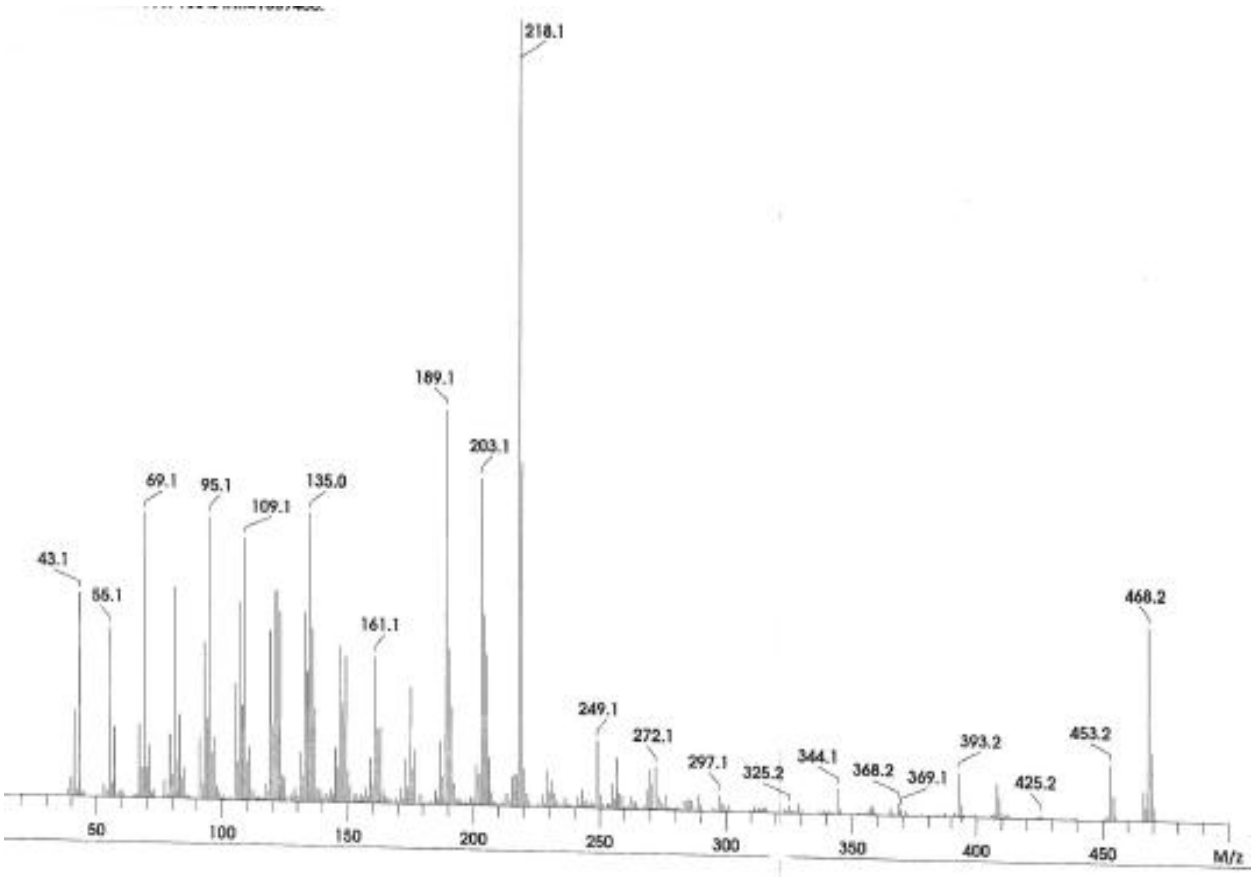
Appendix 21b: ^{1}H NMR spectrum for compound 215



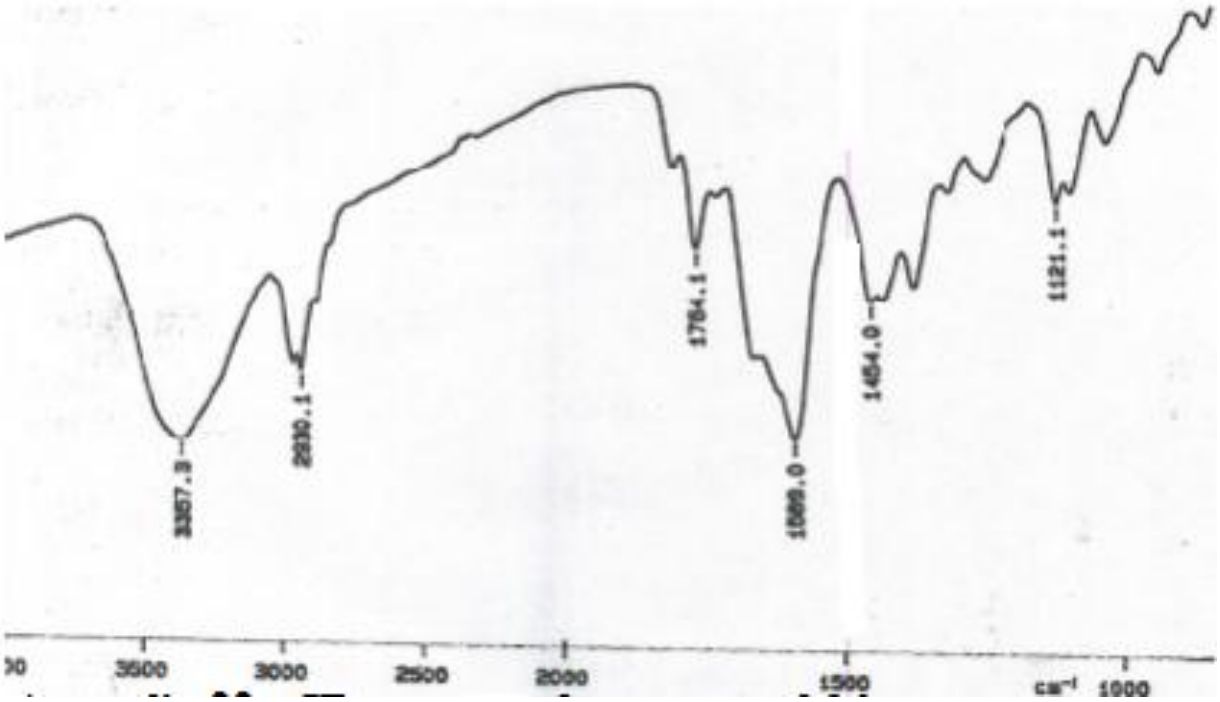
Appendix 21c: ^{13}C NMR spectrum for compound 215



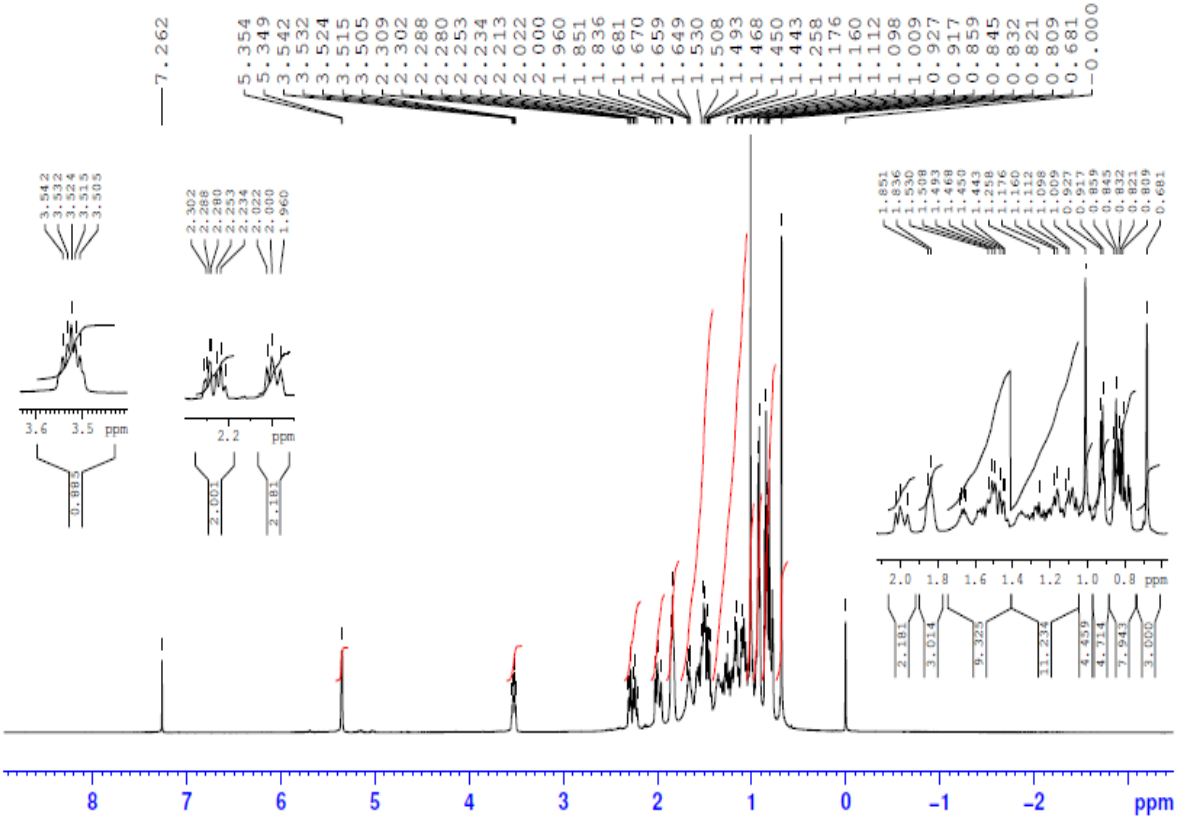
Appendix 21d: DEPT-135 spectrum for compound 215



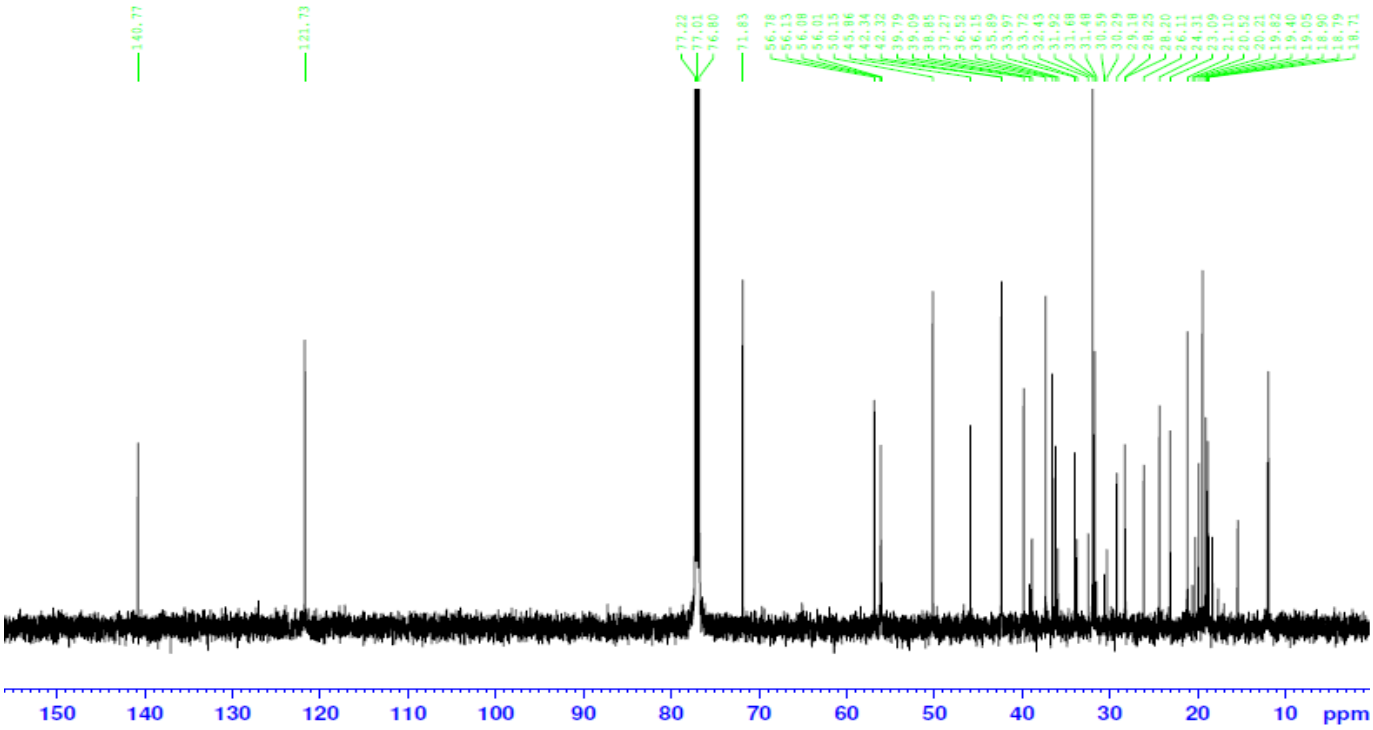
Appendix 21e: EI-MS spectrum for compound 215



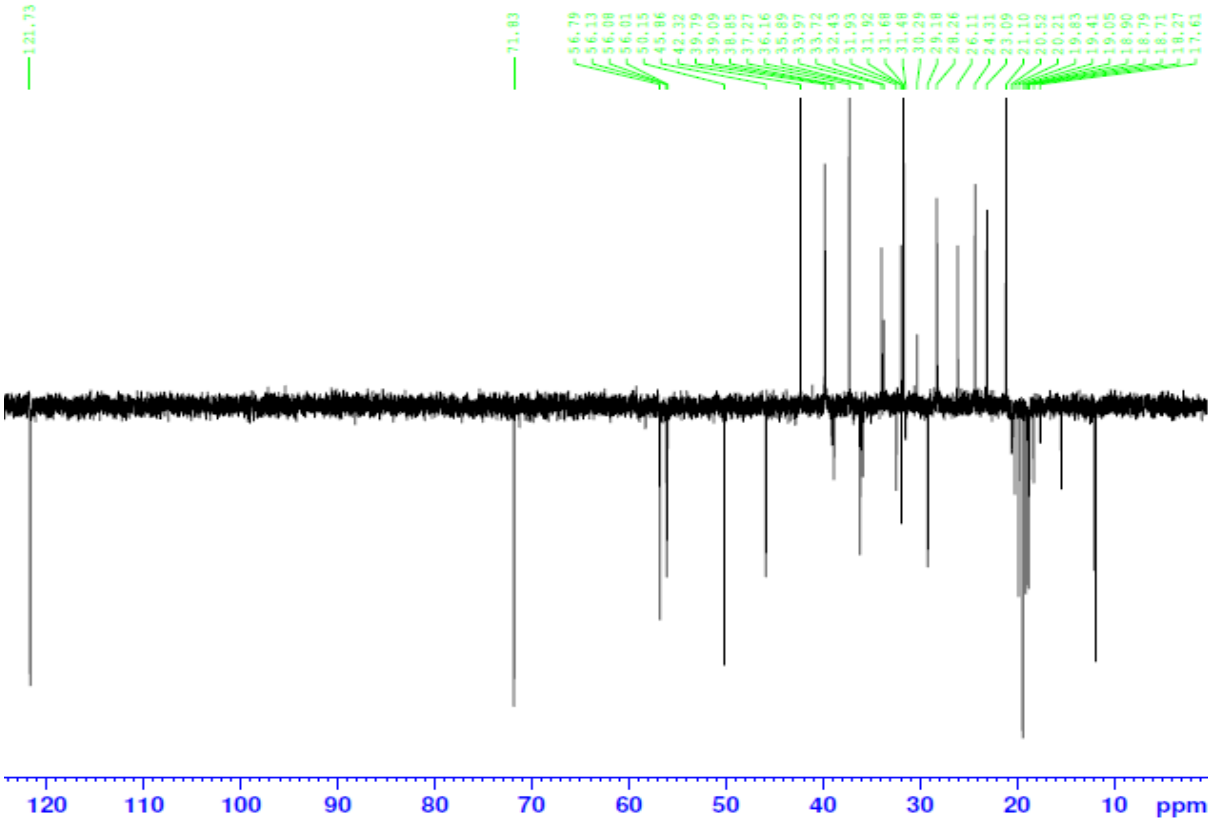
Appendix 22a: IR spectrum for compound 95



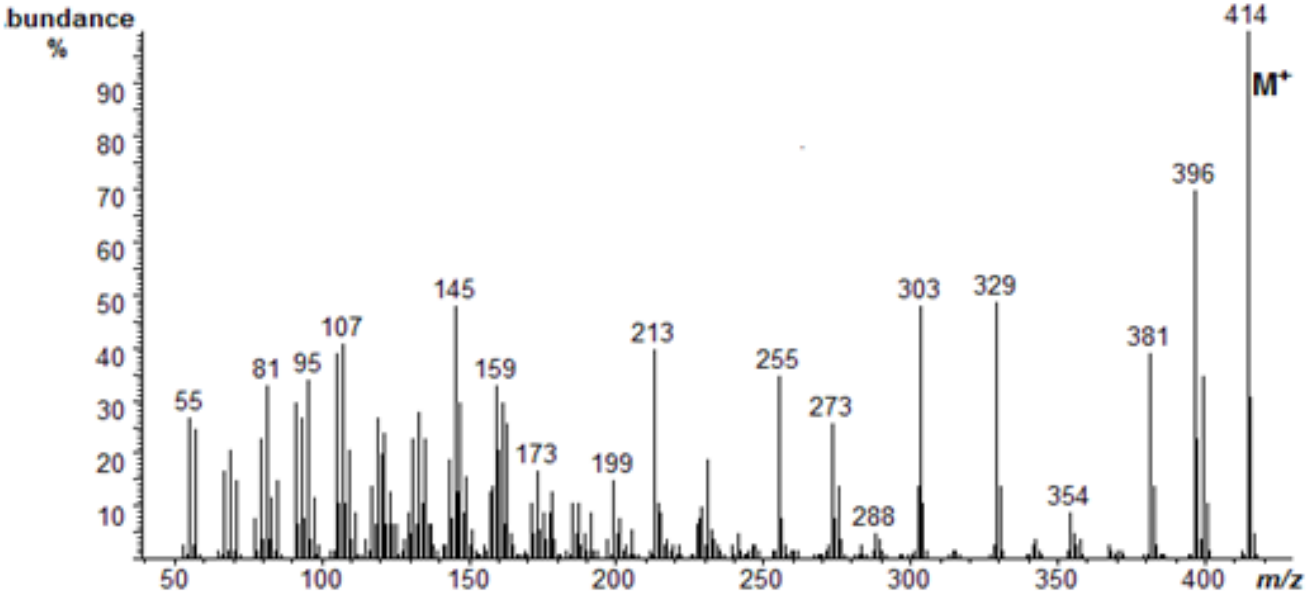
Appendix 22b:^{1}HNMR spectrum for compound 95



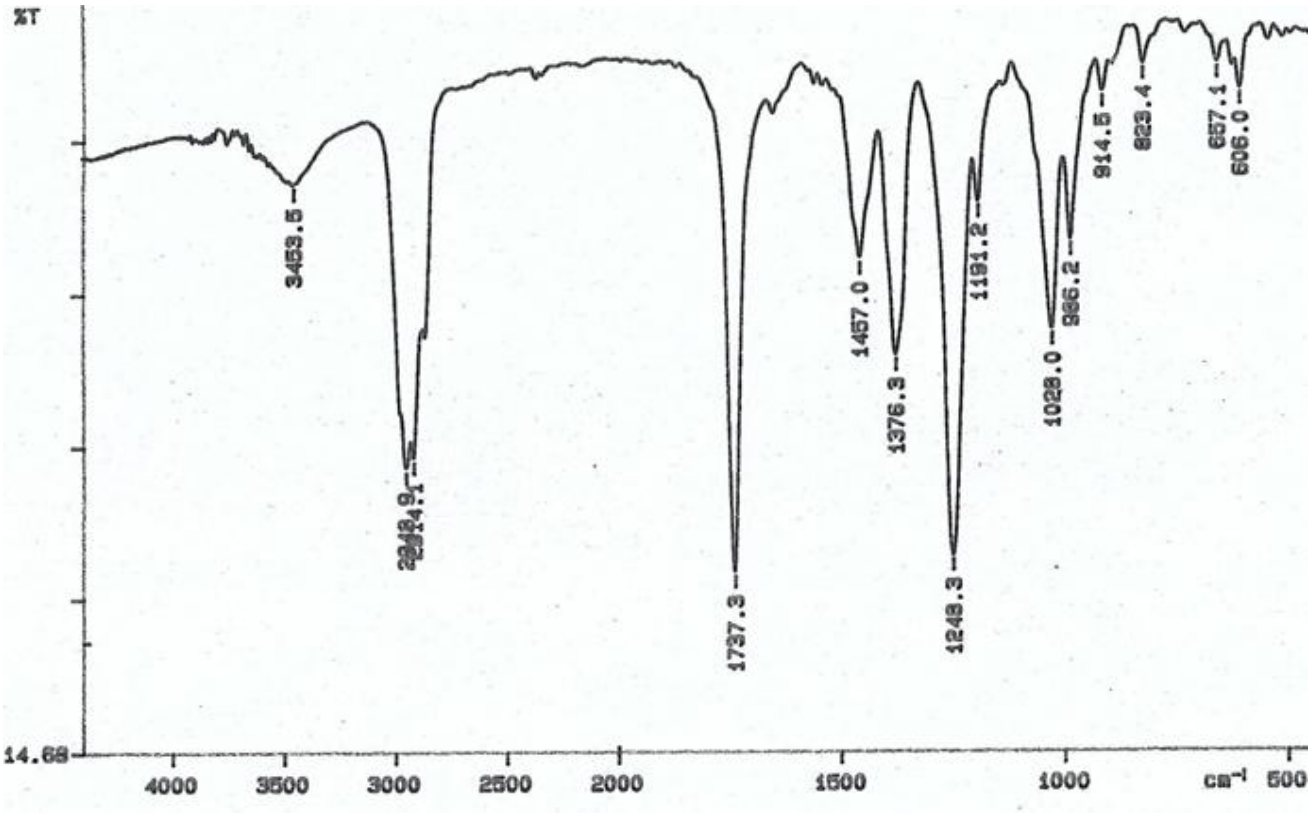
Appendix 22c: ^{13}C NMR spectrum for compound 95



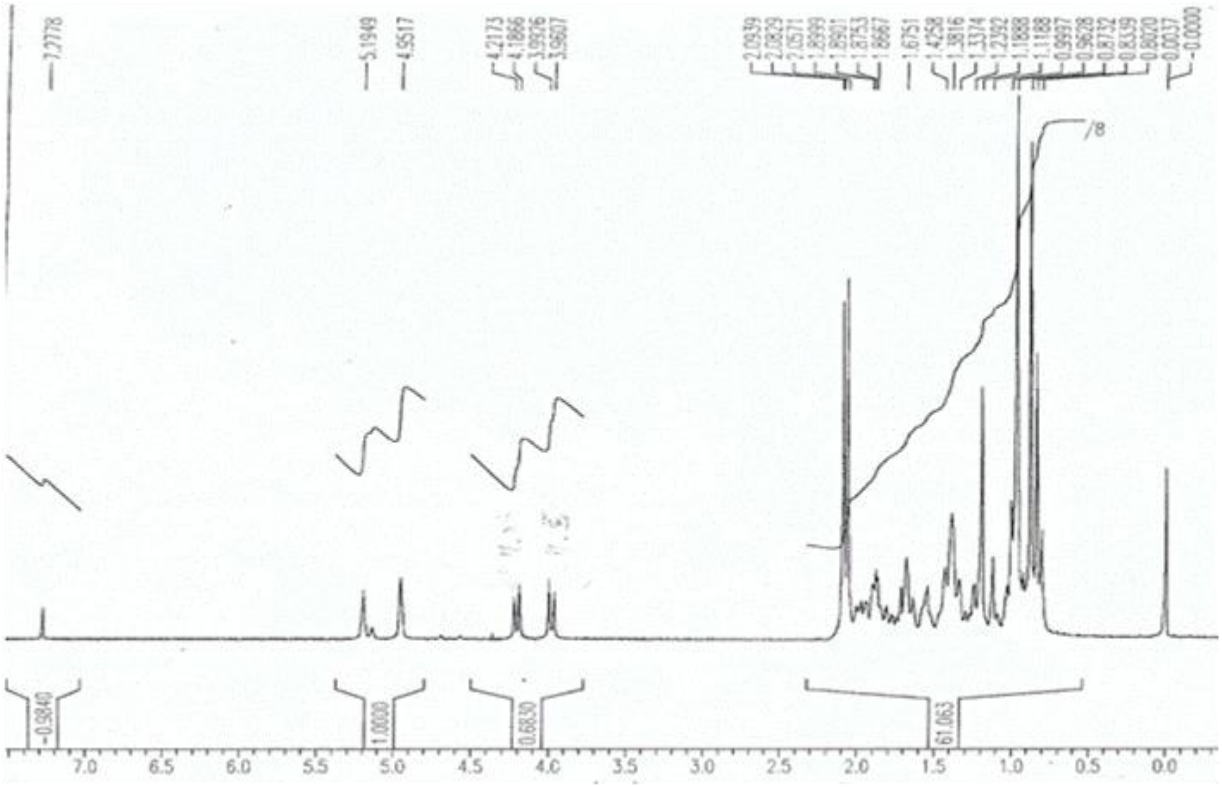
Appendix 22d: DEPT-135 spectrum for compound 95



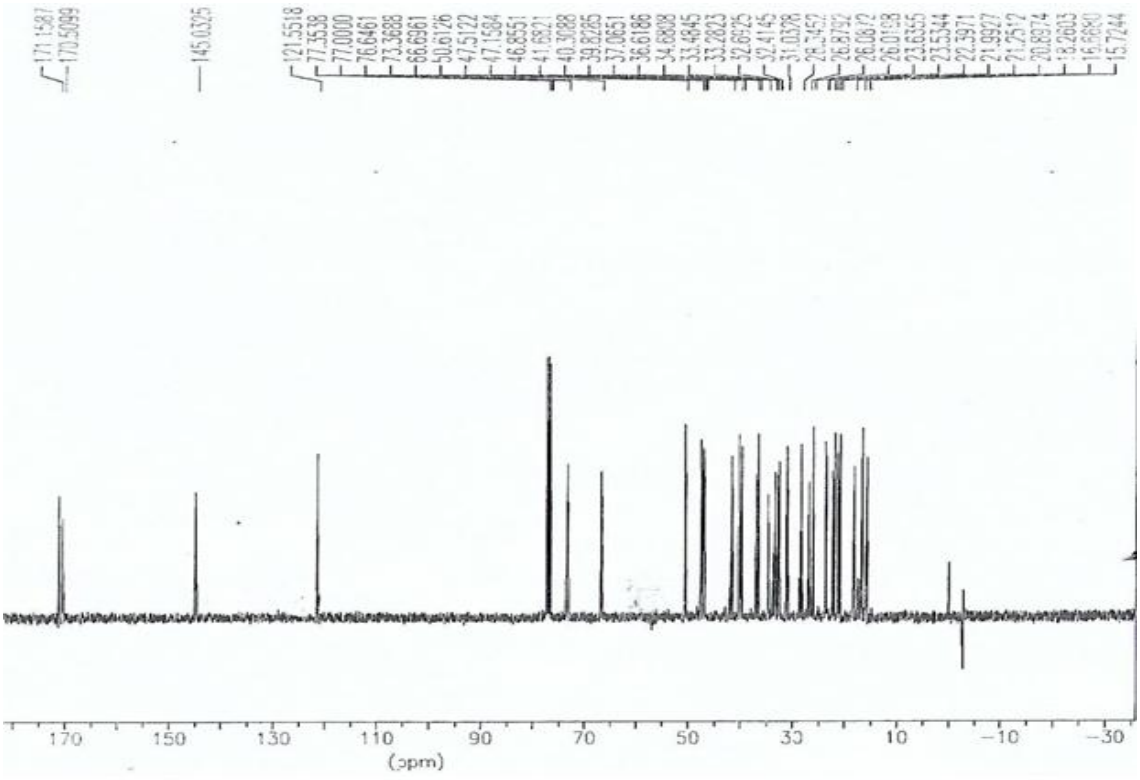
Appendix 22e: EI-MS spectrum for compound 95



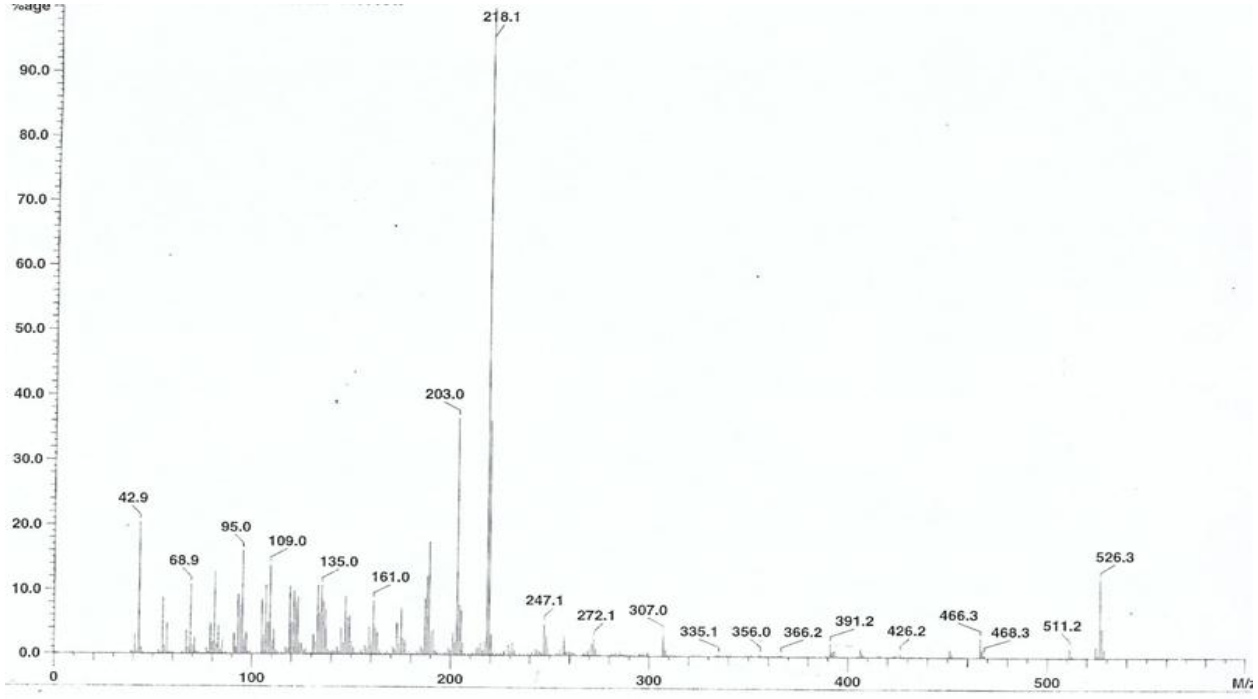
Appendix 23a: IR spectrum for compound 216



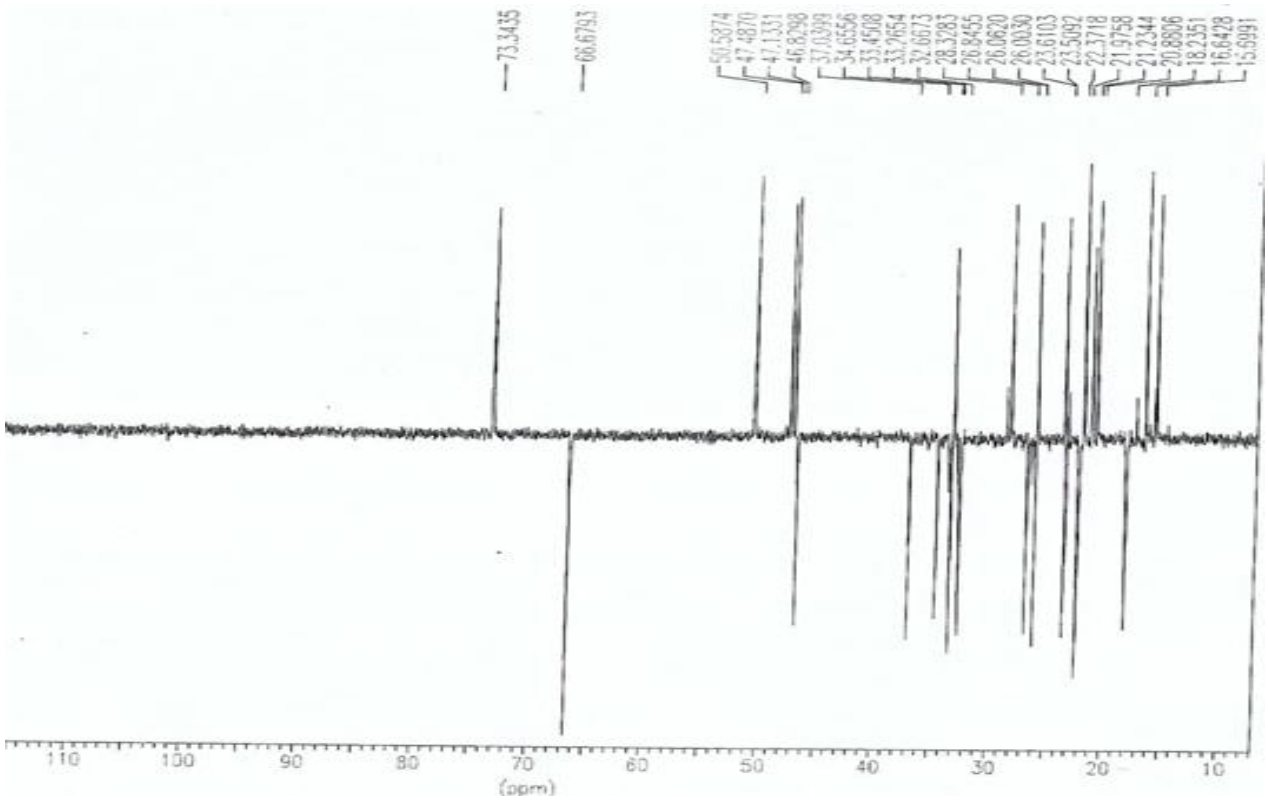
Appendix 23b: ^{1}H NMR spectrum for compound 216



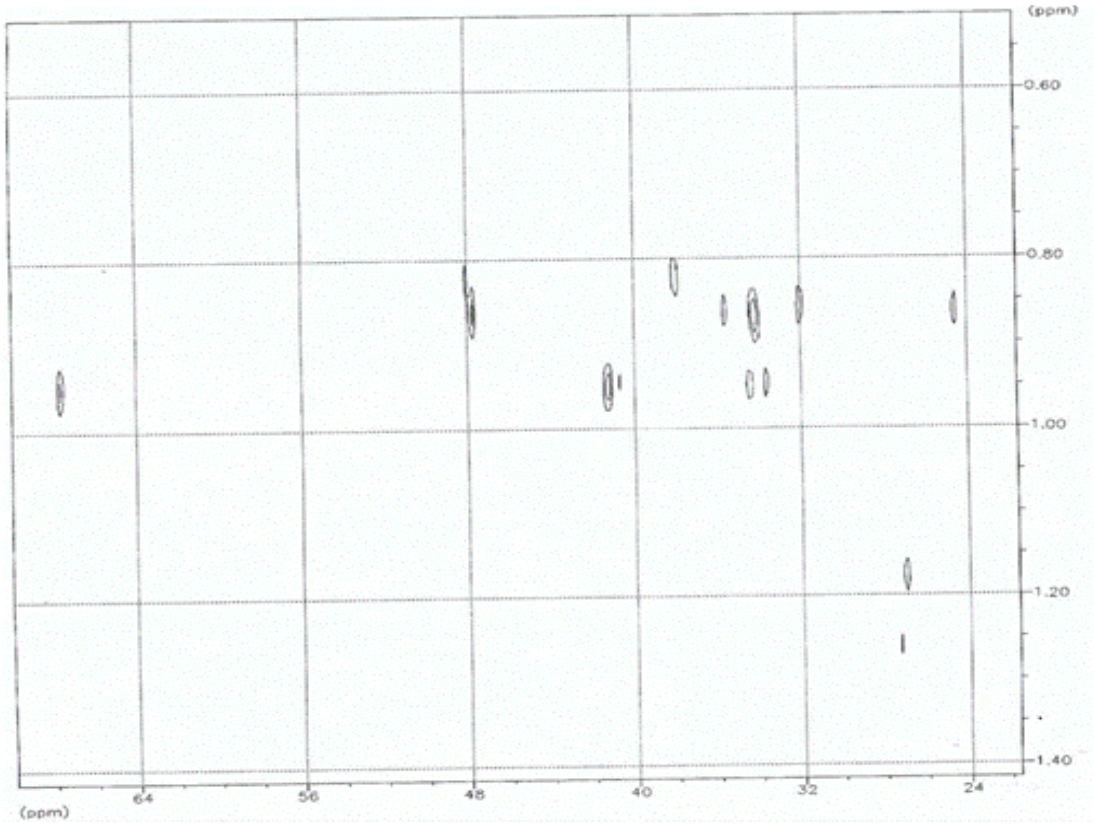
Appendix 23c: ^{13}C NMR spectrum for compound 216



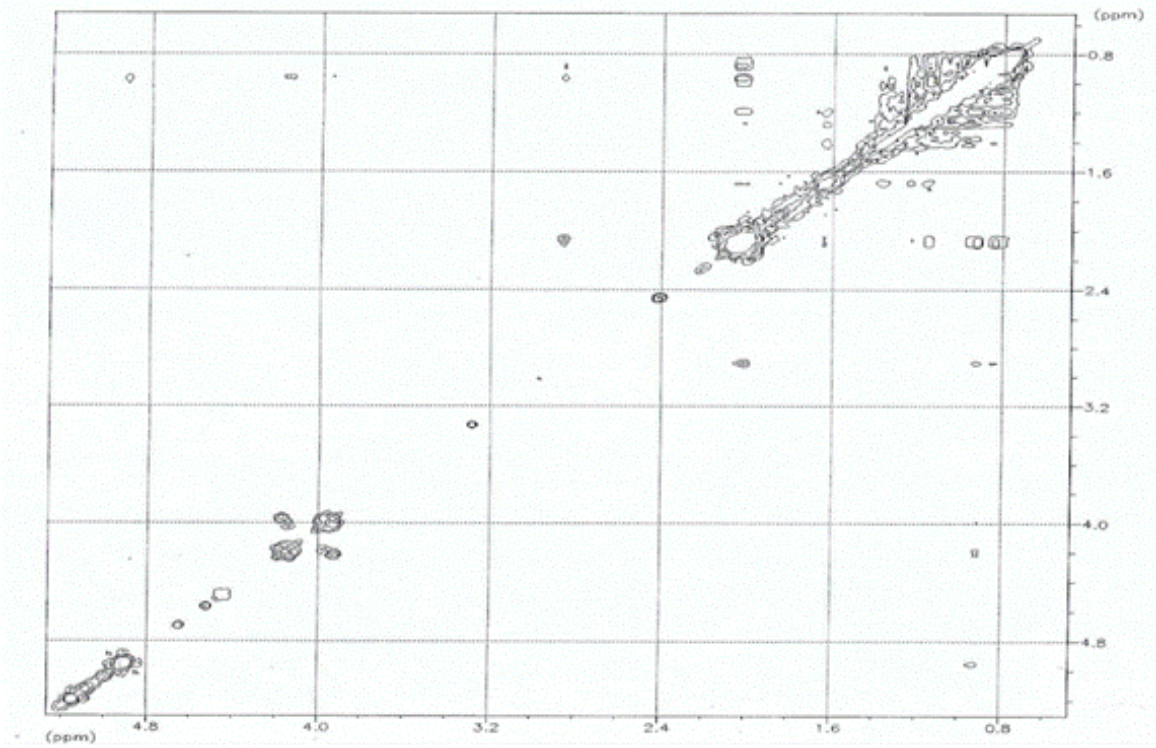
Appendix 23d: EI-MS spectrum for compound 216



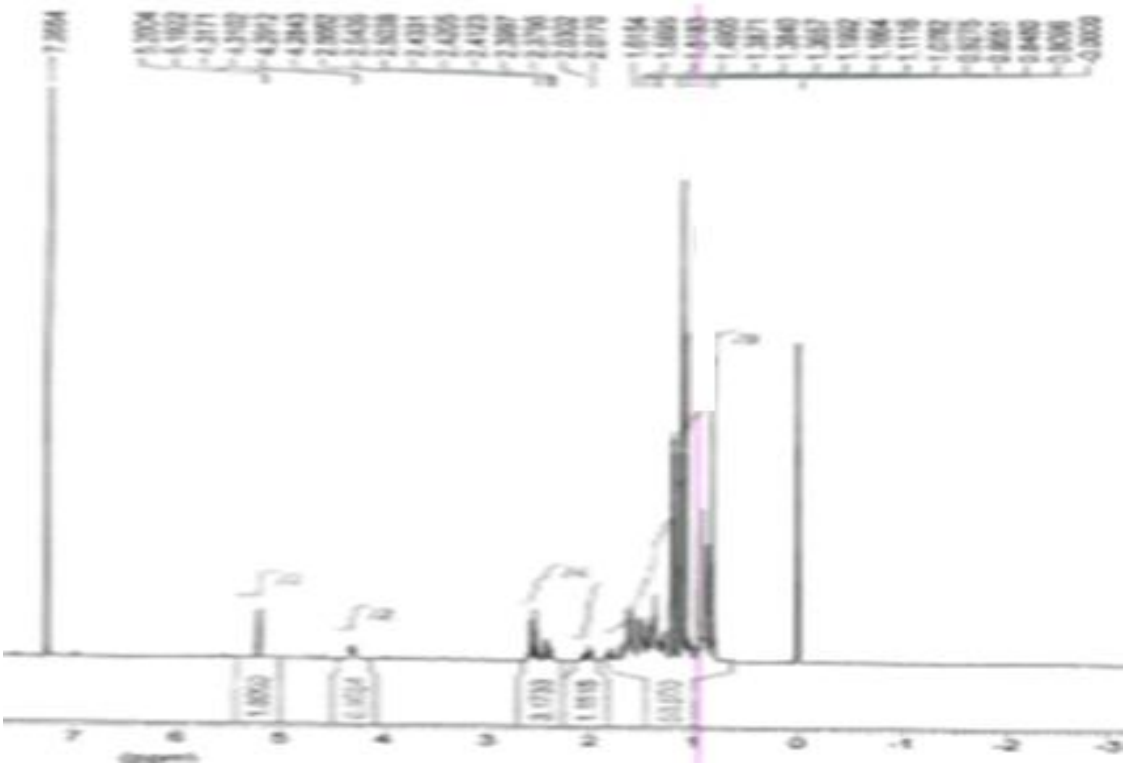
Appendix 23e: DEPT-135 spectrum for compound 216



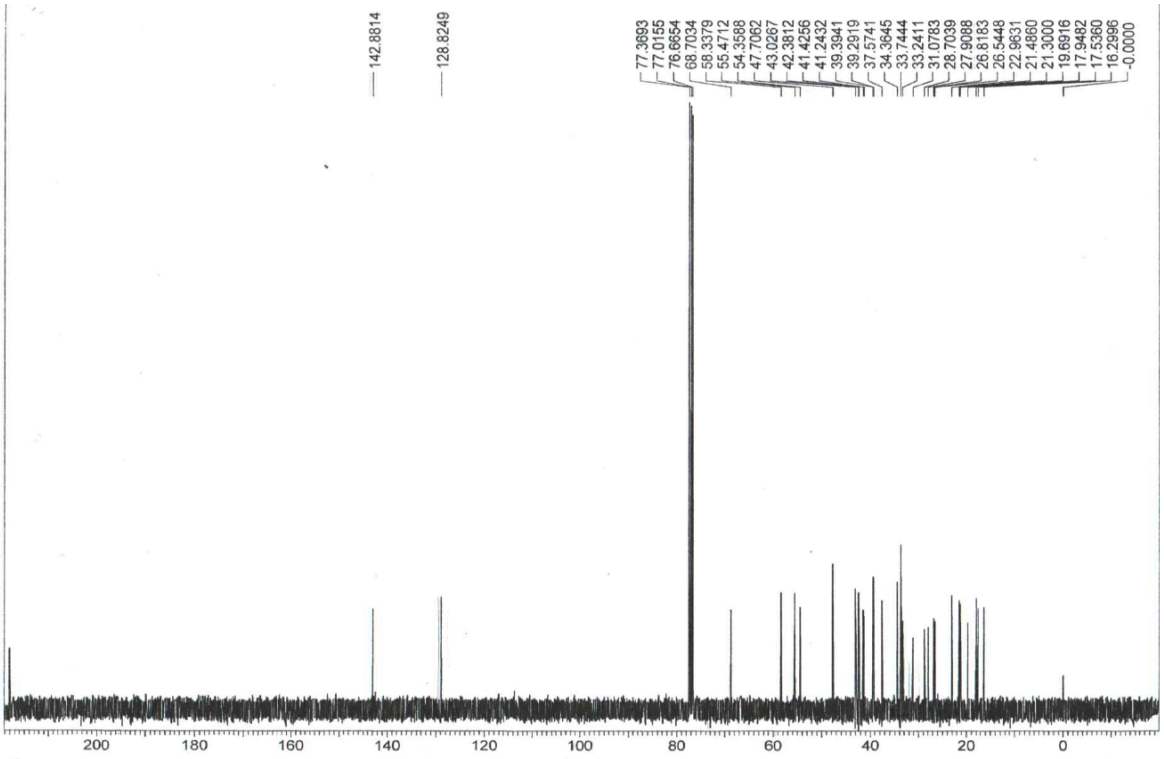
Appendix 23f (i): HMBCNMR spectrum for compound 216



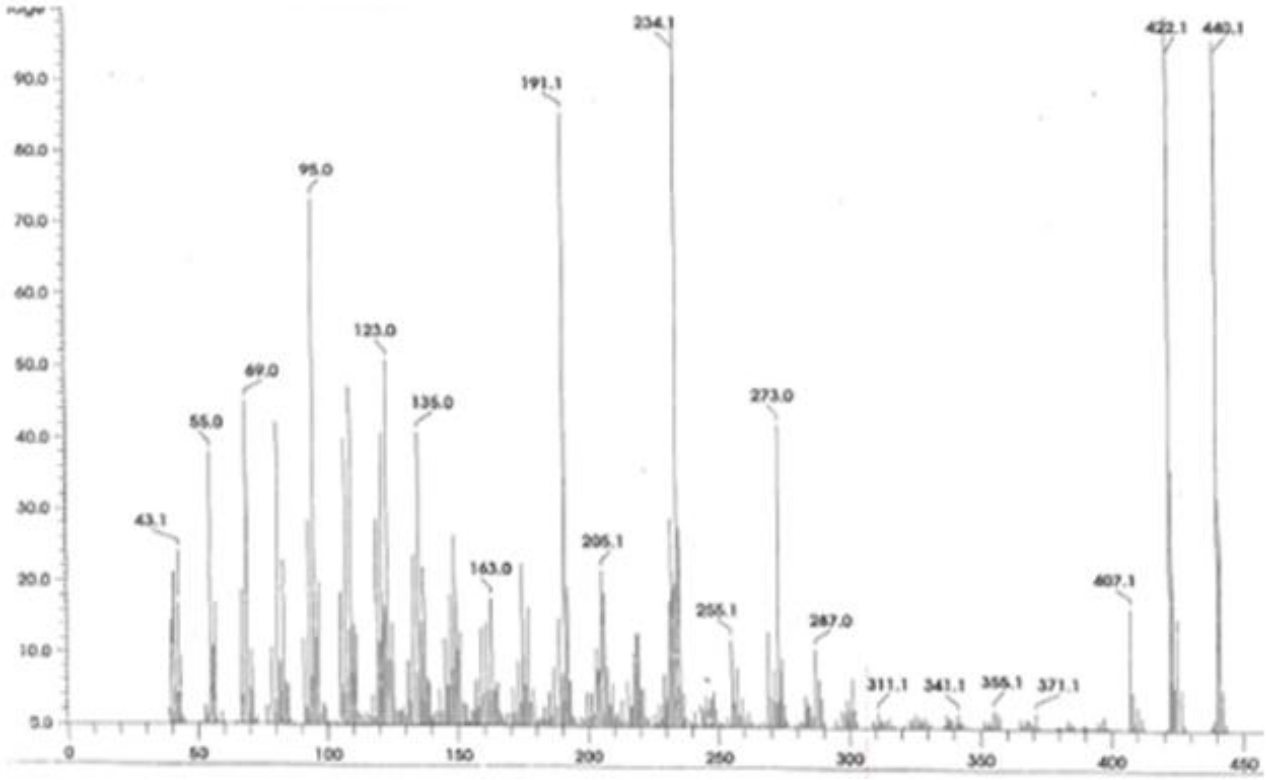
Appendix 23g: ROESY NMR spectrum for compound 216



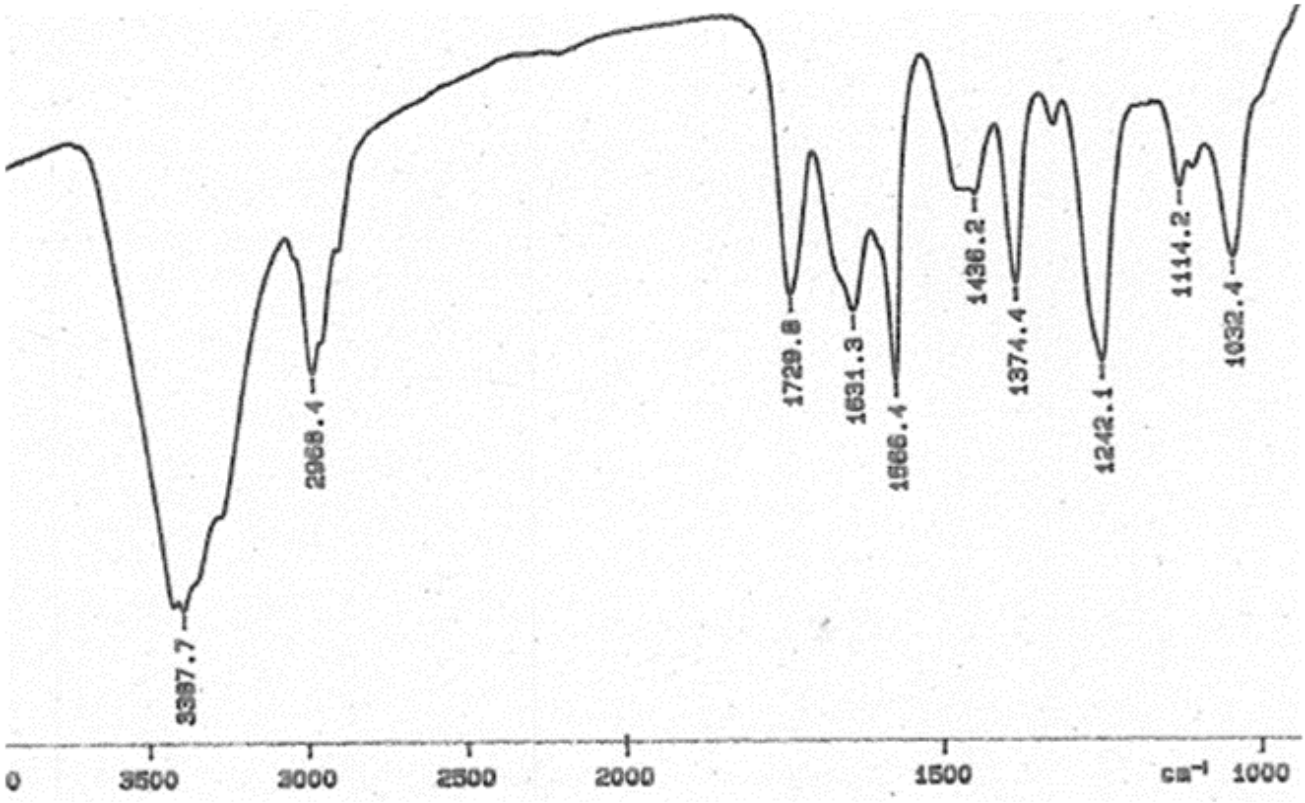
Appendix 24a: ^{1}H NMR spectrum for compound 217



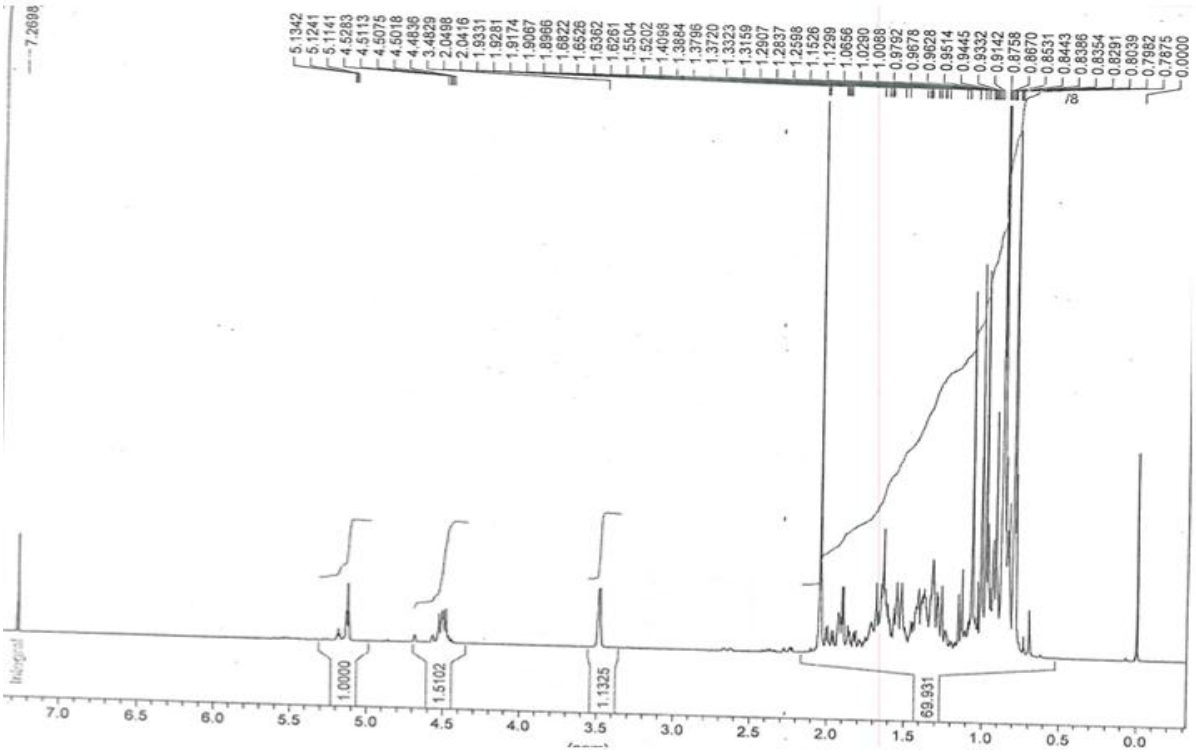
Appendix 24b: ^{13}C NMR spectrum for compound 217



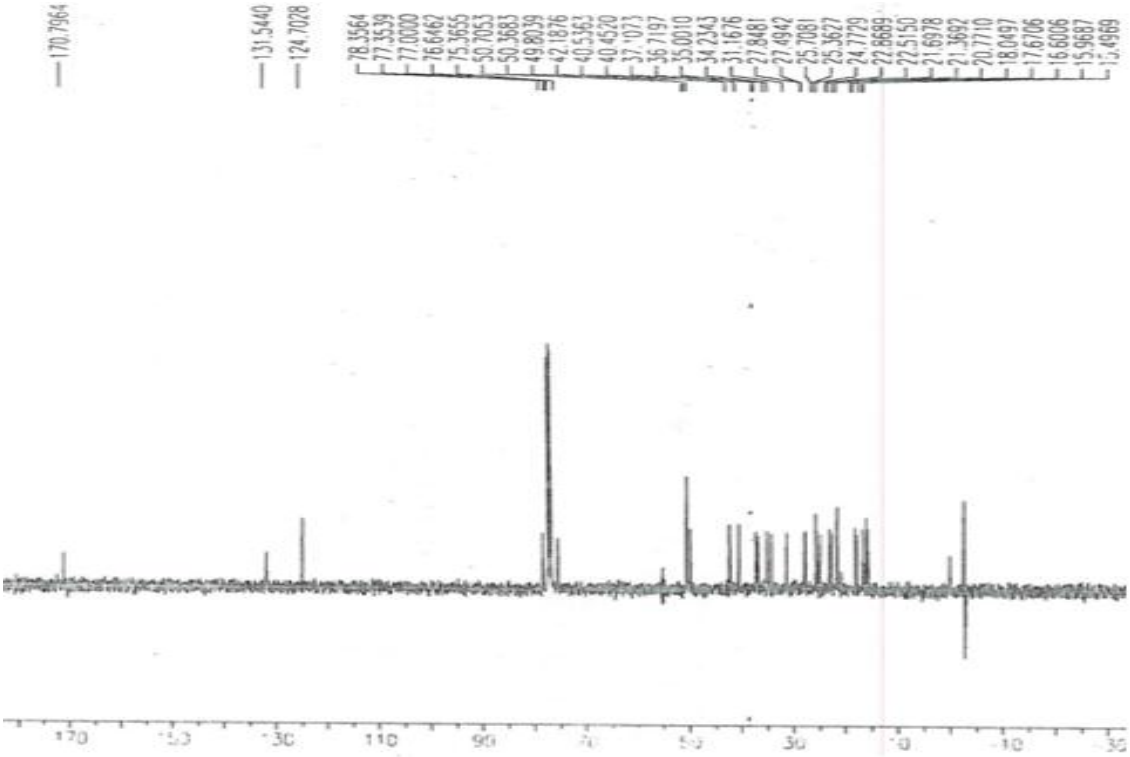
Appendix 24c: EI-MS spectrum for compound 217



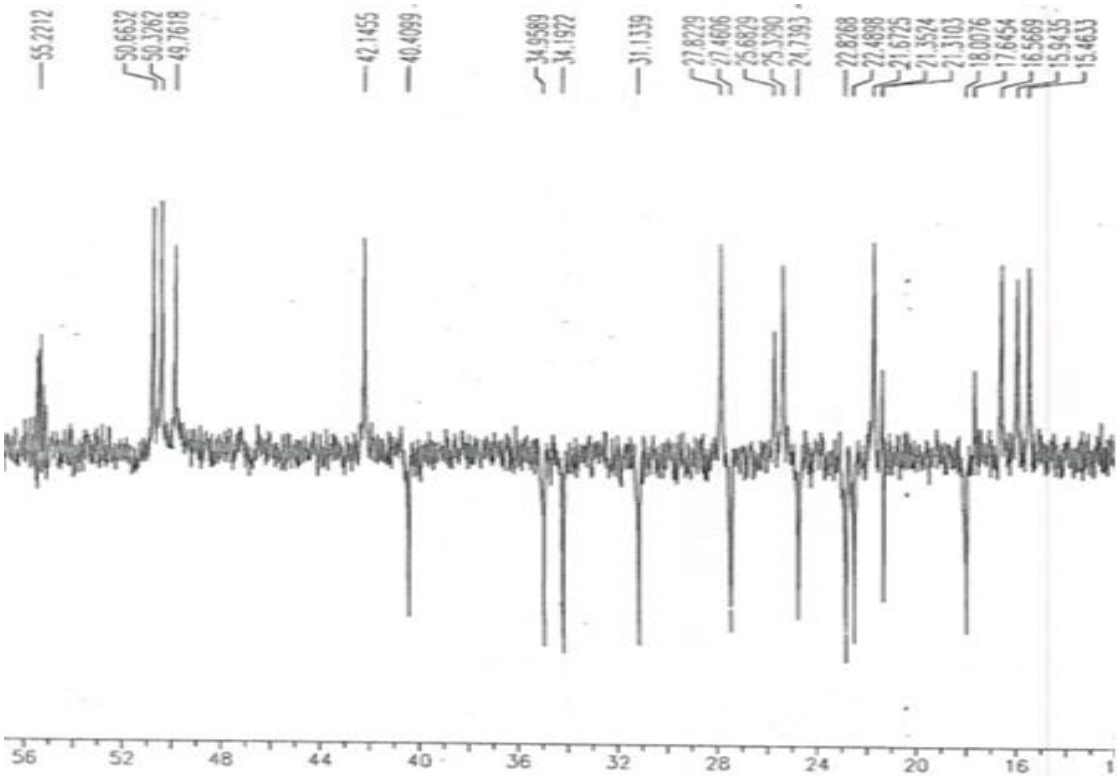
Appendix 25a: IR spectrum for compound 218



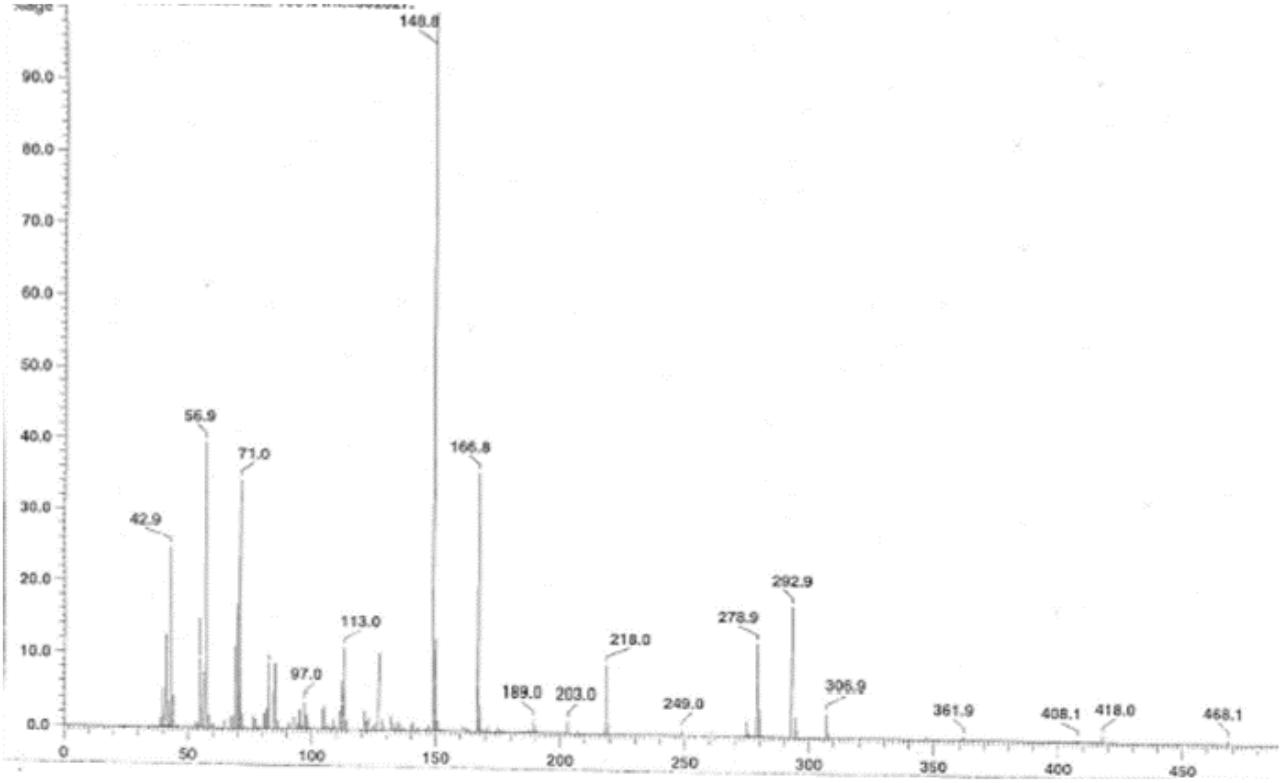
Appendix 25b: ^{1}H NMR spectrum for compound 218



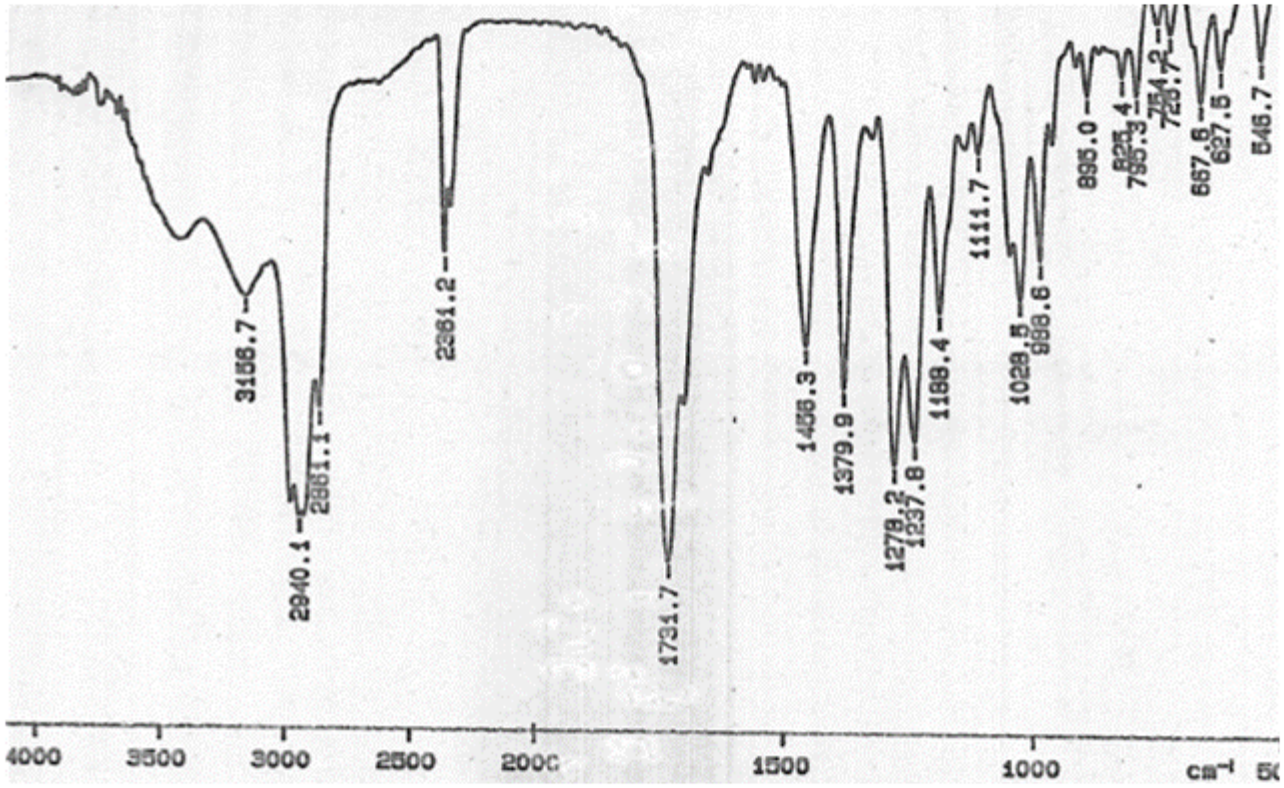
Appendix 25c: ^{13}C NMR spectrum for compound 218



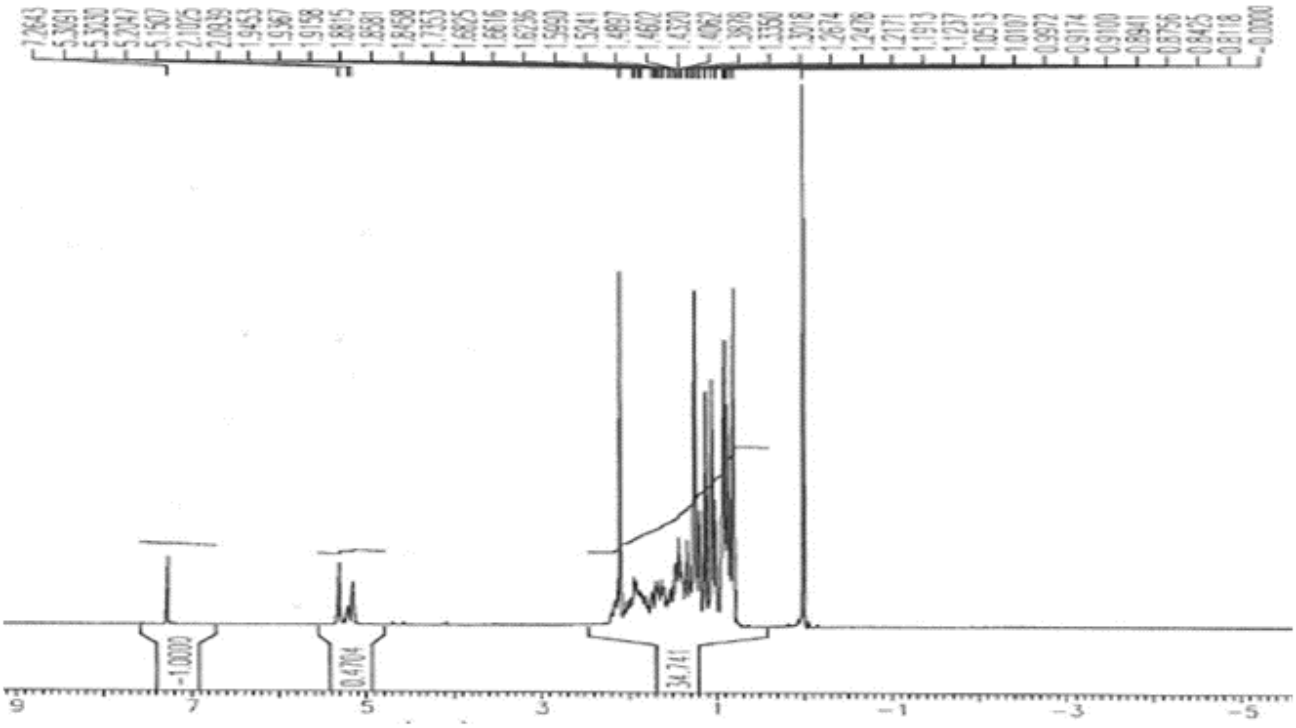
Appendix 25d: DEPT 135 NMR spectrum for compound 218



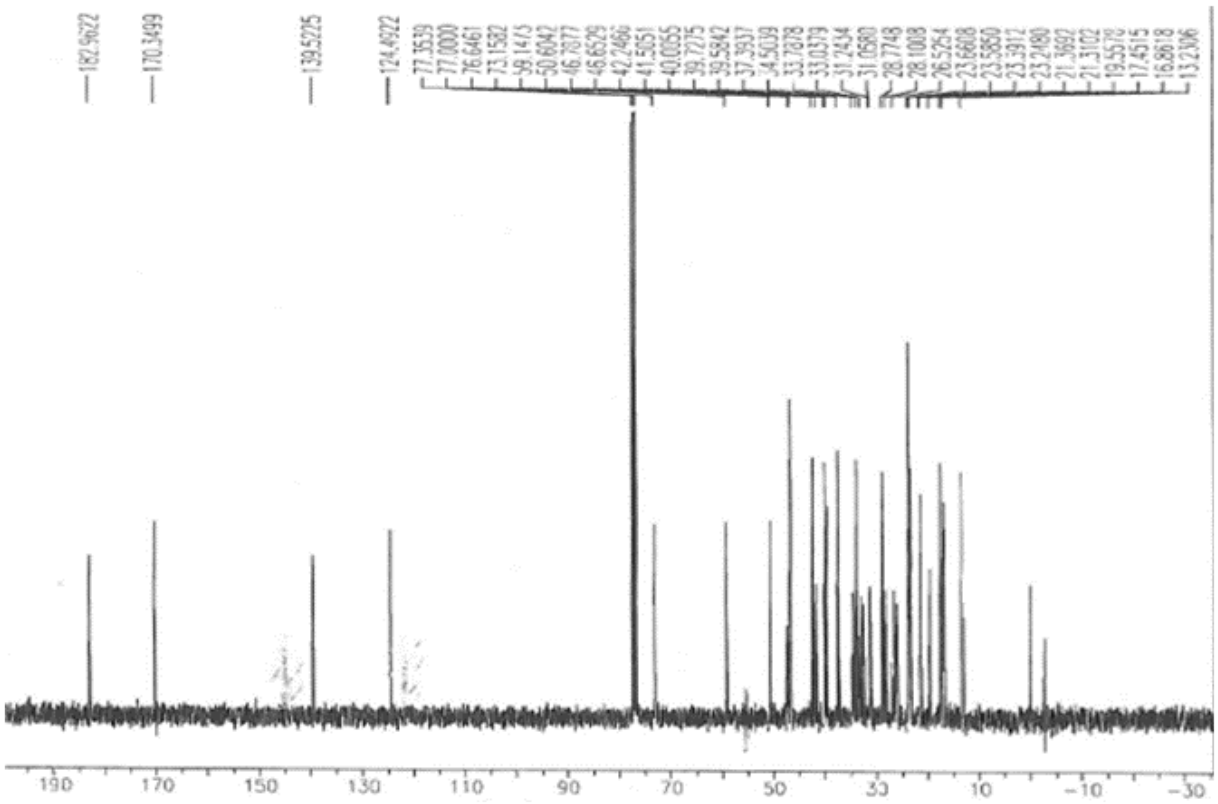
Appendix 25e: EI-MS spectrum for compound 218



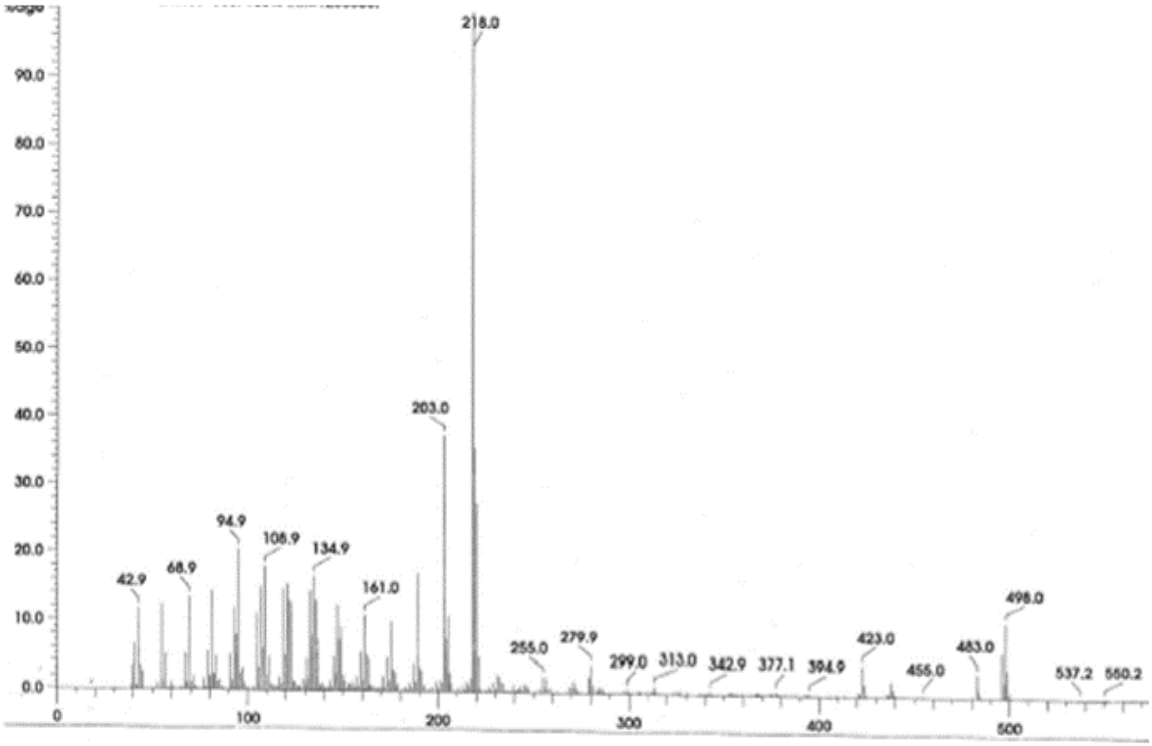
Appendix 26a: IR spectrum for compound 219



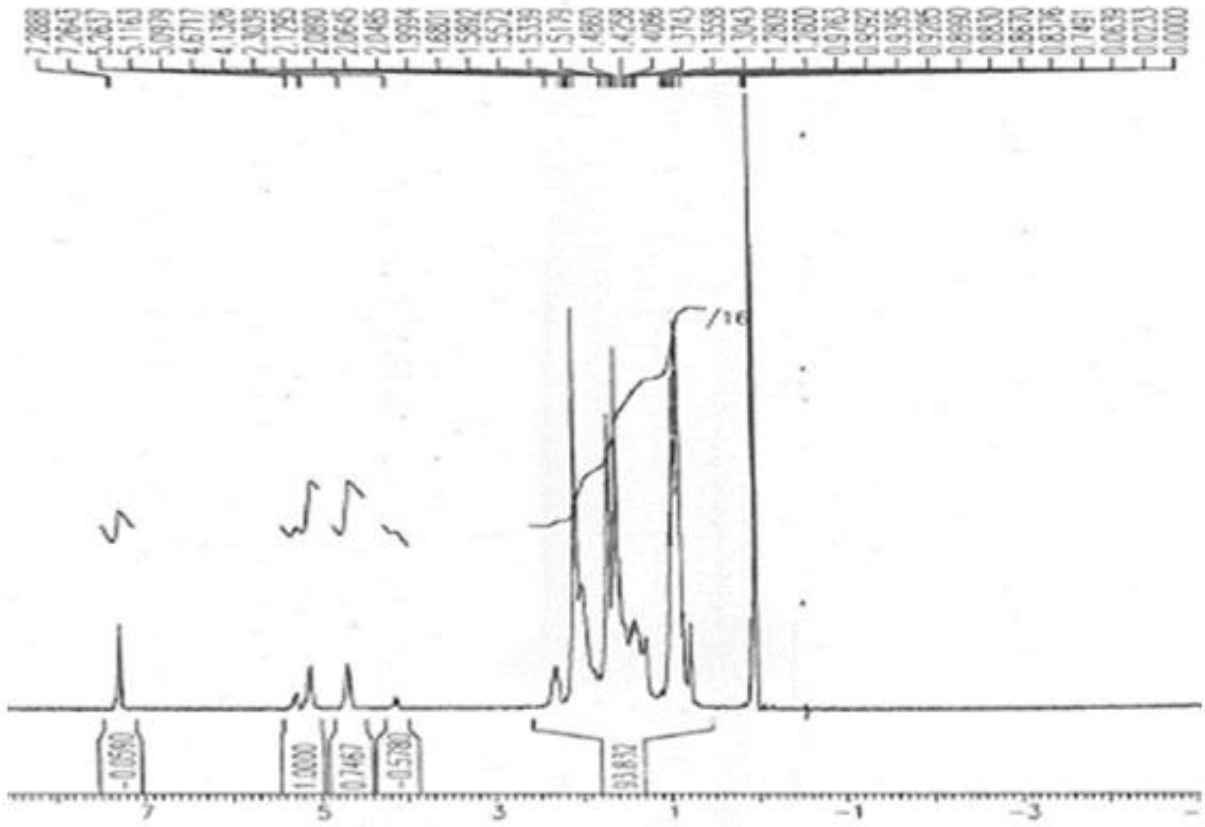
Appendix 26b: ^{1}H NMR spectrum for compound 219



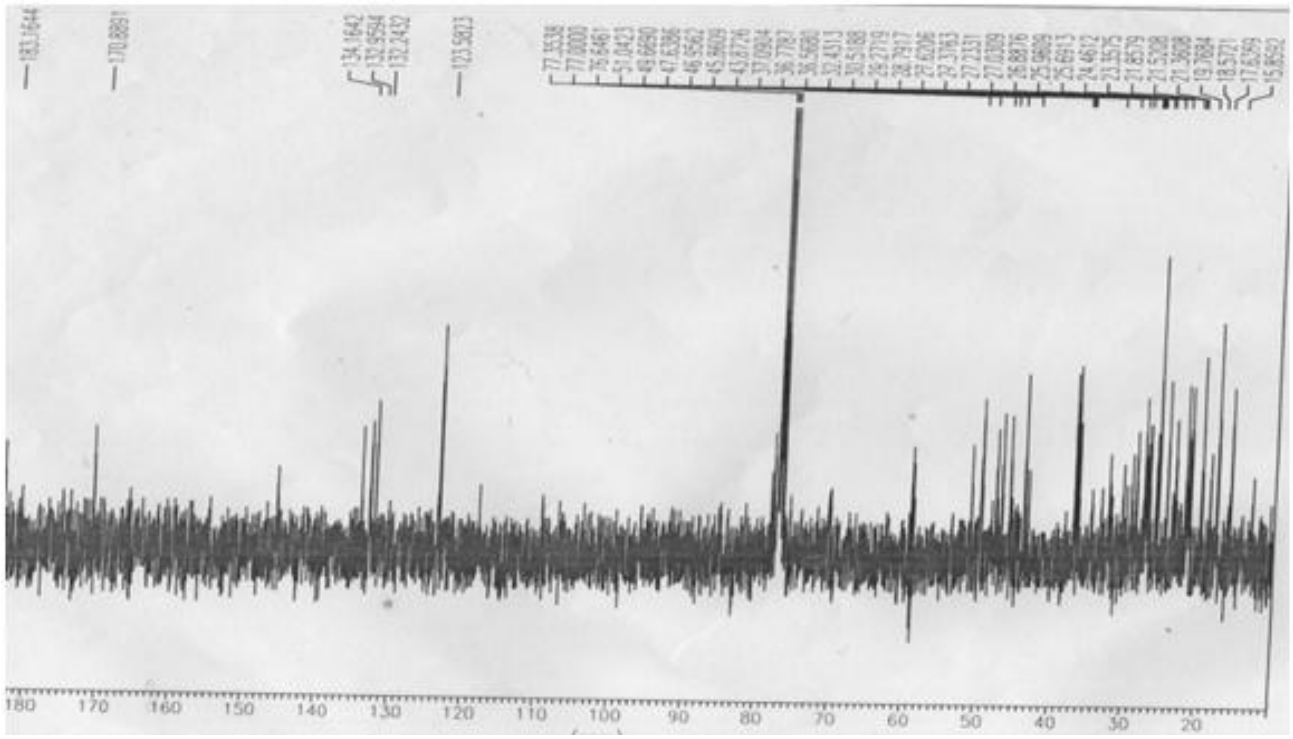
Appendix 26c: ^{13}C NMR spectrum for compound 219



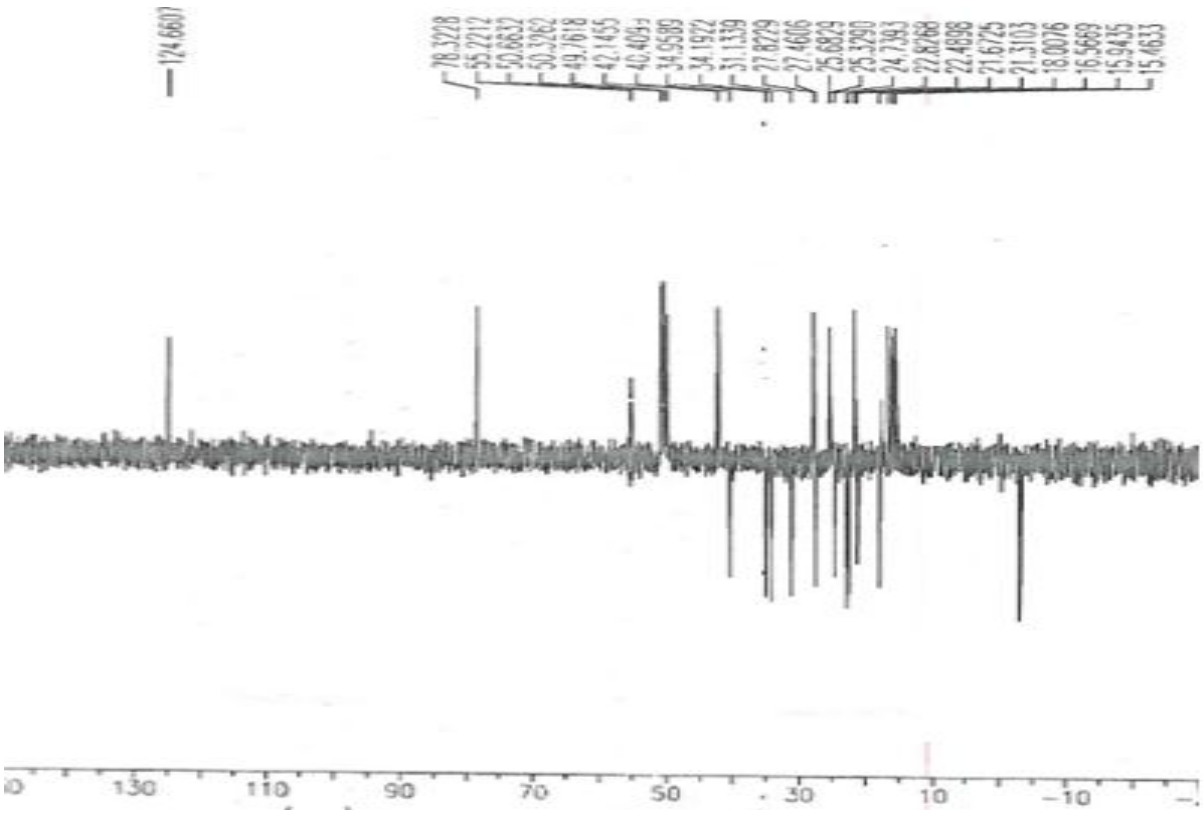
Appendix 26d: ESI-MS spectrum for compound 219



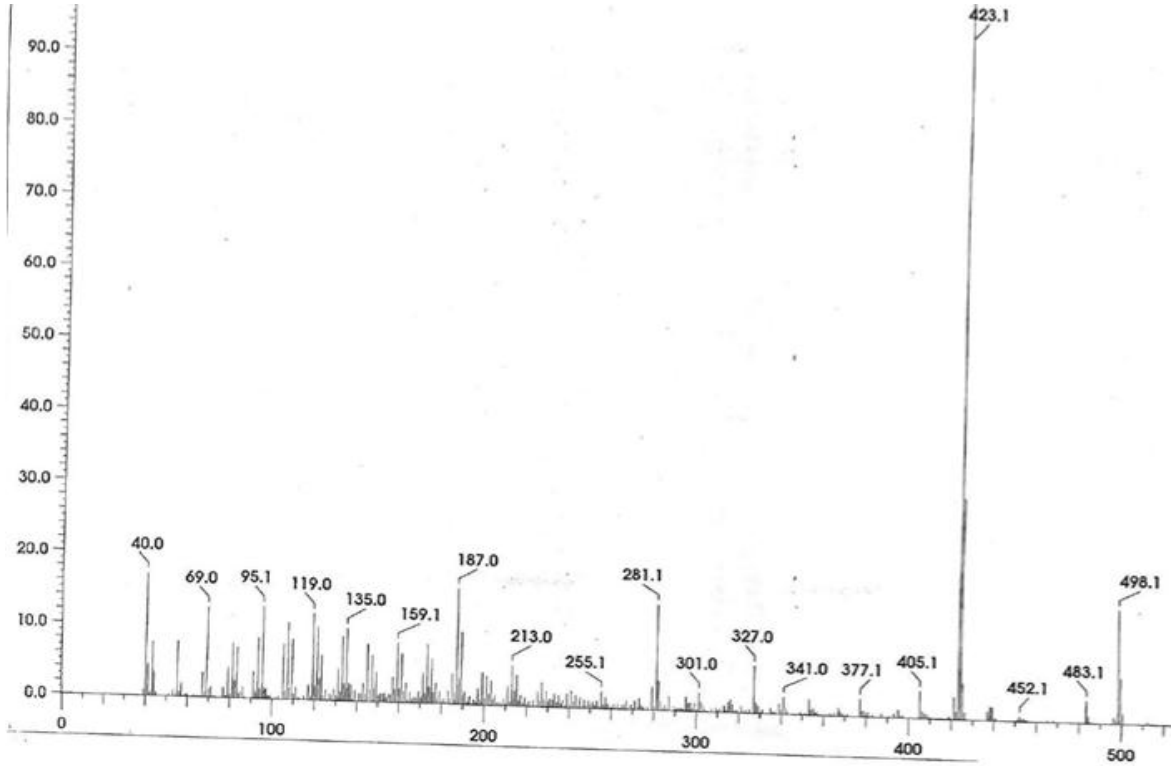
Appendix 27a: ^{1}H NMR spectrum for compound 220



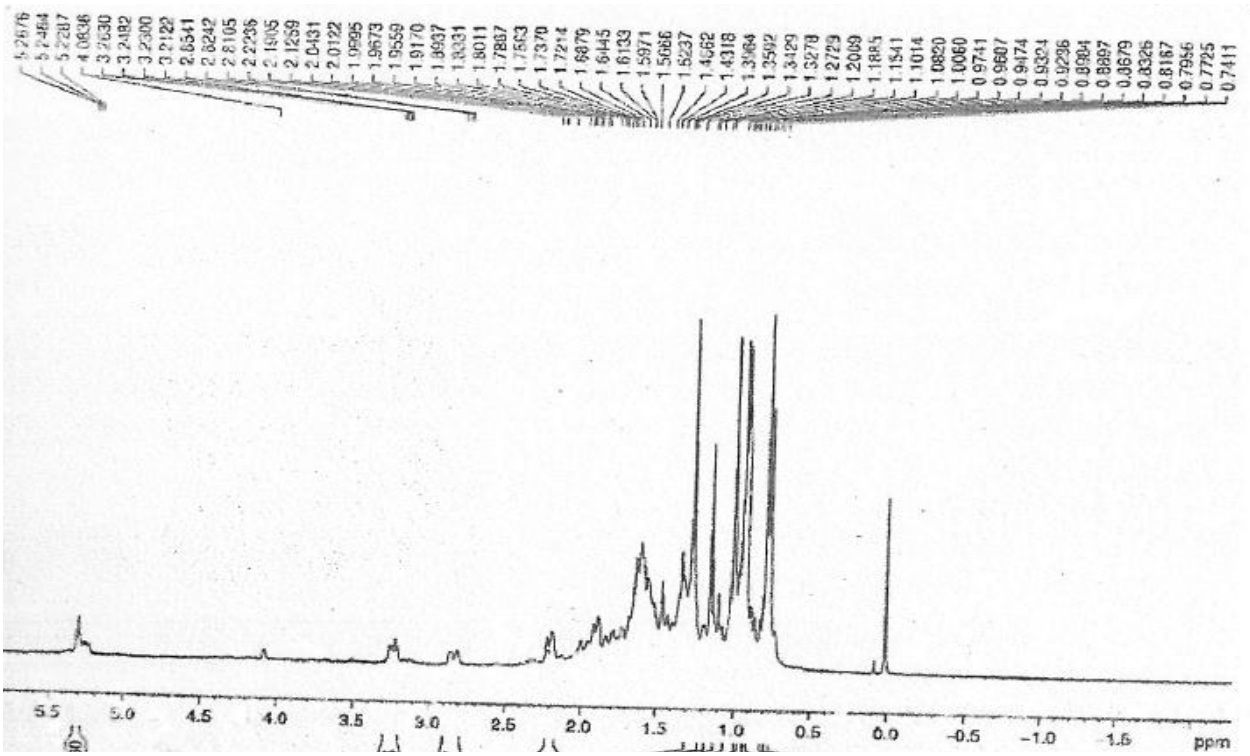
Appendix 27b: ^{13}C NMR spectrum for compound 220



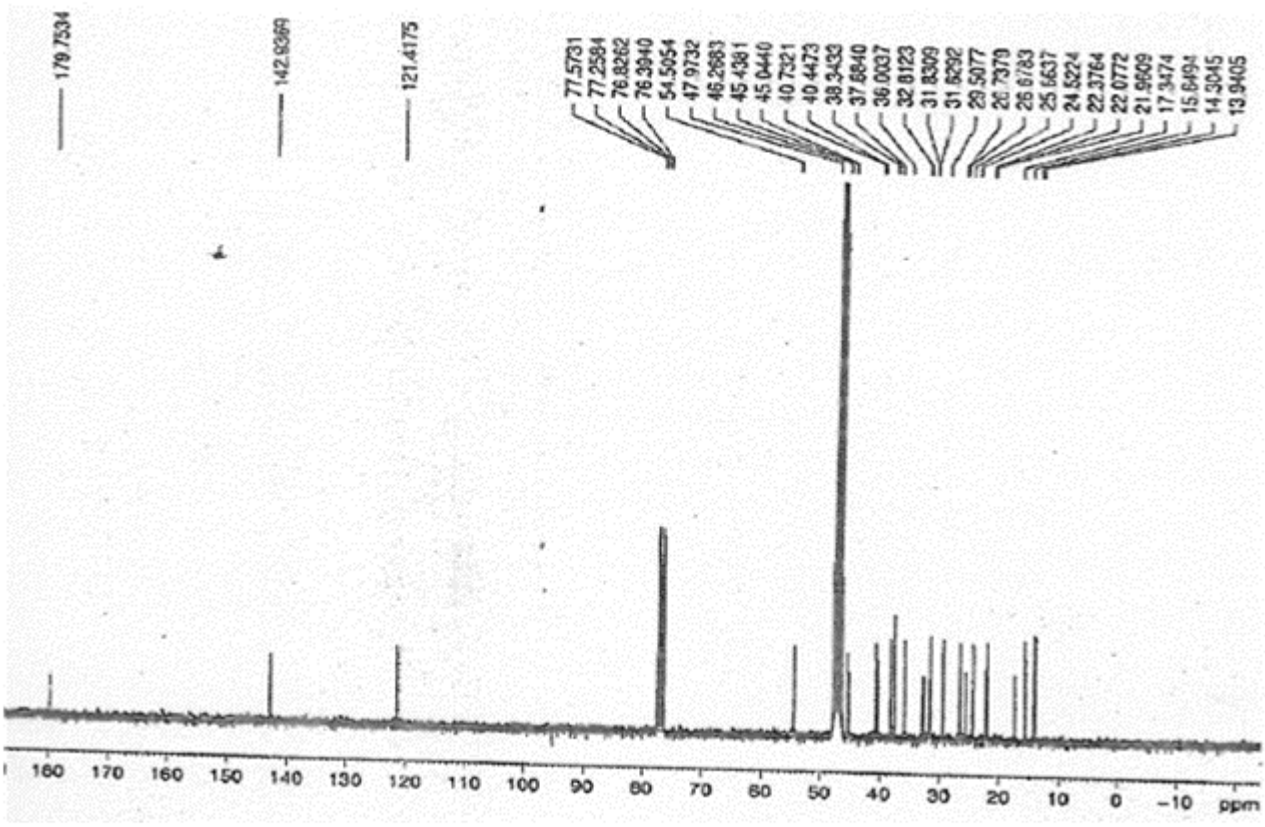
Appendix 27c: DEPT-135 spectrum for compound 220



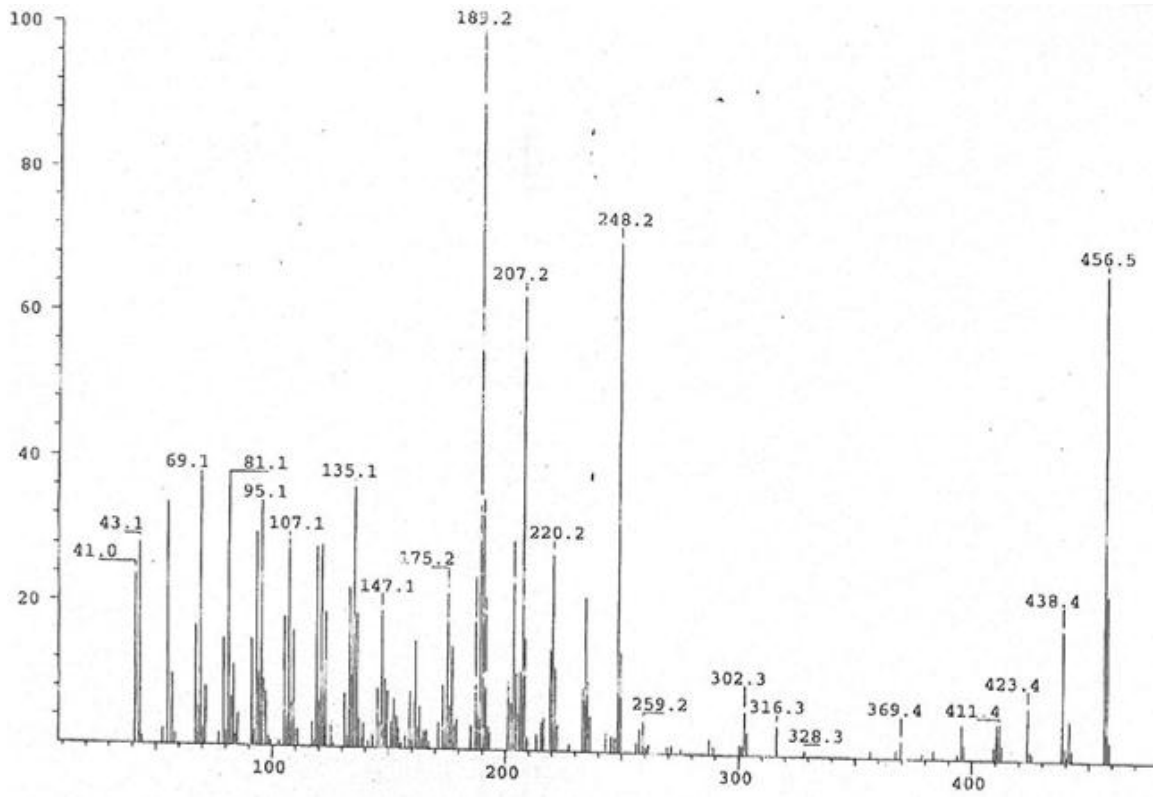
Appendix 27d: EI-MS spectrum for compound 220



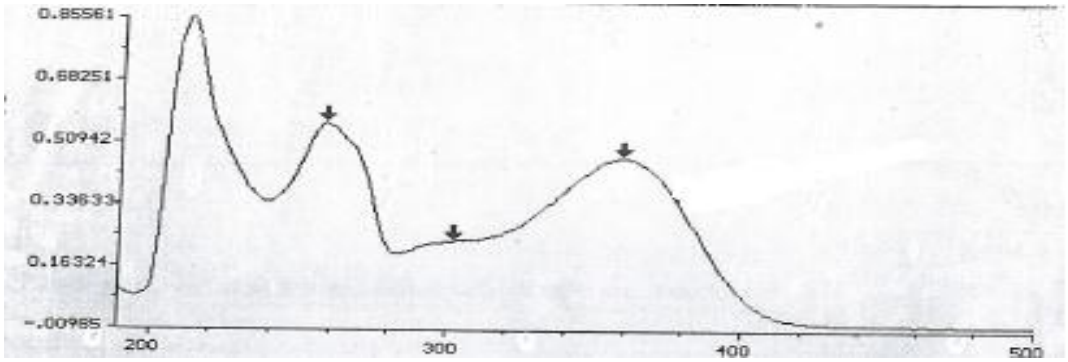
Appendix 28a: ^{1}H NMR spectrum for compound 4



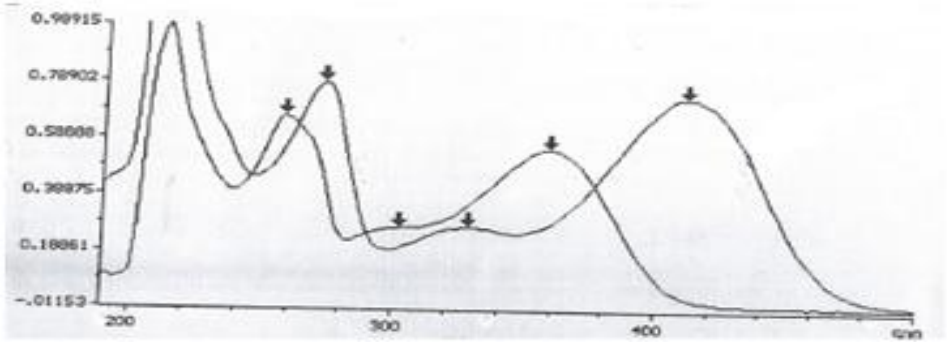
Appendix 28b: ^{13}C NMR spectrum for compound 4



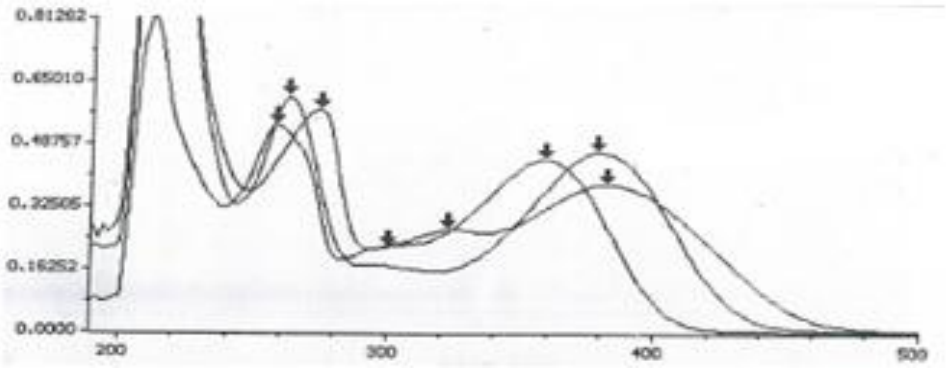
Appendix 28c: EI-MS spectrum for compound 4



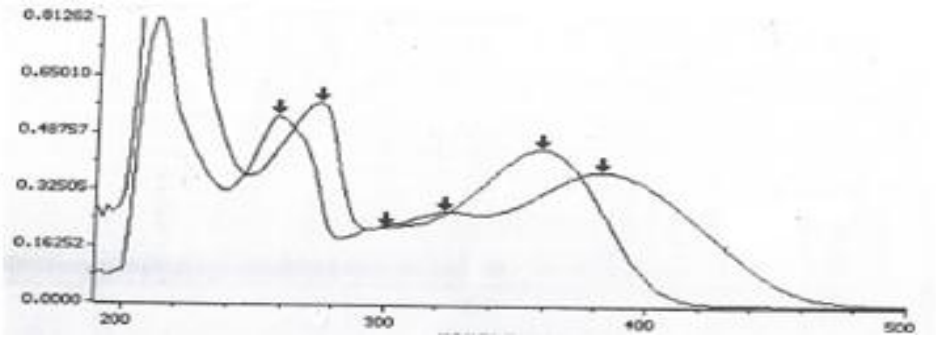
Appendix 29a: Major UV absorption bands for compound 221



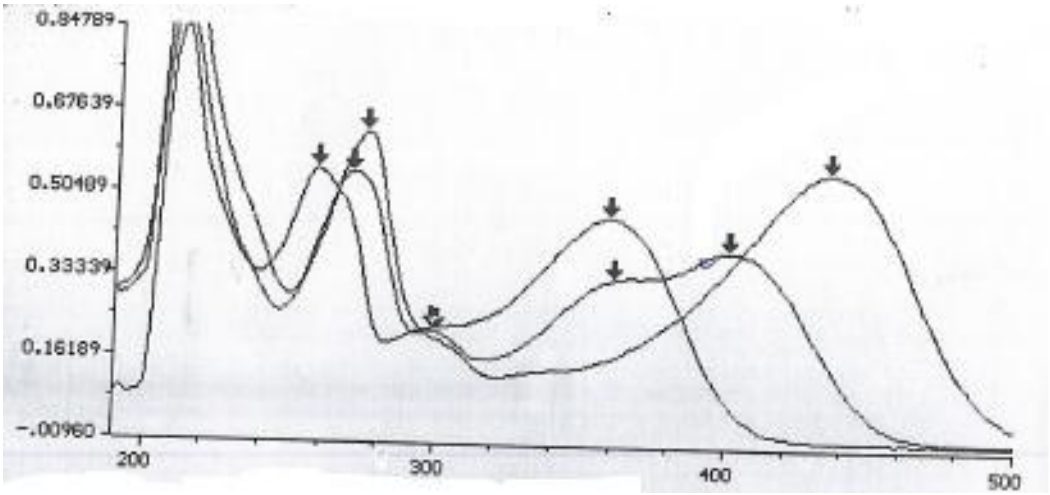
Appendix 29b: UV spectrum in NaOMe/ MeOH for compound 221



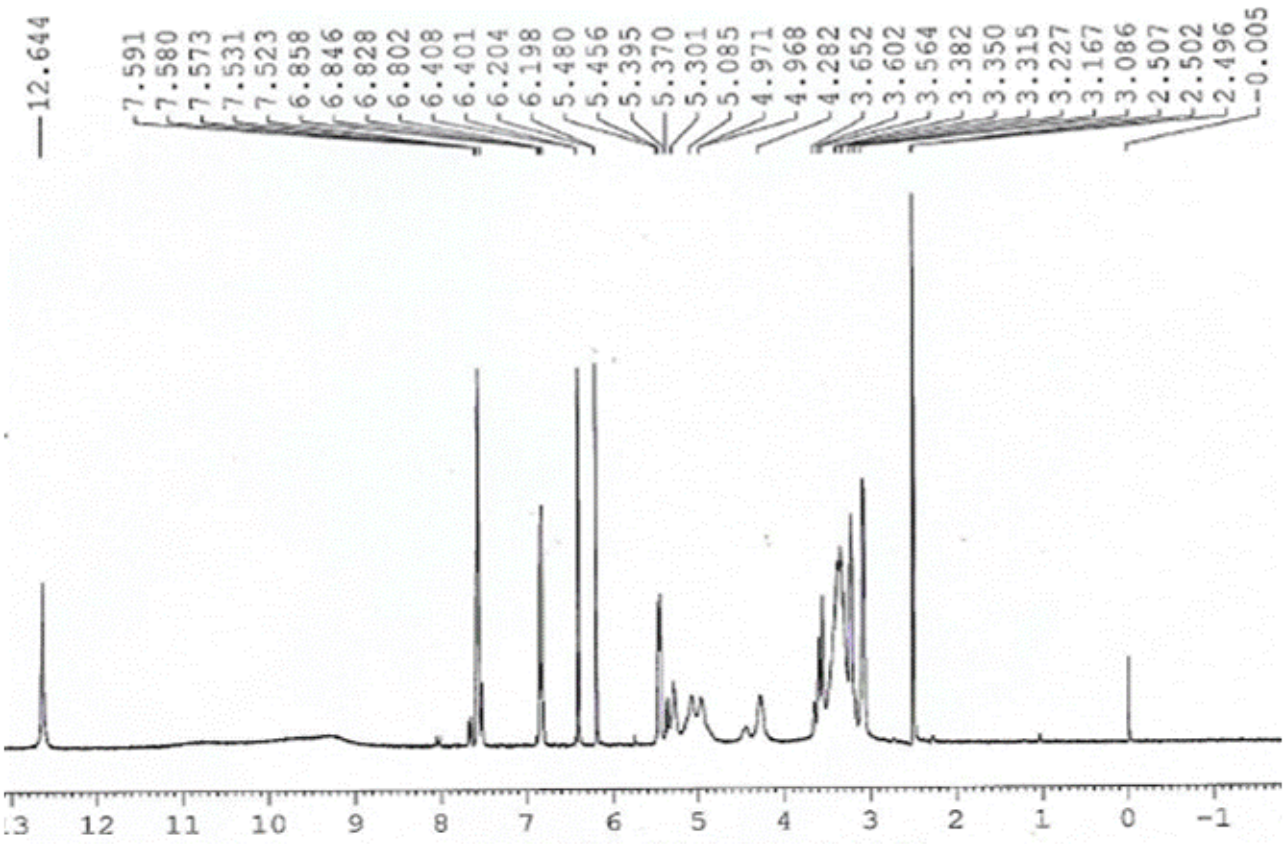
Appendix 29c: Bathochromic shift in NaOAc/ H_3BO_3 for compound 221



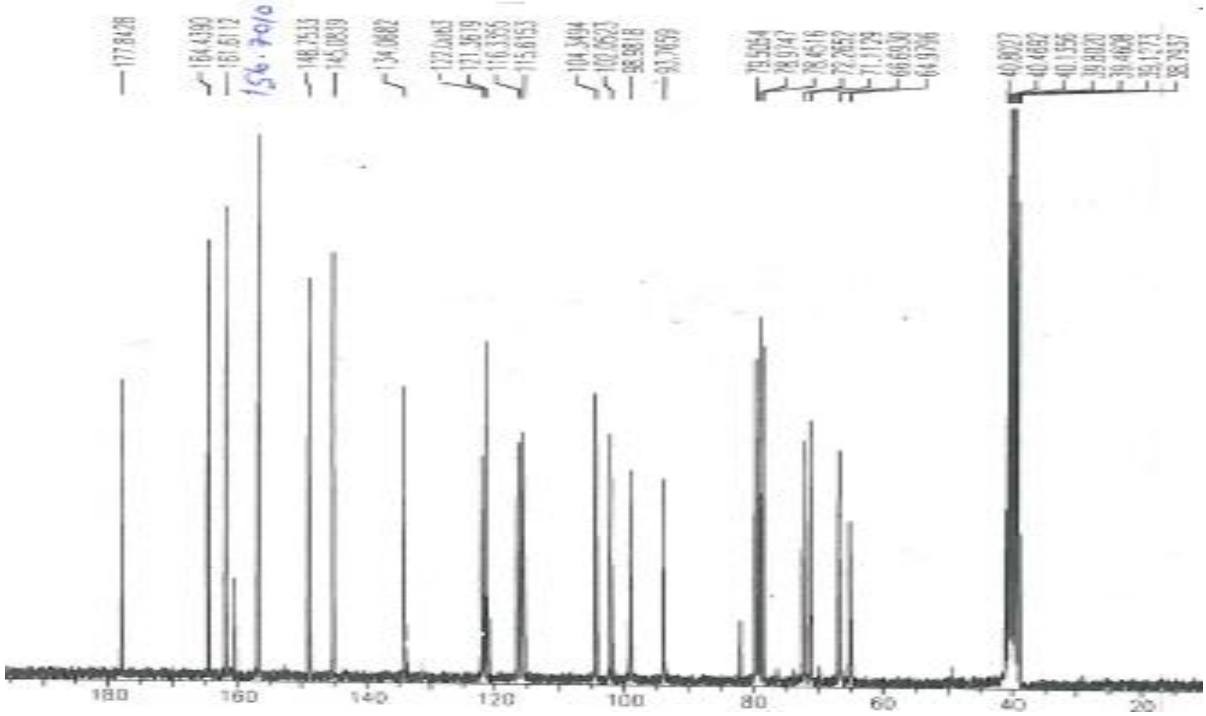
Appendix 29d: Bathochromic shift in NaOAc/ MeOH for compound 221



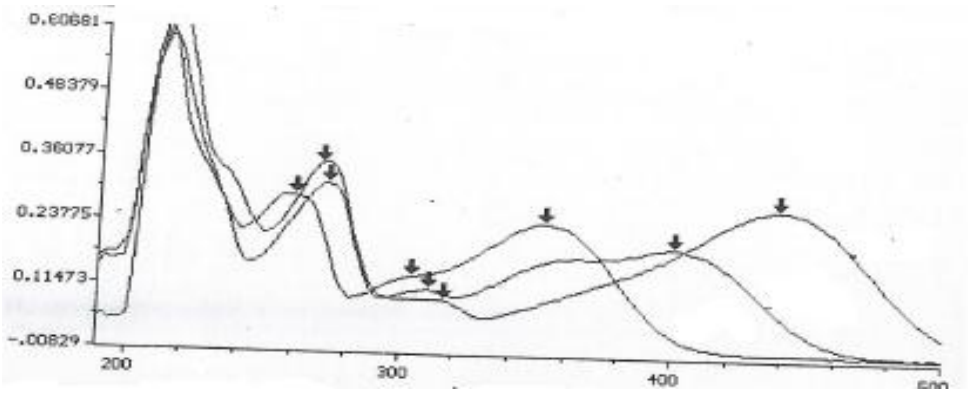
Appendix 29e: Bathochromic shift in $AlCl_3$ / HCl for compound 221



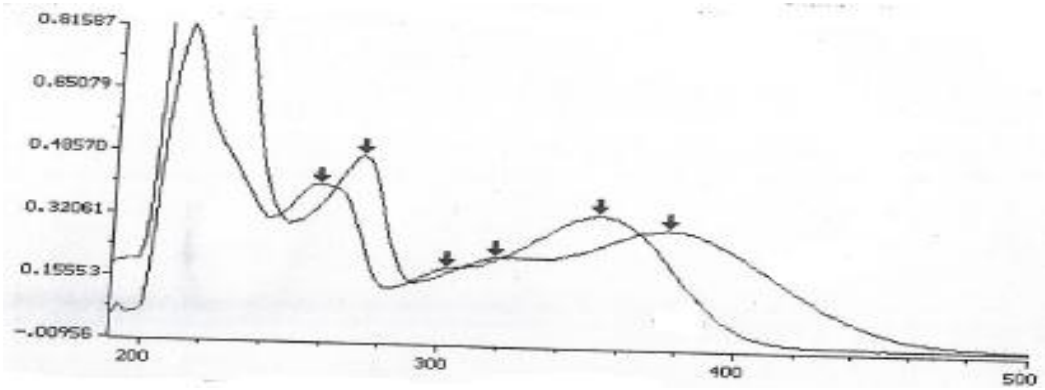
Appendix 29f: ^{1}H NMR spectrum for compound 221



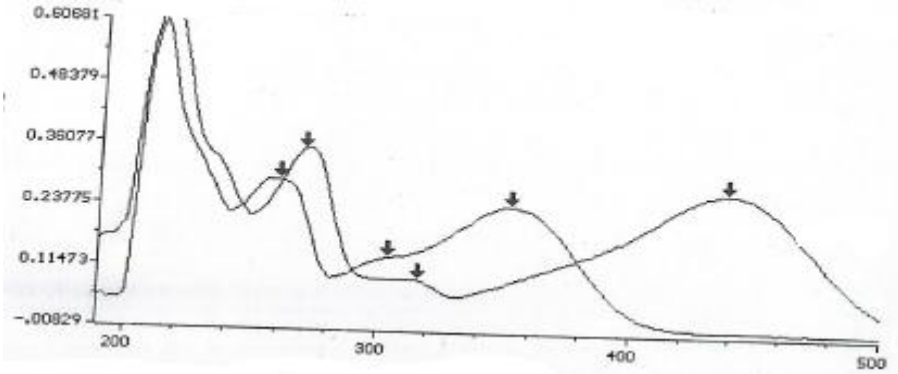
Appendix 29g: ^{13}C NMR spectrum for compound 221



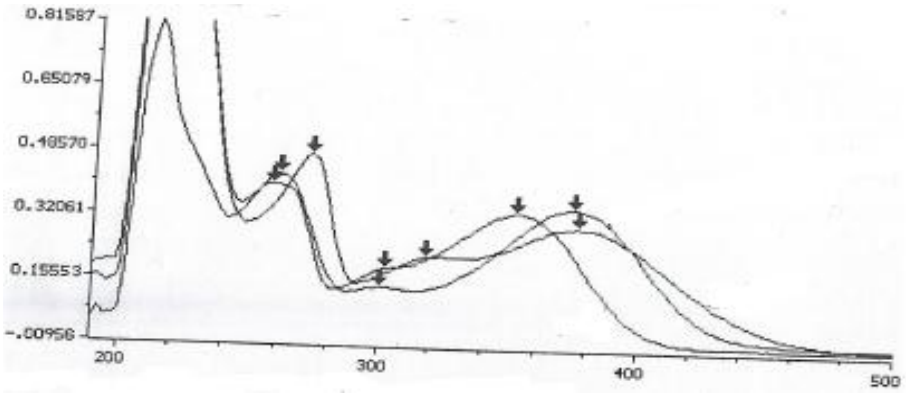
Appendix 30a: UV spectrum in $AlCl_3$ / HCl for compound 222



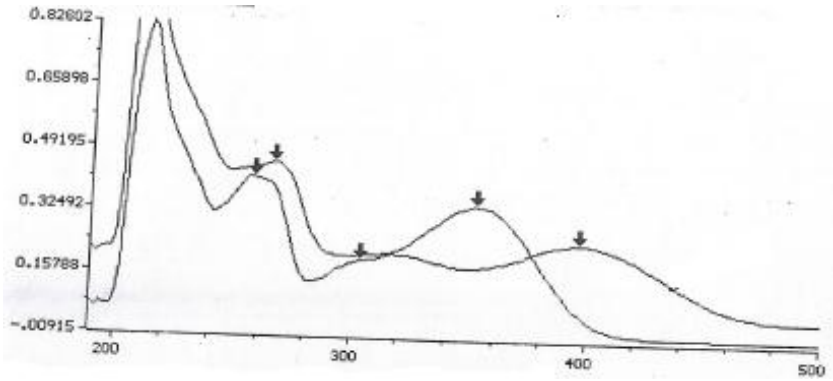
Appendix 30b: UV spectrum in NaOAc for compound 222



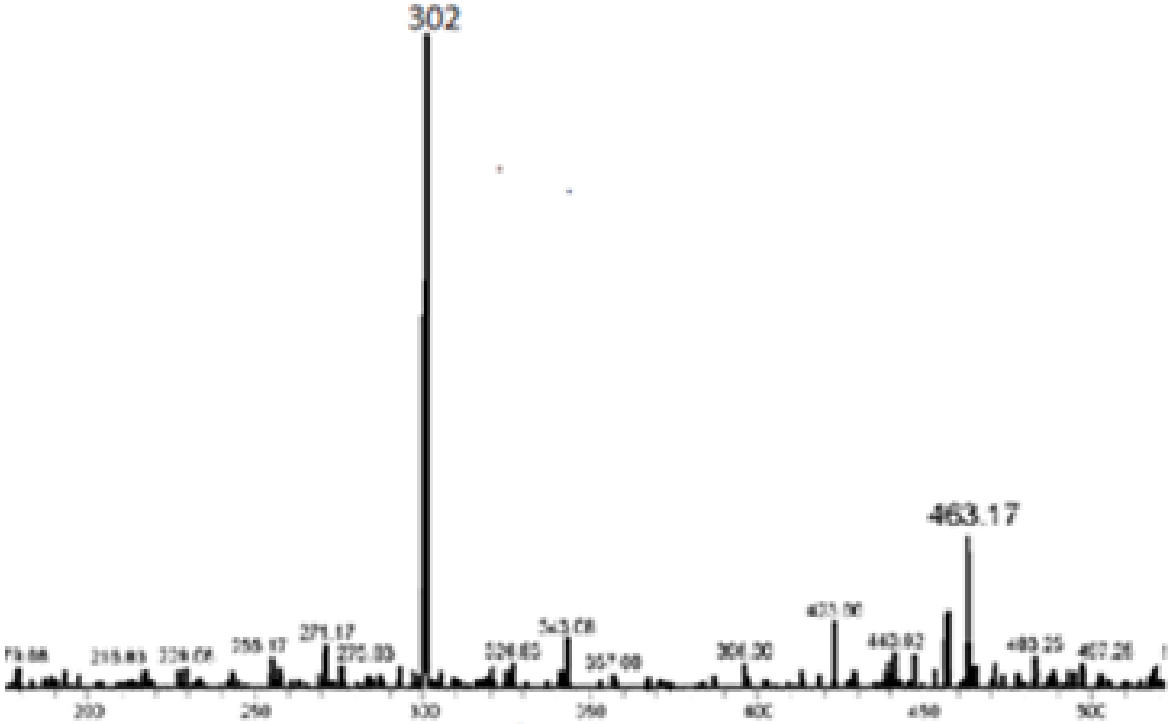
Appendix 30c: Bathochromic shift in NaOMe for compound 222



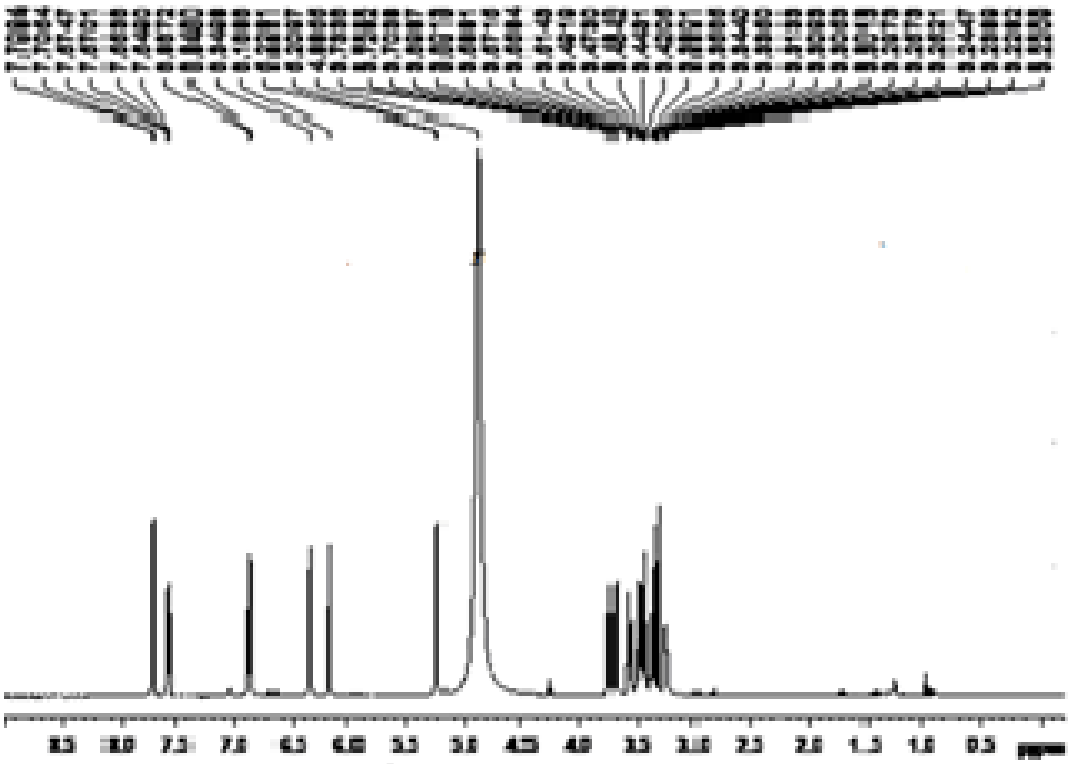
Appendix 30d: Bathochromic shift in NaOAc/ H_3BO_3 for compound 222



Appendix 30e: UV spectrum in NaOMe for compound 222



Appendix 30f: ESI-MS spectrum for compound 222



Appendix 30g: ^{1}H NMR spectrum for compound 222