ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL STUDIES OF *TURRAEA ABYSSINICA, MEYNA TETRAPHYLLA* AND *LEONOTIS MOLLISSIMA*

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A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Doctor of Philosophy Degree in Chemistry of Egerton University

EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted for examination in any institution.

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SD11/0379/13

Recommendation

This thesis has been submitted with our recommendation as the University Supervisors.

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DEDICATION

This thesis is dedicated to my late parents Mr and Mrs Morrison Njenga, my husband George Kinuthia, our children, Anne Kinuthia, Beth Kinuthia, Grace Kinuthia and grandson Addy.

ACKNOWLEDGEMENT

I wish to extend my sincere thanks to the Almighty God for this far He has taken me and to all those who made this work a success. I would like to thank Egerton University for giving me a chance to pursue this degree. This gave me an opportunity to reach this scholarly level of achievement.

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ABSTRACT

Due to the high bills involved in the importation of modern medicinal drugs, about 80% of the African population use traditional medicine from plants to treat common infectious diseases caused by microorganisms. The main objective of this research was to determine the antimicrobial activity of crude extracts and isolated compounds from *Turraea abyssinica, Meyna tetraphylla* (Abyssinian coral tree) and *Leonotis mollissima* (Lion's ear) from Meliaceae, Rubiaceae and Lamiaceae families respectively. They were studied in this research due to their wide use by local communities of Kenya for medicinal remedies. Plant materials were sampled from Kirinyaga East, Narok North, Baringo South, Tharaka Nthi Maua, Laikipia University and Mau Narok in Kenya. They were identified and voucher specimen kept for reference. All the plants crude extracts showed significant antimicrobial activity on all the test microorganism (*Bacillus cereus, Staphylococcus aureus, Escherichia coli,Salmonella typhimurium* and *Candida albicans*) at a concentration of 1 mg/ml despite been sampled from different regions of Kenya. They had lower MIC (Minimum Inhibition Concentration) as compared to the Amoxil® and Doxycycline® antibiotics that were used as positive control for comparison. From *Turraea abyssinica* stem bark dichloromethane crude extract (52.42 g), three compounds **176** (Sitosterol, 4.60 mg), **177** (Scopoletin, 6.00 mg) and **178** [2-(1',2'-Dihydroxypropyl)tetradecanoic acid, 5.65 mg] were isolated. Of the three compounds only compound **176** showed significant activity on *Bacillus cereus, Staphylococcus aureus,* and *Candida albicans*) at a concentration of 2.5 mg/mL to 4.0 mg/mL. *Meyna tetraphylla* leaves dichloromethane crude extract (45.24 g) gave compounds **179** (Phaeophytin, 9.40 mg), **180** (Enantiomer, 5.80 mg), **118** (α-Amyrin, 5.65 mg) and **60** (Sitigmasterol, 5.82 mg). The Structures of the compounds were elucidated using 1D-and 2D NMR. Experiments. Compound **(179)** showed significant activity on *Escherichia coli* and *Salmonella typhimurium* at a concentration of 4.0 mg/mL while α-Amyrin **(118)** had significant activity on *Salmonella typhimurium* at a concentration of 4.0 mg/mL. *Leonotis mollissima* leaves dichloromethane crude extract (79.69 g) yielded compounds **181** (Sederin, 7.70 mg), **182** (20-hydroxylucidenic acid D2, 7.10 mg) and **183** [(13R)-19α,13α-epoxylabda-6β(19).16(15)-dioldilactone, 21.20 mg]. Only compound (**182)** showed significant antimicrobial activity on *Escherichia coli* at a concentration of 0.4 mg/mL. This was a confirmation that the three plants contain compounds that can be isolated and used as drugs to treat various diseases including microbial infectious diseases.

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ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immunodeficiency Syndromes
AR	Analytical grade
BC	*Bacillus cereus*
^{13}C	Carbon-13 Nuclear Magnetic Resonance spectroscopy
CA	*Candida albicans*
CC	Column Chromatography
COSY	Correlation Spectroscopy
d	doublet
dd	doublet of doublet
dt	doublet of triplet
DCM	Dichloromethane
DEPT	Distortionless Enhancement by Polarisation Transfer
DEE	Diethyl ether
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EtOAc	Ethyl acetate
EC	*Escherichia coli*
FT-IR	Fourier-Transform Infrared
^{1}H NMR	Proton Nuclear Magnetic Resonance spectroscopy
Hex	Hexane
HMBC	Heteronuclear Multiple-Bond Correlation
HSQC	Heteronuclear Single Quantum Correlation
H_2O	Water
Hz	Hertz
IC_{50}	Inhibition Concentration that reduces the effect of microorganisms by 50%
LD_{50}	Lethal Dose that is sufficient to kill 50% of population of animals within a certain time
LM	*Leonotis mollissima*
MeOH	Methanol
m	multiplet
MIC	Minimum Inhibitory Concentration
MS	Mass Spectrometry

MT	*Meyna tetraphylla*
ppm	part per million
NACOSTI	National Commission for Science, Technology and Innovation
NMR	Nuclear Magnetic Resonance
NO^+	Nitric Oxide
NOESY	Nuclear Overhauser Effect Spectroscopy
R_f	Retardation factor
s	singlet
SA	*Staphylococcus aureus*
ST	*Salmonella typhimurium*
t	triplet
TA	*Turraea abyssinica*
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
USA	United States of America
UV	Ultra Violet
WHO	World Health Organization

CHAPTER ONE
INTRODUCTION

1.1 Background information

Plants are extremely important in the lives of people throughout the world and many people depend on them to satisfy basic human needs such as food, clothing, shelter and health care. Historically, plant medicines were discovered by trial and error. Our ancestors noticed that aches and pains went away when they drank tea made from the bark of a willow tree. Later, scientists found that the willow bark contained salicylic acid, the active ingredient in aspirin® that relieves pain (Facchini *et al*., 2000).

Many higher plants have been the source of medical agents since the earliest times and today they continue to play a dominant role in the primary health care of about 80% of the world's population (Addae-Mensah, 1992). In Africa, up to 60% of the population consult one of an estimated 200,000 traditional healers especially in rural areas where these healers are more numerous and accessible than allopathic physicians (Van Wyk *et al*., 2000). The people in Asia, North and South America, Australia and New Zealand have used concoctions prepared from a wide range of medicinal plants for treating the sick. The information on which plant and what part of the plant cures what disease was passed on from generation to generation. This rich heritage of traditional medicinal practices was looked down upon following the slicing of third World countries into fragmented pockets with European spheres of influence. It was branded as primitive although many pharmaceutical drugs and medicinal syrups administered to patients in modern hospitals are of plant origin (De Sa' Ferreira and Ferrao, 1999).

Medicinal agents derived from plants are also an essential feature in the health care system of the remaining 80% of the population residing mainly in developing countries. Of the worlds twenty-five best-selling pharmaceutical agents, twelve are derived from natural products, which continue to play an important role in drug discovery programs of the pharmaceutical industry and other research organizations (Akerele, 1991). Without plants, most medicines taken would not exist. Over 40% of medicines now prescribed in U.S.A. contain chemicals derived from plants (Facchini *et al*., 2000).

Throughout the world, botanists and chemists search the plant kingdom for new medicines. For example, the native Pacific yew was burned as trash generated by logging operations in the Pacific Northwest. In 1975, a substance in its bark, taxol, was found to reduce the production of cancerous tumours (Facchini *et al*., 2000). A comprehensive search of known plants for medicinal chemicals is an enormous task. Of the estimated 250,000 plant

species on earth, only 2% have been thoroughly screened for chemicals with potential medicinal use. Many native plant habitats are destroyed almost daily and therefore many medicinally valuable plants will be gone before scientists can investigate them (Facchini *et al*., 2000). Although plant extracts have been used in the treatment of diseases, research has shown some secondary metabolites present in these medicinal plants to be potentially toxic and carcinogenic, thus care should be taken before use (De Sa' Ferraira and Ferrao, 1999). Secondary metabolites are molecules that are not necessary for the growth and reproduction of a plant. They may serve some role in herbivore deterrence due to astringency or they may act as phytoalexins, killing bacteria that the plant recognizes as a threat. They are often involved in key interactions between plants and their abiotic and biotic environments that influence them (Facchini *et al*., 2000).

Turraea abyssinica belong to the *Turraea* genus of the Meliaceae family and is used by the *Samburu*s for making *rungus*, firewood and as fruits to induce vomiting (Amit and Shailendra, 2006). This family has been known to exhibit a wide variety of biological properties (Amit and Shailendra, 2006). Though not much work has been done on it, its root methanol extract showed some antiplasmodial activity (Ndung'u, 2002). *Meyna tetraphylla* of the Rubiaceae family is used by the *Pokots* in Kenya to treat infected hooves of goats and camels. It is also used as an animal fodder and the root decoction is given to pregnant women to alleviate pain during labour (Beentje, 1994). Some of the species in this genus are used by the villagers as food, anticancer, anti-inflammatory, antidysentery, treatment of kidney stones, hepatic disorders, gastrointestinal problems and abdominal distention (Majaz and Khurshid, 2014, Borah *et al*., 2015). No scientific research has been done on this plant so far. *Leonotis mollissima* belong to the Lamiaceae family that is known to treat cold, cough, fever, headache and asthma. It's root decoction is used by the *Marakwets* of Kenya and Tanzanians to treat malaria and stomach problems (Kokwaro, 1976; Fowler 2006).

1.2 Statement of the problem

Most indigenous people do not have easy access to modern medicine for themselves or their livestock due to inaccessibility and unaffordability. Therefore, the communities use traditional herbal medicine to treat themselves and their animals. However, some are toxic and the composition and the efficacy of these traditional herbal medicines have not been scientifically tested. Hence, the need of evaluating the medicinal properties of *Turraea abyssinica*, *Meyna tetraphylla* and *Leonotis mollissima* species. This project is aimed to

determine the antimicrobial activity of crude extracts and pure compounds from these plants that were sampled from different regions of Kenya.

1.3 Objectives

1.3.1 General objective

To determine the antimicrobial activity of crude extracts and compounds isolated from *Turraea abyssinica*, *Meyna tetraphylla* and *Leonotis mollissima* from different regions of Kenya.

1.3.2 Specific objectives

i. To determine the antimicrobial activity of the crude extracts of *Turraea abyssinica*, *Meyna tetraphylla* and *Leonotis mollissima* collected from different ecological zones
ii. To determine structures of the isolated compounds using spectroscopic instruments and to determine the antimicrobial activity of the pure compounds isolated from the plants.

1.4 Hypothesis

i. *Turraea abyssinica*, *Meyna tetraphylla* and *Leonotis mollissima* plants are found in different ecological zones of Kenya.
ii. The pure compounds isolated from *Turraea abyssinica*, *Meyna tetraphylla* and *Leonotis mollissima* have significant antimicrobial activity.

1.5 Justification

Traditional medicinal practice is very popular in developing countries. This is as a result of the easy access and low cost of traditional herbal medicines as opposed to modern allopathic medicinal drugs. *Turraea abyssinica, Meyna tetraphylla* and *Leonotis mollissima* are traditionally used in Kenya as herbal medicines. Personal communications with traditional herbal practioners imply that these medicinal plants cure malaria and microbial diseases. The aim of the study was to determine scientifically the antimicrobial activity of *Turraea abyssinica, Meyna tetraphylla* and *Leonotis mollissima* species from different regions of Kenya. Natural products are a significant source of drugs and leads to drug development through structural modification. The three plants were found to be biologically active and therefore they will be a source of new antimicrobial agents. The bioactivity of *Turraea abyssinica*, *Meyna tetraphylla* and *Leonotis mollissima* crude extracts and pure compounds confirmed their use as herbal medicinal plants by the Kenyans.

CHAPTER TWO

LITERATURE REVIEW

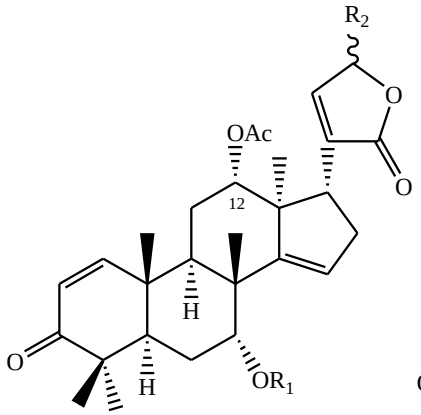
2.1 Medicinal plants

Since ancient times, people have been discovering the nature particularly plants in search of new drugs. This has resulted in the use of a large number of medicinal plants, with healing properties to treat various diseases (Savithramma *et al*., 2011). Nearly 80% of the world's population depends on traditional medicines for primary health care, most of which involve the use of plant extracts (Verpoorte, 1998).

2.2 The Genus *Turraea*

Turraea genus is from the Meliaceae family that consists of about 50 genera and 1400 species (Leonardo *et al*., 2002). This family is characterized chemically by the presence of tetra*nor*triterpenoids (Limonoids) compounds. The search for limonoids started long ago when scientists started looking for the factor responsible for bitterness in fruits. The term limonoids was derived from the limonin, the first tetra*nor*triterpenoid obtained from citrus bitter principle. They are highly oxygenated, modified terpenoids and have lately attracted attention because compounds from this group have exhibited a range of biological activities like insecticidal, insect antifeedant and growth regulating activity on insects. They also have some some other biological activity like antifungal, antibacterial, antimalarial, anticancer, antiviral and a number of other pharmacological activities on humans (Amit and Shailendra, 2006).

Turrraea parvifolia is used by the *Pokots* of Kenya as an emetic. This is a small shrub that is found in East Africa and is characterized by white small flowers, smooth dark grey stems with droping branches. Seven triterpenoids, Turrapavin A **(1)**, Turrapavin B **(2)**, 12α-Acetoxyazadironolide **(3),** Turrapavin C **(4),** 11-*epi*-21-Hydroxytoonacilide **(5)**, 11-*epi*-21, 23-Hydroxytoonacilide **(6)** and Turrapavin D **(7)** have been isolated from methanol extract of the seeds (Cheplogoi and Mulholland, 2003).

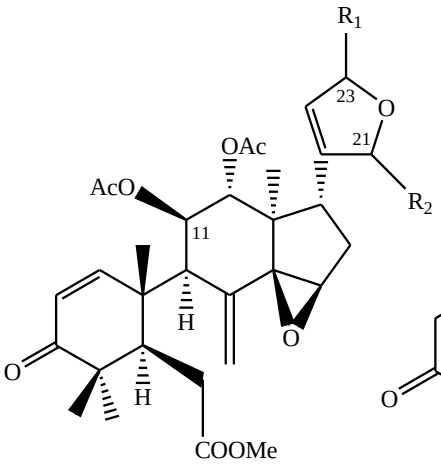


R_1 =H, R_2 = OH **1**

R_1 = Ac, R_2 = OH **2**

R_1 = H, R_2 = O **3**

R_1 = Ac, R_2 = O **4**



R_1 = O, R_2 = OH **5**

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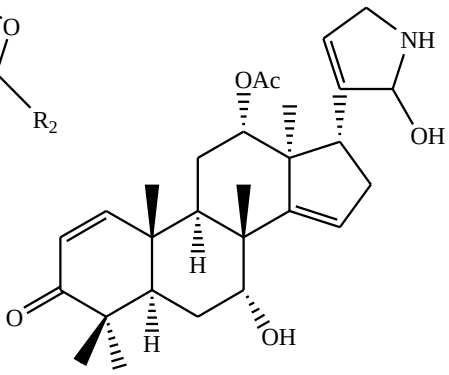

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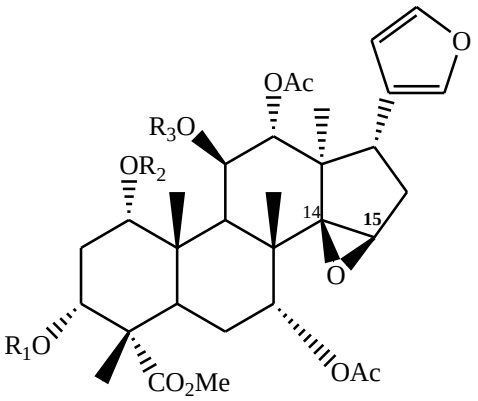
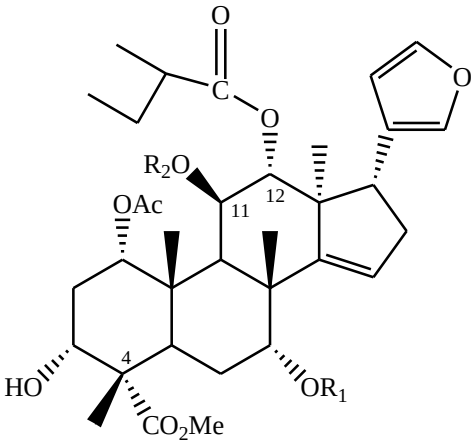
Turraea floribunda's bark is traditionally used as an emetic, while the root and the leaves are used as a purgative (Kakwaro, 1976). Sixteen limonoids, 14*β*,15*β* -Epoxide 1,7,12-*tri*-Ac,Me ester **(8)**, 14*β*,15*β*-Epoxide, 11-(2-methylpropanoyl)-1,7,12-*tri*-Ac,Me ester **(9)**, 14*β*,15*β*-Epoxide-11-(2-methylpropanoyl)-3,7,12-*tri*-Ac,Me ester **(10)**, 28-*nor*-4*α*-carbomethoxy-11*β*-acetoxy-12*α*-(2-methylbutanoyloxy)-14,15-deoxyhavanensin-1,7-diacetate **(11)**, 28-*nor*-4*α*-carbomethoxy-11*β*-hydroxy-12*α*-(2-methylbutanoyloxy)-14,15-deoxyhavanensin-1-acetate **(12)**, 28-*nor*-4*α*-carbomethoxy-11*β*-acetoxy-12*α*-(2-methylbutanoyloxy)-14,15-deoxyhavanensin-1-acetate **(13)**, 28-*nor*-4*α*-carbomethoxy-11*β*-acetoxy-12*α*-(2-methylbutanoyloxy)-14,15-deoxyhavanensin-1-acetate **(14)**, Turraflorin A **(15)**, Turraflorin B **(16)**, Turraflorin C **(17)**, Turraflorin D **(18)**, Turraflorin E **(19)**, Turraflorin F **(20)**, Turraflorin G **(21),** Turraflorin H **(22)** and Turraflorin I **(23)** have been isolated from the bark, seeds and root bark (Akinniyi *et al*., 1986; Fraser *et al*., 1994; Torto *et al*., 1995; MacFarad *et al*., 2004; Ndungu *et al*., 2004).



R_1 = H, R_2 = Ac, R_3 = H **8**

R_1 = H, R_2 = Ac, R_3 = Isobutyrate **9**

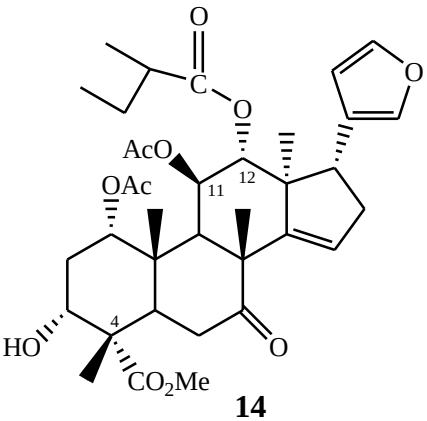
R_1 = Ac, R_2 = H, R_3 = Isobutyrate **10**



R_1 = Ac, R_2 = Ac **11**

R_1 = H, R_2 = H **12**

R_1 = H, R_2 =Ac **13**



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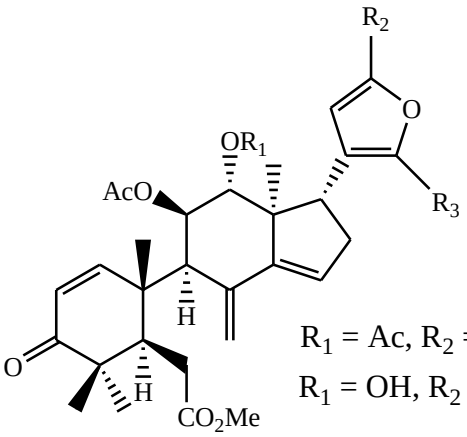
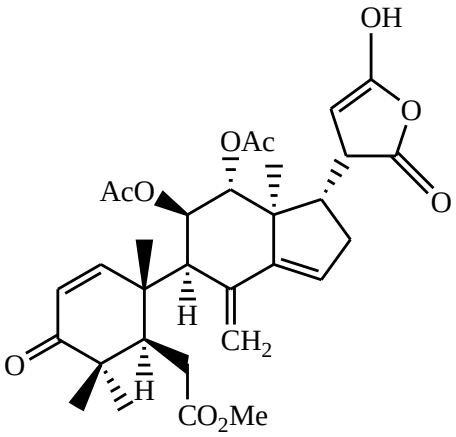


R_1 = Ac, R_2 = H, R_3 = H **15**

R_1 = OH, R_2 = H, R_3 = H **16**



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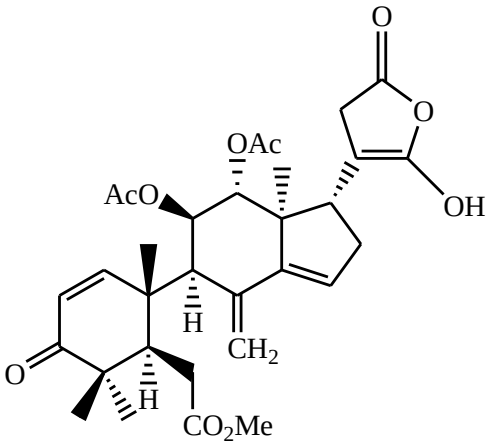

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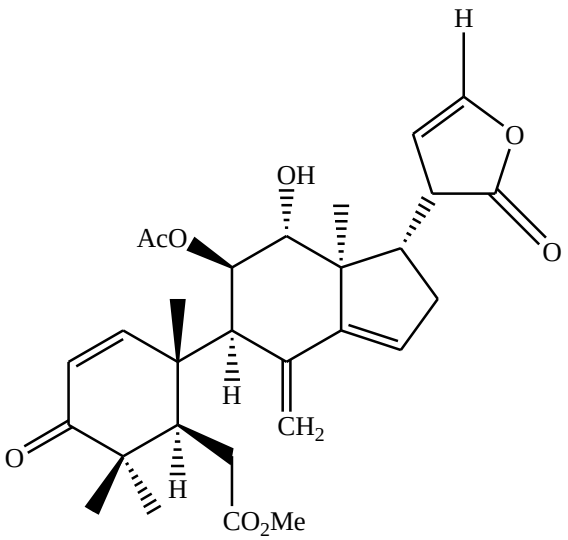

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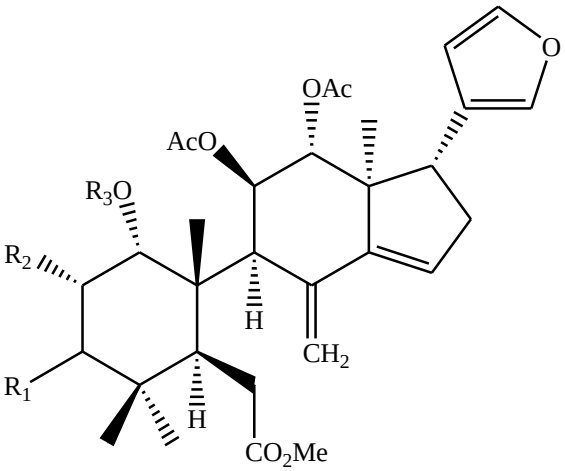
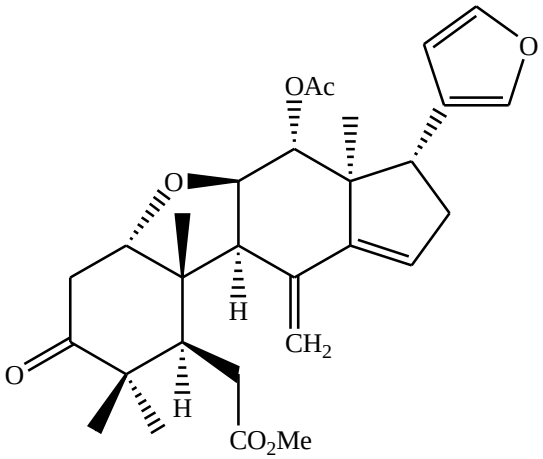


R_1 = O, R_2 = OAc, R_3 = Ac **20**

R_1 = O, R_2 = H, R_3 = OH **21**

R_1 = OAc, R_2 = H, R_3 = Ac **22**



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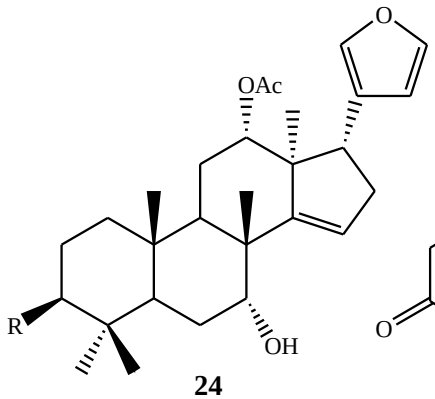
Turraea robusta's roots are used traditionally to treat stomach pain, diarrhoea and other stomach troubles and the leaves are used as an antidote for general poisoning (Kokwaro, 1976). Three limonoids, Mzikonol **(24)** , Mzikonone **(25)** and Turranolide **(26)** have been extracted from it's root bark (Torto *et al.*, 1995).



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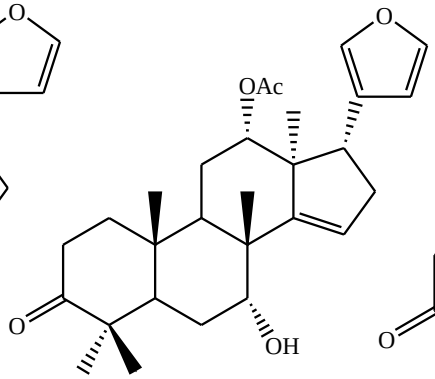

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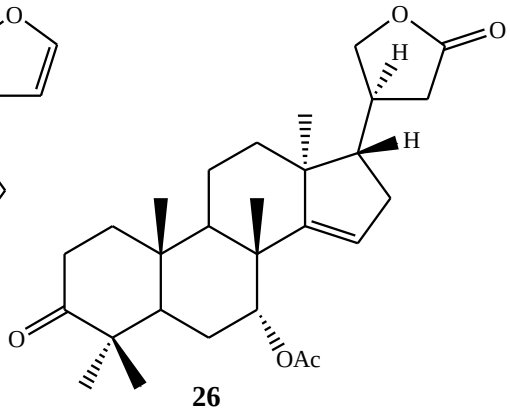

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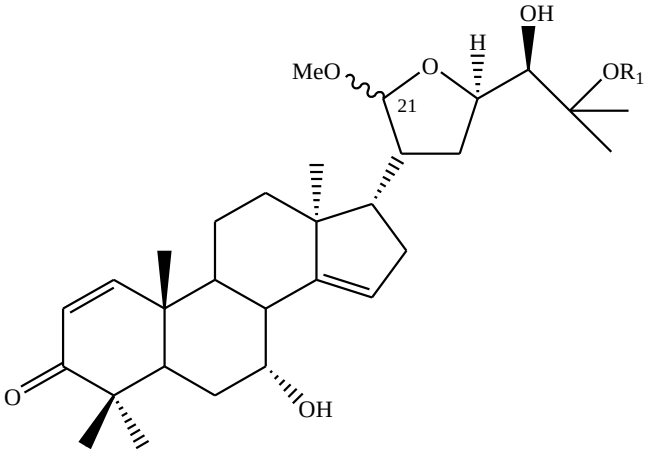
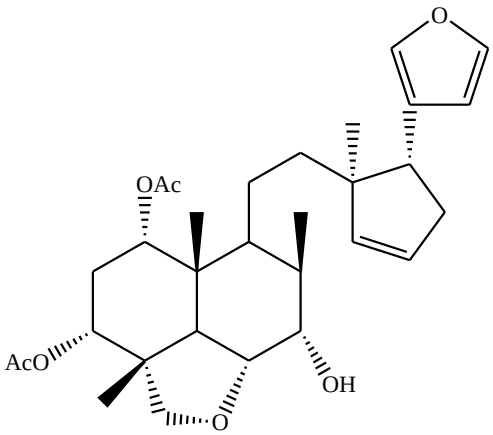
Turraea holstii is used by the *Samburus* to make *rungus*, firewood and the fruits are edible (Rainer, 2006). Eleven triterpenoids, Holstinone A **(27)**, Holstinone B **(28)**, Holstinone C **(29)**, 1,3-Decetylvilasinin **(30)**, 11-*epi*-Toonacillin **(31)**, 11*β*,2*α*-Diacetoxycedrelone **(32)**, 12-*O*-Methylnimbolinin **(33)**, 12*α*-Actoxy-*neo*-trichilinone **(34)**, 1,2-Dihydro-7-acetyl-12*α*-acetoxy-*neo*-trichilinone **(35)**, 1,2-Dihydro-12*α*-acetoxy-*neo*-trichilinone **(36)** and 1,2-Dihydro-11*β*-acetoxy-12*α*-hydroxy-7*α*-acetyl-*neo*-trichilinone **(37)** have been isolated from the stem and root bark **(**Mulholland and Taylor; 1988: Mulholland *et al*., 1999).



$R_1 = CH_3$, 21 = *α*-OMe **27**
R_1 = H, 21 = *β*-OMe **28**
R_1 = H, 21 = *α*-OMe **29**



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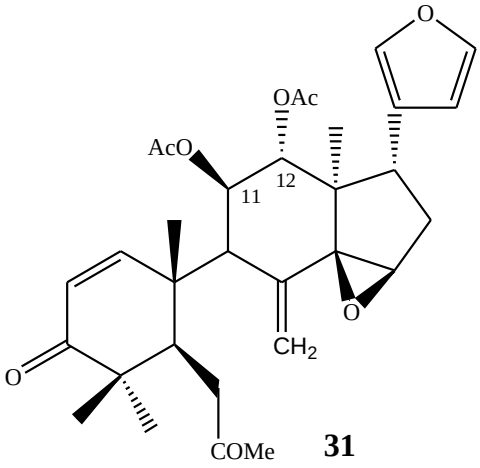

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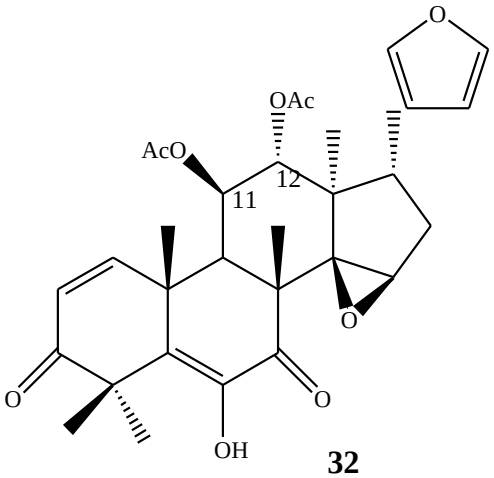

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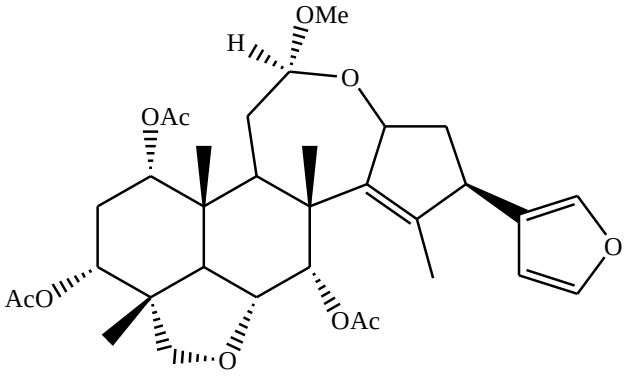

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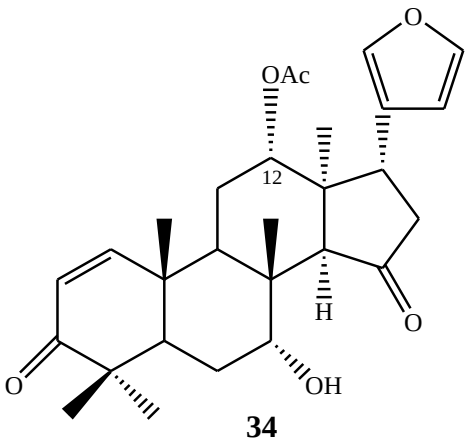

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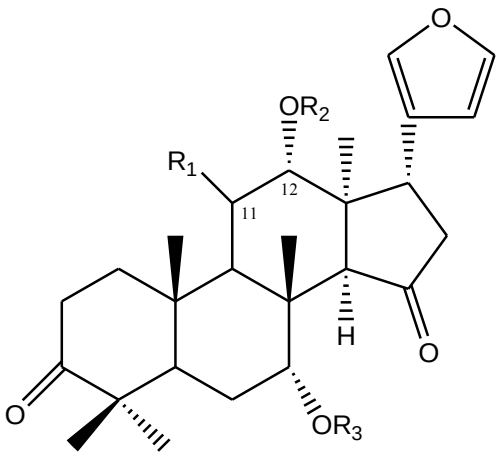
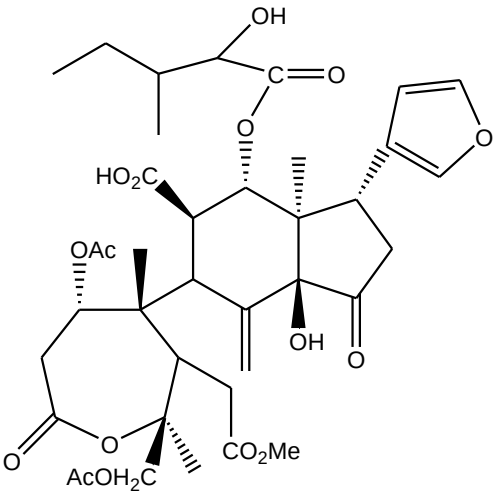


R_1 = H	R_2 = Ac	R_3 = Ac	**35**
R_1 = H	R_2 = Ac	R_3 = H	**36**
R_1 = Ac	R_2 = H	R_3 =Ac	**37**

Turraea obtusifolia's leaves, bark and root bark are used traditionally to treat stomach, intestinal ailments and severe emetic. In Zimbabwe, it is used to prevent fearful dreams associated with heart, rheumatism and swollen painful joints (Rajab *et al*., 1998). Their leaves contain limonoid compounds, which are used in agriculture as antifeedants that is insect repellants to protect plants from insect damage. Extraction of the whole plant gave compound Prieurianin (**38**), a complex limonoid which is a used as a taxonomic indicator

(Akinniyi *et al*., 1986). Compounds Heudelottins A **(39)**, Heudelottins B **(40)** and Hitin **(41)** were also isolated from the plant (Sarker *et al*., 1997; Rajab *et al*., 1998).



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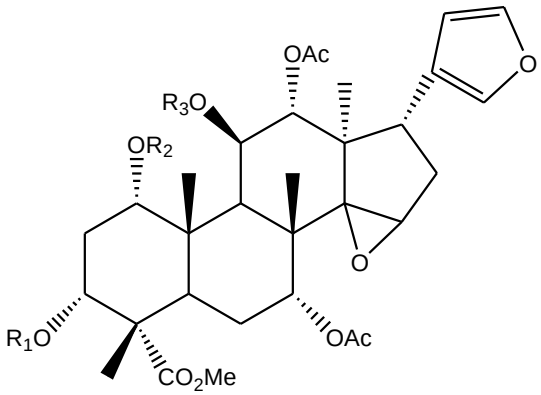
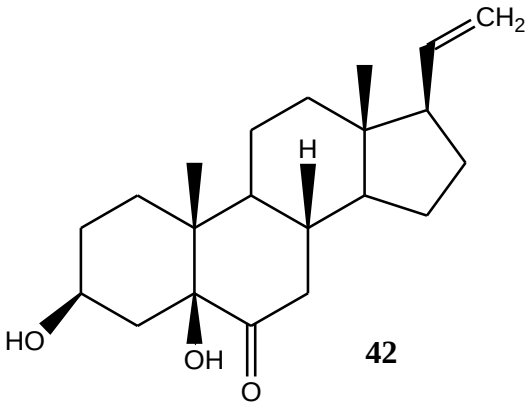


R_1 = H, R_2 = Ac, R_3 = H **39**

R_1 = H, R_2 = Ac, R_3 = COC_3H_7 **40**

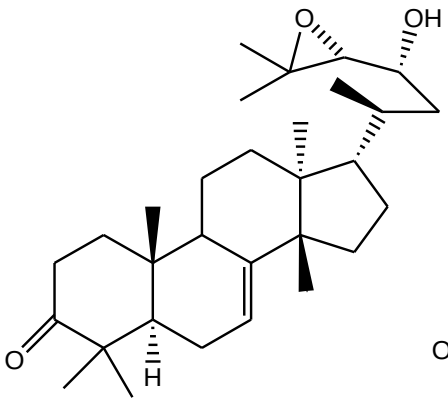
R_1 = Ac, R_2 = H, R_3 = COC_3H_7 **41**

Turraea villossa is traditionally used to treat cancer and diarrhoea. A steroid, Villosterol **(42)** was isolated from it's aerial part (Chiplunkar *et al*., 1993).



42

Turraea nilotica's roots are boiled and the decoction taken if the stomach is upset (Kokwaro, 1976). Isolation of the stem wood and bark yielded a protolimonoid compound Niloticin **(43)** and two closely related compounds Tetra-*nor*-aldehyde (**44)** and 23,24-Dihydroxy-25-methoxy-7-tirucallen-3-one **(45)** Mulholland and Taylor, 1988.



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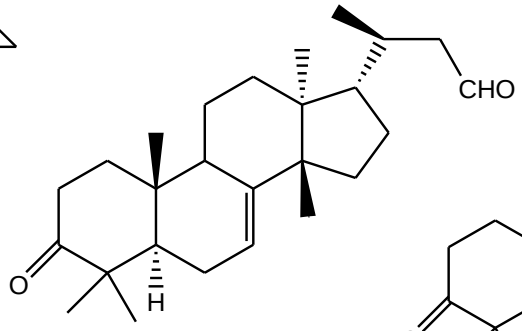

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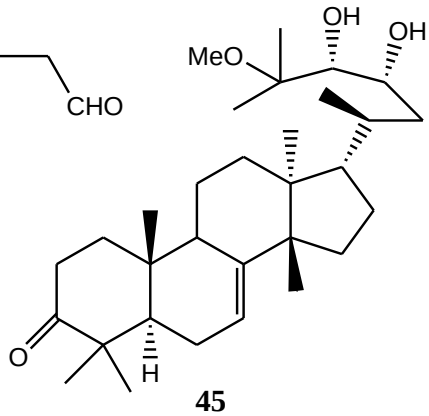
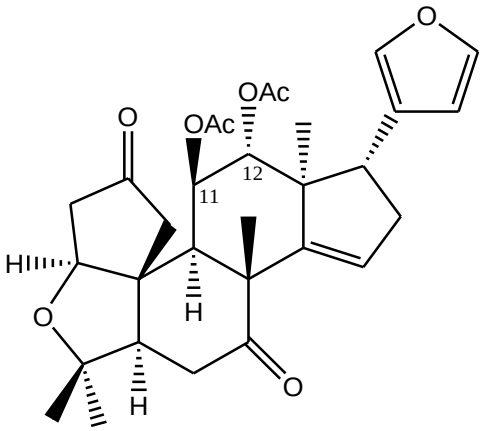


45

Turraea wakefieldii is closely related to *Turraea floribunda*. The bark is used as an emetic, while both root wood and bark are used as a purgative (Kokwaro, 1976). They have limonoids that exhibit a wide variety of biological properties including insect-antifeedant, insecticidal and antimicrobial activity (Ndung'u *et al*., 2003; Ndung'u *et al*., 2004). Five limonoids 11*β*,12*α*-Diacetoxyneotecleanin **(46)**, 7*α*,12*α*-diacetoxy-14*β*,15*β*-epoxy-11*β*-hydroxyneotecleanin **(47)**, 11*β*,12*α*-Diacetoxy-14*β*,15*β*-epoxyneotecleanin **(48)**, 7*α*,12*α*-Diacetoxy-14*β*,15*β*-epoxy-11*β*-hydroxyneotecleanin **(49)** and 11*β*,12*α*-Diacetoxy-1-deoxo-14*β*,15*β*-epoxy-3-hydroxy-2-oxo- neotecleanin **(50)** have been isolated from the root bark. Compounds **46, 47** and **50** exhibited larvicidal activity against larvae of *Anopheles gambiae* (Ndung'u *et al*., 2003; Ndung'u *et al*., 2004).



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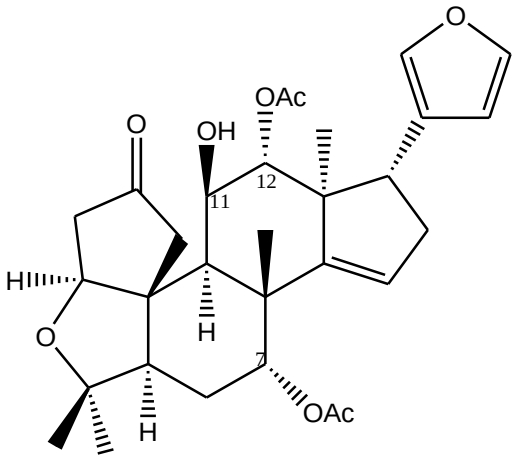

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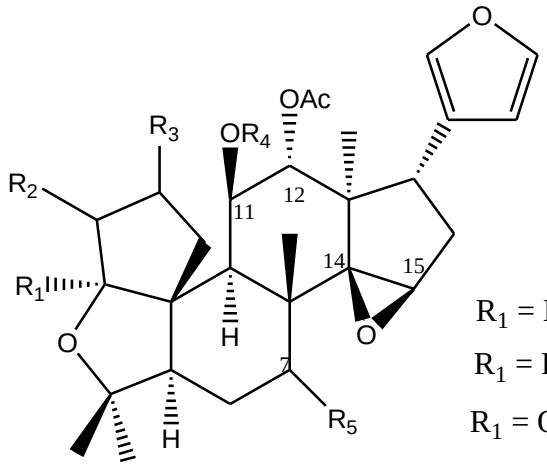
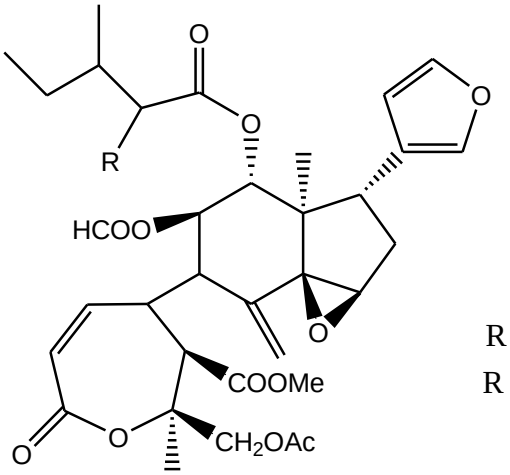


$R_1 = H$ $R_2 = H$ $R_3 = O$ $R_4 = Ac$ $R_5 = O$ **48**

$R_1 = H$ $R_2 = H$ $R_3 = O$ $R_4 = Ac$ $R_5 = OAc$ **49**

$R_1 = OH$ $R_2 = O$ $R_3 = H$ $R_4 = Ac$ $R_5 = O$ **50**

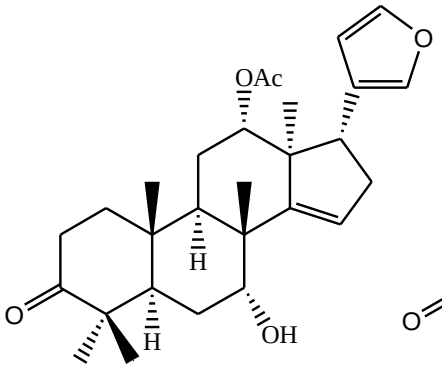
Turraea mombassana root decoction is used by the Masai to treat excess bile, malaria and other fevers (Kokwaro, 1976). Two prieurianin types of limonoids, Mombasol (**51)** andMombasone **(52)** have been isolated from it's stem and roots (Adul *et al*., 1993).



R = OH **51**

R = O **52**

Turraea cornucopia's methanol root bark and chloroform extracts exhibited potent larvicidal and adulticidal activity. For larvicides, the methanol extract was the most active with an LD_{50} values of 202 ppm and the chloroform was the most active as an adulticide with an LD_{50} of 302.1 ppm. Three limonoids were isolate, 12α-acetoxy-1, 2-dihydro-7-deacetylazadiron **(53),** Mzikonone **(54)** and 1α-12β-diacetoxy-1,2-dihydro-7-deacetyl-3β-7α-dihydroxyazadiron **(55)** Owino *et al*., 2014.



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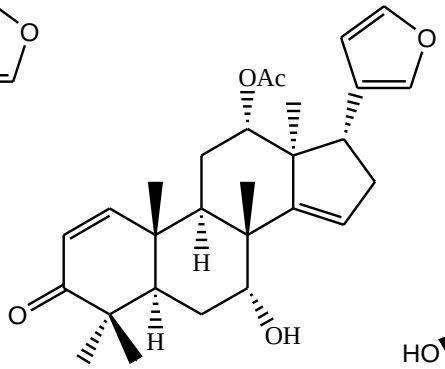

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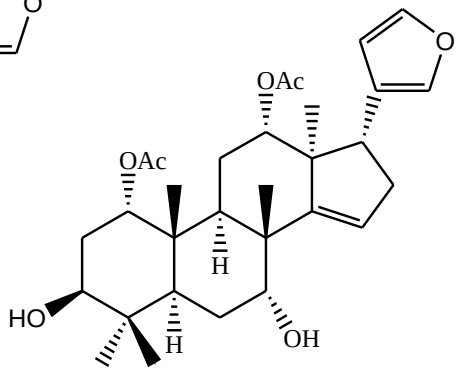
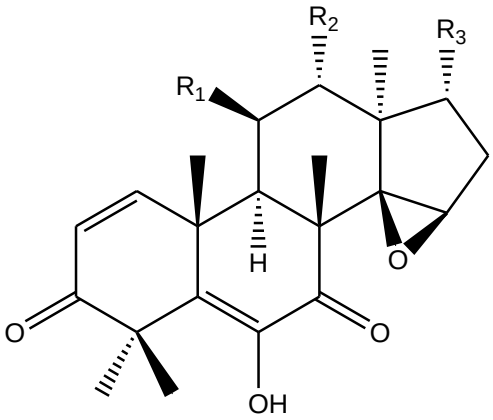
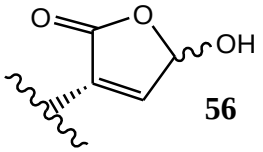


55

Turraea abyssinica (Figure 2.1) is a shrub that is widely found in Narok, Ngong, Kirinyaga and Kakamega (Ivan and Greenway, 1961). It's methanol leave extract had some antiplasmodial activity of 21.9 μg/mL. The extract also possessed significant toxic potential with LD_{50} of 270.7 ppm. Fractionation of this extract gave a limonoid derivative 11β,12α-Diacetoxywalsuranolide **(56)**, three other limonods 11-*epi*-21-Hydroxytoonacilide **(5)**,14β,15β -Epoxide 1,7,12-*tri*-Ac,Me ester **(8),** 11β,12α-Diacetoxycedrelone **(57)** and a tetranortriterpenoid, Walsuranolide **(58)** Essoung*et al*., 2018. The compounds showed some larvicidal activities with an LD_{50} of < 7.0 ppm (Githua, 2006). No antimicrobial activity has been documented on this species neither its compounds.



$R_1 = R_2 = OAc$, $R_3 =$ 

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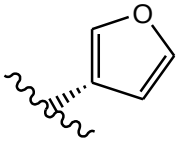
$R_1 = R_2 = OAc$, $R_3 =$ 

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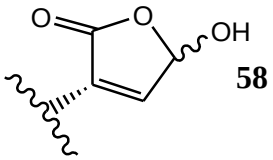
$R_1 = R_2 = OAc$, $R_3 =$ 

58

Figure 2. 1: Whole plant of Narok *Turraea abyssinica*

2.3 The Genus *Meyna*

The Rubiaceae family comprises of about 637 genera and 10,700 species (Mongrand *et al*., 2005). This family is used to treat malaria, headaches, asthma, epilepsy, sore eyes and as an emetic in many developing countries. The genus consists of about 12 species found in Africa and the Indian Ocean islands to the South East Asia. Many of the members of the closely related genera *Keetia*, *Ps*ydrax and *Multidentia* have edible fruits (Maundu and Tengnas, 2005).

Meyna laxiflora methanol seed extract after been assessed for *in vitro* antioxidant activity, was found to possess free radical scavenging property. The IC_{50} values were 84.2±2.1, 91.0±3.0 and 104.5±3.4 μg/ml for DPPH, H_2O and NO radical scavenging respectively (Ganesh *et al*., 2010). Aqueous and methanol extracts of various parts of the plant were also possessed ferric reducing power (Bag *et al*., 2016). A research done at Satpuda hill in India showed that the plant is widely used by the villagers as food, anticancer, anti-inflammatory, anti-dysentery, treatment of kidney stones and abdominal distention (Majaz and Khurshid, 2014). *Meyna spinosa* is also used in India to treat hepatic disorders, gastrointestinal problems, severe skin infections and diabetes (Borah *et al*., 2015).

Meyna tetraphylla (Figure 2.2) is called *Tulungwo* in *Pokot* and *Mutunguru* in Kikuyu. The plant is armed with pained spines above the nodes and the leaves appear to be in fours, actually in pairs on very short spurs at each node. It is a shrub or tree, which is 5-6 m long. It has white or green flowers and its fruits are bluntly 5-angled, 13-17 by 16-20 mm. The buds are sparsely hairy, pedicels densely hairy (Beentje, 1994). Crushed leaves are put

between the infected hooves of goats and camels by the Pokots. It is also used as an animal fodder and the root decoction is given to the pregnant women to alleviate pain (Beentje, 1994). No phytochemical research has been done on this species so far.

Figure 2. 2: Whole plant of Baringo *Meyna tetraphylla*

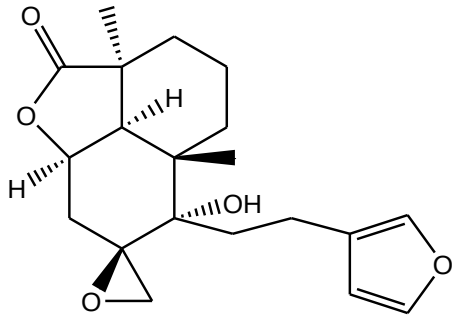
2.4 The Genus *Leonotis*

The genus is from the Lamiaceae family that has 7,200 species distributed in 236 genera. They are known to treat cold, cough, fever, headache and asthma (Fowle, 2006). *Leonotis* genus comprises about ten species (Nurdan and Aysel, 2007).

Leonotis nepetifolia is a very abundant weed in abandoned agricultural land in the whole world. Previous studies have attributed a variety of salutary physiological effects to this species (Boalino and Tinto, 2004). A tea made from its leaves is used to treat coughs, fever, stomach ache, skin ailments, kidney diseases, rheumatism and dysmenorrheal (Kokwaro, 1976; Boalino and Tinto, 2004). In India, the ash of the inflorescence is used to treat burns. Antibacterial activity of the methanol and ethyl acetate extracts of the plant against *Pseudomonas aeruginosa* has been reported (Boalino and Tinto, 2004).

Chemical studies of *Leonotis nepetifolia* led to the isolation of labdanoid, diterpenoids, coumarins and iridoids. The extracts of aerial parts showed anti-inflammatory

activity on TPA-induced edema model. The chromatography of the extracts gave compounds Leonotinin **(59)** and Stigmasterol **(60)** Hortensia *et al*., 2004.



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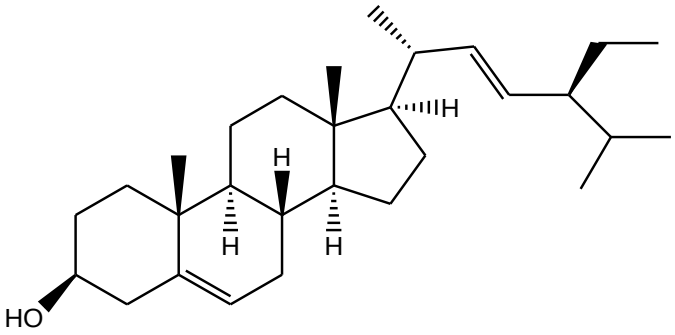

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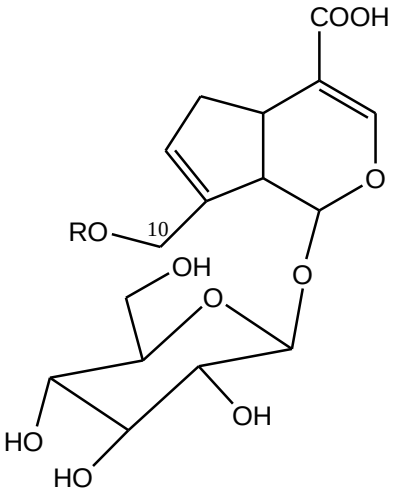
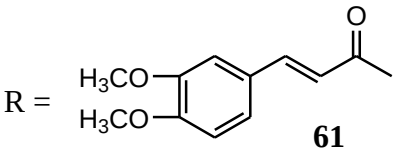
Also two iridoids, 10-*O*-(*trans*-3,4-dimethoxycinnamoyl) geniposidic acid **(61)** and Geniposidic acid **(63)**, along with compounds 10-*O*-(*p*-hydroxybenzoyl) geniposidic acid **(62),**Mussaenoside **(64)** andIxoside **(65)** and three phenylethanoid derivatives have been isolated from the stem of *L*eonotis *nepetifolia*. These compounds were found to have antioxidant activity (Tadahiro *et al*., 1999).





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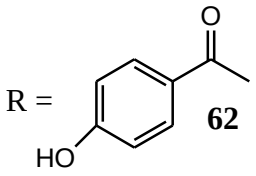
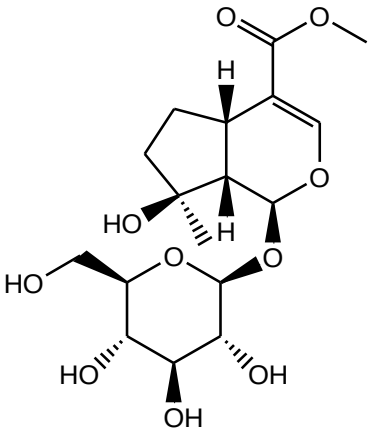


62

R = H **63**



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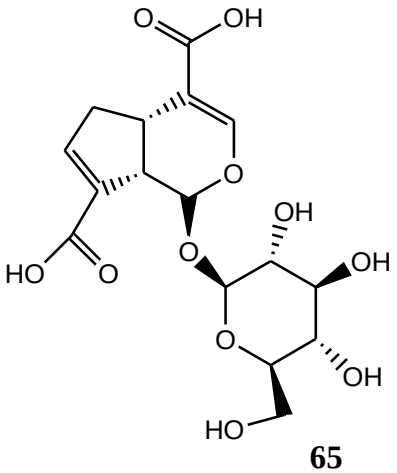
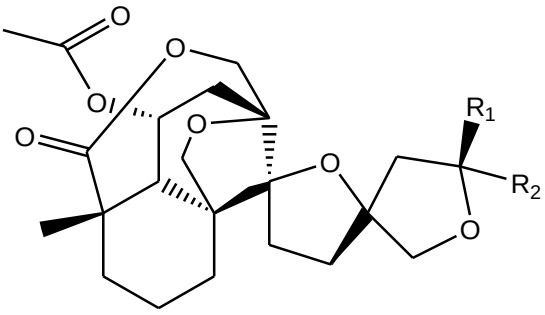


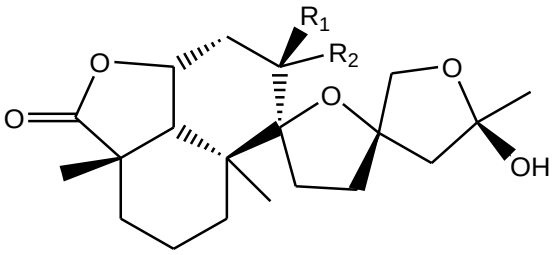
65

Eighteen *bis*-spirolabdane diterpenoids Leonepetaefolins A **(66)**, 15-*epi*-leonepetaefolins A **(67)**, Leonepetaefolins B **(68)**, 15-*epi*-leonepetaefolins B **(69)**, Leonepetaefolins C **(70)**, 15-*epi*-leonepetaefolins C **(71)**, Leonepetaefolins D **(72)**, 15-*epi*-leonepetaefolins D **(73)**, Leonepetaefolins E **(74)**, 5-*epi*-leonepetaefolins E **(75)**, Labdane A **(76)**, Labdane B **(77)**, Labdane C **(78)**, Labdane D **(79)**, Labdane E **(80)**, Labdane F **(81)**, Labdane G **(82),** Labdane H **(83).** Two flavonoids Apigenin **(84)** and Cirsiliol **(85)** were also isolated from the leaves of *Leonotis nepetifolia*. The compounds were assessed for their binding properties in several CNS G protein-coupled receptor assays *in vitro* (Jun *et al*., 2012). A GC-MS analysis on wild and cultivated *Leonotis nepetifolia* showed differences in quality and quantity. Another sixteen compounds Methyl laurate **(86)**, Methyl myristate **(87),** Phytol **(88)**, Methyl palmitate **(89)**, G-undecanolide **(90)**, G-decanolide **(91),** 9,12-Octadecynoic acid methyl ester **(92)** Methyl linoleate **(93)**,6-Octadecynoic methyl ester **(94)**, Methyl stearate **(95)**, Arachidic acid methyl ester **(96)**, Docosanoic acid methyl ester **(97)**, Squalene **(98)**, Stigmast-5-en-3β-ol **(99)**, Stigmast-7-en-3β-ol **(100)** and Stigmasterol **(60)** were isolated totaling 95.13% with Methyl linoleate (46.98%) been the highest compound in quantity. Twenty one compounds 1,3-Diisopropylcyclohexane **(101)**, 1,4-Diisopropylcyclohexane **(102)**, Propanoic acid-2-methyl-2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester Palmitic acid methyl ester **(103)**, Propanoic acid-2-methyl-3-hydroxy-2,4,4-trimethylpentyl ester linolenic acid methyl ester **(104)**, Propanoic acid 2-methyl-1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester **(105)**, Palmitic acid methyl ester **(106)**, Linolenic acid methyl ester **(107)**, 11-Octadecenoic acid methyl ester **(108)**, Stearic acid methyl ester **(109)**, 1,2-Benzenedicarboxylic acid-1,2-bis(2-ethylhexyl) ester **(110)**, 3-Methylheptadecane **(111)**, Docosane **(112)**, Hentriacontane **(113)**, Pentacosane **(114)**, Nonacosane **(115)**, Cycloartenol **(116)**, β-Amyrin **(117)**, α-Amyrin **(118)** together with **92, 94** and **98** were also identified from the cultivated specimen totaling 88.76%. Two isomers propanoic acid-2-methyl-3-hydroxy-2,4,4-trimethylpentyl ester **(119)** 31.97% and Propanoic acid-2-methyl-2,2-dimethyl-1-(2-hydroxy-1-methylethyl)-propyl ester **(120)** 22.78% were identified as the majority constituents in the species (Oliveira *et al*., 2015).



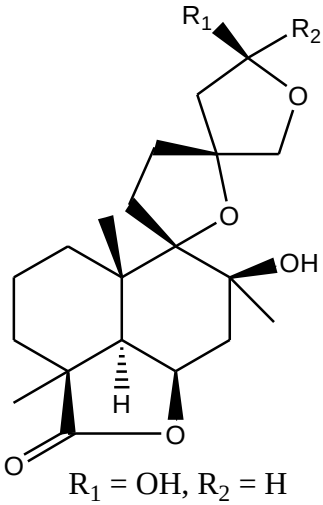
R_1 = OH, R_2 = H **66**

R_1 = H, R_2 = OH **67**



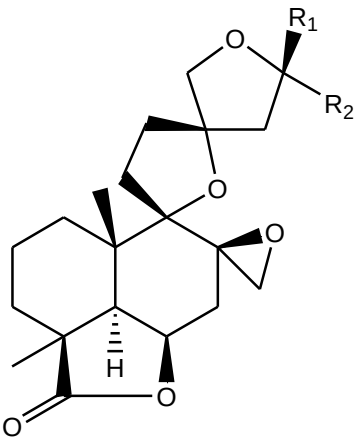
R_1 = OH, R_2 = H, **68**

R_1 = H, R_2 = OH, **69**



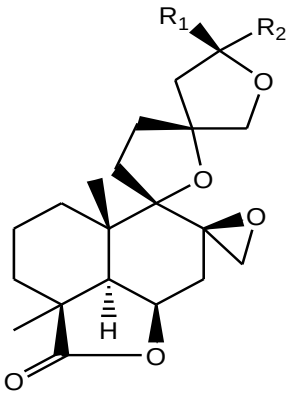
R_1 = OH, R_2 = H **70**

R_1 = H, R_2 =OH **71**



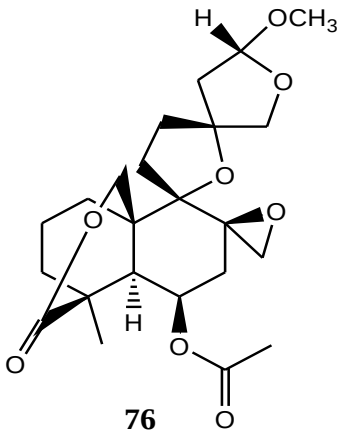
R_1 = OH, R_2 = H **72**

R_1 = H, R_2 = OH **73**



R_1 = OH, R_2 = H **74**

R_1 = H, R_2 = OH **75**



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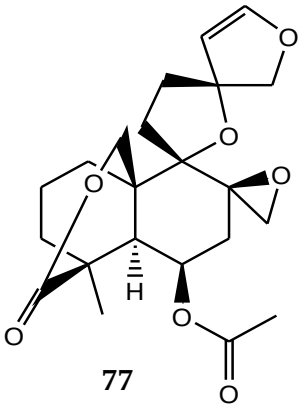

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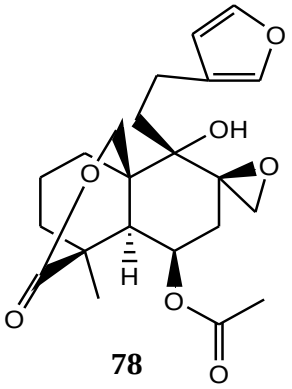

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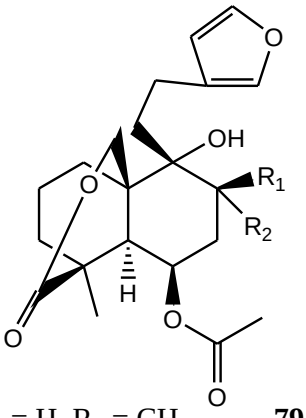
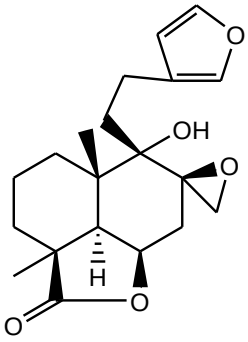


R_1 = H, R_2 = CH_3 **79**

R_1 = OH, R_2 = CH_2Cl **80**



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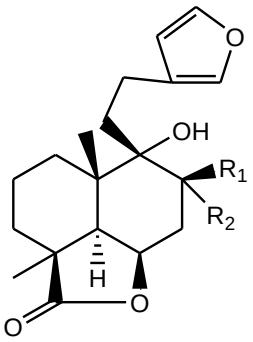
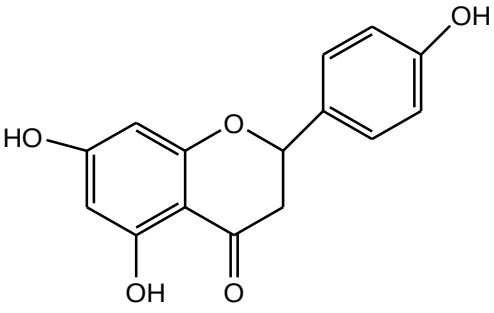


R_1 = OH, R_2 = CH_3 **82**

R_1 = OH, R_2 = CH_2OH **83**



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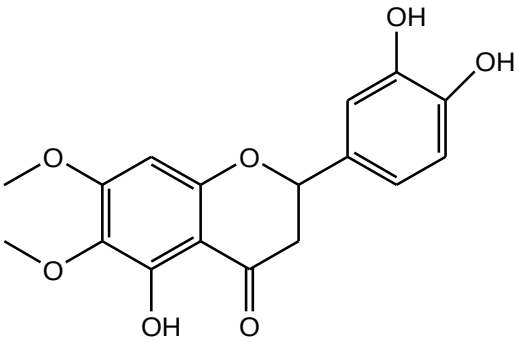

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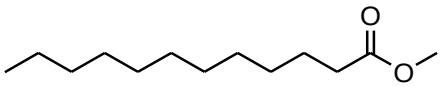

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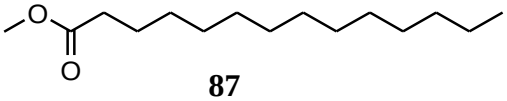

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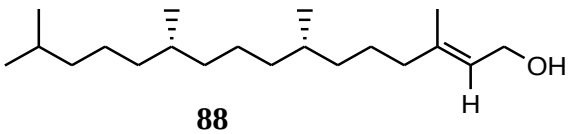

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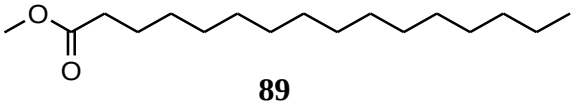

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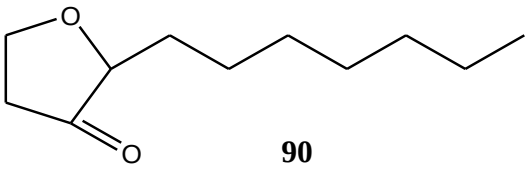

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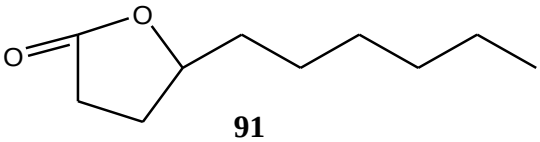

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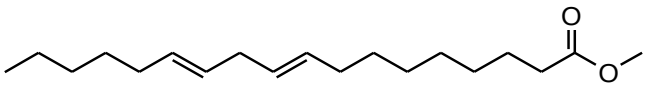

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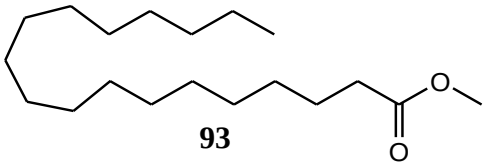

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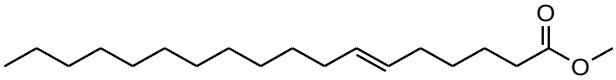

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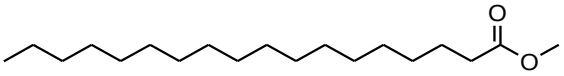

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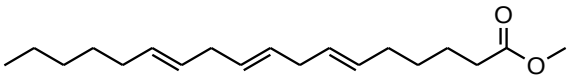

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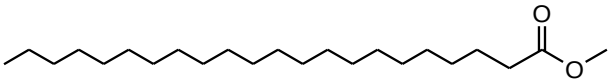

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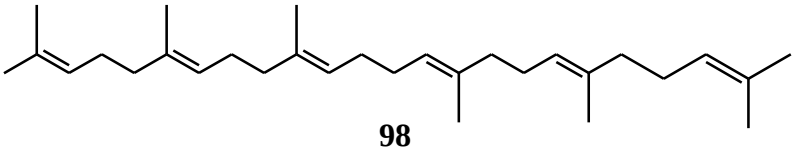

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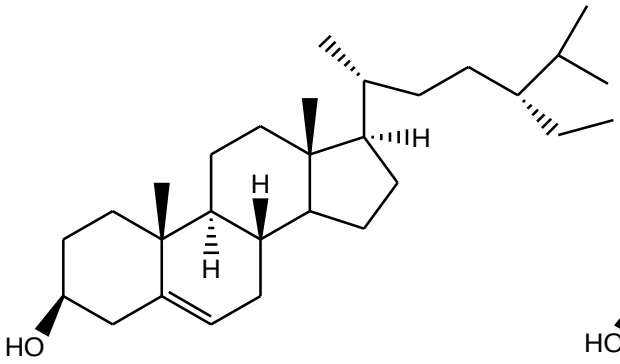

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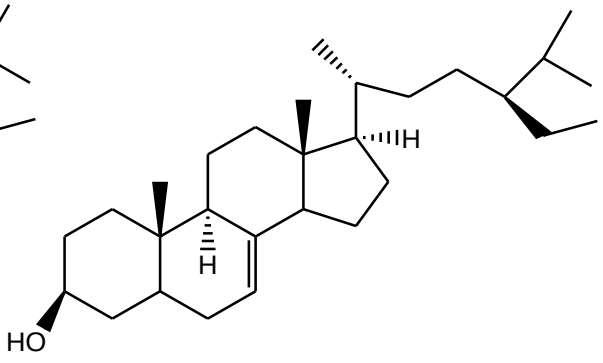

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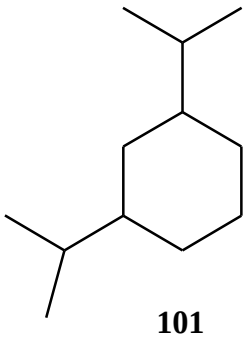

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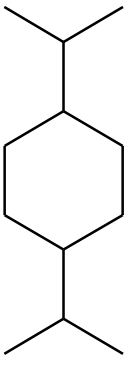

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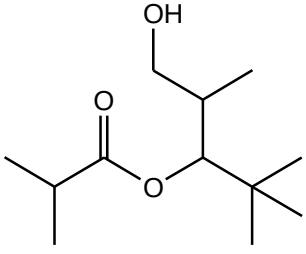

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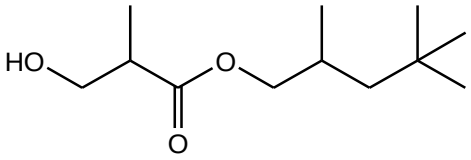

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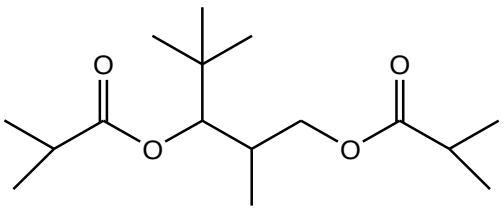

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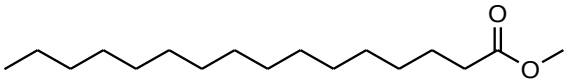

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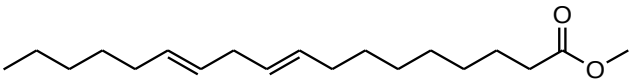

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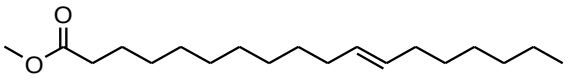

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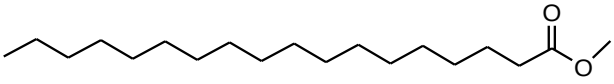

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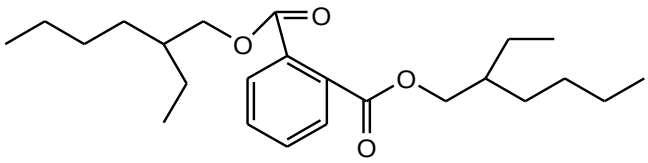

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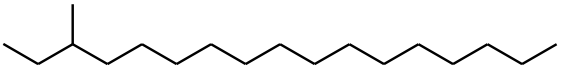

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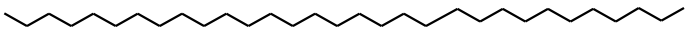

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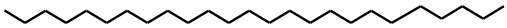

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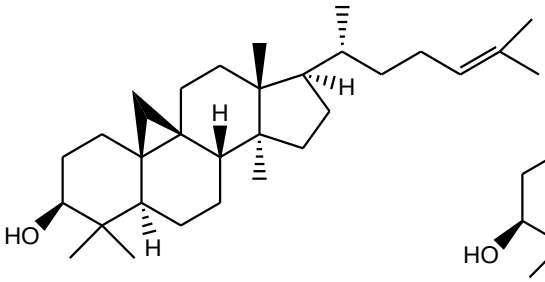

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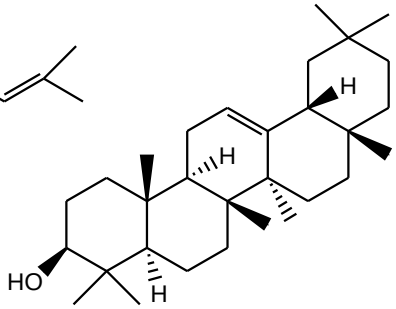

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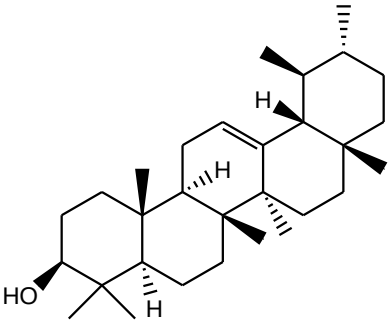

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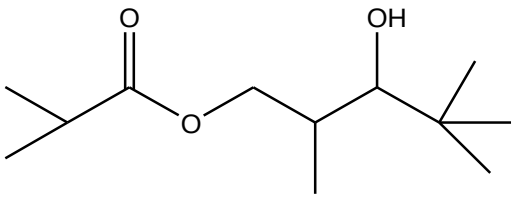

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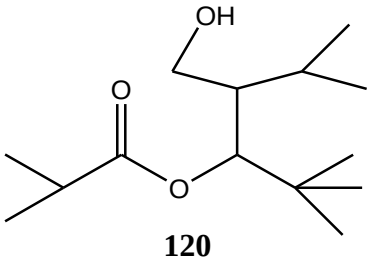


120

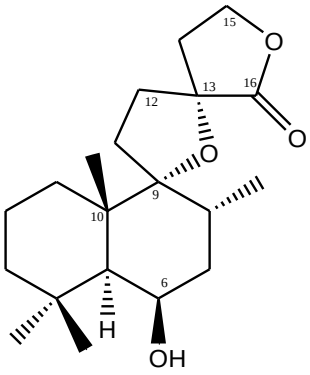
Leonotis leonurus is commonly used in Southern Africa to treat fevers, headaches, dysentery, flu, chest infections, epilepsy, constipation, intestinal worms, spider bites, scorpion stings, hypertension, asthma, arthritis, leprosy and snake bites (Naidoo *et al*., 2011; Mazimba, 2015). It contains substantial amount of nutrients and minerals. It's aqueous leaf extract has also been reported to possess antinociceptive, anti-inflammatory and hypoglycemic properties and activity against type-2 diabetes mellitus (Naidoo *et al*., 2011). Based on it,s well-documented traditional usage profile for respiratory ailments, and it's *in vitro* antibacterial activity, it was identified as a possible source of novel anti-tuberculosis compounds (Stafford *et al*., 2008; Naidoo *et al*., 2011).

The compounds of this plant accounts for the rational use of *Leonotis leonurus* in treating obesity, digestive disorders and muscular cramps. Thirty seven phytochemical compounds have been isolated which are largely constituted of flavonoids, sterols, labdane type diterpenoids, cyclic diterpenes, triterpenoids, tannins, quinines, saponins, iridoids glycosides, alkaloids, dicarboxylic acid and phenolics detected in the acetone and methanol extracts (Naidoo *et al*., 2011; Mazimba, 2015). Their essential oils have high content of monoterpenoids and sesquiterpenoids showing considerable antimicrobial activities. Labdane-type diterpenoids 9,13-Epoxy-6-hydroxy-labdan-16,15-olide **(121)** and 9,13:15,16-Diepoxy-6,16-labdanediol **(122)** have been isolated (Bienvenu *et al*., 2002; Naidoo *et al*., 2011; Mazimba, 2015).

Three Leoleorins, Leoleorin A **(123)**, Leoleorin B **(124)**, Leoleorin C **(125),** eleven Leoleorins Labdane diterpenoids, Leoleorin D **(126)**, Leoleorin E **(127),** Leoleorin F **(128),** Leoleorin G **(129),** Leoleorin H **(130),** Leoleorin I **(131),** Leoleorin J **(132),** Leoleorin K **(133),** Leoleorin L **(134),** Leoleorin M **(135),** Leoleorin N **(136)**, and 16-*Epi*-leoleorin F **(137)** were isolated from the leaves. In a viable binding assay, all isolated compounds showed inhibition in excess of 50% at various CNS receptors. Leoleorin C (**125)** showed adequate binding affinity (Ki = 2.9 lM) for the Sigma 1 receptor (Hankui *et al*., 2013; Mazimba, 2015). Research has also shown that Marrubin (**138)**, a component of *Leonotis leonurus*, lessens diabetic symptoms (Mnonopi *et al*., 2012; Mazimba, 2015).

Thirty eight diterpenes have also been isolated from *Leonotis lenurus*, 13R-premarrubin **(139)**, 13S-premarrubin **(140)**, Leonurun **(141)**, Hispanolone **(142)**, Nepetaefolin **(143)** and polyphenols, Dihydroxyphytyl palmitate **(144)**, Acteoside **(145)**, Geniposidic acid **(146)**, Luteolin **(147)**, Luteolin-7-*O*-*β*-glucoside **(148)**, Apigenin **(149)**, Apigenin-8-*C*-*β*-glucoside **(150)**, Apigenin-7-*O*-*β*-glucoside **(151)**, 4',6-Dimethoxyluteolin **(152)**, 3'-Methoxyluteolin-7-*O*-*β*-glucoside **(153)**, 3,-Methoxyluteolin **(154)**, Apigenin-6-*C*-*α*-

arabinoside-8-*C*-*β*-glucoside **(155)**, Succinic acid **(156)**, Uracil **(157)**, Leonurine **(158)**, Apigenin-6-*C*-*α*-arabinoside-8-*C*-*β*-glucoside, *p*-Cymene **(160)**, Limonene **(161)**, (Z)-*β*-Ocimene **(162)**, (E)-*β*-Ocimene **(163)**, *γ*-Terpinene **(164)**, Terpinolene **(165)**, *β*-Bourbonene **(166)**, *β*-Cubebene **(167)**, *β*-Caryophyllene **(168)**, *α*-Humulene **(169)**, Germacrene D **(170)**, Bicyclogermacrene **(171)**, Caryophyllene oxide **(172)** and Spathulenol **(173)** Mazimba, 2015.



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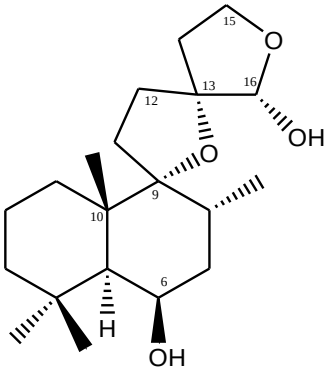

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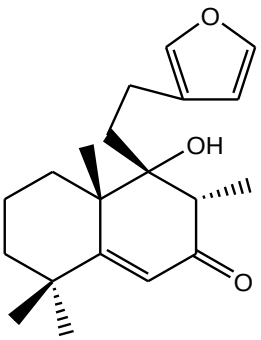

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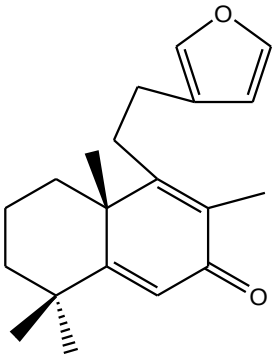

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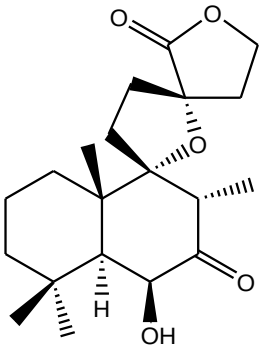

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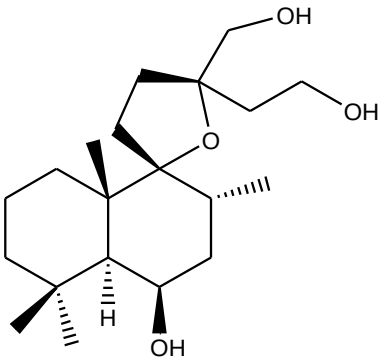

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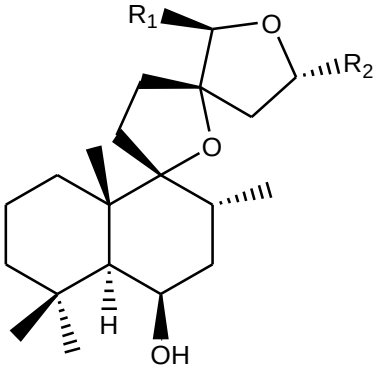
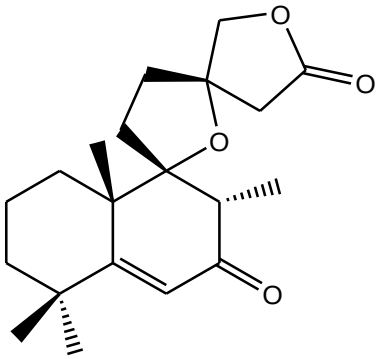


R_1 = H, R_2 = OH **127**

R_1 = OH, R_2 = H **128**



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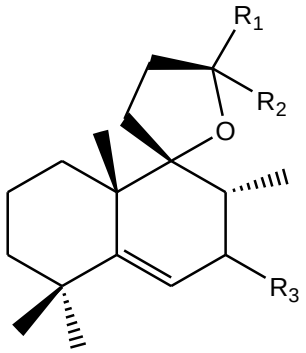
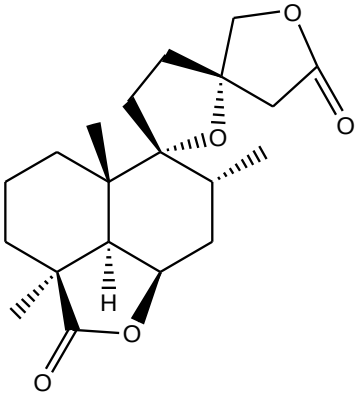


R_1 = CH_2OH, R_2 = $C_2H_4OCOCH_3$, R_3 = O **130**

R_1 = CH_2OH, R_2 = C_2H_4OH, R_3 = O **131**

R_1 = CH_2OH, R_2 = C_2H_4OH, R_3 = β-OH **132**



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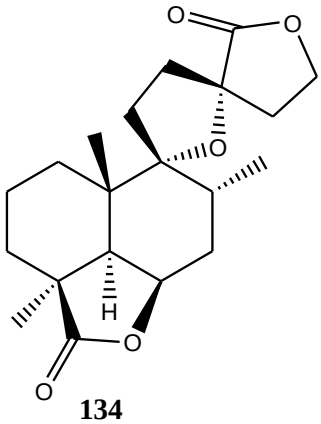

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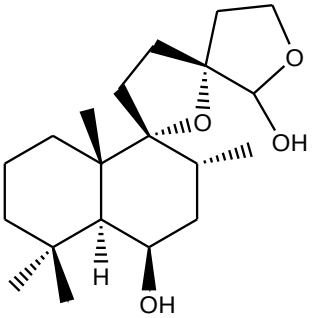

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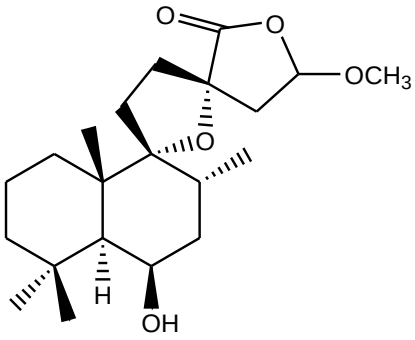

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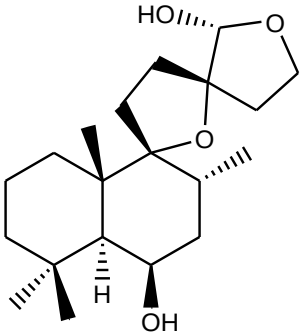

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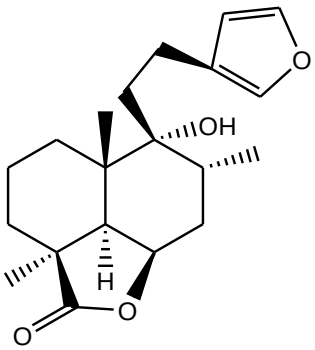

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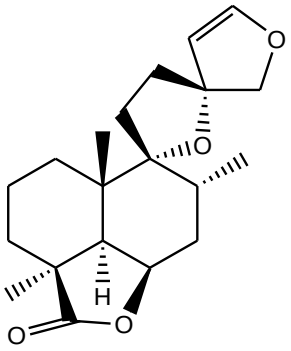

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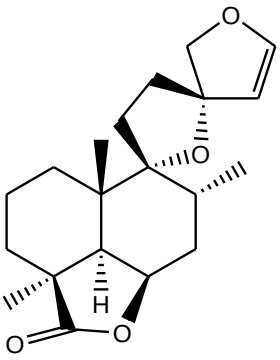

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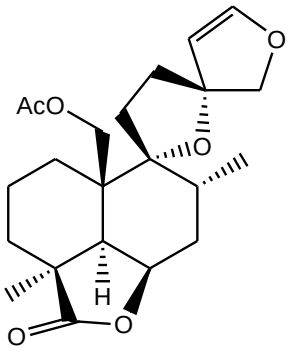

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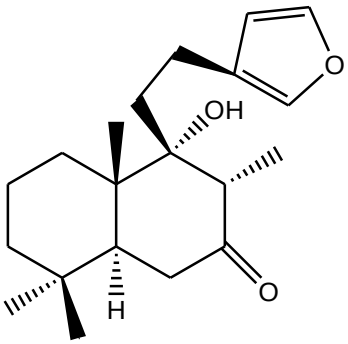

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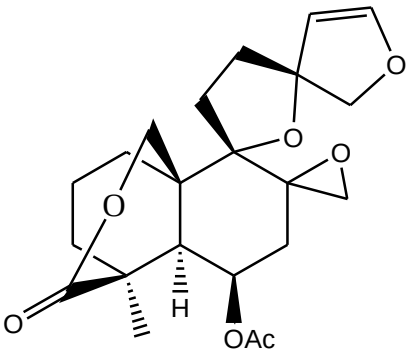

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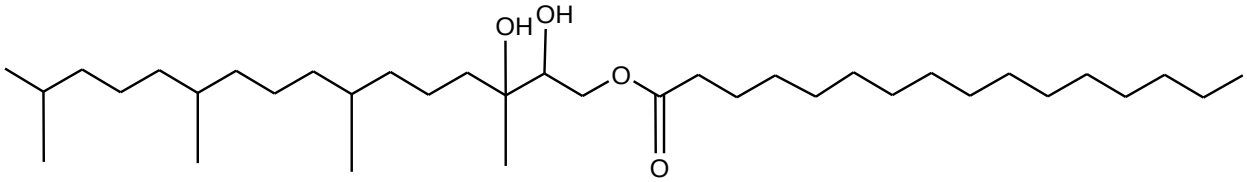

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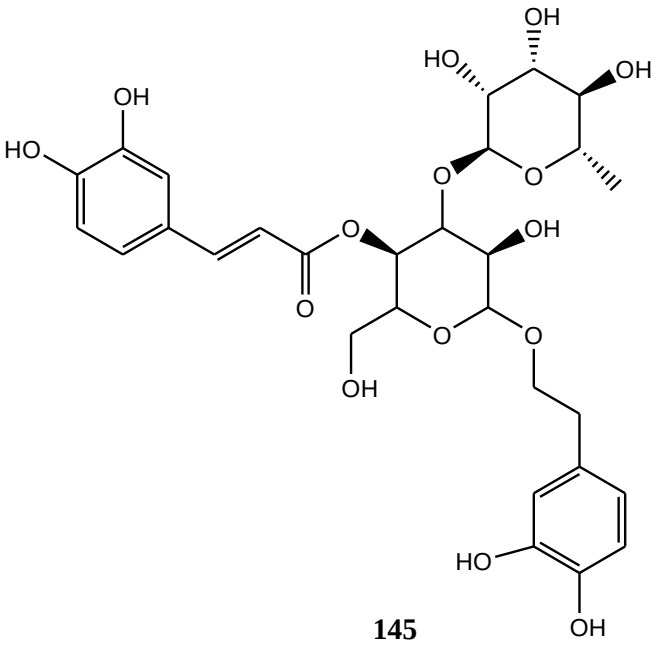

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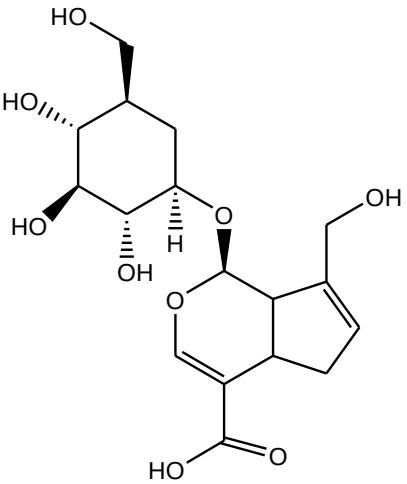

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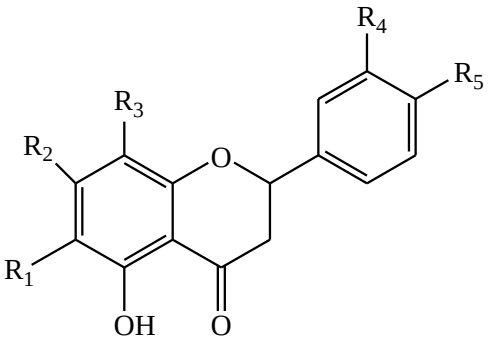


R_1 = H, R_2 = OH, R_3 = H, R_4 = OH, R_5 = OH **147**

R_1 = H, R_2 = *O-gluc*, R_3 = H, R_4 = OH, R_5 = OH **148**

R_1 = H, R_2 = OH, R_3 = H, R_4 = H, R_5 = OH **149**

R_1 = H, R_2 = OH, R_3 = *O-gluc*, R_4 = H, R_5 = OH **150**

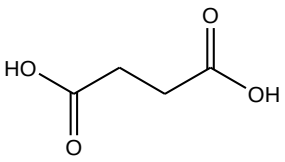
R_1 = H, R_2 = *O-gluc*, R_3 = H, R_4 = H, R_5 = OH **151**

R_1 = OCH_3, R_2 = OCH_3, R_3 = H, R_4 = OH, R_5 = OCH_3 **152**

R_1 = H, R_2 = *O-gluc*, R_3 = H, R_4 = OCH_3, R_5 = OH **153**

R_1 = H, R_2 = OH, R_3 = H, R_4 = OCH_3, R_5 = OH **154**

R_1 = *O-gluc*, R_2 = OH, R_3 = *O-gluc*, R_4 = H, R_5 = OH **155**



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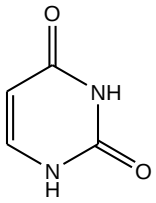

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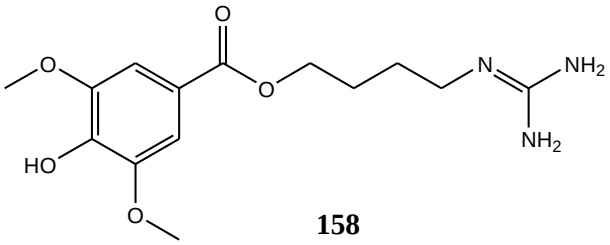

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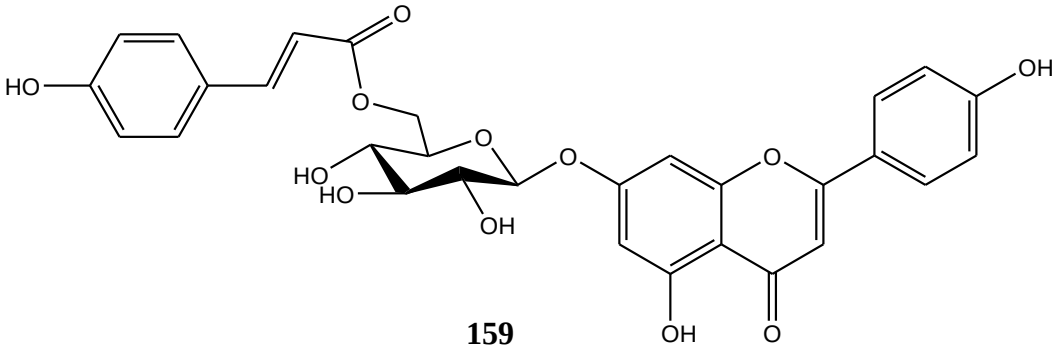

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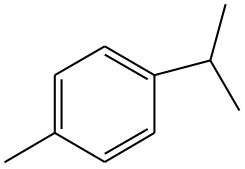

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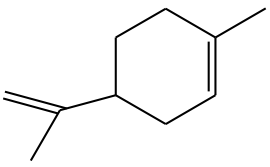

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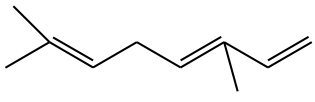

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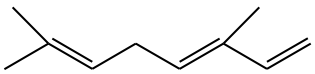

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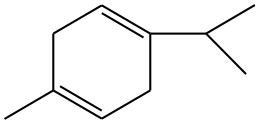

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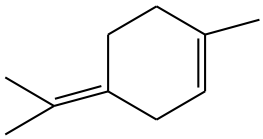

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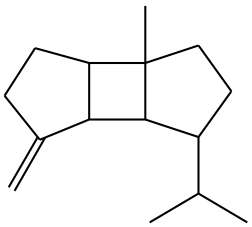

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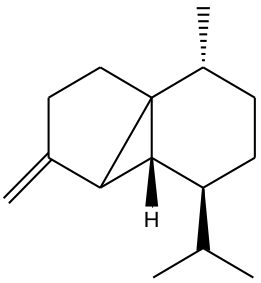

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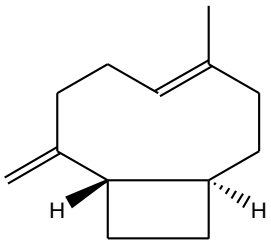

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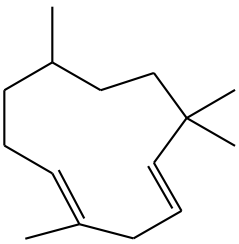

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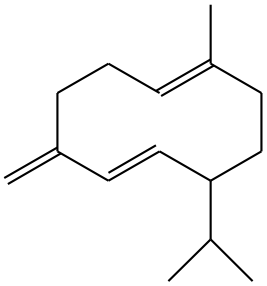

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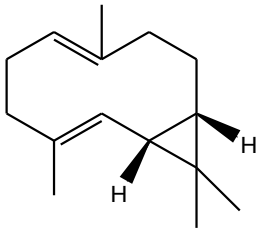

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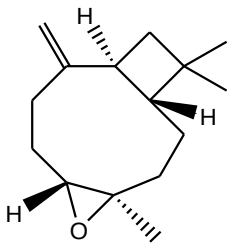

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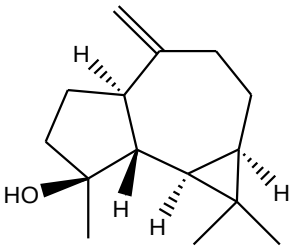

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Leonotis mollissima (Figure 2.3) is known to treat cold, cough, fever, headache and asthma (Fowler 2006). It is called *kipserere* in Marakwet. The root decoction is used by the Marakwets of Kenya to treat wound, festering sore and intestinal worms. Young leaves and buds are used to treat conjunctivitis and indigestion. The leaves are also chewed for cramp in the stomach (Kokwaro, 1976). In Tanzania, the root decoction is used to treat malaria (Fowler 2006). This would be an interesting plant to work on as chemical composition and biological activity has not been reported.

Figure 2. 3: Whole plant of Mau Narok *Leonotis mollissima*

2.5 Spectroscopy

2.5.1 Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance spectroscopy is the absorption and emission of electromagnetic radiation by the nuclei of certain atoms when they are placed in a magnetic field. In order to absorb electromagnetic radiation, nuclei must possess a non-zero magnetic moment. Samples for NMR spectroscopy are typically liquids (or solutions) and solids. The basic components of NMR include a strong magnet into which the sample is placed, a radiofrequency transmitter and a receiver system connected to some type of data display or storage device (Field and Sternhell, 1989; Phillip *et al*., 1998).

The ^{1}H nucleus (proton) is the most commonly studied nucleus by NMR because of the ease of observation, its high natural abundance and the fact that it is invariably present in the majority of samples. Despite its low natural abundance (1.1%), ^{13}C is also an important nucleus because carbon forms the backbone of all organic compounds and structural information can be obtained by NMR spectroscopy. With modern instrumentation, NMR spectra can be obtained routinely on most isotopes. An NMR spectrum is normally presented as a graph of absorption intensity against the frequency of radiation absorbed by the nuclei in a sample. This method is quantitative in that the integrated intensity of a signal is proportional to the concentration of nuclei giving rise to it and for this reason NMR

spectroscopy is a powerful technique for establishing the relative concentrations of components in mixtures (Field and Sternhell, 1989; Phillip *et al*., 1998).

Often one dimensional (1-D) NMR data obtained at the highest available magnetic field do not provide enough information to complete a structure analysis or to assign the resonances in a complex spectrum. Today a variety of multipulse sequences is applied in investigations of complex molecules and such techniques are available on most new NMR instruments. The most important benefit of these methods is that individual chemical shifts and all coupling constants can be measured unequivocally even when multiplets are overlapping. Many two dimensional (2-D) experiments are made up of some basic building blocks, for example: COSY, NOESY HMBC and DEPT which were used in this project (Phillip *et al*., 1998).

2.5.1.1 COSY (Correlation Spectroscopy)

It is a two dimensional experiment in NMR that is used to identify nuclei that share a scalar (*J*) coupling. The presence of off-diagonal peaks (cross-peaks) in the spectrum directly correlates the coupled partners. Most often used to analyse coupling relationships between protons (Phillip *et al*., 1998).

2.5.1.2 NOESY (Nuclear Overhauser Effect Spectroscopy)

This is a two dimensional method that is used to map NOE correlations between protons within a molecule. Most popular with, and best suited to, the study of very large molecules such as bio-polymers, although it still has a place in small molecule work. The spectrum has a layout similar to COSY but cross peaks now indicates NOEs between the correlated protons (Phillip *et al*., 1998).

2.5.1.3 HMBC (Heteronuclear Multiple-Bond Correlation)

It is a two dimensional experiment that is used to identify long-range couplings (two to three bonds) between protons and carbons. It has good sensitivity because it utilises proton detection and it is an extremely powerful tool for piecing together organic structures (Phillip *et al*., 1998).

2.5.1.4 DEPT (Distortionless Enhancement by Polarisation Transfer)

It is a one dimensional experiment that is used for enhancing the sensitivity of carbon observation and editing of ^{13}C spectra. The sensitivity gain comes from starting the experiment with proton excitation and subsequently transferring the magnetization onto

carbon (the process known as polarisation transfer). The editing feature alters the amplitude and sign of the carbon resonances according to the number of directly attached protons, allowing the identification of carbon multiplicities. The experiment is typically run using different final proton pulse angle, resulting in differing signs (+ve or –ve) for various carbon resonances (Field and Sternhell, 1989; Phillip *et al*., 1998).

2.6 Microbial diseases

Microbial diseases have been a problem to man for many years (Mead *et al*., 1999). Each year, more than 200 known microbial diseases are transmitted through air, food and water. They cause about fourteen million illnesses, sixty thousand hospitalizations, and one thousand eight hundred deaths every year. Examples are pneumonia, tuberculosis and cholera, which are caused by micro-organisms like *Salmonella typhimurium, Staphylococcus aureus, Escherichia coli* and *Bacillus cereus* bacteria (Mead *et al*., 1999).

2.7 Micro-organisms

These are organisms which are so small that they can only be seen under the microscope. They include bacteria, yeasts, fungi and moulds. They live almost everywhere on earth where there is liquid water, including hot springs on the ocean floor and deep inside rocks in the earth's crust. They are vital to humans and the environment, as they participate in the Earth's element cycles such as the carbon and nitrogen cycles. They have also fulfilled other vital roles in virtually all ecosystems, such as recycling other organism's dead remains and waste products through decomposition. They cause many diseases like diarrhoea and cancer (Wolska, 2003). In this research project, some selected micro-organisms (*Escherichia coli, Salmonella typhimurium, Bacillus cereus, Staphylococcus aureus* and *Candidas albicans*) were used for the bioassay test.

2.7.1 *Escherichia coli*

They are Gram-negative bacteria that commonly inhabits in the human intestine. They also live in the intestine of many other animals, wild as well as domestic. They cause severe and life-threatening diarrhoea (Aoki *et al*., 2005).*Turraea abyssinica* and *Leonotis mollissima* are used by the local people to treat Pneimonia and urinary tract infections that are caused by *Escherichia coli.*

2.7.2 *Salmonella typhimurium*

They are Gram-negative bacteria that multiply in the gastrointestinal tract of many animal species where they usually cause no disease. In humans their growth causes gastroenteritis. Six to forty eight hours after ingestion of contaminated water or food (usually poultry or beef), illness may begin with nausea and vomiting, often followed by diarrhoea. Local people from the sampling counties use*Turraea abyssinica* and *Leonotis mollissima* to treat such diseases. Isolations of *Salmonella* causing gastroenteritis in humans have increased in recent years in developed countries, primarily because modern methods of animal husbandry, food preparation, and distribution encourage the spread of *Salmonella* (Menichetti, 2005).

2.7.3 *Staphylococcus aureus*

They are Gram-positive coccus that requires anaerobic conditions for growth. They live on the skin or in the nose of a person and cause a range of illnesses like skin infections such as pimples, boils, and cellulites. They also cause abscesses to life-threatening diseases such as pneumonia, meningitis, endocarditis, Toxic shock syndrome (TSS), and septicemia (Menichetti, 2005). Communities from sampling counties *Leonotis mollissima* to treat the above diseases.

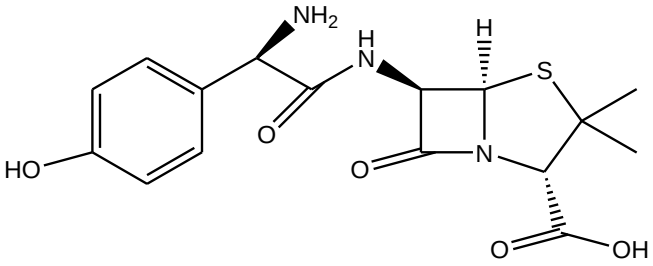
2.7.4 *Candidas albicans*

They are diploid asexual fungus and a causal agent of opportunistic oral and genital infections in humans. Systemic fungal infections (fungemia) have emerged as important causes of morbidity and mortality in immuno-compromised patients (*e.g.*, AIDS, cancer chemotherapy, organ or bone marrow transplantation). Local communities from sampling counties use *Meyna tetraphylla* to treat fungal infections. *Candidas albicans* are among the gut flora, the many organisms that live in the human mouth and gastrointestinal tract. Under normal circumstances, they live in 80% of the human population with no harmful effects, although overgrowth results in candidiasis (Jones *et al*., 2004).

2.8 Antibiotics

Antibiotics are compounds that are produced by living cells and they inhibit in very low concentrations, the growth of micro-organisms such as bacteria, fungi or protozoan. Examples are Amoxil® **(174)** and Doxycycline® **(175)** antibiotics that were used as positive controls in the antibiotic assays in comparison with the plants crude extracts. These

antibiotics are active against many gram positive and gram negative bacteria. Amoxicillin is used to treat pneumonia, skin infections, urinary tract infections and salmonella infections (Simar *et al*., 2011)). Doxycycline treats cancer, eye infection, gonorrhoea, periodontitis among others (Pages *et al*., 2015).



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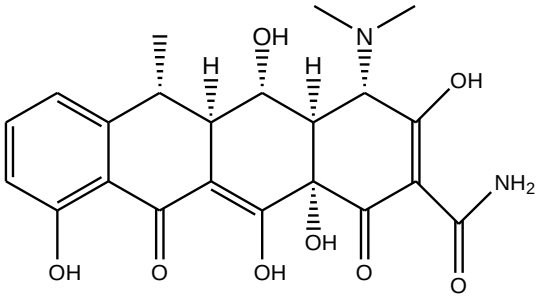

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CHAPTER THREE
MATERIALS AND METHODS

3.1 Collection, Identification and processing of Plant materials

Turraea abysinica (leaves, stem bark and root bark) were sampled from Narok County (North) and Kirinyaga County (East) in June 2015. *Meyna tetraphylla* (leaves and fruits) were sampled from Baringo County (Chemeron) and Tharaka Nthi County (Maua) in June 2014. *Leonotis mollissima* (leaves, stem bark and root bark) were sampled from Laikipia County (Laikipia University) and Nakuru County (Mau Narok) in June 2014 (Figure 3.1). They were identified by Prof. S. T. Kariuki and voucher specimen deposited at the Botany Department, Egerton University. The parts were cut into small pieces and air-dried under shade to a constant weight. They were then ground to fine powder using a grinder at KALRO, Njoro and the masses were taken using a STANTON electronic balance.

Figure 3. 1: Kenya map showing the sampling counties (kwach, 2019)

3.2 General Chromatography

The crude extracts were spotted on a silca gel TLC plates (20 x 20 cm Macharey Nagel Duren). The mobile phases used were varying ratios of hexane, dichloromethane,

ethyl acetate, diethyl ether and methanol (AR, Scharlau). Separations were monitored with inspection under ultraviolet light (UV lamp LF-204-LS, 354 nm and 634 nm) and by spraying the plate with anisaldehyde: sulphuric acid: methanol (1:2:97) mixture. Heating was done in an oven (Electrolux Struers) at 70^{o} C for one minute. The TLC plates that showed compounds with significant Retadation factors (R_f) were used to determine the solvent system for the separation as shown in Figure 3.2.

Figure 3. 2: TLC plates showing compounds with significant R_f

The dichloromethane crude extracts were chosen because they showed significant R_f .They were fractionated by gravity column chromatography on a 2 cm by 30 cm silica gel column (60-200 mesh, Thomas Baker). Further purification was achieved by repeated column chromatography and solvent system checked with TLC (Thin Layer chromatography).

3.3 Nuclear Magnetic Resonance Spectroscopy (NMR)

Identification of pure compounds was achieved by ^{1}H and ^{13}C NMR spectroscopy. NMR spectra were recorded at room temperature on a 500 MHz Bruker AVANCE NMR spectrometer for ^{1}H and ^{13}C respectively at the School of Biomedical and Molecular Sciences, University of Surrey at Guildford UK. About 1 mg to 10 mg of the pure compound was dissolved in 5 ml deuterated solvents (with reference signals at δ_H7.26, δ_C 77.23 for $CDCl_3$ and δ_H 8.74, δ_H 7.58, δ_H 7.22, δ_C 150.31, δ_C 135.93, δ_C 123.95 C_5D_5N) in 5 mm NMR tube. The data was processed by TOPSPIN software. Chemical shifts are in parts per million (ppm) relative to the solvent peaks. One and two dimension NMR spectroscopic experiments were used to interpret the structures and then compared with known compounds reported in literature.

3.4 Extraction and purification of compounds from *Turraea abyssinica*

Dry powder of leaves (1,000 g) was successively and exhaustively extracted with 4 L hexane, 4 L dichloromethane, 4 L ethyl acetate and 4 L methanol for seventy two hours each in a 10 L metal container. Extraction of fruits (100 g) and root bark (500 g) was done in a similar way using 500 mL and 1 L of each solvent respectively. The solvents were evaporated under reduced pressure using a rotary evaporator (Büchi type R-205). The crude extracts were then weighed and kept in 100 g glass sample tubes.

The hexane, dichloromethane, ethyl acetate and methanol of all the crude extracts showed almost similar spots with the dichloromethane extracts having more significant spots and R_f on visualizing with a UV lamp and anisaldehyde spraying reagent. From dichloromethane crude extract of stem bark, three compounds were isolated. Fractions 21-80 eluted with 20 % ethyl acetate in hexane, gave compounds *β*-Sitosterol **(176)**, Scopoletin **(177)** and 2-(1',2'-Dihydroxypropyl)tetradecanoic acid **(178)**. Compound **176** was purified using 20 % methanol in dichloromethane while compounds **177** and **178** were purified with 47.5 % and 5% diethyl ether in dichloromethane respectively as indicted in figure 3.1**.**

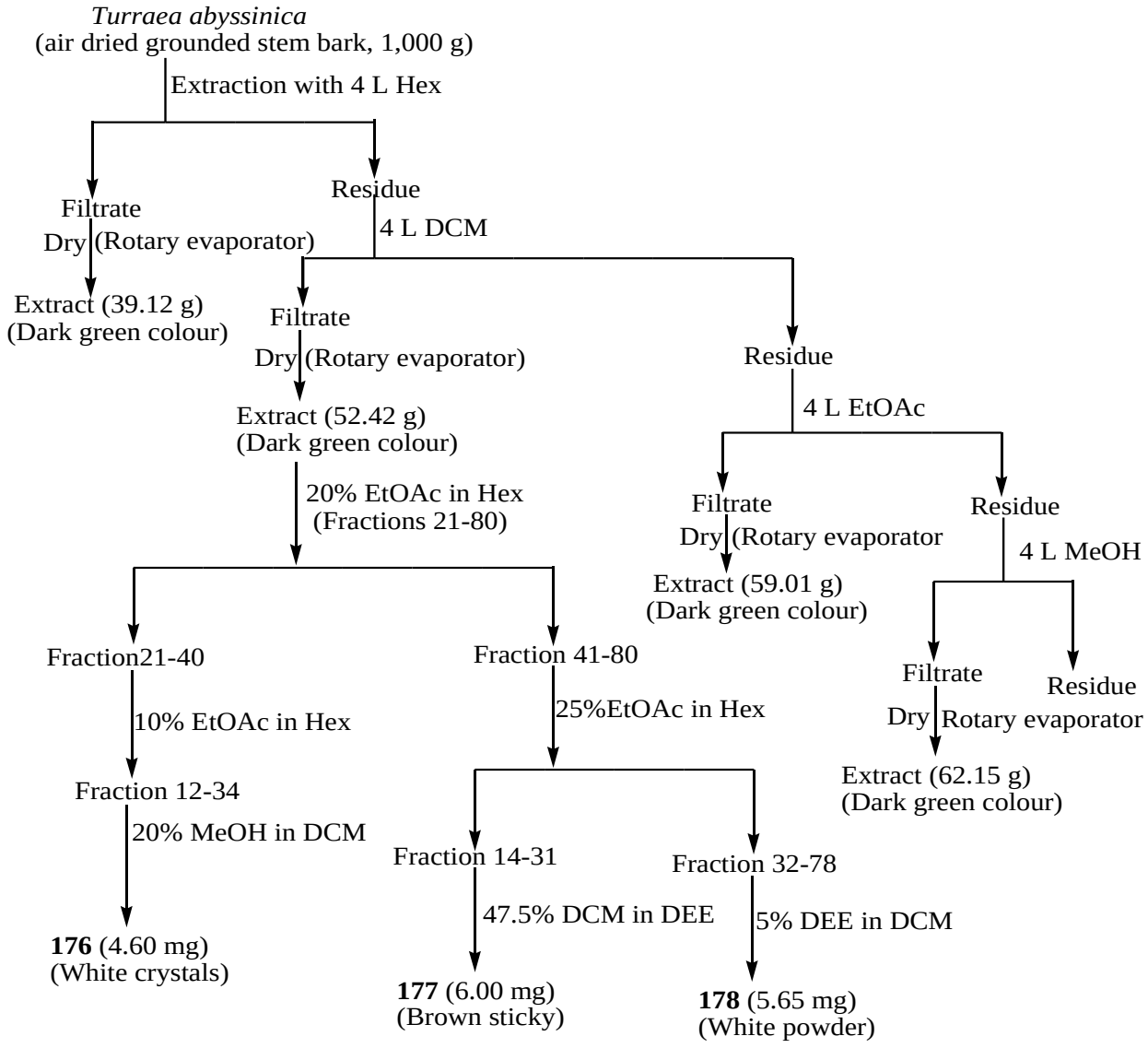


Figure 3.3: Flow chart showing isolation of *Turraea abyssinica* compounds

3.5 Extraction and purification of compounds from *Meyna tetraphylla*

Dry powdered leaves (1,000 g) was sequentially and exhaustively extracted with 4 L hexane, 4 L dichloromethane, 4 L ethyl acetate and 4 L methanol for seventy two hours each in a 10 L metal tin. Extraction of fruits (100 g) was extracted with 500 mL organic solvents using the same procedure. The solvents were evaporated under reduced pressure using a rotary evaporator. The crude extracts were then weighed and kept in sample tubes.

All the leaves crude extracts had significant spots but dichloromethane extract had more spots on visualizing with a UV lamp and anisaldehyde spraying reagent. Four compounds were isolated from dichloromethane crude extract of Baringo leaves. Fractions 13-126 eluted with 20% ethyl acetate in hexane gave compounds Phaeophytin **(179)**,

Enantiomer **(180)**, α-Amyrin **(118)** and Stigmasterol **(60)**. Compound **179** was purified using 20% methanol in dichloromethane while compounds **180, 118** and **60** were purified with 15% and 5% diethyl ether in dichloromethane respectively as indicated in figure 3.4**.**

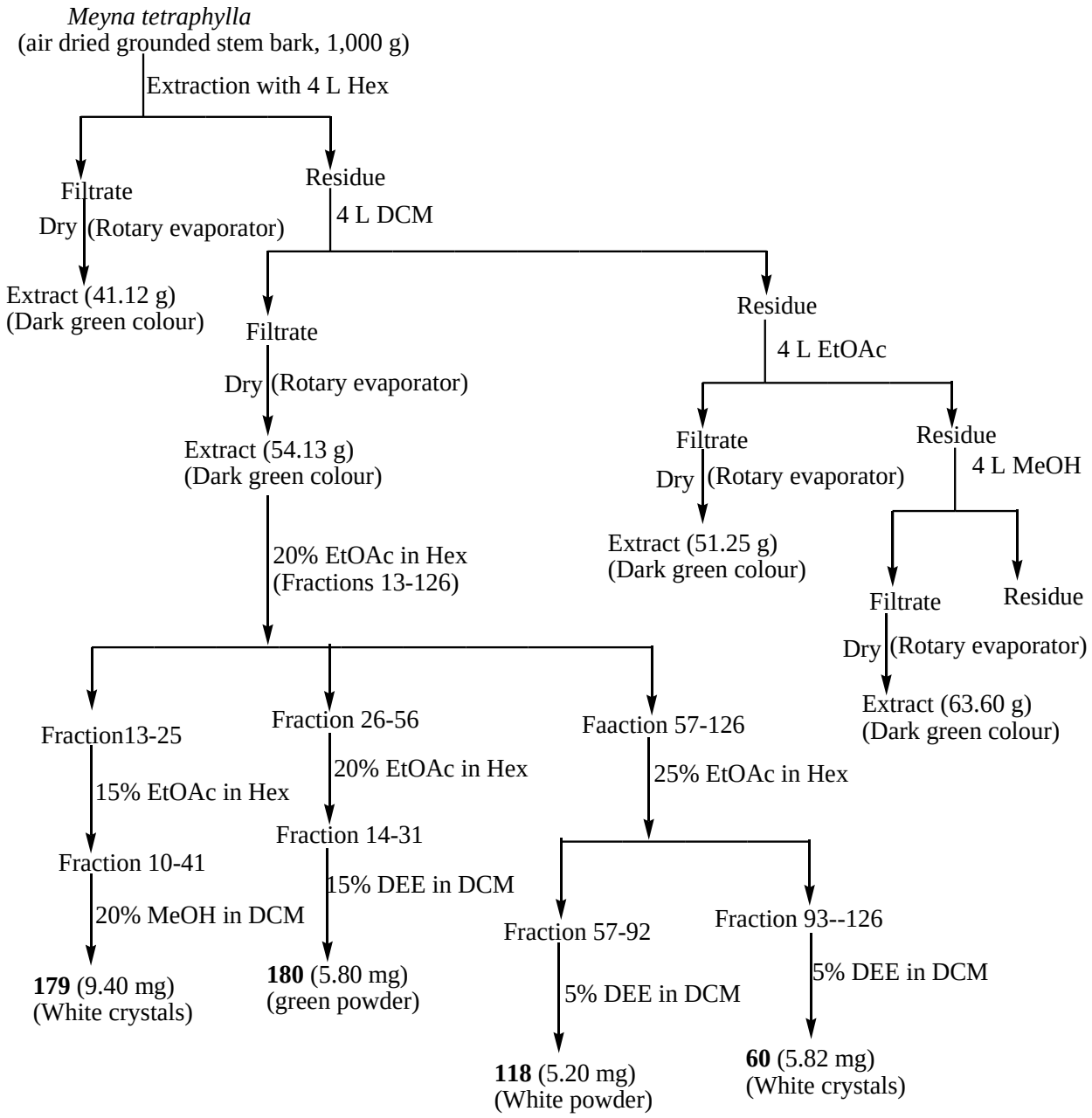


Figure 3. 4: Flow chart showing isolation of *Meyna tetraphylla* compounds

3.6 Extraction and purification of compounds from *Leonotis mollissima*

Dry powder of leaves (1,000 g) was successively and exhaustively extracted with 4 L hexane, 4 L dichloromethane, 4 L ethyl acetate and 4 L methanol for seventy two hours each in a 10 L metal container. Dry powder of root bark (500 g) and stem bark (250 g) were

extracted with 1 L and 500 mL organic solvents using the same procedure. The solvents were evaporated under reduced pressure using a rotary evaporator (Büchi type R-205). The crude extracts were then weighed and kept in 250 mL glass beakers.

Crude extracts from the plants collected in different ecological zone showed significant compounds. Dichloromethane crude extracts of leaves showed more compounds with significant R_fs on visualizing with UV lamp and anisaldehyde reagent. The dichloromethane Laikipia crude extract of leaves was subjected to a solvent step gradient of ethyl acetate: hexane. Fractions containing significant compounds were purified by repeated column chromatography using a solvent step gradient. A solvent gradient of 5% methanol in dichloromethane gave Siderin **(181),** followed by 20-Hydroxylucidenic acid **(182)** in 33% ethyl acetate in hexane while 20% ethyl acetate in hexane gave a Labdane **(183)** compound. A summary of extraction and purification is shown in figure 3.5.

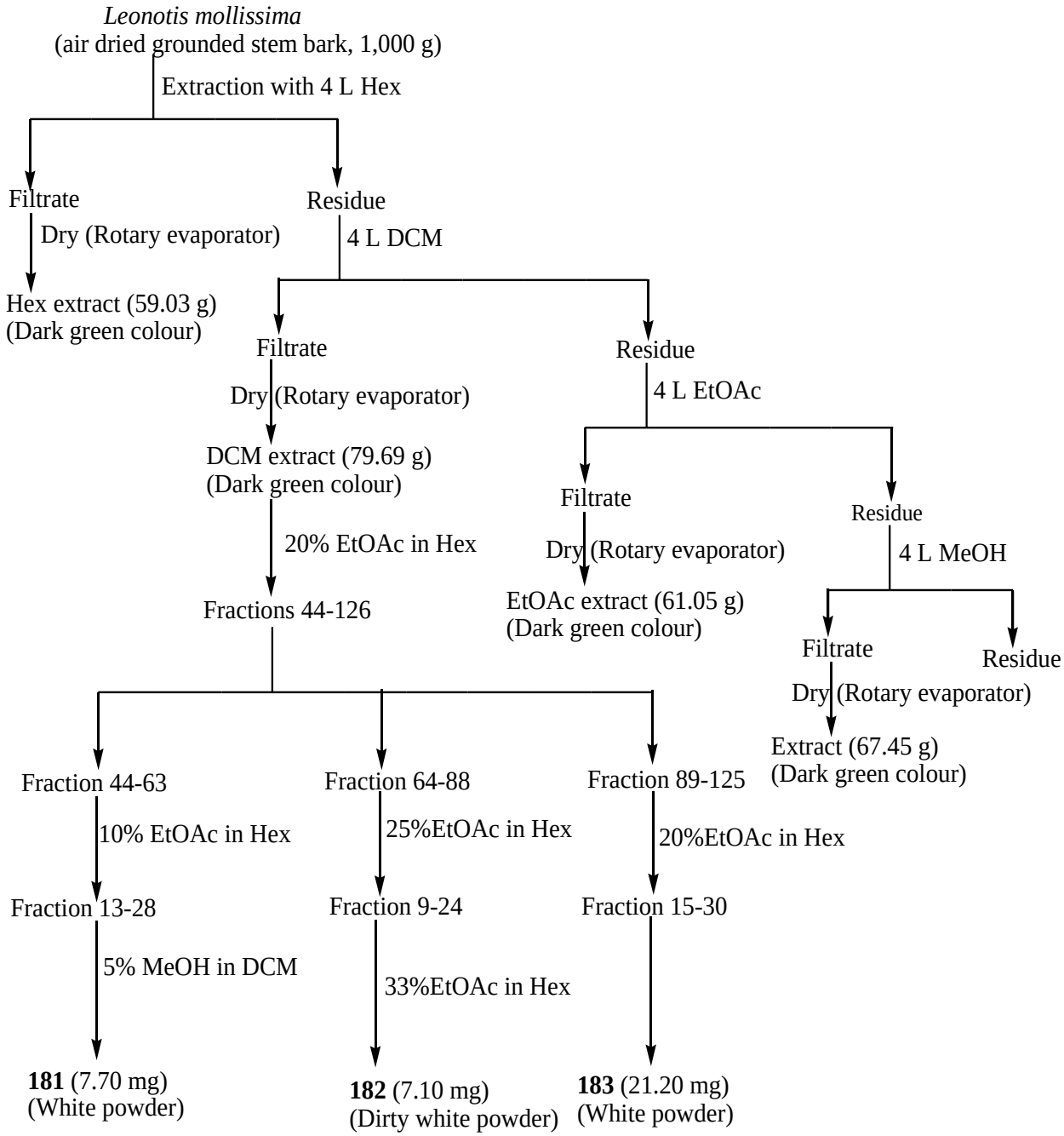


Figure 3. 5: Flow chart showing isolation of *Leonotis mollissima* compounds

3.7 Bioassay tests

3.7.1 Preparation of nutrient agar media

The bioassay test was performed by disc diffusion technique. About 14 g of nutrient agar was weighed, dissolved in 250 mL of distilled water in a 500 mL Erlenmeyer conical flask and sterilized in an autoclave at 121°C for 15 minutes. The nutrient agar was left to cool in a water bath to 40°C, then dispensed into sterile Petri dishes and left to cool in a refrigerator (Mounyr *et al.*, 2016).

3.7.2 Preparation of nutrient broth

About 1.5 g of nutrient broth was weighed, dissolved in 100 mL distilled water in a 250 mL Erlenmeyer flask, sterilized for 15 minutes at 121°C in an autoclave, and then left to cool in a refrigerator (Mounyr *et al*., 2016).

3.7.3 Resuscitation of microorganisms

Escherichia coli ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 10876 from from ChemLab Nairobiand an isolate of *Candida albicans* Scan lab Nakuru were used in this study. Each test microorganism was inoculated in the nutrient broth with a sterilized wire loop, labeled accordingly and the date of preparation indicated. This was then incubated for 24 hours at 37°C (Mounyr *et al*., 2016).

3.7.4 Inoculation and incubation of the resuscitated microorganisms

The resuscitated microorganisms were removed from the incubator and the nutrient broth's turbidity was a sign that growth had occurred. Inoculum were picked using a sterile wire loop, streaked onto respective media (inoculation) on agar plates then followed by incubation at 37°C for 24 hours to get pure cultures (Mounyr *et al*., 2016).

3.7.5 Preparation of test plates

Resuscitated pure cultures of the test microorganism were introduced into the sterilized nutrient agar in the conical flasks and poured into the plates containing the pure cultured microorganism. The surface was scrapped using a sterile loop so that the microorganism are suspended in the media and then poured back into the conical flask containing media. They were thoroughly mixed to obtain homogeneity and the nutrient agar seeded with test microorganism dispensed into the sterile agar plates. (Mounyr *et al*., 2016).

3.7.6 Testing for antimicrobial activity in crude extract

About 100 μL of 10 mg/mL crude extract was applied to the paper discs using adjustable (analogue) volume micropipette and allowed to dry by letting the solvent evaporate for 1 hr. The dry paper discs were carefully placed on the surface of the test plate seeded with the test microorganism. They were labeled, incubated for 24 hours at 37°C and

the inhibition zone measured in millimeters. The procedure was repeated for each test microorganism (Mounyr, *et al*., 2016).

3.7.7 Minimum Inhibitoin Concentration (MIC)

Determination of MIC was carried out for all the crude extracts and isolated compounds using serial dilutions. Exactly 10 μLto 50 μL of 10 mg/mL crude extracts and 5 μL to 40 μL of 4mg/mL isolated compounds were tested for antimicrobial activity in duplicates. Methanol was used as the negative control (Oshomoh, 2012).

3.7.8 Inhibition Concentration at 50% (IC_{50})

Different concentrations of Amoxil® and Doxycycline® antibiotics (10.000 mg/L, 4.000 mg/L, 1.000 mg/L, 0.400 mg/L, 0.100 mg/L, 0.040 mg/L, 0.010 mg/L and 0.004 mg/L in methanol) were prepared using serial dilutions method. The IC_{50} for Amoxil® and Doxycycline® antibiotic was determined using probit analysis software (GraphPad Prism 7 was used to plot inhibition zone against log of concentration of Amoxil® and Doxycycline® antibiotics). The IC_{50} for the crude extracts and the pure compounds were determined in a similar way. The IC_{50} for the crude extracts and the pure compounds were then compared with the IC_{50} for Amoxil® and Doxycycline® antibiotics (Oshomoh, 2012).

3.7.9 Data Analysis

A dose-response curve was drawn using the Graphpad prism program, (GraphPad Prism 7, 2018). The logarithm concentrations of the compound and crude extracts under test were plotted on the x-axis and the inhibition zone on the y-axis. The IC_{50} were determined from the dose response curve. The maximum inhibition (C), the slope and the concentration that provoked the inhibition halfway (B) between A (minimum inhibition) and C (maximum inhibition) was the IC_{50} as shown in figure 3.4 (GraphPad Prism 7, 2018).

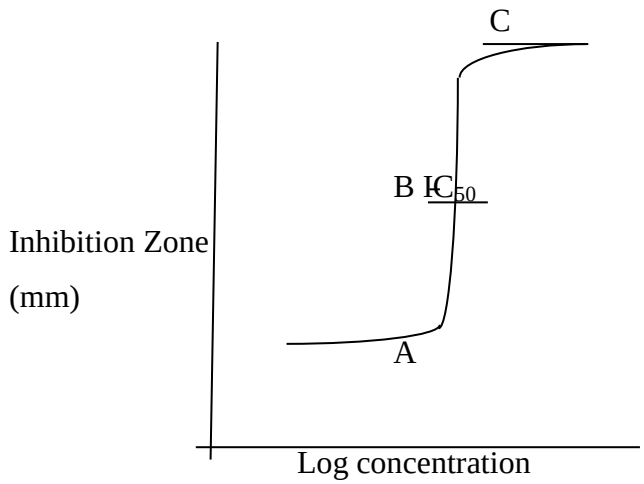


Figure 3. 6: IC_{50} Dose response curve

CHAPTER FOUR

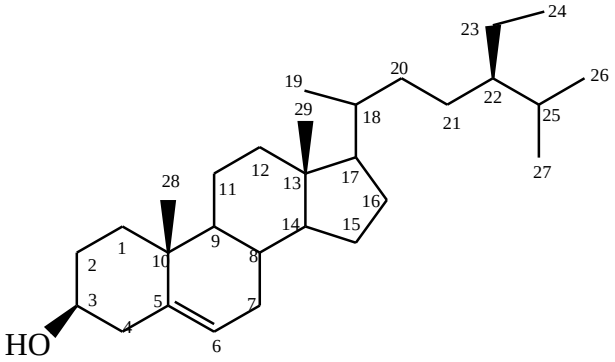
RESULTS AND DISCUSSION

4.1 *Turraea abyssinica* compounds

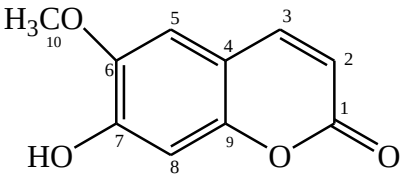
The crude extract of stem back from dichloromethane (52.42 g) was purified with repeated CC and TLC was used to monitor the solvent system. It yielded three compounds *β*-Sitosterol **176** (4.60 mg), Scopoletin **177** (6.00 mg) and 2-(1',2'-Dihydroxypropyl) tetradecanoic acid **178** (5.65 mg).

Table 4. 1 Percentage yield of crude extracts and pure compounds of *Turraea abyssinica*

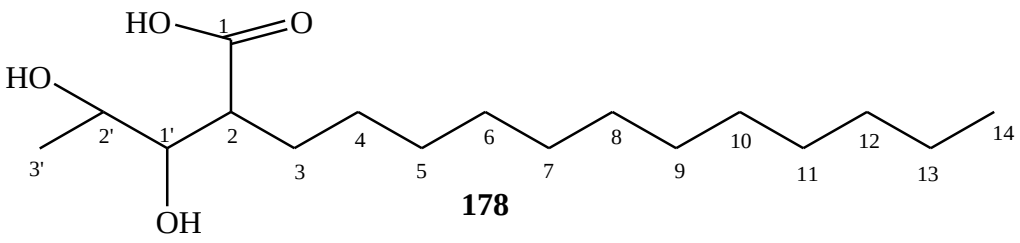
Plant	Weight (g)	Solvent	Crude extract		Pure compound		
Turraea abyssinica (Narok stem bark)	1,000		Weight (g)	% yield (w/w)	Compound name	Weight (mg)	% yield (w/w)
		Hex	39.12	3.91			
		DCM	52.42	5.24	*β*-Sitosterol **(176)**	4.60	0.01
					Scopoletin **(177)**	6.00	0.01
					2-(1',2'-Dihydroxypropyl)tetradecanoic acid **(178)**	5.65	0.01
		EtOAc	59.01	5.90			
		MeOH	62.15	6.22			



176

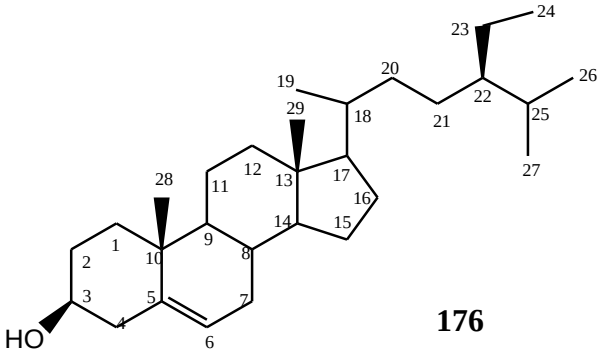


177

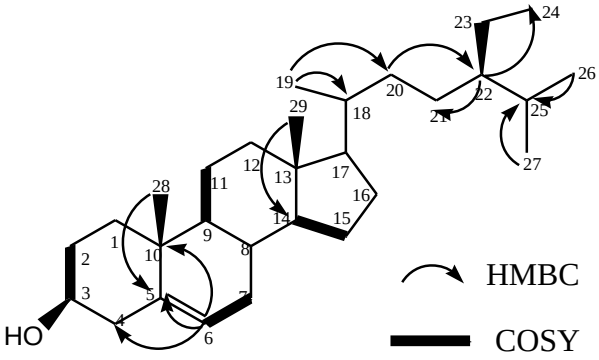


178

4.1.1 Structure elucidation of compound 176 (*β*-Sitosterol)



176



Key: HMBC **H→C** (curved arrows) and **^{1}H–^{1}H** COSY (bold lines) correlations.

Compound **176** (*β*-Sitosterol) was isolated from dichloromethane stem bark of Narok sample as white colourless crystals. The ^{13}C NMR (Appendix 2) spectrum showed 29 carbon signals. The HMBC (Appendix 4)spectrum placed ^{13}C resonance δ_C 140.8 and δ_C 121.9 for C_5=C_6 double bond respectively, δ_C 72.0 for C-3 *β*-hydroxyl group, δ_C 12.1 and δ_C19.0 for angular methyl carbon atoms C_{18} and C_{19} respectively. The ^{1}H NMR (Appendix 1) spectrum signals varied between δ_H 0.83 to δ_H 5.36. The spectrum showed presence of six high intensity peaks indicating presence of six methyl groups at δ_H 0.83, δ_H 0.88, δ_H 0.92 and δ_H 1.01. The position corresponding to the 3Hs of a sterol moiety appeared as a triplet of doublet at δ_H 3.52. A ^{1}H at δ_H 5.36 corresponded to a peak in the region of the ethylene proton suggesting the presence of one proton.

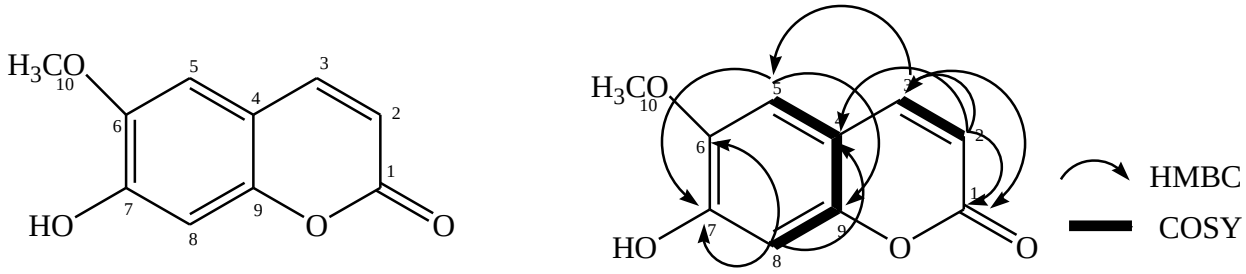
The correlation between ^{1}H and ^{13}C was confirmed by HSQC (Appendix 3) spectrum, COSY (Appendix 5) and NOESY (Appendix 6) spectra also confirmed ^{1}H correlations. All this information (Table 4.2) confirmed that compound **176** was a *β*-Sitosterol (Chaturvedula and Prakash, 2012)..This is the first time that this compound has been isolated fron *Turraea abyssinica* species.

Table 4. 2 NMR data for Compound **176** (*β*-Sitosterol)

Position	^{13}C NMR (125 MHz in $CDCl_3$) *δ* ppm	^{13}C NMR (150 MHz in $CDCl_3$) (Chaturvedula and Prakash, 2012) *δ* ppm	^{1}H NMR (500 MHz in $CDCl_3$) δ ppm	^{1}H NMR (600 MHz in $CDCl_3$) (Chaturvedula and Prakash, 2012) *δ* ppm
1	34.2	37.5 CH_2	1.00, 1.29 (t, 2H, *J* = 7.61 Hz)	
2	31.9	31.9 CH_2	1.57, 1.32 (td, 2H, *J* = 3.84, 2.20 Hz)	
3	72.0	72.0 CH	3.52 (tdd, 1H, *J* = 4.42, 11.11 Hz)	3.53 (tdd, 1H. *J* = 4.5, 4.2, 3.8 Hz)
4	42.5	42.5 CH_2	2.26, 1.99 (d, 2H, *J*=2.62, 5.51 Hz)	
5	141.0	140.9 C		
6	121.9	121.9 CH	5.36 (dd, 1H, *J* = 5.2 Hz)	5.31 (t, 1H, J = 6.4 Hz)
7	31.9	32.1 CH_2	1.83, 1.99 (ddd, 2H, *J* = 3.66, 2.62 Hz)	
8	32.1	32.1 CH	1.43 (dd, 1H, *J* = 4.63 Hz)	
9	50.4	50.3 CH	1.43 (td, 1H, *J* = 4.63 Hz)	
10	36.7	36.7 C		
11	21.3	21.3 CH_2	1.49 (td, 2H, *J* = 3.50 Hz)	
12	40.0	39.9 CH_2	1.83, 1.09 (t, 2H, *J* = 4.24, 7.00 Hz)	
13	42.6	42.6 C		
14	56.3	56.9 CH	1.44 (td, 1H, *J* = 4.63 Hz)	
15	24.5	26.3 CH_2	1.58, 1.05 (td, 2H,	

				J = 3.86, 13.10 Hz)	
16	28.5	28.5	CH_2	1.83, 1.25 (td, 2H, J = 3.66, 6.04 Hz)	
17	57.0	56.3	CH	1.01 (td, 1H, *J* = 4.91 Hz)	
18	36.4	36.3	CH	1.64 (td, 1H, *J* = 4.83 Hz)	
19	19.3	19.2	CH_3	1.01 (d, 3H, *J* = 4.91 Hz)	0.93 (d, 3H, J = 6.5 Hz)
20	34.2	34.2	CH_2	1.25 (td, 3H, *J* = 6.02 Hz)	
21	26.3	26.3	CH_2	1.25 (td, 3H, *J* = 6.02 Hz)	
22	46.1	46.1	CH	0.88 (ttd, 1H, *J* = 6.76 Hz)	
23	23.3	23.3	CH_2	0.91 (m, 2H, *J* = 6.76)	
24	12.2	12.2	CH_3	0.88 (t, 3H, *J* = 6.81Hz)	0.84 (d, 3H, J = 7.2 Hz)
25	29.4	29.4	CH	1.83 (m, 1H, *J* = 3.67 Hz)	
26	20.0	20.1	CH_3	0.83 (d, 3H, *J* = 6.4 Hz)	0.83 (d, 3H, J = 6.4 Hz)
27	19.6	19.6	CH_3	0.83 (d, 3H, *J* = 6.4 Hz)	0.81 (d, 3H, J = 6.4 Hz)
28	19.0	19.0	CH_3	0.92 (s, 3H)	0.68 (s, 3H)
29	12.2	12.0	CH_3	0.83 (s, 3H)	0.1 (s, 3H)

4.1.2 Structure elucidation of compound 177 (Scopoletin)



177

Key: HMBC H→C (curved arrows) and ^{1}H–^{1}H COSY (bold lines) correlations

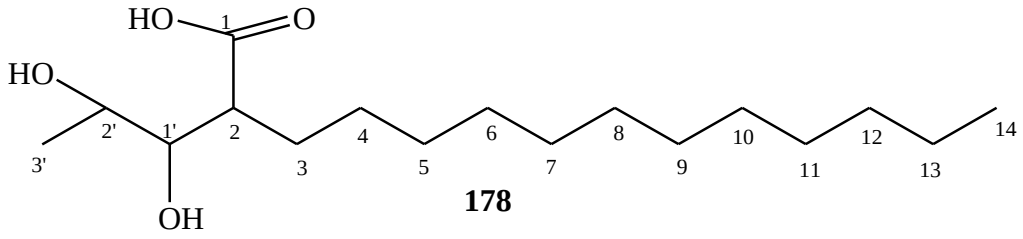
Compound **177** (Scopoletin) was isolated from dichloromethane stem bark of Narok sample crude extract. It was a brown compound with an R_f of 0.2 in 20 % ethyl acetate in hexane. It showed ten carbon resonances in the ^{13}C NMR (Appendix 8) spectrum that confirmed compound **177** as a monoterpenoid. It also showed presence of a methoxy group, four methine groups and five quaternary carbons, one being a carbonyl group.

The ^{1}H NMR (Appendix 7) spectrum showed two methine protons at δ_H 6.26, and δ_H 7.60 both with coupling constant of 9.5 Hz correlating to ^{13}C resonance at δ_C 113.3, δ_C 144.7. Two aromatic singlet protons at δ_H 6.46 and δ_H 6.78 correlating to ^{13}C resonance at δ_C 111.8 and δ_C 111.3 were observed. One methoxy group singlet at δ_H 3.75 attached to the benzene ring correlating to the ^{13}C resonance at δ_C 56.4 was also observed. All this was confirmed by the HMBC, HSQC, COSY and NOESY experiments. The HMBC spectrum (Appendix 10) showed correlation between H-2 resonance δ_H 6.26 (doublet J = 9.45 Hz) with δ_C 162.5, δ_C 143.3, δ_C 129.3, H- 3 resonance δ_H 7.60 (doublet J = 9.48 Hz) with δ_C 162.5, δ_C 111.8 confirming the position of the carbonyl group. The correlation between H-5 resonance δ_H 6.46 with δ_C 143.3, δ_C 144.6, δ_C 144.0, H-8 resonance δ_H 6.78 with δ_C 129.5, δ_C 150.3 and δ_C 144.6 confirmed the position of the hydroxyl group. The NOESY (Appendix 12) and COSY (Appendix 11), correlation between H-2 and H-3, H-5 and H-8 confirmed compound **177** as Scopoletin (Akhmad *et al*, 2012). This is the first tine that Scopoletin has been isolated from *Turraea* genus and *Turraea abyssinica* species. A summary of NMR data is shown in Table 4.3.

Table 4. 3 NMR data for compound **177** (Scopoletein)

Position	^{13}C NMR (125 MHz in $CDCl_3$) δ ppm	^{13}C NMR (500 MHz in CH_3COCH_3) (Akhmad *et al*, 2012) δ ppm	^{1}H NMR (150 MHz in $CDCl_3$) δ ppm	^{1}H NMR (600 MHz in CH_3COCH_3) (Akhmad *et al*, 2012) δ ppm
1	162.5 C	160.5	-	
2	113.4 CH	113.3	6.26, d (1H) J = 9.45 Hz	6.25, d J = 9.75 Hz
3	143.3 CH	144.7	7.60, d (1H) J = 9.48 Hz	7.84, d J = 9.75 Hz
4	129.5 C	112.1	-	
5	111.8 CH	109.9	6.46 s (1H)	7.19 s
6	150.3 C	146.0	-	
7	144.6 C	151.9	-	
8	111.3 CH	103.7	6.78 s (1H)	6.79 s
9	144.0 C	151.2	-	
10	56.4 CH_3	56.7	3.75 s (3H)	3.90 s

4.1.3 Structure elucidation for compound 178 [2-(1',2'-Dihydroxypropyl)tetradecanoic acid]



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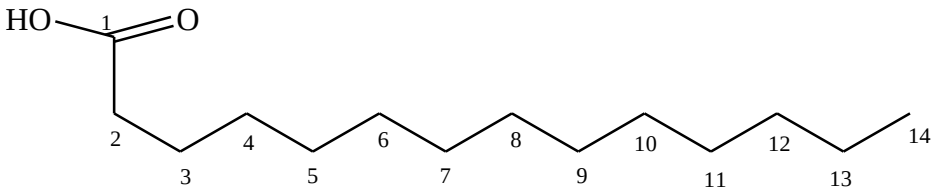
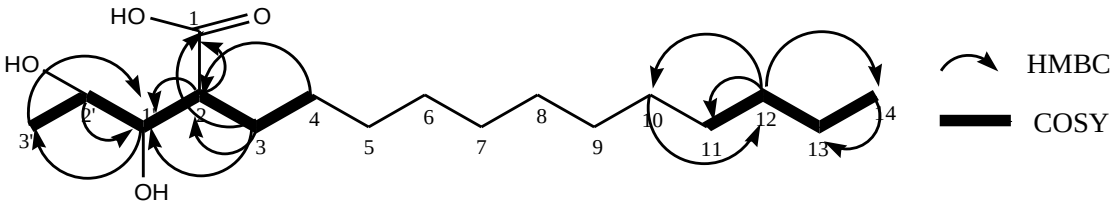


Tetradecanoic acid



Key: HMBC H→C (curved arrows) and ^{1}H–^{1}H COSY (bold lines) correlations

Compound **178** [2-(1',2'-Dihydroxypropyl)tetradecanoic acid] was isolated from dichloromethane stem bark of Narok sample crude extract as white shinny crystals. The ^{13}C NMR (Appendix 14) spectrum of the compound showed presence of 17 carbons with eleven methylene groups, three methine groups, two methyl groups and one carbonyl carbon at δ_C 176.1 indicating presence of a carboxylic acid. They were confirmed by DEPT-135 (Appendix 16) and HSQC (Appendix 15) spectra**.** The ^{1}H NMR (Appendix 13) spectrum showed one triplet methylene proton at δ_H 0.87, δ_H 0.89 with coupling constant of 6.74 Hz correlating to ^{13}C resonance at δ_C 14.3. Ten multiplet methylene proton signals ranging between δ_H 1.26 and δ_H 1.89 correlating to ^{13}C resonance between δ_C 22.7 and δ_C 29.7 indicated a straight chain compound. A doublet triplet methine proton at δ_H 2.53 with coupling constant of 5.10 Hz and a doublet of doublet methine proton at δ_H 3.84 correlating to ^{13}C resonance at δ_C 48.9 and δ_C 80.0 respectively were observed. One doublet methyl proton at δ_H 1.45, δ_H 1.46 and a multiplet methine proton at δ_H 4.21, δ_H 4.20 with coupling constants of 6.21 Hz and 6.54 Hz corresponding to ^{13}C resonances at δ_C 18.5 and δ_C 79.4 respectively were also observed.

The position of the two hydroxyl groups and one carboxyl group were confirmed by HMBC (Appendix 17)**,** COSY (Appendix 18) and NOESY (Appendix 19) spectra. The HMBC spectrum showed correlation between H-2 and H-3 resonance δ_H2.53 and δ_H 1.57 with δ_C 176.1 confirming the position of the carbonyl group. Also HMBC correlation between H-2 and C-1', H-2 and C-I, H-3 and C-1 confirmed the position of the two hydroxyl groups. This was also confirmed by NOESY and COSY correlation between H-2 with H-1', H-3 and H-1' with H-2', H-3'. The ^{1}HNMR and ^{13}C NMR spectra of compound **178** were compared with the spectra of Tetradecanoic acid, indicating that the compound was a straight chain acid with a dihydroxypropyl substituent. All this information (Table 4.4) confirmed the structure of the compound as 2-(1',2'-Dihydroxypropyl)tetradecanoic acid **(**Biological Magnetic Resonance Data Bank). No documented work showed that this compound has been isolated from *Turraea* genus.

Table 4. 4: NMR data for compound **178** (2-(1,2-Dihydroxypropyl)tetradecanoic acid)

Position	^{13}C NMR (150 MHz in $CDCl_3$) δ ppm	^{13}C NMR (500 MHz in $CDCl_3$) (Biological Magnetic Resonance Data Bank) δ ppm	^{1}H NMR (500 MHz in $CDCl_3$) δ ppm	^{1}H NMR (500 MHz in $CDCl_3$) (Biological Magnetic Resonance Data Bank) δ ppm
1	176.1 C	180.6		
2	48.9 CH	34.2	2.53 (dt) J = 5.10 Hz	2.36 (t) J = 7.53 Hz
3	27.0 CH_2	32.0	1.57 (m)	1.64 (m)
4	28.7 CH_2	29.7	1.89 (m)	1.31 (m)
5	29.6 CH_2	29.7	1.26 (m)	1.31 (m)
6	29.6 CH_2	29.7	1.26 (m)	1.31 (m)
7	29.8 CH_2	29.7	1.26 (m)	1.31 (m)
8	29.9 CH_2	29.5	1.26 (m)	1.31 (m)
9	29.9 CH_2	29.4	1.26 (m)	1.31 (m)
10	29.9 CH_2	29.3	1.26 (m)	1.31 (m)
11	29.8 CH_2	29.1	1.26 (m)	1.31 (m)
12	32.1 CH_2	24.7	1.26 (m)	1.31 (m)
13	22.9 CH_2	22.7	1.31 (m)	1.31 (m)
14	14.3 CH_3	14.1	0.87, 0.89 (t) J = 6.74 Hz	0.89 (t) J = 6.86 Hz
1’	80.0 CH		3.84 (dd), J = 4.19 Hz	
2’	79.4 CH		4.21, 4.20 (m) J = 6.541Hz	
3,	18.5 CH_3		1.45, 1.46 (d) J = 6.21 Hz	

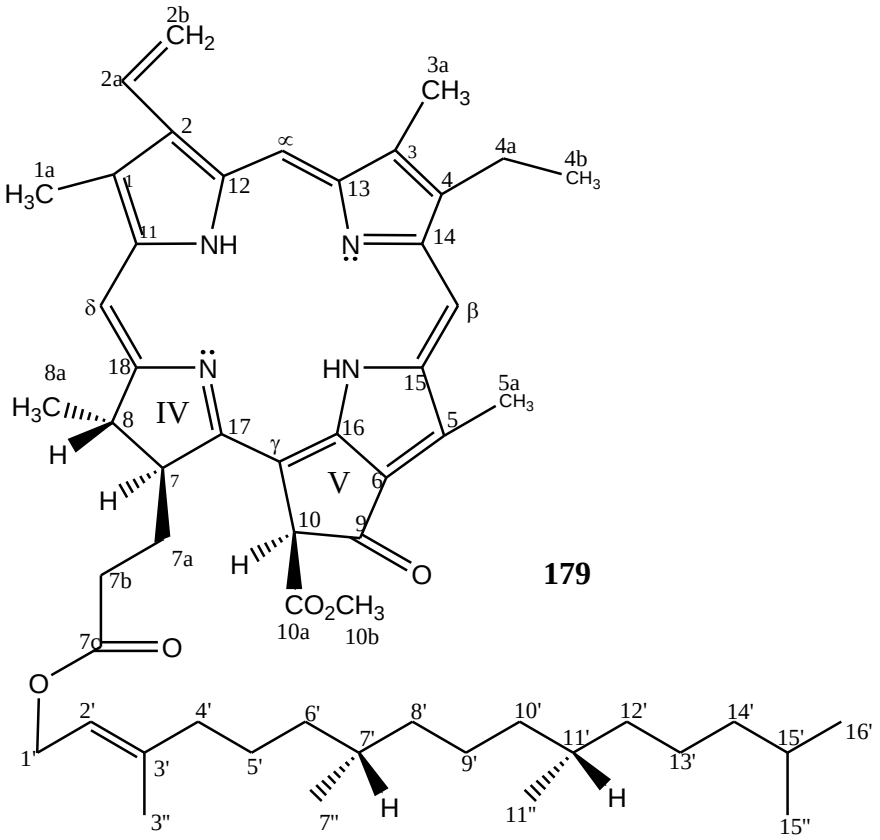
4.2*: Meyna tetraphylla* compounds

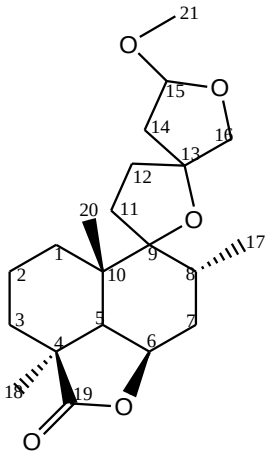
The dichloromethane leaves crude extract (45.24 g) yielded four compounds **179** (Phaephytin, 9.40 mg), **180** (Enantiomer A and B 5.80 mg), **118** (α-Amyrin, 5.20 mg) and **60** (Stigmasterol, 5.82 mg) with repeated CC and monitoring with TLC. Both compounds **60**

and **118** have been isolated from *Leonotis nepetaefolia* species but not in *Meyna* genus nor in *Meyna tetraphylla* species.

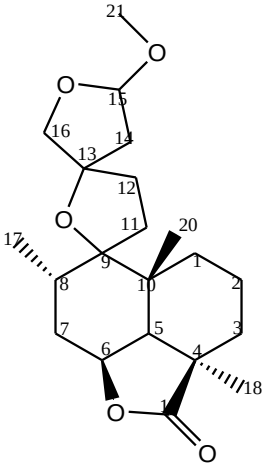
Table 4. 5: Percentage yield of crude extracts and pure compounds of *Meyna tetraphylla*

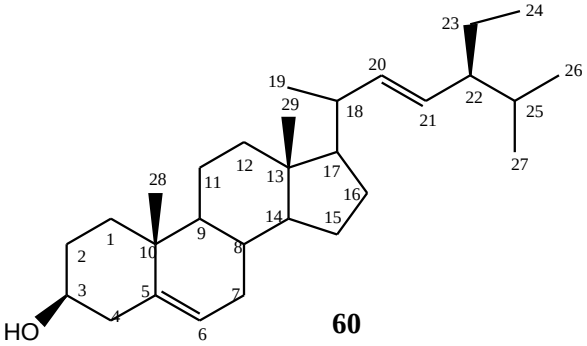
Plant name	Weight (g)	Solvent	Crude extract		Pure compound		
Meyna teyraphyll a (Baringo leaves)	1,000		Weight (g)	% yield (w/w)	Compound name	Weight (mg)	% yield (w/w)
		Hex	41.12	4.11			
		DCM	54.13	5.41	Phaeophytin **179**	9.40	0.02
					Enatiomer **180**	5.80	0.01
					α-Amyrin **118**	5.65	0.01
					Stigmasterol **60**	5.82	0.01
		EtOAc	51.25	5.13			
		MeOH	63.60	6.36			





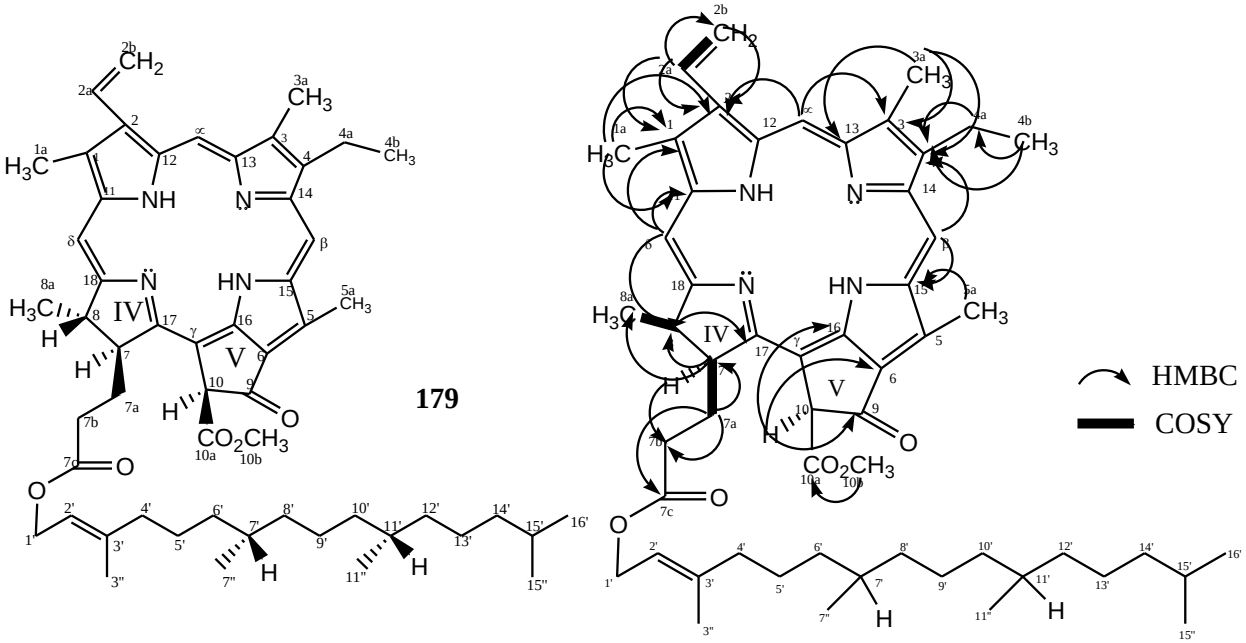
180





60

4.2.1 Structure elucidation of compound **179**(Phaeophytin)



179

Key: HMBC H→C (curved arrows) and ^{1}H–^{1}H COSY (bold lines) correlations

Compound **179** (Phaeophytin) was isolated from dichloromethane Baringo sample leaves crude extract as a green powder. Its structure was assigned by 1-D and 2-D NMR spectroscopy and based on this evidence; it was proposed to be a Phaeophytin with a phytol side chain. The ^{1}H NMR data (Appendix 20) and ^{13}C NMR data (Appendix 21) was compared with the literature data for Phaeophytin a (Sianne *et al*. 1998; Hui *et al*., 2012). The difference in the chemical shifts can be accounted for by the fact that the spectra were run in different MHz from the ones in literature as indicated in Table 4.6.

The degree of unsaturation was twenty. This was accounted for by four pyrrole rings, three carbonyl groups, five vinyl groups, phytol side chain and a cyclopentenone. The ^{13}C NMR spectra gave fifty five carbon resonances. Sixteen of the resonances belong to four pyrrole carbons, one methoxy carbons (δ_C 53.1), eleven methyl carbons ranging between δ_C 11.2 and δ_C 23.3, three carbonyl carbons (δ_C 172.6, δ_C 171.0, δ_C 189.8), sixteen methylene carbons ranging between (δ_C 20.0 and δ_C 142.3), nine methine carbons (δ_C 28.2 - δ_C 132.0) and fifteen quaternary carbon signals. The three carbonyl carbon signals (C-9, C-7c, and C-10a) occurred at the low field of δ_C 171.0 - δ_C 189.8. All the carbon resonances were characterized by DEPT experiments (Appendix 22).

The ^1H resonances at δ_H 1.81, δ_H 3.67 and δ_H 2.52 showed a characteristic of four methyl groups attached to the pyrrole ring corresponding to the ^{13}C NMR resonance at δ_C 23.3, δ_C 12.3 and δ_C 11.3 in the HSQC-DEPT spectrum (Appendix 23). The ^1H and ^{13}C signals at δ_H 3.9 (δ 53.1 ppm) were characteristic of one methoxy group. In the COSY spectrum (Appendix 25) there was a correlation between H-8 (δ_H 4.46 m) and resonance at δ_H 1.81 d (H-8a) and δ_H 1.71 t (H-4b). The spectrum further showed a correlation between H-7a (δ_H2.32 m) and resonance at δ_H 4.21 m (H-7). The ^1H NMR and ^{13}C NMR was compared with the literature that confirmed that compound **179** was a Phaeophytin with a phytol side chain (Sianne *et al*. 1998; Hui *et al*., 2012).

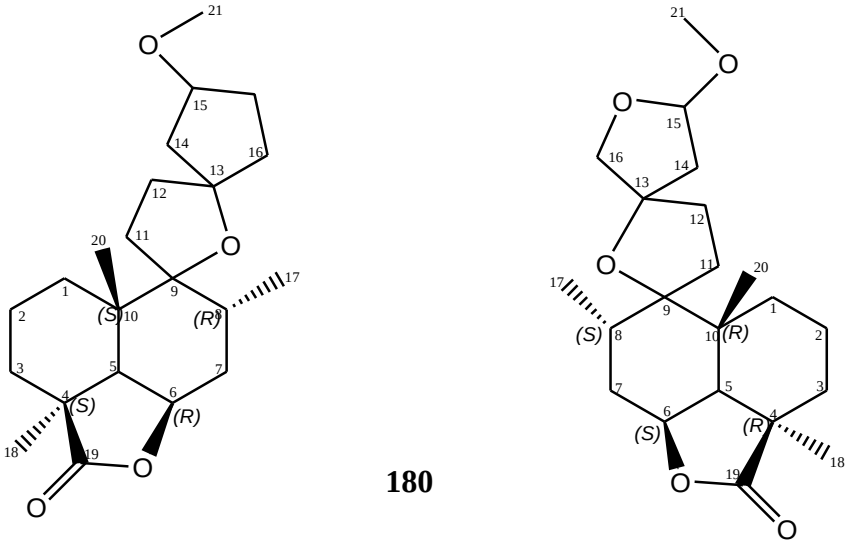
Table 4. 6 NMR data for **(179)** Phaeophytin

No	^{13}C NMR (125 MHz in $CDCl_3$) δ ppm	^{13}C NMR ($CDCl_3$, 75 MHz, Sianne *et al*, 1998) δ ppm	^1H NMR (500 MHz in $CDCl_3$) δ ppm	^1H NMR ($CDCl_3$, 300 MHz, Sianne *et al*, 1998) δ ppm	COSY	HMBC (H→C)
1	132.1 C	131.8	-		-	-
2	136.5 C	136.5	-		-	-
3	136.4 C	136.1	-		-	-
4	145.5 C	145.2	-		-	-
5	129.3 C	129.1	-		-	-
6	129.2 C	129.0	-		-	-
7	51.4 CH	51.1	4.21 m	4.21 ddd	7a	7b,8,8a
8	50.3 CH	50.1	4.46 m	4.46 m	8a	7,7b,8a,17

9	186.9 C	189.6	-		-	-
10	64.9 CH	64.7	6.26 s	6.26 s	-	6,9,10a,16
11	143.1 C	142.9	-			
12	136.4 C	136.2	-			
13	155.9 C	155.5	-			
14	151.2 C	151.0	-			
15	138.2 C	137.9	-			
16	149.9 C	150.0	-			
17	161.5 C	161.3	-			
18	172.4 C	172.2	-			
α	97.8 CH	97.5	9.36 s	9.36 s		2,3
β	104.7 CH	104.4	9.51 s	9.50 s		4,15
γ	105.5 C	105.2	-			-
δ	93.3 CH	93.1	8.56 s	8.55 s		1,8,11
1a	12.3 CH_3	12.1	3.67 s	3.39 s		1,2,11
2a	129.3 CH	129.0	8.10 dd (*J* = 11.90, 17.81 Hz)	7.98 dd	2b	1,2,2b
2b	123.0 CH_2	122.8	7.26 d 6.19 d (*J* = 11.5 Hz)	6.17 dd, 6.28 dd	2a	2
3a	11.5 CH_3	11.2	2.52 s	3.21 s		3,4,13
4a	19.9 CH_2	19.7	3.64 m	3.66 m		4
4b	16.5 CH_3	16.3	1.71 d (*J* = 11.6 Hz)	1.68 t		4,4a
5a	12.3 CH_3	12.2	3.67 s	3.88 s		15
7a	29.9 CH_2	29.8	2.32 m		7	7,7b,7c,7d,8
7b	31.4 CH_2	31.2	3.20 t (*J* = 6.5 Hz)			7,7a,7c, 8
7c	173.2 C	173.0	-			-
8a	22.8 CH_3	22.7	1.81 d (*J* = 7.5 Hz)	1.80 d	8	
10a	169.8 C	173.0	-			

10b	53.1 CH_3	53.0	3.88 s		10a
1’	61.7 CH_2	61.0	4.50 d	4.35 d	
2’	118.0 CH_2	118.0	5.30 t	5.10 t	
3’	142.3 CH_2	142.0			
4’	39.6 CH_2	39.4	1.81 t	1.96 t	
5’	25.2 CH_2	25.0	1.13 m	1.33 m	
6’	37.6 CH_2	37.8	1.13 m	1.25 m	
7’	33.0 CH	33.3	1.60 m	1.65 m	
8’	37.5 CH_2	37.7	1.13 m	1.25 m	
9’	24.6 CH_2	24.7	1.55 m	1.29 m	
10’	37.5 CH_2	37.7	1.12 m	1.25 m	
11’	33.0 CH	33.2	1.70 m	1.65 m	
12’	37.6 CH_2	37.7	1.13 m	1.25 m	
13’	24.6 CH_2	24.4	1.13 m	1.29 m	
14’	40.0 CH_2	39.9	1.13 m	1.25 m	
15’	28.2 CH	28.2	1.81 m	1.83 m	
16’	23.3 CH_3	23.2	1.02 m	1.01 m	
3”	17.6 CH_3	17.1	1.70 s	1.71 s	
7”	22.8 CH_3	21.0	1.11 d	1.06 d	
11”	19.9 CH_3	21.0	1.11 d	1.06 d	
15”	23.3 CH_3	23.2	1.01 d	1.01 d	

4.2.2 Structure elucidation of compound 180 (Enantiomers)



180

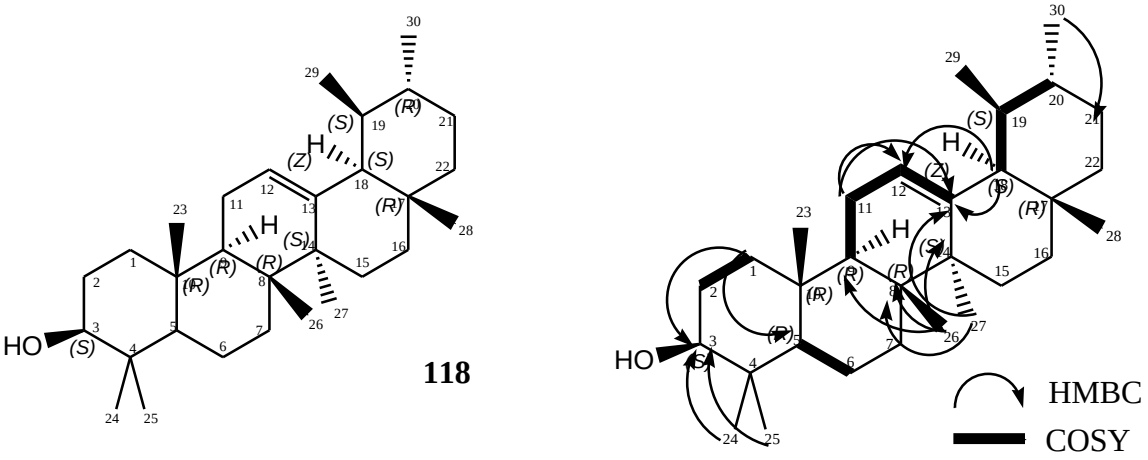
The Enantiomer was isolated from dichloromethane crude extract of Baringo leaves. The acetylated overlapped ^{1}H NMR spectrum (Appendix 26) and ^{13}C NMR spectrum (Appendix 27) of compound **180 (**Enansiomer) was compared with the ^{1}H NMR spectrum (Appendix 51) and ^{13}C NMR spectrum (Appendix 52) of compound **183**, labdane (13R)-19*α*,13*α*-epoxylabda-6*β*(19).16(15)-dioldilactone (Daniela *et al*., 2006). A methyl doublet at δ_H 0.87 was observed corresponding to ^{13}C NMR (Appendix 52) resonance at δ_C 17.3 ppm. Two other methyl groups at δ_H 1.29 ppm and δ_H 1.04 ppm corresponding to ^{13}C NMR resonance at δ_C 23.2 ppm and δ_C 23.6 ppm were also observed attached to the decalin. The ^{1}H NMR signals between δ_H 1.46 ppm and δ_H 3.96 ppm indicated eight methylene groups. A singlet signals at δ_H 3.17 ppm and δ_H 3.96 ppm showed that one methylene was attached to oxygen in a tetrahydrofuran. A singlet signal at δ_H 3.24 ppm indicated the presence of a methoxy group also attached to a tetrahydrofuran. The acetylated overlapped ^{1}H NMR (Appendix 26) spectrum indicated that compound **180** was an Enantiomer.

The^{13}C NMR spectrum of compound **180** (Appendix 27) that was compared with the ^{13}C spectrum of compound **183**, showed a signal at δ_C 48.7 ppm which indicated presence of a methoxy group attached to a tetrahydrofuran. The^{13}C resonance signal at δ_C 183.5 ppm also showed presence of one carbonyl groups in a cyclic ester. In the HMBC spectrum of compound **183** (Appendix 54) the correlations between ^{1}H and ^{13}C confirmed the position of the carbonyl and hydroxyl groups thus confirming that compound **180** was a labdane. Further confirmation of the compound was done using COSY (Appendix 55) and NOESY spectral of compound **183** (Appendix 56). A summary of NMR data for the compound is shown on Table 4.7.

Table 4. 7: Comparison of NMR data for compound **180**

No	^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm of **180**	^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm of **183**	^{1}H NMR (500 MHz in $CDCl_3$) δ ppm of **180**	^{1}H NMR (500 MHz in $CDCl_3$) δ ppm of **183**
	29.5	29.5 CH_2	1.25, 1.30 (m)	1.25, 1.30 (m)
	18.1	18.2 CH_2	1.77, 1.52 (m)	1.77, 1.52 (m)
	29.1	29.1 CH_2	1.46, 2.12 (m)	1.46, 2.12 (m)
	44.1	44.2 C		
	46.3	46.2 CH	2.07 (m)	2.07 (m)
	76.4	76.2 CH	4.70 (m)	4.70 (m)
	32.1	32.1 CH_2	1.63 (dd)	1.63 (m)
	31.9	31.9 CH	1.63 (m)	1.63 (m)
	92.3	92.3 C		
10	39.1	39.1 C		
11	28.2	28.3 CH_2	1.85, 2.11 (m)	1.85, 2.11 (m)
12	37.8	37.2 CH_2	2.12 (m)	2.12 (m)
13	89.6	86.3 C		
14	44.1	42.2 CH_2	2.57, 2.91d, (J =17.3 Hz)	2.57, 2.91d, (J =17.3 Hz)
15	99.4	174.7 CH	5.10 (t)	
16	78.2	78.8 CH_2	3.96, 3.17 (d, J = 8.9 Hz)	4.13, 4.26d,(J = 8.9 Hz)
17	17.3	17.6 CH_3	0.87d,(J=6.3 Hz)	0.87d,(J=6.3 Hz)
18	23.2	23.6 CH_3	1.29 (s)	1.29 (s)
19	183.5	183.6 C		
20	23.6	23.2 CH_3	1.04 (s)	1.04 (s)
21	48.7	47.4 CH_3	3.24 (s)	

4.2.3: Structure elucidation of compound 118 (*α*-Amyrin)



Key: HMBC H→C (curved arrows) and ^{1}H–^{1}H COSY (bold lines) correlations

Compound **118** (*α*-Amyrin) was isolated from dichloromethane crude extract of Baringo sample leaves as a white powder. The thirty carbon resonances observed in the ^{13}C NMR spectrum (Appendix 29) were characterized by DEPT experiment (Appendix 30). This indicated that compound **118** was a triterpenoid with eight methyl groups, nine methylene groups, seven methine groups (one attached to a hydroxyl and one to a double bond) and six quaternary group. The ^{1}H NMR spectrum (Appendix 28) showed presence of eight methyl singlets at δ_H 1.09 (3H-23), δ_H 0.79 (3H-24), δ_H 0.78 (3H-25), δ_H 0.93 (3H-26), δ_H 1.09 (3H-27), δ_H 1.0 9(3H-28), δ_H 0.79 (3H-29), δ_H 0.10 (3H-30). It also showed one olefenic proton at δ_H 5.26 triplet (J = 3.6 Hz) correlating to ^{13}C NMR (Appendix 29) resonance at δ_C 126.1. The ^{13}C NMR spectrum further confirmed the presence of double bond signals at δ_C 126.1 and δ_C 138.2 which were assigned to C-12 and C-13 respectively. A proton at δ_H 3.34 doublet of doublet correlating to δ_C 79.3 was also observed all suggesting an olealane type triterpenoid.

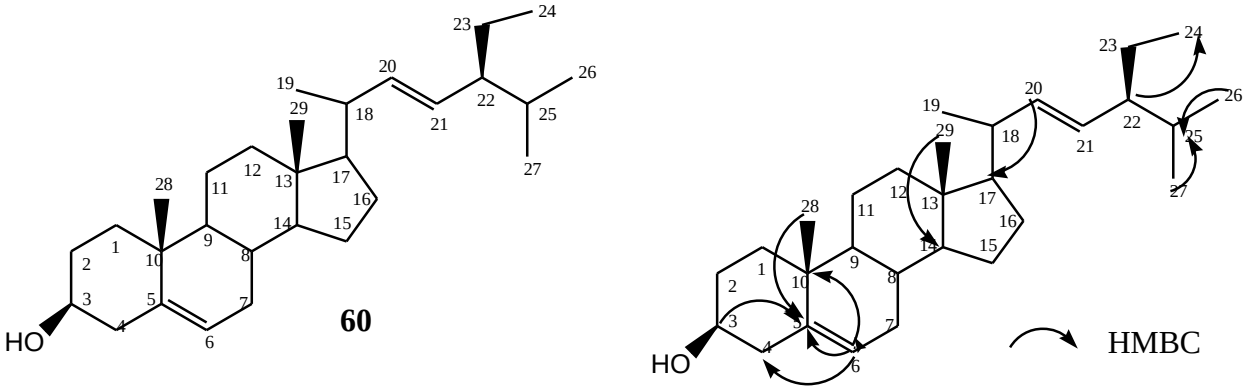
The ^{13}C resonances at C-12, C-13, C-19 and C-20 compared well with the literature values thus confirming the compound as *α*-Amyrin (Liliana *et al*., 2012; Nkeoma *et al*. 2014**).** The six quaternary carbons further confirmed the compound as urs-12-ene (*α*-Amyrin) and not olean-12-ene (*β*-Amyrin) that has seven quaternary carbons. This compound has also been isolated from the leaves of *Leonotis nepetifolia* compound **118** in the literature review (Oliveira *et al*., 2015). A summary of NMR data for compound **118** is shown in Table 4.8.

Table 4. 8: NMR data for compound **118** (α-Amyrin)

No	^{13}C NMR ($CDCl_3$, 150 MHz) δ ppm	^{13}C NMR ($CDCl_3$, 125 MHz) Nkeoma *et al*., 2014; Liliana *et al*., 2012δ ppm	^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm	^{1}H NMR ($CDCl_3$, 500 MHz)Nkeoma *et al*., 2014; Liliana *et al*., 2012) δ ppm
1	38.8 CH_2	38.7	0.99, 1.63 m	1.55, 1.49 m
2	28.2 CH_2	28.7	1.14, 1.87 m	1.52, 1.55 m
3	79.3 CH	79.6	3.34 ddt (J = 11.3o, 4.71 Hz)	3.16 dd, (J = 5.1, 11.2)
4	39.0 C	38.7		
5	55.4 CH	55.1	0.74 m	0.71 m
6	18.5 CH_2	18.4	1.55, 1.37 m	1.53, 1.30
7	33.2 CH_2	32.2	1.48, 1.32 m	
8	39.7 C	40.7		
9	47.7 CH	47.7	1.46 m	1.95
10	37.2 C	36.6		
11	23.5 CH_2	23.3	1.09, 1.91 m	1.84
12	126.1 CH	124.4	5.26 (t. J = 3.6 Hz)	5.06 (t, J = 3.2)
13	138.2 C	139.5		
14	42.2 C	42.0		
15	27.4 CH_2	27.2	1.61 m	1.94 (td, J = 4.5, 13.5 Hβ)
16	24.4 CH_2	26.6	1.66, 2.02 m	1.76 (td, J = 5.0, 13.5 Hβ)
17	37.2 C	33.7		
18	52.9 CH	59.0	2.18 m	1.98
19	39.3 CH	39.6	1.34 m	1.38m
20	39.0 CH	39.6	1.34 m	
21	29.9 CH_2	31.2	1.46, 1.32 m	
22	36.9 CH_2	41.5	1.73 t (J = 3.10, 6.91 Hz)	1.85 (dt, J = 3.0, 7.0)
23	28.3 CH_3	28.1	1.09 s	0.93 s

24	15.7 CH_3	15.6	0.79 s	0.74 s
25	15.8 CH_3	15.6	0.78 s	0.73 s
26	17.2 CH_3	16.8	0.93 s	0.89 s
27	23.8 CH_3	23.2	1.09 s	1.02 s
28	28.3 CH_3	28.1	1.09 s	0.94 s
29	17.3 CH_3	17.4	0.79 d	0.85 (d, J = 6.0)
			(J = 5.80 Hz)	
30	21.4 CH_3	21.4	0.10 d	0.73 (d, J = 7.0)
			(J = 6.91 Hz)	

4.2.4 Structure elucidation of compound 60 (Stigmasterol)



Key: HMBC **H→C** (curved arrows) correlations

Compound **60** (Stigimasterol) was isolated as a white powder from dichloromethane crude extract of Baringo leaves. The ^{1}H-NMR spectrum (Appendix 35) showed six methyl signals at δ_H 1.16 (s), δ_H 1.26 (s), δ_H1.01 (d), δ_H 1.01 (d), δ_H 1.16 (d) and δ_H 0.96 (t) at carbons δ_C 12.2, δ_C 18.9, δ_C 19.6, δ_C 20.0, δ_C 19.0 and δ_C 11.9 confirming that the compound is a sterol. It also showed protons at δ_H 5.01, δ_H 5.48, and δ_H 5.37 at carbon δ_C 140.1, δ_C 128.9 and δ_C 121.9 suggesting the presence of three protons corresponding to that of a trisubstituted and a disubstituted olefinic bond. The HMQC-DEPT (Appendix 37) NMR and HMBC (Appendix 38) suggested a total of 28 carbon signals with one oxygenated carbon signal at δ_C 72.0. It was placed at C-3 due to HMBC spectrum correlations between H-3 with the C-5 resonance δ_C 141.0.

The proton correlating to the H-3 of a sterol moiety appeared as a triplet of doublet of doublets at δ 3.25. The ^{1}H NMR and ^{13}C NMR values for all the protons and carbons were assigned on the basis of DEPT (Appendix 36), HMQC-DEPT (Appendix 37) and HMBC

(Appendix 38) correlations. These spectral data supported the presence of sterol skeleton having a hydroxyl group at C-3 position with two double bonds at C-5/C-6 and C-20/C-21 and six methyl groups supported by the HMBC correlations. Thus, the structure of compound **60** was assigned as stigmasterol (Chaturvedula and Prakash, 2012). Compound **60** (Stigmasterol) has also been isolated from *Leonotis nepetifolia* as indicated in the literature review (Hortensia *et al*., 2004).

Table 4. 9:NMR data for compound **60** (Stigmasterol)

Position	^{13}C NMR (125 MHz in $CDCl_3$) δ ppm	^{13}C NMR (150 MHz in $CDCl_3$) (Chaturvedula and Prakash, 2012) δ ppm	^{1}H NMR (500 MHz in $CDCl_3$) δ ppm	^{1}H NMR (600 MHz in $CDCl_3$) (Chaturvedula and Prakash, 2012) δ ppm
1	34.2 CH_2	37.6	1.38, 1.13 (t, 2H)	
2	31.9 CH_2	32.1	1.57, 1.32 (td, 2H)	
3	72.0 CH	72.1	3.25(tdd,1H, J = 4.57, 11.03 Hz)	3.51(tdd, 1H. *J* = 4.5, 4.2, 3.8 Hz)
4	42.5 CH_2	42.4	2.23, 1.98 (d, 2H)	
5	141.0 C	141.1		
6	121.9 CH	121.8	5.37 (t, 1H, *J* = 5.2 Hz)	5.31 (t, 1H, J = 6.1 Hz)
7	31.9 CH_2	31.8	2.04, 1.79 (dd, 2H)	
8	32.1 CH	31.8	1.45 (tdd, 1H)	
9	50.4 CH	50.2	1.44 (td, 1H)	
10	36.7 C	36.6		
11	21.3 CH_2	21.5	1.52, 1.27 (td, 2H)	
12	40.0 CH_2	39.9	1.49, 1.24 (t, 2H)	
13	42.6 C	42.4		
14	56.3 CH	56.8	1.40 (td, 1H)	
15	24.5 CH_2	24.4	1.60, 1.35 (td, 2H)	
16	28.5 CH_2	29.3	1.60, 1.35 (td, 2H)	
17	57.0 CH	56.2	1.51 (td, 1H)	
18	41.4 CH	40.6	2.33 (s, 1H)	

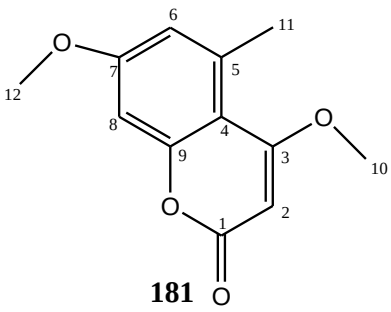
19	19.0 CH_3	21.7	1.16 (d, 3H, *J* = 6.46 Hz)	0.91 (d, 3H, J = 6.2 Hz)
20	140.1 CH	138.7	5.01 (dd, 1H)	4.98 (m, 1H)
21	128.9 CH	129.6	5.48 (dd, 1H)	5.14 (m, 1H)
22	45.7 CH	46.1	1.15 (td, 2H)	
23	23.3 CH_2	25.4	1.33 (m, 2H)	
24	11.9 CH_3	12.1	0.96 (t, 3H, *J* = 6.78 Hz)	0.83 (t, 3H, J = 7.1 Hz)
25	31.87 CH	29.6	1.86 (m, 1H)	
26	20.04 CH_3	20.2	1.01 (d, 3H, J = 6.4 Hz)	0.82 (d, 3H, J = 6.6 Hz)
27	19.61 CH_3	19.8	1.01 (d, 3H)	0.82 (d, 3H, J = 6.6 Hz)
28	19.00 CH_3	18.9	1.26 (s, 3H)	0.71 (s, 3H)
29	12.20 CH_3	12.2	1.16 (s, 3H)	1.03 (s, 3H)

4.3 *Leonotis mollisima* compounds

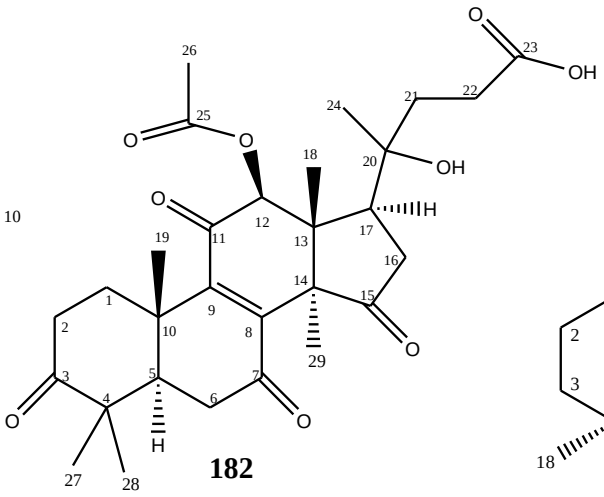
From dichloromethane crude extract of Laikipia leaves sample (79.69 g), three compounds, **181 (**Siderin**), 182** (20-hydroxylucidenicacid D2) and **183**(13R)-19*α*,13*α*-epoxylabda-6*β*(19).16(15)-dioldilactone) were isolated with repeated CC and monitoring with TLC.

Table 4. 10 Percentage yield of crude extracts and pure compounds of *Turraea abyssinica*

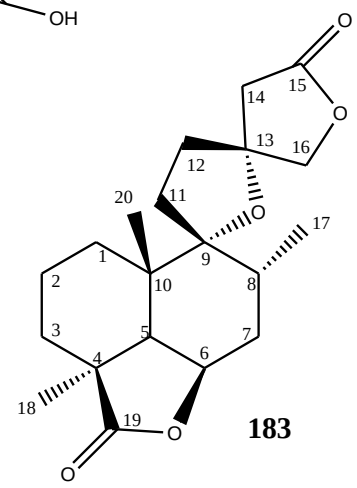
Plant name	Weight (g)	Solvent	Crude extract		Pure compound		
Leonotis mollissima (Laikipia leaves)	1,000		Weight (g)	% yield (w/w)	Compound name	Weight (mg)	% yield (w/w)
		Hex	59.03	5.90			
		DCM	79.69	7.97	Siderin **(181)**	7.70	0.01
					20-hydroxylucidenic acid D2 **(182)**	7.10	0.01
					(13R)-19α,13α-epoxylabda-6β(19).16(15)-dioldilactone **(183)**	21.20	0.03
		EtOAc	61.05	6.11			
		MeOH	67.45	6.75			



181

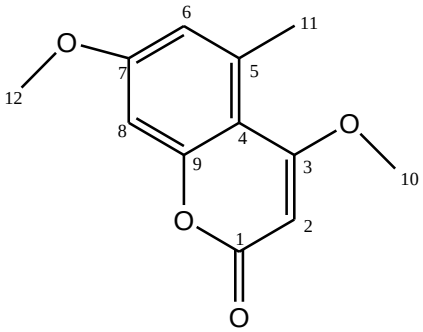


182



183

4.3.1 Structure elucidation of compound 181 (Siderin)



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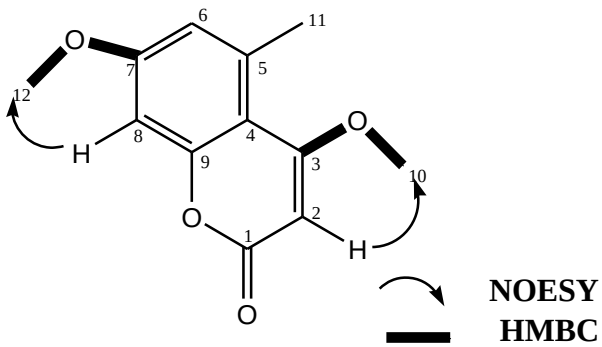


Key: HMBC **H→C** (bold lines) and ^{1}**H–**^{1}**H**NOESY (curved arrows) correlations

Compound **181** (Siderin) was isolated from dichloromethane crude extract of Laikipia leaves as a white powder. This compound showed presence of twelve carbon resonances in the ^{13}C NMR spectrum (Appendix 40). The DEPT-135 spectrum (Appendix 41) showed three methyl groups, three methine groups and six quaternary groups with two attached to methoxy groups. This indicated that the compound is a chromenone with two methoxy moiety and a methyl group. The ^{1}H NMR (Appendix 39) spectrum signals ranged between δ_H 2.58 to δ_H 6.64 showing sp^3 and sp^2 proton respectively. The sp^3proton at δ_H 2.58 correlating to ^{13}C NMR signal at δ_C 23.4 while the two methoxy protons resonances at δ_H 3.90 and δ_H 3.81 correlated to the resonance at δ_C 55.8 and δ_C 56.8 respectively.

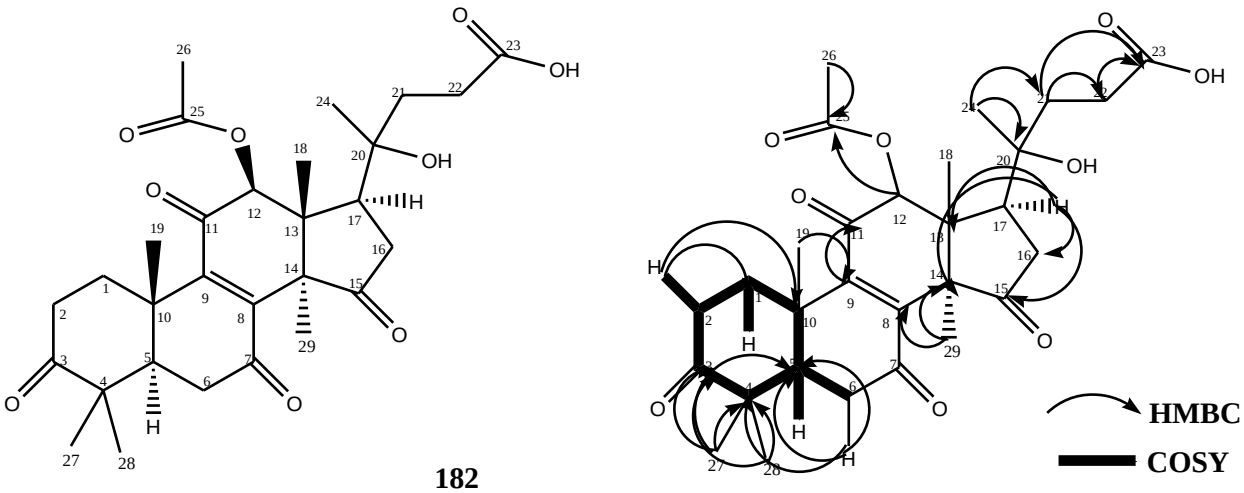
The presence of aromaticity was observed by the presence of six carbons ranging between δ_C115.9 and δ_C 170.0. They were placed at different positions due to HSQC (Appendix 42) and NOESY (Appendix 44) correlations between protons and the carbons. The vinyl protons at δ_H 6.64 and δ_H 6.58 showed *Meta* coupling in aromatic rings of J=2.50 Hz and their positions were identified. This was further confirmed by the COSY spectrum (Appendix 43) showing a coupling between the protons.

The ^{1}H NMR signal at δ_H 5.50 and ^{13}C NMR signals at δ_C 162.1, δ_C 87.8 and δ_C 170.0 suggested the presence of a α,β-unsaturated γ-lactone system in the molecule. The presence and position of the proposed lactone moiety were confirmed by the HMBC correlations between the two methoxy H-10, H-12 with C-3 and C-7 respectively. The NOESY (Appendix 44) spectrum further confirmed of the position of the two methoxy groups. The remaining part of the compound was assigned by comparison with the literature that confirmed compound **181** as Siderin (4,7-Dimethoxy-5-methylchromen-2-one) Usama *et al*., 2012.

Table 4. 11 NMR data for compound **181** (Siderin)

No	^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm	^{13}C NMR ($CDCl_3$, 75 MHz) (Usama *et al.*, 2012) δ ppm	^{1}H NMR(500 MHz) δ ppm	^{1}H NMR ($CDCl_3$, 300 MHz)(Usama *et al.*, 2012) δ ppm
1	162.1 C	163.0		
2	87.8 CH	87.4	5.50 (s)	5.48 (s)
3	170.0 C	169.6		
4	108.1 C	107.7		
5	137.7 C	138.4		
6	115.9 CH	115.6	6.58 (d, *J* = 2.5Hz)	6.54 (d, *J* = 2.6 Hz)
7	162.1 C	161.8		
8	98.9 CH	98.6	6.64 (d, *J* = 2.5Hz)	6.59 (d, *J* = 2.6 Hz)
9	156.9 C	156.6		
10	55.8 CH_3	55.9	3.90 (s)	3.79 (s)
11	23.7 CH_3	23.4	2.58 (s)	2.55 (s)
12	56.8 CH_3	55.4	3.81 (s)	3.88 (s)

4.3.2 Structure elucidation of compound 182 (20-hydroxylucidenicacid D2)



182

Key: HMBC **H→C** (curved arrows) and **^{1}H–^{1}H** COSY (bold lines) correlations

Compound **182** (20-hydroxylucidenicacid D2) was isolated from dichloromethane crude extract of Laikipia University leaves as a dirty white powder. The ^{13}C-NMR spectrum (Appendix 46) combined with the DEPT-135 spectrum (Appendix 47) confirmed the C_{29}

triterpenoid skeleton with seven methyl groups, six methylene groups, three methine groups and thirteen quaternary carbons. The ^{1}H NMR spectrum (Appendix 45) showed presence of seven high intensity peaks indicating presence of seven tertiary methyl group proton singlet at δ_H 1.27 ppm (two), δ_H 1.57, δ_H 2.13, δ_H 1.23 (two), δ_H 1.78 showing a Lanostane type of structure. These correlated to ^{13}C NMR signal at δ_C 15.8, δ_C 21.1, δ_C 23.7, δ_C 21.6, δ_C 17.2 and δ_C 24.9 respectively. Also in ^{13}C NMR, six carbonyl groups, two at δ_C 214.5 and δ_C 198.8, one on the acetoxy group at δ_C 170.6, one on a carboxyl group at δ_C 198.8 and a tertiary hydroxyl at δ_C 69.3 were observed.

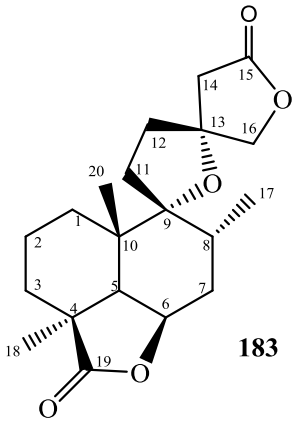
In the HMBC spectrum (Appendix 48), the H-12α resonance (δ_H 5.74) showed correlations with a carbonyl carbon signal (δ_C 170.6, C-25). The two methyl proton both with resonances δ_C 1.23 showed correlation in the HMBC spectrum with the C-4 resonance (δ_C 39.4). In the NOESY spectrum (Appendix 50) the H-17 resonance showed correlations with the H-26 and H-29 resonance (δ_H 2.13, δ_H 1.27) confirming their position. The carbonyl C-25 resonance (δ_C 170.6) was confirmed by HMBC correlation between the H-26 resonances (δ_H 2.13). COSY spectrum (Appendix 49) also showed the correlations between H-5 with Hs-1, 2 and 6. The remaining part of the compound was assigned in comparison with literature (Toshihiro, *et al*., 2005). The spectral analysis and comparison with reported data, led to the proposed structure of compound **182** as 12β-acetoxy-20-hydroxy-3,7,11,15-tetraoxo-25,26,27-trisnorlanost-8-en-24-oic acid (Toshihiro, *et al*., 2005). Compound **182** was run in deuterated dichloromethane at 500 MHz while in the literature it was run with deuterated chloroform at 150 MHz thus the difference in chemical shifts.

Table 4. 12 NMR data for compound **181** (20-hydroxylucidenicacid D2) in $CDCl_3$

No	^{13}C NMR ($CDCl_3$ 125 MHz) δ ppm	^{13}C NMR ($CDCL_3$ 150 MHz) (Toshihiro, *et al*., 2005) δ ppm	^{1}H NMR (500 MHz) δ ppm	^{1}H NMR ($CDCl_3$, 600 MHz) (Toshihiro, *et al*., 2005) δ ppm
1	33.4 CH_2	34.0	2.62, 2.76 ddd (J = 8.84 Hz)	1.73, 2.76 ddd
2	32.1 CH_2	33.6	2.13, 2.35 ddd (J = 10.80 Hz)	2.48, 2.60 ddd
3	214.52 C	214.0		
4	39.4 C	46.9		
5	36.2 CH	50.9	1.96 dd	2.32 dd

6	33.4 CH_2	37.4	2.62, 2.76 dd (J = 8.84)	2.50, 2.75 dd
7	198.8 C	198.4		
8	150.4 C	145.6		
9	150.6 C	149.6		
10	33.4 C	39.3		
11	198.7 C	193.3		
12	77.0 CH	78.6	5.74 s	5.70 s
13	42.3 C	47.9		
14	55.4 C	58.0		
15	214.5 C	203.8		
16	36.2 CH_2	35.4	2.41, 1.96 dd (J = 27.48 Hz)	2.84, 2.27 dd
17	44.9 CH	48.8	3.15 dd (J = 22.20 Hz)	2.95 dd
18	15.8 CH_3	13.0	1.27 s	0.96 s
19	23.7 CH_3	18.7	1.57 s	1.35 s
20	69.3 C	86.4		
21	30.5 CH_2	34.5	2.09, 1.82 ddd (J=10.83, 23,64 Hz)	2.04, 2.10 ddd
22	33.4 CH_2	28.0	2.62, 2.76 ddd (J = 8.84 Hz)	2.56, 2.69 m
23	198.8 C	175.6		
24	24.9 CH_3	26.1	1.78 s	1.49 s
25	170.6 C	170.1		
26	21.6 CH_3	21.0	2.13 s	2.26 s
27	17.2 CH_3	27.6	1.23 s	1.14 s
28	17.2 CH_3	20.4	1.23 s	1.12 s
29	15.8 CH_3	21.1	1.27 s	1.85 s

4.3.3 Structure elucidation of compound 183 (13R)-19*α*,13*α*-epoxylabda-6*β*(19).16(15)-dioldilactone



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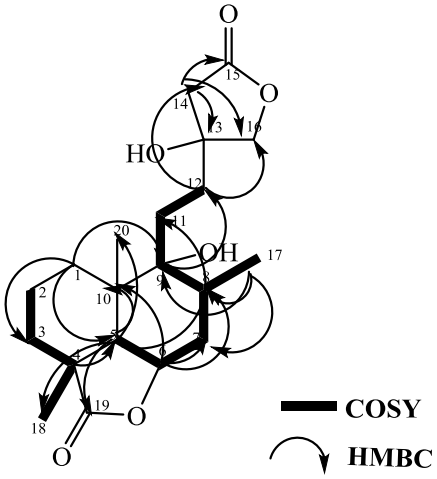

Key: HMBCH→C (curved arrows) and ^{1}H–^{1}H COSY (bold lines) correlations

Compound **183** (13R)-9*α*,13*α*-epoxylabda-6β (19),16(15)-dioldilactanone(13R)-9*α*,13*α*-epoxylabda-6*β* (19),16(15)-dioldilactanone was isolated from dichloromethane crude extract of Laikipia leaves as white crystals. It had twenty carbon resonances in the ^{13}C NMR spectrum (Appendix 52) indicating that it is was Labdane. It showed the presence of three methyl groups, eight methylene groups, three methine groups (one oxygenated) and six quaternary groups. The ^{1}H NMR spectrum (Appendix 51), signals at δ_H 2.07, δ_H 4.70, δ_H 2.57, δ_H 2.91 (doublet J=17.31 Hz), δ_H 4.13, δ_H 4.26 (doublet J = 8.89 Hz) and ^{13}C NMR spectrum (Appendix 52)δ_C 46.2, δ_C 6.2, δ_C 44.2, δ_C 86.3, δ_C 42.1, δ_C 174.7, δ_C 78.8, δ_C 183.6 suggested the presence of two Lactones in the molecule. This also confirmed the presence of two carbonyl groups in the cyclic esters. A methyl doublet at δ_H 0.87 (J = 6.25 Hz) was observed corresponding to ^{13}C NMR resonance at δ_C 17.6. Two other methyl groups at δ_H 1.29 and δ_H 1.04 correlating to ^{13}C NMR resonance at δ_C23.6 and δ_C23.2 respectively were also observed attached to a decahydronaphthalene.

This was all confirmed by the HMBC (Appendix 54) and COSY (Appendix 55) experiments. The HMBC spectrum showed correlation between H-5 resonance δ_H 2.07 with δ_C 44.2, δ_C 76.2, δ_C 183.6 confirming position of one lactone, H-14 resonance δ_H 2.57, δ_H 2.91 (doublet J = 8.89 Hz) with δ_C 37.2, δ_C 86.3, δ_C 174.7, δ_C 78.8 confirming the position of the second lactone. The correlation between H-17 resonance δ_H 0.87 with δ_C 32.1, δ_C 31,9, δ_C 92.3, H-18 resonance δ_H 1.04 with δ_C 29.1, δ_C 46.2, H-20 resonance δ_H 1.29 with δ_C 29.5 and δ_C 39.1 resonances confirmed the position of the three methyl groups. The NOESY (Appendix 56) and COSY (Appendix 55), correlation between H-5 and H-6, H-12 and H-17,

H-2 and H-20 confirmed that compound **183** was labdane (13R)-19*α*,13*α*-epoxylabda-6*β*(19).16(15)-dioldilactone (Daniela *et al*., 2006)**.** A summary of NMR data for the compound is shown in Table 4.11.

Table 4. 13: NMR data for compound **183** (13R)-19*α*,13*α*-epoxylabda-6*β*(19).16(15)-dioldilactone

No	^{13}C NMR ($CDCl_3$, 150 MHz) δ ppm	^{13}C NMR ($CDCL_3$, 400 MHz) (Daniela *et al*., 2006) δ ppm	^{1}H NMR (500 MHz) δ ppm	^{1}H NMR ($CDCl_3$, 400 MHz) (Daniela *et al*., 2006) δ ppm
1	29.5 CH_2	29.3	1.25, 1.30 (m)	1.24 (m)
2	18.2 CH_2	17.9	1.77, 1.52 (m)	1.78, 1.50 (m)
3	29.1 CH_2	28.1	1.46, 2.12 (m)	1.42, 2.11 (m)
4	44.2 C	44.0		
5	46.2 CH	45.9	2.07 (m)	2.08 (m)
6	76.2 CH	75.9	4.70 (m)	4.70 (m)
7	32.1 CH_2	31.6	1.63 (m)	2.08, 1.61 (m)
8	31.9 CH	31.9	1.63 (m)	2.18 (m)
9	92.3 C	92.0		
10	39.1 C	39.0		
11	28.3 CH_2	29.0	1.85, 2.11 (m)	1.83, 2.08 (m)
12	37.2 CH_2	36.9	2.12 (m)	2.10 (m)
13	86.3 C	86.0		
14	42.2 CH_2	43.0	2.57, 2.91 (d, J =17.3 Hz)	2.83 (d, J = 17.2Hz)
15	174.7 C	174.5		
16	78.8 CH_2	78.3	4.13, 4.26 (d, J = 8.9 Hz)	4.40 (d, J = 9.2Hz)
17	17.6 CH_3	17.3	0.87 (d, J=6.3Hz)	0.86 (d, J = 6.4Hz)
18	23.6 CH_3	23.0	1.29 (s)	1.29 (s)
19	183.6 C	183.4		
20	23.2 CH_3	23.4	1.04 (s)	1.05 (s)

4.4 Bioassay tests

4.4.1 *Turraea abyssinica*

The crude extracts from Narok and Kirinyaga counties showed different antimicrobial activity but fairly significant activity at a concentration of 1 mg/mL on all the test microorganism (*Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella typhimurium* and *Candida albicans*) as indicated in Table 4.14. Narok extracts gave more considerable activity than Mount Kenya extracts (Table 4.12). Of all the three compounds **176** (*β*-Sitosterol), **177** (Scopoletin) and **178** [2-(1',2'-Dihydroxypropyl) tetradecanoic acid] that were isolated from Narok *Turraea abyssinica*, only compound **176** gave substantial activity on *BC, SA* and *CA* at a concentration of 2.5 mg/mL to 4.0 mg/mL (Tables 4.15-4.17). *β*-Sitosterol is usually used to treat heart disease, cancer, rheumatoid arthritis, tuberculosis and hair loss (Soodabeh, *et al*., 2014). It also possess good anti diabetic activity (Muhammad *et al*., 2017). Scopoletin has significant pharmacological activities, such as antiarthritic, spasmolytic, antitumor, antidepressant-like, antifungal, antihyperglycemic and antioxidative (Zhou *et al.*, 2012). The saturated carboxylic acid **178** [2-(1',2'-Dihydroxypropyl) tetradecanoic acid] do not exhibit significant antibacterial activity, while the *α,β*-unsaturated carboxylic acids have a broad antimicrobial spectrum and show similar activity against Gram-positive and Gram-negative microorganisms (Giuseppe. *et al*., 2001).

The two antibioctics (Amoxil® and Doxycycline®) that were used as positive controls showed very significant activity on all the test microorganism (Tables 4.18-4.19). All the solvents that were used during extraction (hexane, dichloromethane, ethyl acetate and methanol) showed no activity on all the test microorganism as indicated in table 4.14

Table 4. 14: Inhibition zone (mm) of crude extracts at a concentration of 1mg/ml

Sample	Microoganism					Control
	BC	*SA*	*EC*	*ST*	*CA*	
TA (Narok Leaves} Hex extract	10	19	-	18	15	-
TA (Kirinyaga Leaves) Hex extract	7	12	-	12	10	-
TA (Narok Leaves) DCM extract	10	8	11	-	16	-
TA (Kirinyaga Leaves) DCM extract	-	7	7	11	12	-
TA (Narok Leaves) EtOAc extract	-	10	-	-	8	-
TA (Kirinyaga Leaves) EtOAc extract	-	12	-	-	-	-
TA (Narok Leaves) MeOH extract	7	18	-	11	18	-
TA (Kirinyaga Leaves) MeOH extract	14	10	11	8	12	-

TA (Narok Stem bark) Hex extract	10	14	-	12	20	-
TA (Kirinyaga Stem bark) Hex extract	11	11	-	10	11	-
TA (Narok Stem bark) DCM extract	-	17	11	12	18	-
TA (Kirinyaga Stem bark) DCM extract	-	10	-	-	15	-
TA (Narok Stem bark) EtOAc extract	6	10	6	-	12	-
TA (Kirinyaga Stem bark) EtOAc extract	-	11	-	10	12	-
TA (Narok Stem bark) MeOH extract	10	11	10	18	10	-
TA (Kirinyaga Stem bark) MeOH extract	6	12	-	16	17	-
TA (Narok Root bark} Hex extract	6	19	-	-	-	-
TA (Kirinyaga Root bark) Hex extract	10	10	-	17	11	-
TA (Narok Root bark) DCM extract	-	8	-	-	11	-
TA (Kirinyaga Root bark) DCM extract	-	10	-	-	15	-
TA (Narok Root bark) EtOAc extract	-	10	-	7	10	-
TA (Kirinyaga Root bark) EtOAc extract	-	10	-	-	12	-
TA (Narok Root bark) MeOH extract	8	12	13	20	10	-
TA (Kirinyaga Root bark) MeOH extract	9	7	20	-	11	-

Table 4. 15: Inhibition zone of compound **176** (β-Sitosterol) at different concentrations

	Concentrations							
Microoganism	0.5 mg/mL	1.0 mg/mL	1.5 mg/mL	2.0 mg/mL	2.5 mg/mL	3.0 mg/mL	3.5 mg/mL	4.0 mg/mL
BC	-	-	-	-	6	7	7	8
SA	-	-	-	-	-	6	6	7
EC	-	-	-	-	-	-	-	-
ST	-	-	-	-	-	-	-	-
CA	-	-	-	-	6	7	7	8

Table 4. 16: Inhibition zone of compound **177** (Scopoletin) at different concentrations

	Concentrations							
Microoganism	0.5 mg/mL	1.0 mg/mL	1.5 mg/mL	2.0 mg/mL	2.5 mg/mL	3.0 mg/mL	3.5 mg/mL	4.0 mg/mL
BC	-	-	-	-	-	-	-	-
SA	-	-	-	-	-	-	-	-
EC	-	-	-	-	-	-	-	-
ST	-	-	-	-	-	-	-	-
CA	-	-	-	-	-	-	-	-

Table 4. 17: Inhibition zone of compound **178** [2-(1',2'-Dihydroxy)tetradecanoic acid] at different concentrations

Microoganism	Concentrations 0.5 mg/mL	1.0 mg/mL	1.5 mg/mL	2.0 mg/mL	2.5 mg/mL	3.0 mg/mL	3.5 mg/mL	4.0 mg/mL
BC	-	-	-	-	-	-	-	-
SA	-	-	-	-	-	-	-	-
EC	-	-	-	-	-	-	-	-
ST-	-	-	-	-	-	-	-	-
CA	-	-	-	-	-	-	-	-

Table 4. 18: Inhibition zone (mm) of Amoxil® antibiotic at different concentrations

Concentrations	Microoganism				
μg/mL	*BC*	*SA*	*ST*	*EC*	*CA*
0.004	9	9	11	17	-
0.010	9	10	13	18	-
0.040	10	11	15	19	8
0.100	12	12	15	19	8
0.400	13	13	17	21	9
1.000	15	22	25	22	10
4.000	20	25	30	29	11
10.000	25	30	35	32	12

Table 4. 19: Inhibition zone (mm) of Doxycycline® antibiotic at different concentrations

Concentrations	Microoganism				
mg/mL	*BC*	*SA*	*ST*	*EC*	*CA*
0.004	13	8	10	9	-
0.010	15	10	12	11	-
0.040	21	11	15	16	9
0.100	23	12	18	18	11
0.400	24	15	20	22	15
1.000	26	19	29	25	16
4.000	27	21	33	29	17
10.000	28	24	41	35	30

Tables 4.20-4.24 shows antimicrobial activity of crude extracts at different concentrations. The tables indicated that Narok county crude extracts had significant antimicrobial activity as compared to Kirinyaga county extracts on the tests microorganism. Narok stem bark dichloromethane crude extract that was used to isolate pure compounds gave singnificant activity on all the microogamnism except on *Bacillus cereus.*

Table 4. 20: Inhibition Zone Diameters (mm) of crude extracts at different concentrations

	Bacillus cereus					
	Concentrations					
Sample	0.10 mg/mL	0.20 mg/mL	0.30 mg/mL	0.40 mg/mL	0.50 mg/mL	Control
TA (Narok Leaves} Hex extract	-	-	-	-	-	-
TA (Kirinyaga Leaves) Hex extract	-	-	-	-	-	-
TA (Narok Leaves) DCM extract	-	6	7	7	8	-
TA (Kirinyaga Leaves) DCM extract	-	-	-	-	-	-
TA (Narok Leaves) EtOAc extract	-	-	-	-	-	-
TA (Kirinyaga Leaves) EtOAc extract	-	-	-	-	-	-
TA (Narok Leaves) MeOH extract	-	-	-	-	-	-
TA (Kirinyaga Leaves) MeOH extract	7	8	10	11	12	-
TA (Narok Stem bark) Hex extract	-	-	-	-	-	-
TA (Kirinyaga Stem bark) Hex extract	-	-	7	8	9	-
TA (Narok Stem bark) DCM extract	-	-	-	-	-	-
TA (Kirinyaga Stem bark) DCM extract	-	-	-	-	-	-
TA (Narok Stem bark) EtOAc extract	-	-	-	-	-	-
TA (Kirinyaga Stem bark) EtOAc extract	-	-	-	-	-	-

TA (Narok Stem bark) MeOH extract	-	-	-	-	-	-
TA (Kirinyaga Stem bark) MeOH extract	-	-	-	-	-	-
TA (Narok Root bark} Hex extract	-	-	-	-	-	-
TA (Kirinyaga Root bark) Hex extract	-	-	-	-	-	-
TA (Narok Root bark) DCM extract	-	-	-	-	-	-
TA (Kirinyaga Root bark) DCM extract	-	-	-	-	-	-
TA (Narok Root bark) EtOAc extract	-	-	-	-	-	-
TA (Kirinyaga Root bark) EtOAc extract	-	-	-	-	-	-
TA (Narok Root bark) MeOH extract	-	-	-	-	-	-
TA (Kirinyaga Root bark) MeOH extract	-	6	7	**7**	**8**	-

Table 4. 21: Inhibition Zone Diameters (mm) of crude extracts at different concentrations

Sample	*Staphylococcus aureus* Concentrations					
	0.10 mg/mL	0.20 mg/mL	0.30 mg/mL	0.40 mg/mL	0.50 mg/mL	Control
TA (Narok Leaves} Hex extract	-	6	7	7	9	-
TA (Kirinyaga Leaves) Hex extract	-	-	-	-	-	-
TA (Narok Leaves) DCM extract	-	-	-	-	-	-
TA (Kirinyaga Leaves) DCM extract	-	-	-	-	-	-
TA (Narok Leaves) EtOAc extract	-	-	-	-	-	-
TA (Kirinyaga Leaves) EtOAc extract	-	-	-	-	-	-
TA (Narok Leaves) MeOH	-	6	7	8	10	-

extract						
TA (Kirinyaga Leaves) MeOH extract	-	-	6	7	8	-
TA (Narok Stem bark) Hex extract	-	-	-	6	7	-
TA (Kirinyaga Stem bark) Hex extract	-	-	-	-	-	-
TA (Narok Stem bark) DCM extract	7	10	12	13	15	-
TA (Kirinyaga Stem bark) DCM extract	-	-	-	-	-	-
TA (Narok Stem bark) EtOAc extract	-	-	-	-	-	-
TA (Kirinyaga Stem bark) EtOAc extract	-	-	-	-	-	-
TA (Narok Stem bark) MeOH extract	-	-	-	-	-	-
TA (Kirinyaga Stem bark) MeOH extract	-	-	-	-	-	-
TA (Narok Root bark} Hex extract	-	-	6	6	7	-
TA (Kirinyaga Root bark) Hex extract	-	-	-	-	-	-
TA (Narok Root bark) DCM extract	-	-	6	7	7	-
TA (Kirinyaga Root bark) DCM extract	-	-	6	6	8	-
TA (Narok Root bark) EtOAc extract	-	-	6	7	8	-
TA (Kirinyaga Root bark) EtOAc extract	-	-	-	-	-	-
TA (Narok Root bark) MeOH extract	6	6	7	8	9	-
TA (Kirinyaga Root bark) MeOH extract	-	-	-	-	-	-

Table 4. 22: Inhibition Zone Diameters (mm) of crude extracts at different concentrations

	Escherichia coli					
Sample	Concentrations					
	0.10 mg/mL	0.20 mg/mL	0.30 mg/mL	0.40 mg/mL	0.50 mg/mL	Control
TA (Narok Leaves} Hex extract	-	-	-	-	-	-
TA (Kirinyaga Leaves) Hex extract	-	-	-	-	-	-

TA (Narok Leaves) DCM extract	-	-	7	8	9	-
TA (Kirinyaga Leaves) DCM extract	-	-	-	-	-	-
TA (Narok Leaves) EtOAc extract	-	-	-	-	-	-
TA (Kirinyaga Leaves) EtOAc extract	-	-	-	-	-	-
TA (Narok Leaves) MeOH extract	-	-	-	-	-	-
TA (Kirinyaga Leaves) MeOH extract	-	6	7	7	9	-
TA (Narok Stem bark) Hex extract	-	-	-	-	-	-
TA (Kirinyaga Stem bark) Hex extract	-	-	-	-	-	-
TA (Narok Stem bark) DCM extract	6	7	7	9	10	-
TA (Kirinyaga Stem bark) DCM extract	-	-	-	-	-	-
TA (Narok Stem bark) EtOAc extract	-	-	-	-	-	-
TA (Kirinyaga Stem bark) EtOAc extract	-	-	-	-	-	-
TA (Narok Stem bark) MeOH extract	-	-	-	-	-	-
TA (Kirinyaga Stem bark) MeOH extract	-	-	-	-	-	-
TA (Narok Root bark} Hex extract	-	-	-	-	-	-
TA (Kirinyaga Root bark) Hex extract	-	-	-	-	-	-
TA (Narok Root bark)	-	-	-	-	-	-

DCM extract						
TA (Kirinyaga Root bark) DCM extract	-	-	-	-	-	-
TA (Narok Root bark) EtOAc extract	-	-	-	-	-	-
TA (Kirinyaga Root bark) EtOAc extract	-	-	-	-	-	-
TA (Narok Root bark) MeOH extract	-	-	-	7	10	-
TA (Kirinyaga Root bark) MeOH extract	7	9	11	14	16	-

Table 4. 23: Inhibition Zone Diameters (mm) of crude extracts at different concentrations

	Salmonella typhimurium					
Sample	Concentrations					
	0.10 mg/mL	0.20 mg/mL	0.30 mg/mL	0.40 mg/mL	0.50 mg/mL	Control
TA (Narok Leaves} Hex extract	-	6	7	7	11	-
TA (Kirinyaga Leaves) Hex extract	-	6	6	7	10	-
TA (Narok Leaves) DCM extract	-	-	-	-	-	-
TA (Kirinyaga Leaves) DCM extract	-	-	-	-	-	-
TA (Narok Leaves) EtOAc extract	-	-	-	-	-	-
TA (Kirinyaga Leaves) EtOAc extract	-	-	-	-	-	-
TA (Narok Leaves) MeOH extract	-	7	7	9	10	-
TA (Kirinyaga Leaves) MeOH extract	-	-	-	-	-	-

TA (Narok Stem bark) Hex extract	-	-	7	9	10	-
TA (Kirinyaga Stem bark) Hex extract	-	-	-	-	-	-
TA (Narok Stem bark) DCM extract	-	6	7	8	10	-
TA (Kirinyaga Stem bark) DCM extract	-	-	-	-	-	-
TA (Narok Stem bark) EtOAc extract	-	-	-	-	-	-
TA (Kirinyaga Stem bark) EtOAc extract	-	-	-	-	-	-
TA (Narok Stem bark) MeOH extract	-	7	9	11	13	-
TA (Kirinyaga Stem bark) MeOH extract	-	6	9	10	12	-
TA (Narok Root bark} Hex extract	-	-	-	-	-	-
TA (Kirinyaga Root bark) Hex extract	-	7	7	10	12	-
TA (Narok Root bark) DCM extract	-	-	-	-	-	-
TA (Kirinyaga Root bark) DCM extract	-	-	-	-	-	-
TA (Narok Root bark) EtOAc extract	-	-	-	-	-	-
TA (Kirinyaga Root bark) EtOAc extract	-	-	-	-	-	-
TA (Narok Root bark) MeOH extract	6	7	9	10	12	-
TA (Kirinyaga Root bark) MeOH extract	-	-	-	-	-	-

Table 4. 24: Inhibition Zone Diameters (mm) of crude extracts at different concentrations

	Cadidas albicans					
Sample	Concentrations					
	0.10 mg/mL	0.20 mg/mL	0.30 mg/mL	0.40 mg/mL	0.50 mg/mL	Control
TA (Narok Leaves} Hex extract	7	9	9	12	12	-
TA (Kirinyaga Leaves) Hex extract	-	-	-	-	-	-
TA (Narok Leaves) DCM extract	7	9	9	11	12	-
TA (Kirinyaga Leaves) DCM extract	-	-	6	8	9	-
TA (Narok Leaves) EtOAc extract	-	-	-	-	-	-
TA (Kirinyaga Leaves) EtOAc extract	-	-	-	-	-	-
TA (Narok Leaves) MeOH extract	7	7	9	14	15	-
TA (Kirinyaga Leaves) MeOH extract	-	-	-	-	9	-
TA (Narok Stem bark) Hex extract	-	6	8	15	14	-
TA (Kirinyaga Stem bark) Hex extract	-	-	7	8	9	-
TA (Narok Stem bark) DCM extract	8	11	11	12	12	-
TA (Kirinyaga Stem bark) DCM extract	6	7	8	9	9	-
TA (Narok Stem bark) EtOAc extract	-	-	-	-	7	-
TA (Kirinyaga Stem bark) EtOAc extract	6	8	9	9	10	-

TA (Narok Stem bark) MeOH extract	-	-	-	-	-	-
TA (Kirinyaga Stem bark) MeOH extract	-	-	-	9	10	-
TA (Narok Root bark} Hex extract	-	-	-	-	-	-
TA (Kirinyaga Root bark) Hex extract	-	6	7	8	9	-
TA (Narok Root bark) DCM extract	-	-	-	7	9	-
TA (Kirinyaga Root bark) DCM extract	8	10	11	11	12	-
TA (Narok Root bark) EtOAc extract	-	-	7	7	9	-
TA (Kirinyaga Root bark) EtOAc extract	-	-	8	9	10	-
TA (Narok Root bark) MeOH extract	6	7	7	8	8	-
TA (Kirinyaga Root bark) MeOH extract	-	7	7	8	10	-

Dichloromethane stem bark crude extract of Narok had a Minimum Inhibition Concentration of < 0.1 mg/mL to 0.1 mg/mL on all microorganism except on *Bacillus cereus* which had an MIC of > 0.5 mg/mL. The MIC for the other crude extracts ranged between 0.1 mg/mL and > 0.5 mg/mL**.** Compounds **177** and **178** had an MIC of > 0.16 mg/mL on all the test microorganisms. Compound **176** had an MIC of 0.08 mg/mL on *Bacillus cereus*, 0.10 mg/mL on *Staphylococcus aureus* and > 0.16 mg/mL on *Escherichia coli**,*** *Salmonella typhimurium* and *Candida albicans**.*** All this information is shown inTable 4.25.

Table 4. 25: MIC of crude extracts and pure compounds on test microorganism

Sample	MIC (mg/mL)				
	BC	*SA*	*EC*	*ST*	*CA*
TA (Narok Leaves} Hex extract	> 0.5	0.1	> 0.5	0.1	< 0.1
TA (Kirinyaga Leaves) Hex extract	> 0.5	> 0.5	> 0.5	0.1	> 0.5
TA (Narok Leaves) DCM extract	0.1	> 0.5	0.2	> 0.5	< 0.1
TA (Kirinyaga Leaves) DCM extract	> 0.5	> 0.5	> 0.5	> 0.5	0.2
TA (Narok Leaves) EtOAc extract	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5
TA (Kirinyaga Leaves) EtOAc extract	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5
TA (Narok Leaves) MeOH extract	> 0.5	0.1	> 0.5	0.1	< 0.1
TA (Kirinyaga Leaves) MeOH extract	< 0.1	0.2	0.1	> 0.5	0.4
TA (Narok Stem bark) Hex extract	> 0.5	0.3	> 0.5	0.2	0.1
TA (Kirinyaga Stem bark) Hex extract	0.2	0.5	> 0.5	> 0.5	0.2
TA (Narok Stem bark) DCM extract	> 0.5	< 0.1	< 0.1	0.1	< 0.1
TA (Kirinyaga Stem bark) DCM extract	> 0.5	> 0.5	> 0.5	> 0.5	< 0.1
TA (Narok Stem bark) EtOAc extract	> 0.5	> 0.5	> 0.5	> 0.5	0.4
TA (Kirinyaga Stem bark) EtOAc extract	> 0.5	> 0.5	> 0.5	> 0.5	< 0.1
TA (Narok Stem bark) MeOH extract	> 0.5	> 0.5	> 0.5	0.1	0.5
TA (Kirinyaga Stem bark) MeOH extract	> 0.5	> 0.5	> 0.5	0.1	0.3
TA (Narok Root bark} Hex extract	> 0.5	0.2	> 0.5	> 0.5	0.5
TA (Kirinyaga Root bark) Hex extract	> 0.5	> 0.5	> 0.5	0.1	< 0.1
TA (Narok Root bark) DCM extract	> 0.5	0.2	> 0.5	> 0.5	0.3
TA (Kirinyaga Root bark) DCM extract	> 0.5	0.2	> 0.5	> 0.5	< 0.1
TA (Narok Root bark) EtOAc extract	> 0.5	0.2	> 0.5	> 0.5	0.2
TA (Kirinyaga Root bark) EtOAc extract	> 0.5	> 0.5	> 0.5	> 0.5	0.2
TA (Narok Root bark) MeOH extract	> 0.5	< 0.1	0.3	< 0.1	< 0.1
TA (Kirinyaga Root bark) MeOH extract	0.1	> 0.5	< 0.1	> 0.5	0.1
β-Sitosterol **(176)**	0.08	0.10	> 0.16	> 0.16	> 0.16
Scopoletin **(177)**	> 0.16	> 0.16	> 0.16	> 0.16	> 0.16
2-(1',2'-Dihydroxypropyl) tetradecanoic acid **(178)**	> 0.16	> 0.16	> 0.16	> 0.16	> 0.16

The IC_{50} for Narok stem bark dichloromethane crude extract on *Escherichia coli* (Figure 4.2, Table 4.26), *Salmonella typhimurium* (Figure 4.3, Table 4.26) and *Candida*

albicans (Figure 4.5, Table 4.26) was 0.371 mg/mL, 15.101 mg/mL and 0.001 mg/mL respectively. Mount Kenya dichloromethane leaves (Figure 4.5) and stem bark (Figure 4.6) crude extracts on *Candida albicans* had an IC_{50} of 435.512 mg/mL and 0.255 mg/mL respectively. This was calculated using probit analysis software, Graphpad Prism 7 at different concentrations. Compound **176** (*β*-Sitosterol) had an IC_{50} of 0.141 mg/mL (Figure 4.1, Table 4.26) on *Bacillus cereus* which was 6 times less that of Amoxil® antibiotic (Figure 4.7, Table 4.26) and 162 times less that of Doxycline® antibiotic (Figure 4.8, Table 4.26). Methanol that was used as negative control showed no activity. This is an indication that *Turraea abyssinica* plant has some compounds that can be developed to produce drugs that can be used to treat diseases caused by *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella typhimurium* and *Candida albicans.*

Table 4. 26: IC_{50} mg/mL of crude extracts, pure compounds and positive controls on test microorganism

Sample	Microorganism				
	BC	*SA*	*EC*	*ST*	*CA*
TA (Narok Leaves) DCM extract					435.512
TA (Narok Stem bark) DCM extract			0.371	15.101	0.001
TA (Kirinyaga Stem bark) DCM extract					0.255
β-Sitosterol **(176)**	0.141				
(Scopoletin) **(177)**	-				
(2-(1,2-Dihydroxypropyl) tetradecanal **(178)**	-				
Amoxil® antibiotic	0.775	1.178	1.486	3.811	1.776
Doxycycline® antibiotic	0.044	1.200	233.884	1.276	0.632

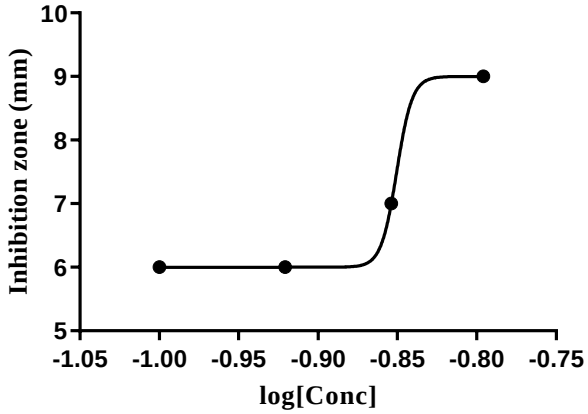

Figure 4. 1: Compound **176** (*β*-Sitosterol). IC_{50} on *BC* = 0.141 mg/mL

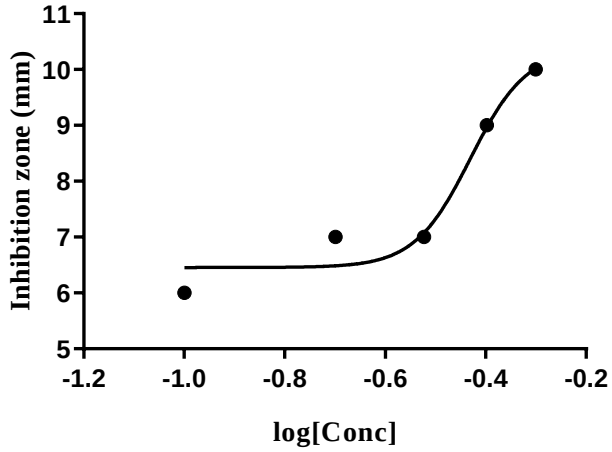

Figure 4. 2: *TA* Narok Stem bark DCM crude extract. IC_{50} on *EC* = 0.371 mg/mL

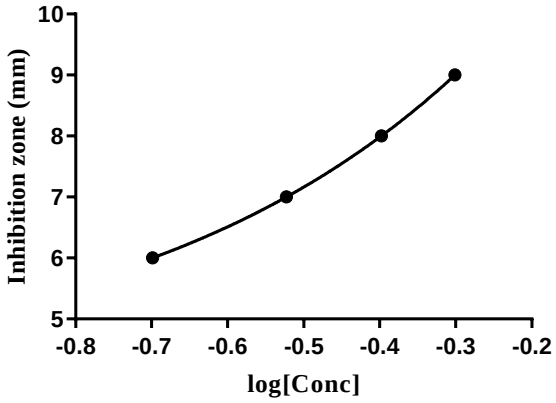

Figure 4. 3: *A* Narok Stem bark DCM crude extract IC_{50} on *ST* = 15.101 mg/mL

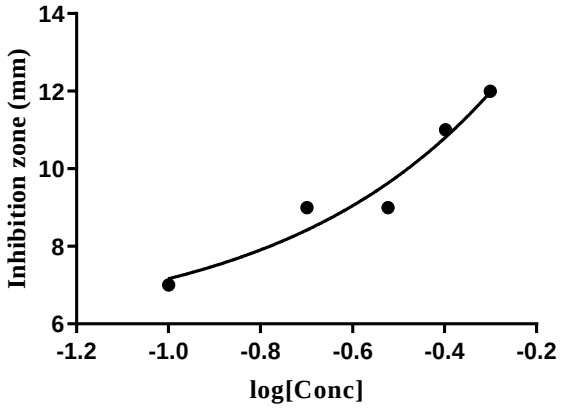

Figure 4. 4: *TA* Kirinyaga Leaves DCM crude extract IC_{50} on *CA* = 435.510 mg/mL

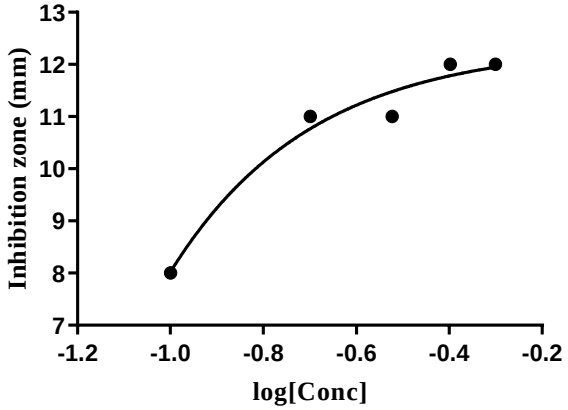

Figure 4. 5: *TA* Narok Stem bark DCM crude extract. IC_{50} on *CA* = 0.001 mg/mL

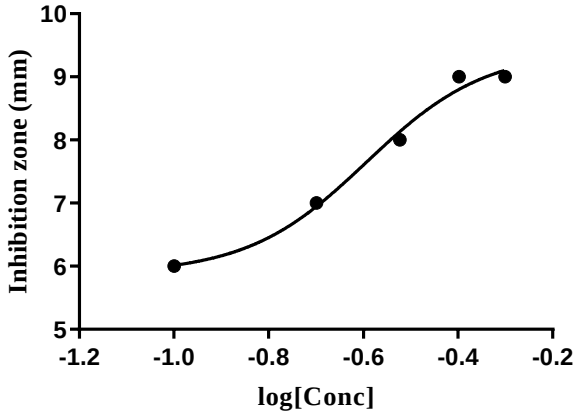

Figure 4. 6: *TA* Kirinyaga Stem bark DCM crude extract. IC_{50} on *CA* = 0.255 mg/mL

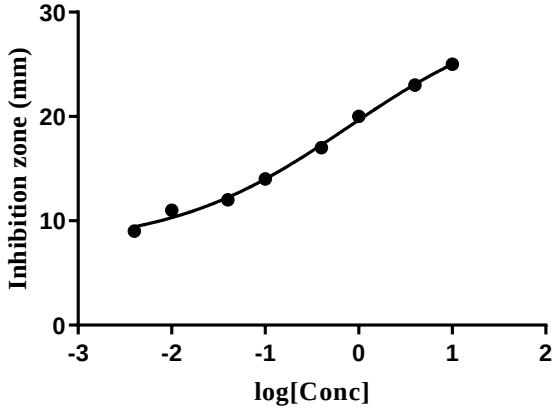

Figure 4. 7: Amoxil® antibiotic IC_{50} on *BC* = 0.775 mg/mL

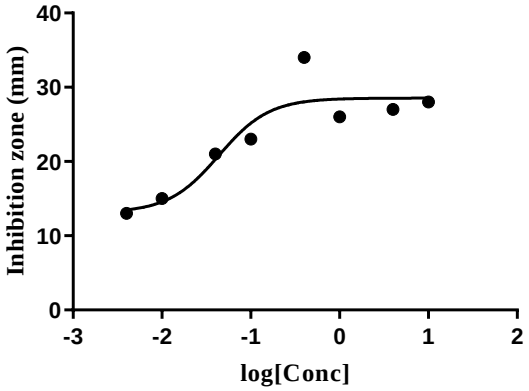

Figure 4. 8: Doxycycline® antibiotic IC_{50} on *BC* = 0.044 mg/mL

4.4.2 *Meyna Tetraphylla*

Crude extracts of Baringo had significant antimicrobial activity on almost all test microoganisms at a concentration of 1 mg/ml as compared to Tharaka Nthi. Tharaka Nthi showed very significant activity on *Candida albicans* at a concentration of 1 mg/ml as indicated in Table 4.27. Extraction solvents that were used as negative control had no activity. Phaeophytin **(179)** showed considerable activity on *Escherichia coli* and *Salmonella typhimurium* at a concentration of 4.0 mg/mL (Table 4.28). Phaeophytin derivatives are known to be cancer preventers. They have antioxidant and antimutagenic activity (Yazı; 2011). *α*-Amyrin **(118)** had significant activity on *Salmonella typhimurium* at a concentration of 4.0 mg/mL (Table 4.30).). Both *α*-amyrin and *β*-amyrin in several plants and the pure compounds have shown anti-microbial and anti-inflammatory (Liliana *et al*., 2012**).** Compound **60** (Stigmasterol) had no significant activity although it is known as a potent and broad-spectrum antibacterial and antifungal agent (Yusuf, *et al*., 2018).

Table 4. 27 Inhibition zone (mm) of crude extracts at a concentration of (1mg/ml)

Sample	Microoganism					Control
	BC	*SA*	*EC*	*ST*	*CA*	
MT (Baringo Leaves) Hex extract	6	12	-	-	-	-
MT (Taraka Nthi Leaves) Hex extract	-	-	-	-	> 30	-
MT (Baringo Fruits) Hex extract	6	10	14	-	9	-
MT (Taraka Nthi Fruits) Hex extract	-	1.5	-	1.5	> 30	-
MT (Baringo root bark) Hex extract	-	-	-	-	8	-
MT (Taraka Nthi root bark) Hex extract	-	-	-	-	> 30	-
MT (Baringo Leaves) DCM extract	6	8	10	12	15	-
MT (Taraka Nthi Leaves) DCM extract	-	-	-	-	> 30	-
MT (Baringo Fruits) DCM extract	10	10	-	12	17	-
MT (Taraka Nthi Fruits) DCM extract	-	-	-	-	> 30	-
MT (Baringo root bark) DCM extract	-	-	-	-	7	
MT (Taraka Nthi root bark) DCM extract	-	-	-	-	> 30	-
MT (Baringo Leaves) EtOAc extract	-	10	-	20	14	-
MT (Taraka Nthi Leaves) EtOAc extract	-	-	-	-	> 30	-
MT (Baringo Fruits) EtOAc extract	11	20	6	11	15	-
MT (Taraka Nthi Fruits) EtOAc extract	-	1.7	-	1.5	> 30	-
MT (Baringo root bark) EtOAc extract	-	-	-	-	7	-
MT (Taraka Nthi root bark) EtOAc extract	-	-	-	-	> 30	-
MT (Baringo Leaves) MeOH extract	10	8	-	15	15	-
MT (Taraka Nthi Leaves) MeOH extract	25	27	25	25	> 30	-
MT (Baringo Fruits) MeOH extract	10	10	-	9	15	-
MT (Taraka Nthi Fruits) MeOH extract	-	1.5	2.0	2.5	> 30	-
MT (Baringo root bark) MeOH extract	-	-	-	-	8	-
MT (Taraka Nthi root bark) MeOH extract	-	-	-	-	> 30	-

Table 4. 28: Inhibition zone of compound 179 (Phaeophytin) at different concentrations

	Concentrations							
Microoganism	0.5 mg/mL	1.0 mg/mL	1.5 mg/mL	2.0 mg/mL	2.5 mg/mL	3.0 mg/mL	3.5 mg/mL	4.0 mg/mL
BC	-	-	-	-	-	-	-	-
SA	-	-	-	-	-	-	-	-
EC	-	-	-	-	8	8	8	8
ST	-	-	-	-	6	7	7	9
CA	-	-	-	-			7	7

Table 4. 29: Inhibition zone of compound 180 (Enantiomer) at different concentrations

	Concentrations							
Microoganism	0.5 mg/mL	1.0 mg/mL	1.5 mg/mL	2.0 mg/mL	2.5 mg/mL	3.0 mg/mL	3.5 mg/mL	4.0 mg/mL
BC	-	-	-	-	-	-	-	-
SA	-	-	-	-	-	-	-	-
EC	-	-	-	-	-	-	-	-
ST	-	-	-	-	-	-	-	-
CA	-	-	-	-	-	-	-	-

Table 4. 30: Inhibition zone of compound 118 (α-Amyrin) at different concentrations

	Concentrations							
Microoganism	0.5 mg/mL	1.0 mg/mL	1.5 mg/mL	2.0 mg/mL	2.5 mg/mL	3.0 mg/mL	3.5 mg/mL	4.0 mg/mL
BC	-	-	-	-	-	-	-	-
SA	-	-	-	-	-	-	-	-
EC	-	-	-	-	-	-	-	-
ST	-	-	-	-	7	7	8	9
CA	-	-	-	-	-	-	-	-

Table 4. 31: Inhibition zone of compound **60** (Stigmasterol) at different concentrations

	Concentrations							
Microoganism	0.5 mg/mL	1.0 mg/mL	1.5 mg/mL	2.0 mg/mL	2.5 mg/mL	3.0 mg/mL	3.5 mg/mL	4.0 mg/mL
BC	-	-	-	-	-	-	-	-
SA	-	-	-	-	-	-	-	-
EC	-	-	-	-	-	-	-	-
ST-	-	-	-	-	-	-	-	-
CA	-	-	-	-	-	-	-	-

Table 4.32-4.36 shows the activity of the crude extracts at different concentrations on *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella typhimurium* and *Candida albicans.* Table 4.36 indicated that both Baringo and Tharaka Nthi crude extracts had very significant antimicrobial activity on *Candida albicans*. Tables 4.34 and 4.36 also

showed some substantial activy on both *Staphylococcus aureus* and *Salmonella typhimurium* on dichloromethane, ethyl acetate and methanol crude extracts. All the organic solvents had no activity on the test microorganism.

Table 4. 32 : Inhibition Zone Diameters (mm) of crude extracts at different concentrations

	Bacillus cereus					
	Concentrations					Control
Samlpe	0.10 mg/mL	0.20 mg/mL	0.30 mg/mL	0.40 mg/mL	0.50 mg/mL	
MT (Baringo Leaves) Hex extract	-	-	-	-	-	-
MT (Tharaka Nthi Leaves) Hex extract	-	-	-	-	-	-
MT (Baringo Fruits) Hex extract	-	-	-	-	-	-
MT (Tharaka Nthi Fruits) Hex extract	-	-	-	-	-	-
MT (Baringo root bark) Hex extract	-	-	-	-	-	-
MT (Tharaka Nthi root bark) Hex extract	-	-	-	-	-	-
MT (Baringo Leaves) DCM extract	-	-	-	-	-	-
MT (Tharaka Nthi Leaves) D extract	-	-	-	-	-	-
MT (Baringo Fruits) DCM extract	-	-	-	-	-	-
MT (Tharaka Nthi Fruits) DCM extract	-	-	-	-	-	-
MT (Baringo root bark) DCM extract	-	-	-	-	-	-
MT (Tharaka Nthi root bark) DCM extract	-	-	-	-	-	-
MT (Baringo Leaves)	-	-	-	-	-	-

EtOAc extract						
MT (Tharaka Nthi Leaves) EtOAc extract	-	-	-	-	-	-
MT (Baringo Fruits) EtOAc extract	-	-	-	-	-	-
MT (Tharaka Nthi Fruits) EtOAc extract	-	-	-	-	-	-
MT (Baringo root bark) EtOAc extract	-	-	-	-	-	-
MT (Tharaka Nthi root bark) EtOAc extract	-	-	-	-	-	-
MT (Baringo Leaves) MeOH extract	-	-	-	7	8	-
MT (Tharaka Nthi Leaves) MeOH extract	8	10	12	14	15	-
MT (Baringo Fruits) MeOH extract	-	-	-	-	-	-
MT (Tharaka Nthi Fruits) MeOH extract	-	-	-	-	-	-
MT (Baringo root bark) MeOH extract	-	-	-	-	-	-
MT (Tharaka Nthi root bark) MeOH extract	-	-	-	-	-	-

Table 4. 33: Inhibition Zone Diameters (mm) of crude extracts at different concentrations

	Staphylococcus aureus					
Sample	Concentrations					
	0.10 mg/mL	0.20 mg/mL	0.30 mg/mL	0.40 mg/mL	0.50 mg/mL	Control
MT (Baringo Leaves) Hex extract	-	-	-	-	-	-
MT (Tharaka Nthi Leaves) Hex extract	-	-	-	-	-	-

MT (Baringo Fruits) Hex extract	-	-	-	-	-	-
MT (Tharaka Nthi Fruits) Hex extract	7	8	8	9	9	-
MT (Baringo root bark) Hex extract	-	-	-	-	-	-
MT (Tharaka Nthi root bark) Hex extract	-	-	-	-	-	-
MT (Baringo Leaves) DCM extract	-	-	-	-	-	-
MT (Tharaka Nthi Leaves) D extract	-	-	-	-	-	-
MT (Baringo Fruits) DCM extract	-	6	6	7	8	-
MT (Tharaka Nthi Fruits) DCM extract	-	-	-	-	-	-
MT (Baringo root bark) DCM extract	-	-	-	-	-	-
MT (Tharaka Nthi root bark) DCM extract	-	-	-	-	-	-
MT (Baringo Leaves) EtOAc extract	-	6	7	7	8	-
MT (Tharaka Nthi Leaves) EtOAc extract	-	-	-	-	-	-
MT (Baringo Fruits) EtOAc extract	-	-	6	9	10	-
MT (Tharaka Nthi Fruits) EtOAc extract	7	8	9	10	10	-
MT (Baringo root bark) EtOAc extract	-	-	-	-	-	-
MT (Tharaka Nthi root bark) EtOAc extract	-	-	-	-	-	-
MT (Baringo Leaves)	-	-	6	6	8	-

MeOH extract						
MT (Tharaka Nthi Leaves) MeOH extract	8	10	11	13	15	-
MT (Baringo Fruits) MeOH extract	-	-	-	-	-	-
MT (Tharaka Nthi Fruits) MeOH extract	8	8	9	9	10	-
MT (Baringo root bark) MeOH extract	-	-	-	-	-	-
MT (Tharaka Nthi root bark) MeOH extract	-	-	-	-	-	-

Table 4. 34: Inhibition Zone Diameters (mm) of crude extracts at different concentrations

Sample	*Escherichia coli* Concentrations					
	0.10 mg/mL	0.20 mg/mL	0.30 mg/mL	0.40 mg/mL	0.50 mg/mL	control
MT (Baringo Leaves) Hex extract	-	-	-	-	-	-
MT (Tharaka Nthi Leaves) Hex extract	-	6	7	10	12	-
MT (Baringo Fruits) Hex extract	-	-	-	-	-	-
MT (Tharaka Nthi Fruits) Hex extract	-	-	-	-	-	-
MT (Baringo root bark) Hex extract	-	-	-	-	-	-
MT (Tharaka Nthi root bark) Hex extract	-	-	-	-	-	-
MT (Baringo Leaves) DCM extract	-	-	-	-	-	-
MT (Tharaka Nthi Leaves) D extract	-	-	-	-	-	-
MT (Baringo Fruits)	-	-	-	-	-	-

DCM extract						
MT (Tharaka Nthi Fruits) DCM extract	-	-	-	-	-	-
MT (Baringo root bark) DCM extract	-	-	-	-	-	-
MT (Tharaka Nthi root bark) DCM extract	-	-	-	-	-	-
MT (Baringo Leaves) EtOAc extract	-	-	-	-	-	-
MT (Tharaka Nthi Leaves) EtOAc extract	-	-	-	-	-	-
MT (Baringo Fruits) EtOAc extract	-	-	-	-	-	-
MT (Tharaka Nthi Fruits) EtOAc extract	-	-	-	-	-	-
MT (Baringo root bark) EtOAc extract	-	-	-	-	-	-
MT (Tharaka Nthi root bark) EtOAc extract	-	-	-	-	-	-
MT (Baringo Leaves) MeOH extract	-	-	-	-	-	-
MT (Tharaka Nthi Leaves) MeOH extract	-	-	-	-	-	-
MT (Baringo Fruits) MeOH extract	-	-	-	-	-	-
MT (Tharaka Nthi Fruits) MeOH extract	10	12	14	15	20	-
MT (Baringo root bark) MeOH extract	-	7	7	9	10	-
MT (Tharaka Nthi root bark) MeOH extract	-	-	-	-	-	-

Table 4. 35: Inhibition Zone Diameters (mm) of crude extracts at different concentrations

	Salmonella typhimurium					
	Concentrations					
Sample	0.10 mg/mL	0.20 mg/mL	0.30 mg/mL	0.40 mg/mL	0.50 mg/mL	Control
MT (Baringo Leaves) Hex extract	-	-	-	-	-	-
MT (Tharaka Nthi Leaves) Hex extract	-	-	-	-	-	-
MT (Baringo Fruits) Hex extract	-	-	-	-	-	-
MT (Tharaka Nthi Fruits) Hex extract	-	--	-	7	8	-
MT (Baringo root bark) Hex extract	6	7	8	9	10	-
MT (Tharaka Nthi root bark) Hex extract	-	-	-	-	-	-
MT (Baringo Leaves) DCM extract	6	8	9	9	13	-
MT (Tharaka Nthi Leaves) D extract	-	-	-	-	-	-
MT (Baringo Fruits) DCM extract	-	-	-	-	-	-
MT (Tharaka Nthi Fruits) DCM extract	6	8	9	11	12	-
MT (Baringo root bark) DCM extract	-	-	6	7	7	-
MT (Tharaka Nthi root bark) DCM extract	-	-	-	-	-	-
MT (Baringo Leaves) EtOAc extract	-	-	-	-	-	-
MT (Tharaka Nthi Leaves) EtOAc extract	9	9	10	11	12	-

MT (Baringo Fruits) EtOAc extract	-	-	-	-	-	-
MT (Tharaka Nthi Fruits) EtOAc extract	-	-	-	-	-	-
MT (Baringo root bark) EtOAc extract	-	-	-	-	-	-
MT (Tharaka Nthi root bark) EtOAc extract	-	-	-	-	-	-
MT (Baringo Leaves) MeOH extract	-	-	-	-	-	-
MT (Tharaka Nthi Leaves) MeOH extract	7	8	8	9	10	-
MT (Baringo Fruits) MeOH extract	-	-	-	-	-	--
MT (Tharaka Nthi Fruits) MeOH extract	8	10	12	15	20	-
MT (Baringo root bark) MeOH extract	-	8	9	10	15	-
MT (Tharaka Nthi root bark) MeOH extract	-	-	-	-	-	-

Table 4. 36: Inhibition Zone Diameters (mm) of crude extracts at different concentrations

	Cadidas albicans					
	Concentrations					
Sample	0.10 mg/mL	0.20 mg/mL	0.30 mg/mL	0.40 mg/mL	0.50 mg/mL	Control
MT (Baringo Leaves) Hex extract	-	-	-	-	-	-
MT (Tharaka Nthi Leaves) Hex extract	>20	>20	>20	>20	>20	-
MT (Baringo Fruits) Hex extract	7	7	8	9	9	-
MT (Tharaka Nthi Fruits)	>20	>20	>20	>20	>20	-

Hex extract						
MT (Baringo root bark) Hex extract	-	6	7	9	10	-
MT (Tharaka Nthi root bark) Hex extract	>20	>20	>20	>20	>20	-
MT (Baringo Leaves) DCM extract	-	-	6	10	12	-
MT (Tharaka Nthi Leaves) D extract	>20	>20	>20	>20	>20	-
MT (Baringo Fruits) DCM extract	-	-	-	-	-	-
MT (Tharaka Nthi Fruits) DCM extract	>20	>20	>20	>20	>20	-
MT (Baringo root bark) DCM extract	-	-	-	-	-	-
MT (Tharaka Nthi root bark) DCM extract	>20	>20	>20	>20	>20	-
MT (Baringo Leaves) EtOAc extract	-	-	-	-	-	-
MT (Tharaka Nthi Leaves) EtOAc extract	>20	>20	>20	>20	>20	-
MT (Baringo Fruits) EtOAc extract	-	-	-	-	-	-
MT (Tharaka Nthi Fruits) EtOAc extract	>20	>20	>20	>20	>20	-
MT (Baringo root bark) EtOAc extract	-	6	7	7	8	-
MT (Tharaka Nthi root bark) EtOAc extract	>20	>20	>20	>20	>20	-
MT (Baringo Leaves) MeOH extract	8	10	11	`12	12	-
MT (Tharaka Nthi Leaves) MeOH extract	>20	>20	>20	>20	>20	-

MT (Baringo Fruits) MeOH extract	-	7	7	8	10	-
MT (Tharaka Nthi Fruits) MeOH extract	>20	>20	>20	>20	>20	-
MT (Baringo root bark) MeOH extract	-	8	9	9	11	-
MT (Tharaka Nthi root bark) MeOH extract	>20	>20	>20	>20	>20	-

Minimum Inhibition Concentration (MIC) of the crude extracts ranged between < 0.1 to > 0.5 mg/mL. The crude extracts from Tharaka Nthi showed significant activity on *Candida albicans* with an MIC of ≤ 0.1 mg/mL (Table 4.37). This is an indication Tharaka Nthi *Meyna tetraphylla* has more effective compounds that can be developed to treat fungal infection. Compound **179** (Phaeophytin) showed considerable antimicrobial activity on all the test microorganism at an MIC of 0.08 mg/mL on *Staphylococcus aureus, Escherichia coli* and *Salmonella typhimurium*, 0.12 mg/mL on *Candida albicans* and > 0.16 mg/mL on *Bacillus cereus* (Table 4.37). Compounds **180** (Enantiomer) and **118** (α-Amyrin) had an MIC of 0.08 mg/mL on *Salmonella typhimurium* and compound **60** (Stigmasterol) had 0.12 mg/mL on *Bacillus cereus* (Table 4.37).

Table 4. 37: Minimum Inhibition Concentration (MIC) mg/mL of crude extracts and pure compounds.

	Microoganism				
Sample	*BC*	*SA*	*EC*	*ST*	*CA*
MT (Baringo Leaves) Hex extract	> 0.5	> 0.5	0.1	> 0.5	> 0.5
MT (Tharaka Nthi Leaves) Hex extract	> 0.5	> 0.5	> 0.5	> 0.5	< 0.1
MT (Baringo Fruits) Hex extract	> 0.5	> 0.5	> 0.5	> 0.5	0.1
MT (Tharaka Nthi Fruits) Hex extract	> 0.5	> 0.5	> 0.5	< 0.1	< 0.1
MT (Baringo root bark) Hex extract	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5
MT (Tharaka Nthi root bark) Hex extract	> 0.5	> 0.5	> 0.5	> 0.5	< 0.1
MT (Baringo Leaves) DCM extract	> 0.5	> 0.5	> 0.5	> 0.5	< 0.1
MT (Tharaka Nthi Leaves) DCM extract	> 0.5	> 0.5	> 0.5	> 0.5	< 0.1
MT (Baringo Fruits) DCM extract	> 0.5	0.1	> 0.5	< 0.1	< 0.1
MT (Tharaka Nthi Fruits) DCM extract	> 0.5	> 0.5	> 0.5	> 0.5	< 0.1
MT (Baringo root bark) DCM extract	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5

MT (Tharaka Nthi root bark) DCM extract	> 0.5	> 0.5	> 0.5	> 0.5	< 0.1
MT (Baringo Leaves) EtOAc extract	> 0.5	0.1	> 0.5	< 0.1	0.1
MT (Tharaka Nthi Leaves) EtOAc extract	> 0.5	> 0.5	> 0.5	> 0.5	< 0.1
MT (Baringo Fruits) EtOAc extract	> 0.5	0.2	> 0.5	> 0.5	0.1
MT (Tharaka Nthi Fruits) EtOAc extract	> 0.5	< 0.1	> 0.5	< 0.1	< 0.1
MT (Baringo root bark) EtOAc extract	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5
MT (Tharaka Nthi root bark) EtOAc extract	> 0.5	> 0.5	> 0.5	> 0.5	< 0.1
MT (Baringo Leaves) MeOH extract	0.3	0.2	> 0.5	< 0.1	> 0.5
MT (Tharaka Nthi Leaves) MeOH extract	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
MT (Baringo Fruits) MeOH extract	> 0.5	> 0.5	> 0.5	0.2	0.1
MT (Tharaka Nthi Fruits) MeOH extract	> 0.5	< 0.1	0.1	0.1	< 0.1
MT (Baringo root bark) MeOH extract	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5
MT (Tharaka Nthi root bark) MeOH extract	> 0.5	> 0.5	> 0.5	> 0.5	< 0.1
Phaeophytin (**179)**	> 0.16	0.08	0.08	0.08	0.12
Enantiomer (**180)**	> 0.16	> 0.16	> 0.16	0.08	> 0.16
α-Amyrin (**118)**	> 0.16	> 0.16	> 0.16	0.08	> 0.16
Stigmasterol (**60)**	0.12	0.12	> 0.16	> 0.16	> 0.16

The IC_{50} for Amoxil® antibiotic on *Candida albicans* (Figure 4.23) and Doxycycline® antibiotic on *Candida albicans* (Figure 4.24) were used for comparison with Baringo leaves and fruits dichloromethane and ethyl acetate crude extracts (Figure 4.1 -4.17, Table 4.38). This was calculated using probit analysis Graphpad Prism 7 at different concentrations. The IC_{50} for leaves crude extracts on *Candida albicans* was 0.300 mg/ml, 0.357 mg/mL, 6 and 5 times less than that of Amoxil® antibiotic and 2 times that of Doxycycline® antibiotic respectively. For Fruits crude extracts, the IC_{50} was 0.191 mg/mL, 0.406 mg/mL, 9 and 4 times less that of Amoxil® antibiotic (Figure 4.23) and 3 and 2 times that of Doxycycline® antibiotic (Figure 424) respectively. These concentrations were low compared to the two antibiotics. Tharaka Nthi fruits ethyl acetate crude extract showed an IC_{50} of 0.255 mg/mL on *Staphylococcus aureus* (Figure 4.9, Table 4.38), 5 times less that of the antibiotics. Compound **179** had an IC_{50} of 0.129 mg/mL (Figure 4.18, Table 4.38) on *Staphylococcus aureus*, 0.141 mg/mL on *Salmonella typhimurium* (Figure 4.20, Table 4.38) and *Escherichia coli* (Figure 4.19, Table 4.38) respectively, which was 9, 27 and 11 times less that of Amoxil® antibiotic and 9 times that of Doxycycline® antibiotic. Compound **180** and **118** showed activity on *Salmonella typhimurium* with an IC_{50} of 0.129 mg/mL (Figure 4.21, Table

4.38) and 0.120 mg/mL (Figure 4.22, Table 4.38) respectively. This was 30 and 32 times less that of Amoxil® antibiotic, 10 and 11 times that of Doxycycline® antibiotic. This is an indication that *Meyna tetraphylla* contain compounds that can be developed to treat diseases caused by the entire test microorganism.

Table 4. 38: IC_{50} mg/mLof crude extracts and pure compounds on test microorganism

Sample	Microoganism				
	BC	*SA*	*EC*	*ST*	*CA*
MT (Baringo Leaves) DCM extract		138.95			0.300
MT (Baringo Fruits) DCM extract				49.889	0.191
MT (Baringo Leaves) EtOAc extract				21.627	0.357
MT (Baringo Fruits) EtOAc extract					0.406
MT (Tharaka Nthi Fruits) EtOAc extract		0.255		53.088	
Phaeophytin (**179)**		0.129	0.141	0.141	
Enantiomer **180**				0.129	
α-Amyrin (**118)**				0.120	
Stigmasterol (**60)**					
Amoxil® antibiotic	0.775	1.178	1.486	3.811	1.776
Doxycycline® antibiotic	0.044	1.200	233.884	1.276	0.632

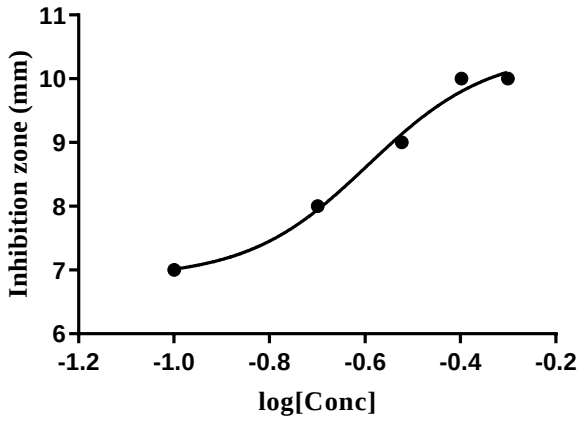

Figure 4. 9: *MT* Tharaka Nthi Fruits EtOAc crude extract IC_{50} on *SA* = 0.255 mg/mL

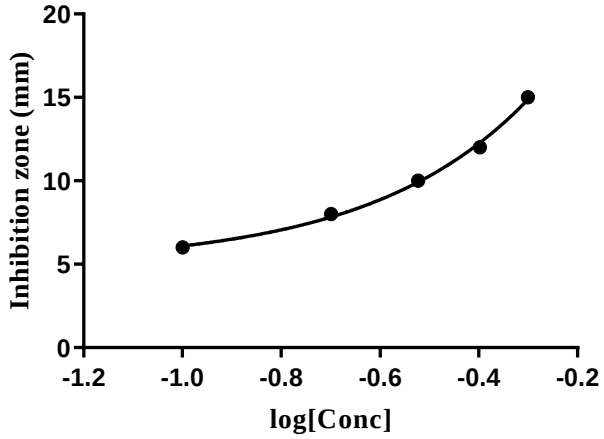

Figure 4. 10: *MT* Baringo DCM crude extract IC_{50} on *SA* = 138.995 mg/mL

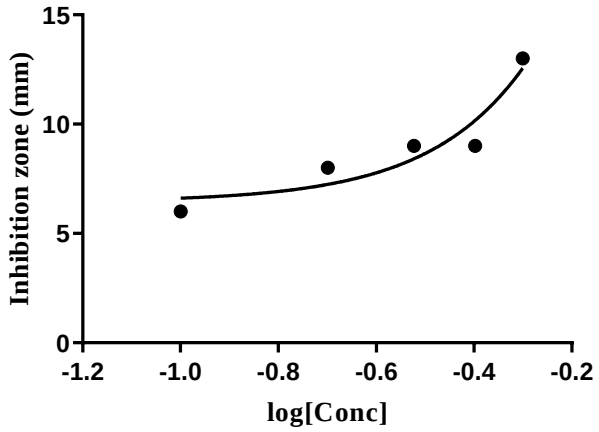

Figure 4. 11: *MT* Baringo Leaves EtOAc crude extract IC_{50} on *ST* = 21.627 mg/mL

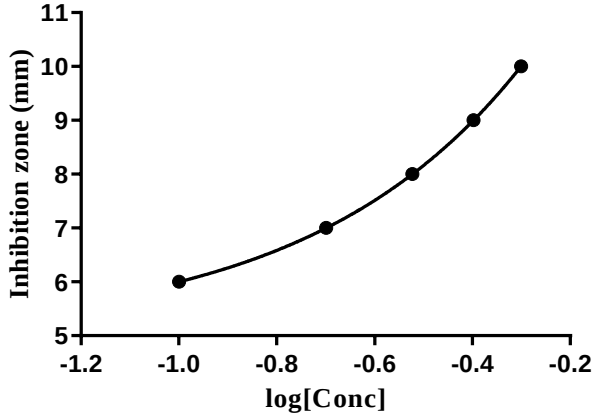

Figure 4. 12: *MT* Baringo Fruits DCM crude extract IC_{50} on *ST* = 49.889 mg/mL

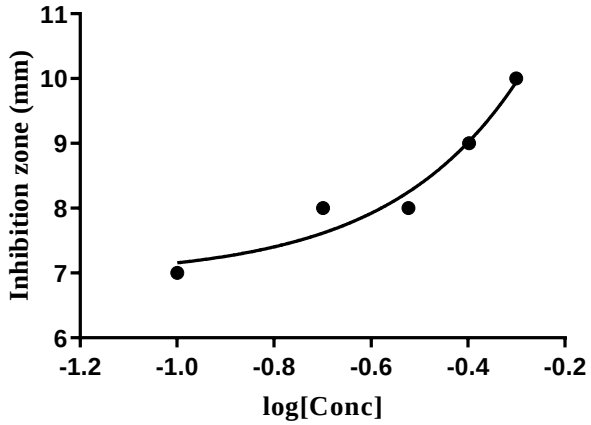

Figure 4. 13: *MT* Tharaka Nthi fruits EtOAc crude extract IC_{50} on *ST* = 53.088 mg/mL

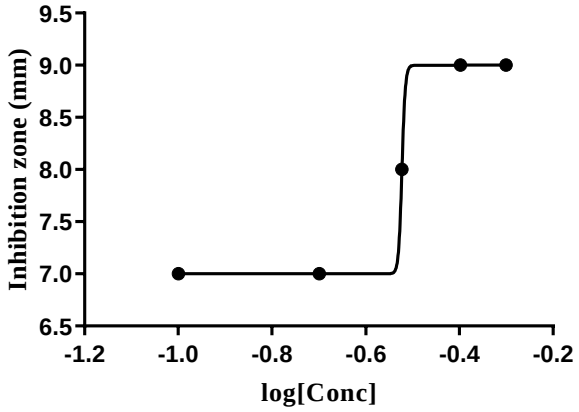

Figure 4. 14: *MT* Baringo Leaves DCM crude extract IC_{50} on *CA* = 0.300 mg/mL

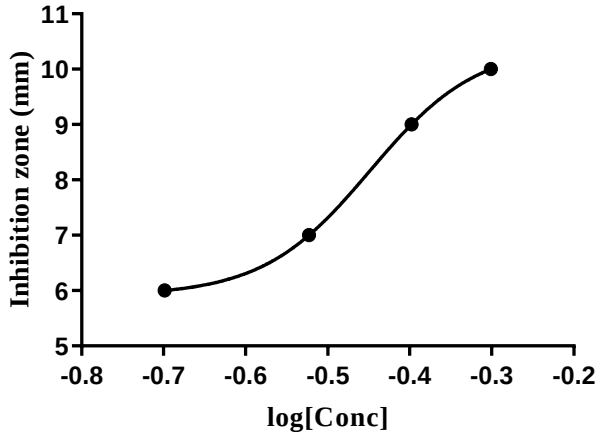

Figure 4. 15: *MT* Baringo Leaves EtOAc crude extract IC_{50} on *CA* = 0.357 mg/mL

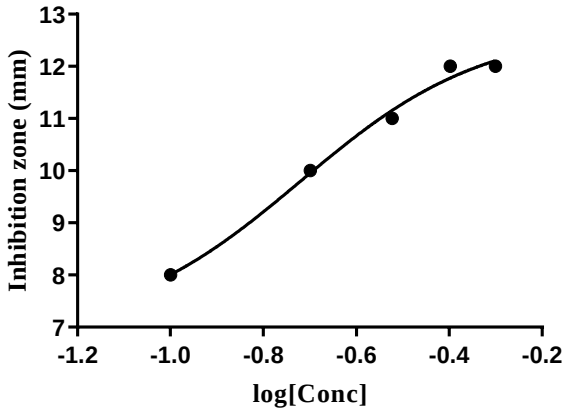

Figure 4. 16: *MT* Baringo Fruits DCM crude extract IC_{50} on *CA* = 0.191 mg/mL

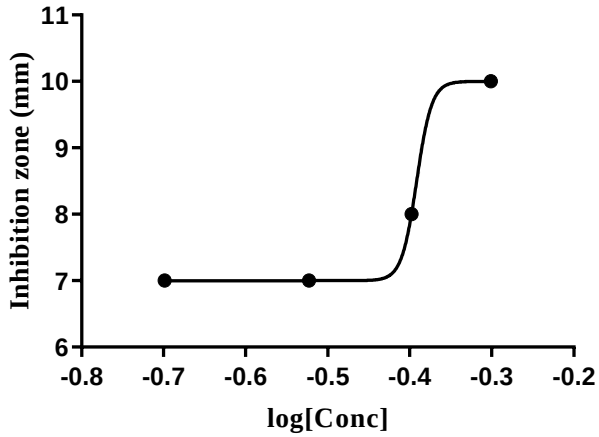

Figure 4. 17: *MT* Baringo Fruits EtOAc crude extract IC_{50} on *CA* = 0.406 mg/mL

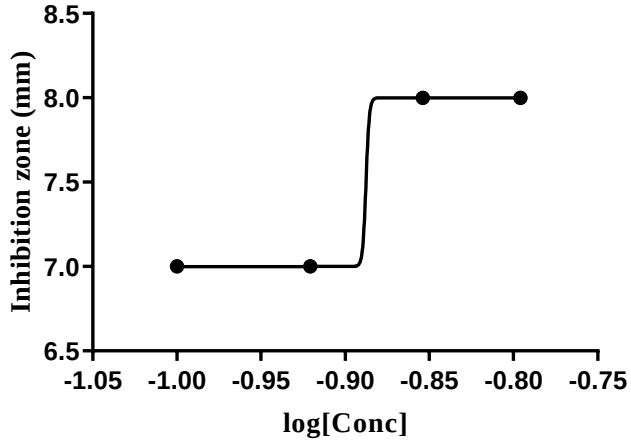

Figure 4. 18: Compound **179** IC_{50} on *SA* = 0.129 mg/mL

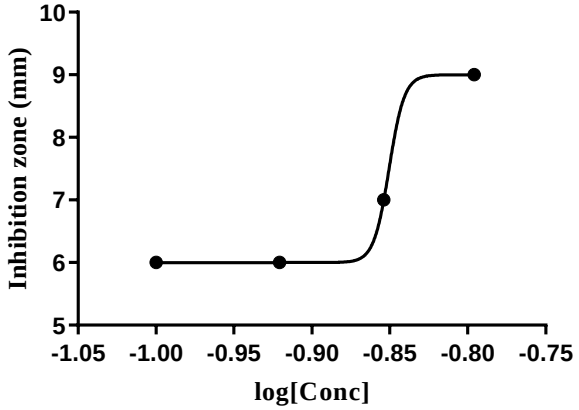

Figure 4. 19: Compound 1**79** IC_{50} on *EC* = 0.141 mg/mL

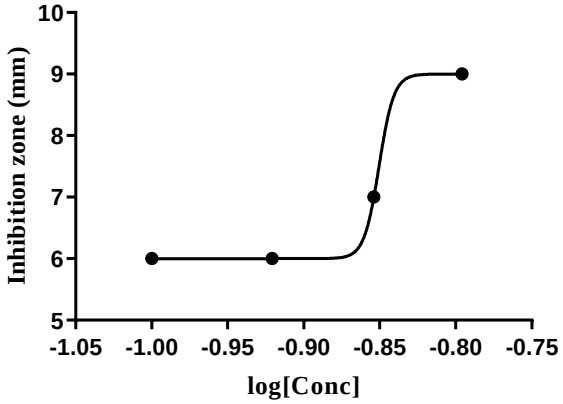

Figure 4. 20: Compound **179** IC_{50} on *ST* = 0.141 mg/mL

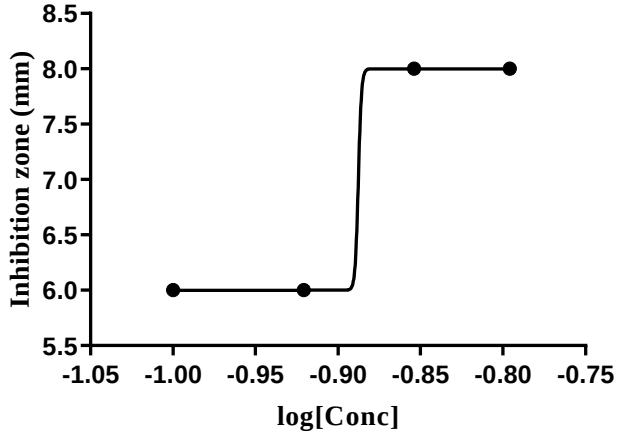

Figure 4. 21: Compound **180** IC_{50} on *ST* = 129 mg/mL

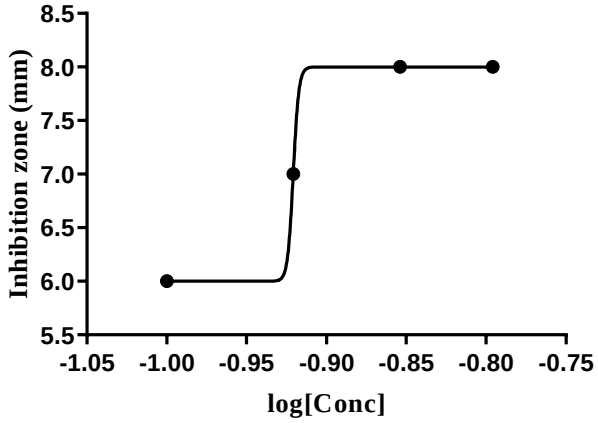

Figure 4. 22: Compound **118** IC_{50} on *ST* = 0.128 mg/mL

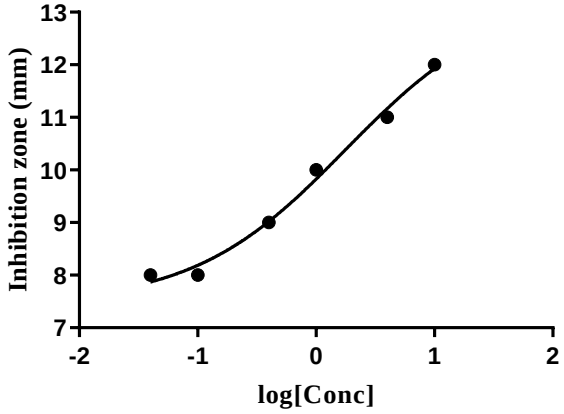

Figure 4. 23: Amoxil® antibiotic IC_{50} on *CA* = 1.776 mg/mL

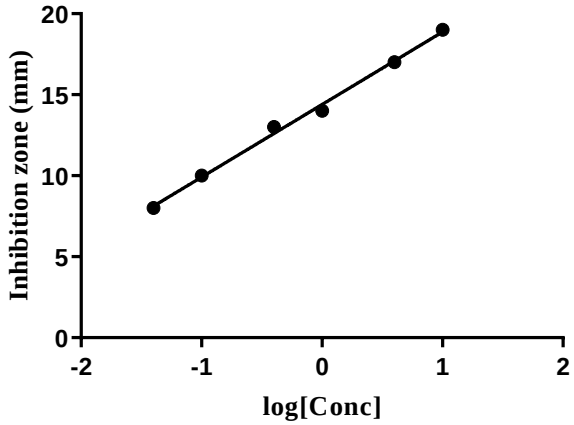

Figure 4. 24: Doxycycline® antibiotic IC_{50} on *CA* = 0.632 mg/mL

4.4.3 *Leonotis mollissima*

Almost all the crude extracts from both Laikipia and Mau Narok *Leonotis mollissima* had very significant antimicrobial activity on *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Bacillus cereus* and *Candida albicans* at a concentration of 1mg/mL as indicated in Table 4.39**.** The crude extracts also had significant activity at concentratios of 0.10 mg/mL to 0.50 mg/mL except on *candida albicans* Tables 4.43-4.47. The organic extraction solvents (hexane, dichloromethane, ethyl acetae and methanol) that were used as negative control did not show any activity. Of all the compounds isolated Siderin **(181)**, 20-hydroxylucidenicacid D2 **(182)** and (13R)-9*α*,13*α*-epoxylabda-6β(19),16(15)-dioldilactanone(13R)-9*α*,13*α*-epoxylabda-6*β* (19),16(15)-dioldilactanone **(183)** only compound (**182)** showed significant antimicrobial activity on *Escherichia coli* at a concentration of 0.4 mg/mL as indicated in tables 4.40-4.42.

Table 4. 39: Inhibition zone (mm) of crude extracts at a concentration of 1 mg/mL

	Microoganism					
Sample	*BC*	*SA*	*EC*	*ST*	*CA*	Control
LM (Laikipia Leaves) Hex extract	16	16	9	17	11	-
LM (Mau Narok Leaves) Hex extract	9	11	-	6	10	-
LM (Laikipia Leaves) DCM extract	12	20	12	20	11	-
LM (Mau Narok Leaves) DCM extract	12	17	15	12	10	-
LM (Laikipia Leaves) EtOAc extract	9	14	12	12	10	-
LM (Mau Narok Leaves) EtOAc extract	-	17	16	11	9	-
LM (Laikipia Leaves) MeOH extract	I0	11	11	15	11	-
LM (Mau Narok Leaves) MeOH extract	6	10	10	15	10	-
LM (Laikipia Root bark) Hex extract	11	12	14	20	15	-
LM (Mau Narok Root bark) Hex extract	9	20	12	14	-	-
LM (Laikipia Root bark) DCM extract	9	15	14	10	-	-
LM (Mau Narok Root bark) DCM extract	10	12	9	11	-	-
LM (Laikipia Root bark) EtOAc extract	9	17	10	13	8	-
LM (Mau Narok Root bark) EtOAc extract	9	20	13	11	-	-
LM (Laikipia Root bark) MeOH extract	10	12	20	18	10	-
LM (Mau Narok Root bark) MeOH extract	9	16	10	16	-	-

Table 4. 40: Inhibition zone of compound Siderin **(181)** at different concentrations

	Concentrations							
Microoganism	0.5 mg/mL	1.0 mg/mL	1.5 mg/mL	2.0 mg/mL	2.5 mg/mL	3.0 mg/mL	3.5 mg/mL	4.0 mg/mL
BC	-	-	-	-	-	-	-	-
SA	-	-	-	-	-	-	-	-
EC	-	-	-	-	-	-	-	-
ST	-	-	-	-	-	-	-	-
CA	-	-	-	-	-	-	-	-

Table 4. 41: Inhibition zone of compound **182** (20-hydroxylucidenicacid D2) at different concentrations

	Concentrations							
Microoganism	0.5 mg/mL	1.0 mg/mL	1.5 mg/mL	2.0 mg/mL	2.5 mg/mL	3.0 mg/mL	3.5 mg/mL	4.0 mg/mL
BC	-	-	-	-	-	-	-	-
SA	-	-	-	-	-	-	-	-
EC	-	-	-	-	6	6	7	9
ST	-	-	-	-	-	-	-	-
CA	-	-	-	-	-	-	-	-

Table 4. 42: Inhibition zone of compound **183** (Labdane) at different concentrations

	Concentrations							
Microoganism	0.5 mg/mL	1.0 mg/mL	1.5 mg/mL	2.0 mg/mL	2.5 mg/mL	3.0 mg/mL	3.5 mg/mL	4.0 mg/mL
BC	-	-	-	-	-	-	7	8
SA	-	-	-	-	-	-	-	-
EC	-	-	-	-	-	-	-	-
ST	-	-	-	-	-	-	-	-
CA	-	-	-	-	-	-	-	-

Table 4. 43 Inhibition Zone Diameters (mm) of crude extracts at different concentrstions

	Bacillus cereus					
	Concentrations					Control
Samlpe	0.10	0.20	0.30	0.40	0.50	

	mg/mL	mg/mL	mg/mL	mg/mL	mg/mL	
LM (Laikipia Leaves) Hex extract	6	7	8	9	10	-
LM (Mau Narok Leaves) Hex extract	-	-	6	7	8	-
LM (Laikipia Leaves) DCM extract	6	8	10	10	11	-
LM (Mau Narok Leaves) DCM extract	6	7	8	10	11	-
LM (Laikipia Leaves) EtOAc extract	-	-	-	-	-	-
LM (Mau Narok Leaves) EtOAc extract	-	-	-	-	-	-
LM (Laikipia Leaves) MeOH extract	-	-	-	-	7	-
LM (Mau Narok Leaves) MeOH extract	-	-	-	-	-	-
LM (Laikipia Root bark) Hex extract	-	6	7	9	10	-
LM (Mau Narok Root bark) Hex extract	-	-	-	7	7	-
LM (Laikipia Root bark) DCM extract	-	6	6	7	7	-
LM (Mau Narok Root bark) DCM extract	-	-	6	7	7	-
LM (Laikipia Root bark) EtOAc extract	-	-	6	7	8	-
LM (Mau Narok Root bark) EtOAc extract	6	6	7	7	8	-
LM (Laikipia Root bark) MeOH extract	-	-	-	-	-	-
LM (Mau Narok Root bark) MeOH extract	-	-	6	7	8	-

Table 4. 44: Inhibition Zone Diameters (mm) of crude extracts at different concentrstions

	Staphylococcus aureus					
	Concentrations					
Sample	0.10 mg/mL	0.20 mg/mL	0.30 mg/mL	0.40 mg/mL	0.50 mg/mL	Control
LM (Laikipia Leaves) Hex extract	-	6	7	8	10	-
LM (Mau Narok Leaves) Hex extract	-	6	6	7	8	-
LM (Laikipia Leaves) DCM extract	6	7	8	9	11	-
LM (Mau Narok Leaves) DCM extract	-	6	7	8	9	-
LM (Laikipia Leaves) EtOAc extract	-	-	-	6	7	-
LM (Mau Narok Leaves) EtOAc extract	6	7	8	8	9	-
LM (Laikipia Leaves) MeOH extract	-	-	-	-	-	-
LM (Mau Narok Leaves) MeOH extract	-	-	-	7	7	-
LM (Laikipia Root bark) Hex extract	6	7	7	8	11	-
LM (Mau Narok Root bark) Hex extract	-	-	-	7	7	-
LM (Laikipia Root bark) DCM extract	-	-	6	7	9	-
LM (Mau Narok Root bark) DCM extract	6	8	9	10	10	-
LM (Laikipia Root bark) EtOAc extract	-	-	-	-	7	-
LM (Mau Narok Root bark) EtOAc extract	-	-	-	7	9	-

LM (Laikipia Root bark) MeOH extract	-	-	6	6	7	-
LM (Mau Narok Root bark) MeOH extract	-	-	6	7	8	-

Table 4. 45: Inhibition Zone Diameters (mm) of crude extracts at different concentrstions

Sample	*Escherichia coli* Concentrations					
	0.10 mg/mL	0.20 mg/mL	0.30 mg/mL	0.40 mg/mL	0.50 mg/mL	Control
LM (Laikipia Leaves) Hex extract	-	6	6	7	8	-
LM (Mau Narok Leaves) Hex extract	-	-	-	-	-	-
LM (Laikipia Leaves) DCM extract	-	6	7	8	10	-
LM (Mau Narok Leaves) DCM extract	6	7	8	10	12	-
LM (Laikipia Leaves) EtOAc extract	6	7	8	9	10	-
LM (Mau Narok Leaves) EtOAc extract	6	6	9	12	13	-
LM (Laikipia Leaves) MeOH extract	6	7	7	9	10	-
LM (Mau Narok Leaves) MeOH extract	-	-	6	6	7	-
LM (Laikipia Root bark) Hex extract	-	-	7	9	12	-
LM (Mau Narok Root bark) Hex extract	7	8	8	9	10	-
LM (Laikipia Root bark) DCM extract	-	6	7	8	9	-
LM (Mau Narok Root bark) DCM extract	-	-	-	-	-	-
LM (Laikipia Root bark) EtOAc extract	-	-	6	6	7	-
LM (Mau Narok Root bark) EtOAc extract	7	8	8	9	10	-
LM (Laikipia Root bark) MeOH extract	-	6	7	8	10	-
LM (Mau Narok Root bark) MeOH extract	-	-	-	-	-	-

Table 4. 46: Inhibition Zone Diameters (mm) of crude extracts at different concentrstions

	Salmonella typhimurium					
	Concentrations					
Sample	0.10 mg/mL)	0.20 mg/mL)	0.30 mg/mL)	0.40 mg/mL)	0.50 mg/mL)	Control
LM (Laikipia Leaves) Hex extract	7	9	10	10	11	-
LM (Mau Narok Leaves) Hex extract	-	-	-	-	-	-
LM (Laikipia Leaves) DCM extract	-	-	7	8	9	-
LM (Mau Narok Leaves) DCM extract	-	6	8	8	9	-
LM (Laikipia Leaves) EtOAc extract	-	-	7	9	10	-
LM (Mau Narok Leaves) EtOAc extract	6	7	9	9	10	-
LM (Laikipia Leaves) MeOH extract	-	7	8	9	10	-
LM (Mau Narok Leaves) MeOH extract	6	7	7	8	9	-
LM (Laikipia Root bark) Hex extract	-	-	6	10	13	-
LM (Mau Narok Root bark) Hex extract	-	7	8	10	10	-
LM (Laikipia Root bark) DCM extract	-	6	6	7	8	-
LM (Mau Narok Root bark) DCM extract	-	6	6	7	7	-
LM (Laikipia Root bark) EtOAc extract	-	-	-	7	8	-
LM (Mau Narok Root bark) EtOAc extract	6	7	7	8	9	-

LM (Laikipia Root bark) MeOH extract	-	7	8	10	11	-
LM (Mau Narok Root bark) MeOH extract	8	8	9	9	10	-

Table 4. 47: Inhibition Zone Diameters (mm) of crude extracts at different concentrstions

	Cadidas albicans					
Sample	Concentrations					
	0.10 mg/mL)	0.20 mg/mL)	0.30 mg/mL)	0.40 mg/mL)	0.50 mg/mL)	Control
LM (Laikipia Leaves) Hex extract	-	-	-	-	-	-
LM (Mau Narok Leaves) Hex extract	-	-	-	-	-	-
LM (Laikipia Leaves) DCM extract	-	-	-	-	-	-
LM (Mau Narok Leaves) DCM extract	-	-	-	-	-	-
LM (Laikipia Leaves) EtOAc extract	-	-	-	-	-	-
LM (Mau Narok Leaves) EtOAc extract	-	-	-	-	-	-
LM (Laikipia Leaves) MeOH extract	-	-	-	-	-	-
LM (Mau Narok Leaves) MeOH extract	-	-	-	-	-	-
LM (Laikipia Root bark) Hex extract	6	8	11	12	12	-
LM (Mau Narok Root bark) Hex extract	-	-	-	-	-	-
LM (Laikipia Root bark) DCM extract	-	-	-	-	-	-
LM (Mau Narok Root	-	-	-	-	-	-

bark) DCM extract						
LM (Laikipia Root bark) EtOAc extract	-	-	-	-	-	-
LM (Mau Narok Root bark) EtOAc extract	-	-	-	-	-	-
LM (Laikipia Root bark) MeOH extract	-	-	-	-	-	-
LM (Mau Narok Root bark) MeOH extract	-	-	-	-	-	-

All the crude extracts had an MIC of < 0.1 mg/mL to > 0.5 mg/mL on all test microorganism despite been sampled from different ecological zone as indicated in Table 4.46. *Candida albicans* had the highest MIC of > 0.5 while *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Salmonella typhimurium* showed almost the same MIC of < 0.1 mg/mL to > 0.5 mg/mL though with some very small differences. Compounds **181** (Siderin) and **183** (Labdane) had an MIC of > 0.16 mg/mL on all microorganisms while compound **182** (20-hydroxylucidenicacid D2) had an MIC of 0.10 mg/mL as indicated in Table 4.48. Siderin is known to have a variety of bioactivities including anticoagulant, estrogenic, dermal photosensitizing, anti-microbial, vasodilator, molluscacidal, antihelmintic, sedative and hypnotic, analgesic and hypothermic activity (Divakar and Parminder, 2017). Lanostene-tetracyclic triterpenes possess anti-tumor, anti-inflammation, antioxidant, antimicrobial and blood fat reducing effects (Qing *et al.,* 2014). Also a variety of biological activities have been encountered in labdane diterpenes such as antibacterial, antifungal, antiprotozoal, enzyme inducing, anti-inflammatory activities and modulation of immune cell functions. They also exhibits significant cytotoxic and cytostatic effects against leukemic cell lines of human origin (Costas and Konstantinos, 2001). This is and indication that *Leonotis mollissima* have compounds that can be developed to treat infectious microbial diseases.

Table 4. 48: MIC of crude extracts and pure compounds on test microorganism

Sample	MIC (mg/mL)				
	BC	*SA*	*EC*	*ST*	*CA*
LM (Laikipia Leaves) Hex extract	< 0.1	0.1	0.1	< 0.1	> 0.5
LM (Mau Narok Leaves) Hex extract	0.2	0.1	> 0.5	> 0.5	>0.5
LM (Laikipia Leaves) DCM extract	< 0.1	< 0.1	0.1	0.2	>0.5
LM (Mau Narok Leaves) DCM extract	< 0.1	0.1	< 0.1	0.1	>0.5
LM (Laikipia Leaves) EtOAc extract	> 0.5	0.3	< 0.1	0.2	>0.5
LM (Mau Narok Leaves) EtOAc extract	> 0.5	< 0.1	< 0.1	< 0.1	>0.5
LM (Laikipia Leaves) MeOH extract	0.4	> 0.5	< 0.1	0.1	>0.5
LM (Mau Narok Leaves) MeOH extract	> 0.5	0.3	0.2	< 0.1	>0.5
LM (Laikipia Root bark) Hex extract	0.1	< 0.1	0.2	0.2	< 0.1
LM (Mau Narok Root bark) Hex extract	0.3	0.3	< 0.1	0.1	> 0.5
LM (Laikipia Root bark) DCM extract	0.1	0.2	0.1	0.1	> 0.5
LM (Mau Narok Root bark) DCM extract	0.2	< 0.1	> 0.5	0.1	> 0.5
LM (Laikipia Root bark) EtOAc extract	0.2	0.4	0.2	0.1	> 0.5
LM (Mau Narok Root bark) EtOAc extract	< 0.1	0.3	< 0.1	< 0.1	> 0.5
LM (Laikipia Root bark) MeOH extract	> 0.5	0.2	0.1	0.1	> 0.5
LM (Mau Narok Root bark) MeOH extract	0.2	0.2	> 0.5	< 0.1	> 0.5
Siderin **(181)**	> 0.16	> 0.16	> 0.16	> 0.16	> 0.16
20-hydroxylucidenicacid D2 **(182)**	> 0.16	> 0.16	0.08	> 0.16	> 0.16
Labdane **(183)**	> 0.16	> 0.16	> 0.16	> 0.16	> 0.16

The IC_{50} for Laikipia (Figure 4.25) and Mau Narok (Figure 4.26) dichloromethane leaves crude extracts (Table 4.49) was 4 and 12 times less that Amoxil® antibiotic on *Bacillus cereus* (Figure 4.39). Also the IC_{50} for Mau Narok ethyl acetate leaves crude extract (Figure 4.36, Table 4.49) was 16 times less that of Amoxil® antibiotic on *Salmonella typhimurium* (Figure 4.41, Table 4.49). The IC_{50} for Doxycycline® antibiotic on *Escherichia coli* (Figure 4.15, Table 4.49) on comparison with both dichloromethane and ethyl acetate leaves crude extracts (Figure 4.32-4.49) was found to be 5,000, 4,000 and 700 times less respectively (Table 4.49). These concentrations were too low compared to those of the two antibiotics. Compound **182** (20-hydroxylucidenicacid D2) had an IC_{50} of 0.141 mg/mL (Figure 4.38, Table 4.49) on *EC* which was 11 times less that of Amoxil® antibiotic (Figure 4.40) and

1,600 times that of Doxycycline® antibiotic. This is an indication that compound *Leonotis mollissima* has compounds that can be used to treat diseases caused by *Escherichia coli*.

Table 4. 49: IC_{50} (mg/mL) of crude extracts and pure compounds on test microorganism

	Microoganism				
Sample	*BC*	*SA*	*EC*	*ST*	*CA*
LM (Laikipia Leaves) DCM extract	0.210	52.602	52.602		
LM (Mau Narok Leaves) DCM extract	0.406	15.101	2.917		
LM (Laikipia Leaves) EtOAc extract			49.889		
LM (Mau Narok Leaves) EtOAc extract			0.314	0.242	
LM (Mau Narok Root bark) DCM extract		0.191			
LM (Laikipia Root bark) EtOAc extract					
LM (Mau Narok Root bark) EtOAc extract	12.388	6,025,596		53.088	
Siderin **(181)**					
(20-hydroxylucidenicacid D2 **(182)**				0.141	
Labdane **(183)**					
Amoxil® antibiotic	0.775	1.178	1.486	3.811	1.776
Doxycycline® antibiotic	0.044	1.200	233.884	1.276	0.632

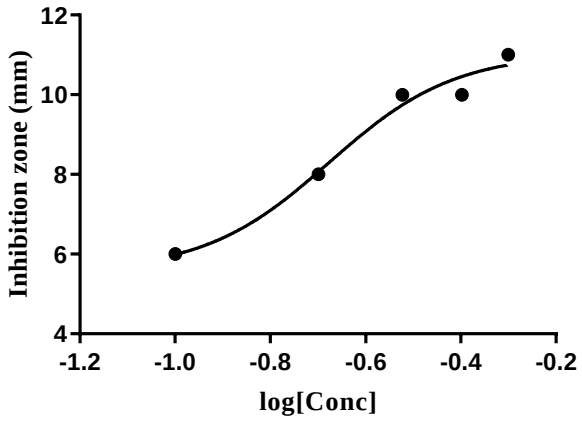

Figure 4. 25: *LM* Laikipia leaves DCM crude extract IC_{50} on *BC* = 0.210 mg/mL

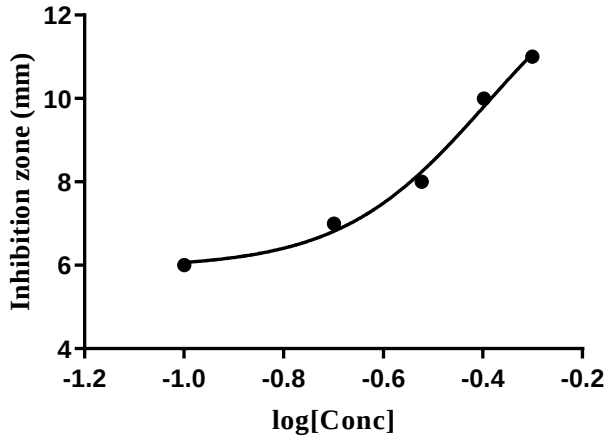

Figure 4. 26: *LM* Mau Narok leaves DCM crude extract IC_{50} on *BC* = 0.406 mg/mL

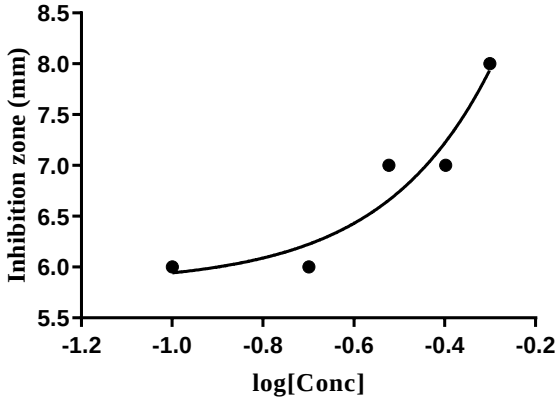

Figure 4. 27: *LM* Mau Narok Root bark EtOAc crude extract IC_{50} on *BC* = 12.388 mg/mL

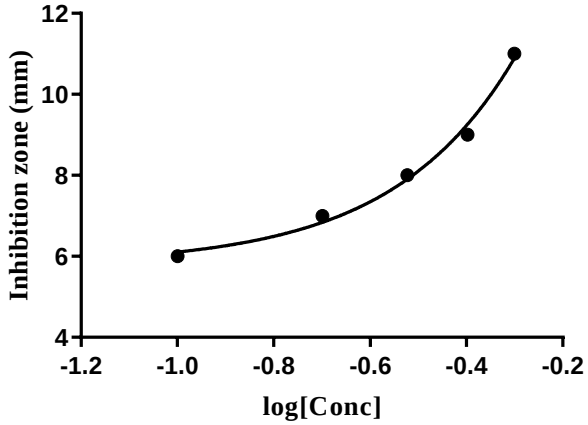

Figure 4. 28: *LM* Laikipia leaves DCM crude extract IC_{50} on *SA* = 52.602 mg/mL

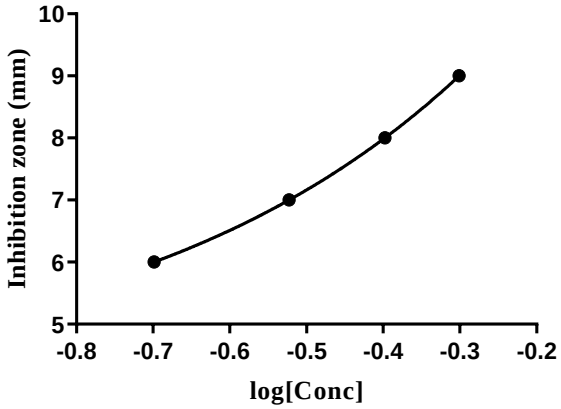

Figure 4. 29: *LM* Mau Narok leaves DCM crude extract IC_{50} on *SA* = 15.101 mg/mL

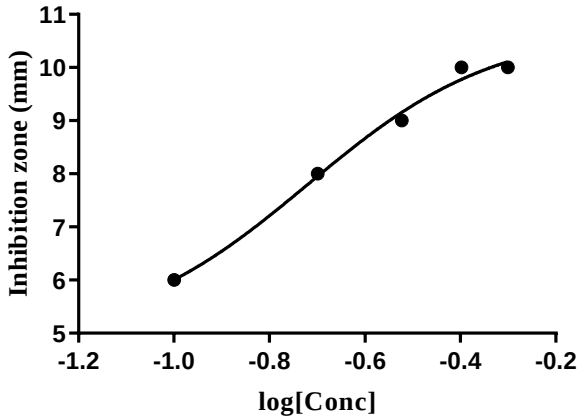

Figure 4. 30: *LM* Mau Narok Root bark DCM crude extract IC_{50} on *SA* = 0.191 mg/mL

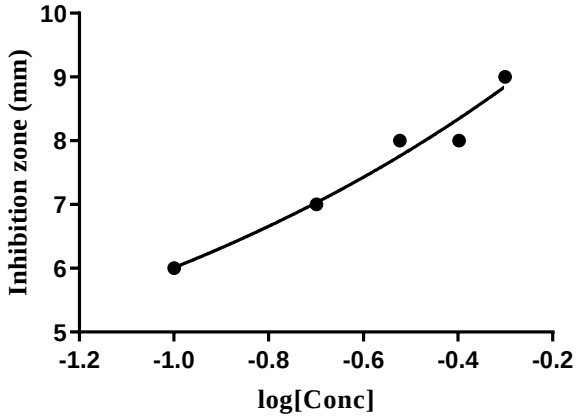

Figure 4. 31: *LM* Mau Narok Root bark EtOAc crude extract IC_{50} on *SA* = 1,025,596 mg/mL

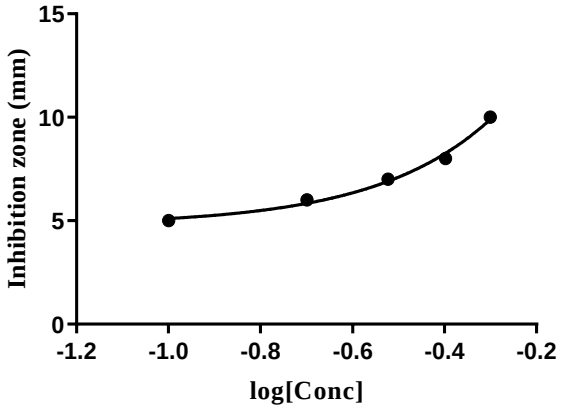

Figure 4. 32: *LM* Laikipia leaves DCM crude extract IC_{50} on *EC* 52.602 mg/mL

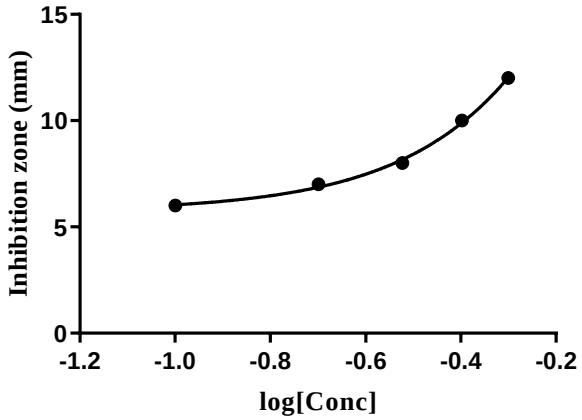

Figure 4. 33: *LM* Mau Narok leaves DCM crude extract IC_{50} on *EC* = 2.917 mg/mL

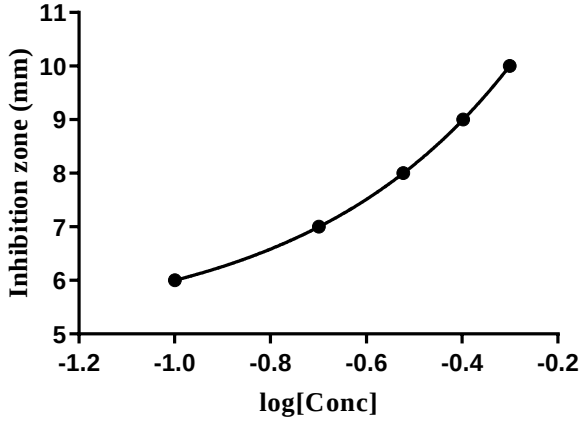

Figure 4. 34: *LM* Laikipia leaves EtOAc crude extract IC50 on *EC* = 49.889 mg/mL

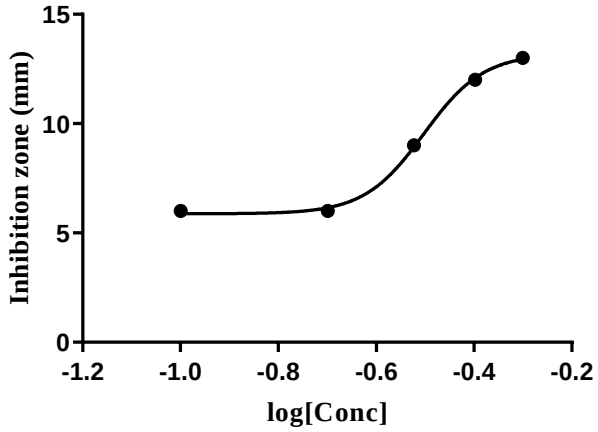

Figure 4. 35: *LM* Mau Narok leaves EtOAc crude extract IC50 on *EC* = 0.314 mg/mL

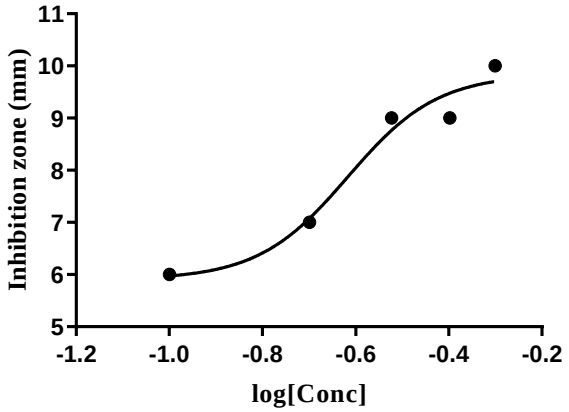

Figure 4. 36: *LM*, Mau Narok Leaves EtOAc crude extract IC50 on *ST* = 0.242 mg/mL

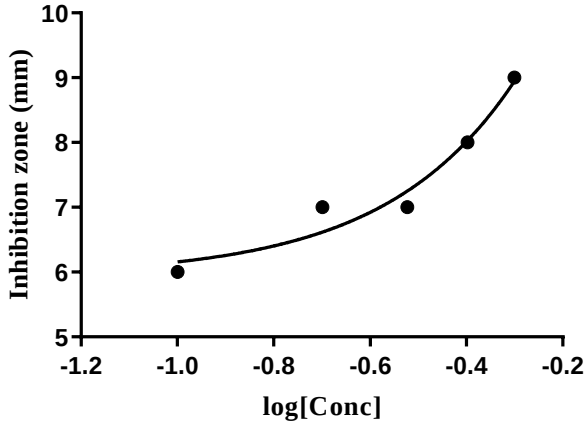

Figure 4. 37: *LM* Mau Narok Root bark EtOAc crude extract IC50 on *ST* = 53.088 mg/mL

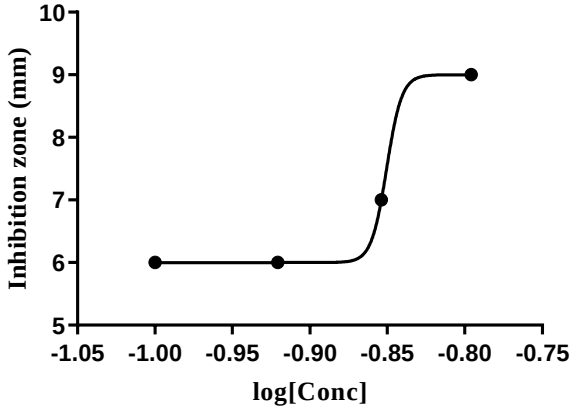

Figure 4. 38: IC_{50} of compound **182** on *EC* = 0.141 mg/mL

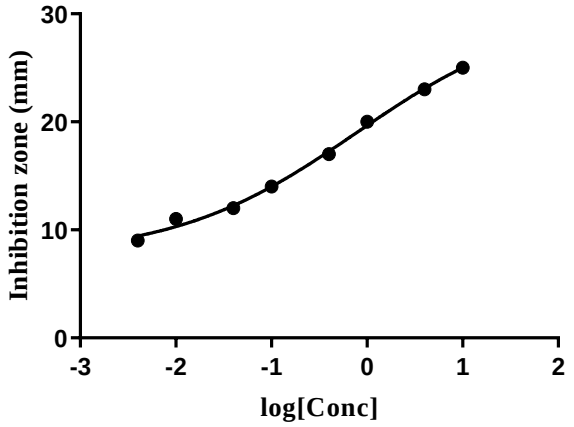

Figure 4. 39: IC_{50} of Amoxil® antibiotic on *BC* = 0.775 mg/mL

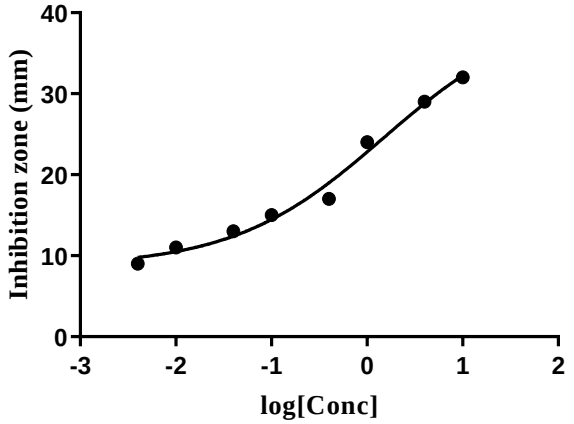

Figure 4. 40: IC_{50} of Amoxil® antibiotic on *EC* = 1.486 mg/mL

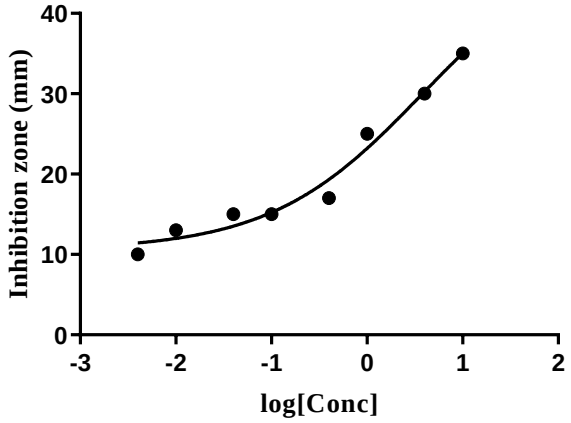

Figure 4. 41: IC_{50} of Amoxil® antibiotic on *ST* = 3.811 mg/Ml

CHAPTER FIVE
CONCLUSION AND RECOMMEDATIONS

5.1 Conclusions

i. All species (*Turraea abysinica, Meyn. tetraphylla* and *Leonoti. mollissima*) crude extracts showed significant antimicrobial activity on all the test microorganism (*Bacillus cereus***,** *Staphylococcus aureus***,** *Escherichia coli, Salmonella typhimurium* and *Candida albicans* at a concentration of 1 mg/ml despite been sampled from different regions of Kenya. Narok *Turraea abyssinica* showed significant antimicrobial activity as compared to the Kirinyaga species at a concentration of 1 mg/mL. Baringo and Tharaka Nthi *Meyna tetraphylla* showed almost the same activity at the same concentrations despite been sampled from different regions of Kenya. Laikipia and Mau Narok *Leonotis mollissima* gave almost the same activity on all the test microorganisms at a concentration of 1 mg/mL. All the crude extracts had lower MIC (Minimum Inhibition Concentration) and IC_{50} (Inhibition Concentration that reduces the effect of microorganisms by 50%) as compared to the Amoxil® and Doxycycline® antibiotics that were used as positive control for comparison. All extraction solvents that were used as negative controls did not show any activity. This is confirmation that the three plants can be used by the Kenyan local people as herbal medicine to treat microbial infectious diseases

ii. From *Turraea abyssinica* dichloromethane stem bark crude extracts, three compounds **176 (***β*-Sitosterol)**, 177 (**Scopoletin) and **178** 2-(1',2'-Dihydroxypropyl)tetradecanoic acid were isolated. Compound **177** showed significant MIC of 0.08 mg/ml on *Bacillus cereus* and 0.10 mg/ml on *Staphylococcus aureus* with an IC_{50} of 0.141 mg/ml on *Bacillus cereus*. Compounds **177** and **178** had an MIC of > 0.16 mg/mL on all microorganism. Their IC_{50} were too low when compared to Amoxil® and Doxycycline® antibiotics that were used as positive control. *Meyna tetraphylla* dichloromethane leaves crude extracts gave four compounds**179 (**Phaeophytin), **180** Enantiomers**, 118 (***α*-Amyrin) and **60** (Stigmasterol). Compound **179** gave significant MIC of 0.08 mg/ml to 0.12 mg/ml on all test microorganism except on *Bacillus cereus*. It also had significant IC_{50} of 0.126 mg/ml and 0.141 mg/ml on *Staphylococcus aureus***,** *Escherichia coli* and *Salmonella typhimurium*. Their IC_{50} were too low when compared to the two antibiotics. From *Leonotis mollissima* dichloromethane leaves crude extract, three compounds **181 (**Siderin)**, 182** (20-

hydroxylucidenicacid D2) and **183** (13R)-19α,13α-epoxylabda-6β(19).16(15)-dioldilactone were isolated. All had an MIC of > 0.16 mg/ml. Compound **182** had significant **IC_{50}** of 0.141 mg/ml on *Salmonella typhimurium*. Their IC_{50} was lower than for Amoxil® and Doxycycline® antibiotics. Methanol was used as negative control for all the compounds isolated from the three plants. Antimicrobial activity of all the compounds isolated from the three plants were lower as compared to the crude extracts. This is a confirmation that the three plants contain compounds that can be isolated and used as drugs to treat various diseases including microbial infectious diseases.

5.2 Recommendations

i. *Turraea abyssinica, Meyna tetraphylla* and *Leonotis mollissima* should be collected from other diiferent regions zones of Kenya and screened for antimicrobial activity for comparison purposes.

ii. More compounds from all parts of *Turraea abyssinica, Meyna tetraphylla* and *Leonotis mollissima* should be isolated and screened for antimicrobial activities.

iii. Since screening was done on only five strains of microorganisms, (*Bacillus cereas, Staphylococuus aureus, Escherichia coli, Salmonella typhimurium*) and *Cadida albicans*, other strains should also be tested.

iv. Since only two antibiotics (Amoxil® and Doxycycline®) were used as positive tests, other antibiotics that are currently in the market should also be used to widenden the research.

v. Availability of Chemical Instruments at Egerton University was the main limitation in this study.

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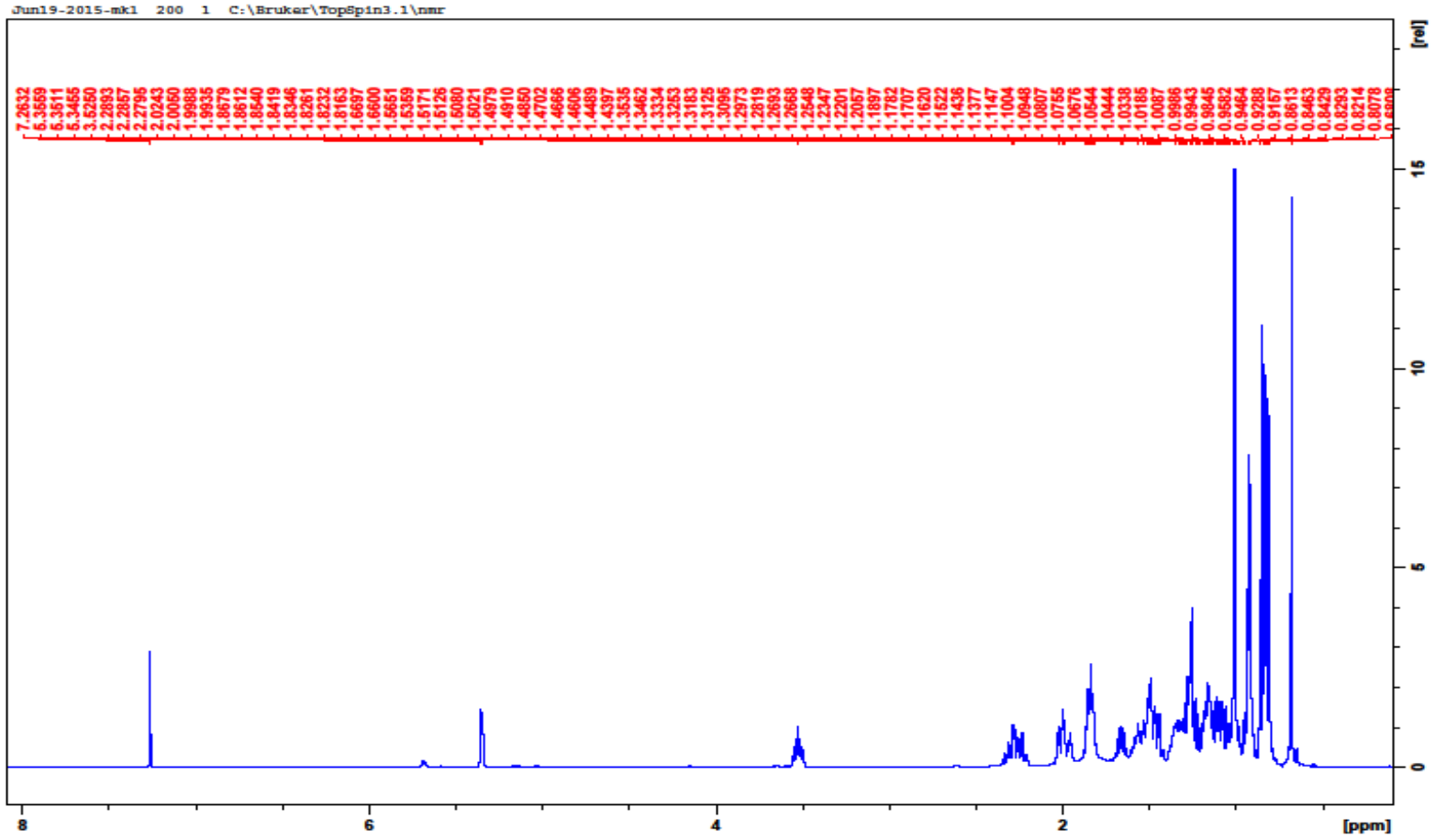
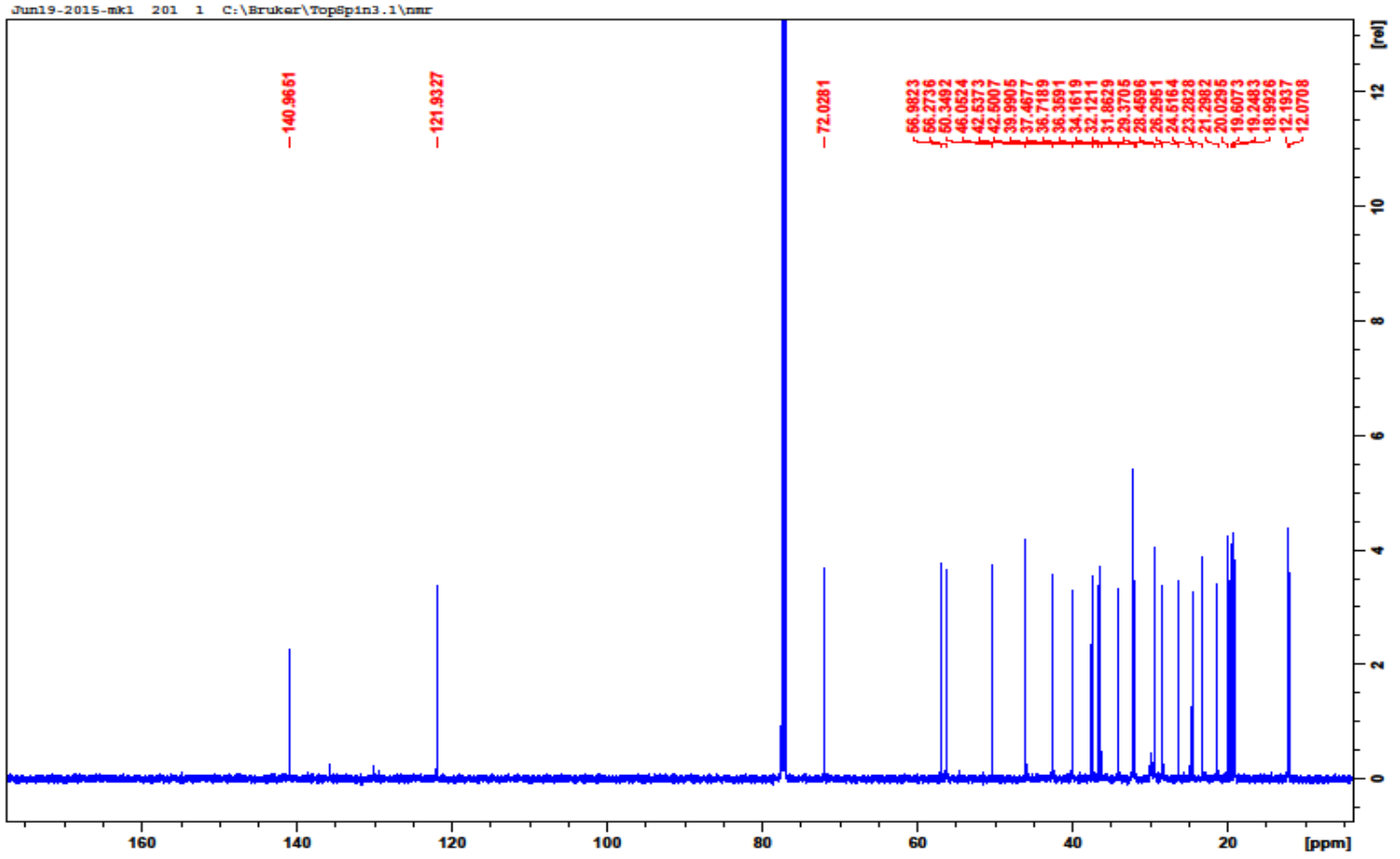
APPENDICES

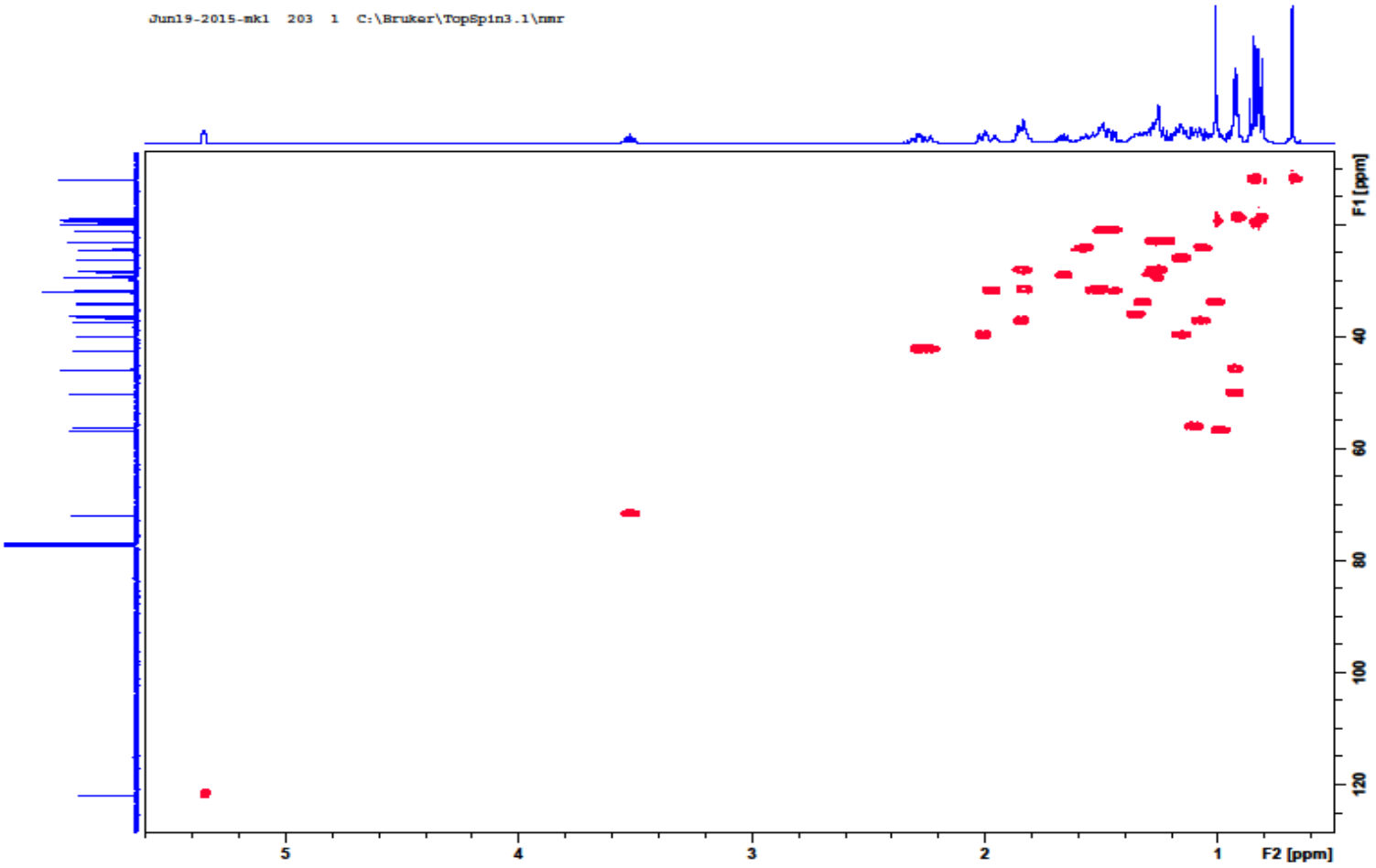
Appendix 1 ^{1}H. Compound **176**



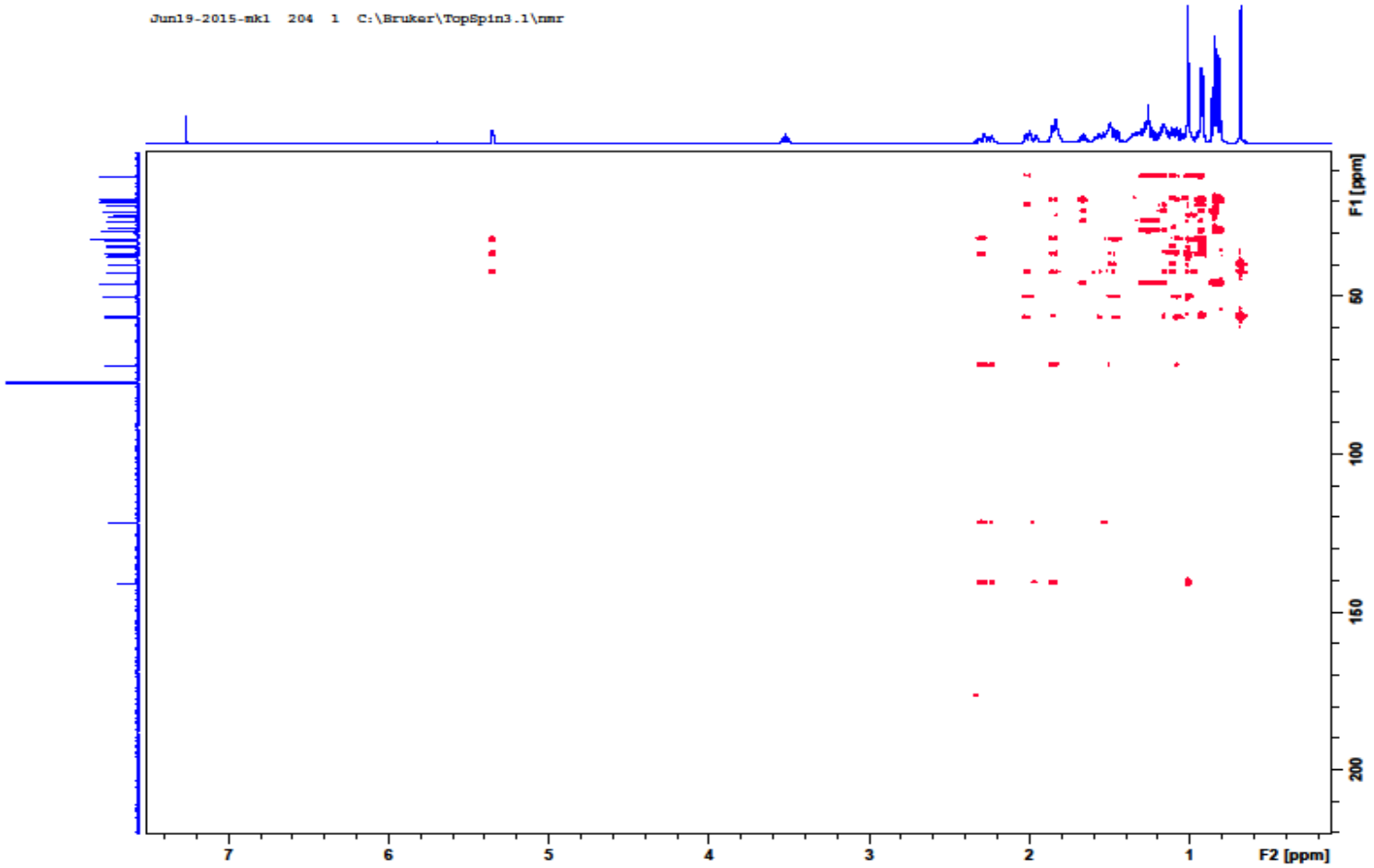
Appendix 2 ^{13}C. Compound **176**



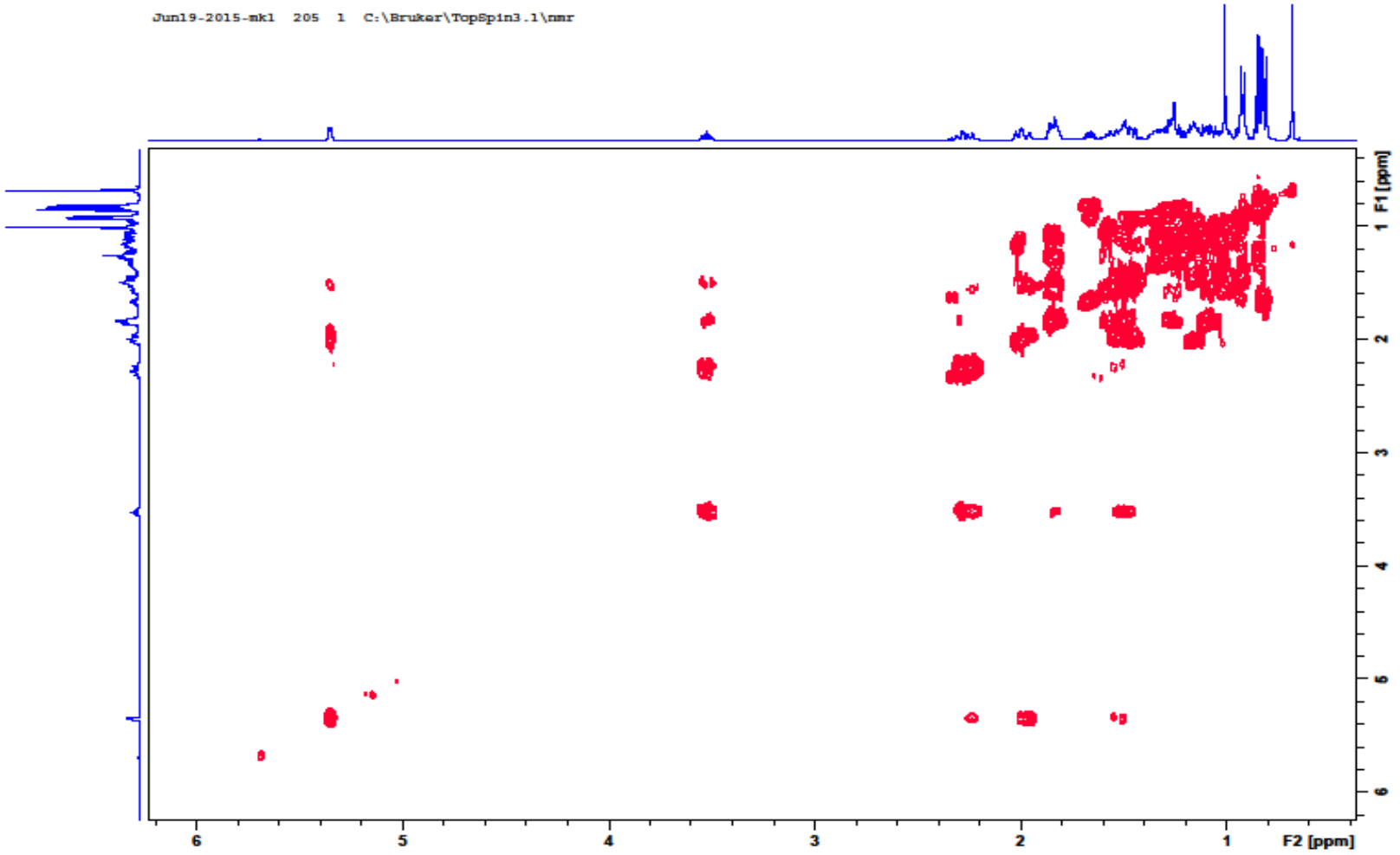
Appendix 3 HSQC. Compound **176**



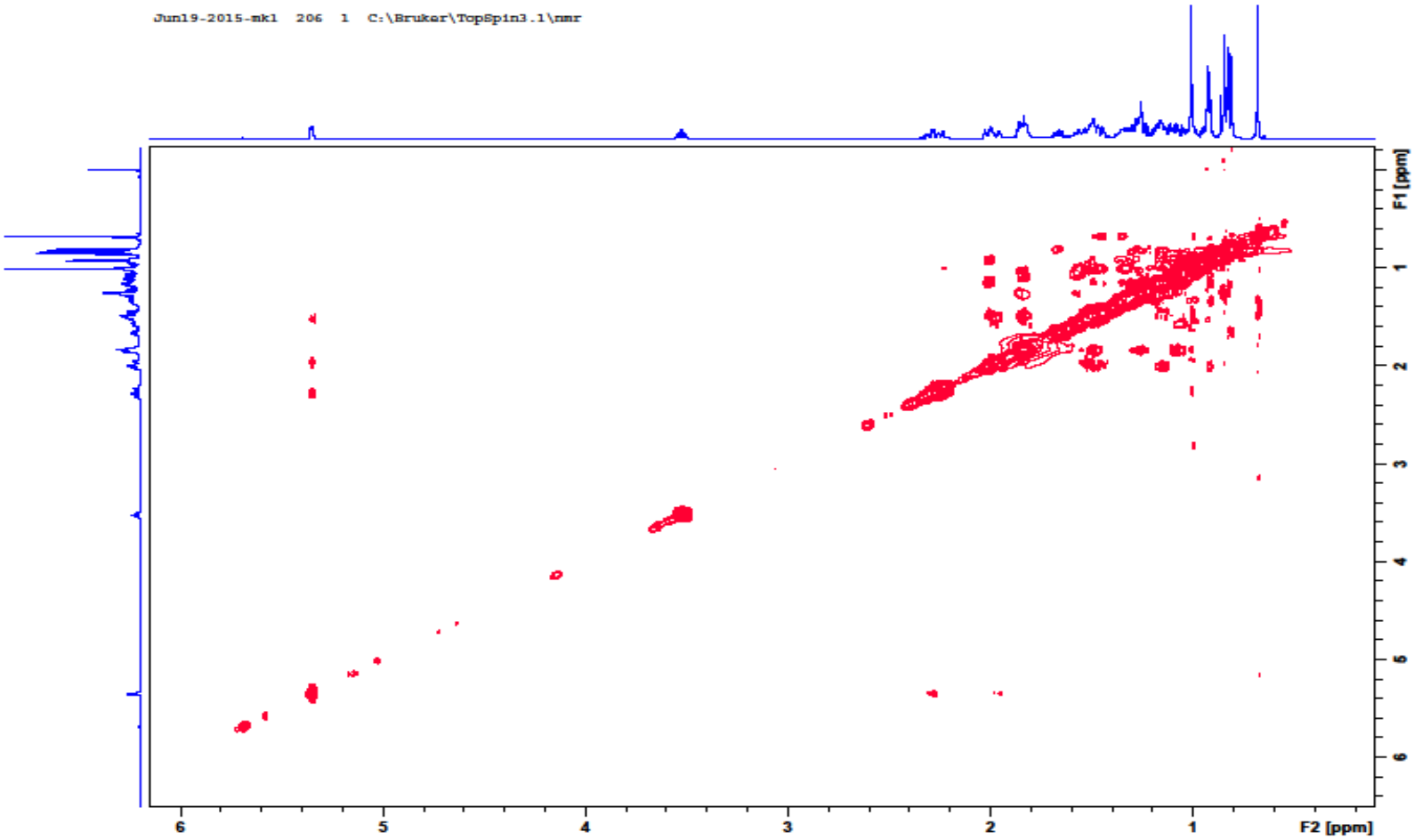
Appendix 4 HMBC. Compound **176**



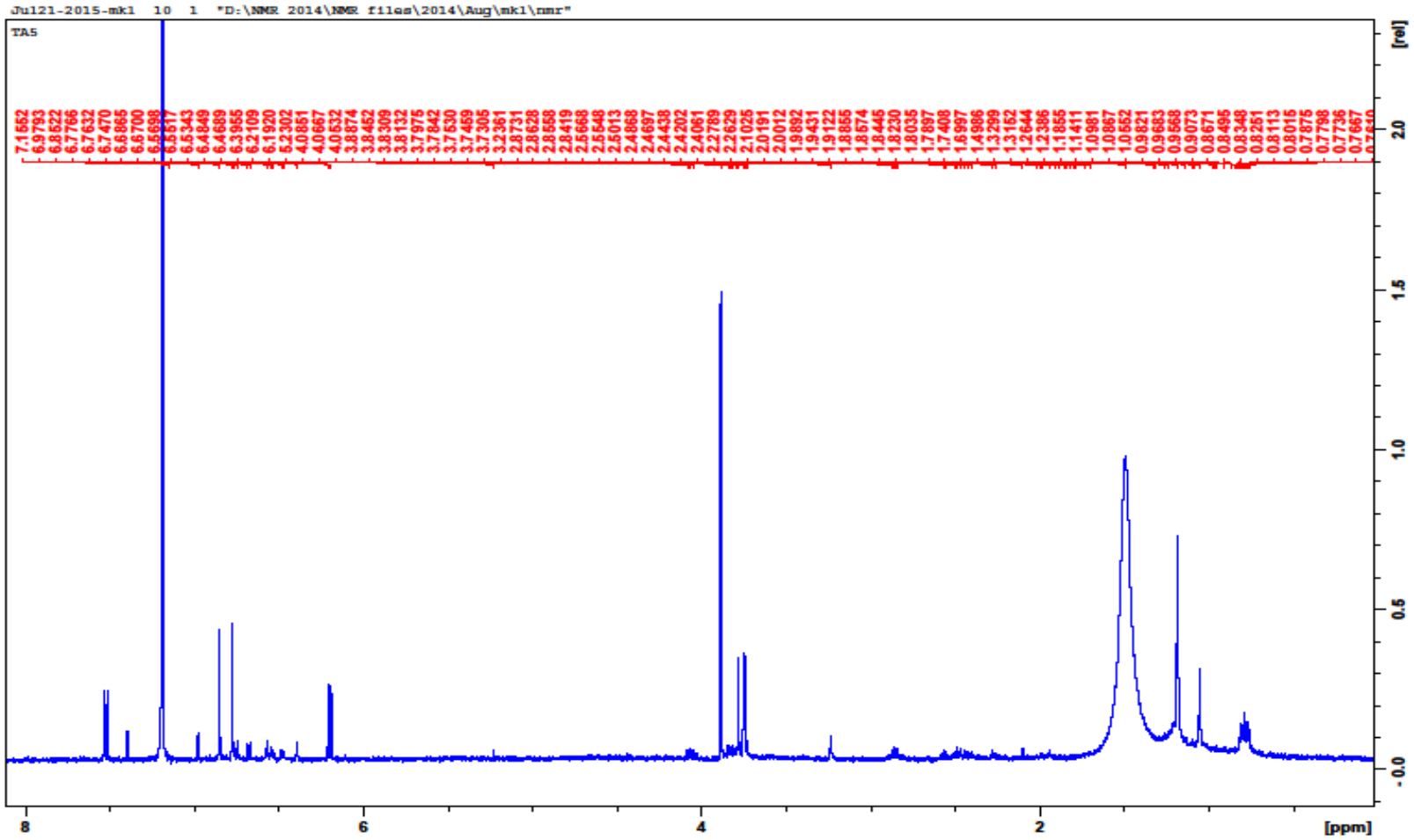
Appendix 5 COSY. Compound176



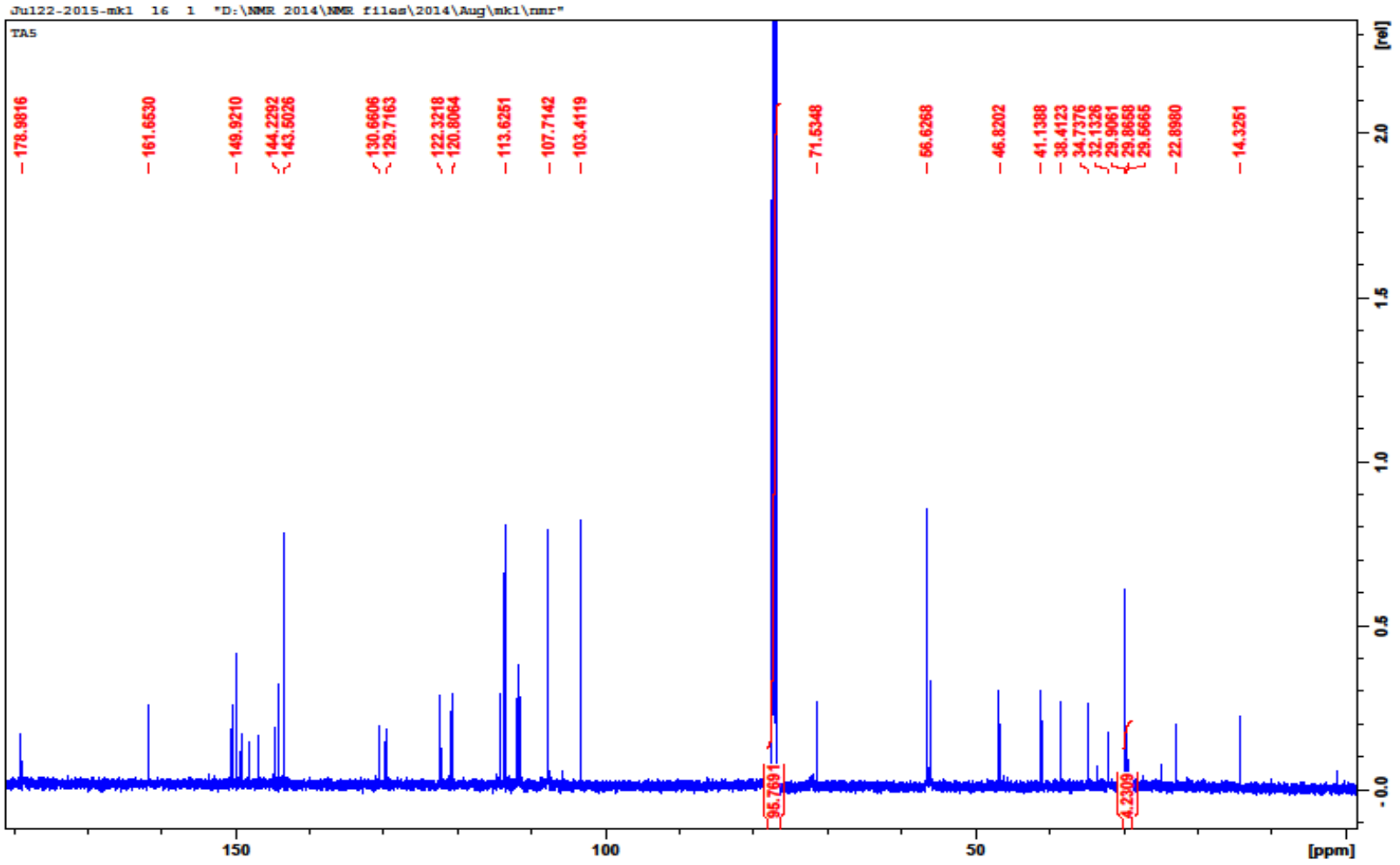
Appendix 6 NOESY. COMPOUND **176**



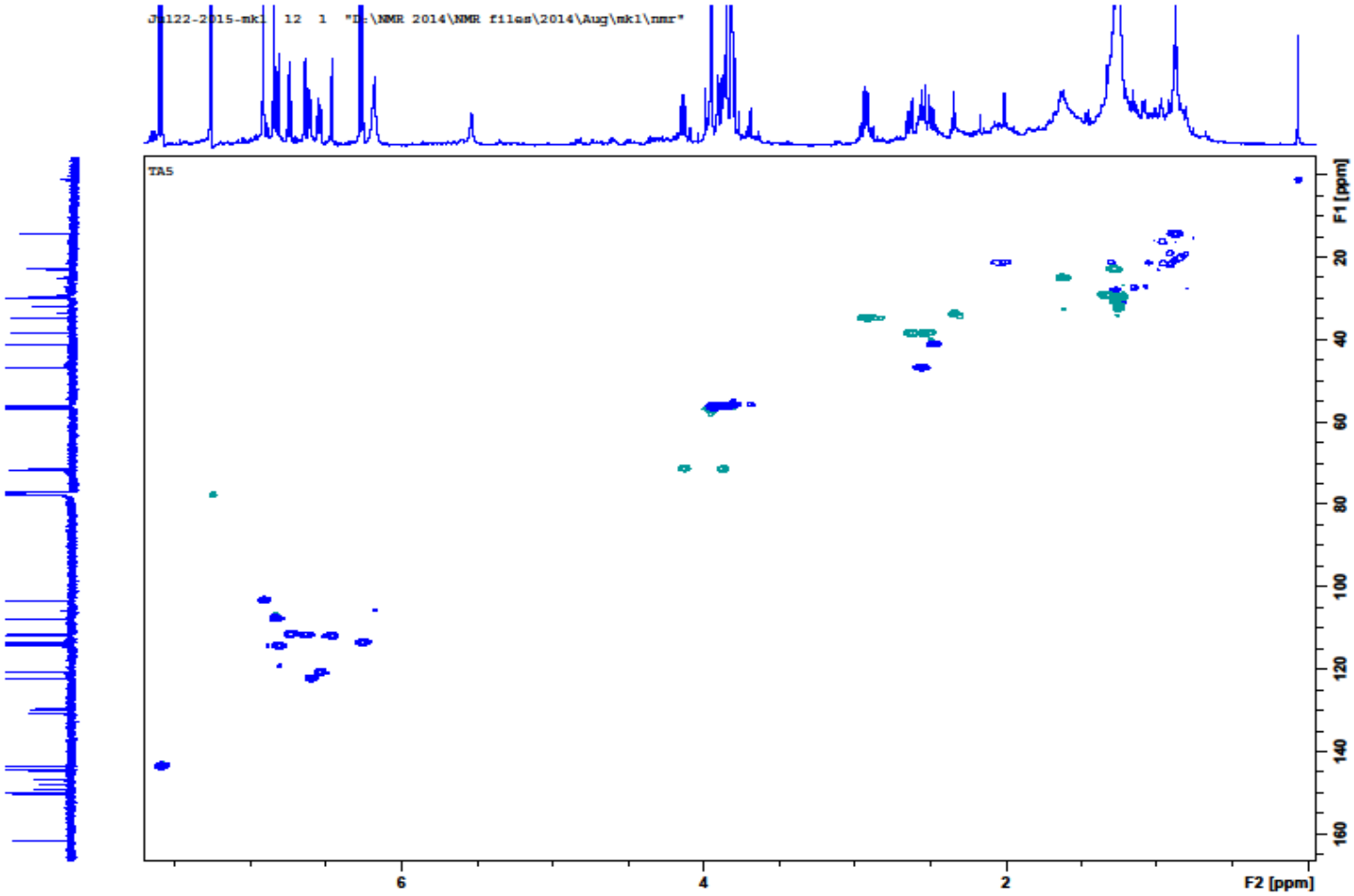
Appendix 7 ^{1}H NMR. Compound**177**



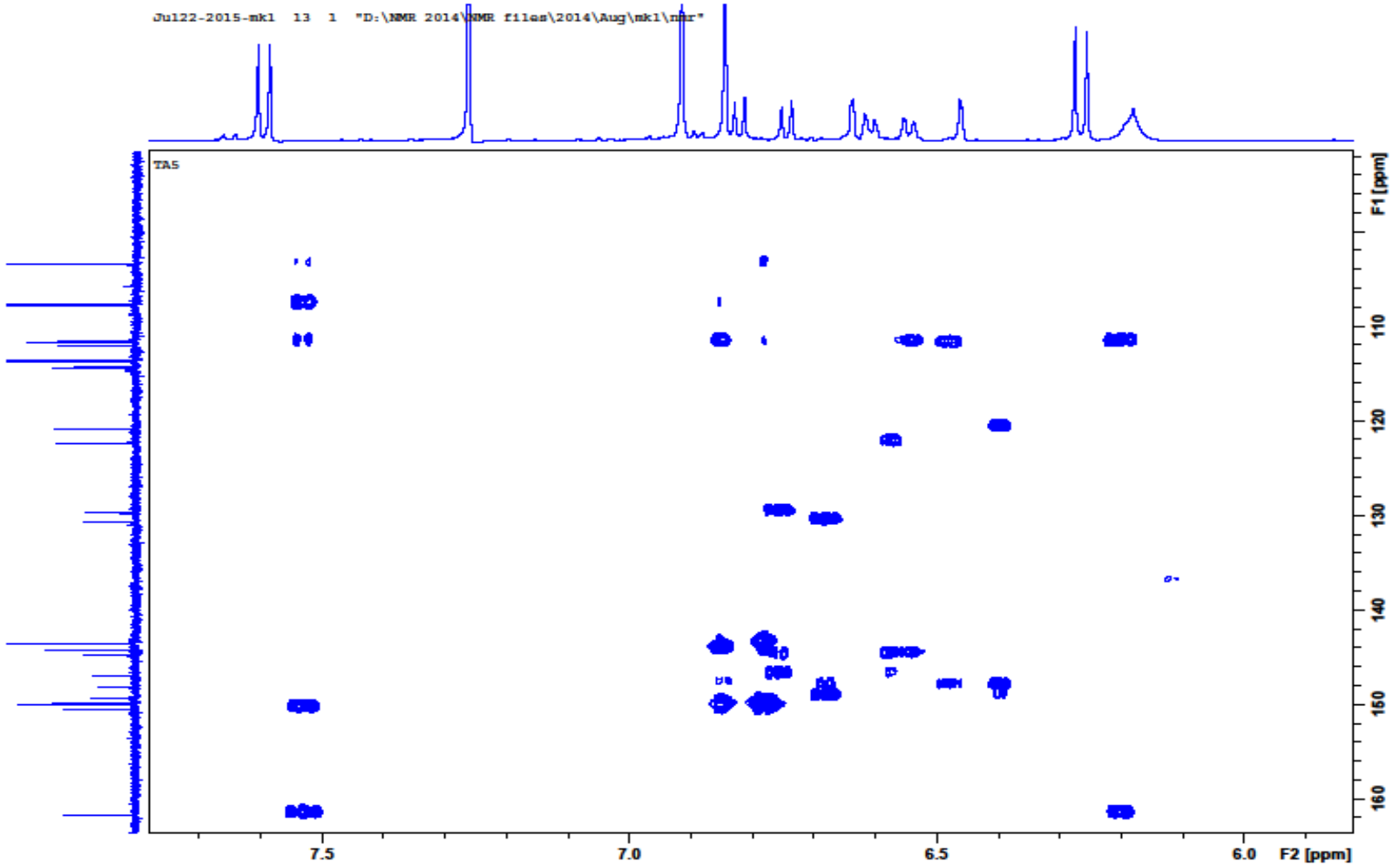
Appendix 8 ^{13}C NMR. Compound**177**



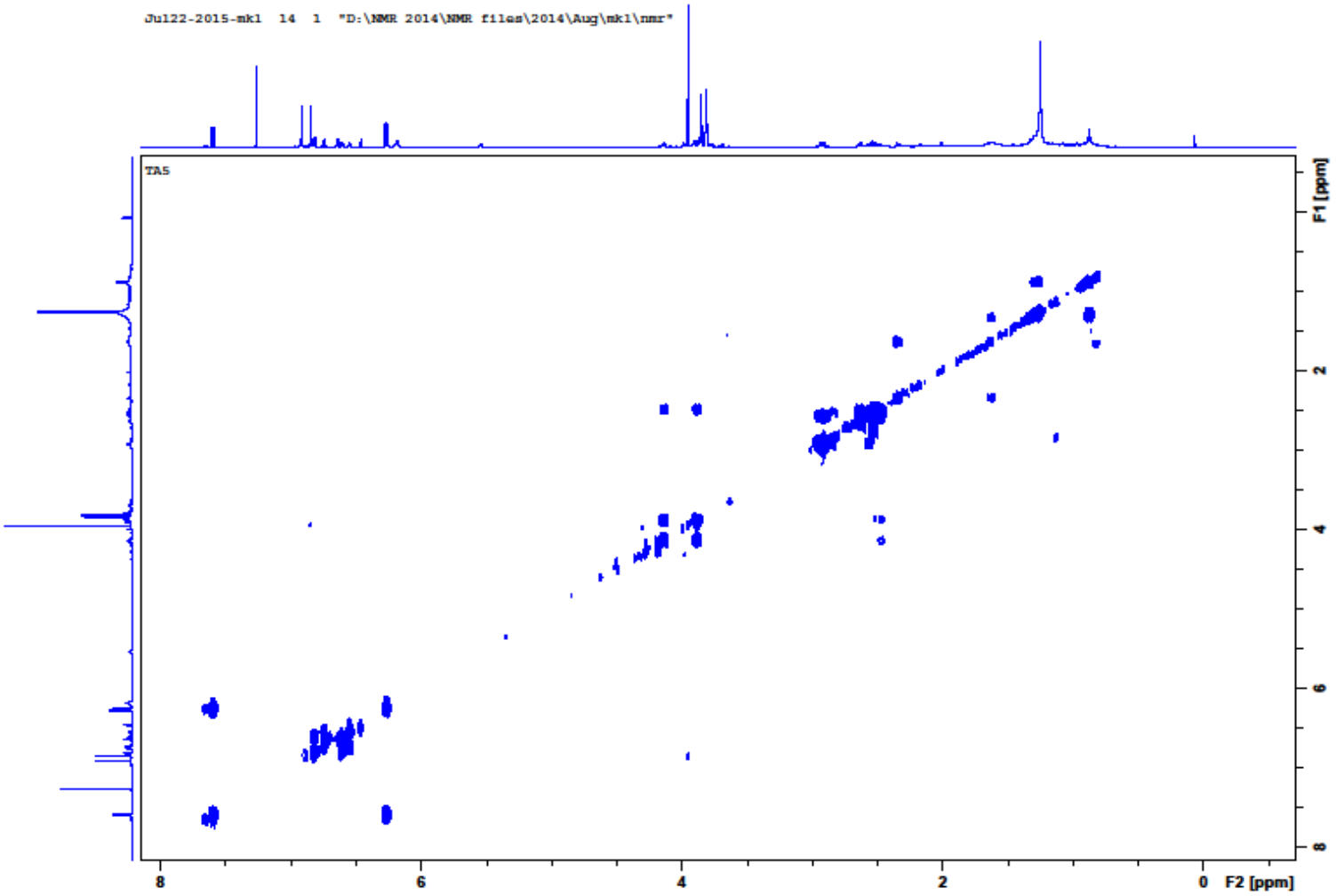
Appendix 9 HSQC Compound**177**



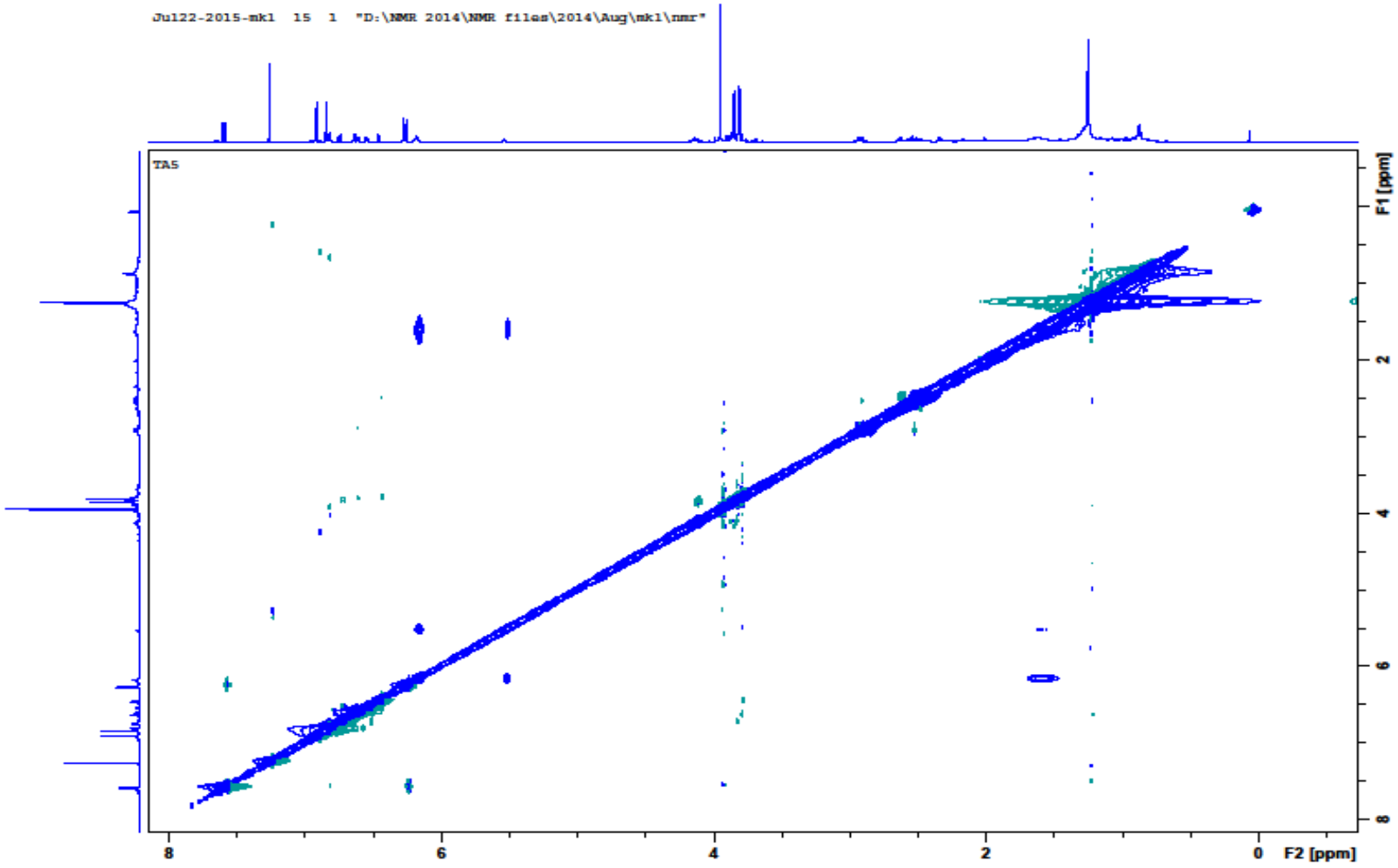
Appendix 10 HMBC. Compound **177**



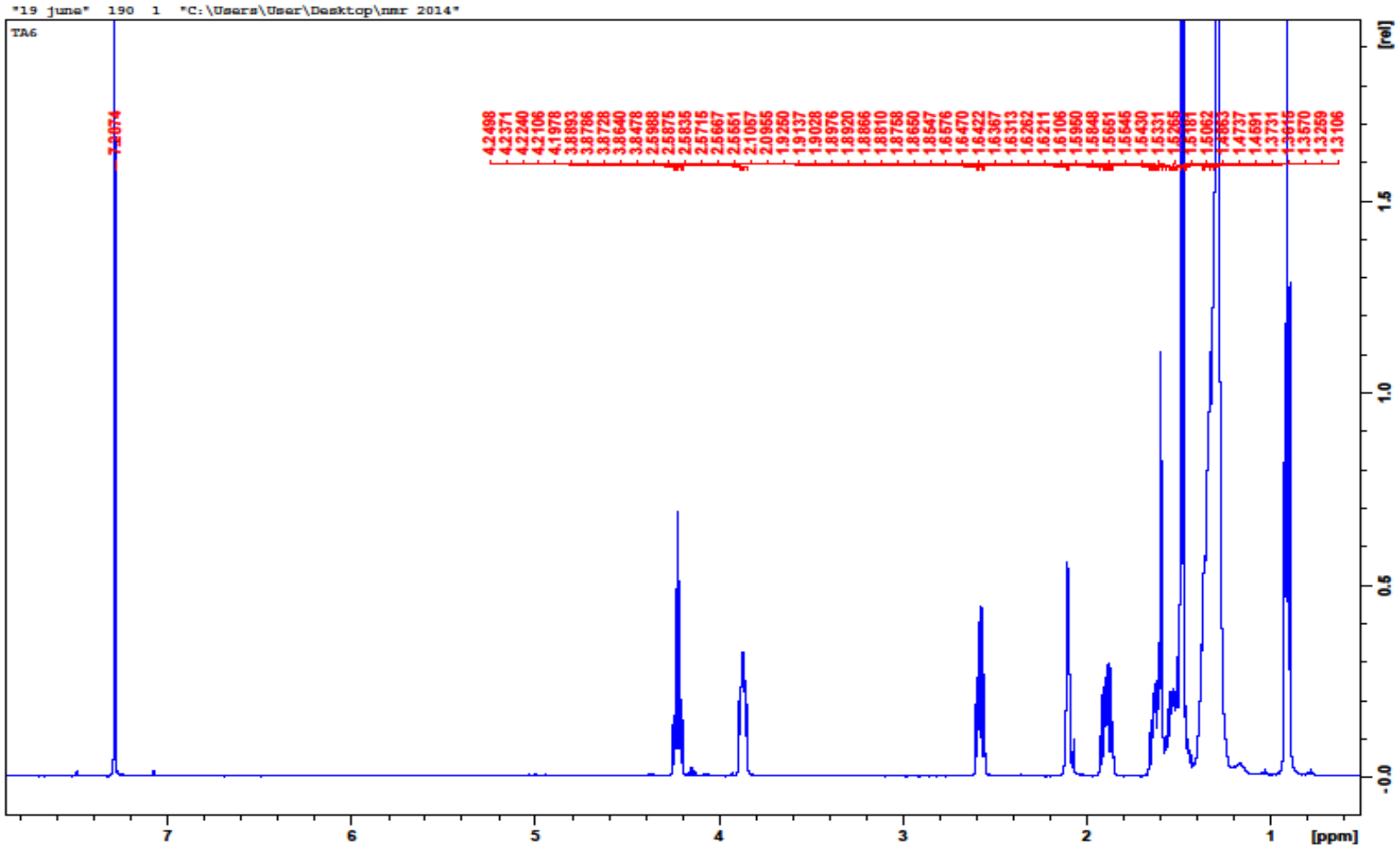
Appendix 11 COSY. Compound **177**



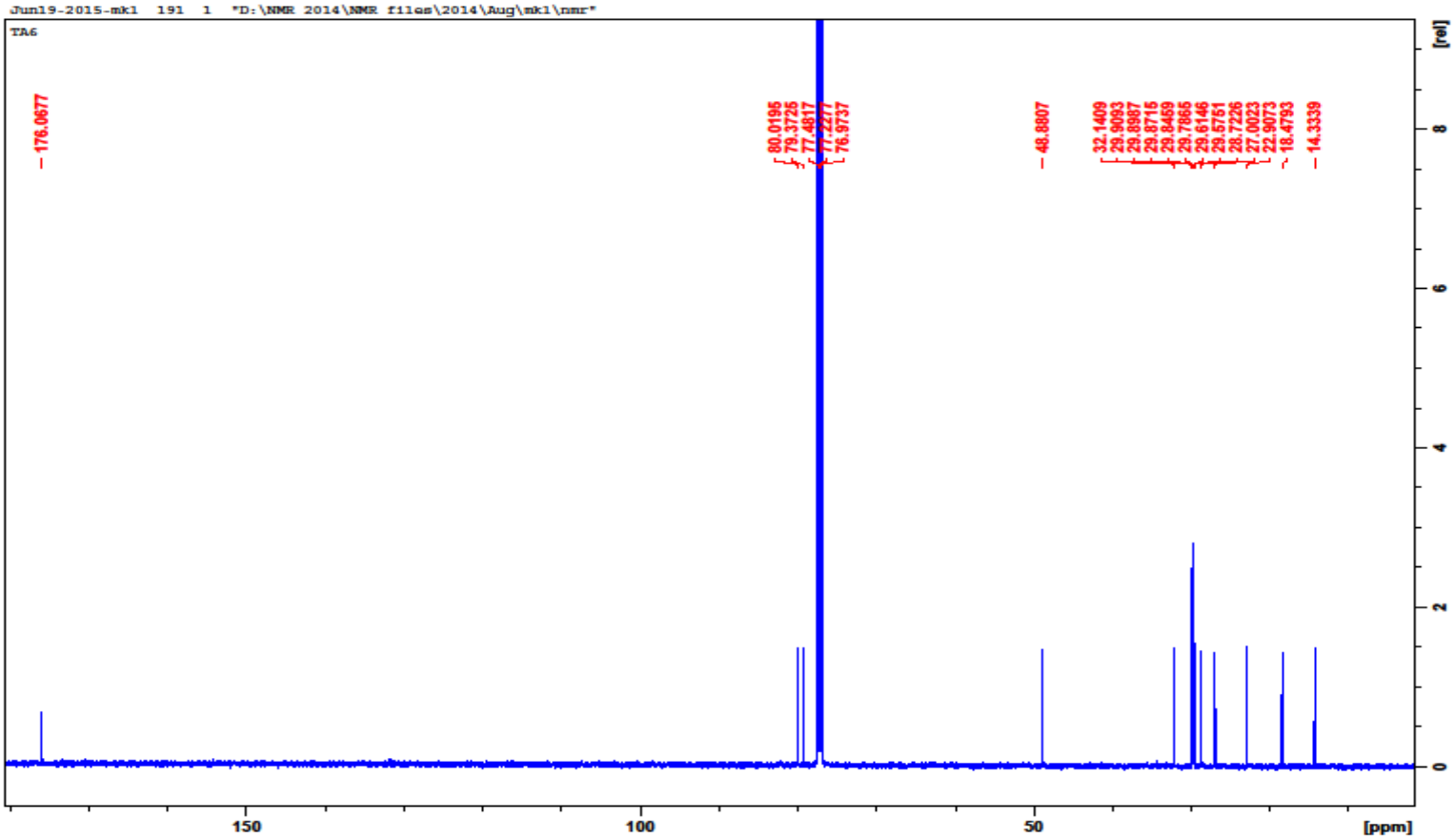
Appendix 12 NOESY. Compound 177



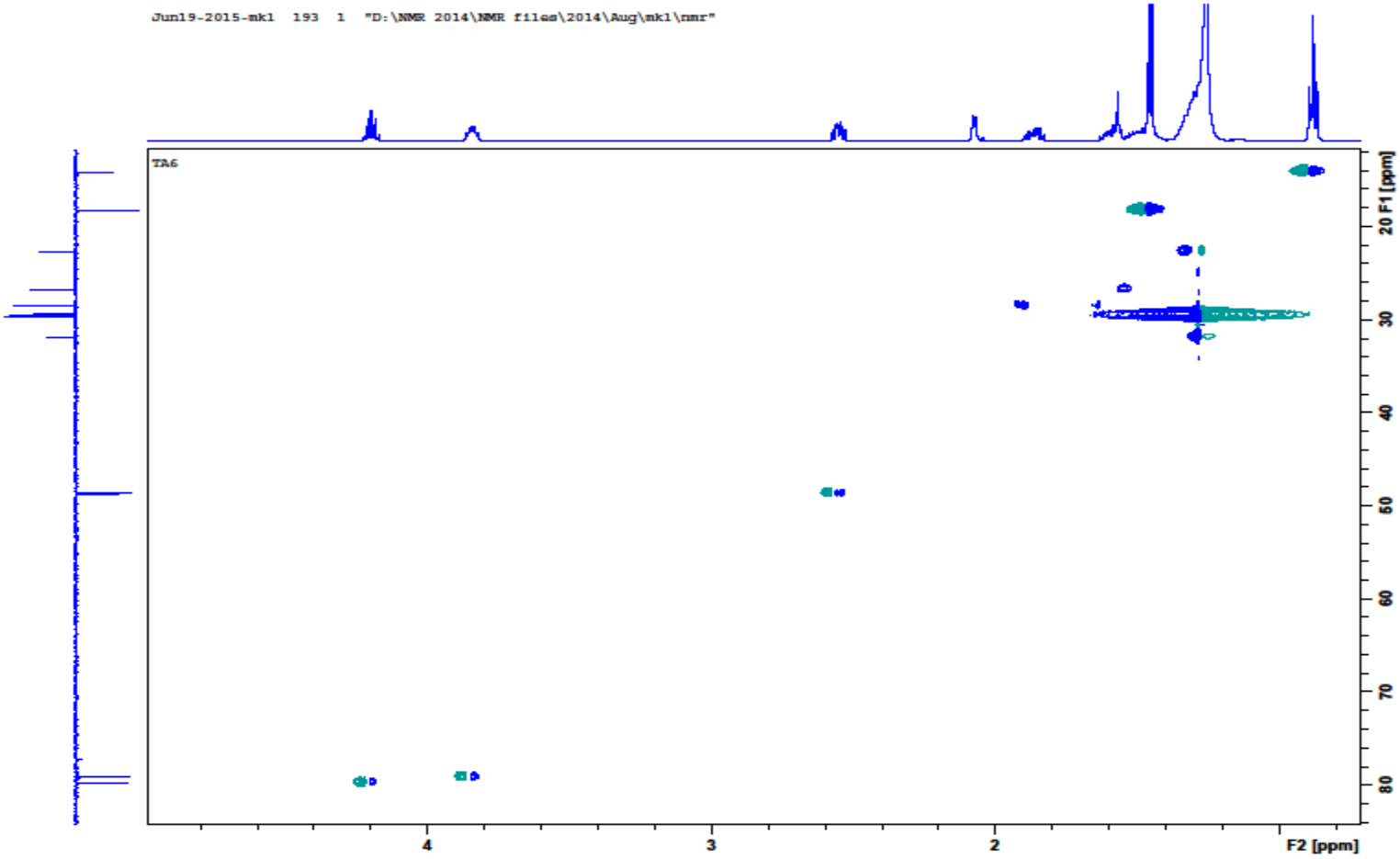
Appendix 13 ^{1}H. NMR. Compound**178**



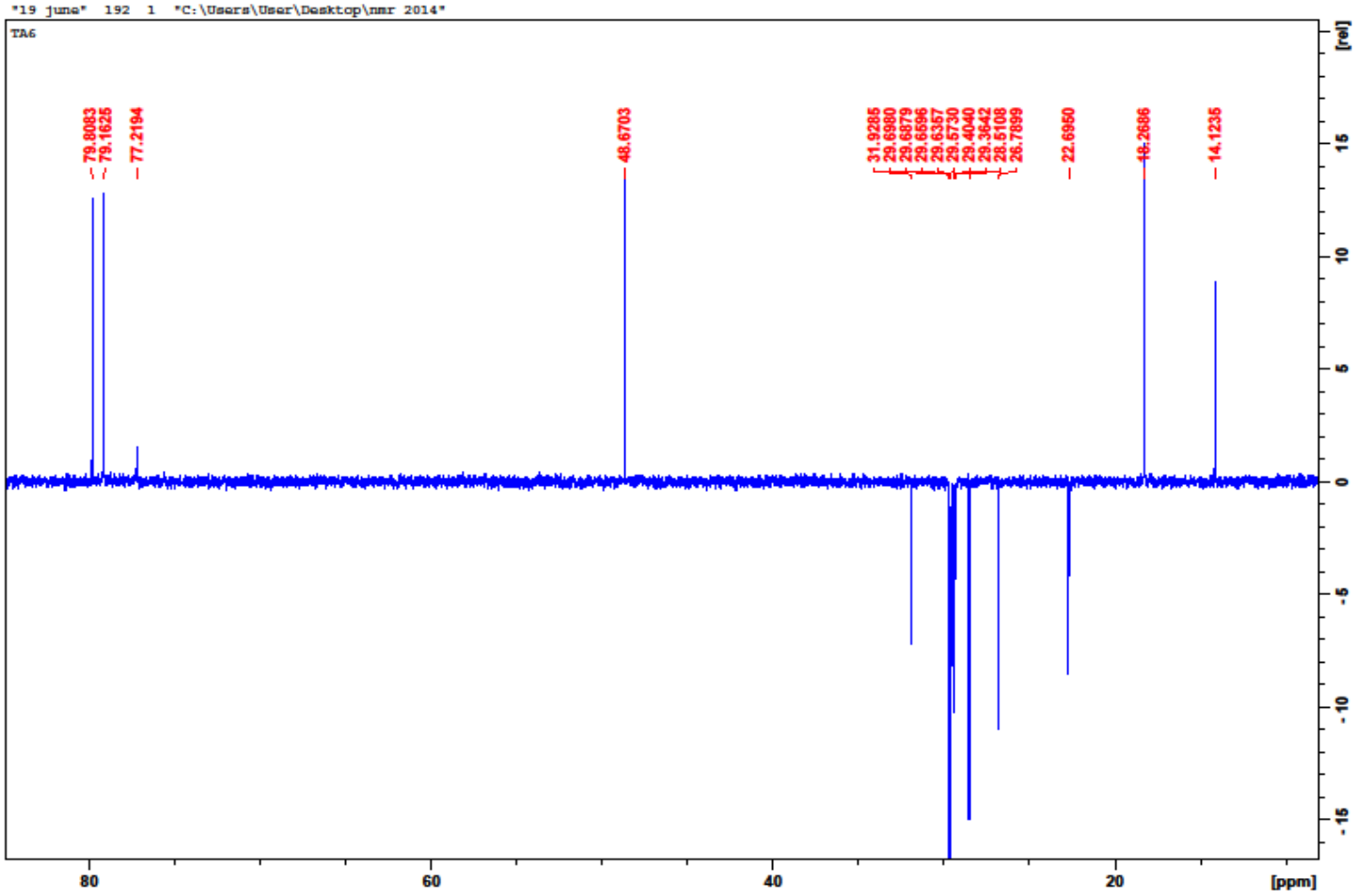
Appendix 14 ^{13}C. NMR. Compound **178**



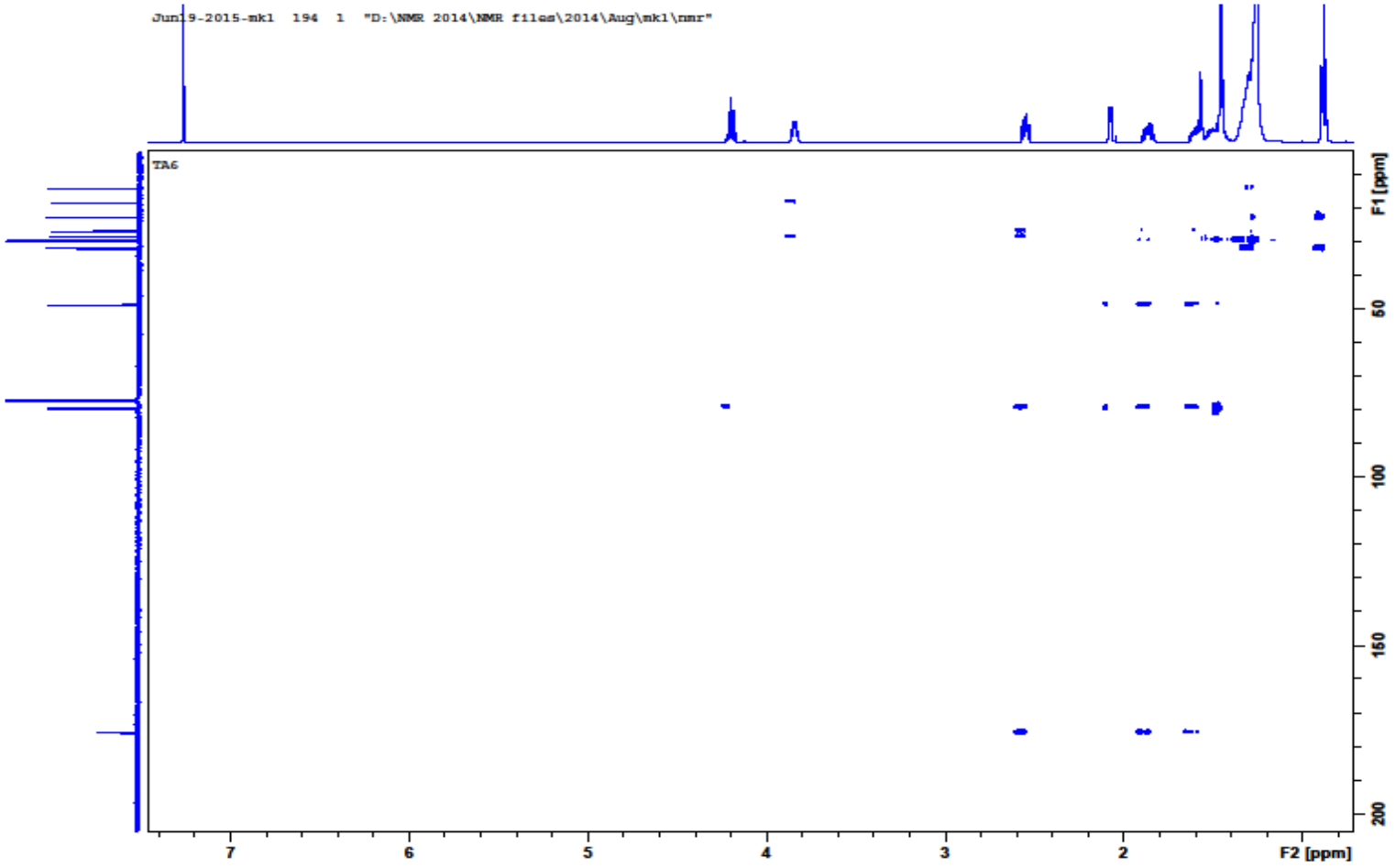
Appendix 15 HSQC. Compound **178**



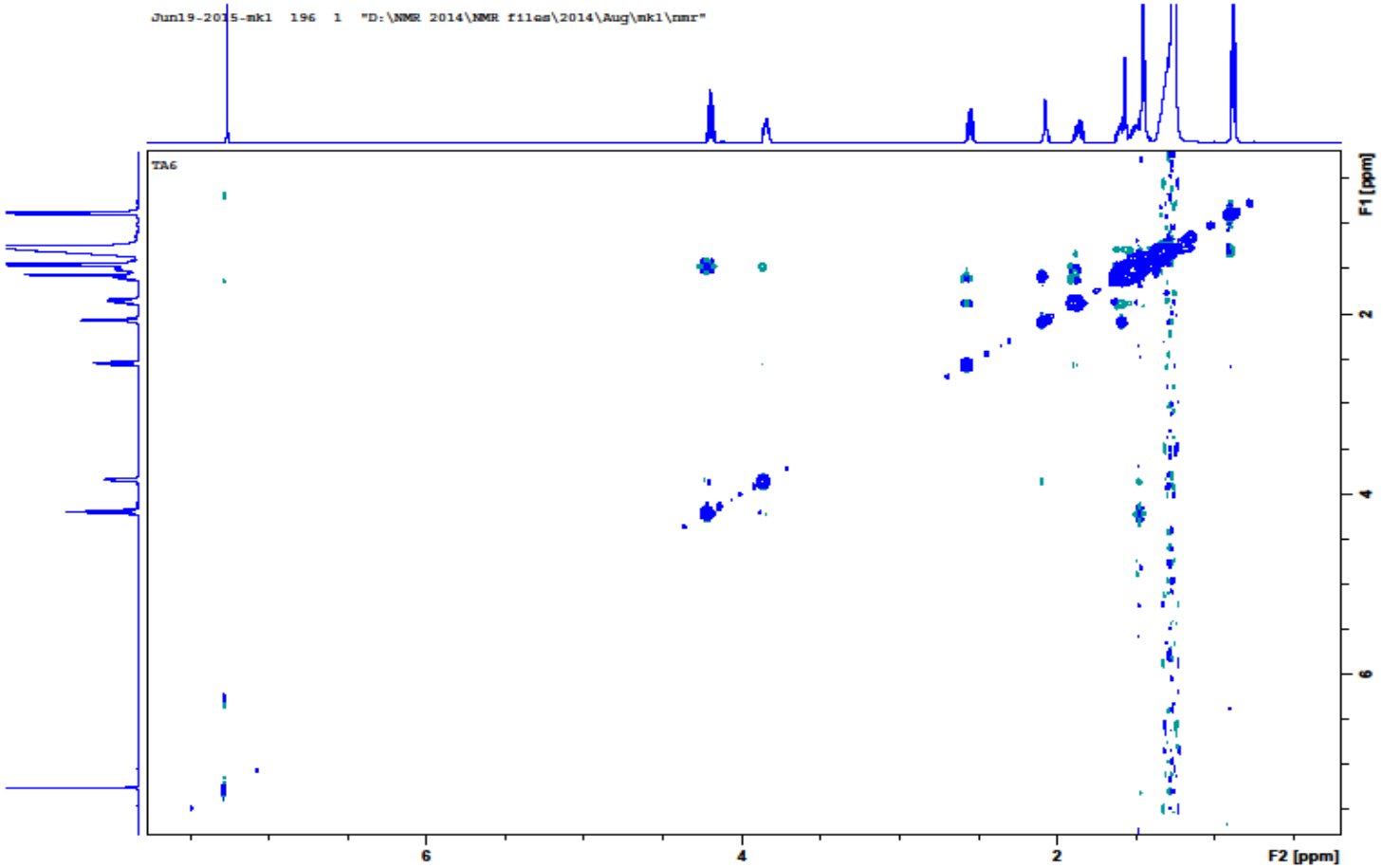
Appendix 16 DEPT. Compound 178



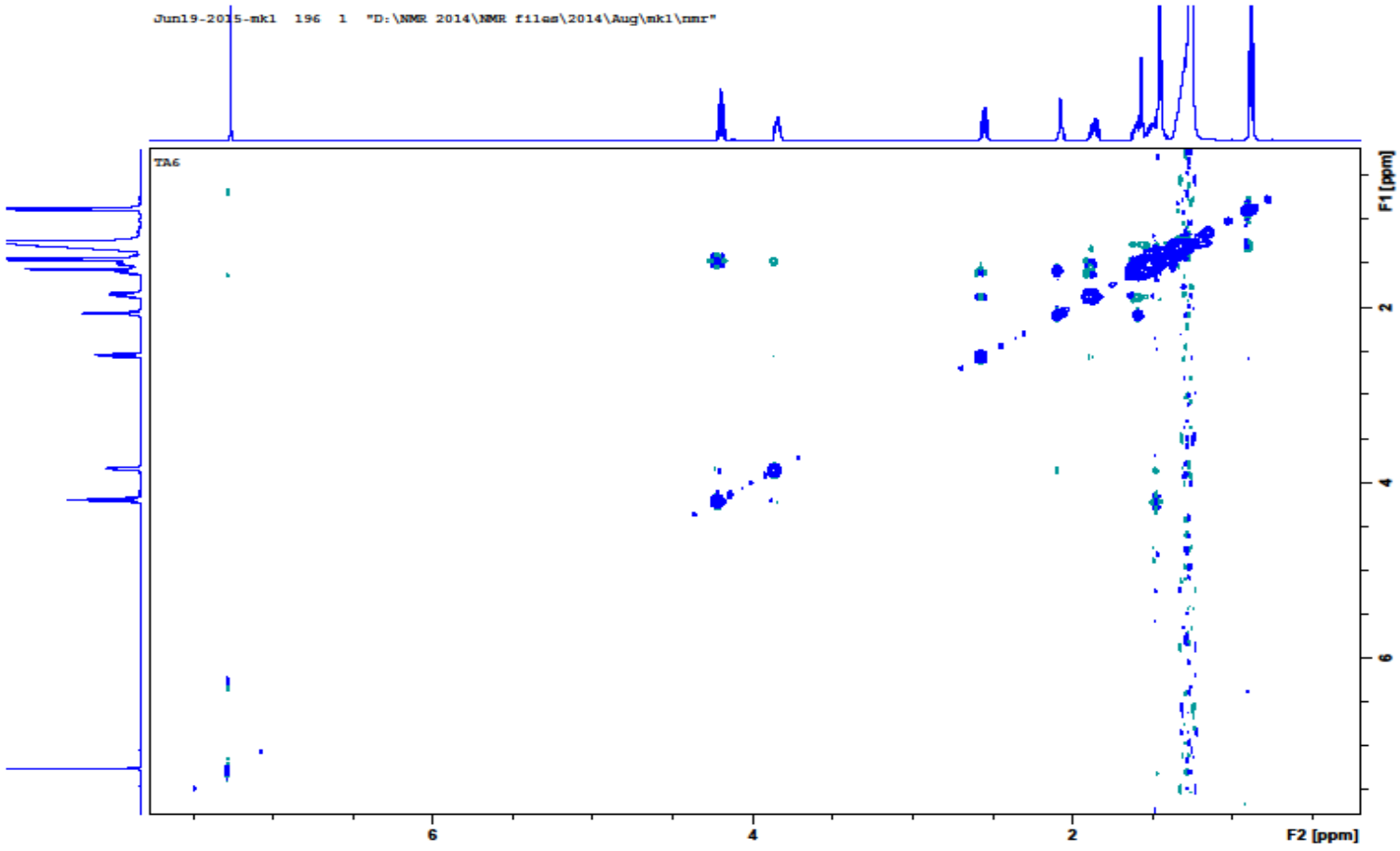
Appendix 17 HMBC. Compound 178



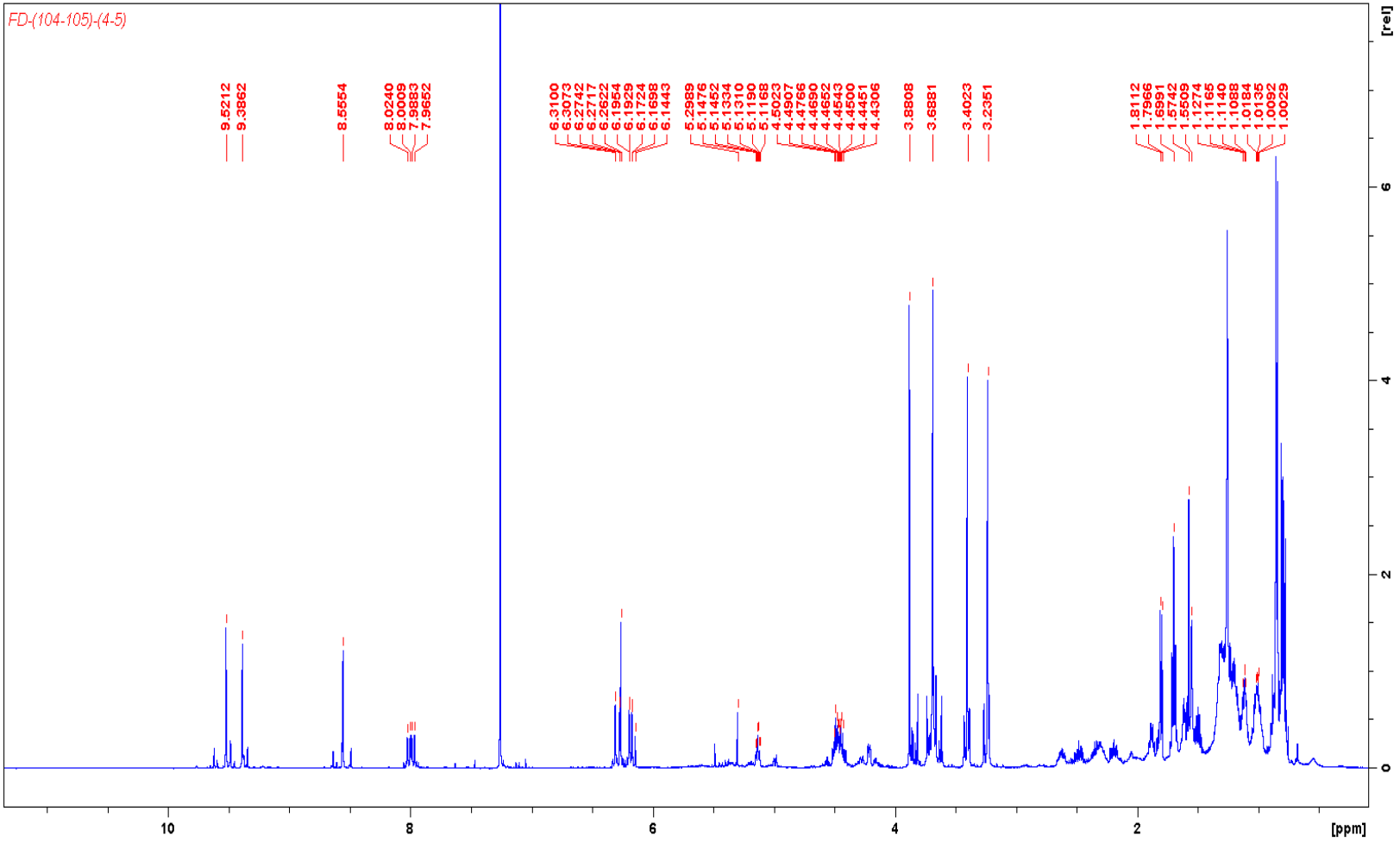
Appendix 18: COSY. Compound **178**



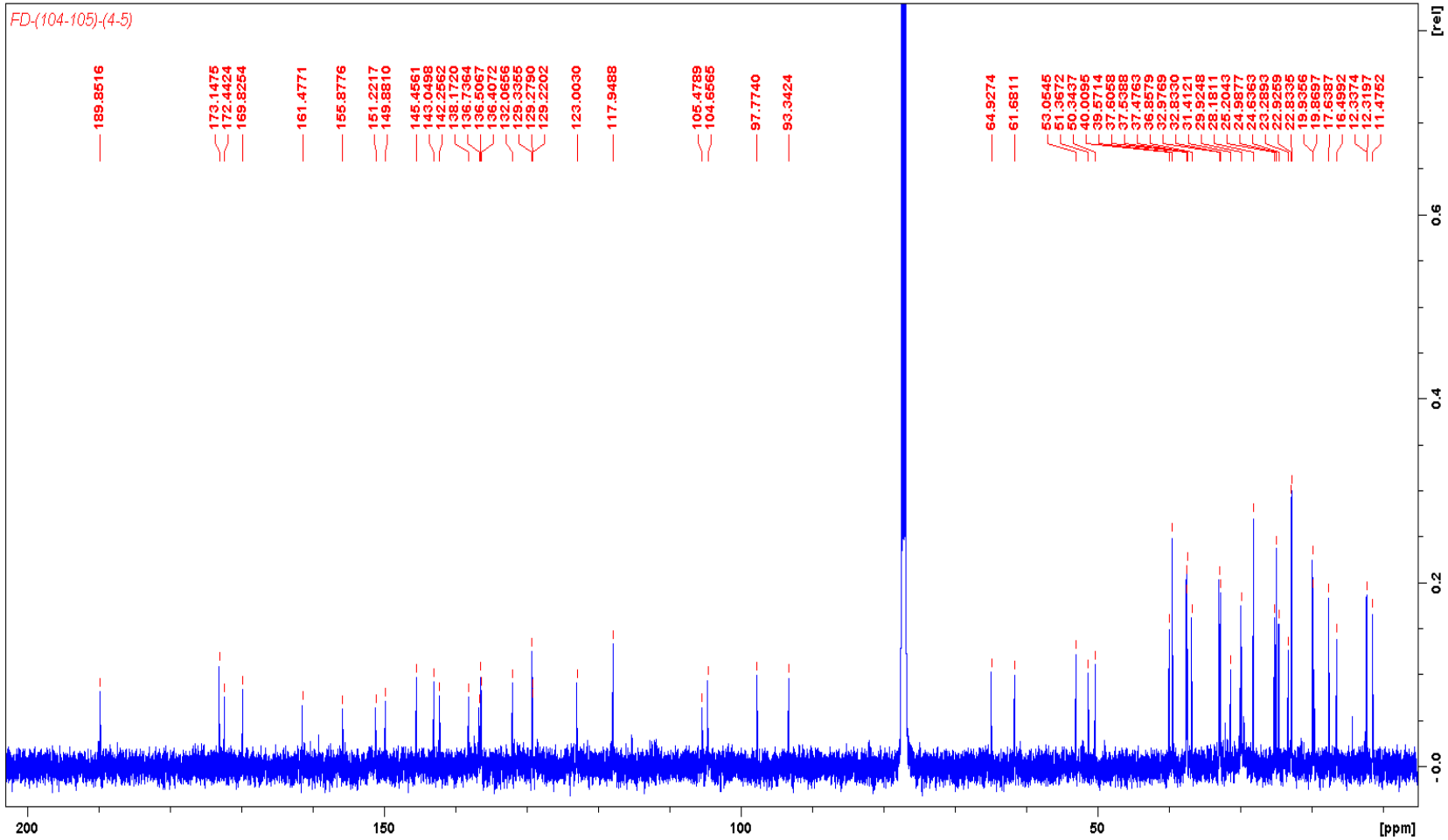
Appendix 19 NOESY. Compound **178**



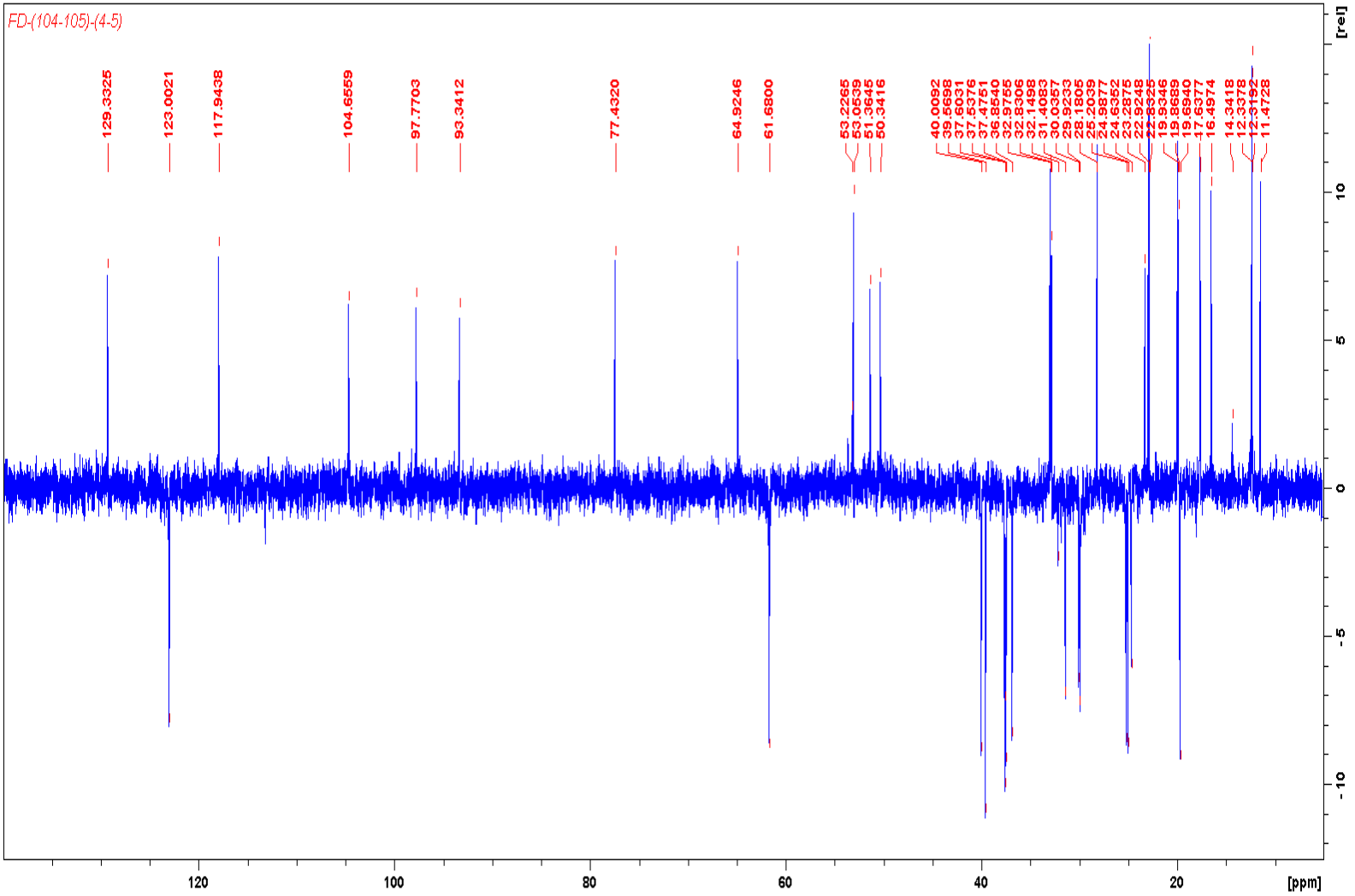
Appendix 20 ^{1}H NMR. Compound **179**



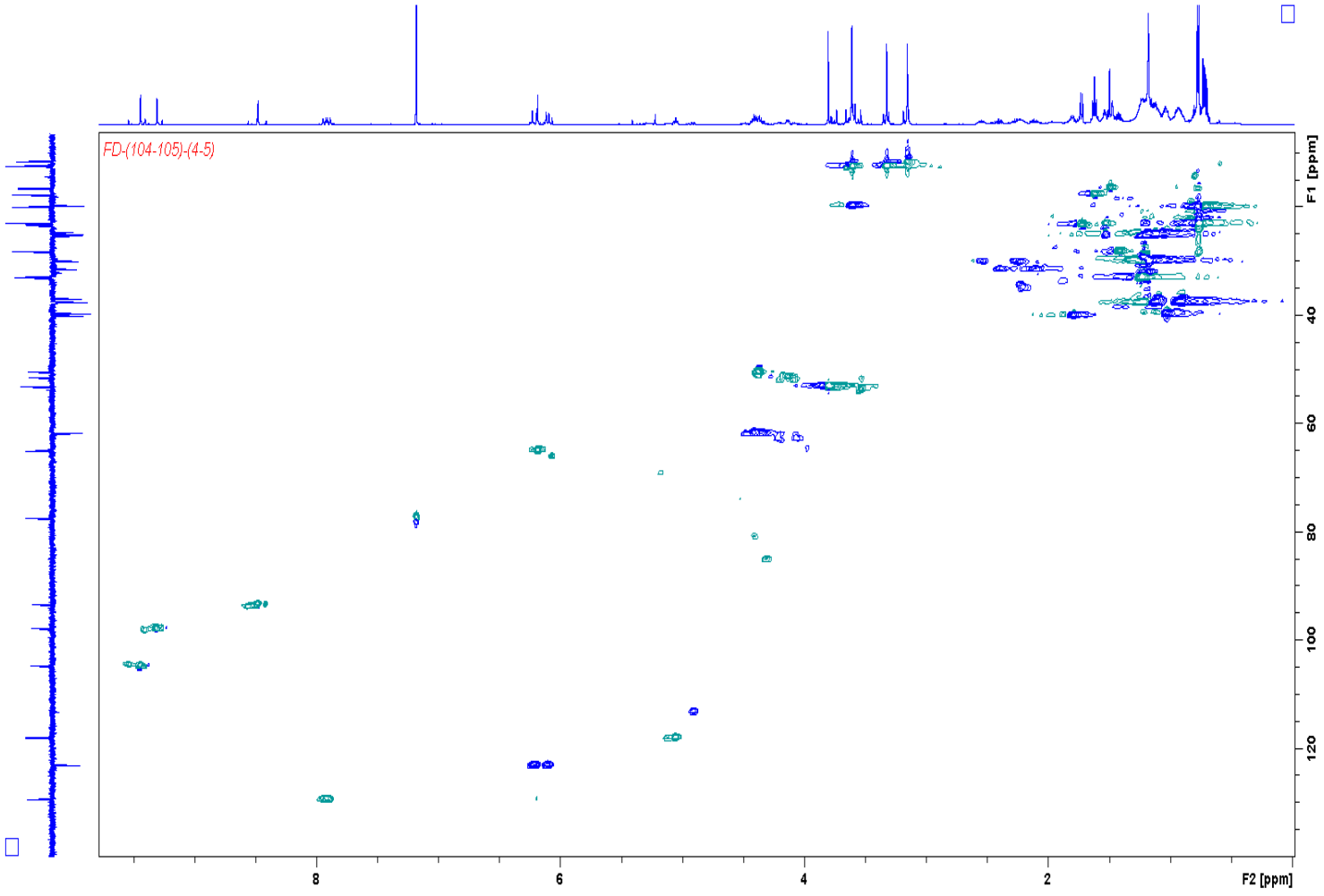
Appendix 21 ^{13}C NMR. Compound **179**



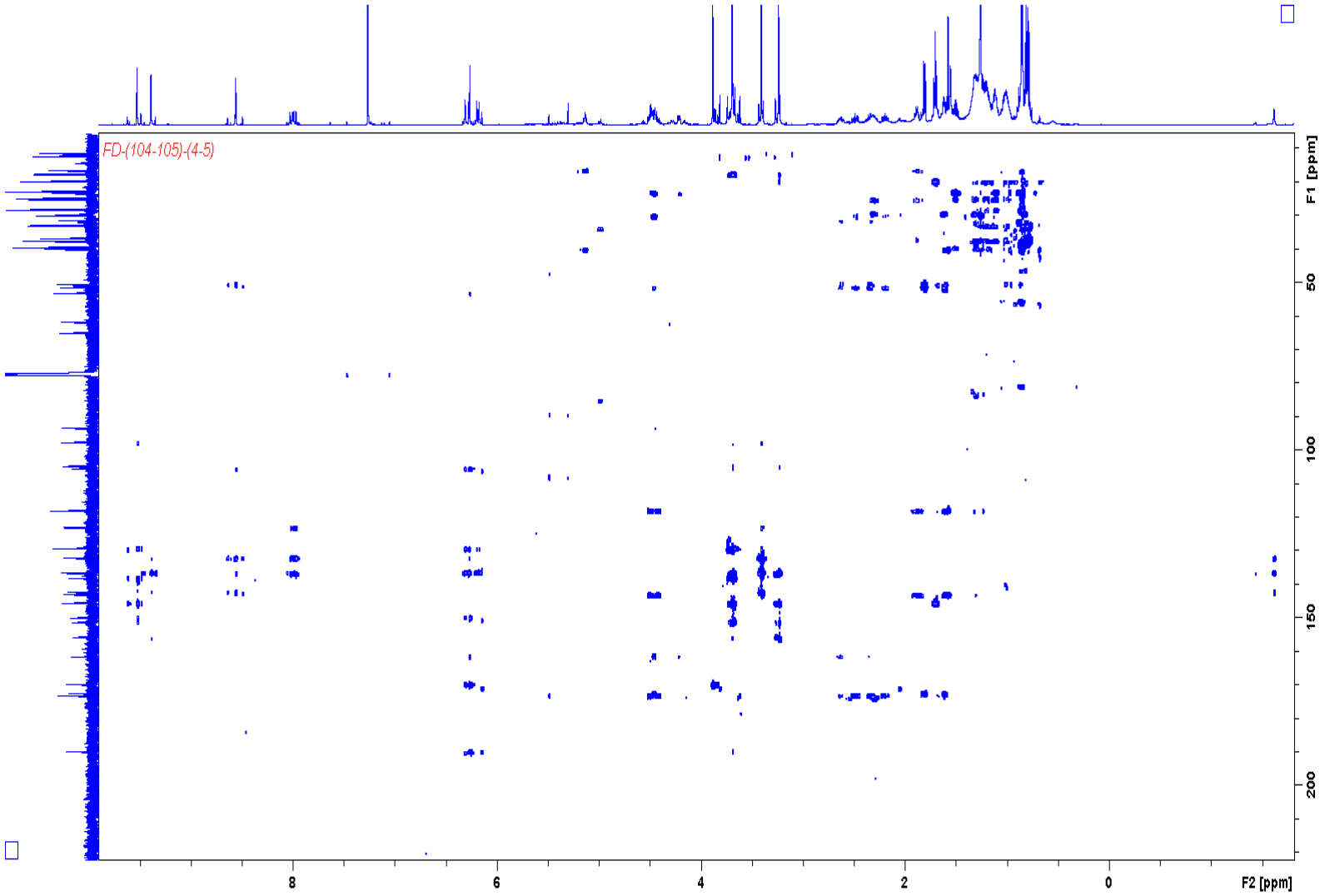
Appendix 22 DEPT. Compound **179**



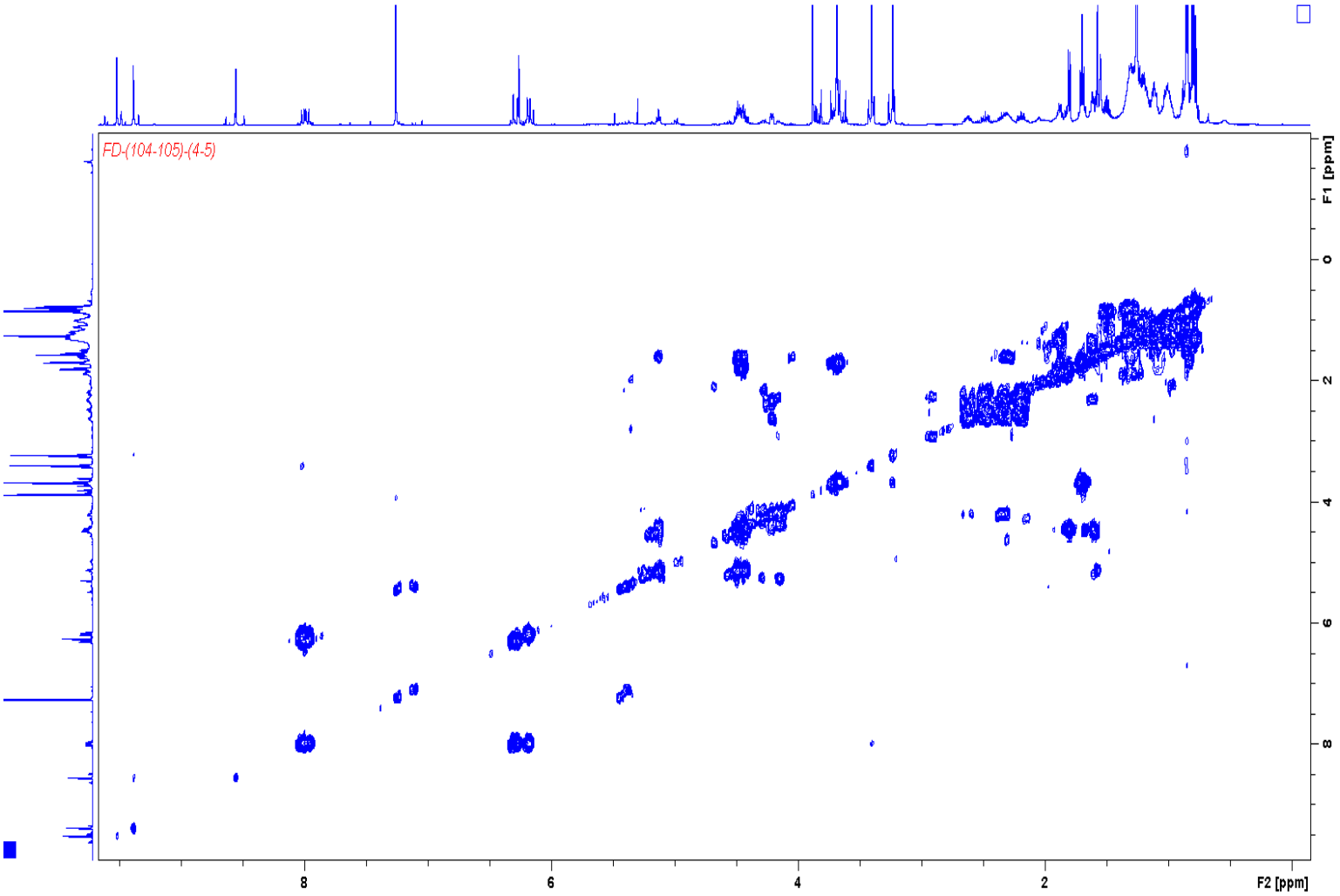
Appendix 23 HSQC-DEPT. Compound **179**



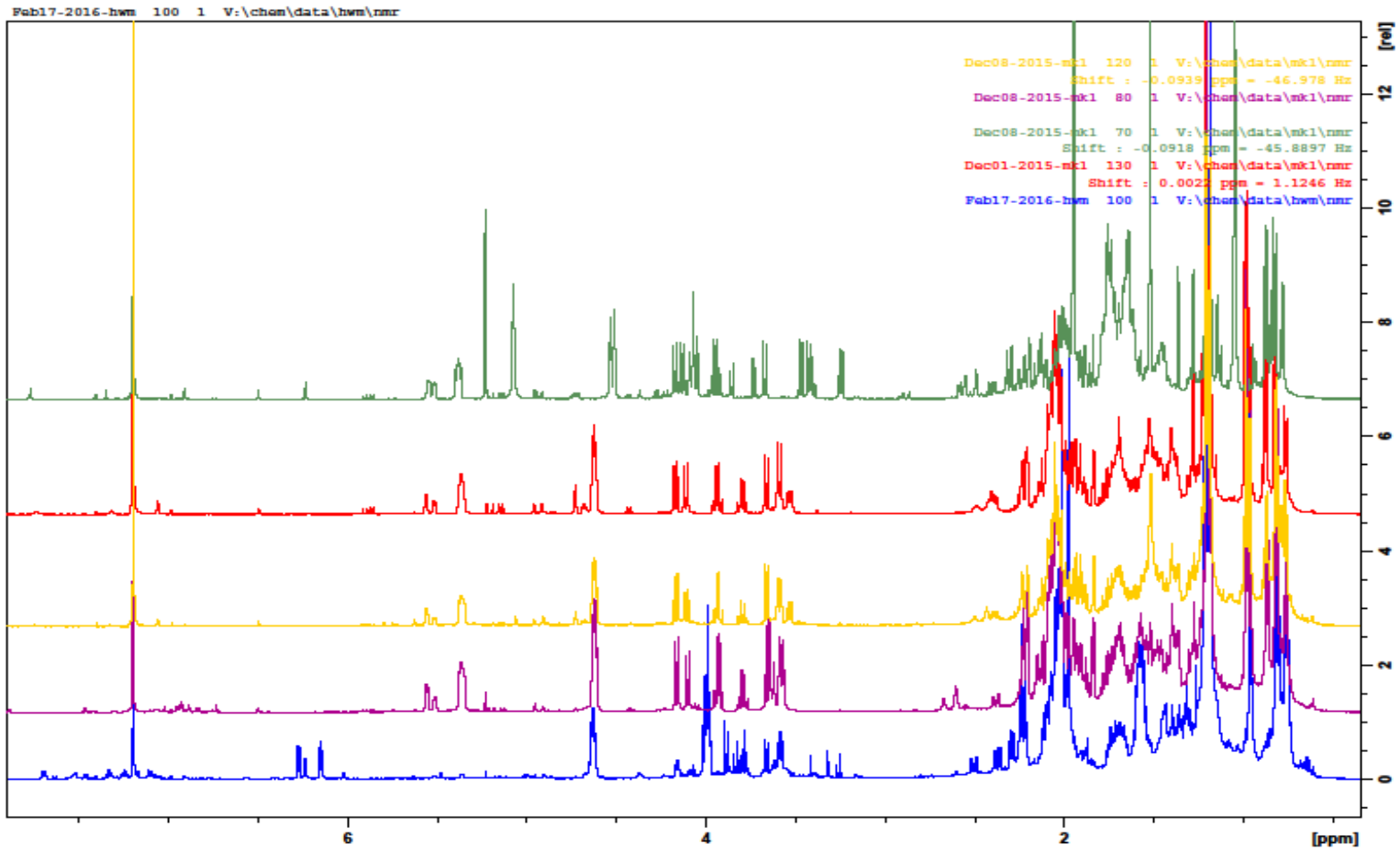
Appendix 24 HMBC. Compound **179**



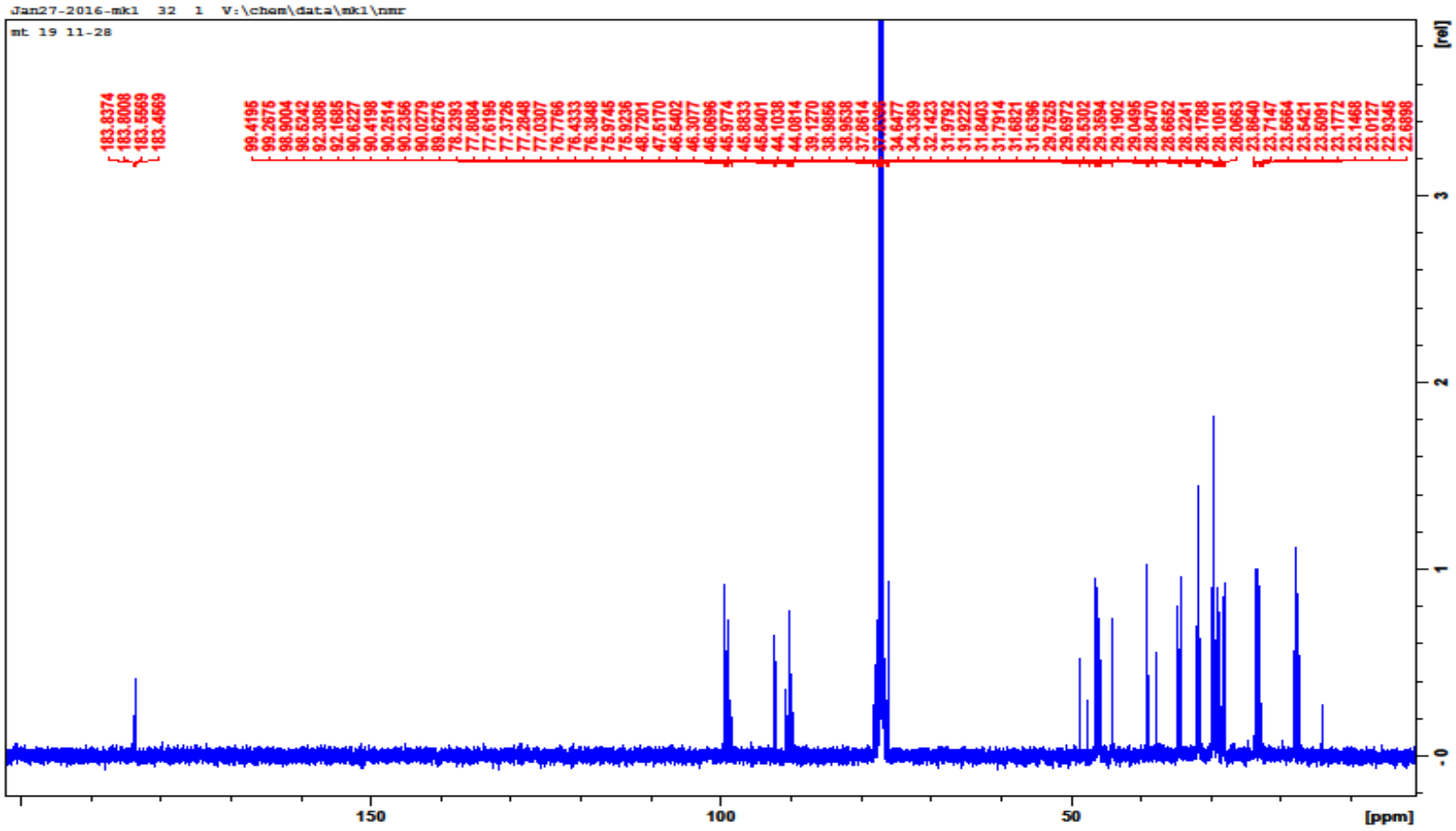
Appendix 25 COSY. Compound **179**



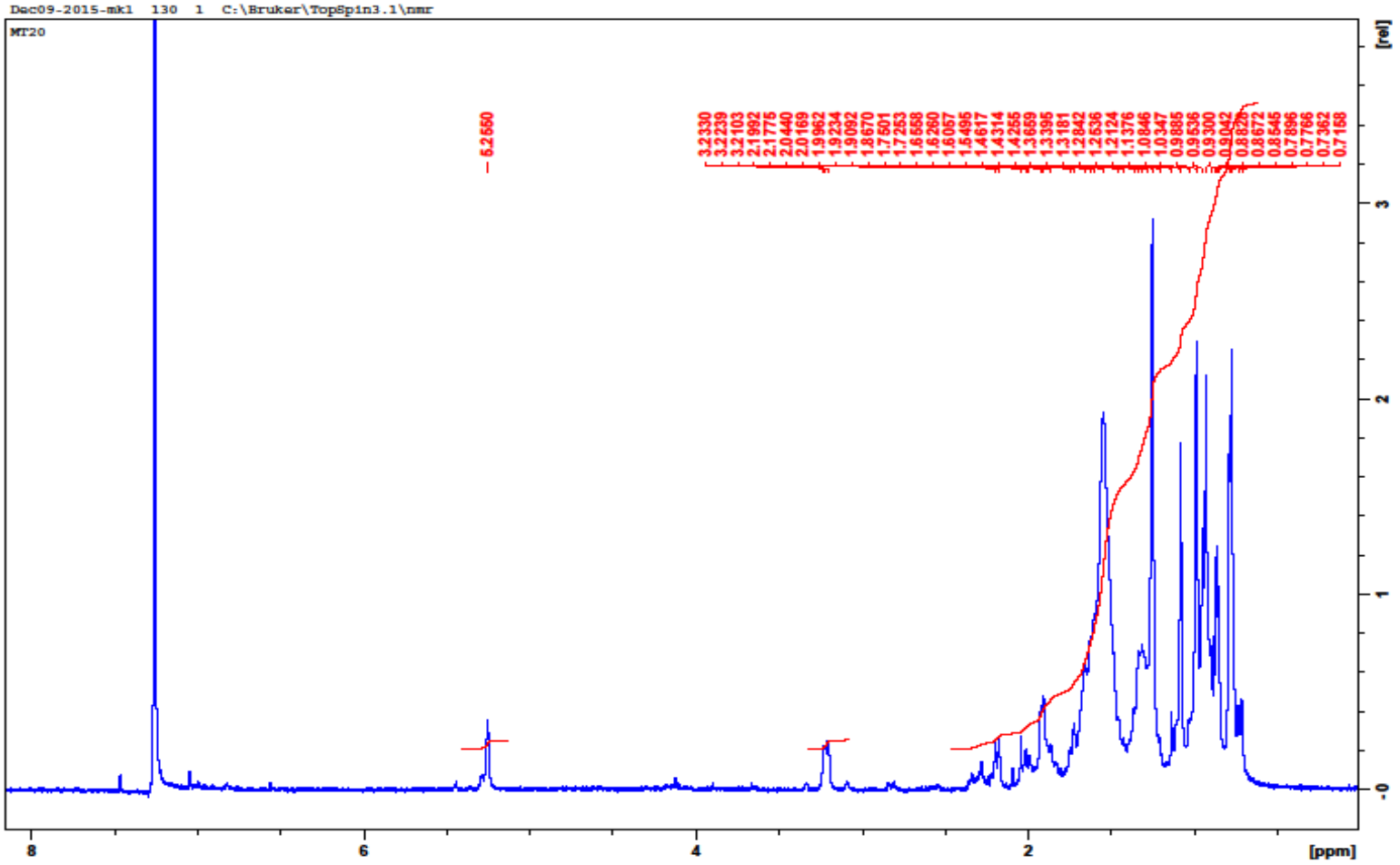
Appendix 26 ^{1}H NMR. Compound **180**



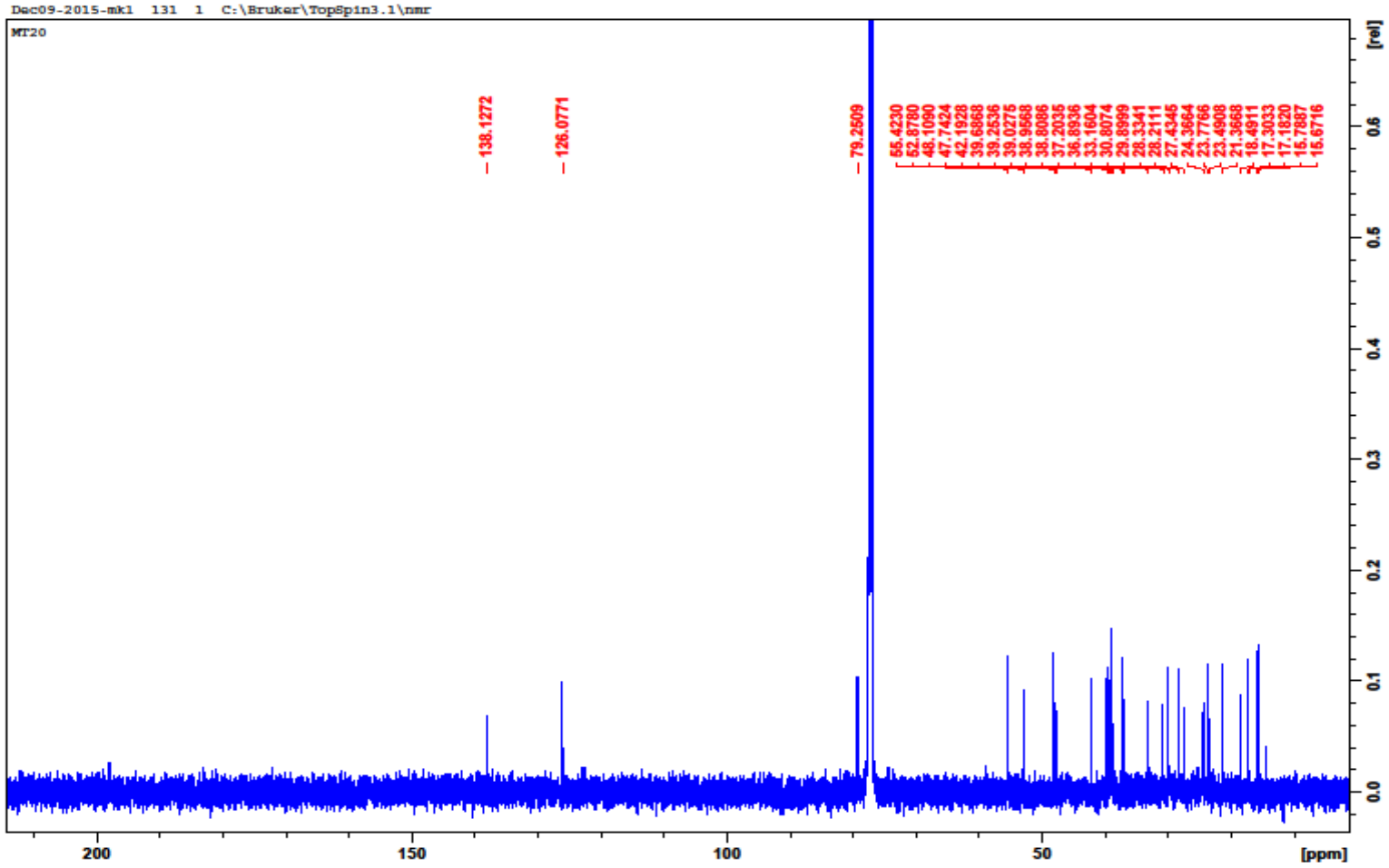
Appendix 27 ^{13}C NMR. Compound **180**



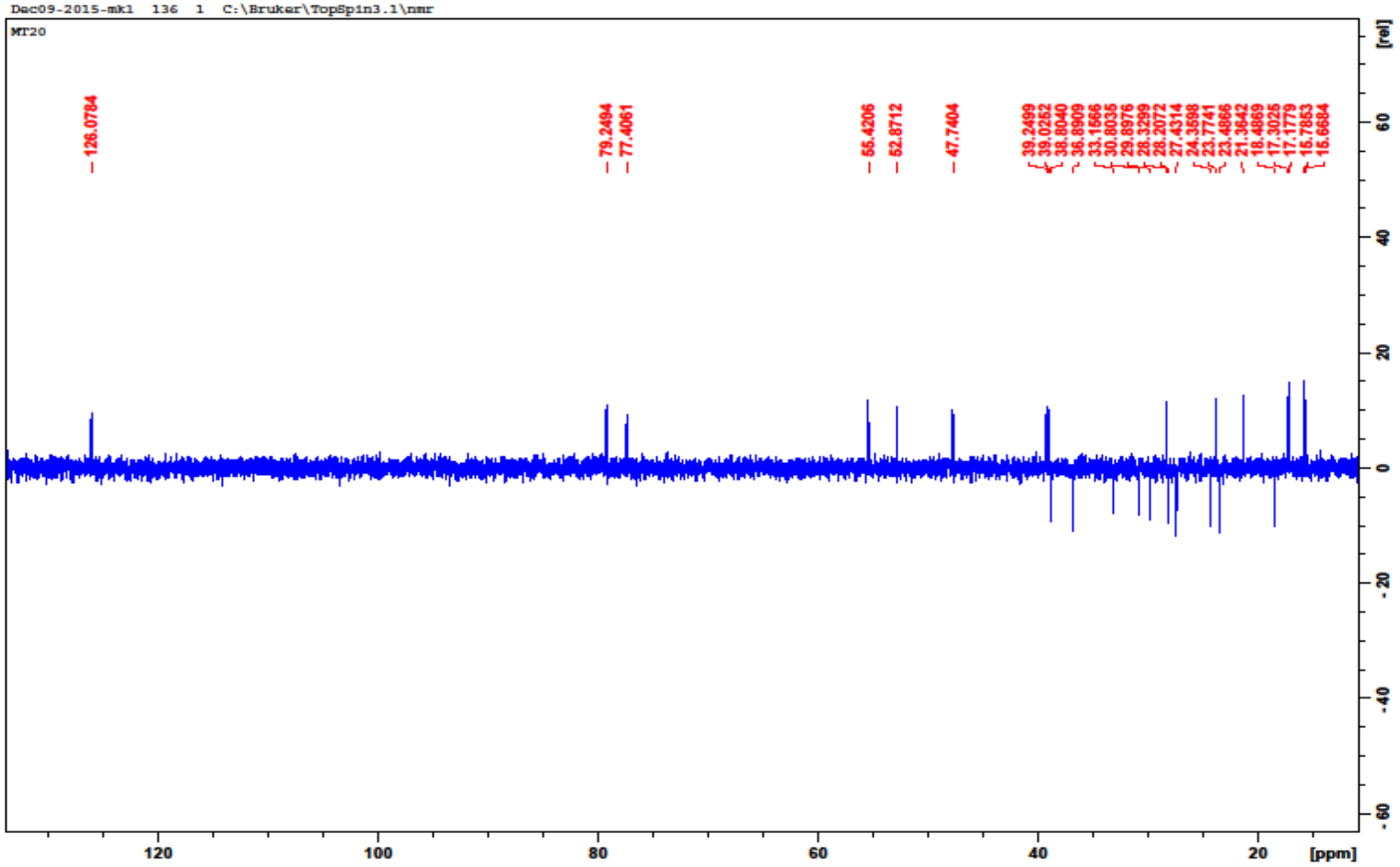
Appendix 28 ^{1}H NMR. Compound **118**



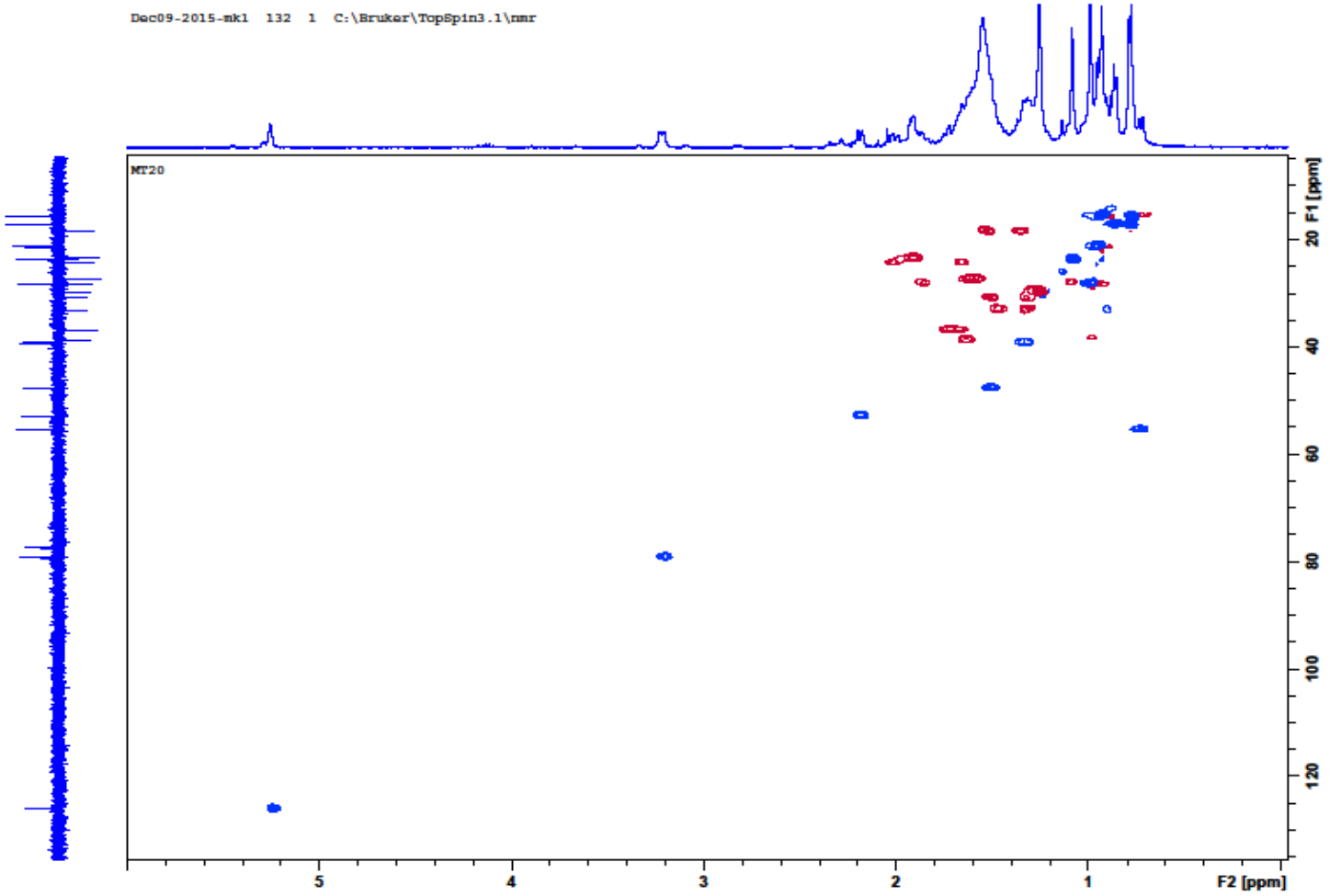
Appendix 29 ^{13}C NMR. Compound **118**



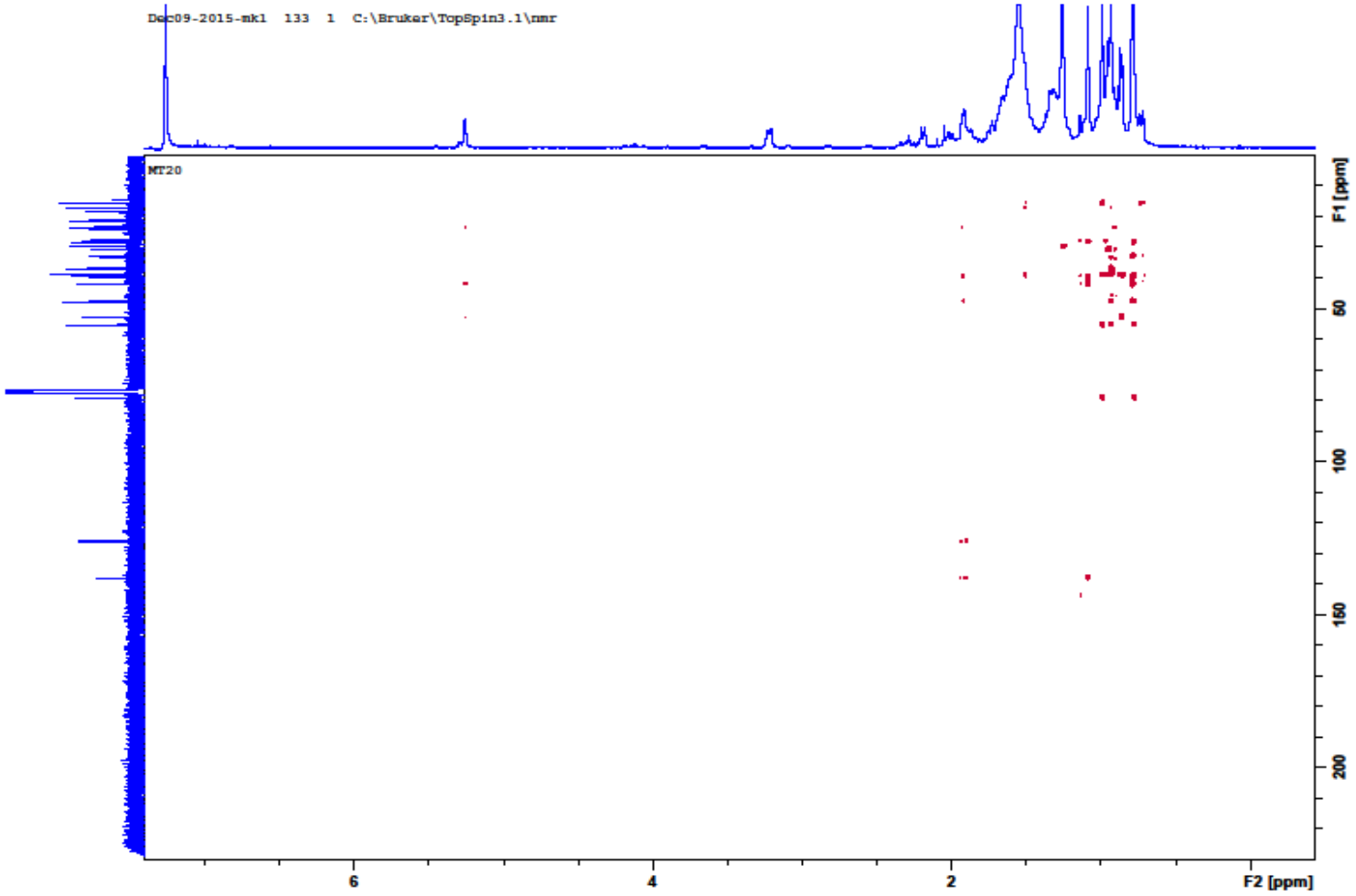
Appendix 30: DEPT. Compound **118**



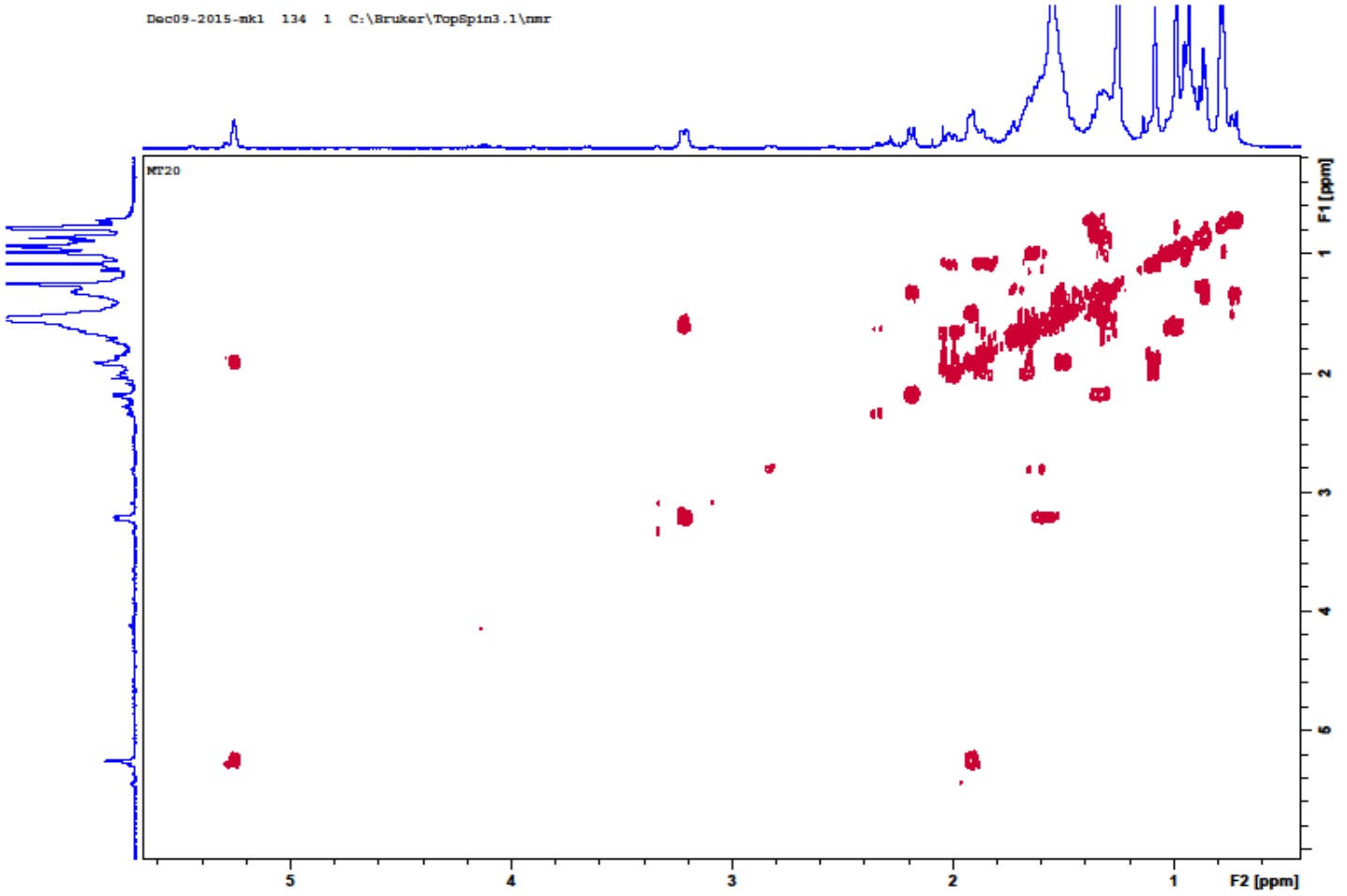
Appendix 31 HSQC-DEPT. Compound **118**



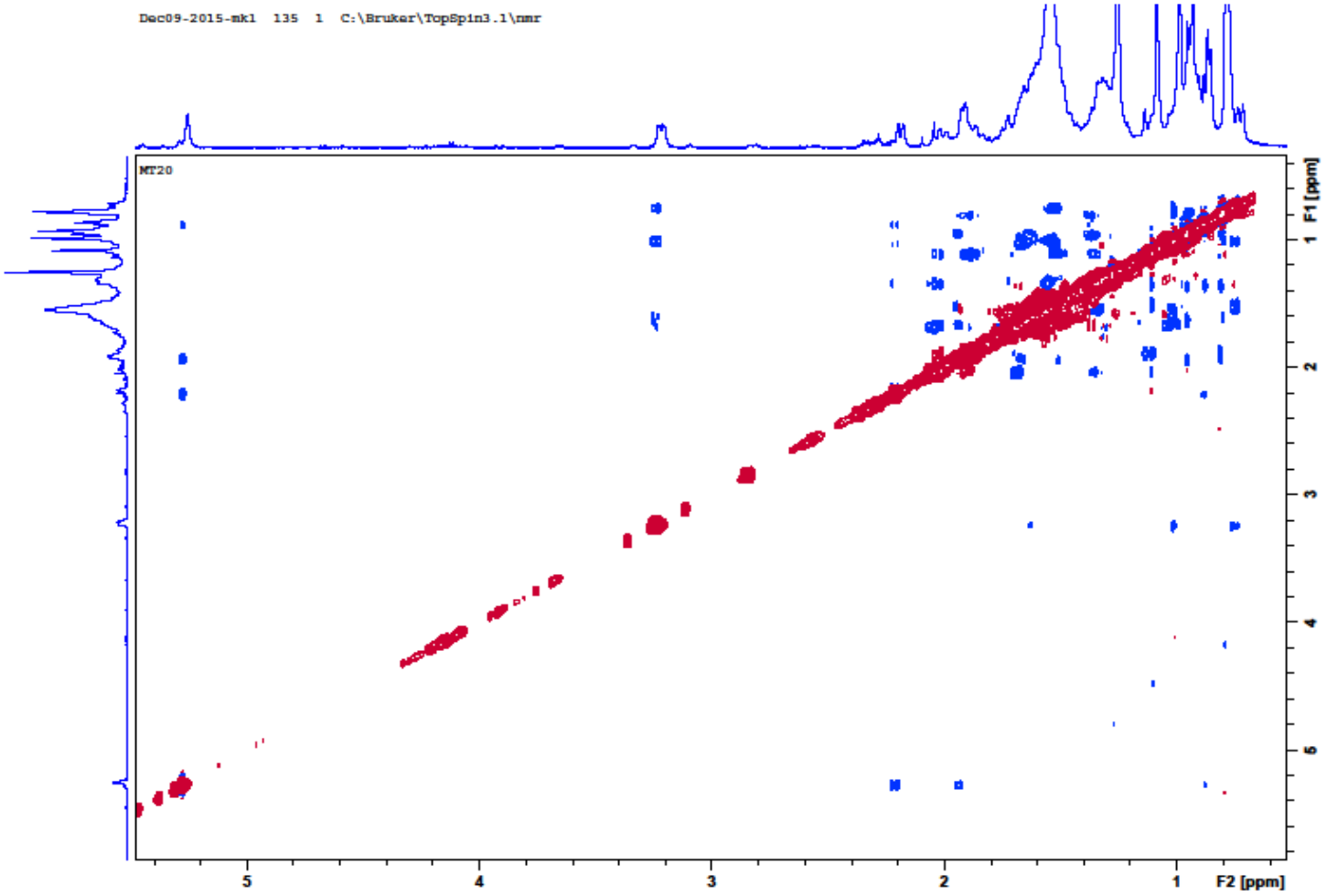
Appendix 32 HMBC. Compound **118**



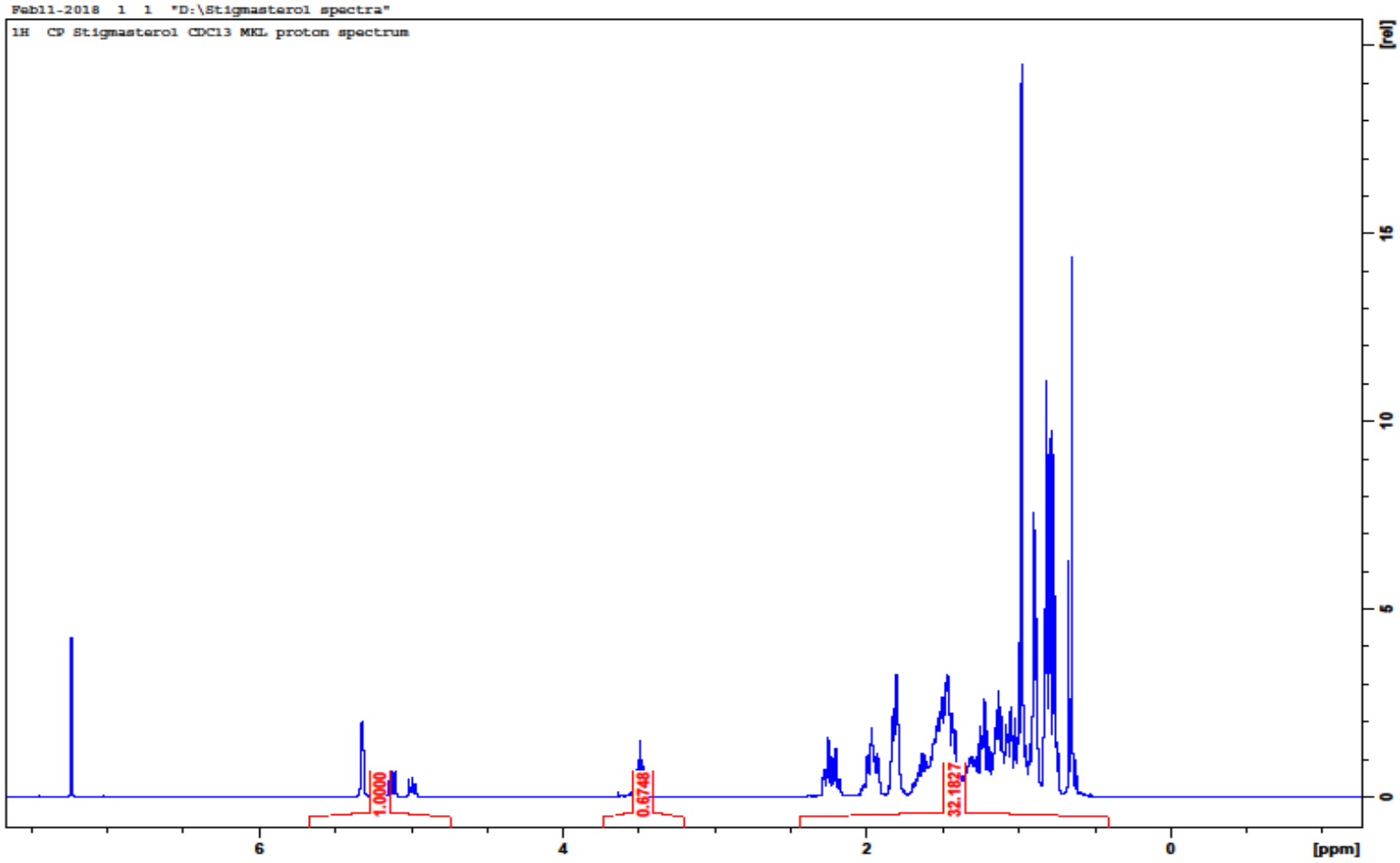
Appendix 33 COSY. Compound **118**



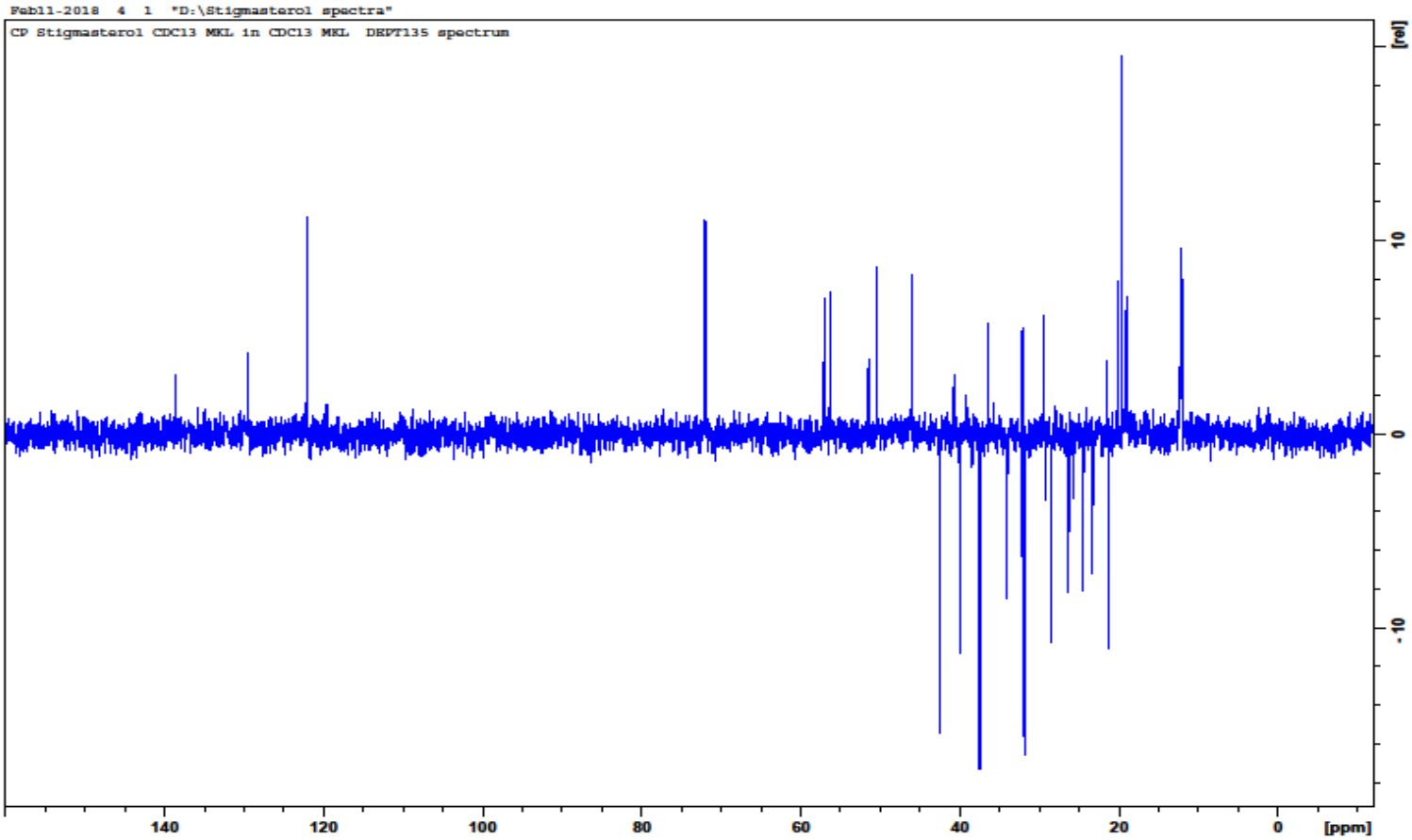
Appendix 34 NOESY. Compound **118**



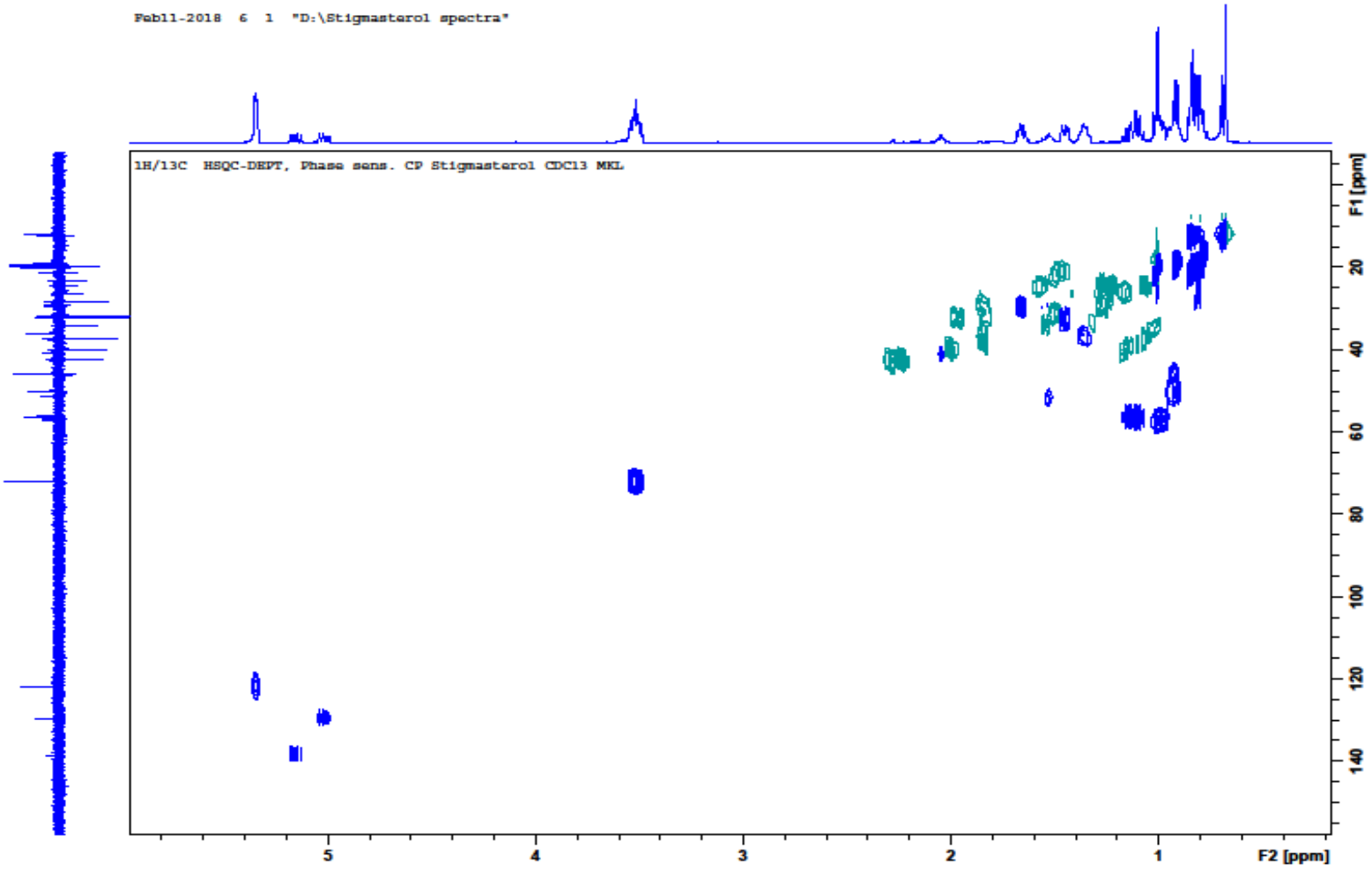
Appendix 35 ^{1}H. Compound **60**



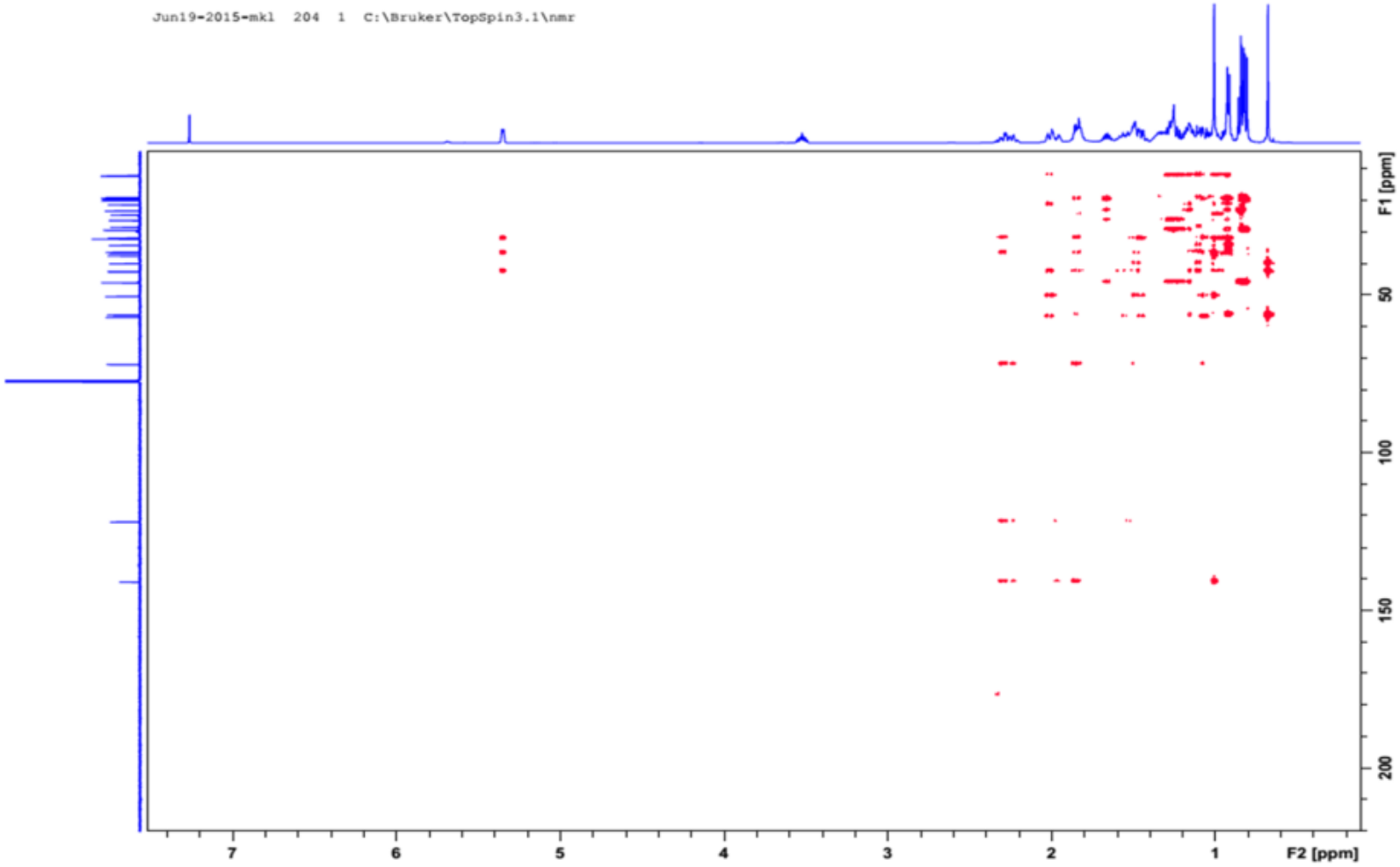
Appendix 36 DEPT. Compound **60**



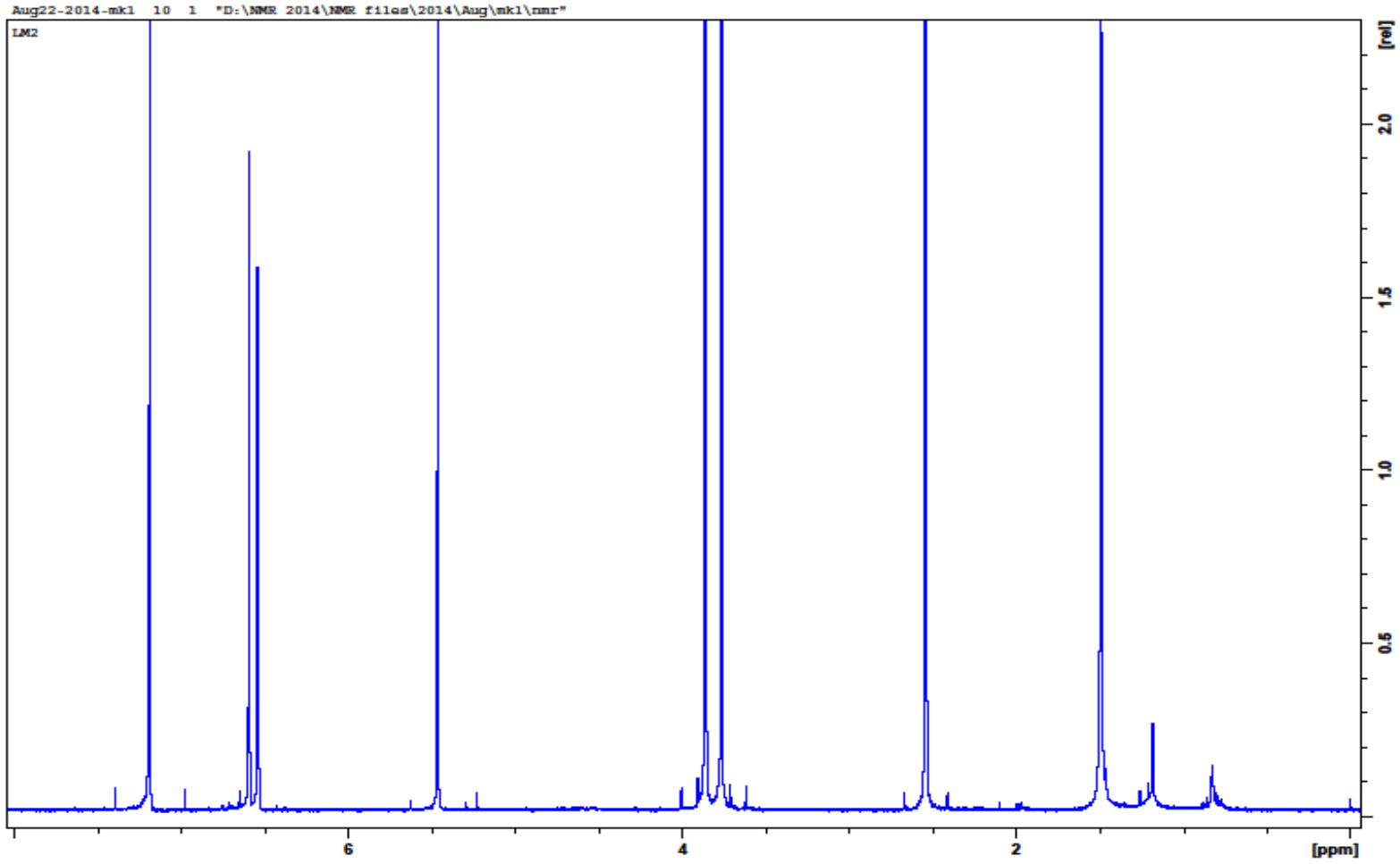
Appendix 37 HSQC-DEPT. Compound **60**



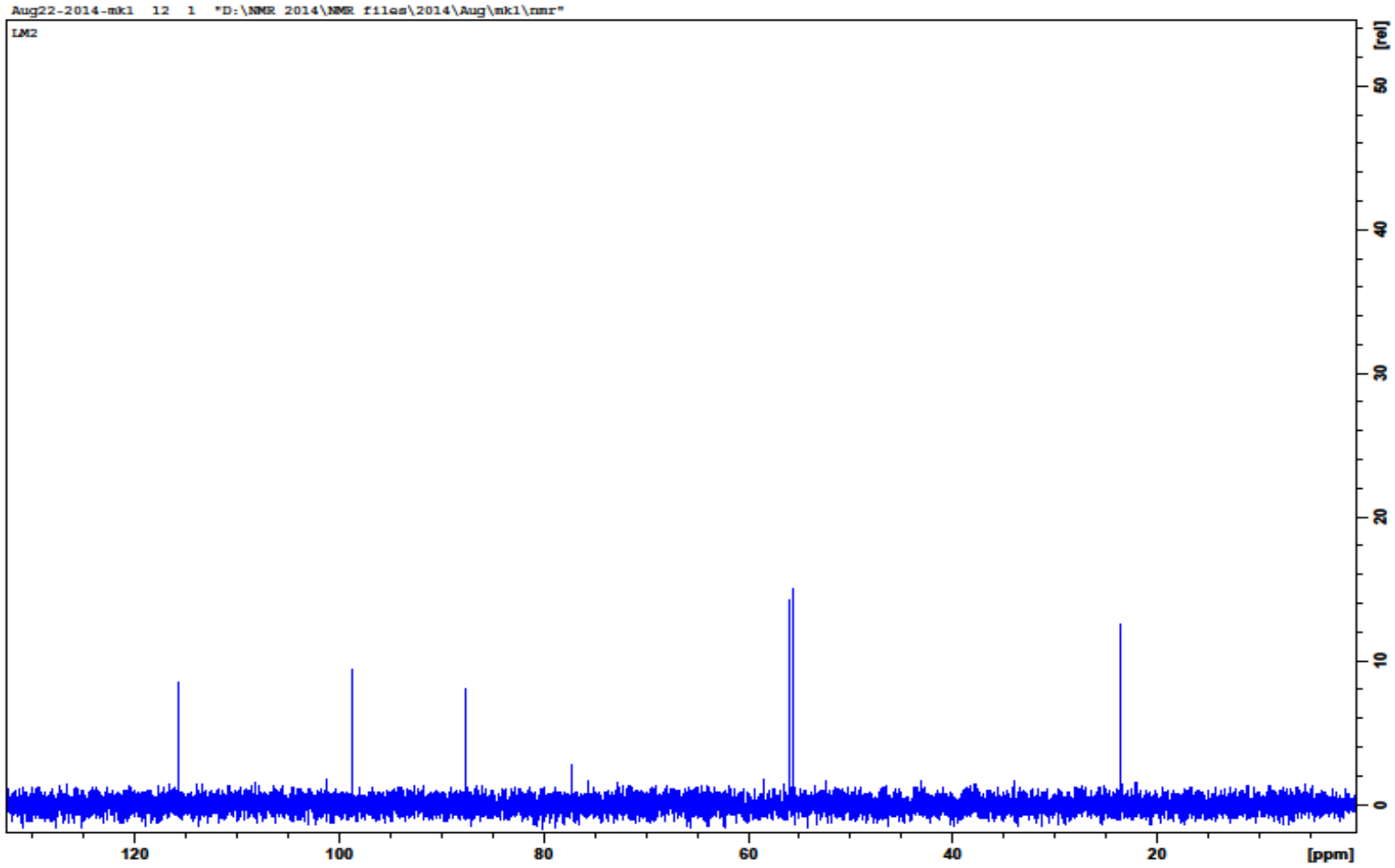
Appendix 38 HMBC. Compound **60**



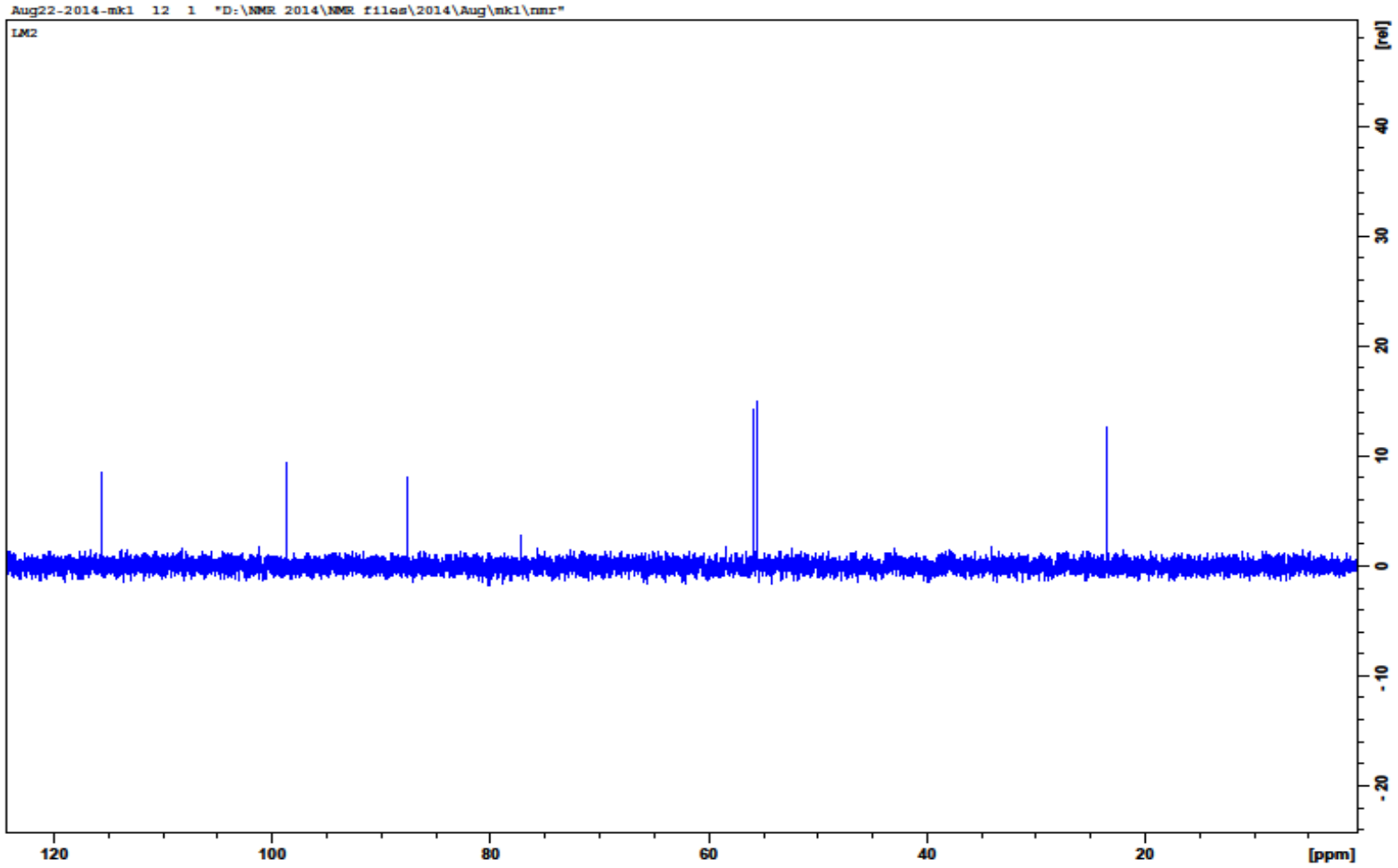
Appendix 39 ^{1}H Compound **181**



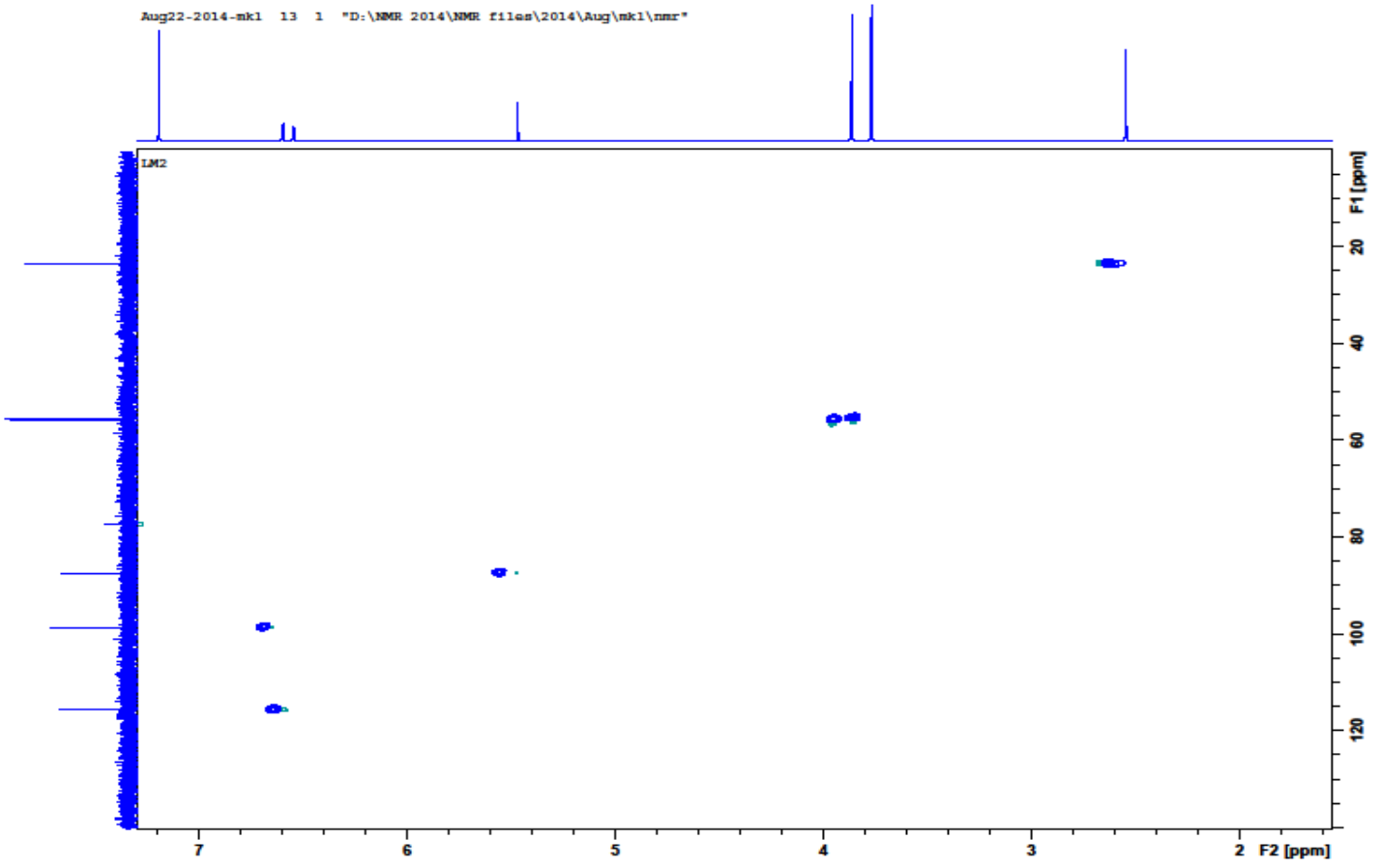
Appendix 40: ^{13}C Compound 181



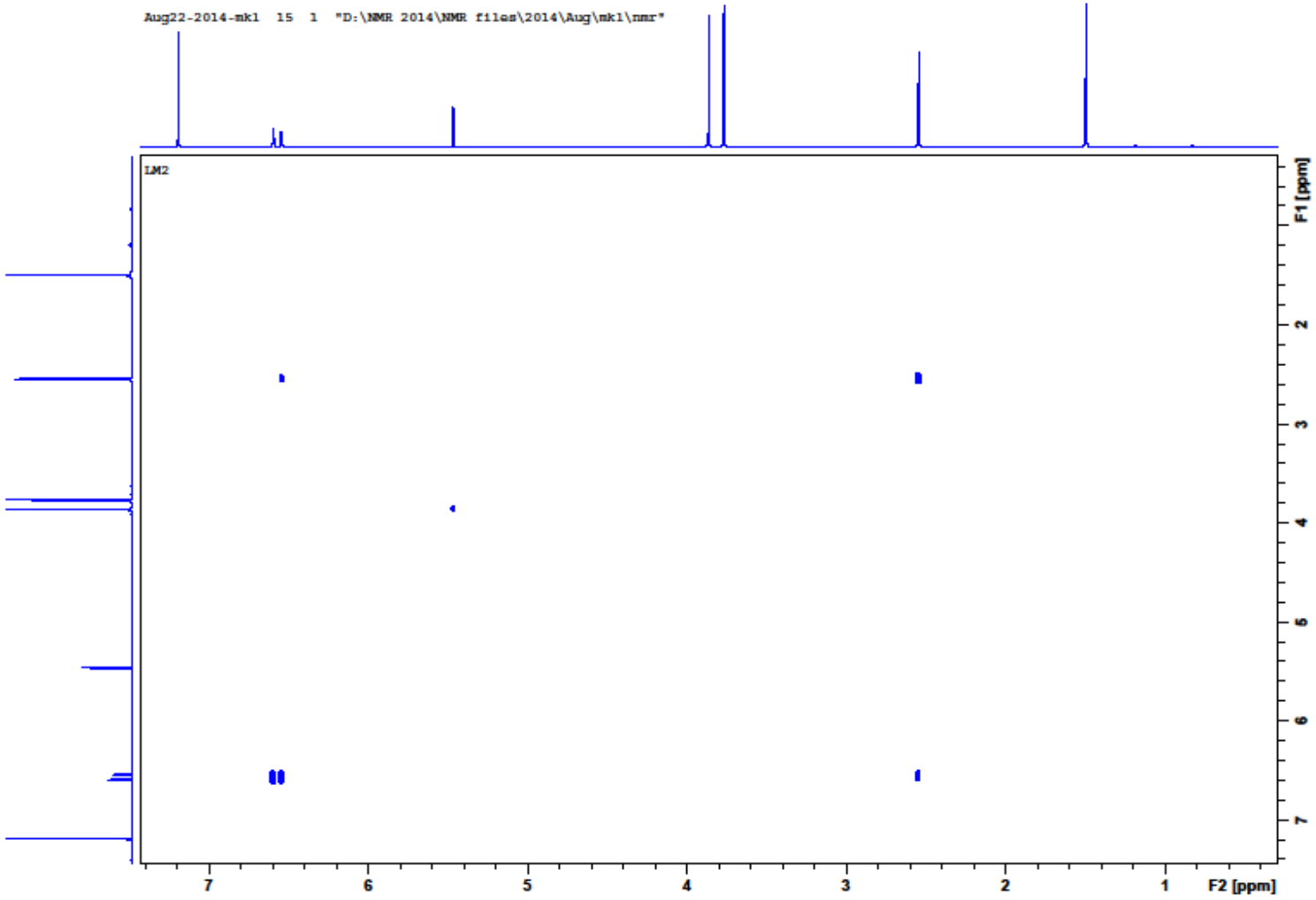
Appendix 41: DEPT Compound **181**



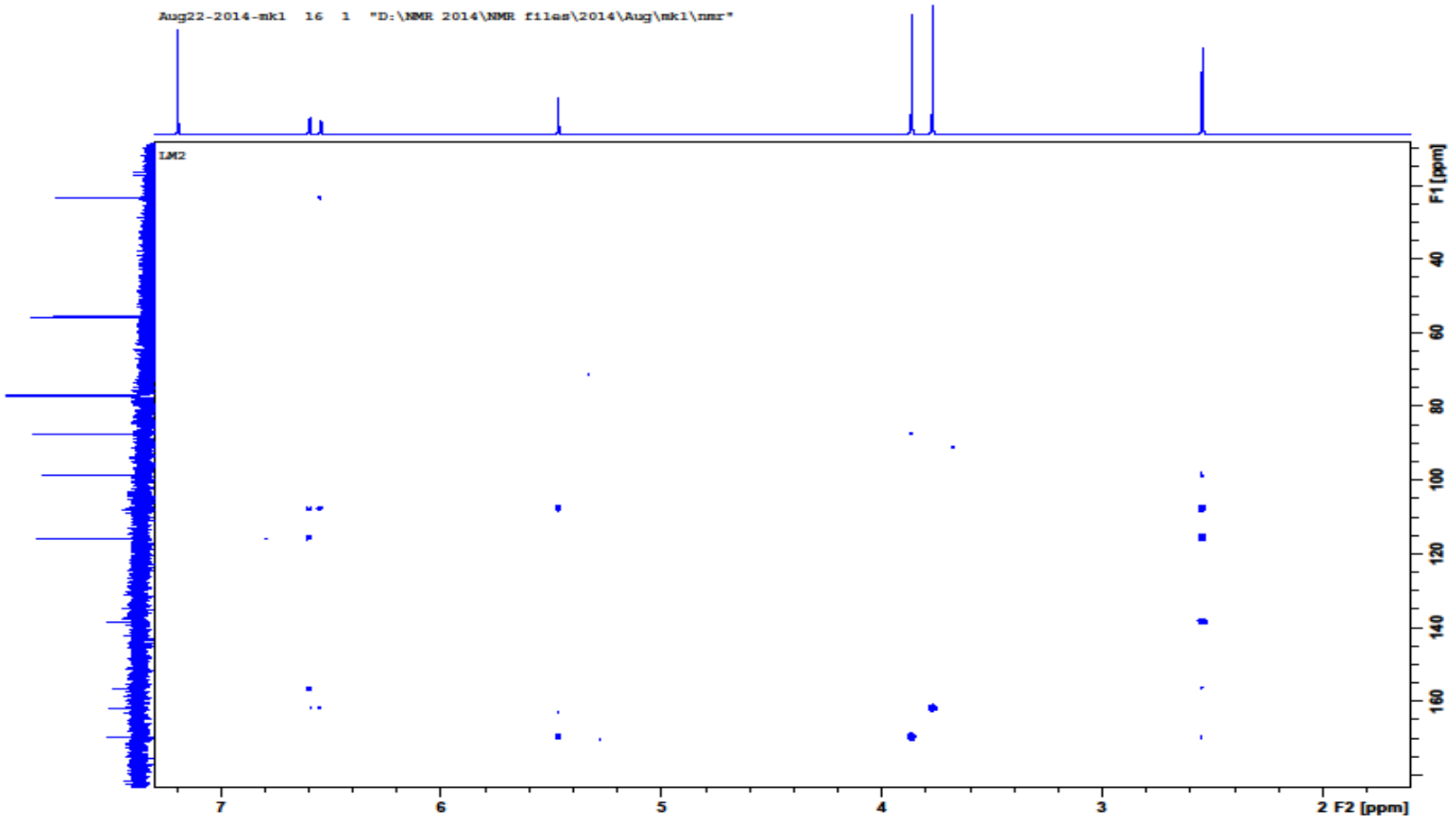
Appendix 42 HSQC Compound 181



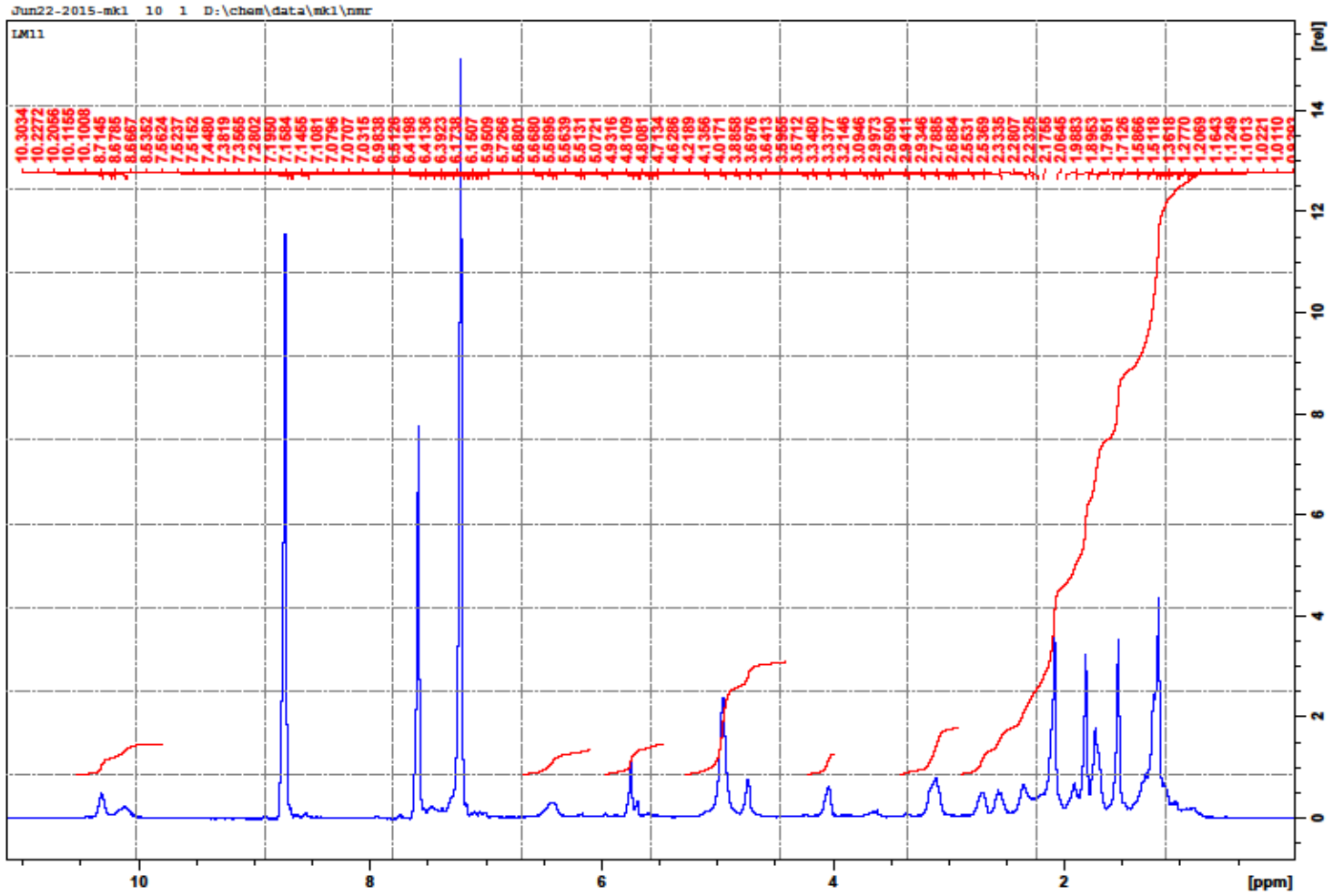
Appendix 43 COSY Compound 181



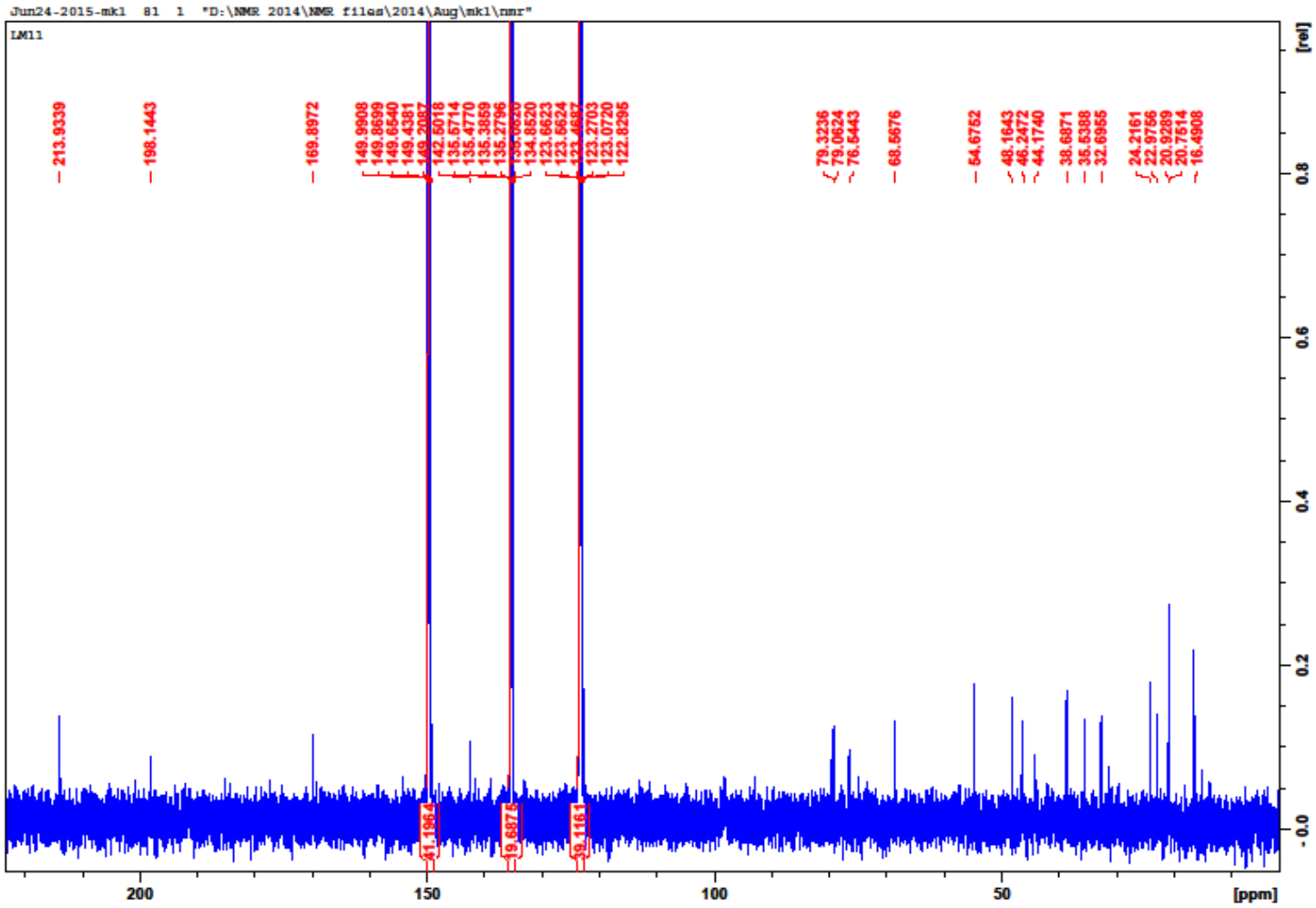
Appendix 44 NOESY Compound **181**



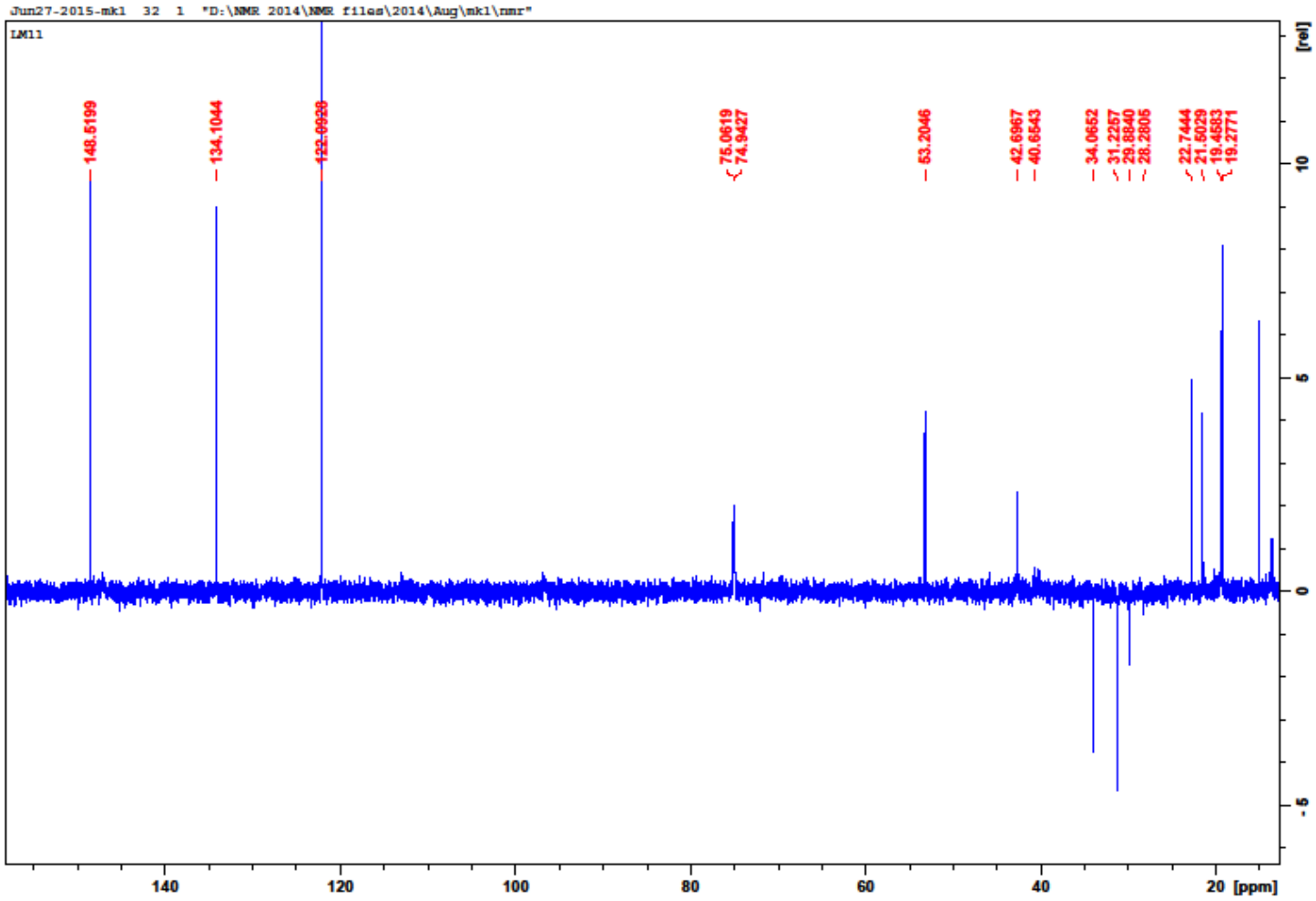
Appendix 45 ^{1}H NMR Compound **182**



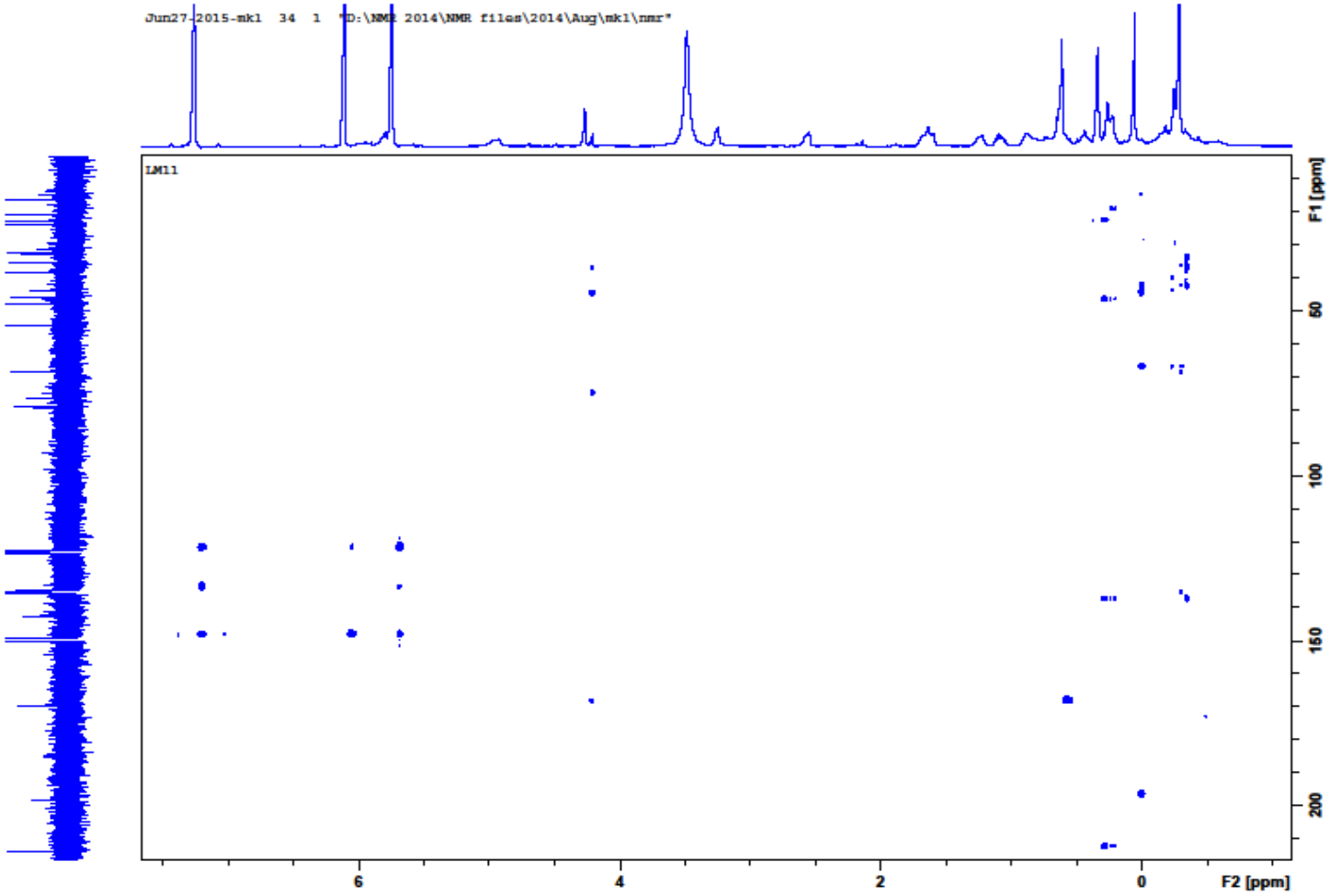
Appendix 46 ^{13}C NMR Compound **182**



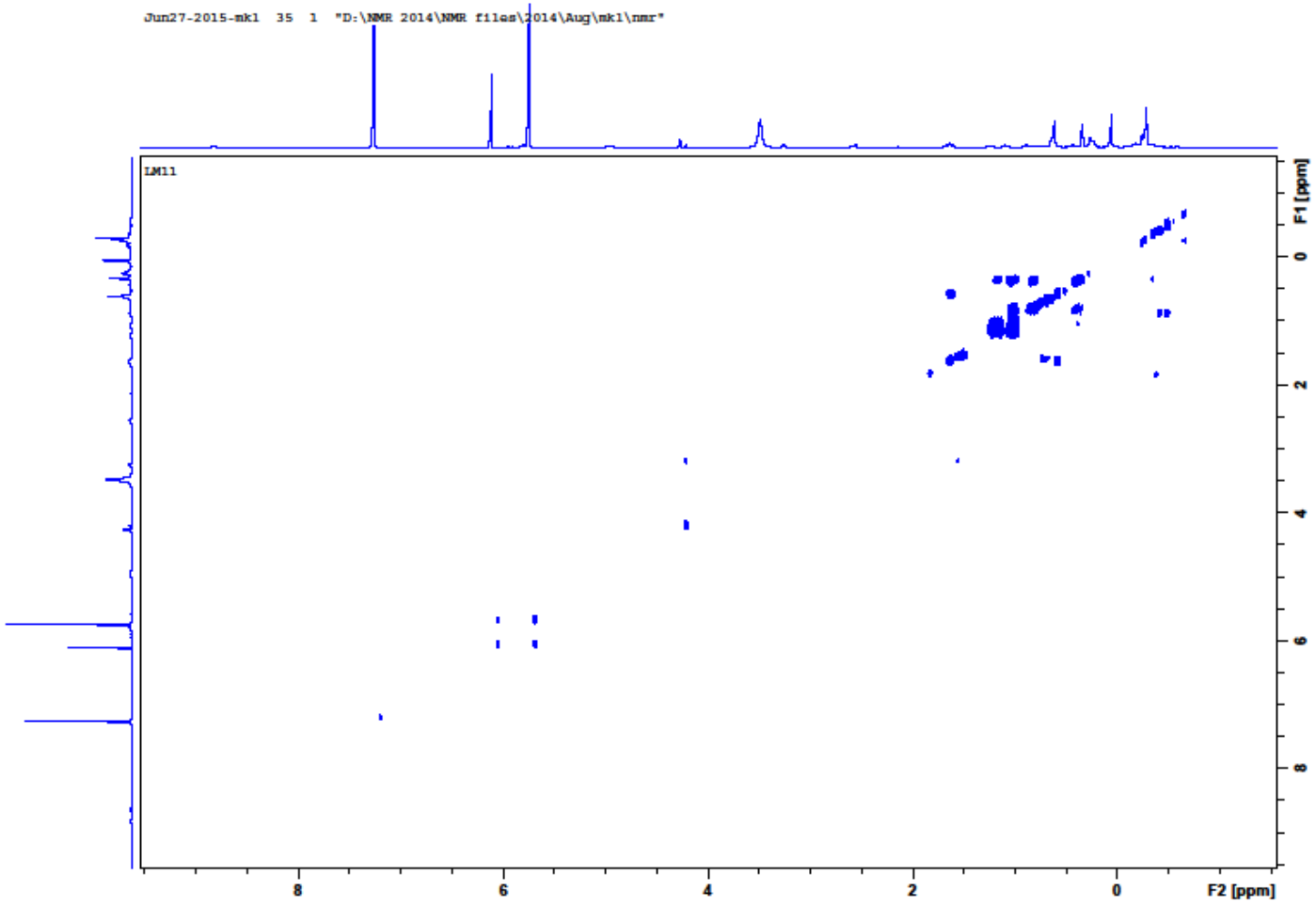
Appendix 47 DEPT Compound **182**



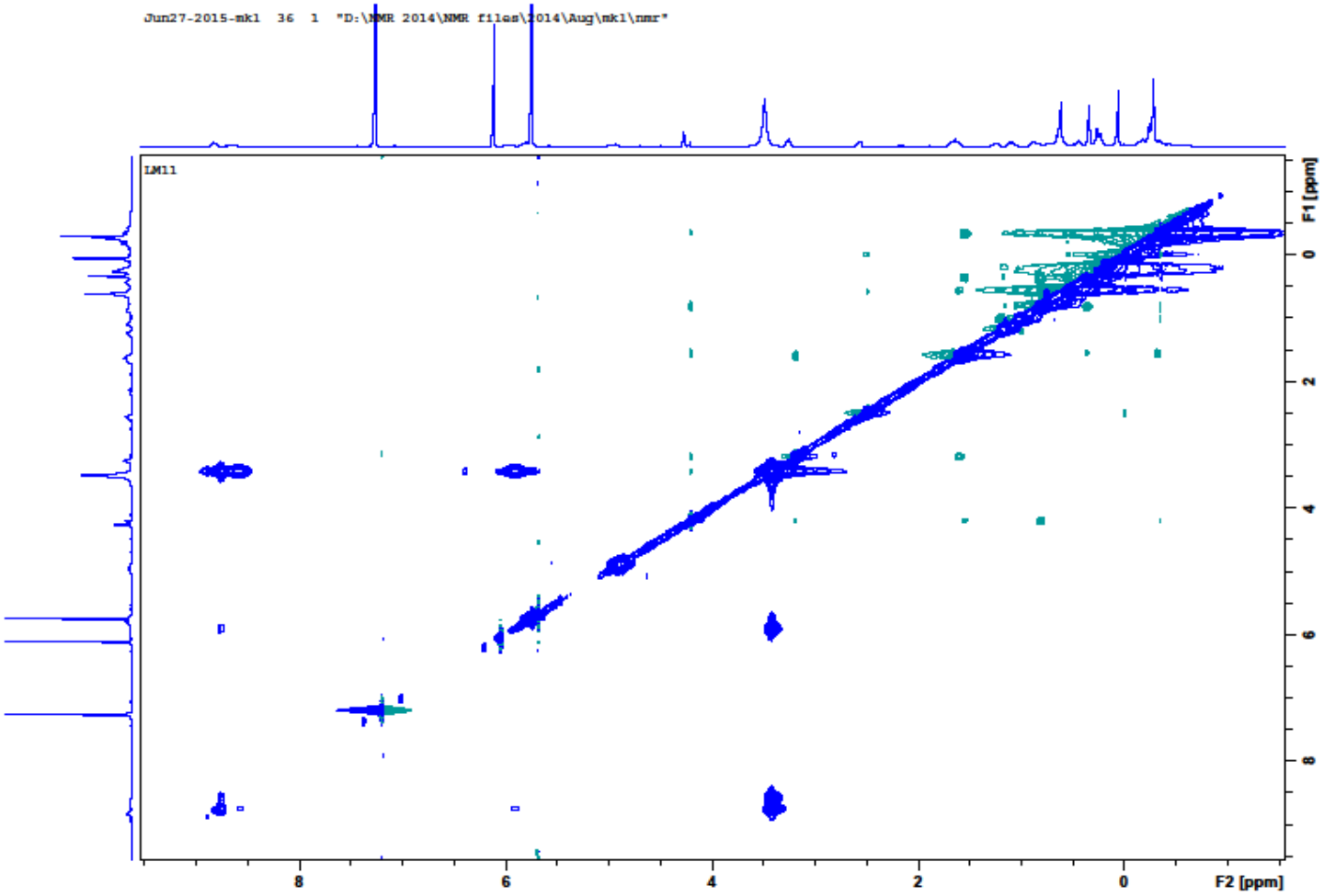
Appendix 48 HMBC Compound **182**



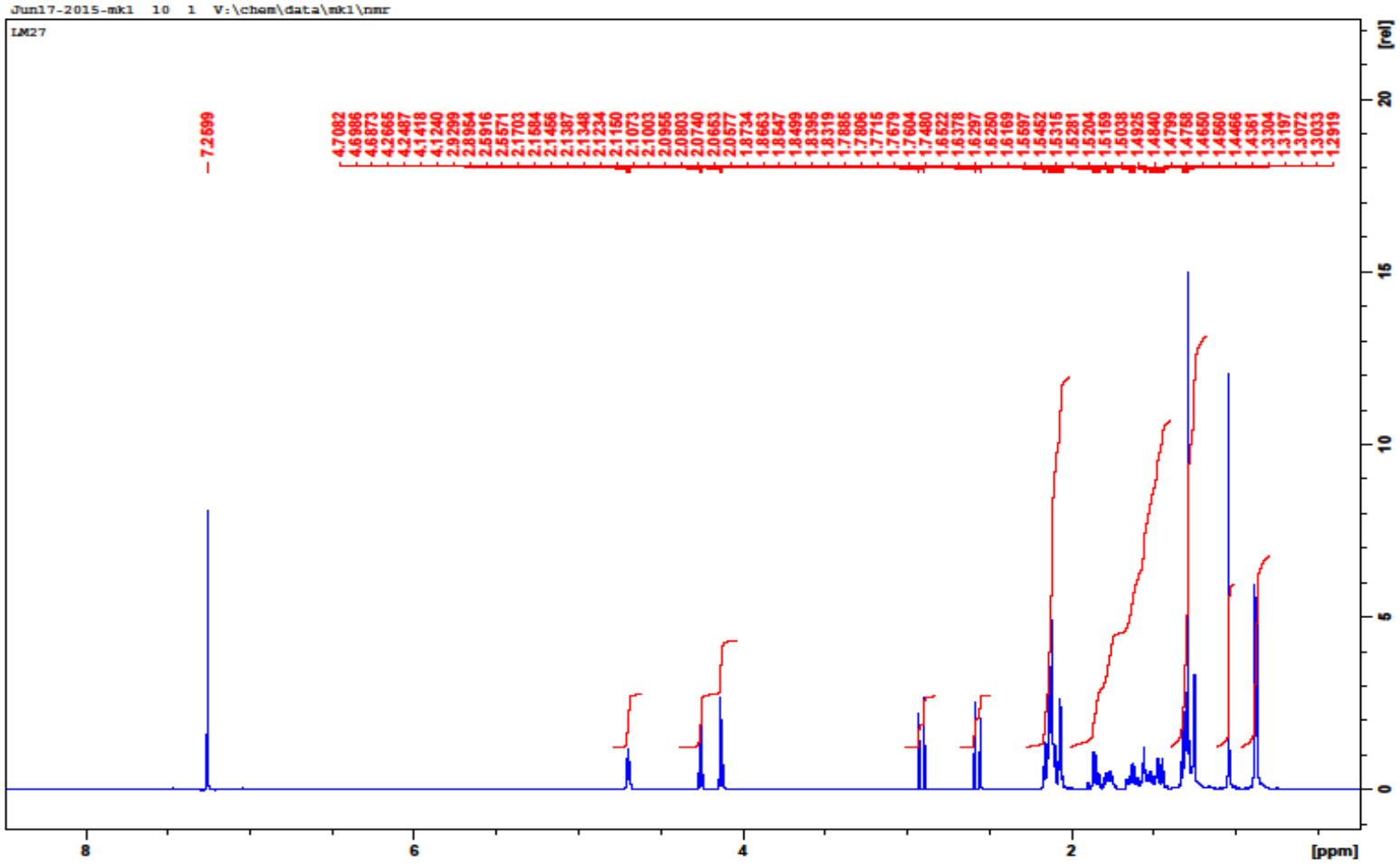
Appendix 49 COSY Compound **182**



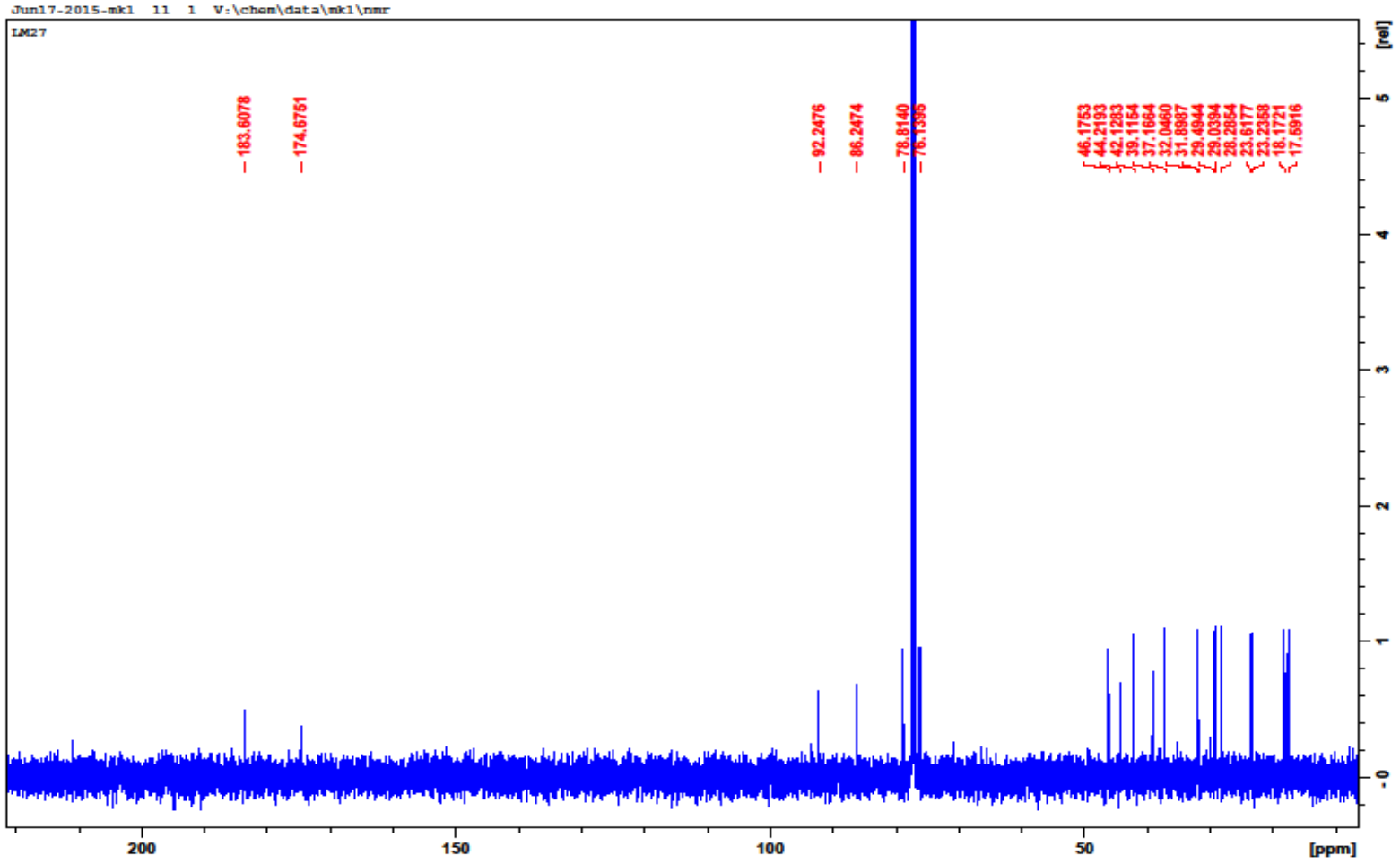
Appendix 50 NOESY Compound **182**



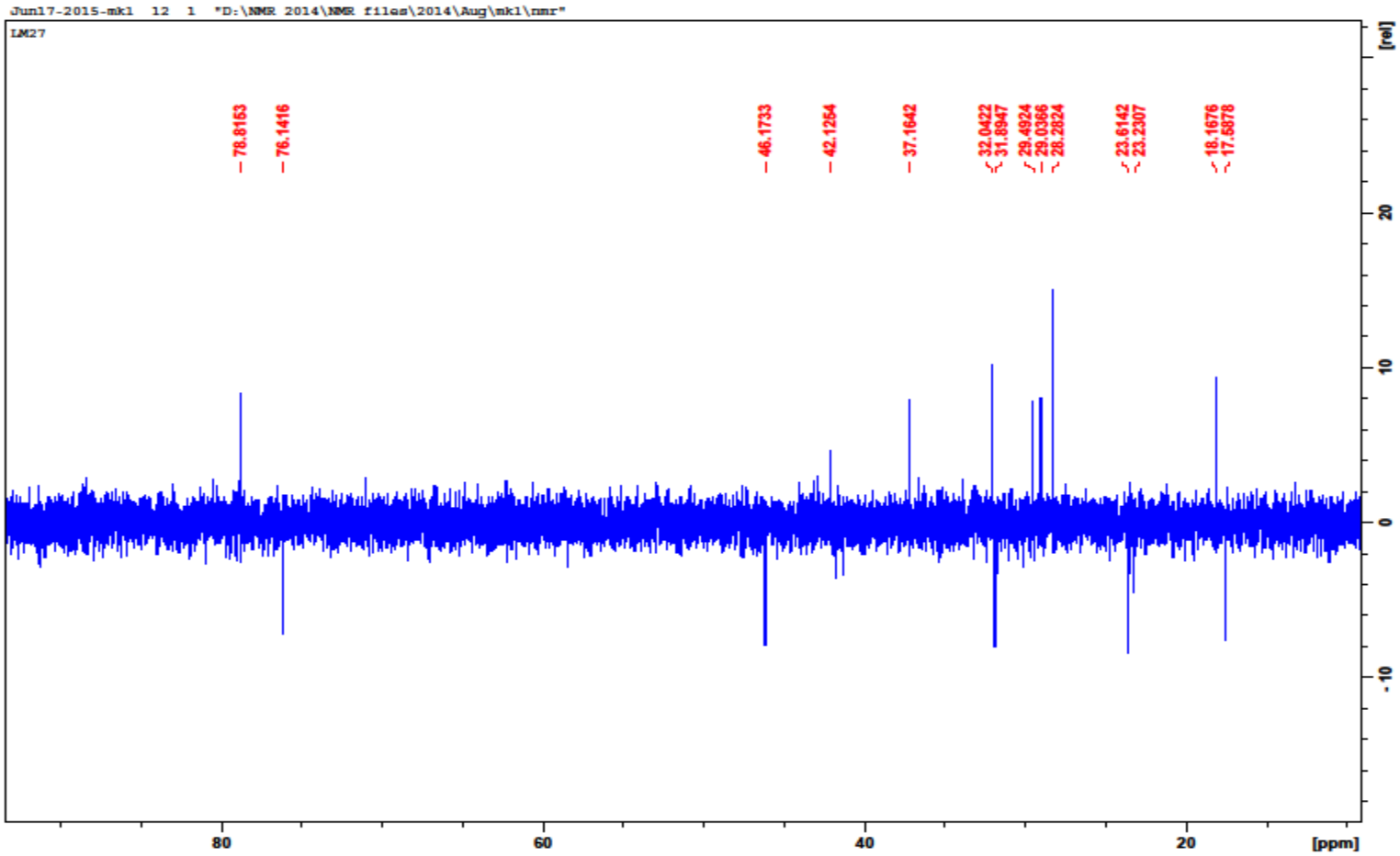
Appendix 51 ^{1}H COMPOUND **183**



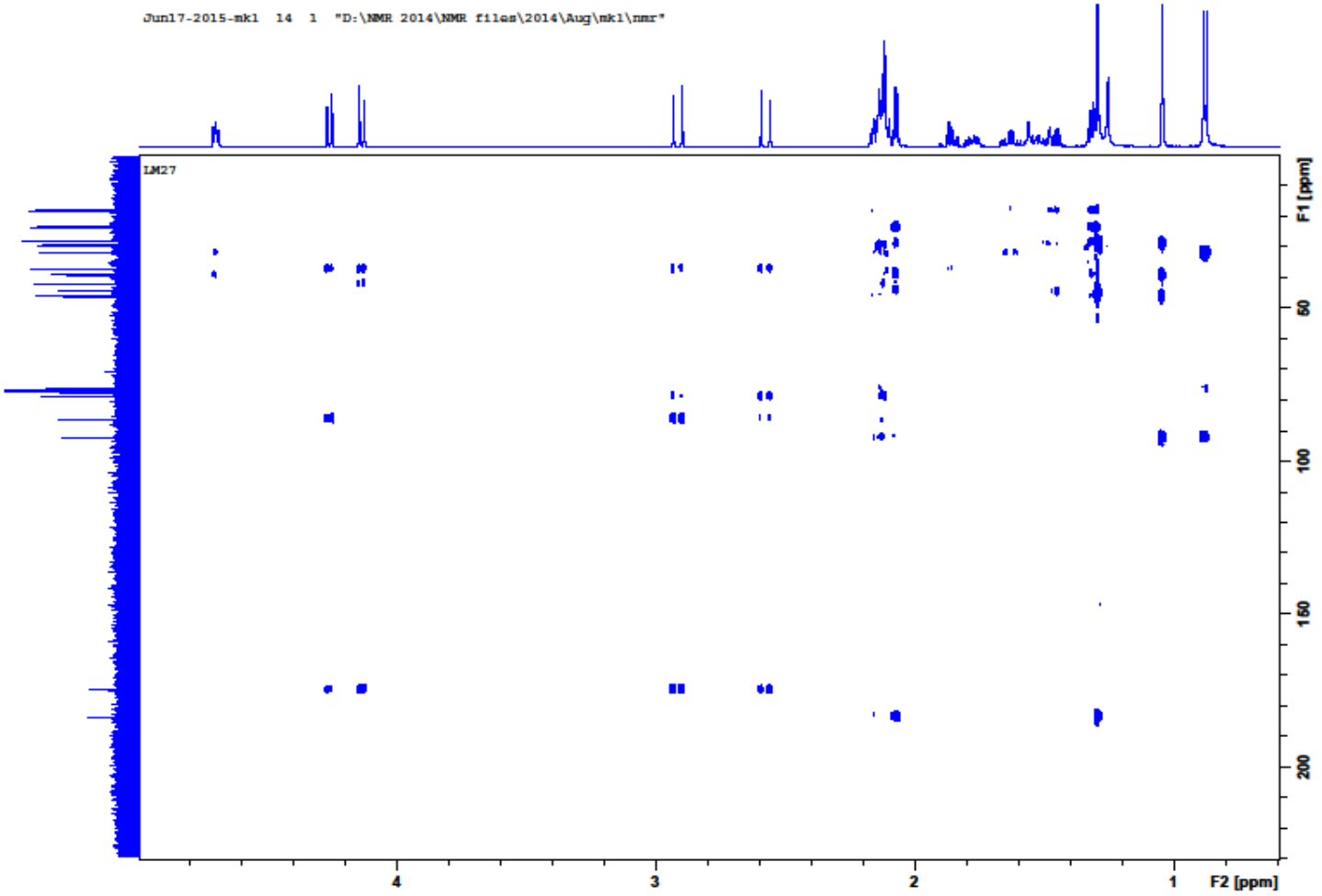
Appendix 52 ^{13}C Compound **183**



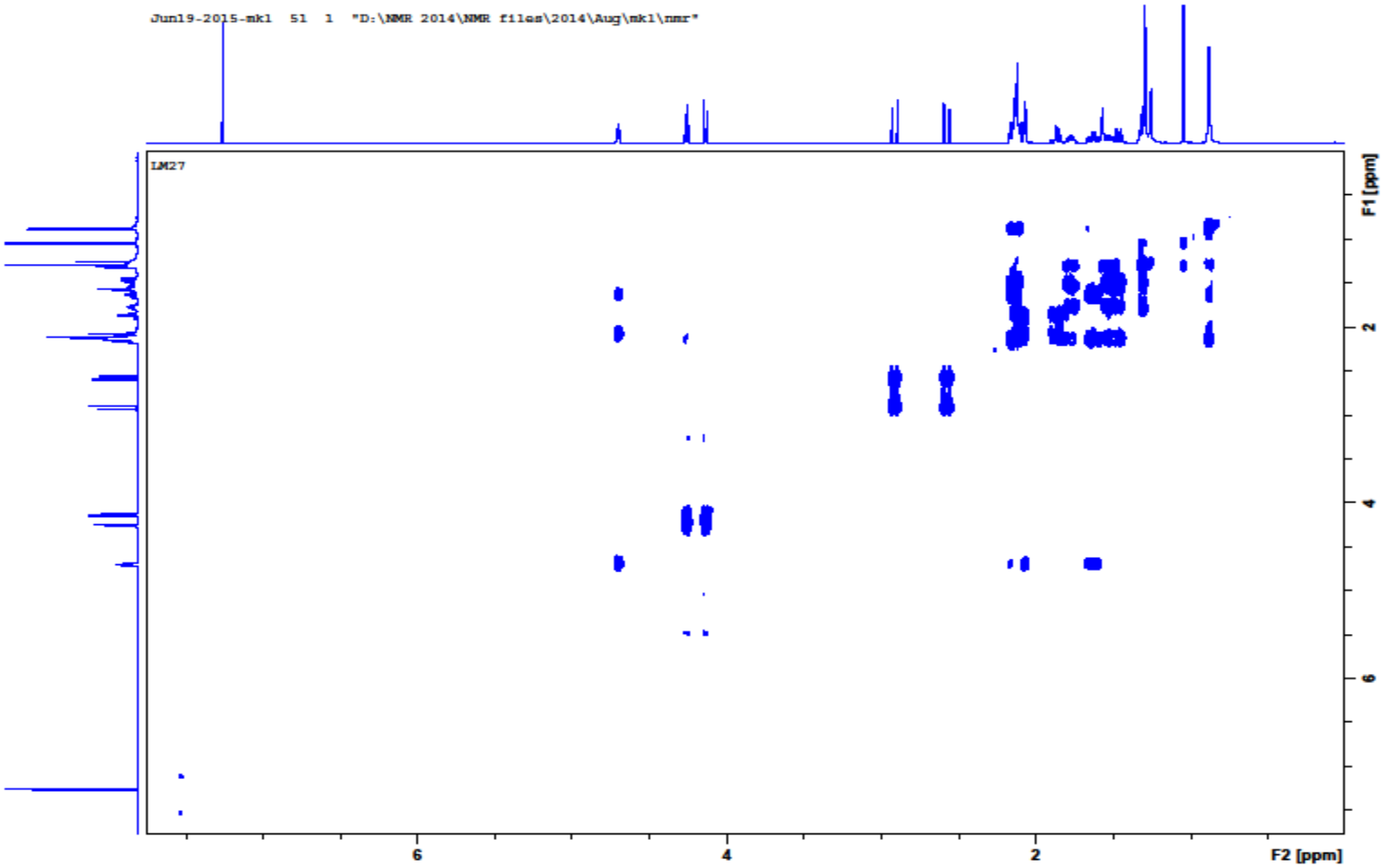
Appendix 53 DEPT Compound **183**



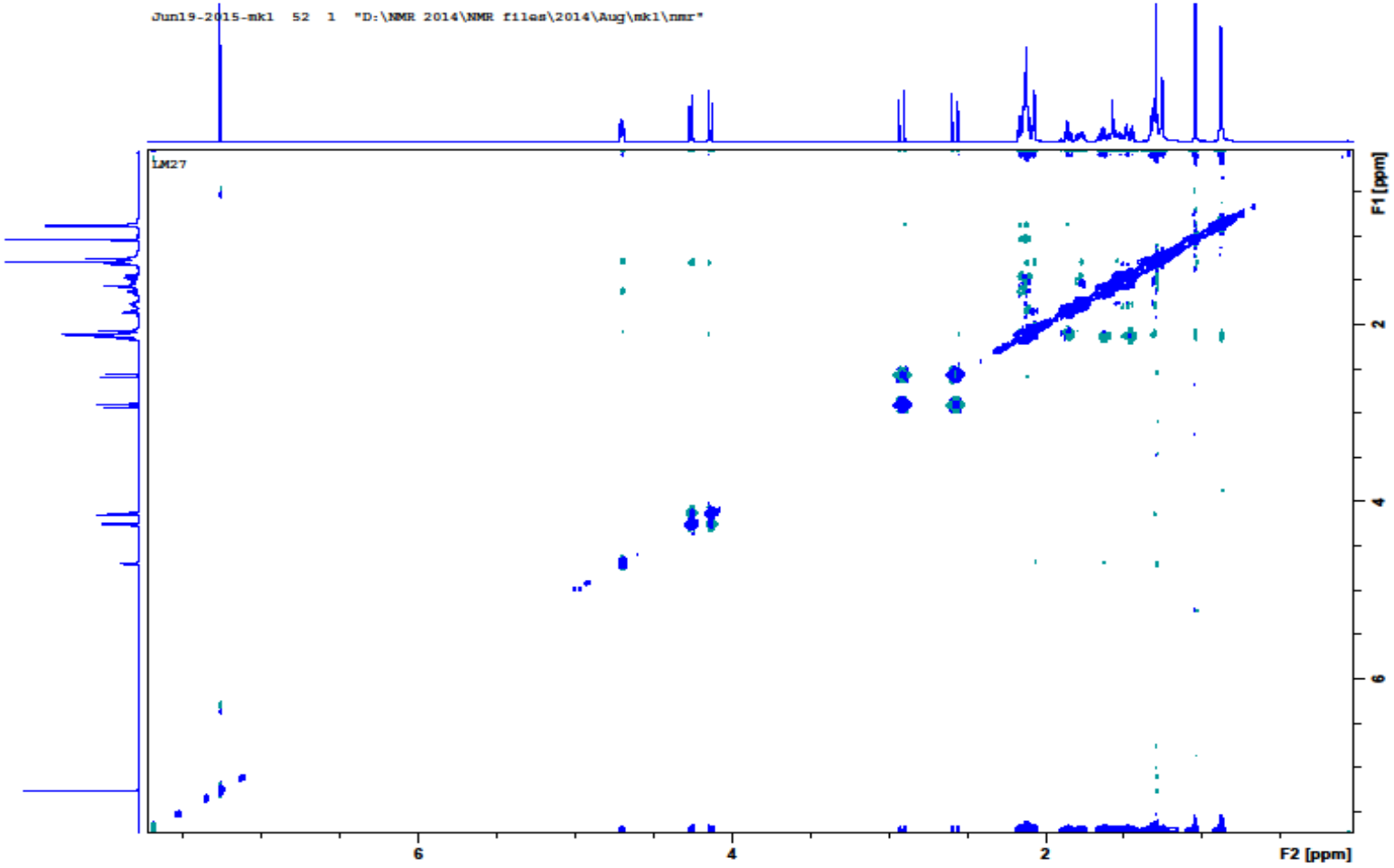
Appendix 54 HMBC Compound **183**



Appendix 55 COSY Compound **183**



Appendix 56 NOESY Compound **183**



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Compounds from *Turraea abyssinica*

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Abstract- ***Turraea abyssinica belong to the Turraea genus of the Meliaceae family and is used by the Samburus of Kenya as rungus, firewood and as fruits to induce vomiting. No phytochemicals have been reported on the Narok Kenya species. The leaves were collected from Narok Kenya, identified and voucher specimen kept for reference in Biological Department, Egerton University, Kenya. Dry powder of stem bark was successively extracted with hexane, dichloromethane, ethyl acetate and methanol for seventy-two hours. The solvents were evaporated under reduced pressure using a rotary evaporator (Büchi type R-205). With repeated column chromatography using a solvent step gradient of 20% methanol in dichloromethane, 47.5% and 95% dichloromethane in diethylether, three compounds, β-Sitosterol(1), Scopoletein(2) and 2-(1,2-Dihydroxypropyl)tetradecanoic acid (3) were isolated. Identification of pure compounds was achieved by ^{1}H and ^{13}C NMR (500 MHz) spectroscopy.***

Index Terms- ***Compounds, Narok, stembark, Turraea abyssinica,***

I. INTRODUCTION

Plants are extremely important in the lives of people throughout the world and many people depend on them to satisfy basic human needs such as food, clothing, shelter and health care. Historically, plant medicines were discovered by trial and error (Facchini et al., 2000). Turraea abyssinica belong to the Turraea genus of the Meliaceae family that comprises of about 50 genera and 1400 species (Leonardo et al., 2002). It is used by the Samburus of Kenya as rungus, firewood and as fruits to induce vomiting. This family has been known to exhibit a wide variety of biological properties (Amit and Shailendra, 2006). Though not much work has been done on it, its root methanol extract showed some antiplasmodial activity (Ndung'u, 2002). No chemical composition has t been reported on the Kenyan Narok Turraea abyssinica. In the course of this research, three compounds were isolated from the Dichloromethane extract of stembark.

II. IDENTIFY, RESEARCH AND COLLECT IDEA

Turraea abyssinica was collected from Narok Kenya, in June 2014, and a voucher specimen deposited at the Department of Biological Sciences Herbarium Egerton University, Njoro Kenya. The leaves were cut into small pieces and air-dried under shade to a constant weight. They were then ground to fine powder using a grinder at KALRO, Njoro Kenya and the masses taken using a Stanton electronic balance.
Dry powder of stem bark (1,000 g) was sequentially and exhaustively extracted with 4 L hexane, 4 L dichloromethane, 4 L ethyl acetate and 4 L methanol for seventy-two hours each in a 10 L metal tin. The solvents were evaporated under reduced pressure using a rotary evaporator. (Büchi type R-205) to give a greenish sticky residue. The dichloromethane leave extract (50 g) was subjected to a solvent step gradient of dichloromethane: methanol. Fractions containing more spots were purified by repeated column chromatography using a solvent step gradient of 20% and 33% ethyl acetate in hexane respectively. The separated components were visualized under UV lamp (254 nm and 365 nm) and then sprayed with anisaldehyde reagent and heated in an oven for one minute at 70°C. The crude extracts were spotted on aluminum TLC plates (20x20 cm Macharey Nagel Duren). The mobile phases used were varying ratios of hexane, dichloromethane, ethyl acetate, diethyl ether and methanol (AR, Scharlau). Separations were monitored with inspection under ultraviolet light (UV lamp LF-204-LS, 354 nm and 634 nm) and by spraying the plate with anisaldehyde: sulphuric acid: methanol (1:2:97) mixture. Heating was done in an oven (Electrolux Struers) at 70°C for one minute. The plates with the best R_f values were used to determine the best solvent system for the separation.

Crude extracts were then fractionated by gravity column chromatography on a 2 cm by 30 cm silica gel column (60-200 mesh Thomas Baker). Further

Compounds from Kenyan *Meyna tetraphylla*

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[2]Ssenior Lecturer in Egerton University, Kenya

Abstract: ***One Phaeophytin with a phytol side chain (1), one triterpenoid [α-Amyrin (2)] and Stigmasterol (3) were isolated from the Dichloromethane extract of the leaves of Meyna tetraphylla. Their structures were elucidated using NMR spectroscopic methods.***

Keywords: Kenyan *Meyna tetraphylla*, Phaeophytin, *α*-Amyrin, Stigmasterol, Leaves

1. Introduction

Meyna *tetraphylla* belongs to Rubiaceae family that comprises of about 637 genera and 10,700 species [4]. This family is mostly used to treat malaria, headaches, asthma, epilepsy, sore eyes and as an emetic in many developing countries. The genus consists of about 12 species found in Africa and the Indian Ocean islands to the South East Asia. Many of the members of the closely related genera Keetia, Psydrax and Multidentia have edible fruits [6].They are used to treat malaria, headaches, asthma, epilepsy, sore eyes and emetic in many developing countries [7].

It is called *Tulungwo* in Pokot and *Mutunguru* in *Kikuyu*. The plants are armed with pained spines above the nodes and the leaves appear to be in fours, actually in pairs on very short spurs at each node. The flowers are in short fascicles on these spurs, corolla lobes 4-5 and the fruit is a berry. It is a shrub or tree, which is 5-6 m long. It has white or green flowers and its fruits are bluntly 5-angled, 13-17 by 16-20 mm. The buds are sparsely hairy, pedicels densely hairy [1]. Crushed leaves are put between the infected hooves of goats and camels by the Pokots. It is also used as an animal fodder and the root decoction is given to the pregnant women to alleviate pain [1].

2. Procedure

Meyna tetraphylla leaves were collected from Baringo and Taraka Nthi counties of Kenyain June 2014. The plant was identified and a voucher specimen deposited at the Botany Department, Egerton University. The leaves were cut into small pieces and air-dried under shade to a constant weight. They were then ground to fine powder using a grinder at KALRO, Njoro Kenya and the masses taken using a STANTON electronic balance.

Exactly 1,000 gm of dry powdered leaves was sequentially and exhaustively extracted with 4 L hexane, 4 L dichloromethane, 4 L ethyl acetate and 4 L methanol for seventy two hours each in a 10 L metal tin. The solvents were evaporated under reduced pressure using a rotary evaporator (Büchi type R-205) to give a greenish sticky residue. The crude extracts were spotted on aluminium TLC plates (20x20 cm Macharey Nagel Duren). The mobile phases used were varying ratios of hexane, dichloromethane, ethyl acetate, diethyl ether and methanol (AR, Scharlau). Separations were monitored with inspection under ultraviolet light (UV lamp LF-204-LS, 354 nm and 634 nm) and by spraying the plate with anisaldehyde: sulphuric acid: methanol (1:2:97) mixture. Heating was done in an oven (ELECTROLUX STRUERS) at 70°C for one minute. The plates with the best R_f values were used to determine the best solvent system for the separation. Exactly 50 gm of the crude extract was then fractionated by gravity column chromatography on a 2 cm by 30 cm silica gel column (60-200 mesh Thomas Baker). Further purification was achieved by repeaed thin layer chromatography and column chromatography.

Identification of pure compounds was achieved by ^{1}H and ^{13}C NMR. NMR spectra were recorded at room temperature on a 500 MHz Bruker AVANCE NMR at the School of Biomedical and Molecular Sciences, University of Surrey at Guildford UK. Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS) as internal standard and coupling (J) are given in Hz.

3. Results

All the leaves crude extracts showed almost similar spots with the dichloromethane extracts having more spots on visualizing with a UV lamp and anisaldehyde spraying reagent. **Compound 1** (9.20 mg) was a green sticky solid with a green spot on visualization with anisaldehyde reagent and UV active with an R_f of 0.5 in 5% diethyl ether in dichloromethane. The ^{13}C NMR spectra gave fifty five carbon resonances. Sixteen of the resonances belong to four pyrrole carbons, one methoxy carbons (δ 53.1), eleven methyl carbons ranging between δ 11.2 ppm and δ 23.3 ppm, three carbonyl carbons (δ 172.6 ppm, δ 171.0 ppm, δ 189.8 ppm), sixteen methylene carbons ranging between (δ 20.0 ppm and δ 142.3 ppm), nine methine carbons (δ 28.2 ppm - δ 132.0 ppm) and fifteen quaternary carbon signals. The three carbonyl carbon signals (C-9, C-7c, and C-10a) occurred at the low field of δ 171.0 ppm-δ 189.8 ppm (Table 1). All the carbon resonances were characterized by DEPT experiments.

The ^{1}H resonances at δ 1.81 ppm, δ 3.67 ppm and δ 2.52 ppm showed a characteristic of four methyl groups attached to the pyrrole ring corresponding to the ^{13}C NMR resonance at δ 23.3 ppm, δ 12.3 ppm and δ 11.3 ppm in the HSQC-DEPT spectrum (Fig 1). The ^{1}H and ^{13}C signals at δ 3.9 ppm (δ 53.1 ppm) were characteristic of one methoxy group. In the COSY spectrum there was a correlation between H-8 (δ 4.46 m) and resonance at δ 1.81 d (H-8a) and δ 1.71 t (H-4b). The spectrum further showed a correlation between H-7a (δ 2.32 m) and resonance at δ 4.21 m (H-7). **Compound 1** was identified as Phaeophytin [8]

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Terpenoids from Kenyan *Leonotis mollissima*

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Abstract
Leonotis mollissima belongs Leonotis genus that comprises of ten species. It is called *kipserere* by Marakwets of Kenya. No phytochemicals have been reported on the Laikipia Kenya species. The leaves were collected from Laikipia University Kenya, identified and voucher specimen kept for reference in Biological Department, Egerton University, Kenya. Dry powder of leaves was successively extracted with hexane, dichloromethane, ethyl acetate and methanol for seventy two hours. The solvents were evaporated under reduced pressure using a rotary evaporator (Büchi type R-205). With repeated column chromatography using a solvent step gradient of 20% and 33% ethyl acetate in hexane three compounds, 4, 7-dimethoxy-5-methylchromen-2-one (1) [an aromatic compound], 12β-acetoxy-20-hydroxy-3, 7, 11, 15-tetraoxo-25, 26, 27-trisnorlanost-8-en-24-oic acid (2) [a triterpenoid] and (13R)-19α, 13α-epoxylabda-6β(19).16(15)-dioldilactone (3)[a diterpenoid] were isolated. Identification of pure compounds was achieved by ^{1}H and ^{13}C NMR (500 MHz) spectroscopy.

Keywords: *Leonotis mollissima*, Siderin, 20-hydroxylucidenicacid d2) labdane, leaves

Introduction
Plants are extremely important in the lives of people throughout the world and many people depend on them to satisfy basic human needs such as food, clothing, shelter and health care. Historically, plant medicines were discovered by trial and error (Facchini *et al*., 2000) [4].
Leonotis mollissima belongs to the genus Leonotis that comprises of about 10 species and to the Lamiaceae family that has 7,200 species distributed in 236 genera (Nurdan and Aysel, 2007) [3]. They are known to treat cold, cough, fever, headache and asthma (Fowler 2006) [1]. The root decoction is used by the Marakwets to treat J. wound, festering sore and intestinal worms. Young leaves and buds are used to treat conjunctivitis and indigestion and are also chewed for cramp in the stomach (Kokwaro, 1976) [2]. No chemical composition and biological activity has not been reported on the Kenyan Laikipia *Leonotis mollissima*. In the course of this research, three compounds were isolated from the Dichloromethane extract of leaves.

Materials and Methods
Leonotis mollissima was collected from Laikipia University Kenya, in June 2014, and a voucher specimen deposited at the Department of Biological Sciences Herbarium Egerton University, Njoro Kenya. The leaves were cut into small pieces and air-dried under shade to a constant weight. They were then ground to fine powder using a grinder at KALRO, Njoro Kenya and the masses taken using a Stanton electronic balance.
Dry powder of leaves (1,000 g) was sequentially and exhaustively extracted with 4 L hexane, 4 L dichloromethane, 4 L ethyl acetate and 4 L methanol for seventy two hours each in a 10 L metal tin. The solvents were evaporated under reduced pressure using a rotary evaporator. (Büchi type R-205) to give a greenish sticky residue. The dichloromethane leave extract (50 g) was subjected to a solvent step gradient of dichloromethane: methanol. Fractions containing more spots were purified by repeated column chromatography using a solvent step gradient of 20% and 33% ethyl acetate in hexane respectively. The separated components were visualized under UV lamp (254 nm and 365 nm) and then sprayed with anisaldehyde reagent and heated in an oven for one minute at 70°C.
The crude extracts were spotted on aluminium TLC plates (20x20 cm Macharey Nagel Duren). The mobile phases used were varying ratios of hexane, dichloromethane, ethyl acetate, diethyl ether and methanol (AR, Scharlau). Separations were monitored with inspection under ultraviolet light (UV lamp LF-204-LS, 354 nm and 634 nm) and by spraying the plate with anisaldehyde: sulphuric acid: methanol (1:2:97) mixture. Heating was done in an oven (Electrolux Struers) at 70°C for one minute. The plates with the best R_f values were used to determine the best solvent system for the separation.
Crude extracts were then fractionated by gravity column chromatography on a 2 cm by 30 cm silica gel column (60-200 mesh Thomas Baker).