JIMMA UNIVERSITY

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DEPARTMENT OF CHEMISTRY

PHYTOCHEMICALINVESTIGATIONOF THEFRUITS OF *LANTANA CAMARA*FOR ANTIBACTERIAL ACTIVITY

BY

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JIMMA, ETHIOPIA

PHYTOCHEMICAL INVESTIGATION OF THE FRUITS OF *LANTANA CAMARA*FORANTIBACTERIAL ACTIVITY

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES,JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

By

SEIFU TEMESGEN

ADVISORS:

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2) Mr. YINEBEB TARIKU (MSc)

DECLARATION

This research is my original work that I have done for the fulfillment of the requirement of Masters of Science in Chemistry, at Jimma University, in the year 2016.

Name: Seifu Temesgen

Sign: ----------

Date: ----------

Advisors

1) Dr. Tsegaye Deyou

Sign: ---------- Date: ----------

2) Mr. Yinebeb Tariku

Sign: ---------- Date: ----------

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Abbreviations/Acronyms

CFU : Colony forming unit

DMSO: Dimethyl Sulfoxide

IR: Infrared spectroscopy

MBC: Minimum Bacterial concentration

MHB: Mueller Hinton Broth

NMR: Nuclear magnetic resonance spectroscopy

TLC: Thin layer chromatography

COSY: Correlation Spectroscopy

HMBC: Heteronuclear Multiple Bond Coherence

HSQC: Heteronuclear Single Quantum Coherence

Acknowledgement

I would like to express my sincere gratitude to my advisors Dr. Tsegaye Deyou and Mr.Yinebeb Tariku for their guidance and support during this research work. I also thank Dr. Legesse Adane my ex-supervisor in setting this research work and put me in the line of research. I would also to extend my thanks to Dr. Negera Abdissa and Lijalem Ayele, for generating spectroscopic data of my samples. Dr. Yared Merdassa, Dr. Abera Gure are also thanked for their moral support during ups and down period I had been facing. My gratitude also goes to all postgraduate students to have wonderful time together. Mr. Girma Geleta from Biology department is thanked for assisting me during generation of biological data of my samples. Finally, I am indebted to Jimma University community, specifically, staff of Department of Chemistry for bringing me all the way to this level.

ABSTRACT

The aim of this research is to carry out phytochemical investigation and evaluation of antibacterial activity of the crude extract and isolated compounds from fruits of *Lantana camara* (Verbenaceae). In this regard, the dried and powdered fruits of *Lantana camara* were subjected to sequential solvent extraction using petroleum ether, chloroform, acetone and methanol by maceration. The extract in each case was filtered and concentrated on Rota evaporator under reduced pressure at 50°C. Antibacterial evaluation of the crude extracts were done using *in vitro* method against four bacterial strains: *Staphylococcus aureus* (Gram positive), *Escherichia coli* (Gram negative), *Pseudomonas aeruginosa* (Gram negative) and *Bacillus cereus* (Gram negative) with zone of inhibition ranging from 6-15mm. The petroleum ether extract was subjected to chromatographic separation and yielded of two compounds (**37** and **40**). Similar treatment of the acetone extract afforded one compound (**41**). The three isolated compounds were also tested for antibacterial activities against the above bacterial strains. Compound **41** showed good activity with zone of inhibition 15.75 mm which is more than half of that of the reference drug, gentamycin (20.5 mm).

CHAPTER ONE
INTRODUCTION

1.1 General

The past two centuries have seen enormous achievements in control of infectious diseases, previously the leading cause of death, in large measure due to sanitation and food safety, vaccines, antibiotics and improved nutrition. This has led people to put their faith in the notion that medical science would succeed in overcoming the remaining obstacles. Vaccination has eradicated smallpox, nearly eradicated poliomyelitis and greatly reduced many other highly dangerous infections such as diphtheria, tetanus and measles. New diseases such as HIV and new forms of influenza have taken both professional and popular opinion by surprise and have renewed the challenges before the world public health community. Emergence of antibiotic-resistant strains of common organisms due to overuse of antibiotics and lack of vaccines for many dangerous microorganisms poses problems to humanity [1]. This stresses the need for new vaccines, effective antibiotics and strengthened environmental control measures. New knowledge of the microbiological origins of cancers such as that of the cervix, stomach and liver have strengthened primary prevention and brought hope that new cures will be found for other chronic diseases of infectious origin. Infectious diseases are in general, the world's leading cause of premature deaths, killing almost 50,000 people every day [2].

Morbidity and mortality due to diaharea continues to be a major problem in many developing countries, especially amongst children's, such as pathogenic *Escherichia coli, V. cholera, Aoromonas Spp., Shigella Spp.,* and *S. aureuse* are most common [2]. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world [1, 2, 3], with the continues use of antibiotics, microorganisms have become resistance. In addition to this problem, antibiotics are sometimes associated with adverse effects on host, which include hyper sensitivity, depletion of beneficial gut and mucosal microorganisms, immune suppression and allergic reactions [4]. This has created immense clinical problem in the treatment of infectious disease [5]. Therefore, a need to development alternative antimicrobial drugs for the treatment of infectious disease, one approach is to screen local medicinal plants for possible antimicrobial properties. Plant materials remain an important resource to combat serious diseases in the world [6]. According to WHO the world's population is dependent on the traditional medicine and a major part of the traditional therapies involves the use of plant extracts or their active constituents, yet a

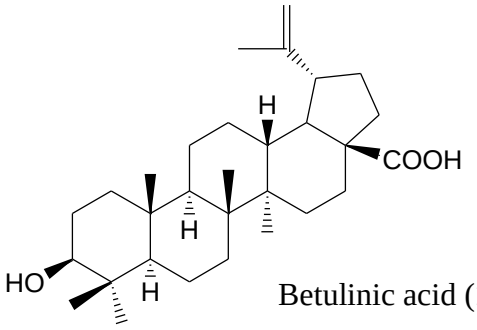
scientific study of plant to determine their active compounds is a comparatively new field. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health care needs in the developing countries. Research and acquisition of new knowledge, risk communication, application of currently available means and fair distribution will be great challenges to public health in the coming decades.

1.2 Traditional Medicine and Ethnopharmacology

Indigenous people and ancient civilizations experimented with various plant and animal parts to determine what effect they might have on humans. Traditional healers found that some had healing power through trial and error. These represented the first crude drugs and knowledge passed down through the generations and systematized, for example in traditional Chinese Medicine and Ayurveda [7]. Many of these traditional medicines have real, beneficial effects; and extracts of these crude drugs led to the discovery of their active ingredients and eventually to the development of modern chemically pure drugs [8]. The use of medicinal herbs and herbal medicine is an old human tradition and the recent progress in modern therapeutics has stimulated the use of natural products worldwide for diverse ailments and diseases. According to WHO[9], traditional medicine is popular in all regions of the world and its use is rapidly expanding even in developed countries. For example, in China, traditional herbal preparations account for 30–50% of the total medicinal consumption and before thirty five years ago the annual global market for herbal medicine is over 60 billion USD [10].

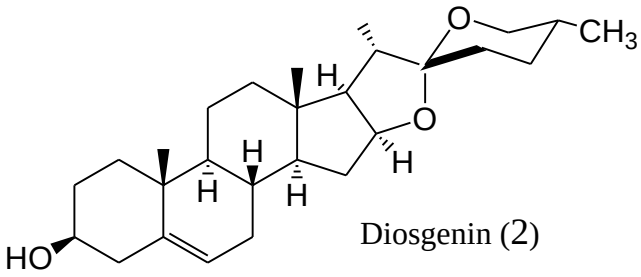
1.3 Pharmacological Drugs from Medicinal Plants.

Betulinic acid (**1**) is a lupane-type triterpene that is widely distributed in the plant kingdom, and this compound, along with various derivatives, has been shown to have anticancer, antibacterial, antimalarial, anti-HIV, anthelminthic, anti-inflammatory, and antioxidant properties [11].



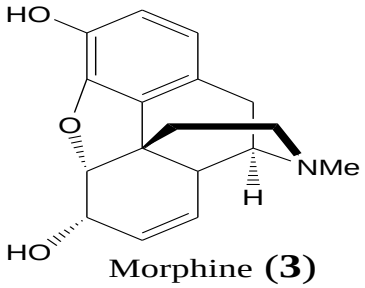
Betulinic acid (**1**)

It is also important to note that, Diosgenin (**2**), a steroidal sapogenin obtained from the tubers of various *Dioscorea* species that grow in Mexico and Central America, can be converted chemically in several steps into progesterone; progesterone is an important anti-inflammatory drug [12].



Diosgenin (2)

Morphine (**3**), an opiate analgesic alkaloids from *Papaver Somniferum*, is a drug that is still used widely today for the alleviation of severe pain [13].



Morphine (3)

1.4 Traditional Uses of *Lantana camara*

The genus *Lantana*is used in many parts of the world to treat a wide range of disorders [10]. For instance, its leaves and flower have been used traditionally in folk remedies for treating fever, influenza and the whole plant was reported to have remedies against cold, rheumatism, asthma and high blood pressure, the leaves alone is used to treat sores, chicken pox, measles (leaves), stomach ache (powdered root in milk), cough (decoction of leaves) [14], tetanus, malaria and ataxia of abdominal viscera (whole plant) [15], antiseptic for wounds (leaf oil) [16]. It was reported that *Lantana camara* has good potential to treat fistula, pustules, tumors and rheumatism (fruits/berries)[14,17-19], antilymphocytic, immunosuppressive, hepatoprotective, thrombin inhibitory, termiticidal, ant motility, antifilarial, *in vitro* cytotoxic and antimicrobial activity (different parts of the plant) [20-24]. Moreover, the leaf extract has been reported to possess the wound healing and ant diabetic property [20]. As in the rest part of the world, medicinal plants have been also used in Ethiopia for many centuries, the use of which has become an integral part of the different cultures [25]. Various ailments among people in different parts of the country, in using

knowledge and usage of herbal medicine for different treatment is still a major part of their life and culture. For example, in Libo-Kemekem district in north Ethiopia, *Lantana camara* is medicinal plants and play an important role in the healthcare system of the community in the district [26]. Traditional medicinal plants were harvested mostly from natural vegetation area followed by home gardens[26]. *Lantana camara*isan important medicinal plant, locally known as '*Yewof-qolo*' in Amharic and '*Midhan dubara*' in Afan Oromo. It is found mostly on fertile sandy and light clay soil of all regions of Ethiopia, running water and birds has been played a major role for their spreading which feed on it. Fruits are edible (fresh and ripe) eaten by children in dry area. Different parts of this plant species contain many chemical constituents; therefore, it can contribute for its medicinal values and other industrial purposes like perfumery [27].Powder of stem of *L. camara* in water has potential in the treatment of diarrhea [28].

CHAPTER TWO
REVIEW OF LITERATURE

2.1 Botanical Information

Lantana camara (Verbenacea*)* comprises 2600 species in100 genera. It can grow in compact clumps, dense thickets is heavily branched andevergreen shrub. Stems are square, with small prickles, hairy when young. The leaves are broadly oval in shape with toothed edges arranged in opposite pairs. Flowers are a mixture of cream, pink or orange numerous small rounded heads. Fruits are flesh green in cluster Changed to black, when ripened [14].

Figure 1. Aerial parts of *Lantana camara* plant (Picture by Seifu, April 2015)

2.2Biological Activities of Crude Extracts of *Lantana camara*

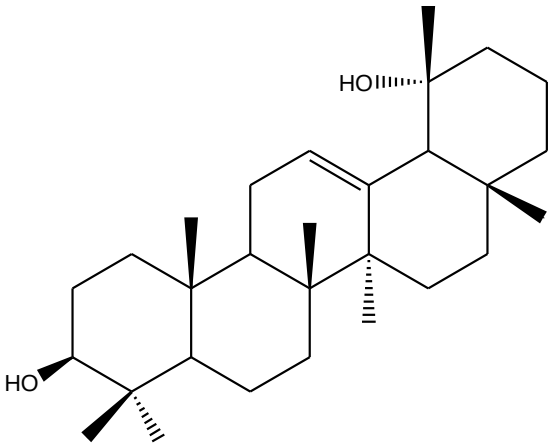
Different parts of this plant are being used in various parts of the world to treat several human illnesses. This fact led scientists to carry out scientific investigations in order to find out the chemical agents that could possibly be responsible for the observed therapeutic activities. Itis a reservoir of several important bioactive molecules. And considered as one of the important medicinal plants of the world [29]. The plant is said to possess constituents like alkaloids, terpenoids [30-32], flavonoids [33] steroids [30-33], and phenolic [34]. Several classes of compounds such as mono and sesquiterpenes, triterpenes, iridoid glycosides, furanonaphthoquinones, and phenylethanoide glycosides have been also reported from this species in genus [22].For many years, natural products from *Lantana camara* have been used in the prevention and cure of many serious diseases all over the world [34].

The essential oil and extracts of *L. camara* are used in herbal medicines for the treatment of various human diseases such as skin itches, leprosy, cancer, chicken pox, measles, asthma, ulcers, tumours, high blood pressure, tetanus, rheumatism, etc. The leaves extracts has been also reported to have antifungal[35], antiproliferative[36], antibacterial[37], nematicidal [38], anthelmintic [39] and anticancer activities [40].Extracts of stems and compounds isolated from the seedlings of *Lantana camara* were assessed for their antifilarial activity *in vitro* and *in vivo* [43]. The dichloromethane extract of leaves (pink flower) was also found to show very promising activity *in vitro* against cultures of chloroquine-sensitive (3D7), chloroquine-sensitive strain (D10) and chloroquine resistant (W2) strains of *Plasmodium falciparum*, and also showed *in vitro* cytotoxicity against human WI-38 fibroblasts [44-45].The non-polar extract of root-bark also displayed high anti-malarial activity against the multidrug resistant K1 strain [46]. Its methanol and ethanol extracts of the leaves and flowers of also showed mosquito larvicidal activity against 3rd and 4th instarlar vae of *Aedes aegypti* and *Culex quinquefasciatus* [47]. Chloroform fractions obtained from *Lantana camara* flowers showed repellent properties against *Aedes* mosquitoes (*Aedesalbopictus, Aedesvittatus* and *Aedesaegypti*) [48]. The petroleum ether and methanol extracts of the aerial part of *Lantana camara* have been reported to be toxic to *Calloso bruchuschinensis* [49]. Premature leaves of *Lantana camara* showed antioxidant activity [50]. An investigation of acetone extracts from leaves of *Lantana camara* showed growth inhibitory effects against two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram-positive (*B.cereuse*and *S. aureus*) bacteria [51]. A crude extract of *Lantana camara* leaves had a cytotoxic

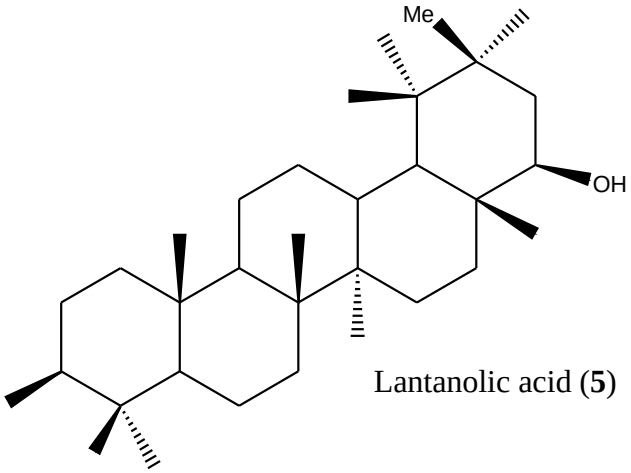
effect on HeLa cells, by employing the 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay that an increase in the concentration or duration of the extract treatment was effective in killing cancer cells[52].The crude methanol extract of leaves of it showed antidiabetic activity [51].Methanol extracts prepared from the leaves of it were found to inhibit human R-thrombin [52].The crude Chloroform extract of its fruits showed antibacterial activities against *Pseudomonas aeruginosa, Salmonella thyphimurium, Staphylococcus aureus and Escherichia coli* [52].

2.3Compounds from *Lantana camara*and their Biological Activities

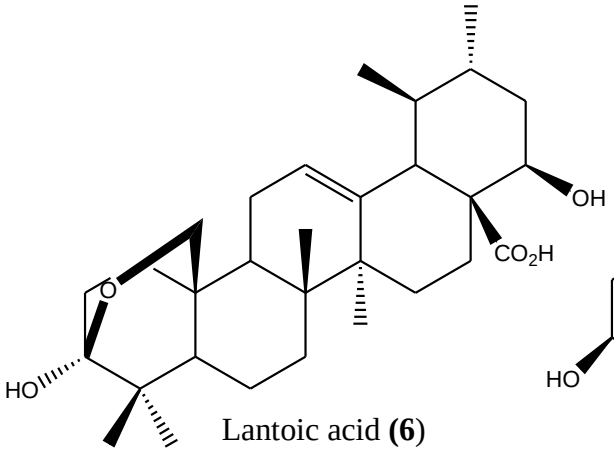
Compounds isolated from the aerial parts of *Lantana camara* such as pomolicacid (**4**)(from root and stem), lantanolic acid (**5**)(from roots) and lantoic acid (**6**)(from leaves), lantacin (**7**), camarin (**8**)(from leaves), camarinin (**9**)(from stem)[47]. Oleanonic acid (**10**)(from leaves, stem), and oleanolic acid (**11**)(from leave, stem, root), lantadene A (**12**) (from leaves, stem, root) found to be very toxic to brine shrimp larvae [55], compound **12** also exhibited antitumor activity on two-stage squamous cell carcinogenesis [56]. Ester derivatives of compound **11** (from roots) showed anticancer activity [57]. Lantic acid (1**3**) was found to possess strong antibacterial activity against *Escherichia coli* and *Bacillus cereus*[58].The compound lantadene B (**15**) , lantadene C (**16**) and 22β-dimethylacryloyloxy-24-hydroxy-3-oxo-olean-12-en-28-oic acid (**17**) from the leaves of *Lantana camara* showed cytotoxic activities against four cancer cell lines namely; human oral epidermoid carcinoma, human colon cancer (HCT-116), human breast cancer (MCF-7), and mouse lymphocytic leukemia (L1210) [59]. While camarinic acid (**14**) again from the leaves was found to be active against *Staphylococcus aureus*and *Salmonella typhi* it also showed high antimutagenic activity [60].



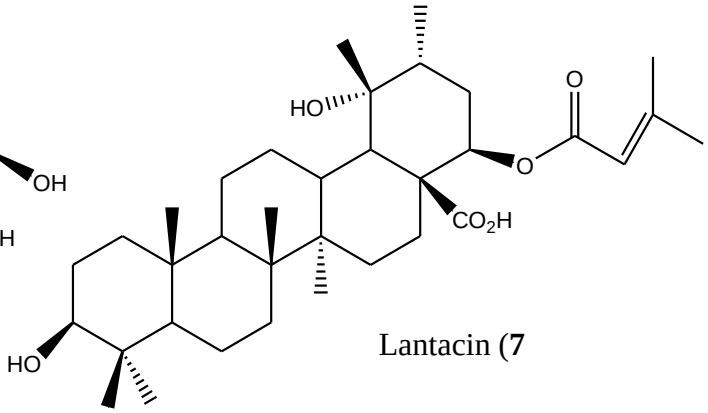
Pomolic Acid (**4**)



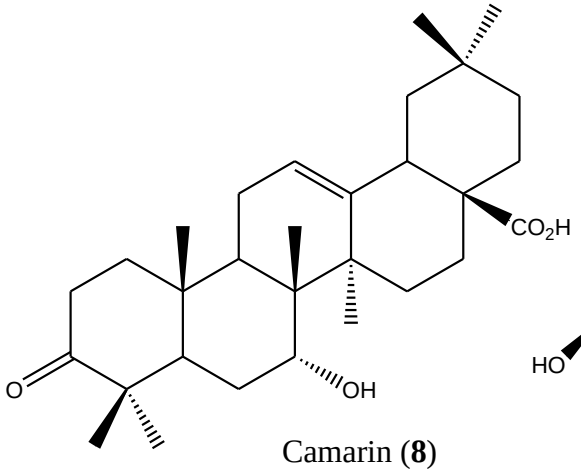
Lantanolic acid (**5**)



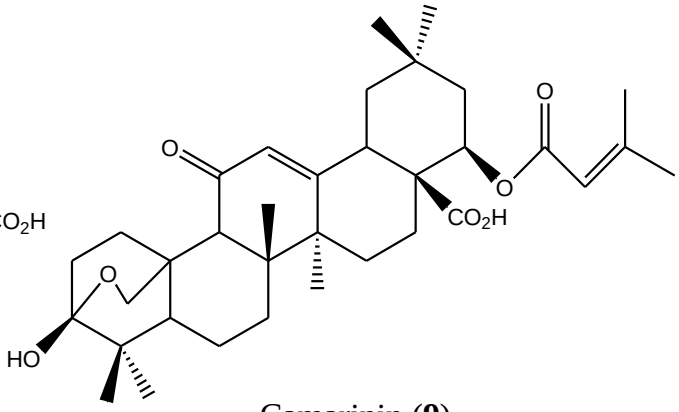
Lantoic acid **(6**)



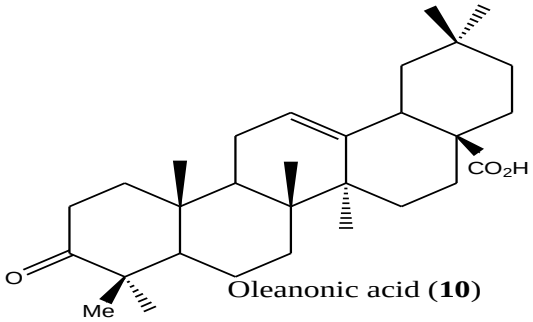
Lantacin (**7**



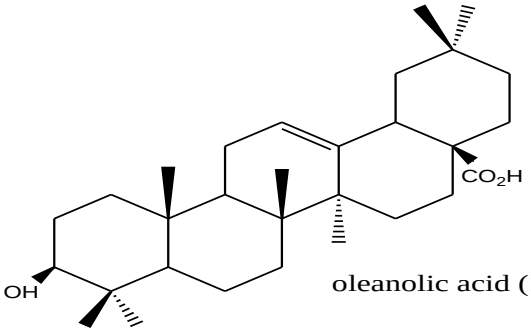
Camarin (**8**)



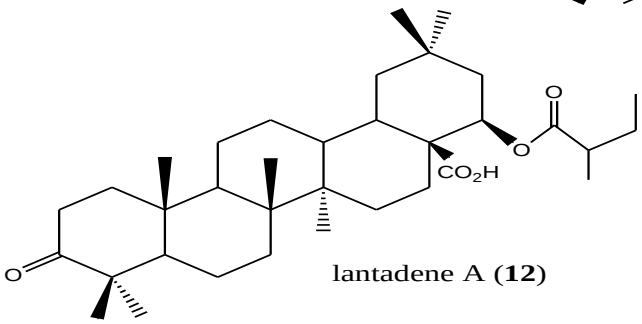
Camarinin (**9**)



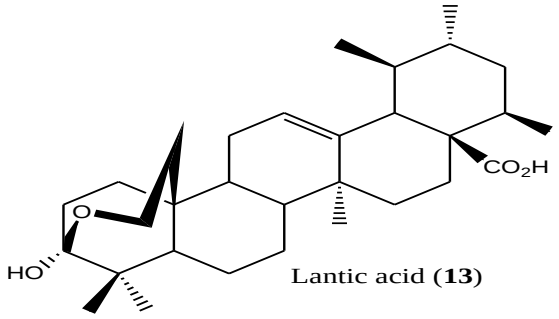
Oleanonic acid (**10**)



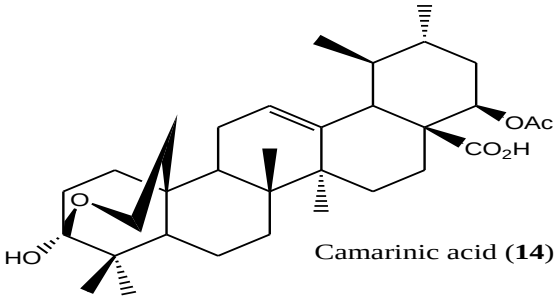
oleanolic acid (**11**)



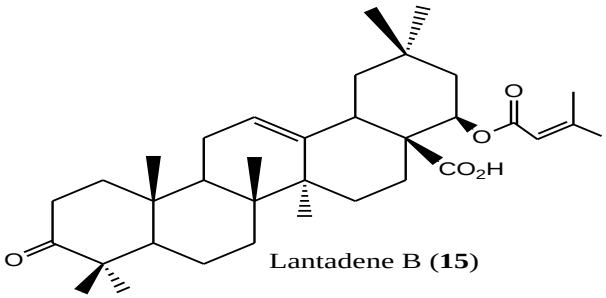
lantadene A (**12**)



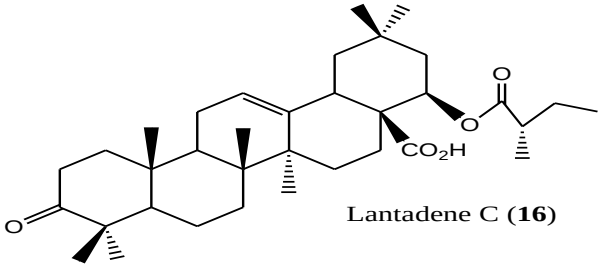
Lantic acid (**13**)



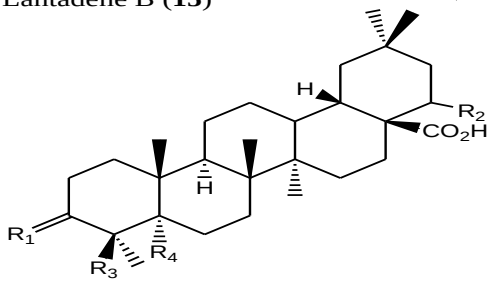
Camarinic acid (**14**)



Lantadene B (**15**)



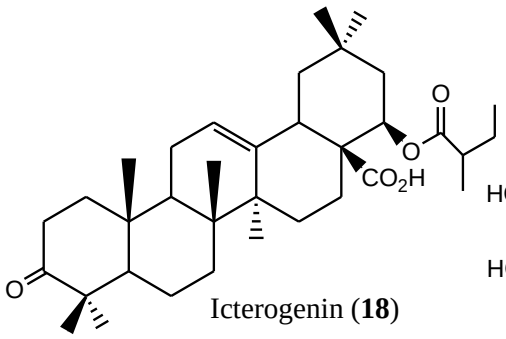
Lantadene C (**16**)



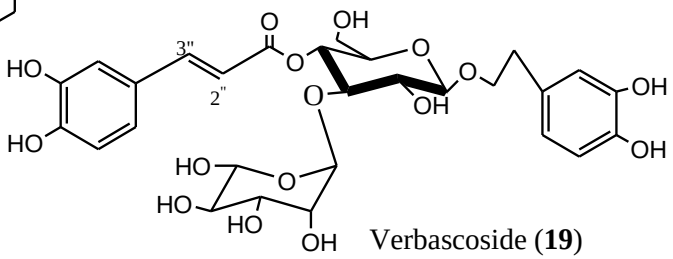
R_1=O,R_2=B-OCOCH=C$(CH_3)_2$,R_3=CH_2OH,R_4=CH_3

22β-dimethylacryloyloxy-24-hydroxy-3-oxo-olean12-en-28-oic acid (**17**)

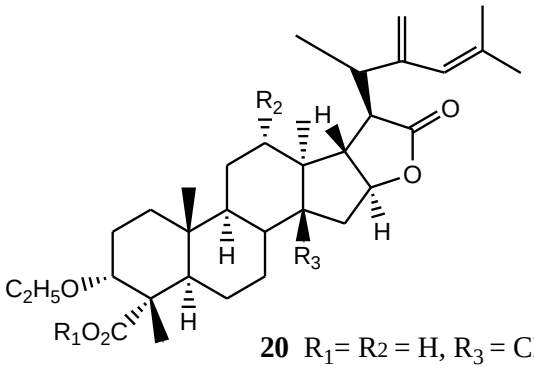
Compounds 22β-dimethylacryloyloxy-24-hydroxy-3-oxo-olean-12-en-28-oic acid (**17**) and icterogenin (**18)** from the leaves of *Lantana camara*, act as antagonists of the Bcl-xL/Bak association [54]. Lantanilic acid (**23**)(from leave, stem, root) showed antimutagenic activity [61].Verbascoside (**19**)(from dried leaves) isolated from *Lantana camara* found to be an inhibitor of protein kinase C (PKC) [62]. Euphane lactone A **(20),** Euphane lactone B **(21)** and euphane lactone C (**22**) found to show anticoagulant activity [63].



Icterogenin (**18**)

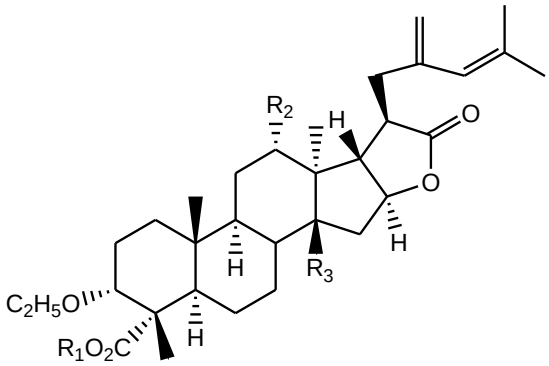


Verbascoside (**19**)



20 R_1= R_2 = H, R_3 = CH_3

21 R_1= H, R_2 = OH, R_3 = CH_3

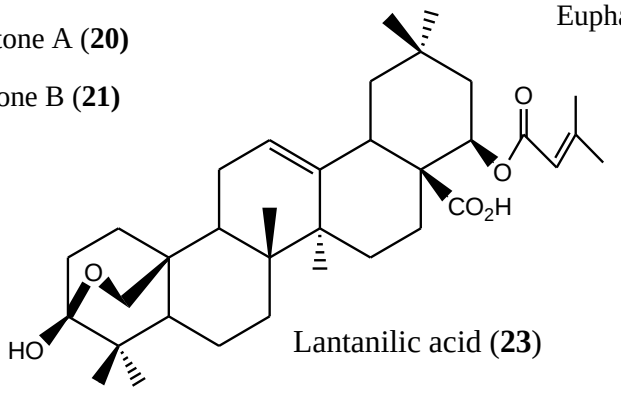


R_1= β-DGlu, R_2 = OH, R_3 = CH_2OH

Euphane lactone C (**22**)

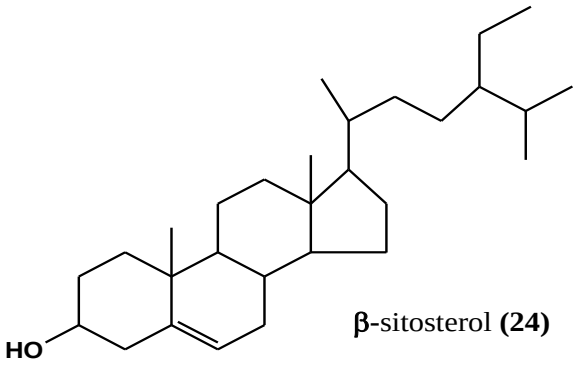
Euphane lactone A (**20)**

Euphane lactone B (**21)**

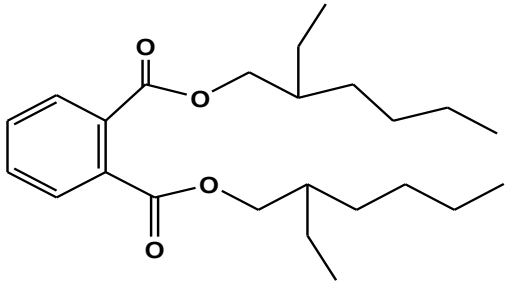


Lantanilic acid (**23**)

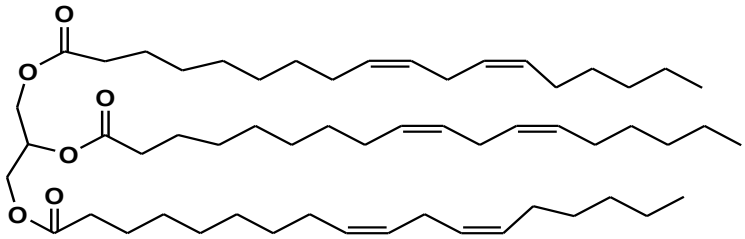
Compounds such as β-sitosterol (**24**) and di-(2-ethylhexyl) phthalate (**25**), trilinolien (**26**) were isolated from crude chloroform extract of fruits of *Lantana camara* and found to show good antibacterial activities [52].



β-sitosterol **(24)**



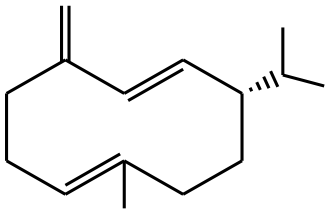
di-(2-ethylhexyl)phtalate **(25)**



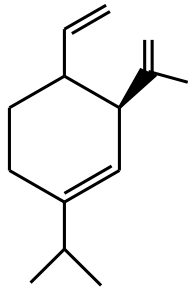
trilinolien **(26)**

A similar study also revealed that sesquiterpene hydrocarbons afforded a major portion of the oil with *trans*-caryophyllene, bicyclogermacrene, α-curcumene and germacrene D as the most abundant classes of compounds [63]. Monoterpene hydrocarbons, the second major class of compounds constituted of the oil of *Lantana camara* with sabinene, α-pinene and γ-terpinene as the major components, whereas oxygenated monoterpenes comprised only , having (*E*)-citral , (*Z*)-citral and 1,8 cineole in appreciable amounts [64]. Yellow flowering stem of *Lantana camara* afford phytosterols, 3β-hydroxystigmast-5-en-7-one, increasing concentrations of acetone yields 22β-angeloyloxylantanolic acid and lantanilic acid and concentrated with chloroform and methanol as eluent gives β-sitosterol-3-O-β-D-glucoside [64].

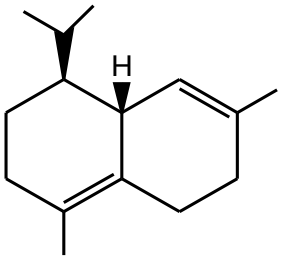
The leaves of *Lantana camara* is rich in essential oil, some of them are germacrene D (**27**),σ-elemene (**28**),σ-cadenene (**29**), β-elemene (**30**), espatulenol (**31**) and aromadendrene (32) are some of the essential oils in the leaves of *Lantana camara* [65]



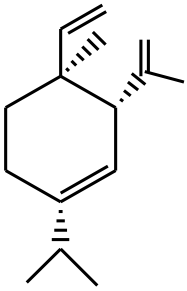
Germacrene D (**27**)



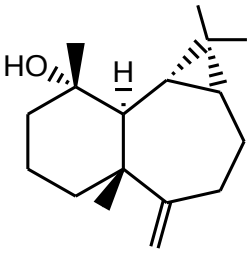
α-elemene (**28**)



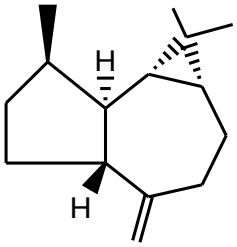
σ-cadenene (**29**)



β-elemene (**30**)

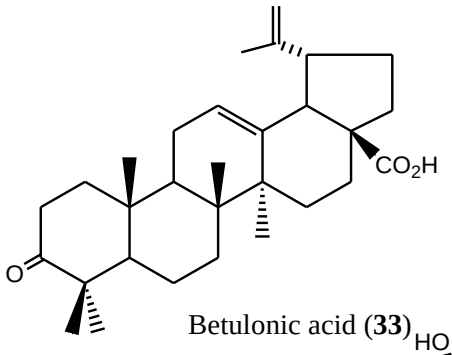


Espatulenol (**31**)

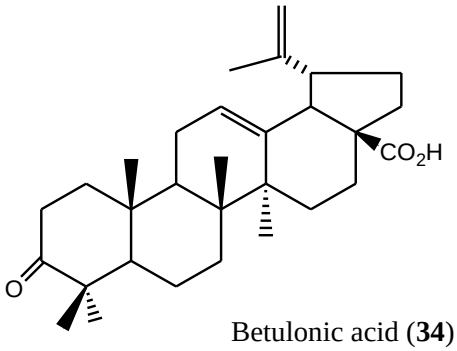


Aromadendrene (**32**)

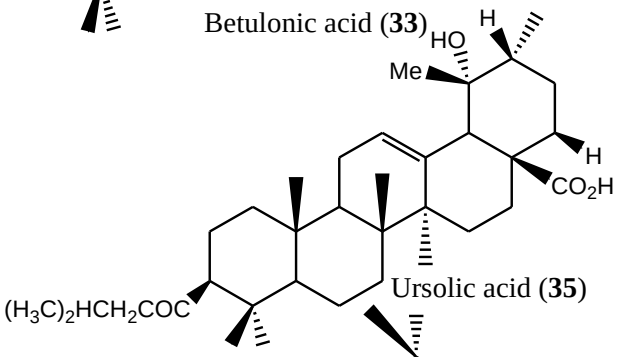
The stem was found to contain pentacyclictriterpenoids such as betulonic acid (**33**), oleanolic acid (**11**), ursolic acid (**35**), and flavonoids like camaroside (**36**)[14].Betulonic acid (**34**), oleanonic acid (**10**) and lantadene A (**12**) lantadeneB (**15**) lantanilic acid (**23**) [66].Known triterpenoids, a mixture of β-amyrin(**37**), α-amyrin(**38**) and pomolic acid (**1**)were isolated from the stems of *Lantana camara*[67].



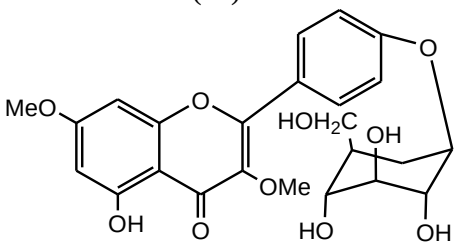
Betulonic acid (**33**)



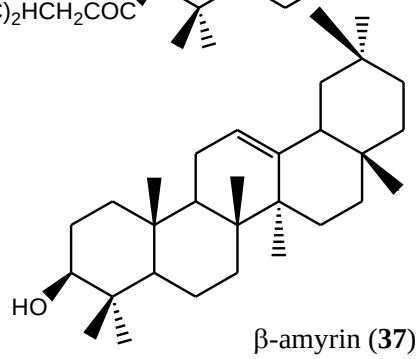
Betulonic acid (**34**)



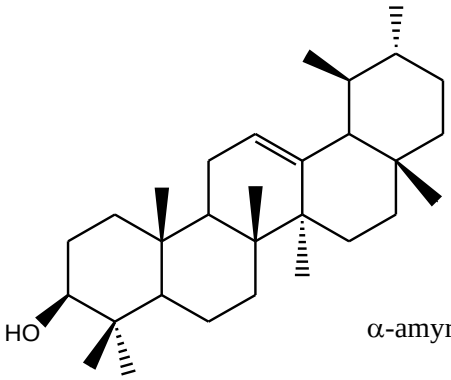
Ursolic acid (**35**)



Camaroside (**36**)



β-amyrin (**37**)



α-amyrin (**38**)

2.4 Statement of the Problem

Microbial infection is a major challenge to public health. This could be partly due to the emergency of drug resistance by microbes and unaffordable high cost of drugs. There are recorded side effects of synthetic drugs which amplified the burden of microbial infection. On the other hand, due to culturally linked traditions, the community trust of using traditional medicine is high. *Lantana camara* is one of the plants grown in Ethiopia and an ornamental plant used in traditional medicine for the treatment of various diseases and it is known for the presence of many biologically active compounds. Therefore, the present study is aimed at the phytochemical investigation and evaluation of antibacterial activity of compounds isolated from fruits of *Lantana camara* (*Verbenaceae*).

2.5 Objectives of the Study

2.5.1 General objective

The general objective of this study was to identify secondary metabolites from fruits of *Lantana camara* for antibacterial activities.

2.5.2 Specific objectives

- To extract fruits of *Lantana camara* sequentially using petroleum ether, chloroform, acetone and methanol.
- To evaluate the antibacterial activities of crude extracts and isolated compounds against four bacterial strains (*Staphylococcus aureus* (ATCC 6538), *Escherichia coli (*ATCC 8739*),Pseudomonas aeruginosa* (ATCC 15442) and *Bacillus cereus* (ATCC 14579)) and
- To isolate and elucidate structures of the isolated compounds using chromatographic and spectroscopic techniques (1D, 2D, NMR and IR).

2.6 Significance of the Study

Lantana camara is one of the medicinal plants in the world. This plant is well known for the treatment of various ailments and other domestic applications. In Ethiopia, different parts of this plant are used for the treatments of various ailments. However, there are not much scientific investigations both phytochemically and biologically on the plant found in Ethiopia which motivated us to investigate the chemical compositions and their biological activities. Therefore, this study is focused on adding to knowledge on the phytochemical constituents of fruits of *L. camara*, provide information about antibacterial activities of isolated compounds that could be used as hit in the discovery of antibacterial agents and develop an original insight about the chemical composition in the fruits of *Lantana camara* around Jimma**.**

CHAPTER THREE
MATERIALS AND METHODS

3.1 Chemicals

Methanol, acetone, chloroform, hexane and ethyl acetate were used as organic solvents. Iodine for detection of spots on TLC, Silica gel (60-120mm mesh size) was also used. Dimethyl sulfoxide (DMSO), Mueller Hinton agar and Nutrient broth as culture were used for antibacterial control in this study. All the chemicals and reagents were of analytical grade used.

3.2 Apparatus and Equipments

Rota vapors (LABOROTA-4000,Hedolf,Germany), TLC plates, round bottom flask, volumetric flask, measuring cylinder, pestle and mortar, weighing balances, condenser, glass columns for column chromatography and UV-254 and 365 nm chamber for detection of spots on TLC were used for the study. Spectral recording were done using Bruker 400-500 MHz advance NMR spectrometer with TMS as internal standard at Addis Ababa University and University of Belfield, Germany**.**

3.3 Collection and Preparation of Plant Materials

Fresh fruits of *Lantana camara* with pink, white and yellow flower type were collected from Jimma Teachers' College campus, Jimma, Oromia region. The plant materials were identified and voucher specimens were deposited at Herbarium, Department of Biology, at Jimma University. The plant materials were washed with distilled water and shade dried in laboratory at room temperature. The dried berries were ground with manual grinder.

3.4 Extraction and Isolation

The ground plant material which is the combination of pink, white and yellow flower (0.75 Kg) was sequentially extracted with petroleum ether, chloroform, acetone and methanol each time at 25°C for 24 hrs using a shaker. The solvents were removed using rotary evaporator under reduced pressure at 50°C.The crude extracts of petroleum ether, acetone, and the combination of chloroform and methanol were separately subjected to column chromatography after being adsorbed on to silica gel (60-120 mm mesh size). In all cases, the column was initially eluted with 100% *n*-hexane and then followed by gradient increasing amounts of ethyl acetate in *n*-hexane. A total of 100 fractions each *ca*. with 25 mL were collected from petroleum ether and combined into eight (Fr-A-Fr-H)

major fractions after TLC analysis. The major fraction of petroleum ether (Fr-F, eluent: n-hexane/ethyl acetate, 70:30) and Fr-H (65:35) gave white amorphous crystals, labeled as **40** (22.0 mg) and **37** (6.0 mg), respectively. The TLC solvent was 60:40*n*-hexane/ethyl acetate with Rf values of 0.82 and 0.70, respectively. Similarly, the acetone extract gave 190 fractions of each *ca*. 25 mL which were reduced into five (Fr I-Fr M) major fractions after TLC monitoring. The major fraction, labeled as Fr-K eluted at (75:25, *n*-hexane/ethyl acetate) yielded a brown amorphous compound labeled as **41** (20.0 mg) with Rf 0.64 in (77:23, *n*-hexane/ethyl acetate) solvent system for TLC. There was no compound purified from combined extracts chloroform and methanol. The structures of these compounds were elucidated based on combined spectral data and comparison with data in literature.

3.5 Evaluation of antibacterial activity

The antibacterial activity tastes were carried out using *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Bacillus cereus* using standard procedure [68].All bacterial cultures were first grown on 5% sheep red blood agar plates at 37^0C for 24 hrs prior to inoculation on to the nutrient agar. Few colonies (4-5) of similar morphology of the respective bacteria were transferred with a sterile inoculating loop, a nutrient broth liquid medium and this medium and this liquid culture was incubated until adequate growth of turbidity standard was obtained. The turbidity of the actively growing broth culture was adjusted with sterile saline solution to obtain turbidity optically comparable to that of the 0.5 McFarland standards that was resulted in a suspension containing approximately 1-2 X10^8CFU/mL for the taste strain. The respective bacterial culture was streaked on to the Muller-Hinton agar Petri plates using a sterile swab to ensure through coverage of the plates and a uniform thick lawn of growth following incubation. Then 6mm diameter sterile discs of what man N^o-3 paper were placed on the surface of the inoculated agar approximately at equal distance of corners in Petri plates in a 100 mg/mL concentration that were prepared by dissolving 100mg of crude and isolated compounds in 1mL of DMSO using micropipette on to the discs. After addition of taste solution on the discs, it was allowed to diffuse for 5 minutes and the Petri plates then kept in an incubator at 32^0C for 24 Hrs. The antibacterial activity was evaluated after 24 hrs by measuring the diameter of zone of growth inhibition surrounding the discs (in mm) using transparent ruler results were expressed as Mean of tests [8]. Gentamycin and Dimethyl sulfoxide (DMSO) were used as positive and negative controls, respectively. All the taste strains were obtained from Biology department, Jimma University.

CHAPTER FOUR

RESULT AND DISCUSION

The crude extracts were shown in the Table 4.1 as petroleum ether, chloroform, acetone and methanol extract.

Table 4.1.Crude extracts obtained from fruits of *Lantana camara* using four different solvents

Solvents	Mass in g	% yield
Petroleum ether	6.71	0.91
Chloroform	4.43	0.596
Acetone	4.38	0.616
Methanol	13.64	1.835

The four crude extracts were subjected to biological activity against Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram-positive (*B. cereuse* and *S. aureus*) bacteria and showed antibacterial activities with different zone of inhibition at a concentration of 100 mg/ml as given in Table 4.2.

Table 4.2. Zone inhibition of crude extracts of *Lantana camara* fruits against four bacterial strains.

Strain	Conc. mg/mL	Diameter of zone of inhibition in mm					
		PET	$CHCl_3$	Acetone	MeOH	Genta.	DMSO
S. aureus	100	8	10	11	-	20	NI
B. cerues	100	9	8	15	7	19	NI
E. coli	100	11	10	9	9	20	NI
P. auregenosa	100	9	11	8	8	18	NI

NI: No Inhibition at 100 mg/mL

It follows that the crude extract of acetone is found to be more active on *Bacillus cereus and moderately on S. aureuse.* The methanol extract is also observed to be the least active relative to the standard gentamycin on all tasted bacterial strain except *B. cereus.*

The petroleum ether and acetone extracts were then subjected to column chromatography over silica gel for its chemical constituents. The characterization and activities of these compounds are discussed in the subsequent section.

4.1 Characterization of Compounds from Petroleum Ether Extract of Fruits of *L. camara*

The air-dried and ground fruit of *L. camara* was exhaustively extracted with petroleum ether to afford a crude extract (6.71g). A chromatographic separation of this crude extract led to the identification of two compounds and their structural characterization is discussed in this section.

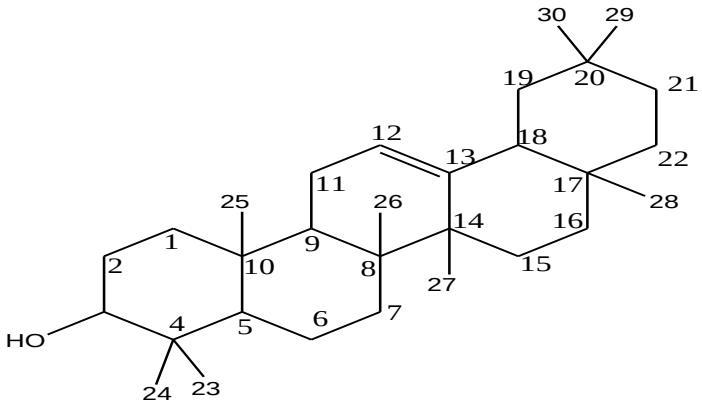
4.1.1Characterization of compound 37

Compound**37** was isolated as a colorless amorphous solid with mp 191 ^{o}C. The UV λ_{max} was observed at 274and 229 nm. The ^{13}CNMR data revealed the presence of thirty carbon atoms indicating that the compound has a triterpenoid skeleton [70].

The ^{1}HNMRspectrumof compound **37** exhibited eight methyl signals at δ 0.75 (s, 3H), 0.76 (s, 3H) 0.76 (s, 3H), 0.79 (s, 3H), 1.13 (s, 3H) 0.94 (s, 3H), 0.87 (s, 3H), 0.75 (s, 3H), and0.97 (s,3H) ppm indicating the presence of protons of aliphatic methyl groups while a proton appeared as a triplet of double doublet (t, 3.22 ppm) is observed for oxygenated aliphatic proton and the downfield proton signals at 5.18 ppm showed protons of a methylene group of olefinic carbon. Therefore, from the observed ^{1}HNMR data it is evident that the compound has a skeleton of sterol.

The ^{1}H-^{1}H COSY spectrum of compound **37**showed that a proton at δ 5.18 coupled with 1.56, whereas a proton δ at 3.22 coupled with 1.86 and the proton at δ0.75 coupled with 0.97. It was also observed that a proton at δ 1.86 coupled with 1.56 which this gave a piece of information that these respective protons are *vicinal* of each other in the structure. The thirty signals in ^{13}C NMR spectrum is classified as primary, secondary, tertiary and quaternary. The proton attached carbon atoms were identified from HSQC spectrum which showed cross peaks between proton and its carbon atoms. he up field and intense peaks of carbon showed HSQC cross peaks with proton at δ 0.97 (s) (C-23), 0.75 (s) (C-24), 0.87 (s) (C-25), 0.94 (s) (C-26), 1.13 (s) (C-27), 0.79 (s) (C-28), 0.76 (s) (C-29) and 0.75 ppm (s) (C-30) are consistent with eight methyl protons. Furthermore, from the HSQC data

carbon atoms at δ 39.9 (C-4), 38.9 (C-8), 37.1 (C-10), 145.3 (13), 41.8 (C-14), 32.8 (C-17), and 31.2 ppm (C-20) are quaternary carbons as there is no correlation peaks to attached protons. The carbon signal at δ 79.1 ppm indicates an oxygenated carbon in the compound. The two downfield signals at δ 121.8 and δ 145.3 ppm are due to olefinic carbon and assignable to C-12 and C-13. Overall, compound **37** has ten methyl, nine methylene and five methine groups and seven quaternary carbon atoms. Based on the spectroscopic data (Table 4.3/ Appendix A, Literature 71), compound **37** was characterized as β-amyrin which has been previously identified from the stem part of *L. camara.*



β –Amyrin (37)

Table 4.3.NMR data of β-amyrin (**37**) with reported data (500 MHz, $CDCl_3$)

Position	**^{13}CNMR**	**^{13}C NMR (Literature)**	**^{1}HNMR**	**^{1}HNMR (Literature)**	**Nature of Carbon**
1	38.9	38.7	1.61 (t)	1.61	CH_2
			0.97 (m)	0.98	
2	27.3	27.3	1.56 (t)	1.59	CH_2
			0.76 (t)	0.77	
3	79.1	79.1	3.22 (t)	3.20	CH
4	39.9	39.9	-	-	C
5	55.3	55.3	0.75 (t)	0.73	CH
6	18.5	18.5	1.54 (m)	1.52	CH_2
7	32.6	32.6	1.43 (t)	1.49	CH_2
8	38.7	38.9	-	-	C
9	47.7	47.7	1.56 (t)	1.58	CH
10	37.1	37.0	-	-	C
11	23.6	23.6	1.86 (d)	1.86	CH_2
12	121.8	121.8	5.18 (s)	5.16	CH
13	145.3	145.3	-	-	C
14	41.8	41.8	-	-	C

15	26.3	26.2	1.68 (m)	1.74	CH_2
16	27.0	27.0	1.96 (m)	1.97	CH_2
17	32.8	32.6	-	-	C
18	47.3	47.2	1.94 (t)	1.94	CH
19	46.9	46.9	1.64 (d)	1.64	CH_2
				1.01	
20	31.2	31.2	-	-	C
21	34.8	34.8	1.13 (t)	1.31	CH_2
22	37.2	37.2	1.00 (t)	1.09	CH_2
23	28.5	28.2	0.97 (s)	0.98	CH_3
24	15.7	15.7	0.75 (s)	0.75	CH_3
25	15.6	15.6	0.87 (s)	0.92	CH_3
26	16.9	16.9	0.94 (s)	0.95	CH_3
27	26.1	26.1	1.13 (s)	1.12	CH_3
28	28.5	28.5	0.79 (s)	0.81	CH_3
29	33.5	33.5	0.76 (s)	0.85	CH_3
30	23.8	23.8	0.75 (s)	0.85	CH_3

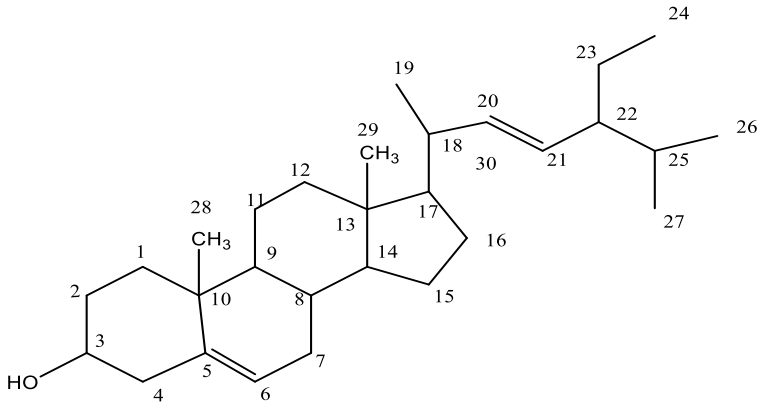
4.1.2 Characterization of compound 40

Compound **40** was isolated as colorless amorphous solid with IR spectrum (bands at 3326 cm^{-1} OH stretching) indicated the presence of alcohol functionality. Moderately, intense band at 1199 cm^{-1} at 689 cm^{-1} shows OH bond vibration of hydroxyl groups. The peak at 1642 cm^{-1} shows C=C vibrations. The band at 2950 cm^{-1} is most likely an alkene C-H stretching and the band at 2842 cm^{-1} and medium band at 1470 cm^{-1} shows C-H stretching of methylene groups.

The ^{1}HNMRspectrumof compound **40** exhibited six methyl signals at δ 0.71 (s, 3H), 0.81 (d, 3H) 0.82 (d, 3H), 0.90 (d, 2H), 0.83 (t, 3H) and 1.03 (s,3H) ppm indicates presence of protons of aliphatic methyls while a proton appeared as a triplet of double doublet (tdd 3.41 ppm) is for oxygenated aliphatic proton and the downfield proton signals at 4.99, 5.38 and 5.15 ppm showed that protons of a olefinic carbon. Therefore, from the observed IR and ^{1}HNMR data it is evident that the compound has a skeleton of sterol.

Furthermore, the ^{13}CNMRspectrum gave 29 signals classified as; six methyls, nine methylenes, eleven methine and three quaternary carbon atoms which is supported by DEPT-135 spectrum. The peaks at 42.5 and 36.5 ppm were assignable to saturated quaternary carbon atoms, which are confirmed by absence of corresponding signals from the DEPT-135 spectrum. Whereas, the observed peaks at 141.3, 121.8, 138.7 and 129.4 revealed the presence of olefinic carbon in the structure. From the DEPT-135 a peak at 12.2, 18.9, 19.8, 20.2, 12.1 and 21.7ppm are consistent

with six methyl carbon atoms. Peaks at 37.6, 32.2, 42.5, 31.8, 21.5, 39.8, 24.4, 29.6, 25.4ppm showed methylene carbon atoms. Furthermore, peaks at 72.3, 121.8, 31.8, 56.9, 56.2, 40.6, 138.7, 129.4 and 46.0ppm showed methine carbon atoms. Therefore, from the given data, the structure of the compound has sterol skeleton. Based on spectroscopic data (Appendix **B**) and comparison with the reported literature (Table 4.3) [68, 69 and 71] the compound was characterized as stigmasterol. It is a compound identified from *Lantana camara* fruit for the first time.



Stigmasterol (40)

Table4.4. NMR data of compound **40**and Literature reported data (400 MHz, $CDCl_3$).

Position	**^{13}CNMR**	**^{13}CNMR (Literature)**	**^{1}HNMR**	**^{1}H-NMR (Literature)**	**Nature of carbon**
1	37.7	37.6			CH_2
2	32.1	32.1			CH_2
3	72.3	72.1	3.41	3.51(tdd)	CH
4	42.5	42.4			CH_2
5	141.3	141.1	-	-	C
6	121.8	121.8	5.38	5.32 (t)	CH
7	31.8	31.8			CH_2
8	31.8	31.8			CH
9	50.2	50.2			CH
10	36.5	36.6	-	-	C

11	21.5	21.5			CH_2
12	39.8	39.9			CH_2
13	42.5	42.4	-	-	C
14	56.9	56.8			CH
15	24.4	24.4			CH_2
16	29.6	29.3			CH_2
17	56.2	56.2			CH
18	40.6	40.6			CH
19	21.8	21.7	0.90	0.91 (d)	CH_3
20	138.7	138.7	4.99	4.98 (m)	CH
21	129.4	129.6	5.15	5.14 (m)	CH
22	46.0	46.1			CH
23	25.4	25.4			CH_2
24	12.1	12.1	0.83	0.83 (t)	CH_3
25	29.7	29.6			CH
26	20.2	20.2	0.82	0.82 (d)	CH_3
27	19.9	19.8	0.81	0.80 (d)	CH_3
28	18.9	18.9	0.71	0.71 (s)	CH_3
29	12.3	12.2	1.03	1.03 (s)	CH_3

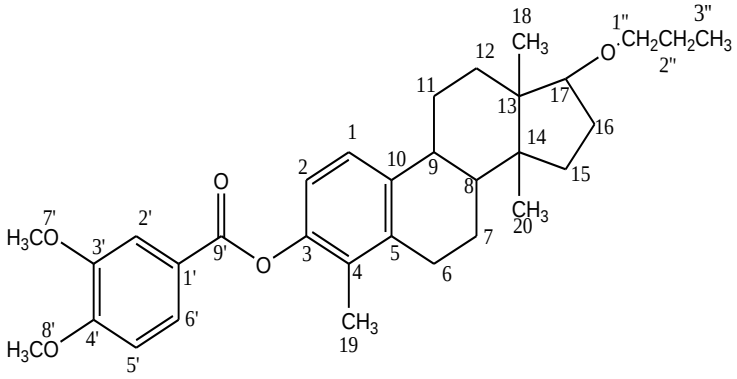
4.2 Characterization of Compounds from Acetone extract of Fruits of *L. camara*

4.2.1Characterization of compound 41

Analysis of **IR** spectrum of compound **41**(Appendix **C**)indicated that there is aromatic functional group due to the presence of weak bands between 2000 and 1650 cm^{-1}.And also the peak at 1602 cm^{-1} shows aromatic C=C vibrations. Due to the presence of bands at 1704 cm^{-1} indicates that the compound has carbonyl group while the peak at 1100-1300 cm^{-1} shows a C-O stretching of acetate.

The ^{1}HNMR of this compound exhibited four methyl signals at 0.53(s,3H),0.49(s,3H),0.41(s,3H) and0.40ppm(s,3H).The peak at 3.91(s,3H),and at 3.87ppm(s,3H) shows methoxy methyl and there are eight methylene protonsat4.01(m,2H),4.05(m,2H),4.39(m,2H),4.35(t,2H),4.39(m,2H),4.39(q, 2H), 4.49 (m,2H) and 4.73ppm(m,2H) indicates protons of aliphatic methylene and a proton appeared at 5.03 (d, 1H), 5.20 (d, 1H), 6.10 (s, 1H) , 6.08 (d,1H) , and 6.11ppm(d,1H) shows aromatic protons because aromatic protons are observed between 5.00-7.00ppm. Therefore, from the observed IR and ^{1}HNMR data it is evident that the compound has a steroidal skeleton.

Moreover, the ^{13}CNMRspectrum gave 32 signals; six methyl, eight methylene, eight methyne and ten quaternary carbons from the data by DEPT-135 spectrum. The peaks at 119.9, 121.7, 123.2, 124.0, 29.1, 33.0, 126.1, 129.2and 129.9 ppm showed the quaternary carbon atoms, the peak at 166.8 ppm shows quaternary carbonyl Carbon, it is confirmed by absence of their signals from the DEPT-135 spectrum. The peaks observed at 129.0, 130.9, 131.4, 116.4 ppm and 117.4 ppm shows aromatic carbons of methine and at 25.4 and 26.1 ppm also shows aliphatic methine. The peaks at 18.0, 18.1, 19.5, 21.6, 2.7, 23.9, 24.7 and 24.4 ppm showed aliphatic Methylene carbons. And peaks at 67.8 ppm and 73.5 ppm showed methoxy carbons and the peak at 10.2 ppm, 10.9 ppm, 11.2 ppm and at 13.2 ppm showed methyl groups. Therefore, from the given data, the structure of the compound looks to have steroidal skeleton.



Estra-17-propoxy, 3', 4' - dimethoxy-3yl benzoate**(41)**

Table4.5. NMR data of **41** with reported data of Estra-17-propoxy, 3', 4'-dimethoxy-3yl benzoate (400 MHz, $CDCl_3$)

Position	**^{13}CNMR**	**^{13}CNMR (Literature)**	**^{1}H NMR**	**^{1}H-NMR (Literature)**	**Nature of carbon**
1	116.4	116.34	5.04	5.03(d)	CH
2	117.4	117.42	5.20	5.18(d)	CH
3	119.9	119.95		-	C
4	121.6	121.59		-	C
5	123.2	123.38		-	C
6	18.0	18.00	4.01	4.01(m)	CH_2
7	18.1	18.16	4.05	4.05(m)	CH_2
8	25.4	25.37	4.87	4.87(m)	CH
9	26.1	26.07	4.89	4.89(t)	CH
10	124.0	124.00		-	C
11	19.5	19.53	4.38	4.39(m)	CH_2
12	21.6	21.56	4.35	4.39(t)	CH_2
13	29.1	29.03		-	C
14	33.0	33.00		-	C
15	22.7	22.78	4.39	4.39(m)	CH_2
16	23.9	23.95	4.39	4.39(q)	CH_2
17	33.4	33.39	4.91	4.92(s)	CH
18	10.2	10.26	0.40	0.40(s)	CH_3
19	10.9	10.96	0.41	0.41(s)	CH_3
20	11.2	11.20	0.50	0.49(s)	CH_3
1'	126.1	126.09		-	C

2’	129.0	129.00	6.09	6.09(s)	CH
3’	129.2	129.21		-	C
4’	129.8	129.89		-	C
5’	130.9	130.94	6.08	6.08(d)	CH
6’	131.4	131.42	6.11	6.12(d)	CH
7’	67.8	67.85	3.87	3.88(s)	OCH_3
8’	73.5	73.50	3.91	3.94(s)	OCH_3
9’	166.8	166.75		-	C=O
1”	24.7	24.50	4.49	4.50(m)	CH_2
2”	24.4	24.50	4.73	4.72(m)	CH_2
3”	13.2	13.24	0.54	0.53(s)	CH_3

The ^{1}HNMR, ^{13}CNMR, DEPT-135 and IR data of compound **41**and that of estra-17-propoxy, 3’, 4’-dimethoxy-3yl benzoate of literature are the same [72]. Thus, based on the similarities of spectral data, the chemical structure of **41** was proposed to be identical with that of estra-17-propoxy, 3’, 4’-dimethoxy-3yl benzoate. Based on spectroscopic data (Appendix **C**) and comparison with the reported literature (Table 4.5) the compound was characterized as Estra-17-propoxy, 3’, 4’-dimethoxy-3yl benzoate. Therefore, the compound identified from *Lantana camara* fruit is Estra-17-propoxy, 3’, 4’-dimethoxy-3yl benzoate.

4.3Evaluation of Antibacterial Activities of the Isolated Compounds

Three isolated compounds (**37**, **40**and**41**) were evaluated for their antibacterial activities using the same bacterial species as for the crude extract: *Pseudomonas aeruginosa, Bacillus cereus, Pseudomonas aureus* and *Escherichia coli* by disc diffusion method. The growth inhibition zones (in mm) of the compounds were given in Table 4.6.The result indicated that compound **41**showed comparable activities with that of standard drugs against *P. aeruginosa* (19 mm) and *E. coli (*19 mm*).*This compound also showed moderate activity (16 mm) on *S. aureuse*. Compounds **40** and **37** showed nearly similar activity (Appendix **E**).

Table 4.6.Results of antibacterial activities of the isolated compounds in zone of inhibition in mm.

	Diameter of zone of inhibition in mm				
Bacteria strain	**40**	**37**	**41**	**Gentamycin**	**DMSO**
P. aeruginosa	11	13	19	21	NI
B. cereus	16	15	9	18	NI
E. coli	15	14	19	22	NI
S. aureus	13	15	16	21	NI

NI =No inhibition

Compound **41**showed comparatively higher antibacterial activity than other compounds on the tested bacteria strains (Appendix **F**). The most susceptible organisms are *Escherichia coli and P. auregenosa,* whereas *Bacillus cereus* was found to be most resistant bacteria against compound**41** Table4.3.The overall results of this study provide evidence that *Lantana camara* fruits extract as well as the isolated compounds exhibit antibacterial activity for both gram negative and gram positive pathogens. Thus, the observed antibacterial activities of the crude extracts and the isolated compounds could justify the traditional use of the plant for the treatment of different bacterial infections. The observed antibacterial activities of the isolated compounds as hit compound in the development of antibacterial drugs. However, further tests are recommended on large number of bacterial strains to decide their potential as candidates in development of antibacterial drugs.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

From the fruits *of L. camara* three compounds were isolated and characterized as β-amyrin (**37)**, stigmasterol (**40**) andestra-17-propoxy, 3', 4'-dimethoxy-3yl benzoate (**41**). *In vitro* antibacterial activity test results showed that compound **41** is potentially active especially, against *E. coli and Ps. auregenosa*. Whereas compounds **37** and **40** showed a moderate activities than the reference drug (Gentamycin). The observed antibacterial activities of the isolated compounds could give insight about the potentials of the compounds as hit compound in development of antibacterial lead drugs.

5.2 Recommendations

- Further study on chemical composition including the edible fruit of *Lantana camara* for future use is important for the development and production of new drugs.
- Further purification should be carried out on the fruits parts of *L. camara.*
- Absolute configuration of the isolated compounds should be established.
- The biological activity should be also done on another bacterial strain.

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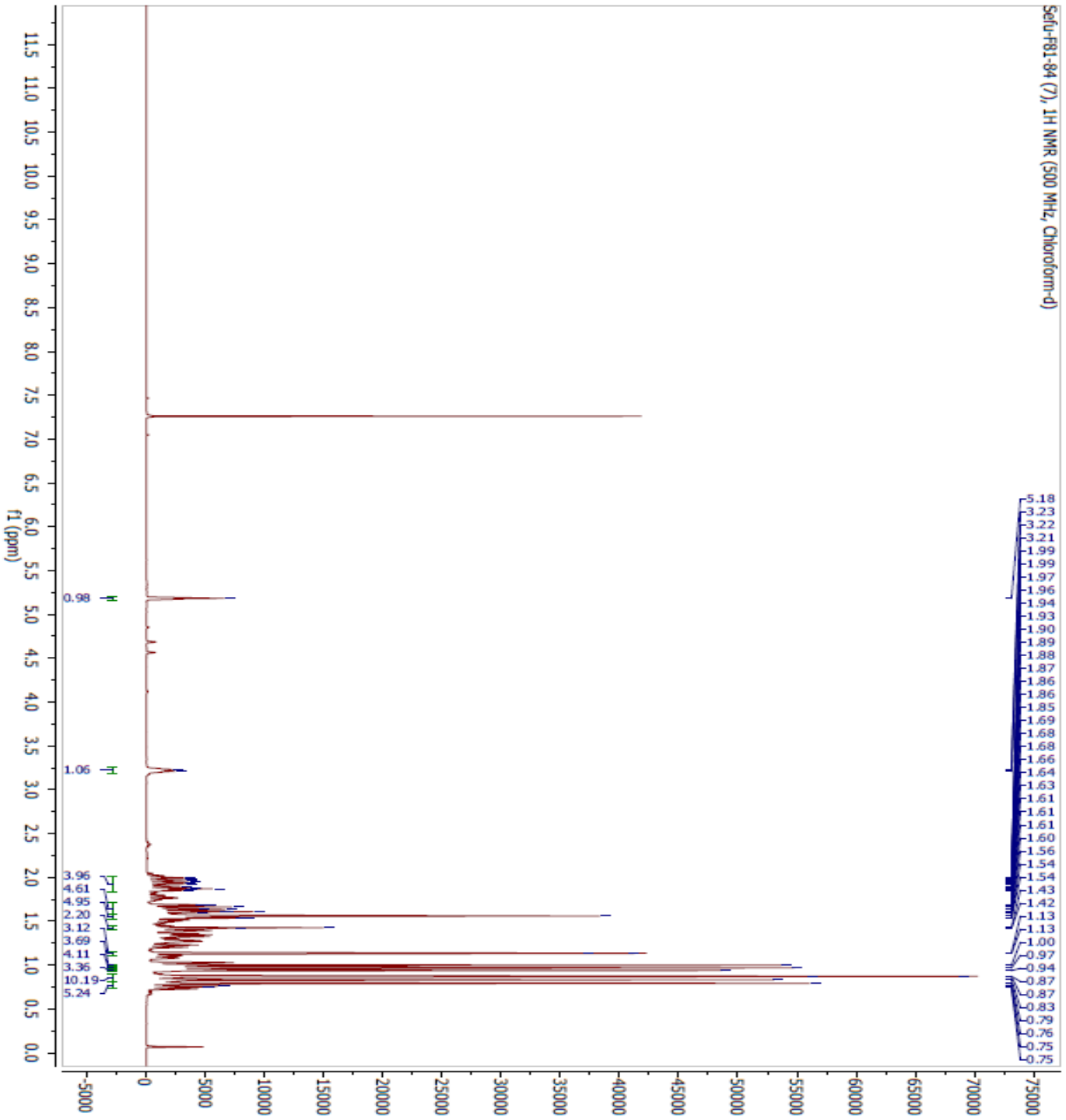

Appendix A. 1H NMR - Spectra of Compound 37

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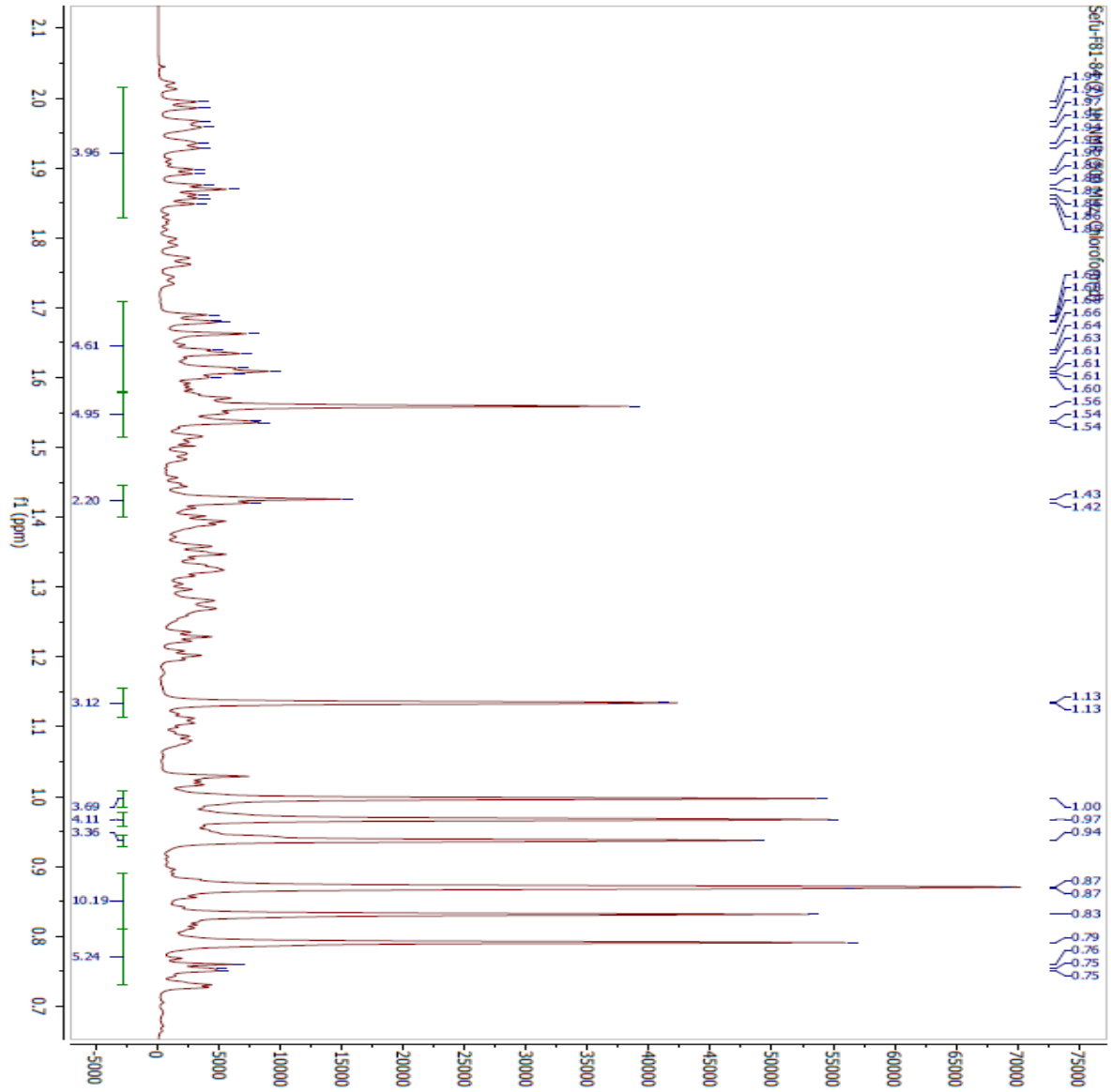
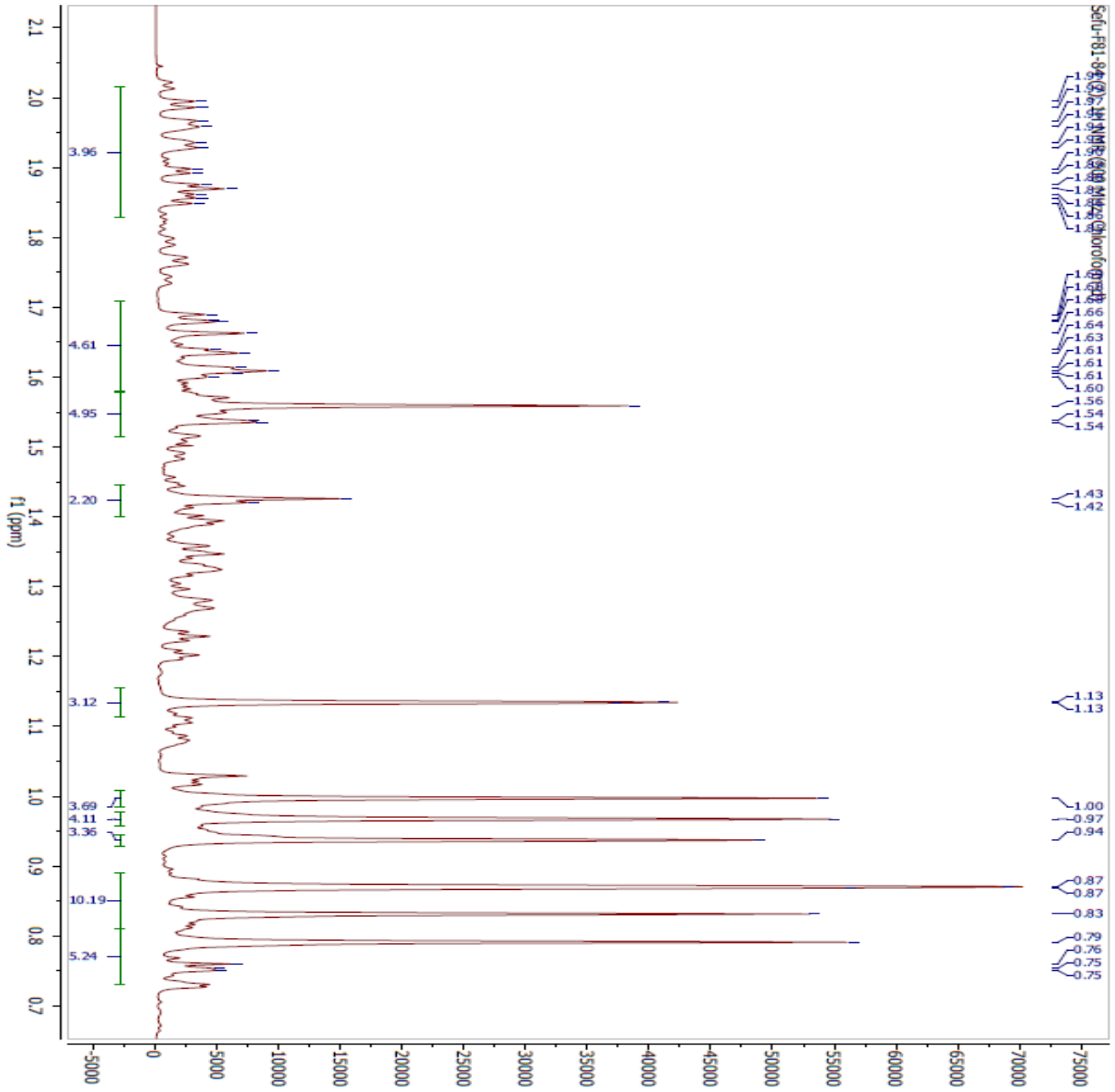
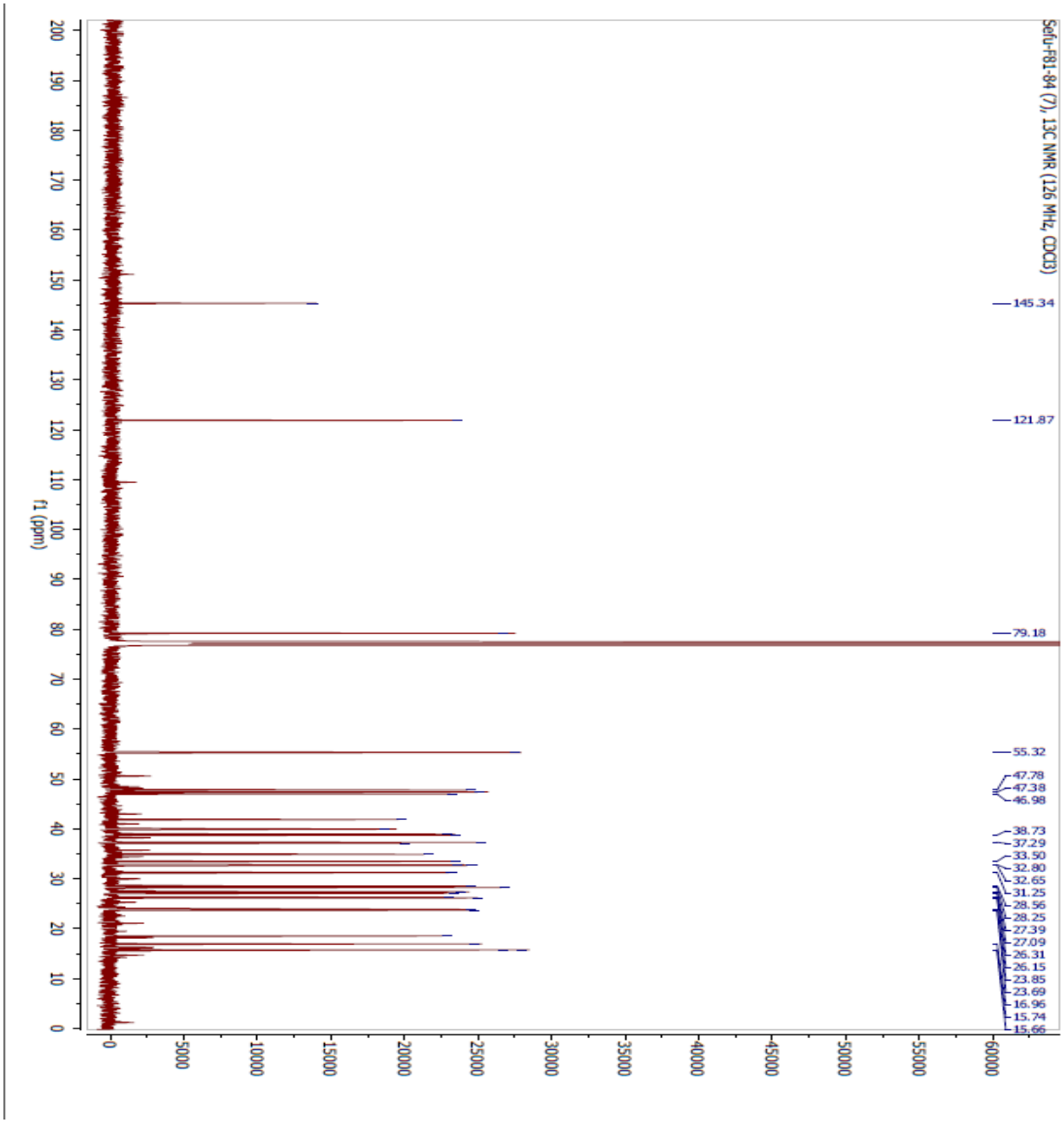


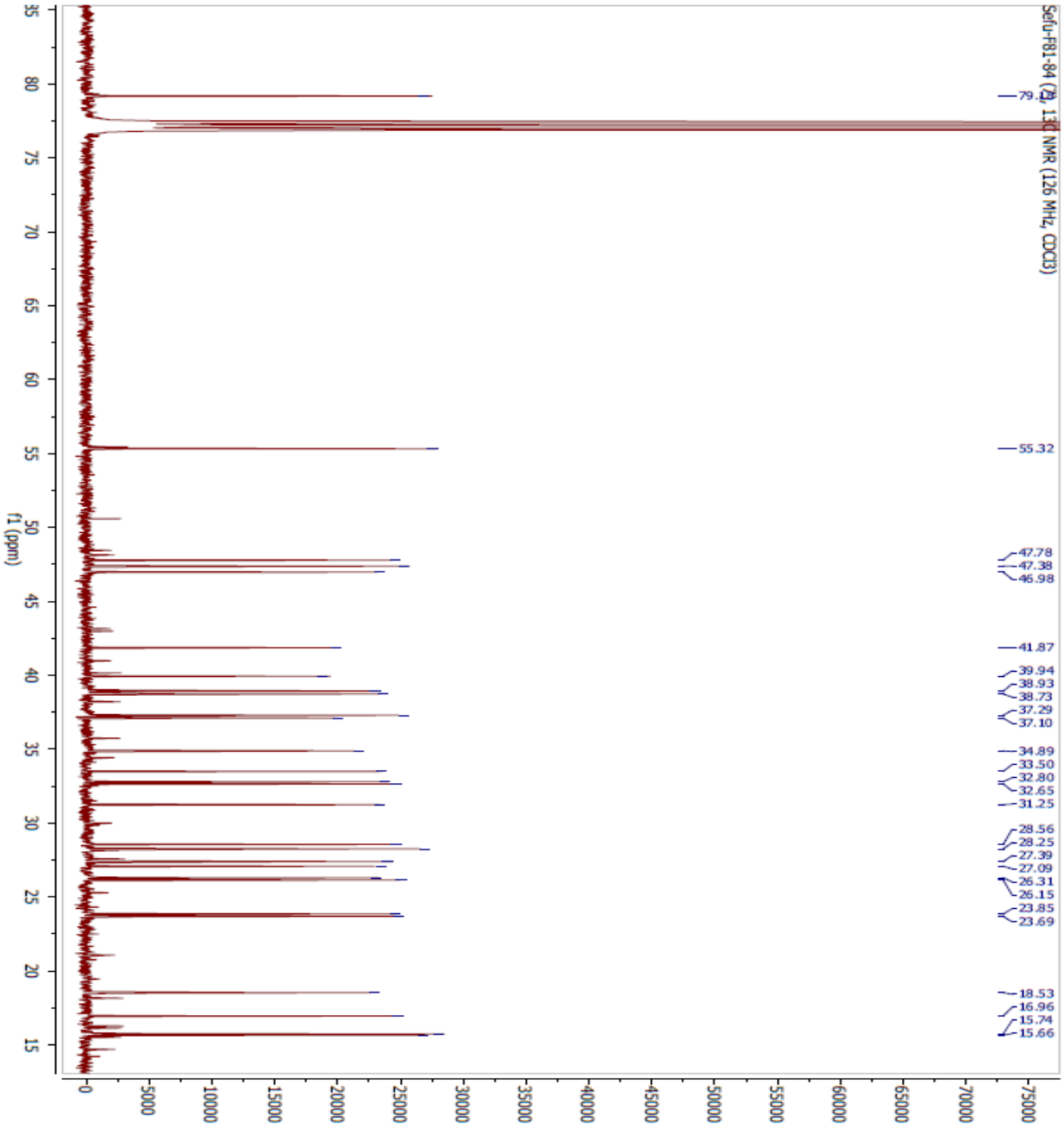
Appendix A. 1H NMR - Spectra of Compound 37



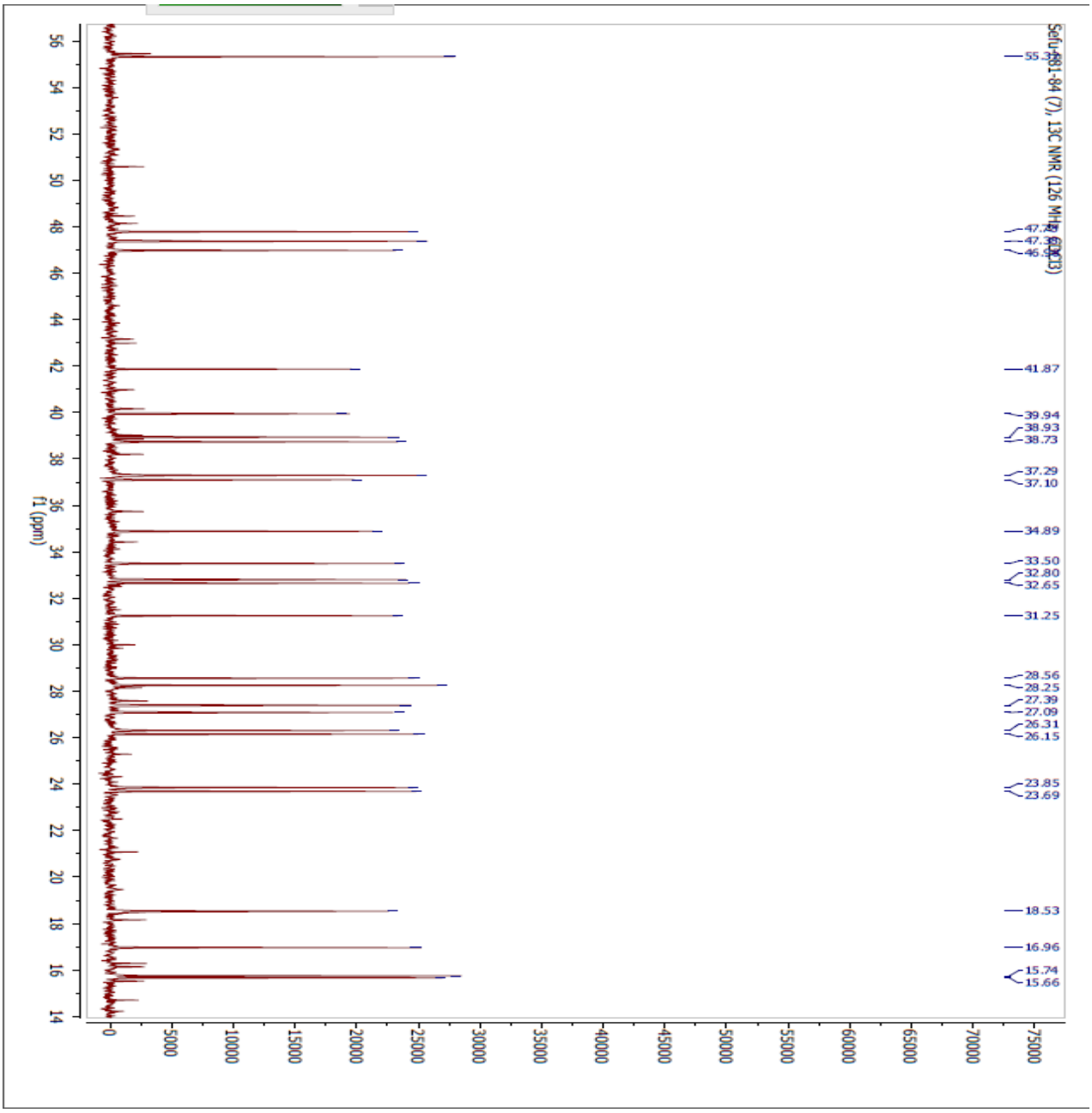
Appendix A. 13C NMR - Spectra of Compound 37



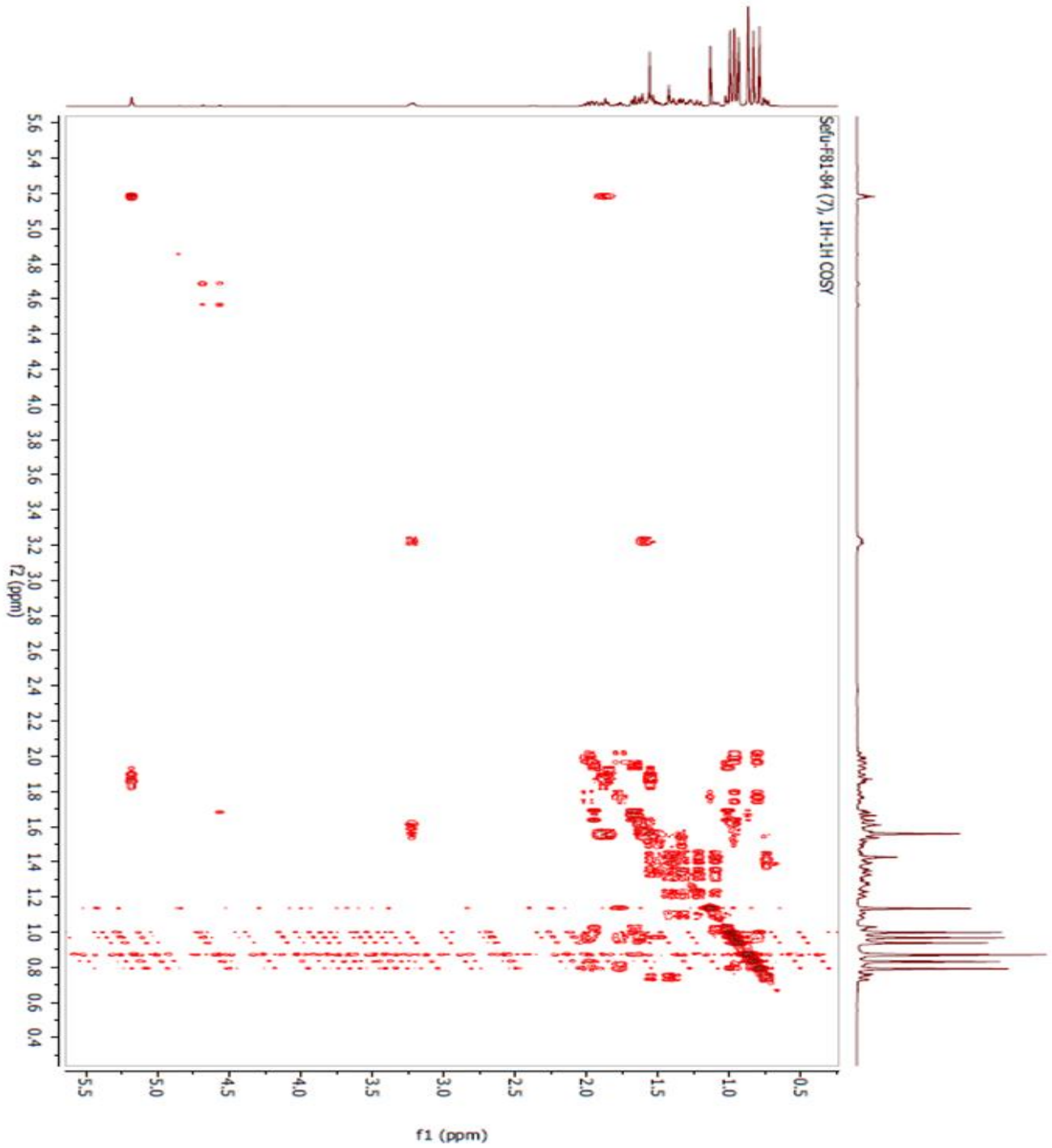
Appendix A. 13C NMR - Spectra of Compound 37



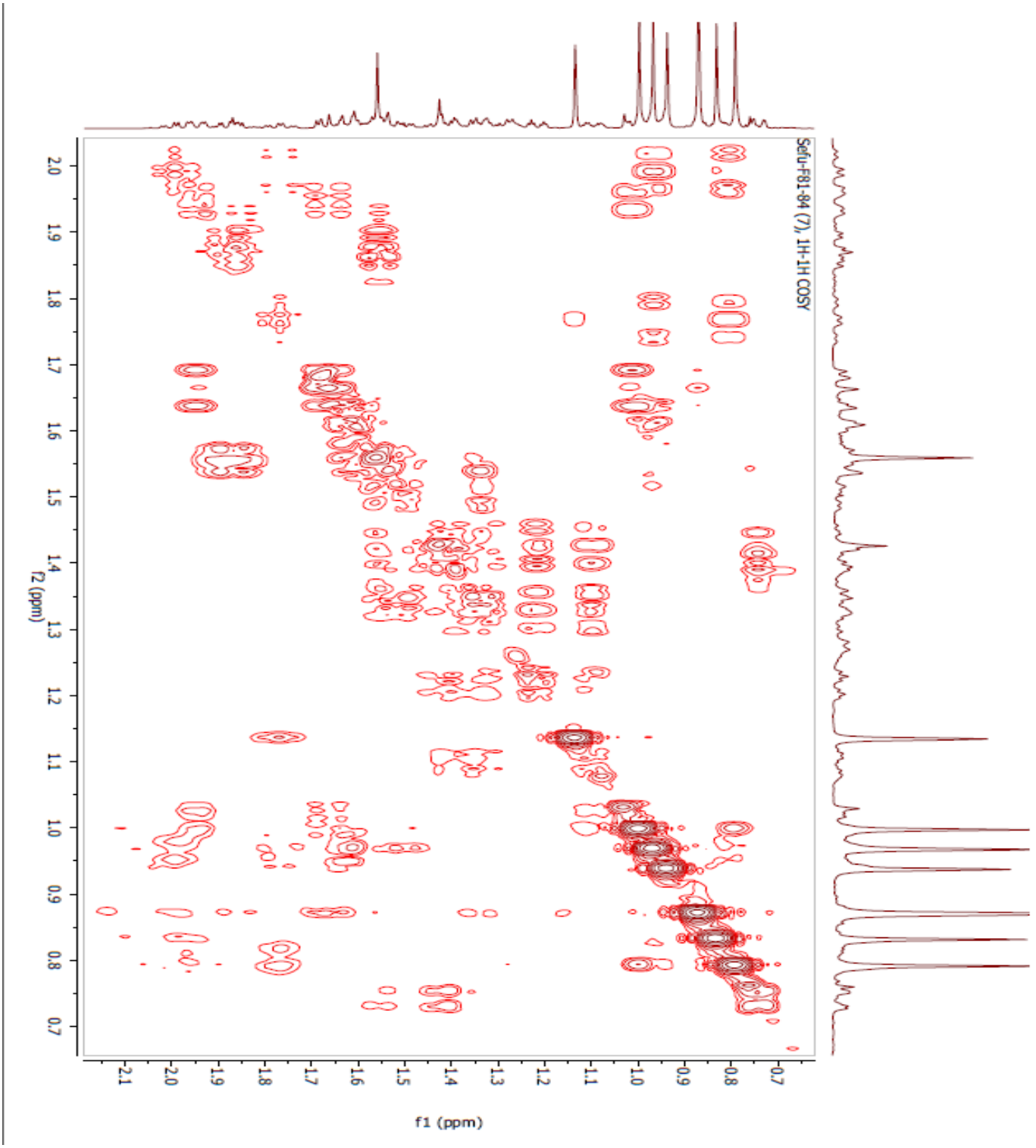
Appendix A. 13C NMR - Spectra of Compound 37



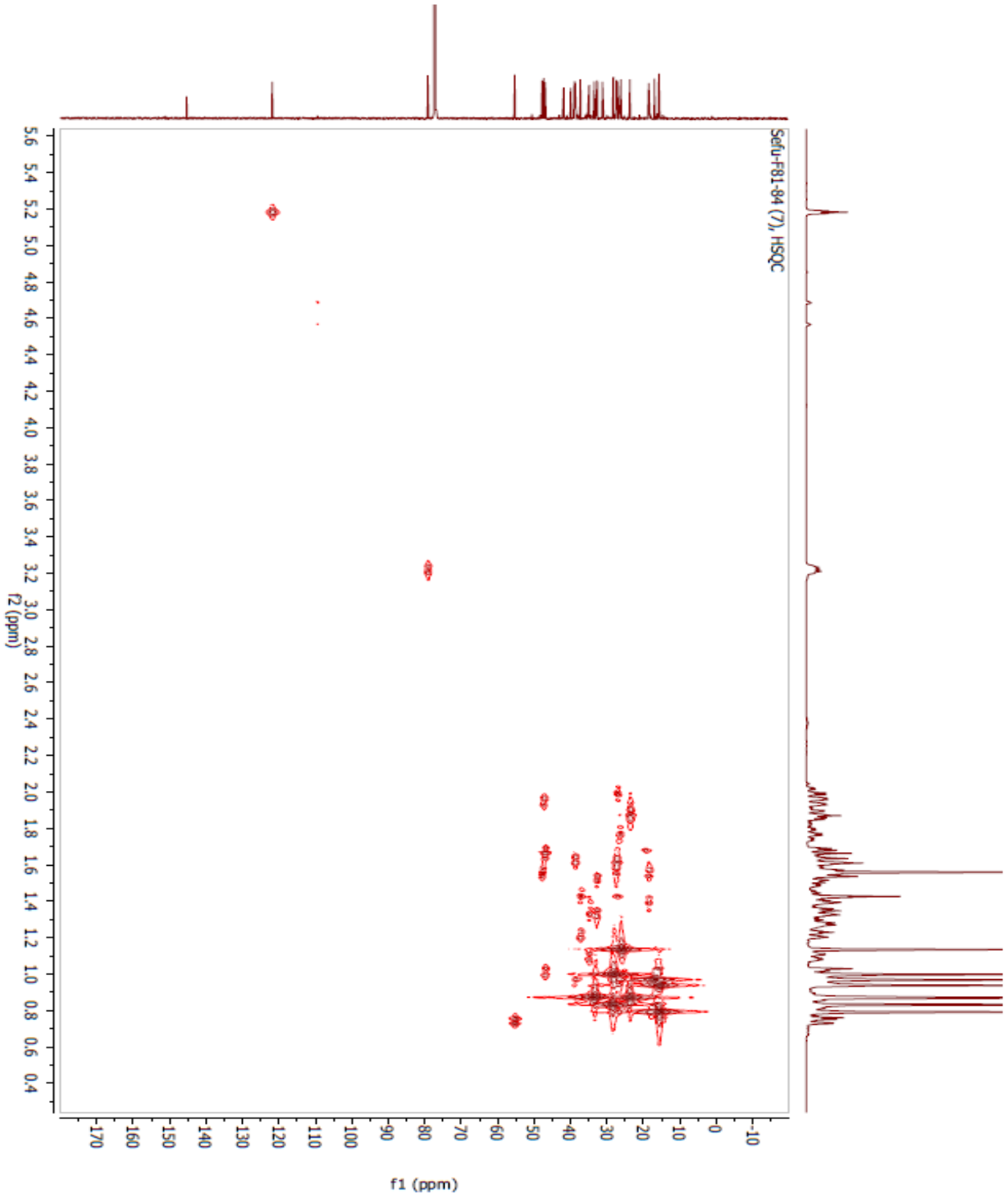
Appendix A. 1H-1H COSY - Spectra of Compound 37



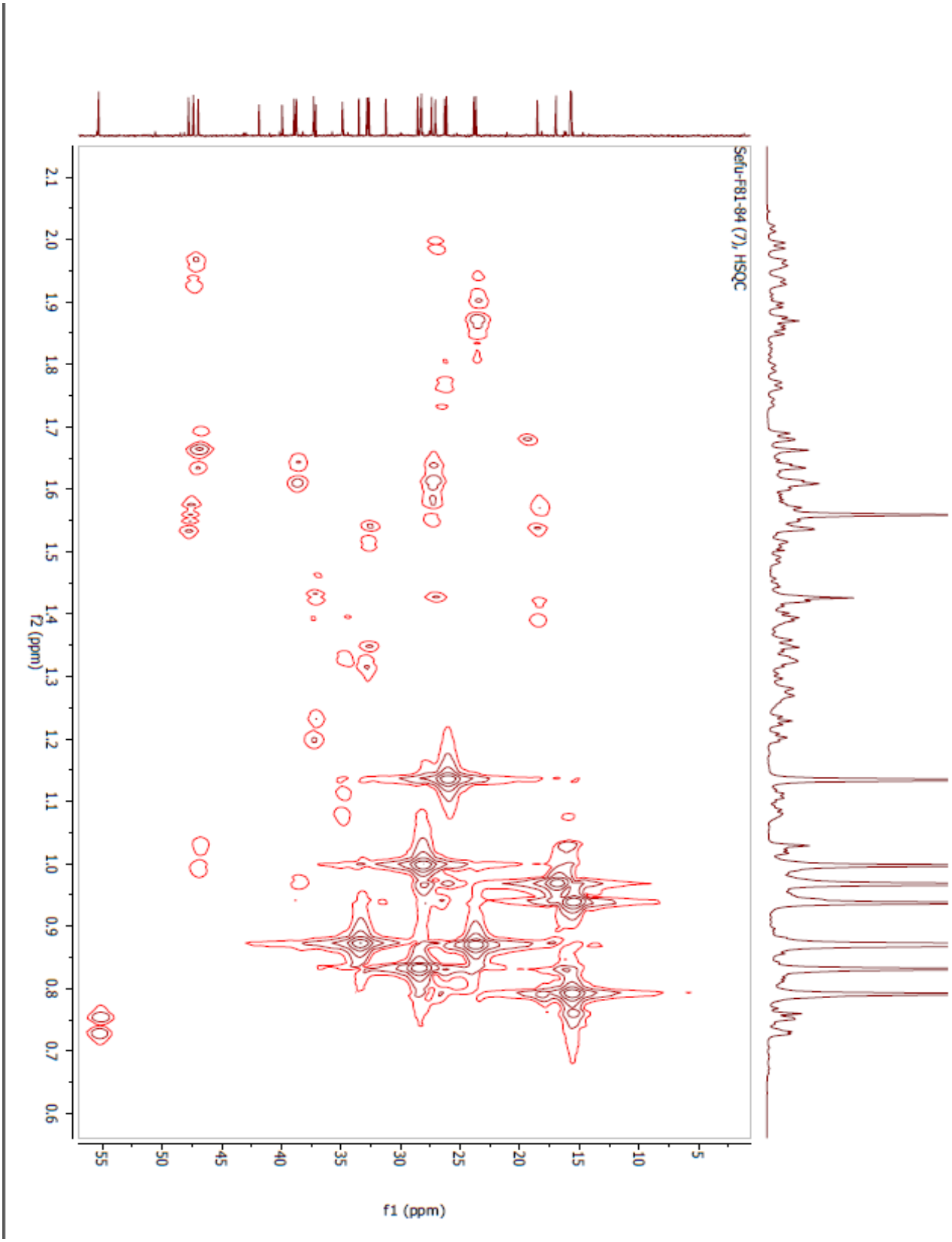
Appendix A. 1H-1H COSY - Spectra of Compound 37



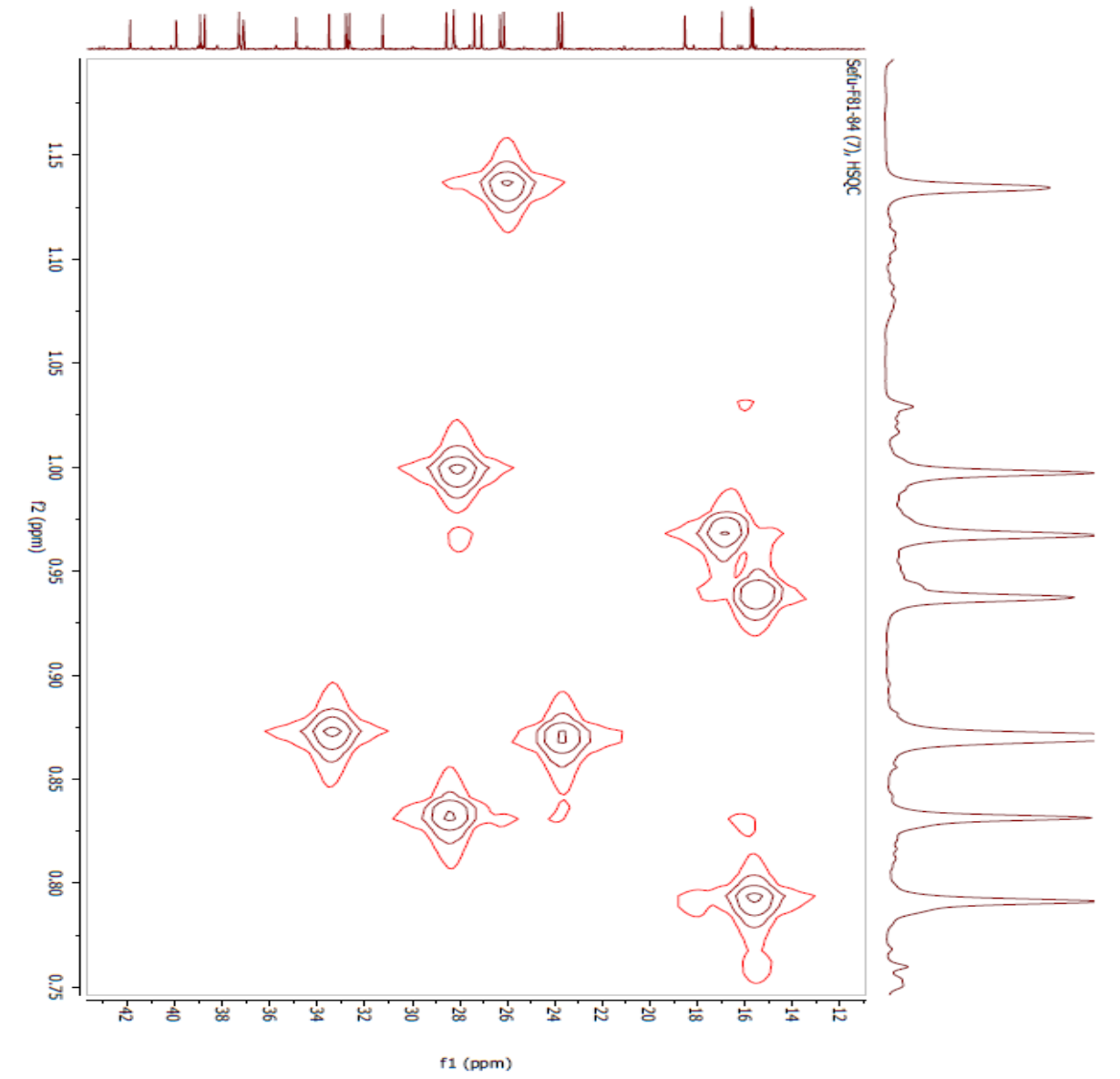
Appendix A. HSQC - Spectra of Compound 37



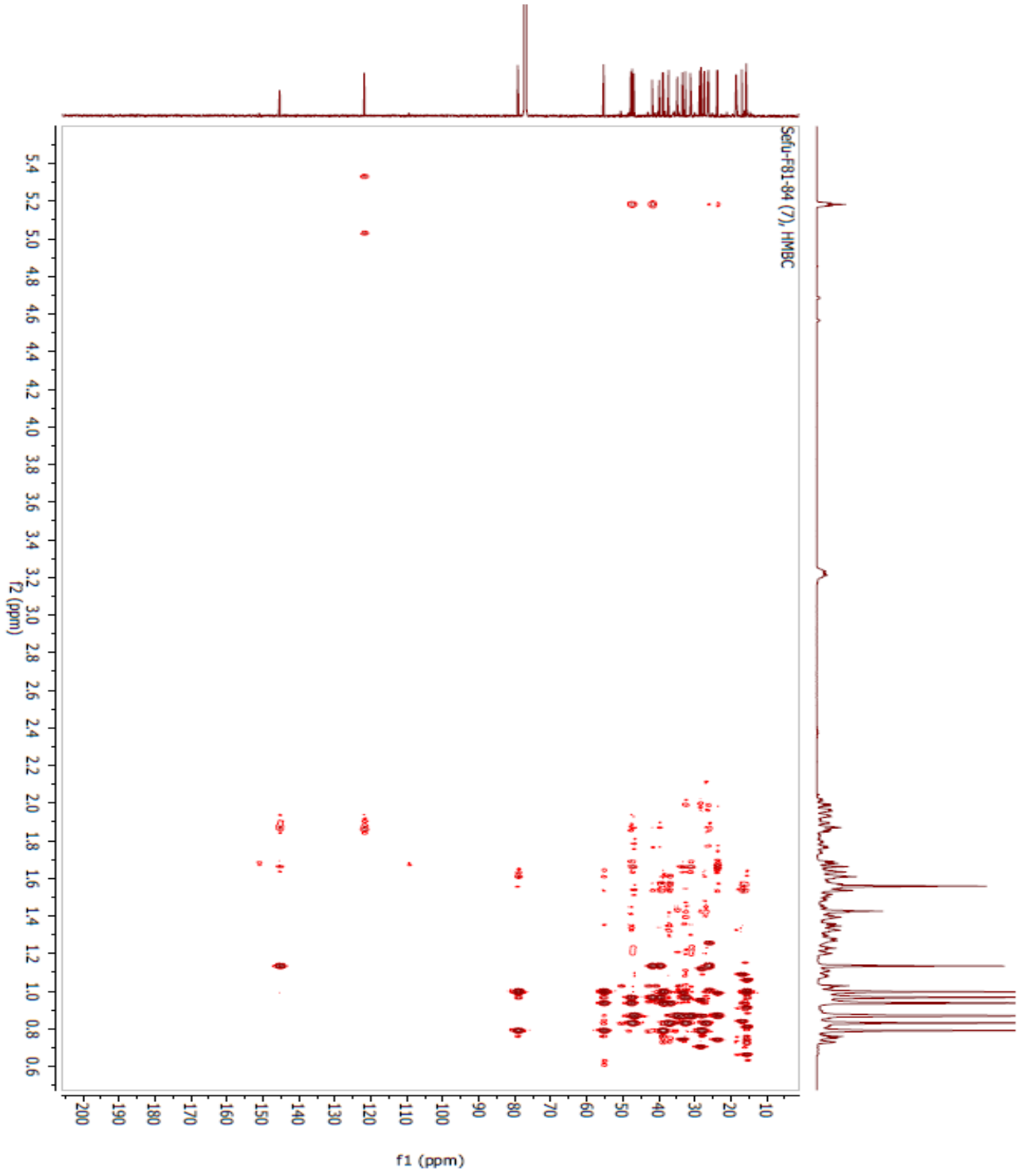
Appendix A. HSQC - Spectra of Compound 37



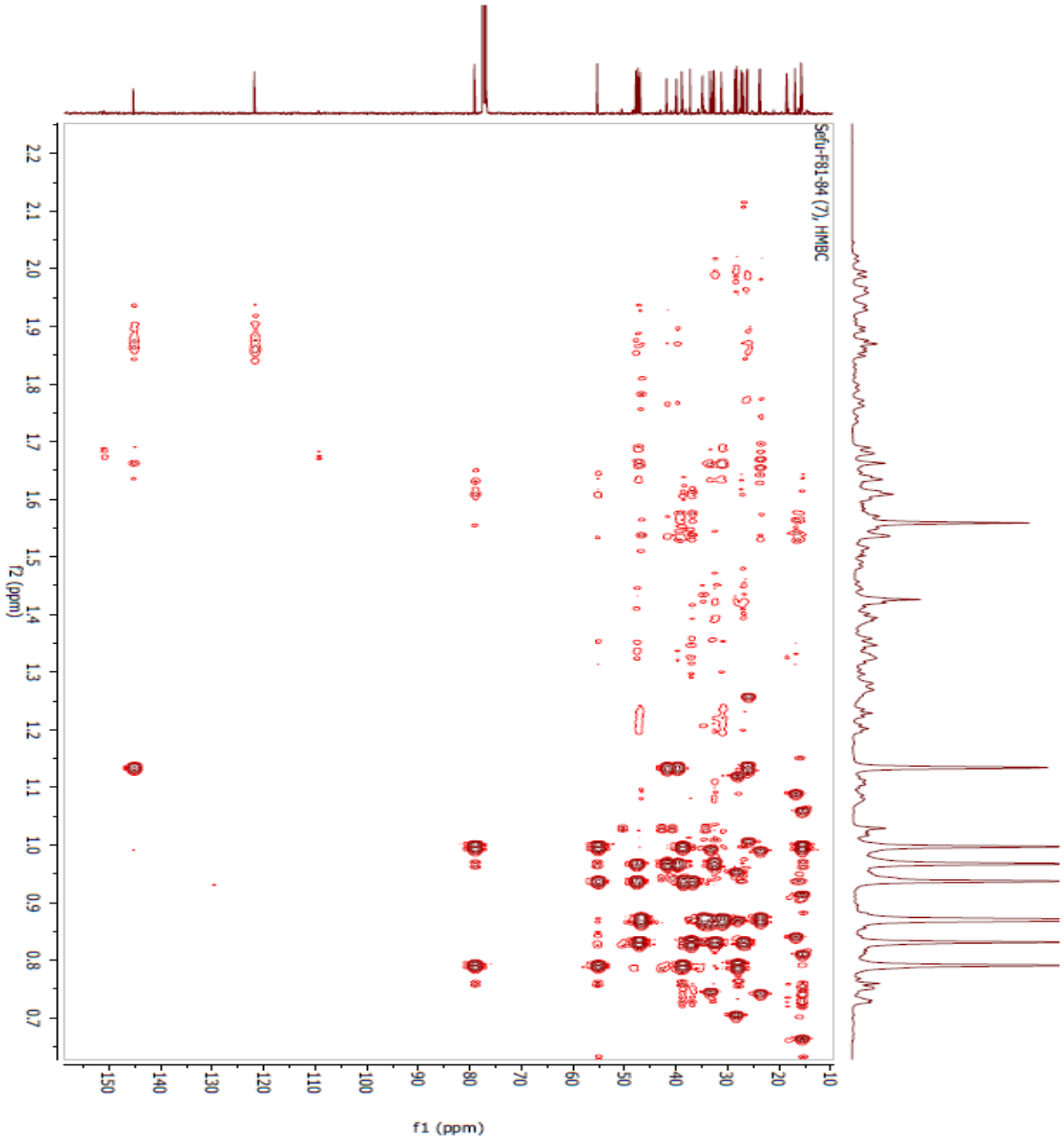
Appendix A. HSQC - Spectra of Compound 37



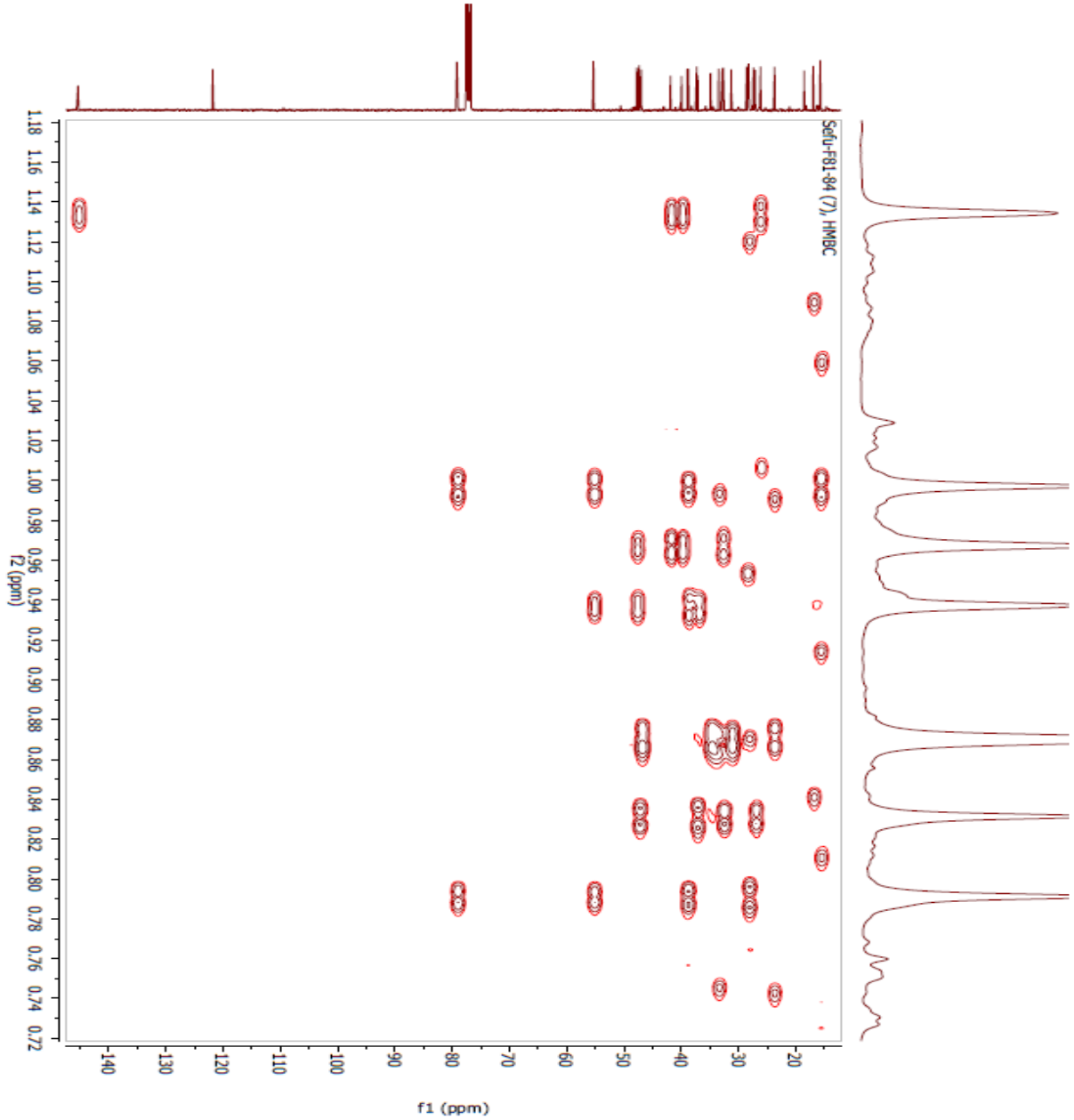
Appendix A. HMBC - Spectra of Compound 37



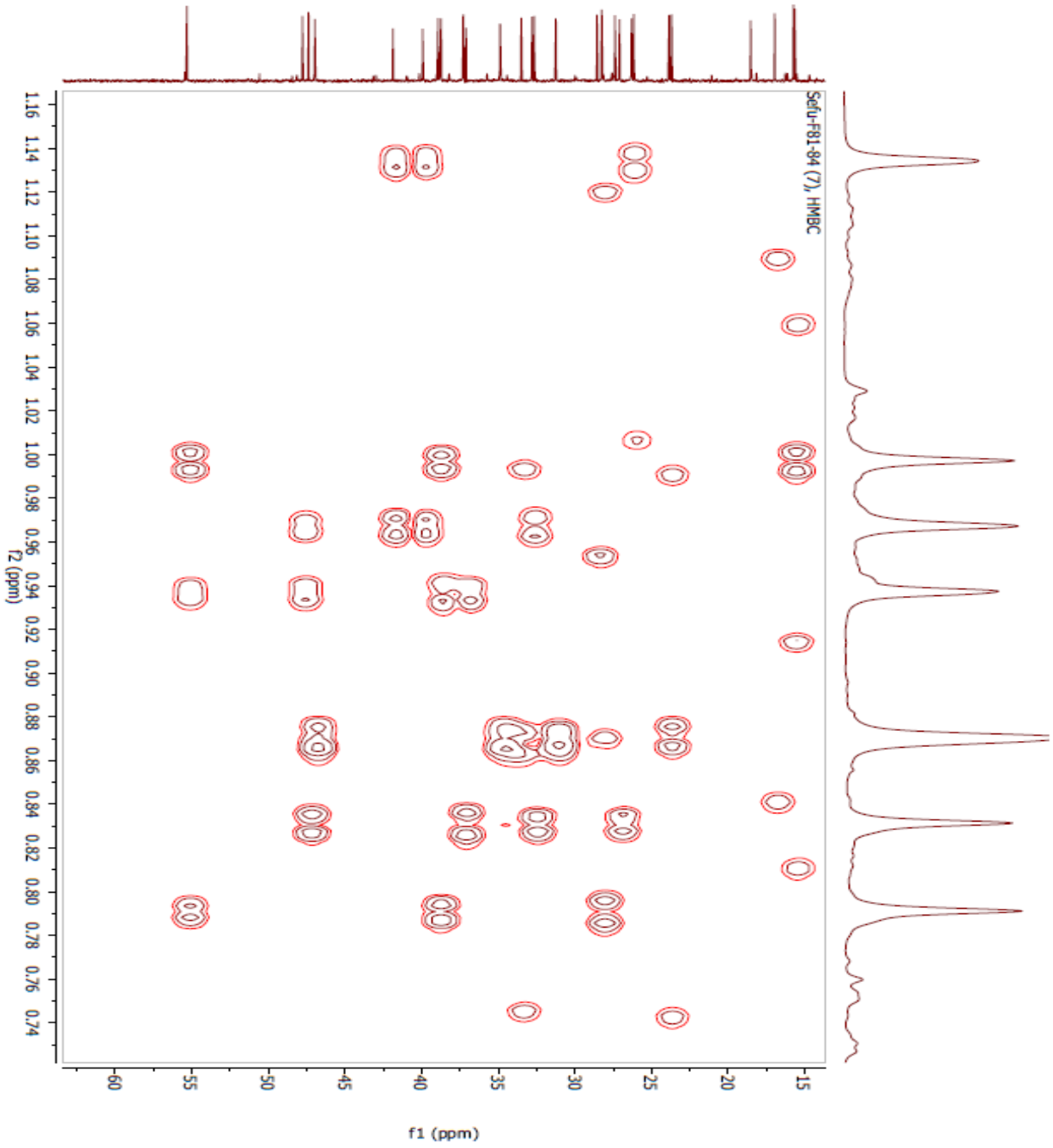
Appendix A. HMBC - Spectra of Compound 37



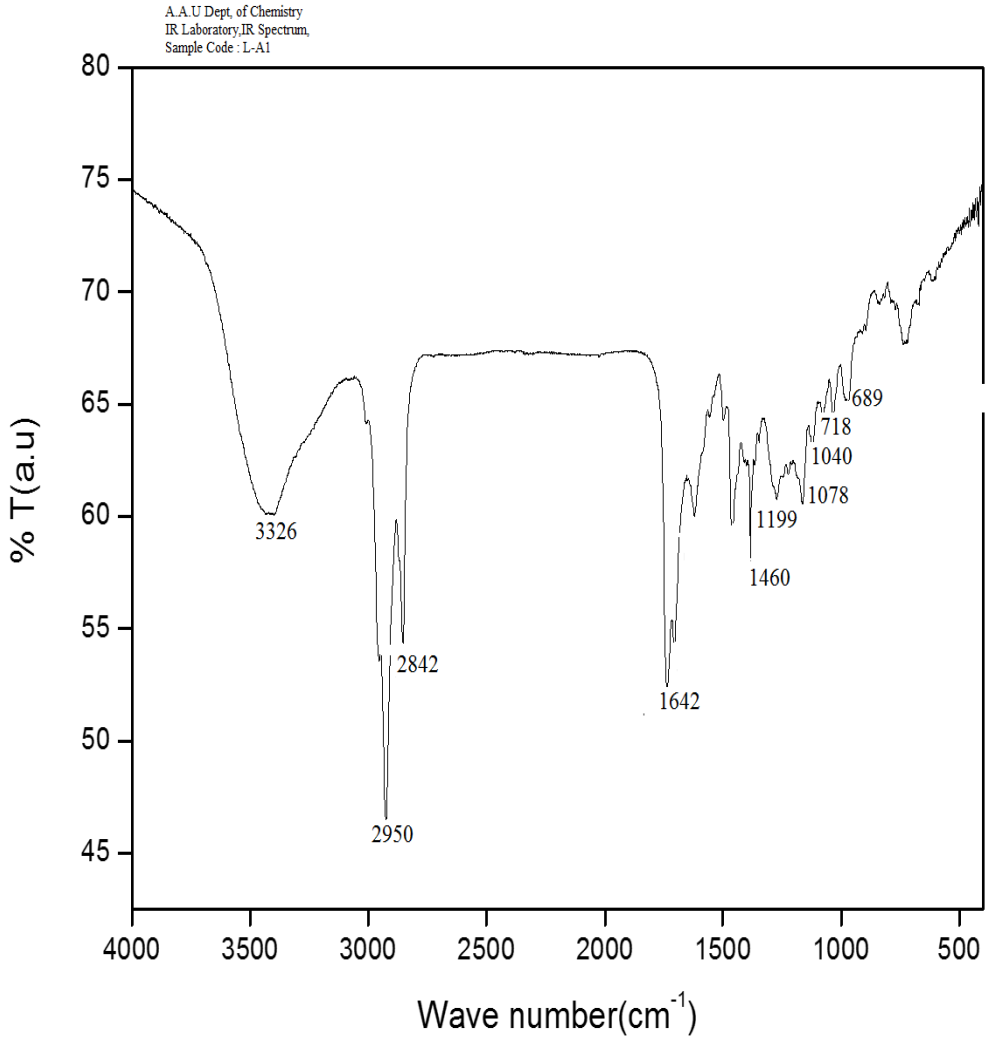
Appendix A. HMBC - Spectra of Compound 37



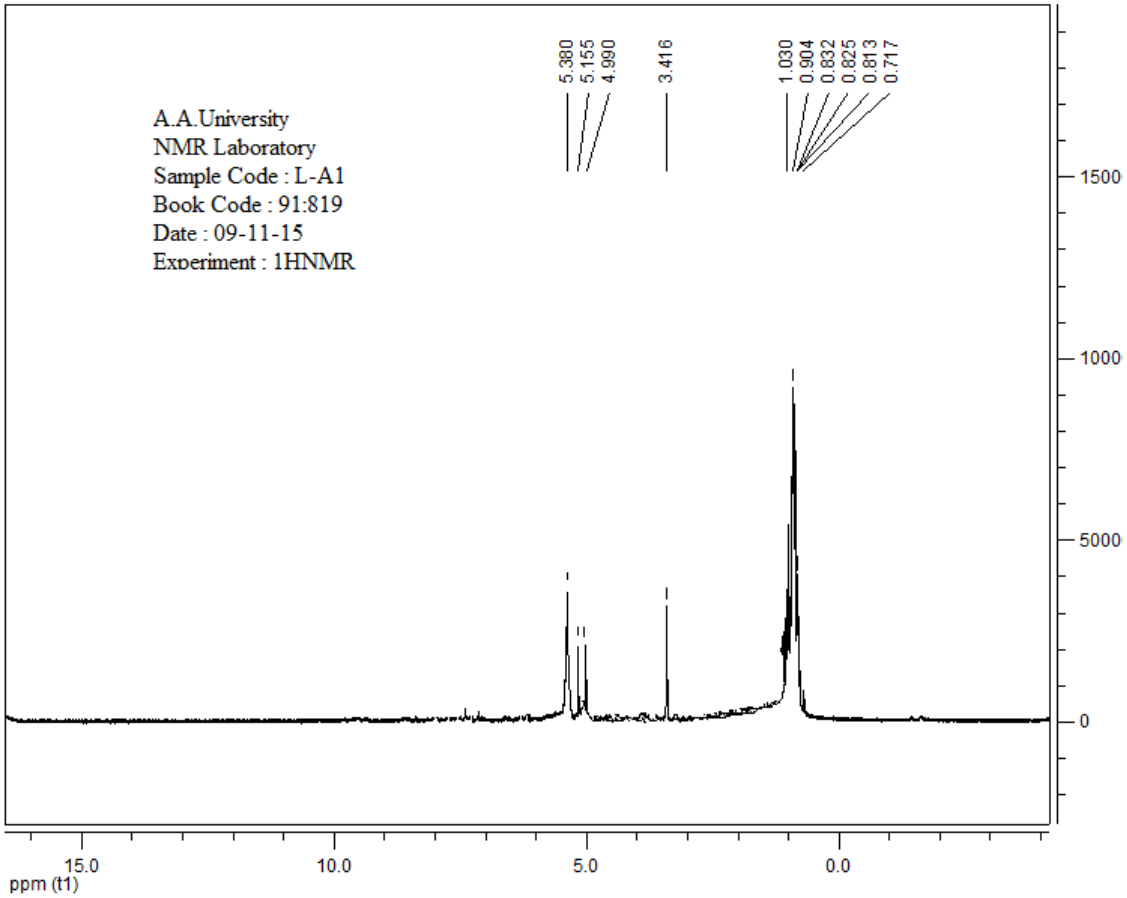
Appendix A. HMBC - Spectra of Compound 37



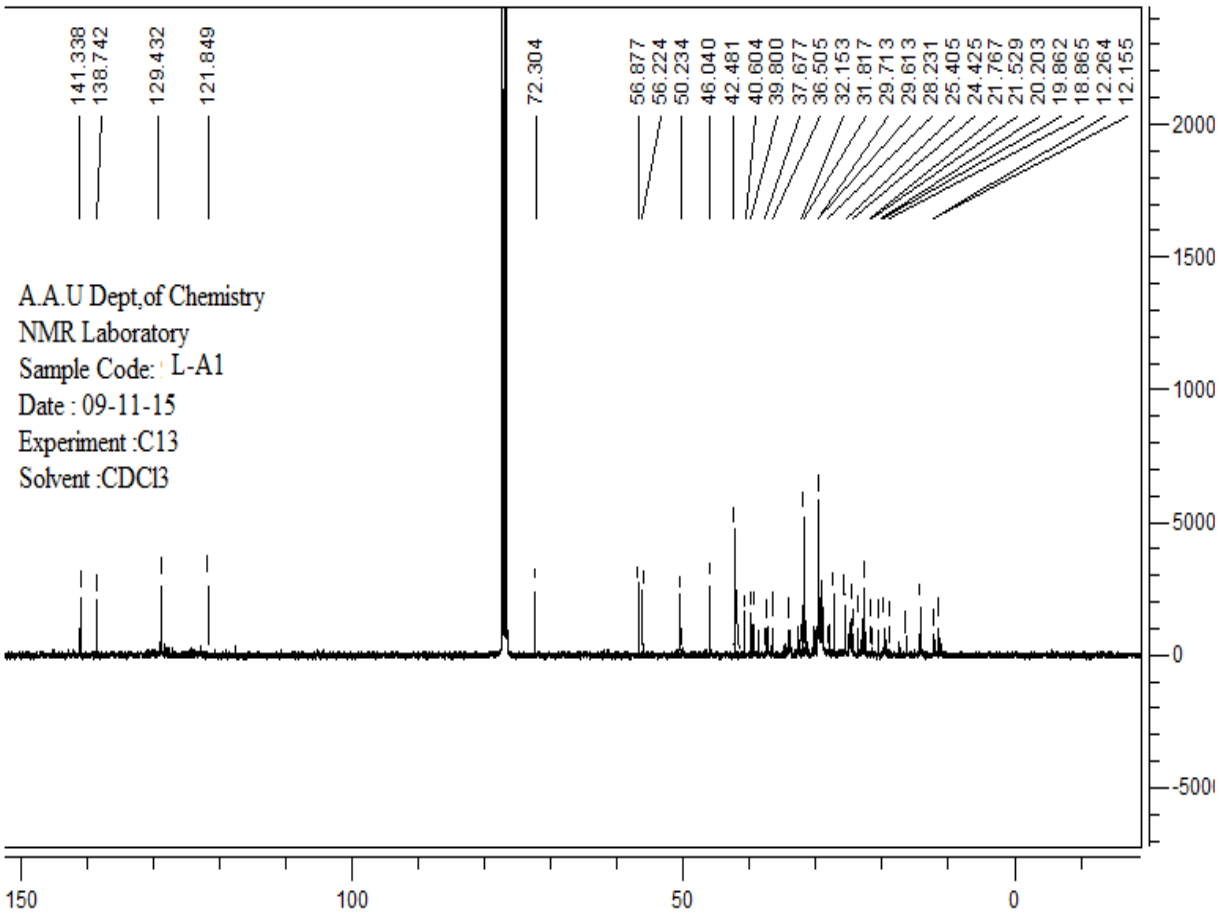
Appendix **B**. IR-Spectra of Compound 40



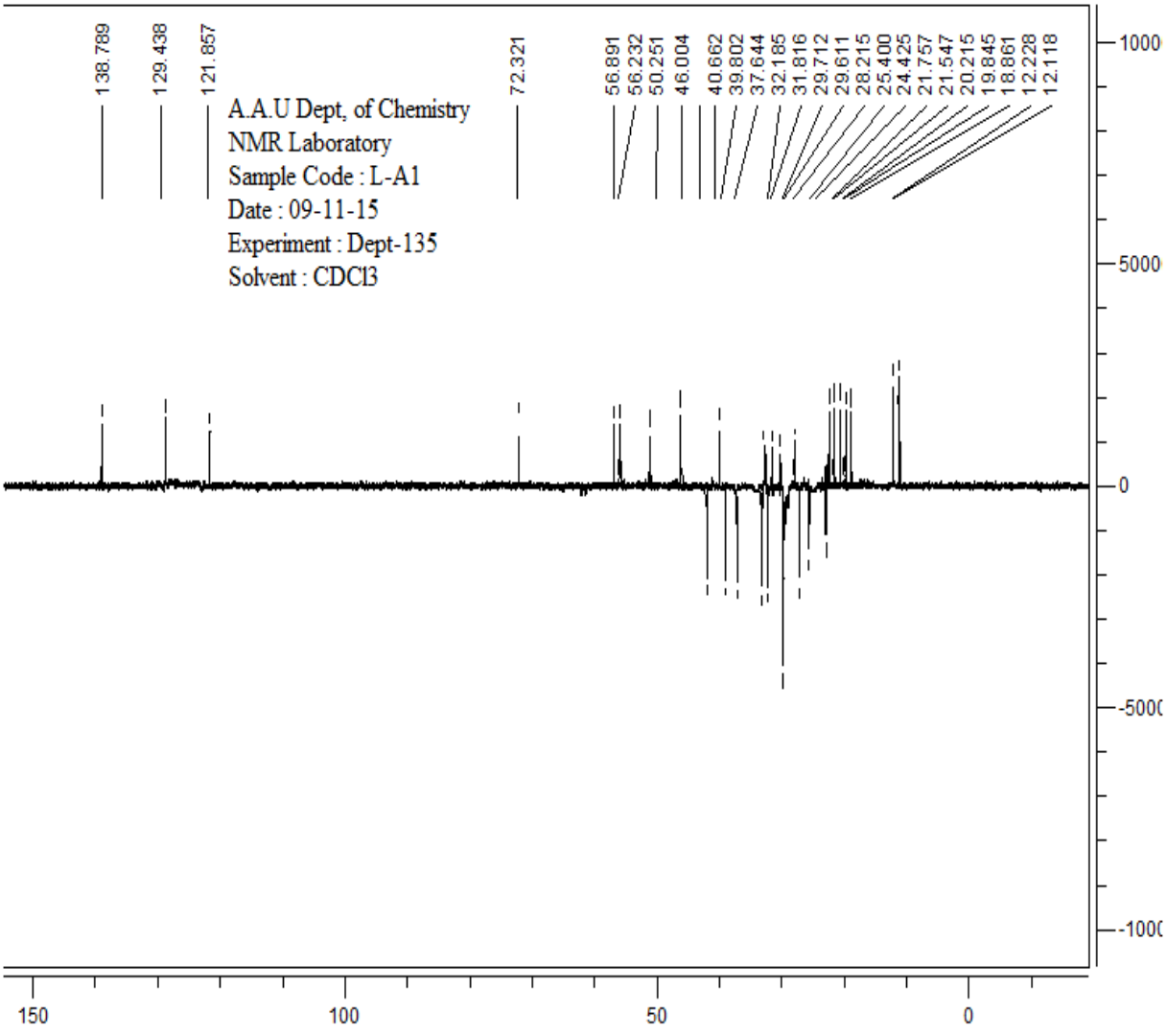
Appendix B. 1H NMR - Spectra of Compound 40



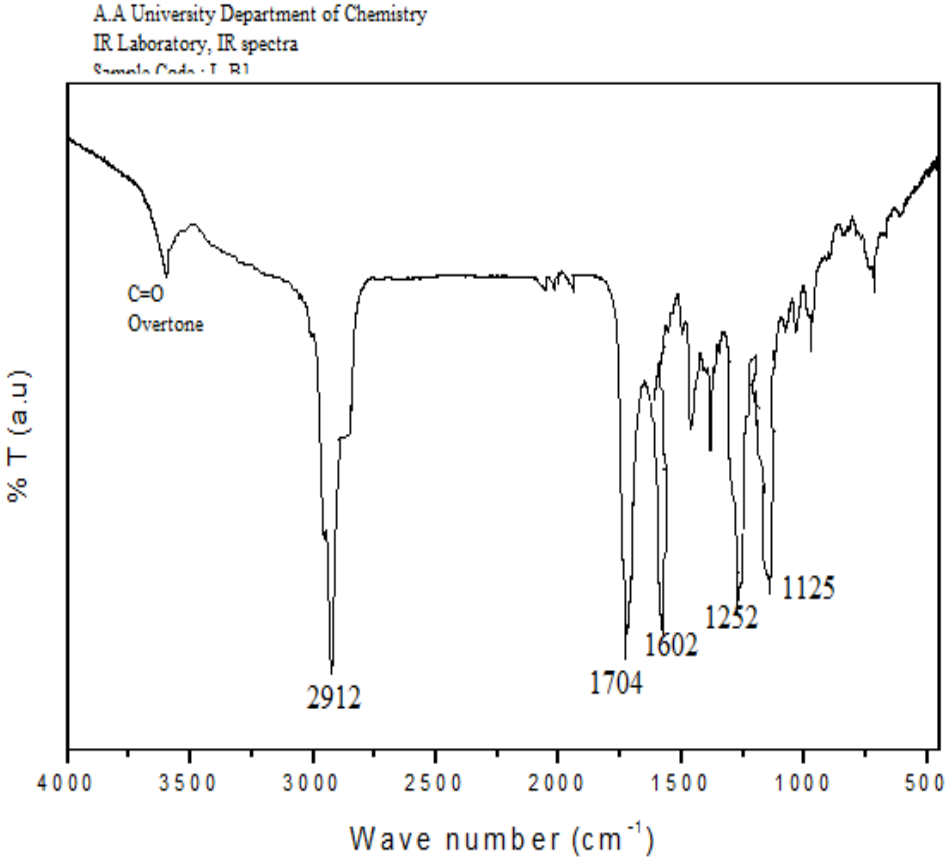
Appendix B. 13C NMR Spectra of compound 40



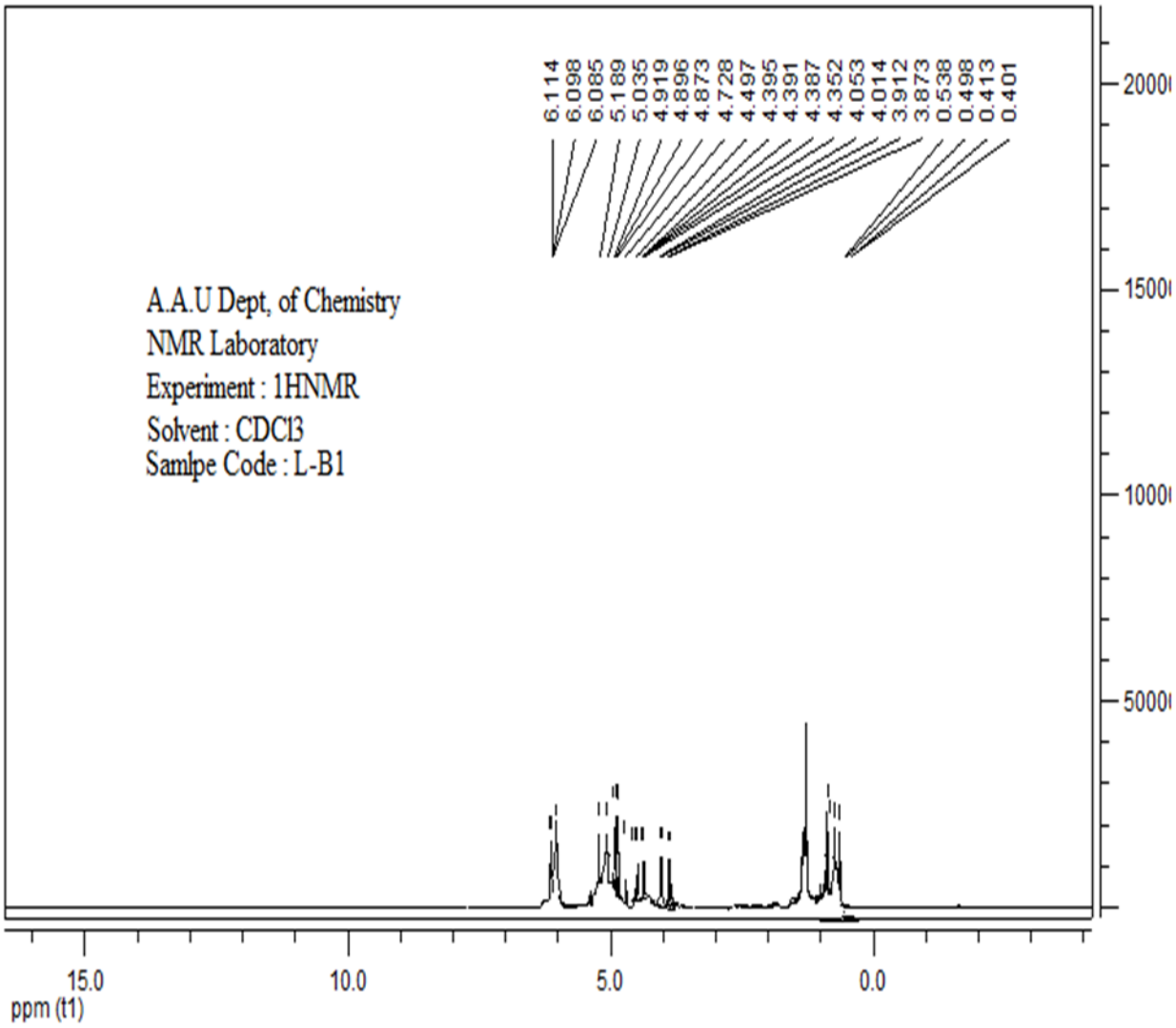
Appendix B. DEPT - 135 Spectra of Compound 40



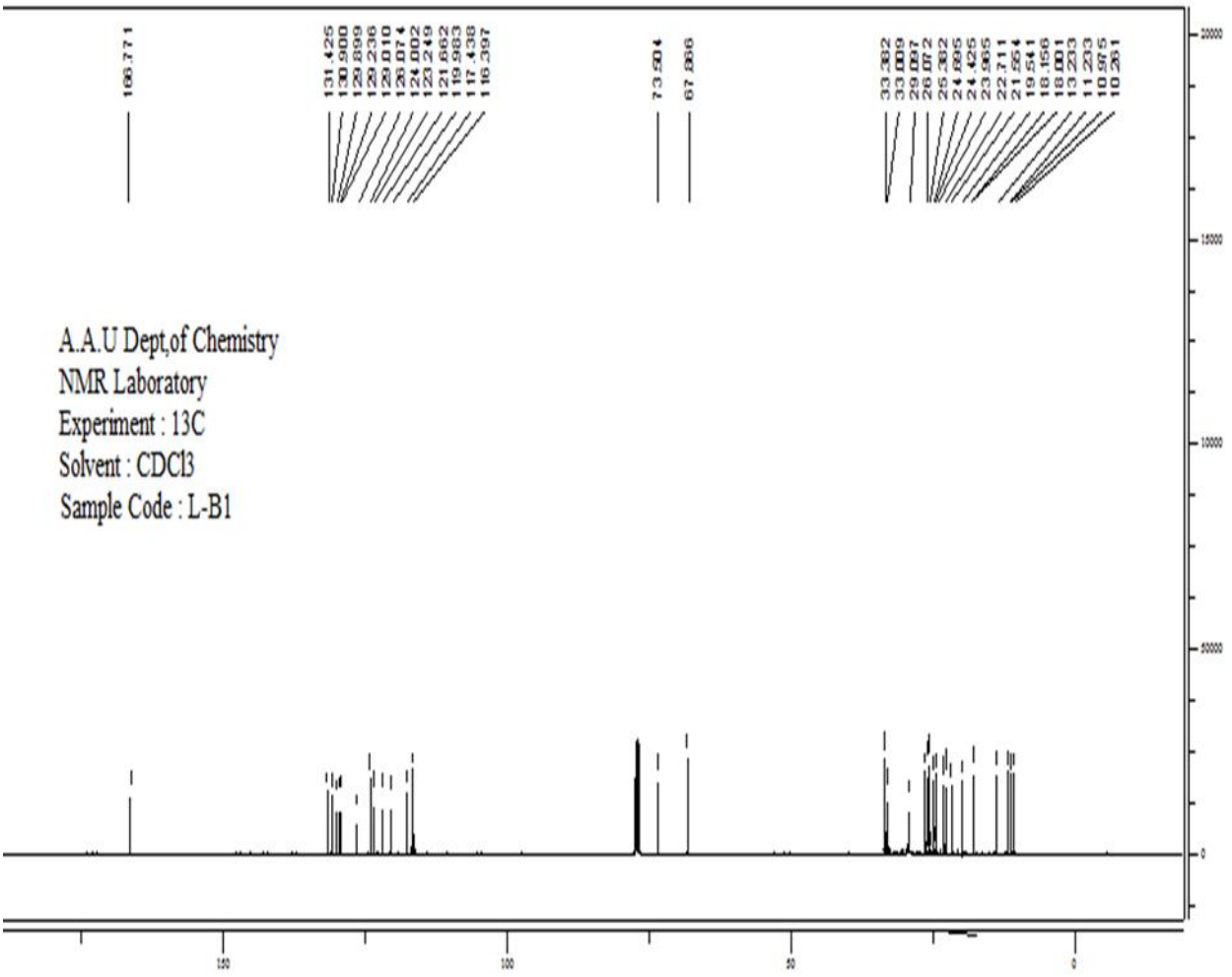
Appendix C. IR - Spectra of Compound 41



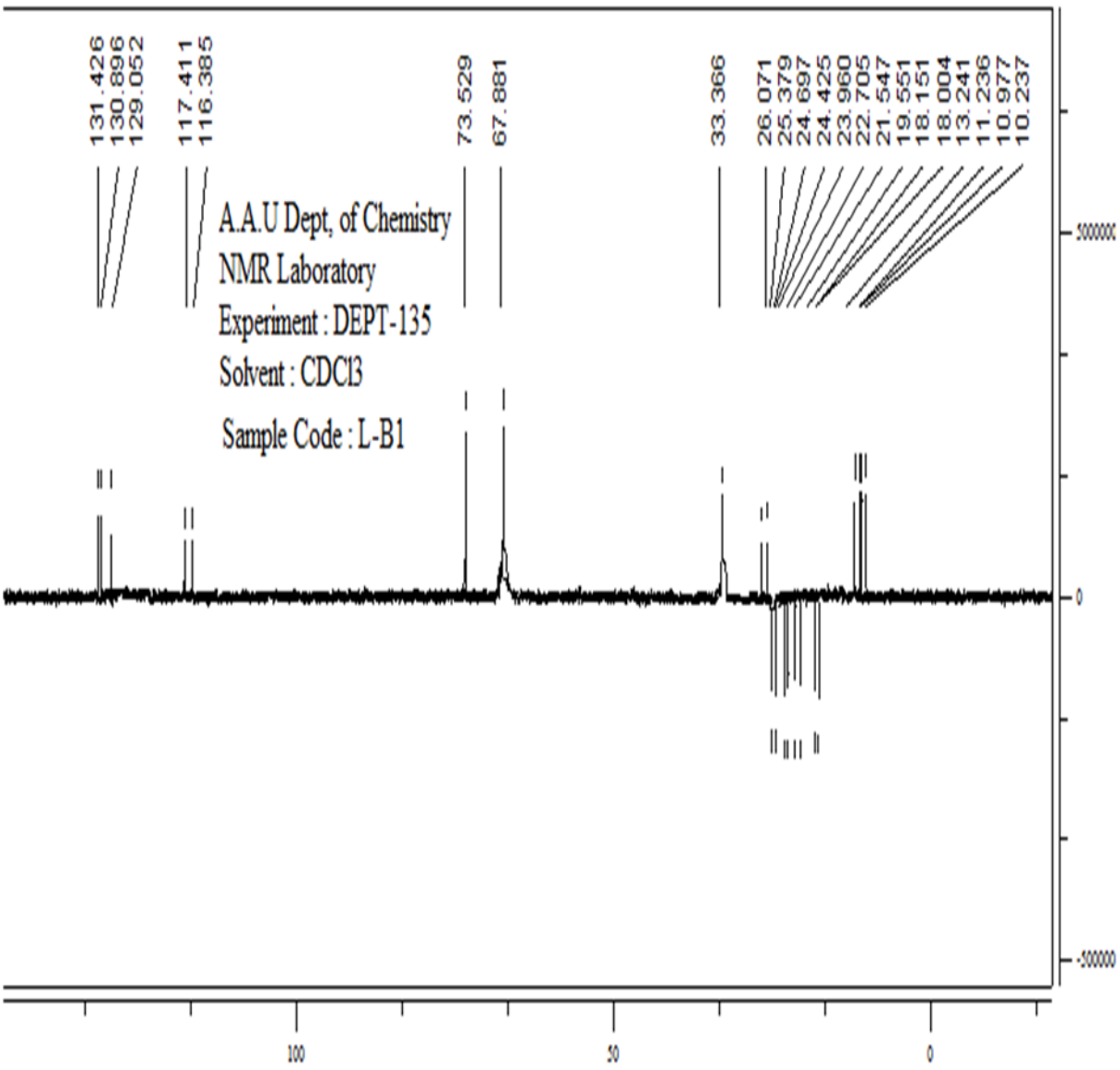
Appendix C. 13H NMR - Spectra of Compound 41



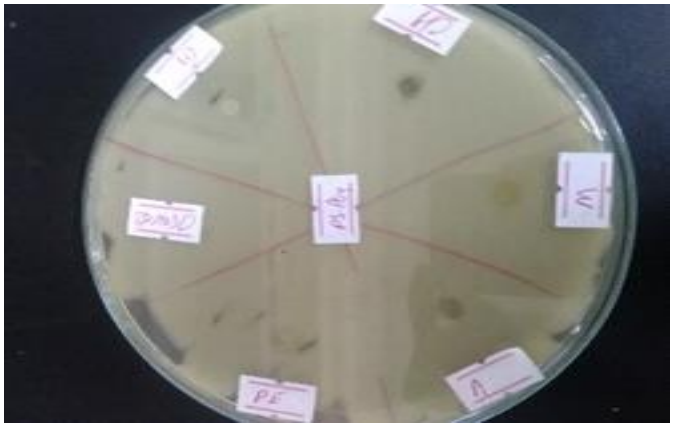
Appendix C. 13C NMR of compound 41



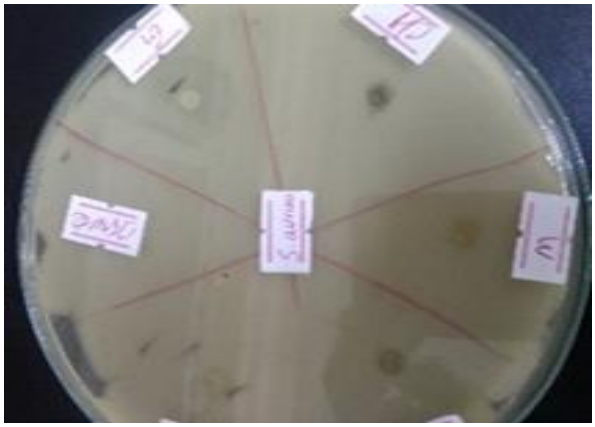
Appendix C. DEPT-135 NMR of compound 41



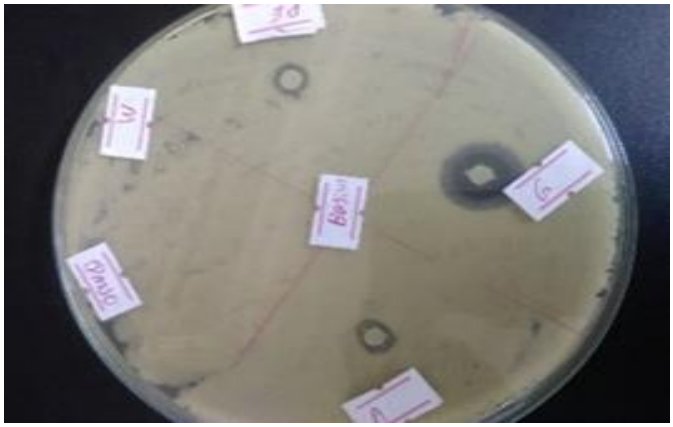
Appendix D. Zone inhibition of crude extracts of solvents on four bacterial strains.



Ps.aurus



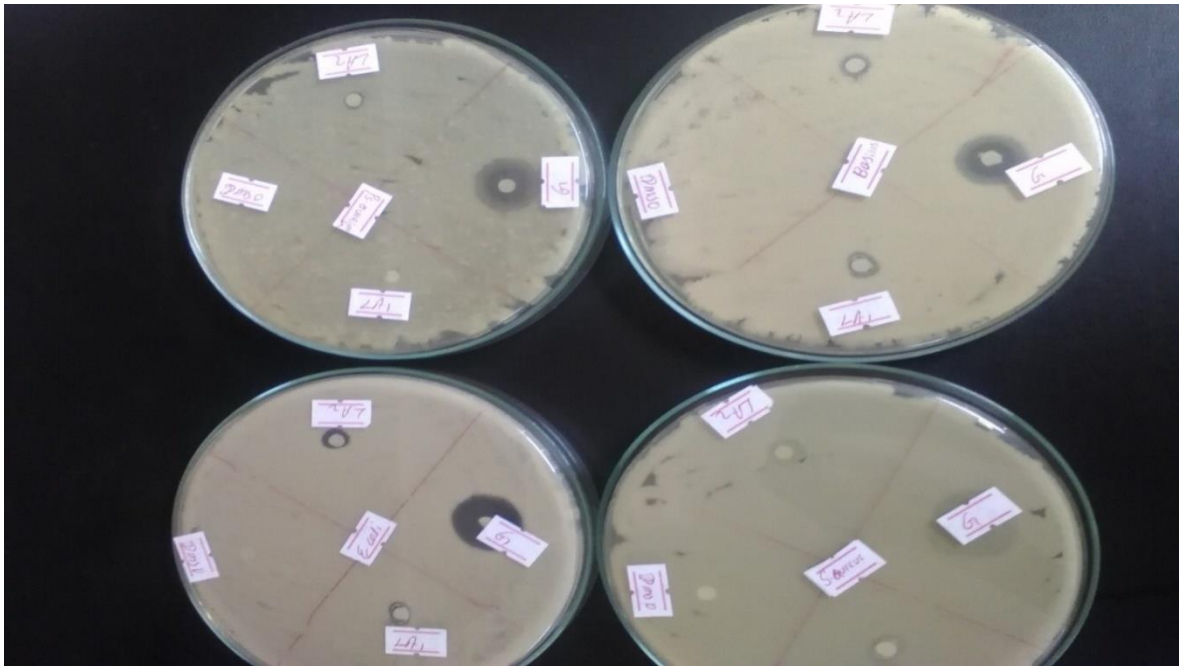
S.aureuse



B. ceruese

E.coli

Appendix E. Zone inhibition of Compound 41 extracts of solvents on four bacterial strains.



Appendix **F**. Zone inhibition of Compound **37** and **40** extracts of solvents on four bacterial strains.