

**PHYTOCHEMICAL INVESTIGATION AND BIOLOGICAL ACTIVITIES OF  
SELECTED SPECIES FROM GENUS *CUCUMIS* IN ETHIOPIA: A COMBINED  
EXPERIMENTAL AND COMPUTATIONAL STUDY**



**TESHALE ASSEFA BIRU**

**A Dissertation Submitted to  
the Department of Applied Chemistry  
School of Applied Natural Science**

**Presented in Fulfillment of the Requirement for the Degree of Doctor of Philosophy in  
Applied Chemistry (Natural Product Chemistry)**

**Office of Graduate Studies  
Adama Science and Technology University**

**June, 2024  
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## DECLARATION

I hereby declare that this Dissertation entitled” Phytochemical Investigation and Biological Activities of Selected Species from genus *Cucumis* in Ethiopia: A Combined Experimental and Computational Study” is my original work that was completed under the guidance of Professor Yadessa Melaku and Doctor Hailemichael Tesso. It has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials used for this dissertation have been duly acknowledged through appropriate citations.

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## RECOMMENDATION

We, the supervisor(s) of this dissertation, hereby certify that we have read and revised the dissertation entitled "Phytochemical Investigation and Biological Activities of Selected Species from Genus *Cucumis* in Ethiopia: A Combined Experimental and Computational Study" prepared under our guidance by Teshale Assefa Biru submitted in fulfillment of the requirements for the degree of Doctor of Philosophy in Applied Chemistry (Natural Product Chemistry) Therefore, we recommend the submission of the dissertation to the department for further review and defense.

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## **APPROVAL SHEET FOR SUBMITTING FINAL DISSERTATION**

As members of the Board of Examining of the final PhD dissertation open defense, we hereby certify that the recommendations and suggestions made by the board of examiners are appropriately incorporated into the final version of the dissertation entitled” Phytochemical Investigation and Biological Activities of Selected Species from Genus *Cucumis* in Ethiopia: A Combined Experimental and Computational Study” by Teshale Assefa Biru.

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We, the undersigned, members of the Board of Examiners of the dissertation open defense by Teshale Assefa Biru have read and evaluated the dissertation entitled” Phytochemical Investigation and Biological Activities of Selected Species from Genus *Cucumis* in Ethiopia: A Combined Experimental and Computational Study” and examined the candidate during open defense. This is, therefore, to certify that the dissertation is accepted for partial fulfillment of the requirement of the degree of Doctor of Philosophy in Applied Chemistry (Natural Product Chemistry)

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### List of Abbreviations and Acronyms

|                  |                                             |        |                                                  |
|------------------|---------------------------------------------|--------|--------------------------------------------------|
| ADT              | AutoDock Tools                              | MS     | Mass Spectrometry                                |
| BBB              | Blood-brain barrier                         | MTT    | Mean Transit Time                                |
| CFU              | Colony Forming Unit                         | NCCS   | National Centre for Cell Science                 |
| CYP              | Cytochrome-P                                | NMR    | Nuclear Magnetic Resonance                       |
| DMEM             | Dulbecco's Modified Eagle<br>Medium         | NRB    | Number of Rotatable Bonds                        |
| DNA              | Deoxyribonucleic Acids                      | OGD    | OesophagoGastroDuodenoscopy                      |
| DNPH             | Dinitrophenylhydrazine                      | Pca    | Prostate Cancer                                  |
| DPPH             | 2,2'-diphenyl-1-picrylhydrazyl              | PDB    | Protein Data Bank                                |
| FBS              | Fasting Blood Sugar                         | P-gp   | Permeability glycoprotein                        |
| GI               | Gastrointestinal                            | ppm    | Parts Per Million                                |
| GC/MS            | Gas chromatography-Mass<br>Spectrometry     | PTLC   | Preparative Thin Layer<br>Chromatography         |
| GUI              | Graphic User Interface                      | Rf     | Retardation factor                               |
| HBD              | Hydrogen-bond donors                        | RI     | Retention Indices                                |
| HDI              | Low human development index                 | RNS    | Reactive nitrogen species                        |
| HMBC             | Heteronuclear Multiple Bond<br>Correlation  | ROS    | Reactive oxygen species                          |
| HSQC             | Heteronuclear Single Quantum<br>Correlation | SMILE  | Simplified molecular-input line-<br>entry system |
| IC <sub>50</sub> | Concentration of 50% Inhibition             | SOD    | Superoxide Dismutase                             |
| IR               | Infrared Radiation                          | TB     | Tuberculosis Bacteria                            |
| KSR              | kinase Suppressor Ras                       | TLC    | Thin layer chromatography                        |
| MCF              | Michigan Cancer Foundation                  | TPSA   | Total Polar Surface Area                         |
| MIC              | Minimum Inhibitory Concentration            | UV-Vis | Ultra violet-visible spectrometer                |
| MHA              | Mueller-Hinton Agar                         | WHO    | World health Organization                        |

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## ABSTRACT

The Genus *Cucumis* has been used in Ethiopia in a traditional medicine for the treatment of many diseases including cancer, cholera, diabetes, infectious diseases, malaria and respiratory tract disorders. In view of its traditional use, the leaves, stems, fruits and roots of *C. ficifolius*, fruits of *C. dipsaceus* and leaves of *C. prophetarum* were successively extracted with *n*-hexane/petroleum ether, EtOAc and methanol furnish 25 compounds. The compounds were identified using spectroscopic methods as cucurbitacin B (**24**), cucurbitacin D (**26**),  $\alpha$ -spinasterol (**58**), isocucurbitacin D (**100**), 23,24-dihydroisocucurbitacin D (**102**), lignoceric acid (**104**), eicosane (**105**), stearic acid (**106**), 12-hydroxyoctacosanoic acid (**107**), 13-hydroxyoctadecanoic acid (**108**), kuguaglycoside E-3-acetate (**109**), octacosane (**110**), docosane (**111**), 17-(5-ethyl-2,6-dihydroxy-6-methylhept-3-en-2-yl)-9-(hydroxymethyl)-13-methyl-1H-cyclopenta[ $\alpha$ ]phenanthren-3-ol (**112**), erythrodiol (**113**), (9,12)-propyl icosanoate (**114**), 2-heptylcyclohexanol (**115**), decanoic acid (**116**) and 1,3-dihydroxypropan-2-yl dodecanoate (**117**). Most of the compounds were new to the genus except compound **24**, **26**, **58**, **100**, **102** and **116**. Compounds **157** (57.08%), **149** (54.90%), **166** (99.76%) and **105** (37.8%) were identified as a major constituent from the essential oils of the leaves, stems, fruits and roots of *C. ficifolius*, respectively. The essential oils of the leaves, stems and fruits of *C. dipsaceus* furnished hydrocarbons including compound **170** (33.40%), **173** (98.93%) and **166** (96.65%) as a major constituent, respectively whereas compound **166** (99.75%), **160** (96.31%) and **105** (51.79%) were identified from the essential oils of the leaves, stems and roots of *C. prophetarum*. The essential oils and isolated compounds were evaluated for their *in vitro* antibacterial activity using disc diffusion method. The essential oil of the leaves of *C. dipsaceus* exhibited better inhibition zone diameter (IZD) of  $11.93 \pm 0.42$  mm against *E. coli* while the other displayed modest activities compared with the positive control (ciprofloxacin). The *n*-hexane extract of the stem of *C. ficifolius* exhibited IZD of about  $14.00 \pm 0.20$  mm,  $13.50 \pm 0.50$  mm and  $13.00 \pm 0.50$  mm against *P. aeruginosa*, *S. pyogenes* and *E. coli* at  $400 \mu\text{g mL}^{-1}$  while root extract also displayed activity against *P. aeruginosa* and *S. aureus* with IZD of  $14.00 \pm 0.10$  mm and  $13.67 \pm 0.53$  mm respectively. Compound **58** showed IZD of  $13.67 \pm 0.57$ ,  $15.00 \pm 0.10$  and  $13.33 \pm 0.57$  mm against *E. coli*, *P. aeruginosa* and *S. pyogenes* respectively.

The results of MTT assay against MCF-7 breast cancer cell lines revealed that EtOAc extract of the stem of *C. dipsaceus* showed better cytotoxicity relative to the other samples at low

micromolar concentrations ( $IC_{50} = 10.41 \mu\text{g/mL}$ ) and comparable to standard drug ( $IC_{50} = 7.96 \mu\text{g/mL}$ ).

Compounds **109**, **113**, **24**, **112**, (**26 & 100**) and **102** exhibited better DPPH radical scavenging activities with  $IC_{50}$  values of 4.83, 7.09, 7.24, 7.99, 8.84 and  $8.95 \mu\text{g mL}^{-1}$ , respectively. The result was close to with ascorbic acid ( $IC_{50} = 2.31 \mu\text{g/mL}$ ). The *in silico* molecular docking analysis of isolated compounds were done using AutoDock vina against DNA gyrase, protein kinase and human topoisomerase II $\beta$ . Compounds **24** and **26** displayed binding affinity of  $-9.1$  and  $-9.8$  kcal/mol against DNA gyrase, respectively. Compounds **58** ( $-8.0$  kcal/mol) and **112** ( $-7.6$  kcal/mol) displayed better binding affinity against DNA gyrase compared to ciprofloxacin ( $-7.3$  kcal/mol). The binding affinities of compounds **58**, **26** and **109** were found to be  $-9.6$ ,  $-9.4$  and  $-9.4$  kcal/mol against human topoisomerase II $\beta$ , respectively. Most of the isolated compounds obeyed Lipinski's rule of five. Swiss ADME prediction has shown that compounds **104-108**, **111** and **115** inhibited CYP1A2 cytochromes, compounds **106**, **107** and **108** inhibited CYP2C9 cytochromes, compounds **107** and **108** inhibited CYP2D6 cytochromes, while compounds **24**, **26**, **100** and **102** were found to inhibit CYP3A4 and substrate of permeability glycoprotein (P-gp). The toxicological prediction gave results of endpoints such as hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity. The studied compounds were predicted to be non-hepatotoxic, non-mutageni, non-cytotoxicity and non-irritant. Therefore, the antibacterial, cytotoxic and radical scavenging activities displayed by the constituents of *C. ficifolius*, *C. dipsaceus* and *C. prophetarum* corroborate the traditional use of this plant against bacteria and cancer.

**Key words:** Cucurbitaceae, *Cucumis*, *Cucumis ficifolius*, *Cucumis dipsaceus*, *Cucumis prophetarum* Antibacteria, Cytotoxicity, Radical Scavenging, Molecular Docking.

# 1. INTRODUCTION

## 1.1 Background

Since ancient times, medicinal plants and herbs have been used as the primary sources of medicine for people throughout the world. The global health needs of the entire population could not be met by the current healthcare system alone (WHO, 2023). As a result, humans have long used chemicals produced from medicinal plants to treat a wide range of illnesses. This is corroborated by a WHO report stating that 80% of people living in developing countries get their major medical care from plants (Gezahegn Z., *et al.*, 2015).

Modern medicine makes considerable use of natural product. There is still a great deal of undiscovered compounds in the plant world that may have therapeutic value, and very few known plant species have been examined from a phytochemical or pharmacological perspective (Friss, I., *et al.*, 2010). Many medications utilized in modern medicine are mostly sourced from natural resources or their chemical counterparts. Hence, medicinal plants are still a major source of novel bioactive compounds and many of them are used as hit in drug design and discovery (Abuajah, C.I., *et al.*, 2015). Furthermore, the pharmaceutical business has a tremendous demand for phytocompounds originating from plants (Swamy, M., and Sinniah, U., 2015).

Numerous ailments in humans and animals including fever, diarrhea, coccidiosis, diarrhea, TB, skin disorders, hyperlipidemia, and salmonellosis, can be treated using a wide range of African herbs (Usman, J.G., *et al.*, 2011). These ailments are common in tropical areas (Usman, J.G., *et al.*, 2011). For hundreds of years, African people have utilized plants to extract medicinal compounds, treat a variety of diseases, and improve their overall health. Due to the diversity of Ethiopia's physical characteristics, climate, geography, habitat, vegetation types, and wildlife, the country possesses abundant biodiversity resources (Friss, I., *et al.*, 2010). Due to difficulties to access modern health care facilities, traditional healers are accepted in Ethiopian culture. Traditional medicines are also inexpensive compared to the currently existing modern drug. Ethiopian plants have demonstrated a high level of therapeutic efficacy for a number of human and domestic animal maladies; as a result, knowledge of the usage of medicinal plants plays a critical role in meeting the nation's needs for livestock and human health care (Endashaw, B., 2007).

Studies on the phytochemistry of plants, animals, and microbes aid in our knowledge of the fundamental mechanisms guiding the growth, development, and differentiation of their chemical products. Numerous compounds are produced by plants and animals (Bizuayehu, Z., 2008). In this way, natural products are the potential resources for identification of leads in order to develop novel medicinal molecules. This is due to the low cost, great therapeutic efficacy, and negligible to nonexistent side effects of natural products. The customary knowledge that is currently available forms the basis of the experts' investigations into the therapeutic benefits of plants (Mohanty, S.K., *et al.*, 2014).

The genus *Cucumis* (Cucurbitaceae) comprises of 62 species distributed mainly in Africa, Asia, Australia and some islands in the Pacific (Rajasree, R.S *et al.*; 2016; Dwivedi, N.K., *et al.*, 2010). *Cucumis* is a genus of twining, tendril-bearing plant producing annual stems up to 2.5 meters long (Abebe, W., 2016). Some species of the genus *Cucumis* are traditionally been used in various parts of the world against wounds, burns, cancer, cholera, diabetes, ear disorders, eye disorders, fever, stomachache, gastro-intestinal, malaria, menstrual disorders, tetanus, tuberculosis, typhoid, and vomiting (Dwivedi, N.K., *et al.*, 2010). Pharmacological studies conducted on various extracts of *Cucumis* species and their isolates have shown antibacterial, antihyperglycemic, antiviral, and anticancer activities (Hernández, N., *et al.*, 2022; Kapoor, N., *et al.*, 2020; Rios, J.L., *et al.*, 2012; Shaizey, A.M., *et al.*, 2014). A lot of works that have been done by the researchers throughout the world on various plants of this genus indicated the presence of triterpenes like compounds named as cucurbitacins (Rajasree, R.S., *et al* ,2016).

In Ethiopia there exist different species of *Cucumis* including *Cucumis ficifolius*, *Cucumis dipsaceus*, *Cucumis prophetarum*, *Cucumis metuliferus*, *Cucumis jeffreyanus*, *Cucumis aculeatus*, *Cucumis pustulatus*, *Cucumis humifructus*, *Cucumis insignis*, *Cucumis abyssinicus*, and *Cucumis kelleri*. Despite all the efforts made by researchers, infectious diseases caused by microorganisms continue to pose a threatening challenge to public health. The problem is exacerbated by increasing rate of resistance of disease-causing microorganisms to the currently existing antibiotics (Bouyahya, A., *et al.*, 2017). Moreover, Cancer is a serious health problem in all populations, regardless of wealth or social status (WHO, 2023). These days, the issue is getting worse in low and middle-income nations (WHO, 2020). This is evident as the incidence is currently rising in developing countries mainly due to the rapid growth in the human population and increment in the prevalence of

risk factors associated with economic transition (Tesfaye, S., *et al.*, 2021). This, therefore, calls for continuous search for affordable, safe and effective drugs from natural products that can be used against bacteria and cancer.

Despite the tremendous use *C. ficifolius*, *C. dipsaceus*, and *C. prophetarum* in Ethiopian folk medicine to cure a variety of illnesses, a thorough analysis of the literature revealed that little is known about their phytochemical constituents, antibacterial, cytotoxic, and radical scavenging properties. Therefore, this dissertation describes the results of the investigation of phytochemical studies, antibacterial, cytotoxic, radical scavenging activities and *in silico* molecular docking analysis of the constituents of the stems, roots, fruits, and leaves of *C. ficifolius*, fruits of *C. dipsaceus*, and leaves of *C. prophetarum*.

## **1.2 Statement of the Problem**

Despite the great progress in medicine, infectious diseases caused by bacteria, fungi, viruses and parasites continue to pose a threatening challenge to public health. These diseases account for approximately one-half of all deaths in developing countries due to poverty, unavailability of medicines and the emergence of widespread resistance of pathogens to the currently existing antimicrobial drugs. According to the Global Cancer Observatory database, there will be over 21.6 million additional cancer-related cases worldwide between 2020 and 2025. Oxidative stress has been identified as a crucial component in the development and progression of various life-threatening illnesses including cancer. The ongoing search for an inexpensive, safe, and potent herbal remedy is necessitated by the high cost of synthetic drugs, the rising incidence of cancer, and the resistance of microorganisms to the currently existing antibiotics. In view of this, plants with documented traditional use might be considered as rich sources of new drugs or lead compounds. Plants of the genus *Cucumis* including *C. ficifolius*, *C. dipsaceus* and *C. prophetarum* are traditionally used to treat wounds, cancer, cholera, diabetes, ear and eye disorders, fever, stomachache, gastro-intestinal, goiter, heart disorders, hepatic disorders, infections, infertility, malaria, menstrual disorders, respiratory tract disorders, sexually transmitted diseases, tetanus, tuberculosis and typhoid (Dwivedi, N.K., *et al.*, 2010; Hernández, N., *et al.*, 2022; Kapoor, N., *et al.*, 2020; Shaizey, A.M., *et al.*, 2014; Ríos, J.L., *et al.*, 2012; Sharma, R. and Arya, V., 2011). Despite the traditional use of these plants and a close look at the literature review indicated as that the chemistry and biological activities of some species in the genus *Cucumis* in Ethiopia were not well explored. Hence, this dissertation

was designed to investigate the chemical constituents, antibacterial, cytotoxic, radical scavenging activities and *in silico* molecular docking analysis of constituents of the stems, roots, fruits, and leaves of *C. ficifolius*, fruits of *C. dipsaceus*, and leaves of *C. prophetarum*.

### 1.3 Objectives of the Study

#### 1.3.1 General Objective

The overall objective of this study was phytochemical analysis, biological activities, and computational study of compounds from some species of the genus *Cucumis* grown in Ethiopia.

#### 1.3.2 Specific Objectives

The specific objectives of this dissertation were to:

- i. extract and analyze essential oils from stems, leaves, fruits and roots of *C. ficifolius*, *C. dipsaceus*, and *C. prophetarum* by hydro-distillation using Clevenger type apparatus and GC-MS, respectively
- ii. successively extract the roots, stems, leaves and fruits of *C. ficifolius*, fruits of *C. dipsaceus*, and leaves of *C. prophetarum* using *n*-hexane/petroleum ether, ethyl acetate and methanol by maceration
- iii. isolate compounds from the extracts using chromatographic techniques including silica gel column chromatography and PTLC.
- iv. characterize the structures of the isolated compounds using NMR and in some case by comparison with the data reported in the literature for the same compound.
- v. test the antibacterial activities of the extracts and isolated compounds against two Gram negative (*E. coli* and *P. aeruginosa*) and two Gram positive (*S. aureus* and *S. pyogenes*) bacterial strains using agar disc diffusion method.
- vi. evaluate radical scavenging activities of the extracts and isolated compounds using DPPH assay.
- vii. examine cytotoxicity of the extracts on human breast cancer cell line MCF-7 using MTT assay.
- viii. assess *in silico* molecular docking analysis of the isolated compounds against DNA gyrase B, human topoisomerase II $\beta$  and KSR2:MEK1 protein targets using AutoDock vina version 4.2 software.
- ix. carry out drug-likeness properties of the isolated compounds using Swiss ADME.
- x. assess the ADMET analysis of the isolated compounds by using Swiss ADMET.

#### 1.4 Significance of the study

The results of this study might:

- help to identify active compounds from the extracts of the roots, stems, leaves and fruits of *C. ficifolius*, fruits of *C. dipsaceus*, and leaves of *C. prophetarum*.
- help to get lead compound in the treatment of some pathogenic bacteria and cancer.
- provide molecular modeling data to supports the *in vitro* experiment for better understanding of ligand–protein interaction and pharmacokinetic properties of the isolated compounds.
- serve as a spring board for further study on these plants for their phytochemicals constituents and biological activities.
- bridge the gap between modern medicine and traditional medicine.

## 2. LITERATURE REVIEW

### 2.1 Natural Products Against Infectious Diseases, Oxidative Stress and Cancer

#### 2.1.1 Natural Products Against Infectious Diseases

A disease that occurs through the invasion of a host by a foreign agent whose activities harm the normal functioning of the host's organs and/or systems are referred to as infectious disease (Tibayrenc, M., 2007). The diseases are caused by distinctive pathogens such as bacteria, virus, fungus or protozoan. They occur when the pathogens make their entry into the host cell and then, the pathogens initiate its replication process. The duration taken by the pathogen to cause the disease is completely depends on the virulence capacity of the causative agent (Mehnaz, A., 2009). Damage to tissues of the host mainly results from the growth and metabolic processes of infectious agents intracellular or within body fluids, with the production and release of toxins or enzymes that interfere with the normal functions of organs and/or systems of the host. Even though, there are much advancement in diagnostic techniques and treatment procedure, still infectious diseases continue to be serious threat to human beings as many of the people especially children in the developing countries do not have access to these advances' methods (Al-Worafi, Y.M., 2024).

The common signs and symptoms of infectious diseases are fever, rashes, petechia, diarrhea and vomiting (Zychlinsky, A., 1993). By understanding the dynamics of disease and the means of contracting it, methods of fighting, preventing, and controlling are essentially important (Tibayrenc, M., 2007; Walker, M.J., *et al.*, 2014). However, some pathogens, after apparent elimination and a period of dormancy, are able to acquire properties that enable them to reinfect their original or new hosts, usually in increasingly alarming proportions (Barber, N.C., *et al.*, 2015).

Infectious illnesses are ongoing difficulties to the world. Therefore, to help create appropriate public health responses, there is a need for raising awareness of infectious diseases and advancements in control efforts (Barber, N.C., *et al.*, 2015). Many medicinal plant extracts and secondary metabolites have been investigated for their effectiveness against these infections over time (Bhatia, P., *et al.*, 2021). Many drugs including penicillin (1), amoxicillin (2), tetracycline (3), doxycycline (4) and azithromycin (5) have been discovered from natural origin (Figure 1).

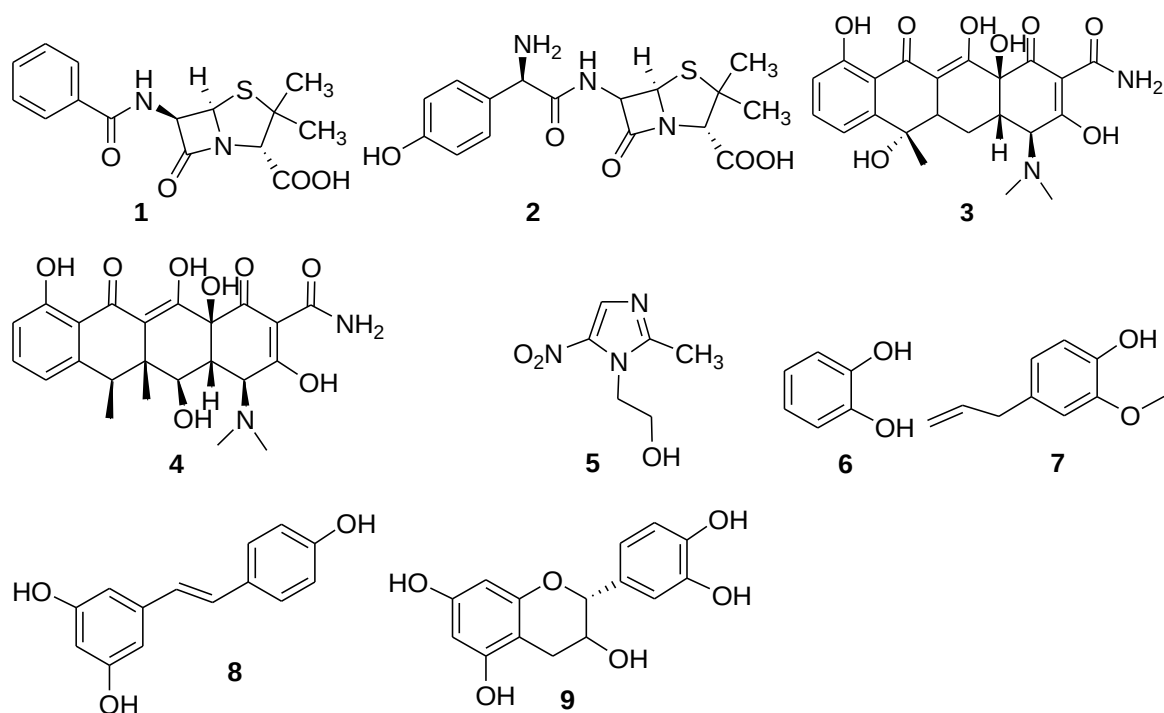


**Figure 1:** Antibacterial drugs from natural source

Natural products continue to be a valuable source of novel medications and essential building blocks for the creation of new pharmaceuticals (Kang-Ju, *et al.*, 2016). Among the antibacterial compounds that have been reported from plants are catechol (**6**) from *Diospyros kaki* Thunb (Jeong, E.Y., *et al.*, 2009), eugenol (**7**) from clove leaves *Syzygium aromaticum* (Renur, N.M., *et al.*, 2021), resveratrol (**8**) from *Veratrum grandiflorum* (Kaur, A., *et al.*, 2022), and catechin (**9**) from tea (Vuong, Q.V., *et al.*, 2010) (figure 1).

### 2.1.2 Natural Products Against Oxidative Stress

Oxidative stress induced by reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anions, and singlet oxygen is believed to be a primary factor in various diseases as well as in the normal process of aging (Stohs, S.J., 1995). ROS can initiate, the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. The elimination or inactivation of reactive oxygen species, or the prevention of their cellular formation by antioxidants and free-radical scavengers, is considered to be a practical approach to reducing the risk of these diseases. The importance of antioxidants in human health has become increasingly clear due to spectacular advances in understanding the mechanisms of their reaction with oxidants (Sporn, M.B., and Suh, N., 2000).

Plants contain a wide variety of chemicals that have potent biological effects (Sporn, M.B., and Suh, N., 2000). As a result, there has been a growing interest in the use of herbs as a

source of therapeutic drugs. By Bergman, M., *et al.*, (2003) has isolated and characterized water-soluble antioxidants from spinach (Bergman, M., *et al.*, 2003, and Lomnitski, L., *et al.*, 2003). Some of antioxidants originated from nature includes ascorbic acid (**10**) from *Casuarina equisetifolia* (Wapwera, J.A., 2021), lipoic acid (**11**) from potato (Saha, M., *et al.*, 2018), masoprocoul (**12**) from *Larrea nitida* (Javaid, S., *et al.*, 2019) (Figure 2).

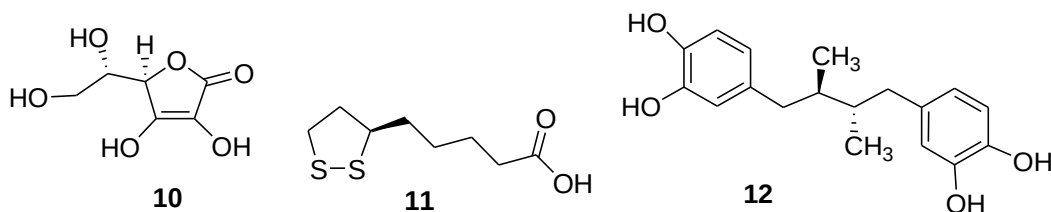


**Figure 2:** Antioxidants from natural sources

Most of the natural antioxidants from plant materials are mainly polyphenols (phenolic acids, flavonoids, anthocyanins, lignans and stilbenes), carotenoids (xanthophylls and carotenes) and vitamins (vitamin E,  $\alpha$ -tocopherol and Vitamin C (ascorbic acid)) (Baiano, A., *et al.*, 2015). These natural antioxidants including polyphenols and carotenoids exhibit a wide range of biological effects, such as anti-inflammatory, antibacterial, antiviral, anti-aging, and anticancer (Zhang, Y.J., *et al.*, 2015, Balmus, I.M., *et al.*, 2016).

Flavonoids (Figure 3) including quercetin (**13**), isorhamnetin (**14**), morin (**15**), myricetin (**16**), apigenin (**17**) and luteolin (**18**) also reported to have antioxidant activities (Choi, Y.H., 2016; Dilshara, M.G., *et al.*, 2016; Paredes, G., *et al.*, 2015).

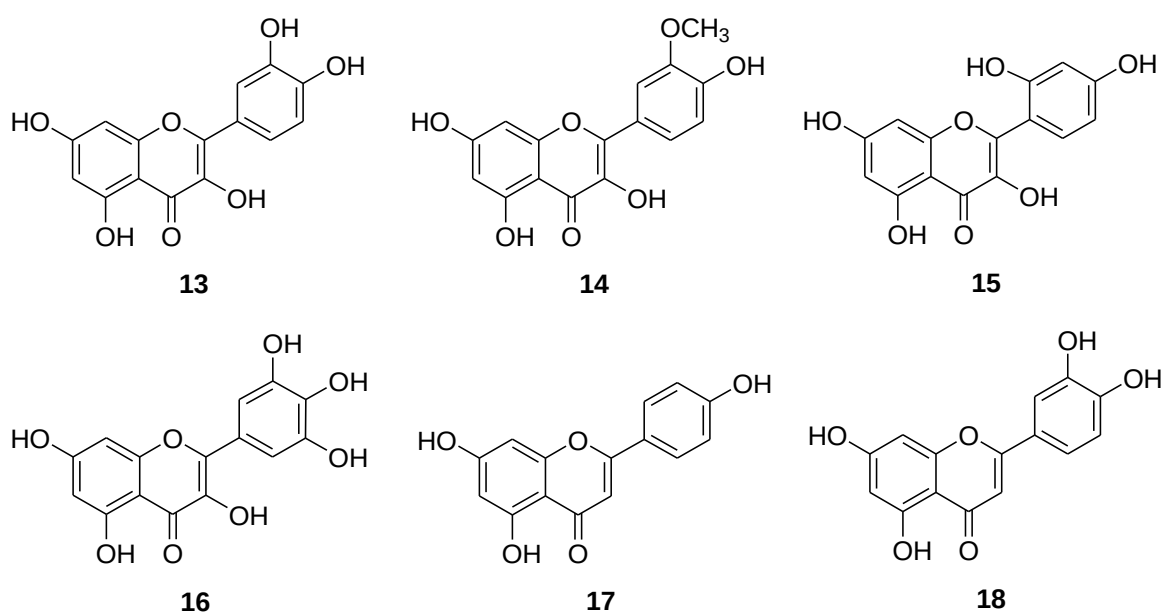


**Figure 3:** Antioxidant flavonoids from plants

### 2.1.3 Natural Products Against aCancer

Cancer is a global public health issue that arises from aberrant cell proliferation that has the potential to spread and replicate endlessly (Ali, E.S., *et al.*, 2022). Vital organs such as the esophagus, stomach, colorectum, liver, lung, and breast are repeatedly affected by this most deadly disease. Cancer remains as a major public health issue in many nations around the world, despite recent improvements in survival rates brought about by advancements in early identification and the quality of accessible treatment (Siegel, R.L., *et al.*, 2016, Miller, K.D., *et al.*, 2016). For example, in the case of the United States in 2016, nearly 1.7 million new cases of cancer are predicted to occur, in addition to over 595,000 cancer deaths (Siegel, R.L., *et al.*, 2016).

According to the Global Cancer Observatory database, there would be 10.2 million incidences of cancer in women and 11.4 million cases in men, a 12.1% increase in new cases. There will be a 12.1% rise in new cancer cases, with 11.4 million cases in males and 10.2 million in females by 2025 (Cancer Tomorrow, 2023). There were an estimated 10 million deaths because of cancer in 2020. According to projected estimates, there will be 47% more cancer cases by 2040, low human development index (HDI) countries will contribute to 95% of cases and medium HDI countries will contribute to 64% of cases (Sung, H., *et al.*, 2021).

In the United States, the three most prevalent cancer kinds in women are breast, uterine corpus and colon. In men the top three are prostate, colon, and melanoma (Miller K.D., *et al.*, 2016). Unfortunately, death rates are increasing for cancer of the liver, pancreas, and uterine corpus (Siegel, R.L., *et al.*, 2016). In China, it was predicted that lung cancer was the primary cause of three million cancer deaths in 2015. Stomach, esophageal, and liver cancer were also frequently diagnosed cancers in China (Chen, W, *et al.*, 2016). In Ethiopia, breast cancer is the leading cause of cancer-morbidity among adult women, accounting for one-third of all cancer cases among women and one in five of all cancer cases (Timotewos, G., *et al.*, 2018). An estimated 16,133 new breast cancer cases and 9061 breast cancer deaths occur annually in the country (Bray, F., *et al.*, 2018). Breast cancer frequently occurs in a young reproductive age group, and results in a long duration of illness (Wondimu, A., *et al.*, 2022).

Natural products have been a major source of beneficial compounds for cancer chemotherapy, supplying new lead compounds and proven medications for synthetic optimization, as well as materials for examining the cellular and molecular mechanisms of action associated with cancer inhibition (Cragg, G.M., *et al.*, 2012; Newman, D.J., *et al.*, 2016). Thus, over 49% of the 175 small-molecule anticancer medications that were brought to the market in Western nations over a roughly 70-year span were either synthesized from natural chemicals or directly sourced from organisms (Butler, M.S., *et al.*, 2014). As a result, there are four primary classes of higher plant-derived anticancer agents that are used in clinical settings in the United States and Europe. These include the semi-synthetic epipodophyllotoxins, taxanes, vinca, and bisindole alkaloids (Cragg, G.M., *et al.*, 2012). Plants have been used for hundreds of years to treat a wide range of illnesses, extract therapeutic molecules, and enhance general health. To this day, plants continue to be a major source of novel active chemicals and numerous blockbuster medications. Malignant cancer is the second leading cause of mortality globally, taking lives and destroying people's health.

Researchers studying epidemiology have shown that eating certain fruits and vegetables can lower risk of developing a number of diseases, including cancer (Abuajah, C.I., *et al.*, 2015). Over 50% of anticancer drugs were produced from natural sources, especially from plants (Mann, 2002; Kim, J., and Park, E.J., 2002). Some plants may have anticancer qualities because of their antioxidant content. Apoptosis in cancer cells can also be induced by numerous plant antioxidants, which are employed as anticancer medications (Michels, G., *et al.*, 2006). Paclitaxel, often known as Taxol (**19**), is a well-known plant-based anticancer drug. It is derived from the *Taxus brevifolia* Nutt. (western yew) plant. Paclitaxel works by promoting the polymerization of microtubules, which stops the growth of new cells (Ardalani, H., and Ghayour, M., 2017). Podophyllotoxin derivatives, especially etoposide (**20**) and teniposide (**21**), act as inhibitors of DNA Topoisomerase II, leading the cell to apoptosis (Gallego-Jara, J., *et al.*, 2020). The hydrophobic property of curcumin (**22**) continues to be the major obstacle to developing effective medicines for cancer treatment (Ayubi, M., *et al.*, 2019). Parthenolide (PTL) (**23**) (Figure 4) also has low bioavailability due to its poor solubility in water (Karam, L., *et al.*, 2021). Neurotoxicity caused by vinca alkaloids is a significant side effect of using these alkaloids as cancer medication (Arora, R.D., & Menezes, R.G., 2021).

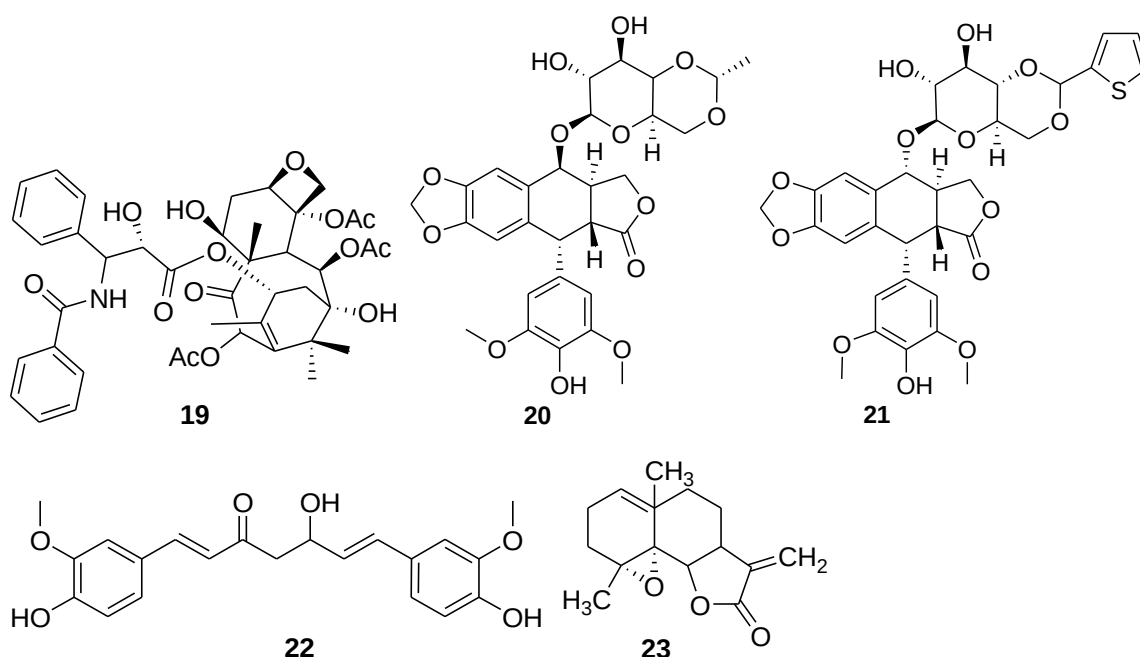


**Figure 4:** Some anticancer agent from natural sources

Plants the genus *Cucumis* are known to have anticancer properties owing to the presence of cucurbitacins, highly unsaturated tetracyclic triterpenoids produced mainly by plants belonging to the family Cucurbitaceae (Piao, X.M., *et al.*, 2018). Some researchers have shown that cucurbitacins have hepatoprotective, anti-inflammatory, antidiabetic, and anticancer properties (Jayaprakasam, B., *et al.*, 2003; Rios, J.L., *et al.*, 2012). They can be administered in combination with various chemotherapeutic drugs to reduce tumor resistance and enhance the efficacy of anticancer drugs (Yang, T., *et al.*, 2017; Yar, S., *et al.*, 2016). Recent studies have focused on the anticancer effects of cucurbitacins, including cucurbitacins B (**24**), E (**25**), D (**26**), and I (**27**) (Figure5) which are the most common cucurbitacin derivatives isolated from Cucurbitaceae plants (Wakimoto, N., *et al.*, 2008).

Among the cucurbitacin, **24** possesses promising inhibitory effect towards numerous cancer cell lines. It exhibited potent anticancer effects towards the various breast cancer cell lines (Wakimoto, N., *et al.*, 2008; Zhang, M., *et al.*, 2010). Moreover, this compound also demonstrates cytotoxic effects towards numerous cancer cells (Matsuda, H. *et al.*, 2010; Yasuda, S., *et al.*, 2010). Additionally, **24** was active towards T98G, U118, U93, U373, U343, Hep-2 (Yin, D., *et al.*, 2008). Most importantly, **24** possesses significant cytotoxic effects towards over 100 cancer cell lines (Garg, S., *et al.*, 2018). Cucurbitacin D also possesses potent anticancer effects towards the various cancer cells (Ding, N., *et al.*, 2016). Moreover, **25** and **27** possess anticancer effects (Jayaprakasam, B., *et al.*, 2003).

Cucurbitacin E demonstrated cytotoxicity towards acute U937, HL-60, HT1080 (Nakashima, S., *et al.*, 2010; Huang, W.W., *et al.*, 2012). Cucurbitacin I also showed cytotoxicity towards CNE-2, HK1 (Lui, V.W. *et al.*, 2009).

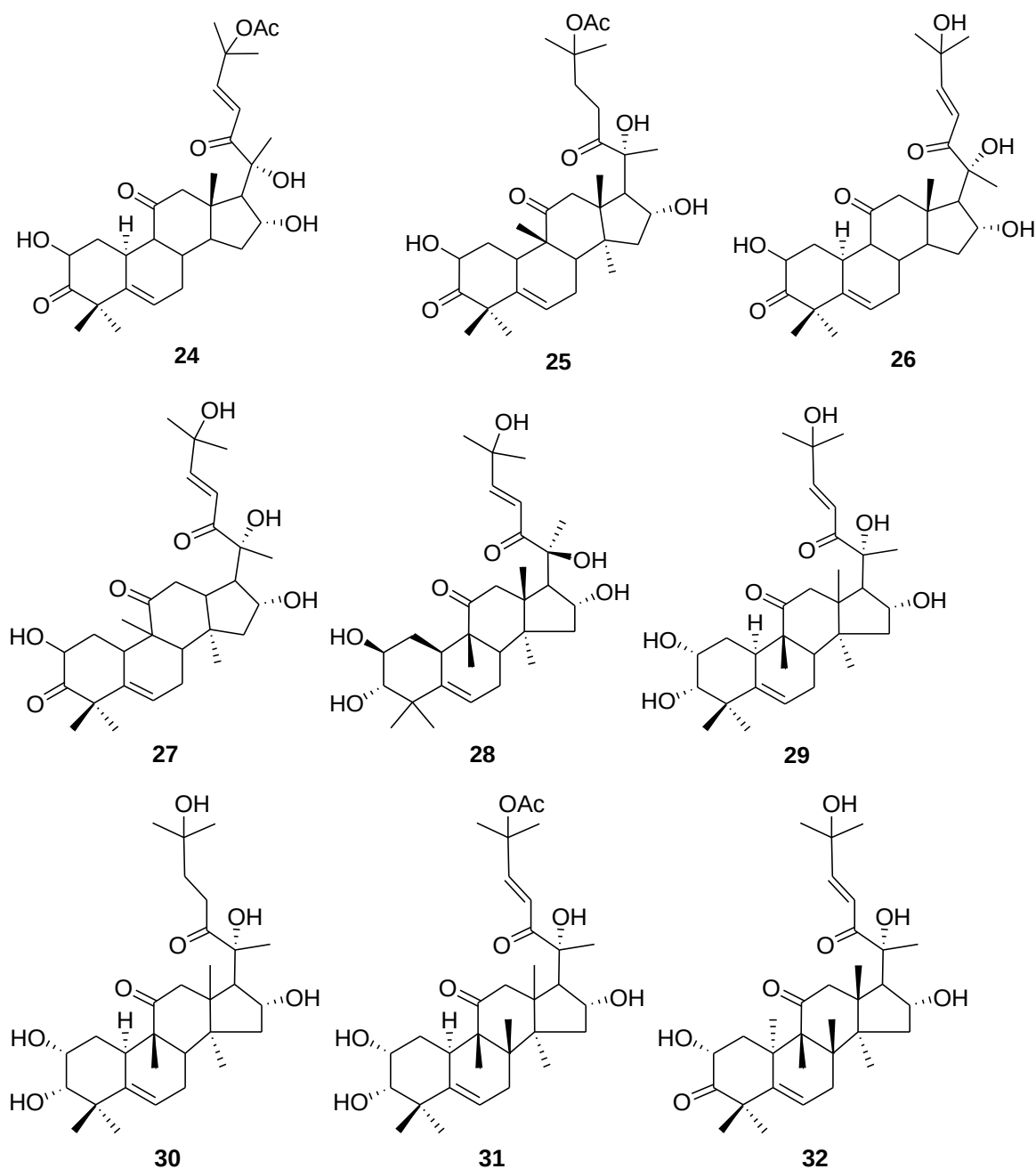


**Figure 5:** Cucurbitacins having anticancer properties

Cucurbitacins F (28), O (29), P (30), Q (31), and R (32) (Figure 5) possess promising anticancer effects towards various cancer cells *in vitro* (Chen, J.C., *et al.*, 2005; Kupchan, S.M., *et al.*, 1970; Escandell, J.M., *et al.*, 2008). Many *in vitro* studies have shown that cucurbitacins inhibit cell proliferation, migration, and invasion and induce G2/M phase cell

cycle arrest, autophagy, and apoptosis through different molecular mechanisms in various tumor cells (Liu, T., *et al.*, 2008; Garg, S., *et al.*, 2018). In addition, animal experiments have shown that cucurbitacins significantly inhibit tumor growth and metastasis *in vivo* (Piao, X.M., *et al.*, 2018; Zhang, T., *et al.*, 2012; Deng, C., *et al.*, 2016; Sikander, M., *et al.*, 2016) indicating that these compounds exert a wide range of anticancer effects. Hence, it is eventually important to find cucurbitacin derivatives as anticancer agents.

## 2.2 Cucurbitaceae Family

The family Cucurbitaceae is grown widely around the world as crops and includes a large group of plants which are medicinally valuable. The family is comprised of about 130 genera and 825 species that are primarily cold sensitive, annual/perennial vines (Ismail, A., *et al.*, 2015). The Cucurbitaceae is a remarkable plant family, deserving much attention because of its economic, aesthetic, cultural, medicinal, and botanical significance. In the Worlds, cucurbits have been associated with human nutrition and culture for more than 12,000 years (Broth well, D. and Broth well, P., 1969). The flesh of the fruits of many species is eaten as fruits or vegetables. Many cucurbits are important components of traditional medicines, and some may have modern pharmaceutical applications (Janick, J., *et al.*, 1993). Seeds or fruit parts of some *Cucurbits* are reported to possess purgatives, emetics and anthelmintic properties due to the secondary metabolite cucurbitacin content. A number of compounds of this group have been investigated for their cytotoxic, hepatoprotective, anti-inflammatory and cardiovascular effects (Jeffery, C., *et al.*, 1990). Mature fruits can also be strikingly ornamental or can serve as containers or as musical instruments. Within this family, the genera *Cucumis*, *Cucurbita*, and *Citrullus* are considered to have high economic importance (Raven, P.H., *et al.*, 1993). Some plants of the family Cucurbitaceae have shown anti-diabetic activity, these are *Cocinia indica*, *Momordica cymbalaria*, *Momordica dioica*, *Cucumis trigonus* and *Luffa tuberosa* (Sharma, R. and Arya, V., 2011). *Bryonia alba* L. for rheumatic pain (Yeşilada, E., *et al.*, 1995), *Luffa operculata* Cogn. for sinusitis, *Corallocarpus epigaeus* is used for wounds, obesity, skin disease, tumours, cough and bronchitis (Nandagopalan, V., *et al.*, 2011).

## 2.3 The Genus *Cucumis*

*Cucumis* is a genus of twining, tendril-bearing plant in the Cucurbitaceae family (Kirkbride, J., 1993). It consists of more than 62 species, indigenous mainly to tropical Africa, also Asia, Australia and some islands in the Pacific. *Cucumis* are medium-sized, climbing, hairy plants and well represented in the moist and moderately dry tropics of the world, particularly

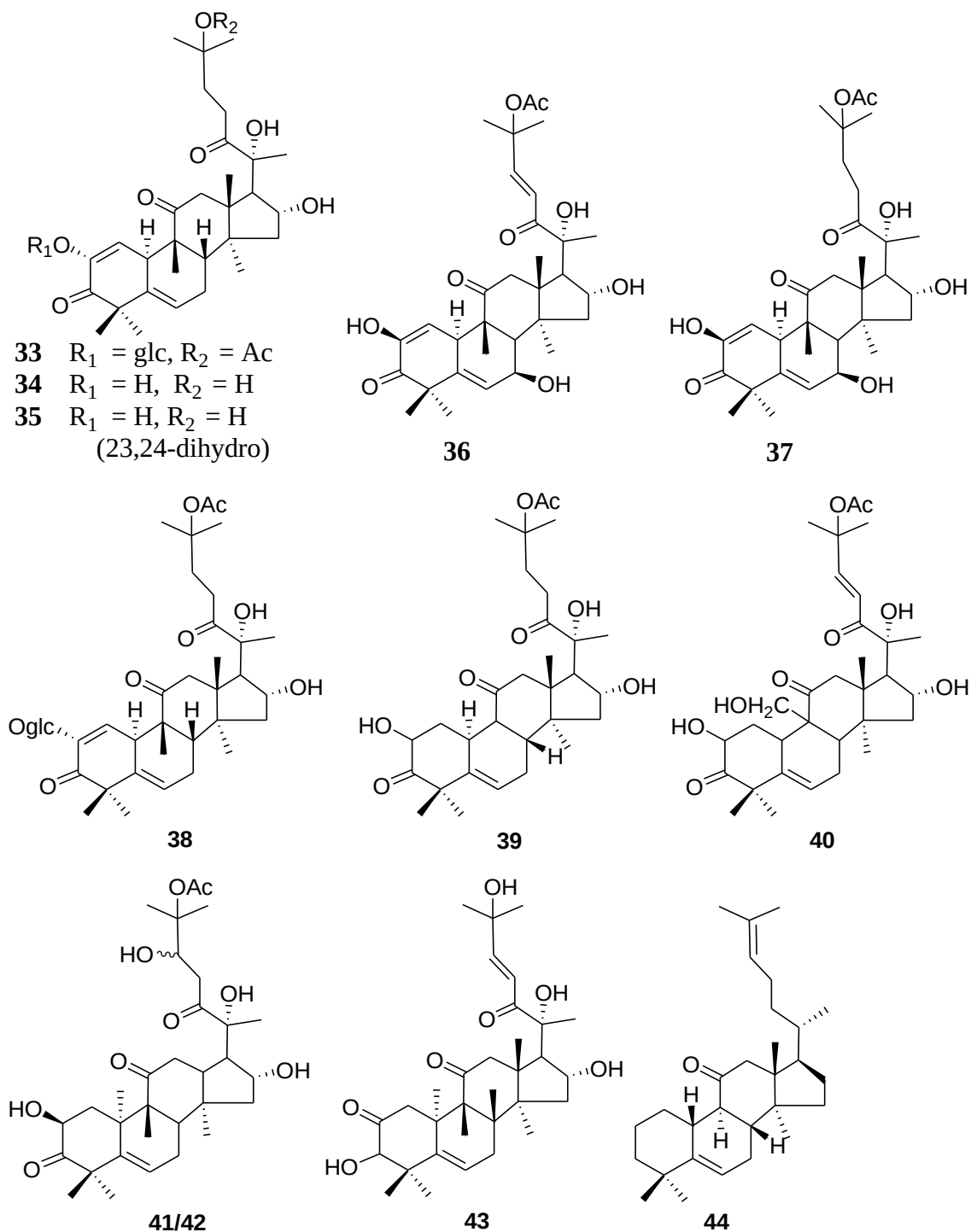
in grass and bush land areas of Africa. Some species occur in semi-desert or even desert vegetation (Abebe, W., 2016). Root systems are rarely woody (*C. trigonus*) and extensive, but usually shallow and rarely tuberous (*C. kalahariensis*); stems are angled, sulcate, not aculeate or rarely aculeate (*C. aculeatus* and *C. ficifolius*), and variously pubescent or rarely glabrous, with non-breakaway hairs or rarely breakaway hairs (*C. saculeuxii*); nodes are geniculate or not geniculate. Each node has a single leaf and a simple tendril (sometimes curling), except that of *C. humifructus*, which has a fascicle of five to eight tendrils, and that of *C. rigidus*, which lacks them; tendrils of *C. insignis* are either simple or bifid. Tendrils are variously pubescent, rarely glabrous, or rarely aculeate (Chittaranjan, K., 2011). A lot of works that have been done by the researchers throughout the world on various plants of the genus which has led to the isolation a number of cucurbitacin's (triterpenes like compounds) (Rajasree, R.S *et al* ,2016; Dwivedi, N.K., *et al.*, 2010).

### **2.3.1 Ethnobotanical Uses**

Results obtained in the survey conducted by different researchers indicated that *Cucumis* are being used to treat wounds, burns, cancer, cholera, diabetes, ear disorders, eye disorders, fever, stomachache, gastro-intestinal, goiter, heart disorders, hepatic disorders, infertility, inflammation, malaria, menstrual disorders, mumps, paralysis, pox, respiratory tract disorders, skin disorders, sexual disorders, sexually transmitted diseases, sun stroke, tetanus, tuberculosis and typhoid (Dwivedi, N.K., *et al.*, 2010; Hernández, N., *et al.*, 2022; Kapoor, N., *et al.*, 2020; Ríos, J.L., *et al.*, 2012). The whole plant parts of bitter gourd are used in the treatment of malaria in the south-western regions of Nigeria. The plant is reported to possess anti-fungal, anti-inflammatory, anti-parasitic and act as a digestive stimulant (Choudhury, M.D., *et al.*, 2010). Different researchers reported the wound healing potential of *C. sativus* (Shrivastava, A., *et al.*, 2013). Regular intake of cucumber fruit promotes healthy hair growth. It is useful in skin problems, sunburn and also for curing swelling under the eye. Its juice is also efficient to soften the skin texture. Placing two slices of cucumber on eyes for 10 minutes can decrease the inflammation and cure skin infection significantly. Its fruit is also considered traditionally for weight loss, intestinal worms and tapeworms. Leaves are boiled, mixed with cumin seeds, roasted, powdered and administered for throat infections. Due to its elevated content of potassium (50-80 mg/100g), cucumber can also significantly reduce blood pressure (Shrivastava, A., *et al.*, 2013).

### 2.3.2 Chemical Constituents of Some Species in the Genus *cucumis*

Phytochemical investigation of the stems of *C. melo* led to the isolation and identification of cucurbitane-type triterpenoids including compounds **33** to **38**, cucurbitacin B (**24**), 23,24-dihydrocucurbitacin B (**39**), cucurbitacin A (**40**), cucurbitacin G (**41**), cucurbitacin H (**42**), cucurbitacin R (**32**), isocucurbitacin R (**43**), 19-norlanosta-5, 24-dien-11-one (**44**), hexanorcucurbitacin D (**45**) and dihydro isocucurbitacin B (**46**) (Figure 6) (Chuan, Ch., *et al.*, 2009).



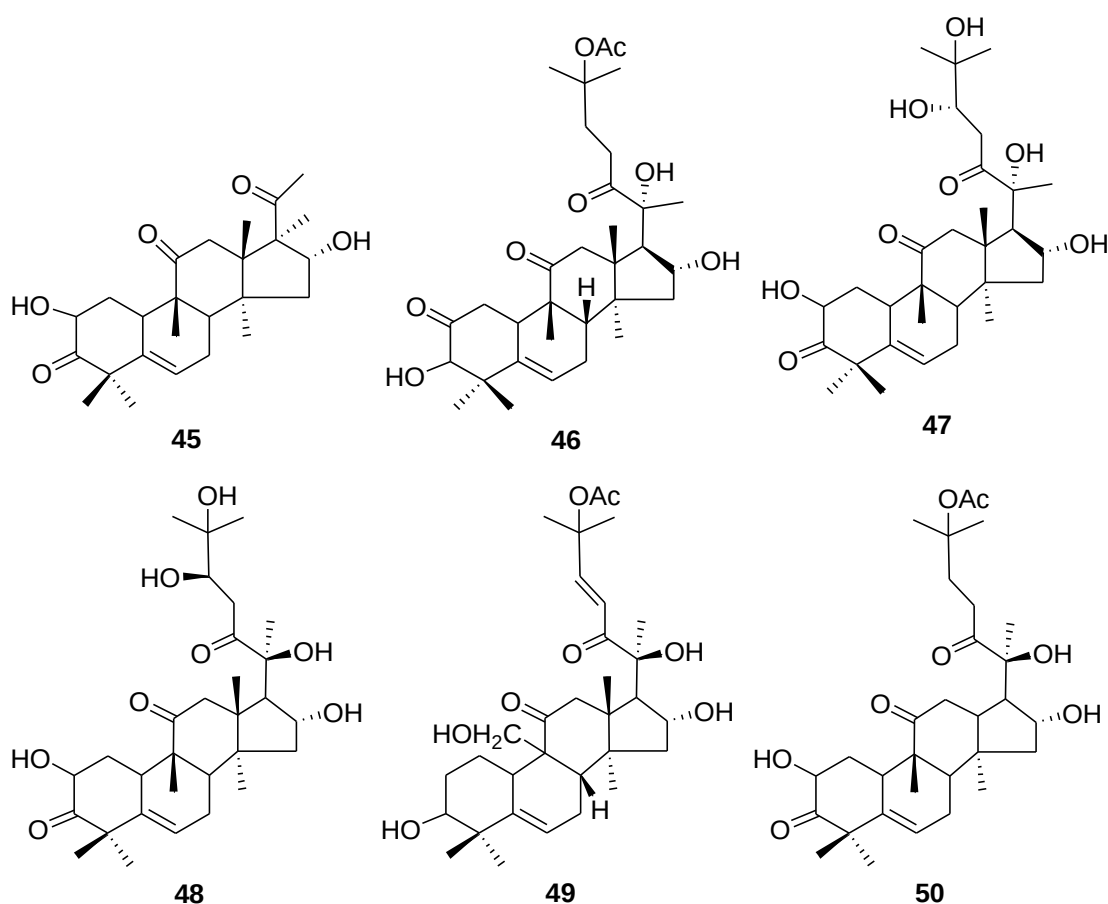


**Figure 6:** The structure of cucurbitane-type triterpenoids reported from stems and fruits of *C. melo*

Other cucurbitacin derivatives, hexanorcucubitaicin D (45), cucurbitacin E (25), cucurbitacin J (47), cucurbitacin K (48), cucurbitacin A (40), cucurbitacin C (49), cucurbitacin I (27) and cucurbitacin L (50) (Figure 6) were also reported from the fruits of *C. melo* (Matsuda, H., *et al.*, 2010). Furthermore, curcumin S [(R)-5,7-dihydroxy-2- [1-hydroxy-2-(4-hydroxy-3- methoxyphenyl) ethyl] chromone] (51), 5,7-dihydroxy-2- [2 (4-hydroxyphenyl) ethyl] chromone (52), 5,7-dihydroxy-2-[2-(3,4-dihydroxyphenyl) ethyl] chromone (53), luteolin (18), quercetin (13), glucosyloxy-5-hydroxy-2-[2-(4-hydroxyphenyl) ethyl] chromone (54) and Luteolin-7-O- $\beta$ -glucuronide (55) 7- were isolated from the EtOAc fraction of seeds of *C. melo* (Figure 7) (Matsuda, H., *et al.*, 2010).

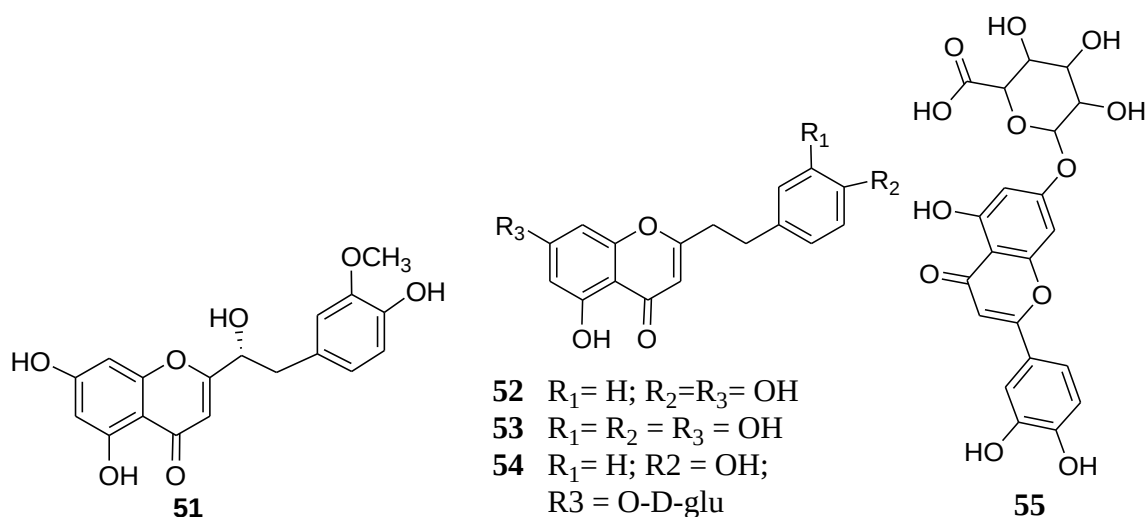


**Figure 7:** The structure of phenyl chromenones from seeds of *C. melo*

The MeOH extract of the seeds of *Cucumis melo* L. var. *Reticulatus* (Cucurbitaceae) led to the isolation of 5,7-dihydroxy-2-[2-(3-methoxy-4-hydroxyphenyl) ethyl] chromone (**56**), a triglyceride (1,3-di-(6,9)-docosa-6,9-dienoyl-2-(6) hexacos-6-enoylglycerol (**57**),  $\alpha$ -spinasterol (**58**), stigmasta-7,22,25-trien-3-ol (**59**), and D:B-friedoolean-5-ene-3- $\beta$ -ol (**60**) (Sabrin R. and Ibrahim, M., 2014).

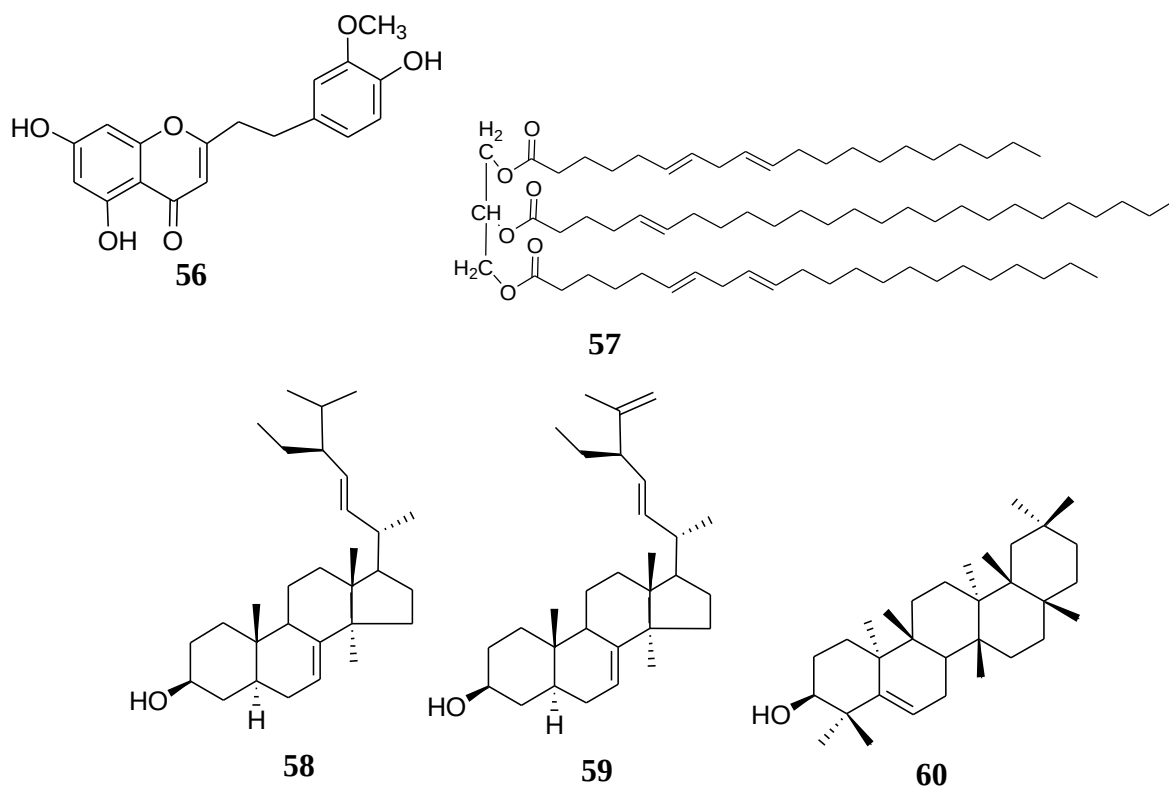


**Figure 8:** The compounds reported from the seeds of *C. melo*

*C. sativus* is a widely cultivated plant in the genus *Cucumis*. It is a creeping vine bearing cylindrical fruits that used as culinary vegetables. The yellow solid compound which was soluble in hot water and organic solvents but insoluble in cold water has been identified from flowers of *C. sativus* (5,7-di-O-methyl luteolin-6-C-(3-O-benzoyl)- $\beta$ -dxyloside) (**61**) (Senthamilselvi, M.M., *et al.*, 2016).

Extract of cucumber leaves tissue expressing induced resistance against powdery mildew fungi revealed the presence of two major C-glycosyl flavonoid namely cucumerin A (**62**) and cucumerin B (**63**). The C-glycosyl flavonoids such as vitexin (**64**), isovitexin (**65**), orientin, (**66**), and isoorientin, (**67**) were isolated from the resistant plant in addition to *p*-coumaric acid, (**68**) and its methyl ester (**69**) (Figure 9) (David, J., *et al.*, 2003).

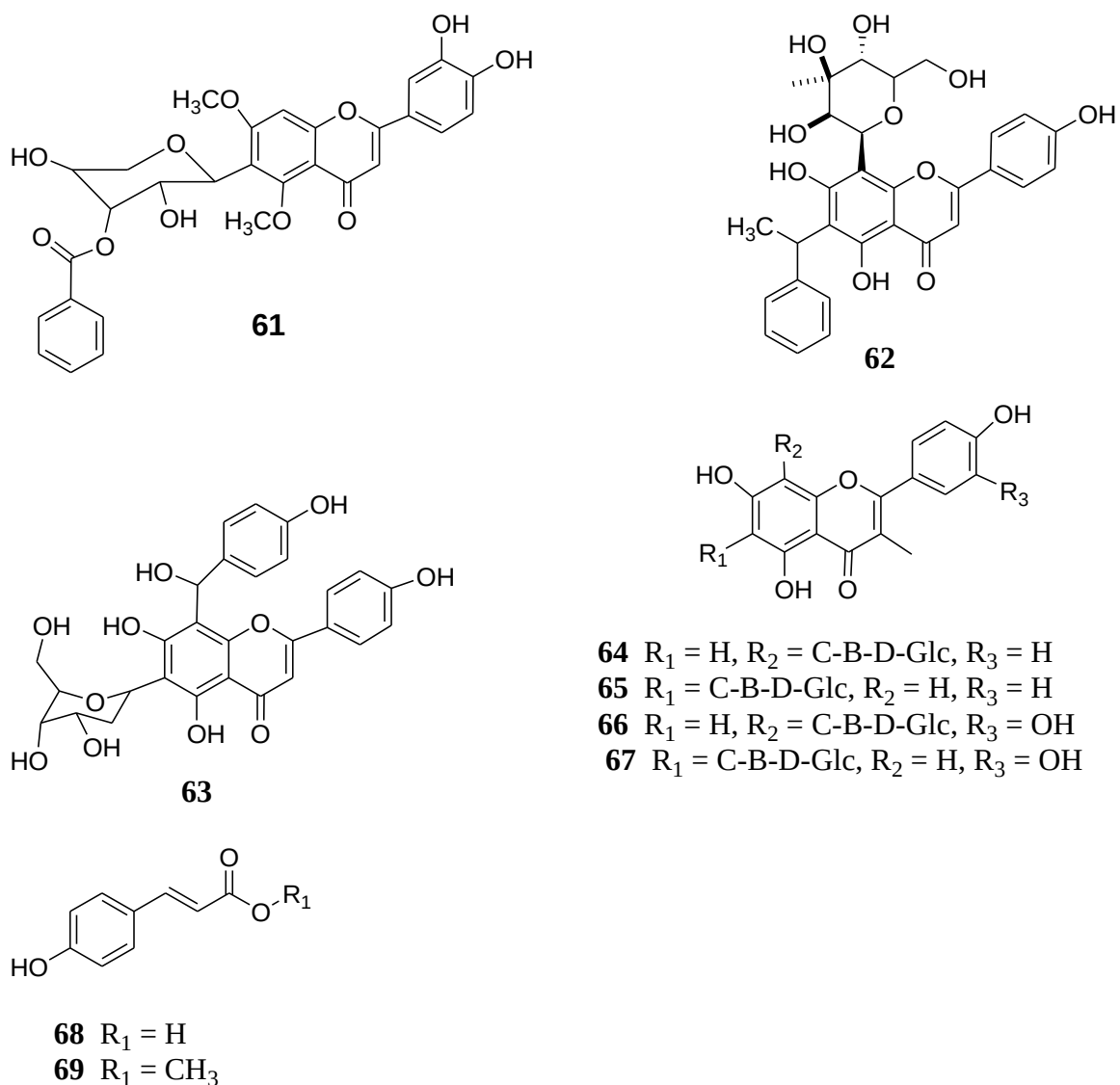


**Figure 9:** The compounds reported from flowers and leaves tissue of *C. sativus*

*C. maxima* commonly known as squash is widely used as vegetable and a source of vitamin A, iron, phosphorus, and calcium. Compounds such as  $\alpha$ -spinasterol (**58**) and 24-ethyl-5 $\alpha$ -cholesta-7,22,25-trien-3 $\beta$ -ol (**59**) possessing anti-carcinogenic and anti-genotoxic properties were isolated from the flowers of *C. maxima* (Ragasa, C.Y. and Lim, K., 2005) (Figure 8).

Seventeen compounds (Figure 10) have been identified by paper chromatography from 15 *Cucumis* species such as *C. africanus*, *C. heptadactylus*, *C. melo* and *C. myriocarpus*, *C. anguria*, *C. dipsaceus*, *C. ficifolius*, *C. prophetarum*, *C. pustulatus*, and *C. zeyheri*. *C. hirsutus*, *C. humifructus*, *C. metuliferus*, *C. sagittatus*, and *C. sativus* (Petrus, A.J.A., 2014).

| No | Compounds                                               |                |                |                |                |                |                |
|----|---------------------------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
|    |                                                         | R <sup>1</sup> | R <sup>2</sup> | R <sup>3</sup> | R <sup>4</sup> | R <sup>5</sup> | R <sup>6</sup> |
| 70 | 6-C- $\beta$ -D-glucopyranosylapigenin (isovitexin)     | Gl             | H              | H              | H              | H              | H              |
| 71 | 8-C- $\beta$ -D-glucopyranosylapigenin (vitexin)        | H              | Gl             | H              | H              | H              | H              |
| 72 | 7-O- $\beta$ -D-glucopyranosylisovitexin (Saponarin)    | Gl             | H              | Gl             | H              | H              | H              |
| 73 | 4-O- $\beta$ -D-glucopyranosylsaponarin                 | Gl             | H              | Gl             | Gl             | H              | H              |
| 74 | 2-O- $\beta$ -D-glucopyranosylisovitexin                | Gl             | H              | H              | H              | H              | Gl             |
| 75 | 6-(4-hydroxy-1-ethylbenzene) vitexin                    | Gl             | H              | H              | Gl-Gl          | H              | H              |
| 76 | 8-(4-hydroxy-1-ethylbenzene) isovitexin                 | Gl             | HEB            | H              | H              | H              | H              |
| 77 | 6-(4-hydroxy-1-ethylbenzene) vitexin                    | HEB            | Gl             | H              | H              | H              | H              |
| 78 | 6,8-di-C- $\beta$ -D-glucopyranosylapigenin (vicenin-2) | Gl             | Gl             | H              | H              | H              | H              |

|    |                                                                                                                                            |        |    |    |       |    |    |
|----|--------------------------------------------------------------------------------------------------------------------------------------------|--------|----|----|-------|----|----|
| 79 | 6-C- $\beta$ -D-glucopyranosylluteolin<br>(homooreintin)                                                                                   | Gl     | H  | H  | H     | OH | H  |
| 80 | 8-C- $\beta$ -D-glucopyranosylluteolin<br>(oreintin)                                                                                       | H      | Gl | H  | H     | OH | H  |
| 81 | 7-O- $\beta$ -D-<br>glucopyranosylhomooreintin<br>(lutoranin).                                                                             | H      | Gl | Gl | H     | OH | H  |
| 82 | 6-C- $\beta$ -D-glucopyranosyl-3, 4, 5-<br>trihydroxy-7-methoxy-flavone-4-X-<br>di-O-glucopyranoside (4-X-di-O-<br>glucosylswertiajaponin) | Gl     | H  | OH | Gl-Gl | H  | H  |
| 83 | 6-C-diglucopyranosylapigenin                                                                                                               | Gl-Gl  | H  | H  | H     | H  | OH |
| 84 | 6-C-diglucopyranosylorientin                                                                                                               | Gl- Gl | H  | H  | H     | OH | H  |
| 85 | X-cafeoyl-6-C-<br>diglucopyranosylapigenin                                                                                                 | Gl- Gl | H  | H  | H     | H  | OH |
| 86 | X-cafeoyl-6-C-<br>diglucopyranosylorientin                                                                                                 | Gl- Gl | H  | H  | H     | OH | H  |

**Figure 10:** The structures of the flavonoids reported from the genus *Cucumis*

### 2.3.3 Biological Activities of the Genus *Cucumis*

The fruits of *C. melo* are useful in chronic eczema and medicinally used to promote skin hydration, to treat light burns and scrapes or wounds (Shrivastava, A., *et al.*, 2013). Dose dependent cytotoxic activities were observed by aqueous fruit extract of *C. melo* in human prostate carcinoma cells. As the dose of the extract increased, the number of viable cells decreased. This shows the anticancer and cytotoxic potential of the fruit of *C. melo* (Ittiyavirah, S.P., *et al.*, 2013). A seed powder is mixed with water and used as a vermifuge (Shrivastava, A., *et al.*, 2013). The fruit extract has a high superoxide dismutase activity (SOD). The SOD activity is responsible for the *in vitro* antioxidant and anti-inflammatory properties of the extract (Vouldoukis, I., *et al.*, 2004). The therapeutic potential of *C. melo* might be due to the presence of cucurbitacin E (Ayyad, S.N., *et al.*, 2011). Therapeutic potential of cucurbitacin E could be limited by the bitter taste of this compound but special pharmaceutical formulation is highly recommended to overcome this issue. As different reports showed the cytotoxicity of chloroform extract of seeds and isolated compounds **24**

of *C. prophetarum* against human cancer cell lines showed a positive result. Compound **24** is most widely used for in vitro studies on tumor inhibition (Ayyad, S.N., *et al.*, 2011).

Numerous evidences have shown that **24** inhibits the growth of many human cancer cell lines including breast, prostate, lung, uterine cervix, liver, skin, and brain cancers. Combination therapy with multiple drugs is a common practice in the treatment of cancer to get an additive or synergistic effect and to reduce toxicity to the host (Liu, K., *et al.*, 2008).

The antimicrobial activity of seeds extracts of *C. sativus* (Cucumber) revealed that all the seeds extracts were found to be active against *E. coli*, *S. thermophilous*, *F. sporium*, and *T. mareesei* (Sood, A., Gupta, R., 2012). Studies on the antifungal activities of the ethanol extract of *C. sativus* showed that the ethanol extracts of *C. sativus* were effective against six fungi after diameter of zone inhibition were compared with the activity of the standard drug (Mallik, J., *et al.*, 2013). The wound healing properties of aqueous extracts of *C. sativus* were reported (Patil, M.V.K., *et al.*, 2011). The extracts of *C. sativus* were shown to have various pharmacological activities such as anti-bacterial, antifungal, cytotoxic, antacid, carminative, hepatoprotective, hypoglycemic and hypolipidemic and wound healing (Ankita, S., *et al.*, 2012).

## **2.4 Cucumis in Ethiopia**

### **2.4.1 Botanical Description of Some Species of Cucumis in Ethiopia**

#### **2.4.1.1 Cucumis ficifolius**

*Cucumis ficifolius* (Cucurbitaceae) belonging to the genus *Cucumis* is known locally in Ethiopia as “Holotoo” in Afan Oromo and “Yemidir Embuay” in Amharic. It is a perennial herb climbing to 2.5 m long; stems are hairy with short fine hairs; older basal stems thickened and with whitish somewhat scaly fissured bark, leaves are blade ovate to broadly rotundate in outline, scabrid 30-76 mm long, 24-80 mm broad, 0.8-1.3 times as long as broad. Lobes rounded above, narrowed or sometimes not narrowed below with the lateral and with the central lobes broadly rounded or broadly ovate (Mesfin, T., *et al.*, 2006). Fruit on a 30-70 mm long stout stalk thickened up wards to above insertion on the fruit bluntly ellipsoid (52-60 mm) long; 34-50 mm across, 1-2 mm high each ending in hard blunt broad-based hair; bright yellow and seeds are elliptic (Ayyad, S.N., *et al.*, 2011). The habitat for this plant species is grassland, wooded grassland, *Acacia* woodland, rocky slopes, secondary

vegetation and cultivated places. In Ethiopia the plant is mainly distributed in Tigray, Gojam, Shewa, Wollega, Gamo Gofa, Arsi and Harerge (Mesfin, T., *et al.*, 1994).



**Figure 11:** The picture of *C. ficifolius* (photo by Teshale A. from Dera, Arsi, Oromia, Ethiopia, 2020)

#### 2.4.1.2 *Cucumis dipsaceus*

*Cucumis dipsaceus* Ehrenb. ex Spach is a species of annual climbing and flowering plant belonging to genus *Cucumis*, and family Cucurbitaceae. *C. dipsaceus* fruit is mono-colored, pale yellow. It is 3- 6.5cm long and 2.5-4cm in diameter. It is densely aculeate and glabrous blunt at the apex. Seeds is elliptic, unwinged and 4-5 mm long× 2 mm diameter x 1mm thick. It is cylindrical 1-3(-4) cm long with non-breakaway hairs and hispid (Vansanth, K.G., *et al.*, 2013). It has its origin in Ethiopia and distributed in other African countries including Kenya, Somalia, Tanzania, Uganda, Sudan and Southern Egypt. The plant is also found in Asia (Mabberley, D.J., 2008; Nivedhini, V., *et al.*, 2014).



**Figure 12:** The picture of *C. dipsaceus* (photo by Teshale A from Dera, Arsi, Oromia, Ethiopia, 2020)

#### 2.4.1.3 *Cucumis prophetarum*

*Cucumis prophetarum* Linnaeus subsp. dissectus (Naud.) C. Jeffrey is intemodes 4-7(-9) cm long and its leaves is petioles 1.5-6.5 cm long. Leaves blades cordate at the base; with a basal sinus; 2-7(-14) X 1.8-6.5(-8) cm. Male flowers: Pedicel 4-8(-12) mm long. Calyx lobes broadly triangular, or oblong to narrowly oblong in outline. Fruit spines up to 3 mm

long in subsp. *prophetarum* and bright green coloration of plants, rather slender more or less spreading hairs, and fruit spines 4-6 mm long. The plant is distributed in Chad, Egypt, Ethiopia, Kenya, Mauritania, Niger, Rwanda, Somalia, Tanzania, Uganda, Saudi Arabia, South Yemen, and Yemen (Kirkbride, J., 1993).



**Figure 13:** The picture of *C. prophetarum* L plant (photo by Teshale A from Dera, Arsi, Oromia, Ethiopia, 2020)

## 2.4.2 Ethnobotanical Uses of Some Species of *Cucumis* in Ethiopia

### 2.4.2.1 *Cucumis ficifolius*

*C. ficifolius* has been used for many years in traditional medicine to treat dysentery and diarrhea. In Niger, the fruits are used for the treatment of measles. It is a popular hedge plant in Ethiopia traditionally been used for the treatment of diarrhea, teeth-ache, wound, skin infections, cancer, coughing, rabies in dogs, bad breath, TB, and eye disease (Abebe D., *et al.*, 2003). Different parts of the plant are used for the treatment of different ailments including skin cancer (Tadesse, B., *et al.*, 2014). Local people usually grind the roots of *C. ficifolius* and tie on the affected body of the patient to treat wound. The powdered root mixed with *Teff kita* is eaten to treat rabies (Admasu, P. and Mekonnen, Y., 2014). The fruit is used as an abortifacient for women and to hasten expulsion of the placenta for cows in Ethiopia.

In Nigeria and Ethiopia, the fresh fruit is applied to address inflamed fingers. The fruit has veterinary use as a vermifuge with the addition of natron for horses by the Hausa. It is also used as a medicine for fowls. In some places it is an ingredient of medicine for syphilis, “Kuruba”, “Chiffa”, “Mageriat geter” (meningitis), “nessir” (epistaxis), “wefbeshita” (Teklehaymanot, T., and Giday, M., 2007) and also the roots are remedy for malaria. The seeds are oil bearing. The root extract of *C. ficifolius* is recorded to be used in local honey-wine or “Tej” to make beverage more intoxicating (Jeffery, C., 1995).

Roots of *C. ficifolius*, leaves of *Ocimum gratissimum* and leaves of *Calpurnia aurea* are pounded together and mixed with cold water and a cup of coffee given to human to treat fibril illness. Sap of fruit of *C. ficifolius* added to ear canal to cure ear pain. A piece of root of *C. ficifolius* chewed with salt and swallowed by human for stomach complication. The leaves are used to cure tetanus. The dried powdered bark and leaves of *C. ficifolius* mixed with alcohol are taken by human to treat sudden sickness (Endalew, A., 2007).

#### **2.4.2.2 *Cucumis dipsaceus***

The fruit *Cucumis dipsaceus* used for gastrointestinal diseases, diarrhea, stomach pain, constipation and meningitis. Extract of fresh leaves and fruits are used for hemorrhoid and rabies (Prelude, 2001) eye blindness (Kalayu, M., *et al.*, 2013). The seeds are diuretic; the fruit juice is used as a nutritive and as a demulcent in anti-acne lotions (Kumar, D., *et al.*, 2010). Poultice is prepared from leaves and tendrils for the treatment of wounds. Fruit juice act as an antidote in poisoning case (Christopher, K., & Ruffo, A.B.T., 2002). The fruit is used against snake bite, insect bite, stomach pain/ diarrhea, dry cough, abdominal pain and gonorrhea (Temesgen, A., *et al.*, 2019). Its fruit juice is topically applied to prevent hair loss (Busmann, R.W., and Glenn, A., 2010). The stem decoction is used as anti-emetic and roots are used for hepatitis, snakebite, carnivore bite and gallstone. The tender leaves and young shoots are chopped, cooked with coconut milk or groundnut paste used as vegetable and then taken with staple food (Lata, S., *et al.*, 2017).

#### **2.4.2.3 *Cucumis prophetarum***

*C. prophetarum* is used in Saudi folk medicine for the treatment of liver disorders (Abdulrhman, A., *et al.*, 2018). The fruits of *C. prophetarum* is used for inflammatory-related problems and is proved to be possessing anticancer and hepatoprotective effects (Gawli, K., and Lakshmidhevi, N., 2015)

### **2.4.3 Chemical Constituents of Some Species of *Cucumis* in Ethiopia**

The dichloromethane extract of the fruits of *C. ficifolius* was reported to have 2',3'-dihydroxypropyl pentadecanoate (87), pentadecanoic acid (88) and tetradecanoic acid (89) (Tamrat, T., *et al.*, 2021) (Figure 14).

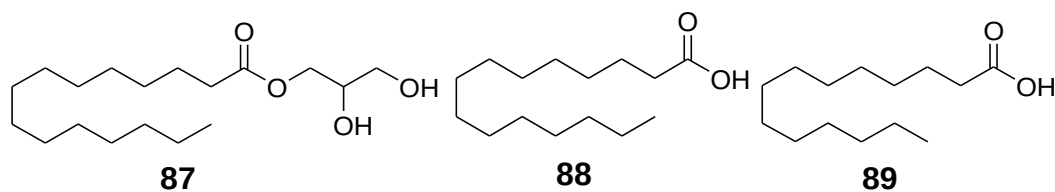


**Figure 14:** The compounds isolated from the fruit of *C. ficifolius*

Phytochemical screening of the 96% ethanolic extracts of the fruits of *C. dipsaceus* revealed the presence of various secondary metabolites including tannins, flavonoids, saponins, terpenes, cardiac glycosides, alkaloids, carbohydrates, and reducing sugars whereas coumarins, sterols, anthraquinones and cyanogenic glycoside were not detected (Osman, A., *et al.*, 2018). The GC-MS analysis of the fruit extracts revealed the presence of octadecanoic acid (**90**), pentadecanoic acid (**88**), eicosanoic acid (**91**), oleic acid (**92**), tetradecanal (**93**), octadecanal (**94**), pentadecanal (**95**), tridecanoic acid (**96**), alkyne and amide (Sowbaraniga, R. and Chitra, M., 2019).

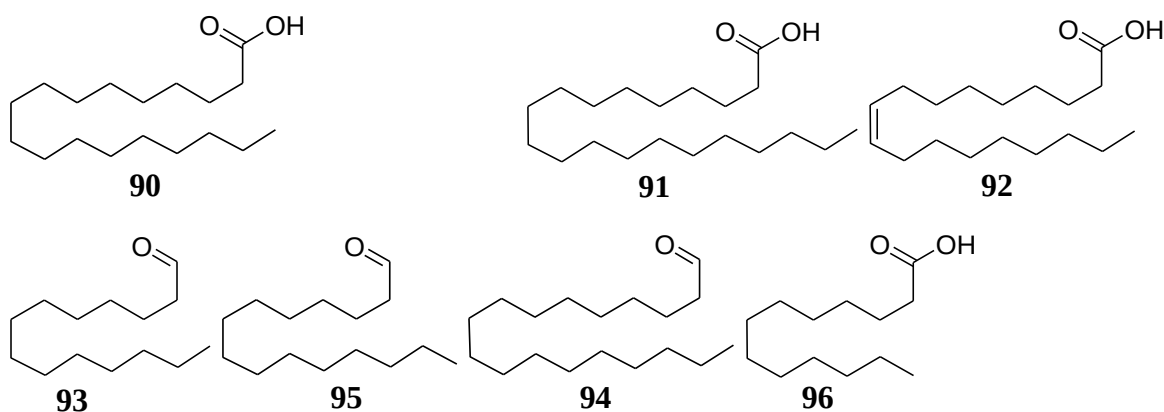


**Figure 15:** The compounds isolated from the fruit of *C. dipsaceus*

Cucurbitacin (B (**24**), E (**25**), I (**27**), O (**29**), P (**30**) and Q (**31**)); dihydrocucurbitacin (D (**97**) and E (**98**)), isocucurbitacin (B (**99**), D (**100**) and E (**101**)) and dihydroisocucurbitacin (D (**102**) and E (**103**)) (Figure 16) have been reported to be isolated from root of *C. prophetarum* L. (Ayyad, S.N., *et al.*, 2011). Phytochemical reports have shown the presence of cucurbitacin A and B (Atta-ur-Rehman, *et al.*, 1973), sterol from aerial parts, and saponins/triterpenes from the fruits (El-Tawil, B.A.H., 1983) of *C. prophetarum*. Different parts of this plant except its leaves were well explored for its phytochemical constituents.

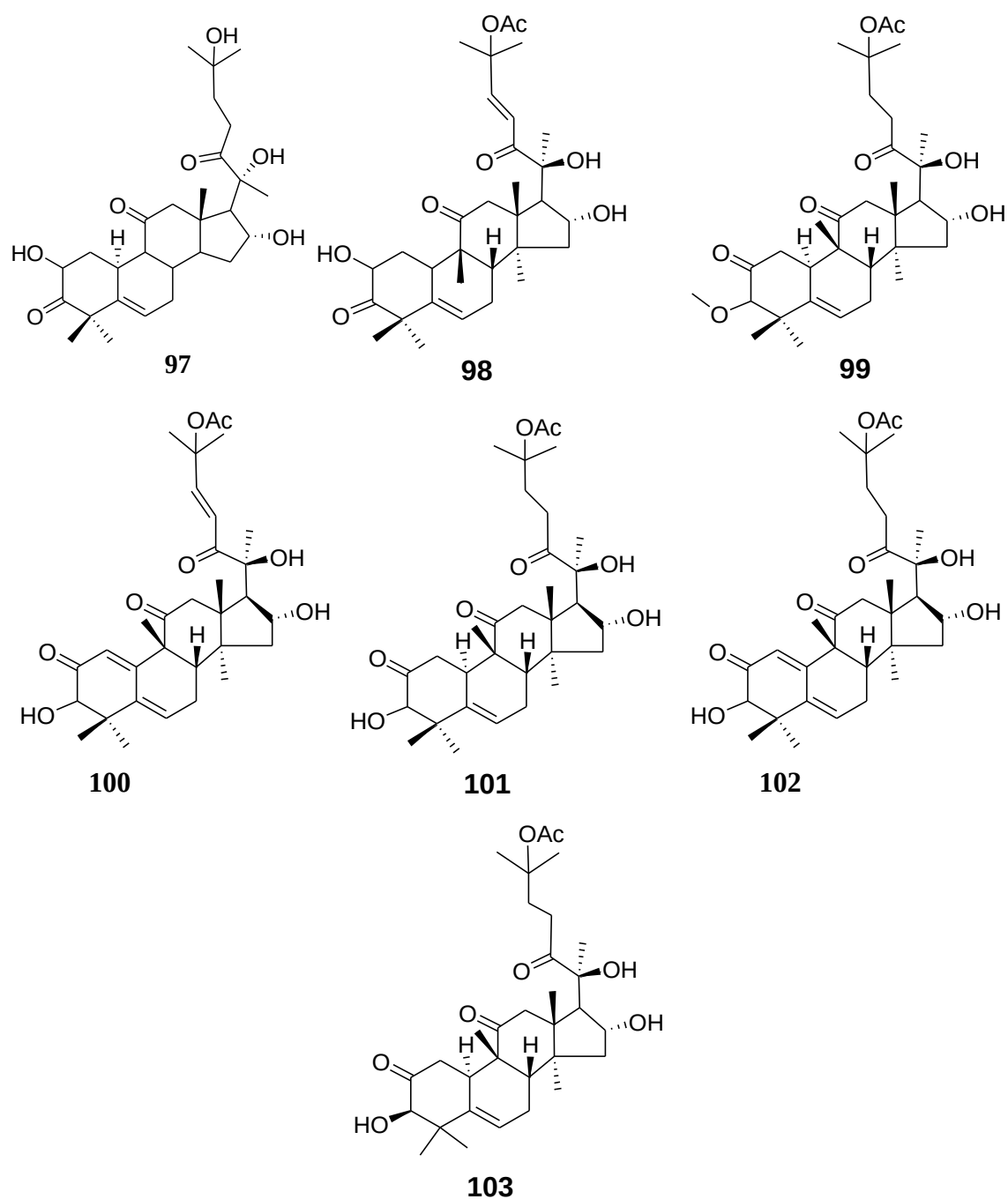


**Figure 16:** The compounds isolated from roots of *C. prophetarum*

#### 2.4.4 Biological activities of some species of *Cucumis* in Ethiopia

*C. ficifolius* are reported to possess purgatives and anti-helminthic properties due to the secondary metabolite *cucurbitacin* content (Gabrielsen, M., *et al.*, 2013; Saboo, S.S., 2013 and Sood, A., Gupta, R., 2012). The methanol, *n*-butanol and aqueous solvent extracts of the root of *C. ficifolius* were reported to have anti-nociceptive and anti-inflammatory activities (Desalegn, G.D., *et al.*, 2019).

A number of pharmacological activities including hepatoprotective (Karaye, I.U., *et al.*, 2012), analgesic (Victor, k., *et al.*, 2016), anti-inflammatory (H-Mastuda, T., *et al.*, 2002), and cytotoxic (H. Oh, *et al.*, 2004), antioxidant (Gopalan, C., *et al.*, 2007), antimicrobial and antibacterial (Golani, S., *et al.*, 2012), antiallergic (Anita, S.W., *et al.*, 2018), anticarcinogenic (Kesarkar, S., *et al.*, 2009) and anti-obesity activities (Buer, C.S.N., *et al.*, 2010) were reported on extracts of *C. dipsaceus*. Methanol extracts of the fruits of *C. dipsaceus* was reported to be active against bacteria (Cespedes, C.L., *et al.*, 2001).

The ethanol extract of aerial part of *C. prophetarum* produced contraction of isolated rabbit duodenum, which was antagonized by atropine exhibiting a muscarine type cholinergic activity (Al-Yahya, M.A., *et al.*, 1990). The extract also produced a significant fall in blood pressure in normotensive anaesthetized rabbit, and molluscicidal activity against *Biomphalaria alexandrina* snails in bait formulations (Abdel-Hamid, A.Z., 1997). The chloroform extract of *C. prophetarum* showed neuromuscular blocking activity on muscles in addition to its antimicrobial activity against *Bacillus subtilis* in MIC of 2 mg/mL (Al-Yahya, M.A., *et al.*, 1990). The greatest cytotoxic activity was found to be in the ethyl acetate extract fraction of fruit of *C. prophetarum*. Among the cucurbitacin that were isolated and tested cucurbitacin B and E showed potent cytotoxicity activities against all six human cancer cell lines (Abdulrhman, A., *et al.*, 2018). Cucurbitacin B and dihydrocucurbitacin B isolated from *C. prophetarum*, were studied for their cytotoxic activity towards human cancer cell lines, mouse embryonic fibroblast (NIH3T3) and virally transformed form (KA3IT) cells (Ayyad, S.N., *et al.*, 2011). The antidiabetic and antioxidant activities of the different fractions of fruits of *C. prophetarum* has been reported (Gawli, K., and Lakshmidhevi, N., 2015).

### 3. MATERIALS AND METHODS

#### 3.1 General

The solvents used in the extraction and column chromatography was HPLC grade include *n*-hexane (99%), petroleum ether (40-60 °C), chloroform (99.8%), ethyl acetate (99.5%) and methanol (99.8%) of Alchem chemical PVT. LTD, US. Reagents used include ascorbic acid, DPPH, and ciprofloxacin. Milling machine and Whatman filter paper were used for powdering sample and filtration, respectively. Rotary evaporator (Heidolph Laborata 4000) was used for evaporation of solvent from extracts. Thin layer chromatographic (TLC) analyses were carried out on TLC plates (0.2 mm Merck silica gel 60 mesh) coated on aluminum foil and pre-coated Silica gel plates (Merck, Keisel gel 60 F254, 0.25mm). Compounds on TLC were detected using UV light (254 nm and 365 nm) and in some cases after spraying with vanillin/H<sub>2</sub>SO<sub>4</sub>; while column chromatography was performed using silica gel (200-400 mesh, Merck). The <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and DEPT-135 spectra were recorded on a Bruker avance spectrometer operating at 400 MHz FT-NMR (Bruker (AVANCE III)) spectrometers using appropriate solvent. The chemical shifts were reported in  $\delta$  (ppm) units. The melting point was measured using melting point apparatus. UV-Visible spectrometry, GC-MS instruments, PTLC and Clevenger apparatus were used in this dissertation.

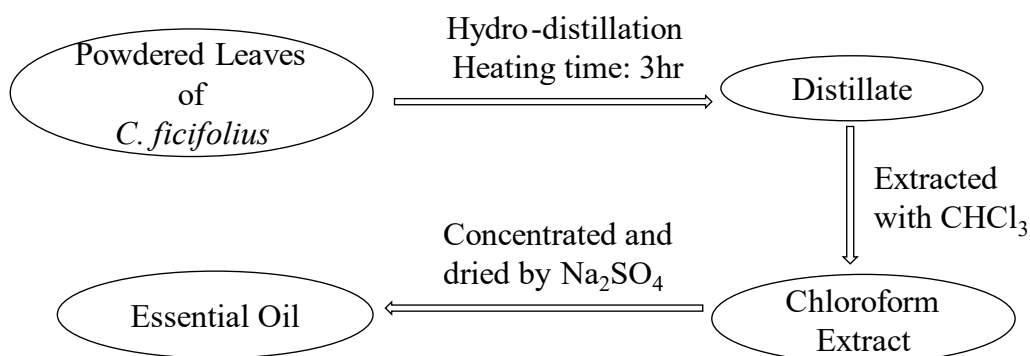
#### 3.2 Plant Material Collection, Authentication and Preparation

The plant parts of *Cucumis ficifolius*, *Cucumis dipsaceus* and *Cucumis prophetarum* L were collected from Dera, Arsi, Oromia, Ethiopia, in October 2021. The plant materials were authenticated by Mr. Melaku Wendaafirash and deposited at the National Herbarium of Addis Ababa University with voucher specimen number CF001, CD002 and CP003 on January 2020 G.C for *C. ficifolius*, *C. dipsaceus* and *C. prophetarum*, respectively. The plant material was washed with distilled water. The dried leaves, fruits, stems and roots were separately grounded into powder using a Milling machine. The samples were then stored in polyethylene bag prior to extraction.

#### 3.3 Extraction of Essential Oil

The air-dried ground leaves of *C. ficifolius* (50 g) were hydrodistilled using Clevenger type apparatus for 3 hr. The essential oil was extracted from the distillate using chloroform and separated by separatory funnel. The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated to afford essential oil (scheme1). It was preserved in a sealed sample tube and stored under refrigeration at 4°C until analysis. This was repeated for the fruits, roots, stems of *C. ficifolius*, *C. dipsaceus* and *C. prophetarum*.

The general flow chart showing the extraction of essential oil by hydro-distillation was depicted in Scheme1.



**Scheme 1:** Process flow chart for extraction of essential oil

### 3.3.1 GC-MS Analysis of Essential Oils

The analysis of the essential oils of *C. ficifolius*, *C. prophetarum* and *C. dipsaceus* were done using Gas Chromatography-Mass Spectrometry (GC-MS) Agilent 7950B series GC equipped with mass selective detector [(MSD), Agilent 5977 series operated in the EI mode (electron energy = 70 eV), Scan range = 40 -500 amu and scan rate = 10 scans/ sec] and an Agilent chem station data system. The GC column was an Agilent-5 fused silica capillary with a (5% phenyl)- polymethyl siloxane stationary phase, film thickness of 0.25  $\mu\text{m}$ , a length of 30m, and an internal diameter of 0.25mm. The carrier gas was helium with a column head pressure of 48.7 kpa and a constant flow rate of 1 mL/min. Inlet (injector) temperature was 200  $^{\circ}\text{C}$ ; Ion-source (interface) temperature was 280  $^{\circ}\text{C}$ . The GC oven temperature program was used as follows: 50  $^{\circ}\text{C}$  initial temperature, hold for 2min with an increase of 2  $^{\circ}\text{C}/\text{min}$ , to 150  $^{\circ}\text{C}$ , increased 5  $^{\circ}\text{C}/\text{min}$ , to 220  $^{\circ}\text{C}$ , increased 10  $^{\circ}\text{C}/\text{min}$ , to 250  $^{\circ}\text{C}$  and isothermal at 280  $^{\circ}\text{C}$ . Total GC running time was 70 min. A 1% w/v solution of the sample in n-hexane was prepared and an injection volume of 1  $\mu\text{L}$  was employed using a split ratio of 1:20.

### 3.3.2 Retention Indices (RI)

Retention indices (RI) using *n*-alkanes were used as the basis. The percent of each compound was based on the peak area divided by the total area of component peaks. The temperature range was from 50-250 °C, with a temperature program rate as follows: 50 °C initial temperature, hold for 2 min with an increase of 2 °C/min, to 150 °C, increased 5 °C/min, to 220 °C, increased 10 °C/min, to 250 °C and isothermal at 280 °C starting at three minutes and finishing at 70 minutes. The pressure applied in this experiment was 48.7 kpa with a total flow of 50 mL/min and 1 mL/min of column flow. The injection, ion source and the interface temperatures were 200 °C, and 280 °C respectively. RI were calculated using the following formula:

$$\text{Retention indices (RI)} = 100 \left[ \frac{(tR - tR_z)}{(tR_{(z+1)} - tR_z)} + z \right]$$

Where:

RI: Retention index value of unknown compound (peak)

z: the number of carbons in the alkane preceding unknown compound

tR: the retention time of unknown compound

tR<sub>z</sub>: the retention time of the preceding alkane

tR<sub>(z+1)</sub>: the retention time of the following alkane

### 3.3.3 Identification of Components

The components of essential oil were identified on the basis of comparison of their retention indices and mass spectra with published data (Babushok, V.I., *et al*, 2011) and computer matching with WILEY 275 and National Institute of Standards and Technology (NIST3.0) libraries provided with computer controlling the GC-MS system, in Adama Science and Technology University, Ethiopia. The spectrum of the unknown component was compared with the spectrum of the known components stored in the library. The name, molecular weight and structure of the components of the test materials were ascertained. Furthermore, the RIs of each compound was compared with the same compound reported in the literature

## 3.4 Extraction and Isolation

### 3.4.1 Extraction

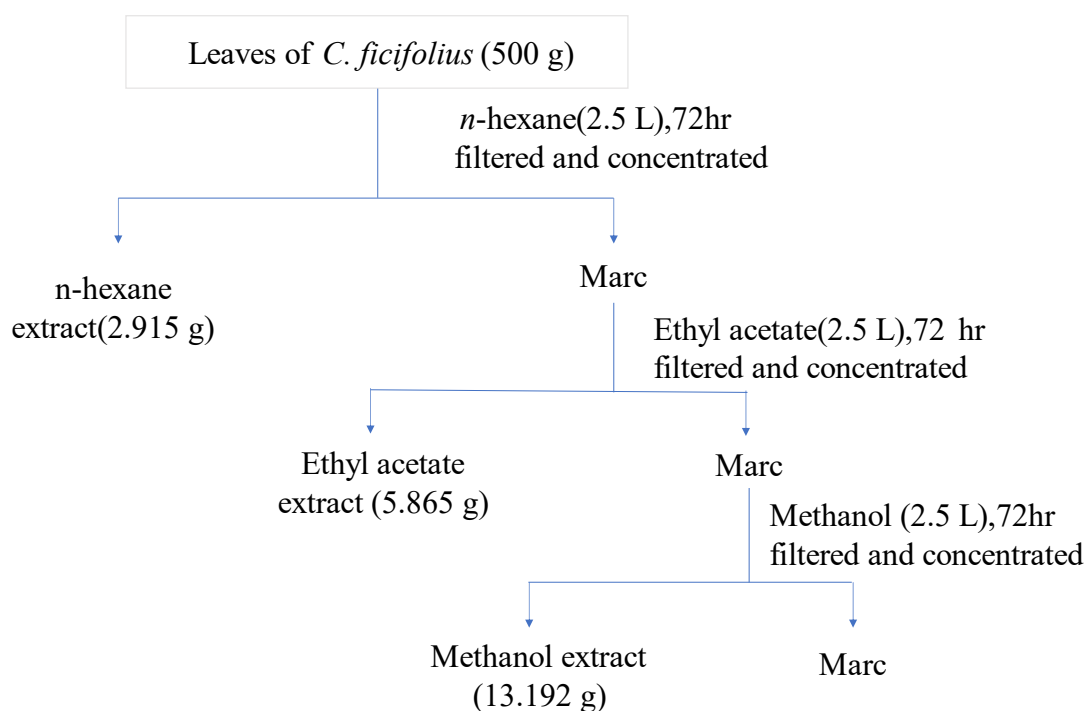
The air-dried powdered stems (300 g), roots (500 g), leaves (500 g) and fruits (500 g) of *C. ficifolius* were successively extracted with each 2.5 L of *n*-hexane or petroleum ether, ethyl acetate (EtOAc) and methanol on maceration for 72 hr. Each extract was filtered and

concentrated under reduced pressure at 40 °C using rotary evaporator using *n*-hexane/petroleum ether, EtOAc and methanol respectively.

Likewise, the dried powdered fruits of *C. dipsaceus* (500 g) was successively extracted with each 2.5 L of petroleum ether, EtOAc and methanol on maceration for 72 hr. Each extract was filtered and concentrated under reduced pressure at 40 °C using rotary evaporator. The extracts were analyzed with TLC for isolation and biological activities.

The air dried and powdered leaves of *C. prophetarum* (700 g) was extracted with methanol for 72 hr, filtered and concentrated on rotatory evaporator. The extract was suspended in distilled water (300 mL) and partitioned using petroleum ether, chloroform, ethyl acetate three wise with 1.5 L of each solvent. Each fraction was dried and concentrated under reduced pressure at 40 °C by using rotary evaporator.

The schematic view showing extraction procedure of leaves part of *C. ficifolius* is depicted in Scheme 2 and this was repeated for other parts of *C. ficifolius* and *C. dipsaceus*.



**Scheme 2:** The general extraction procedure for extraction of leaves of *C. ficifolius*.

### 3.4.2 Isolation of Compounds from *Cucumis ficifolius*, *Cucumis dipsaceus* and *Cucumis prophetarum*

#### 3.4.2.1 Stem of *Cucumis ficifolius*

The combined *n*-hexane and ethyl acetate extracts (8.0 g) was adsorbed to the same quantity of silica gel and fractionated over silica gel (150 g) column chromatography using *n*-hexane

for packing. Elution was carried out with gradient increasing polarity of EtOAc in *n*-hexane to afford 52 fractions, each 100 mL. Fraction 16, eluted using *n*-hexane:EtOAc (85:15), was re-chromatographed using *n*-hexane:EtOAc (9:1) to give compound **104** (16.5 mg). Fractions 20-22, eluted with *n*-hexane:EtOAc (85:15), furnished compound **58** (23 mg).

#### 3.4.2.2 Leaves of *Cucumis ficifolius*

The *n*-hexane and EtOAc extracts (8.0 g) of the leaves of *C. ficifolius* was adsorbed and subjected to silica gel column chromatography over silica gel (150 g) using *n*-hexane for packing. Elution was carried out with *n*-hexane:EtOAc of increasing polarity to afford 20 fractions, each 100 mL. Fractions 9-11, eluted with *n*-hexane:EtOAc (85:15), was re-chromatographed over silica gel column chromatography using *n*-hexane:EtOAc (9:1) to afford compound **58** (63.9 mg) as a white crystals.

#### 3.4.2.3 Root of *Cucumis ficifolius*

The *n*-hexane and EtOAc extracts (12.0 g) of the root of *C. ficifolius* was adsorbed to silica gel and subjected to column chromatography over silica gel (150 g) using petroleum ether for packing. Elution was carried out by increasing polarity of EtOAc in petroleum ether to give 375 fractions, 10 mL each. Fractions of the same  $R_f$  values were combined to give 29 fractions. Fractions 41-70, eluted using *n*-hexane:EtOAc (85:15), was re-chromatographed using *n*-hexane:EtOAc (9:1) to give compound **58** (24.1 mg). Fractions 139-159, eluted with *n*-hexane:EtOAc (3:2), furnished compound **24** (20.5 mg) while fractions 354-367, eluted with EtOAc:Methanol (7:3), furnished compound **26** (22.5 mg).

#### 3.4.2.4 Fruits of *Cucumis ficifolius*

The petroleum ether and ethyl acetate extracts (18.0 g) of the fruit of *C. ficifolius* was adsorbed to silica gel and subjected to column chromatography over silica gel (200 g) using petroleum ether for packing. Elution was carried out with increasing polarity of EtOAc in petroleum ether to afford 282 fractions, each 10 mL. Fractions of the same  $R_f$  values were combined to afford 26 fractions. Fractions 1-40, eluted with petroleum ether:EtOAc (9:1) after silica gel column chromatography gave compound **105** (50 mg), while compound **106** (80 mg) was obtained from Fractions 44-59 which was eluted with petroleum ether:EtOAc (9:1). Fractions 81-106, eluted with petroleum ether:EtOAc (8:2), furnished compound **107** (35 mg) whereas fractions 118-136, eluted with petroleum ether:EtOAc (7:3), furnished compound **58** (35 mg). Fractions 205-225, eluted with petroleum ether:EtOAc (6:4), afford

compound **108** (30 mg) and fraction 247-268, eluted with methanol:EtOAc (3:7), afford compound **109** (30 mg).

#### 3.4.2.5 Fruit of *Cucumis dipsaceus*

The petroleum ether extract (16.0 g) was adsorbed to silica gel and subjected to column chromatography (CC) over silica gel (150 g) using increasing polarity of EtOAc in petroleum ether to afford 225 fractions, each 10 mL. Fraction 39-70, eluted using petroleum ether:EtOAc (85:15) was mixed and re-chromatographed by using petroleum ether:EtOAc (9:1) as eluent to give 25 sub fractions (each 10 mL), out of which subfractions 8-10 was identified as compound **110** (28 mg) while subfraction 14 and 15 was found to be compound **111** (15 mg). Fractions **95-110**, eluted using petroleum ether:EtOAc (85:15), was mixed and re-chromatographed by using petroleum ether:EtOAc (9:1) as eluent to give 25 subfractions, out of which fractions 16 was identified as compound **105** (20 mg). Fractions 161-206, eluted using petroleum ether:EtOAc (6:4) were combined and re-chromatographed using petroleum ether:EtOAc (65:35) as eluent to afford 30 subfractions, each 10 mL. Subfraction 24 gave compound **112** (35 mg). Fractions 217-222 were rechromatographed over silica gel using petroleum ether:EtOAc (4:6) as eluent to afford 10 subfractions, 10 mL of which subfraction 4 was identified as compound **113** (20 mg).

The EtOAc extract (7.83 g) was also fractionated over silica gel (150 g) column chromatography using petroleum ether for packing. Elution was carried out with petroleum ether:EtOAc (9:1) of increasing polarity of EtOAc in petroleum ether as mobile phase to afford 130 fractions, each 10 mL. Fractions 1-15, eluted using petroleum ether:EtOAc (85:15), was re-chromatographed using petroleum ether:EtOAc (9:1) to afford 42 subfractions, 10 mL each. Sub-fraction 8 was identified as compound **114** (10 mg). Fractions 18-54, eluted using petroleum ether: EtOAc (4:1), was re-chromatographed using petroleum ether:EtOAc (9:1) to give 73 subfractions (10 mL each), among which subfractions 11-31 were mixed and re-chromatographed to afford compound **58** (30 mg).

Fractions 101-130, eluted using petroleum ether:EtOAc (1:1), was combined and re-chromatographed over silica gel using petroleum ether:EtOAc (3:2) to give 170 fractions (10 mL each) out of which subfractions 85-106 was identified as mixture of compound **26** and **100** (60 mg). Compound **102** (20 mg) was obtained from fractions 145-154.

#### 3.4.2.6 Leaves of *Cucumis prophetarum*

The petroleum ether extract (20 g) was adsorbed on equal quantity of silica gel and subjected to silica gel column chromatography (CC) using increasing polarity of petroleum ether:EtOAc to afford 300 fractions, each 10 mL.

Fractions 45-60 eluted with petroleum ether:EtOAc (8:2), was re-chromatographed using petroleum ether:EtOAc (9:1) as éluent to give compound **115** (20 mg). Likewise, fractions 81-106, eluted with petroleum ether:EtOAc (8:2), was re-chromatographed to give compound **116** (30 mg). Fractions 118-136 eluted with petroleum ether:EtOAc (7:3), was fractionated over silica gel column chromatography to furnish compound **58** (40 mg) and fractions 200-210, eluted with methanol: EtOAc (3:7), was purified using silica gel column chromatography to give compound **117** (25 mg).

### 3.5 Structure Elucidation

The essential oils compositions were analyzed using GC-MS. The structures of the purified compounds were determined using NMR spectroscopic technique. In some cases, the spectral data of the isolated compounds were compared with data of similar compounds reported in the literature

### 3.6 Biological Activity

#### 3.6.1 Antibacterial Activity

Antibacterial activities of the samples were evaluated using disc diffusion method against four bacterial strains (two Gram negative; *Escherichia coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-27853) and two Gram positive; *Staphylococcus aureus* (ATCC-25923) and *Streptococcus pyogenes* (ATCC-19615) obtained from Adama regional laboratory, Ethiopia. Appropriate colonies of required bacterial strains were selected and standardized with 0.5 McFarland standards turbidity. Centrifuged pellets of bacteria from a 24-hr old culture containing  $1.5 \times 10^8$  CFU (colony forming unit) per mL was spread on the surface of the nutrient agar (NaCl 0.5%, tryptone 1%, yeast extract 0.5%, agar 1%, 1000 mL of distilled water, pH 7.0) which was autoclaved at 121 °C for 15 min then cooled to 45 °C, 25 mL poured into each Petri dishes and allowed to settle. Samples were prépare at différent concentrations of 400, 200, 100, 50, 25 and 12.5 µg/mL in dimethyl sulfoxide (DMSO). After solidification of media on Petri dishes, the tested samples with different concentration and ciprofloxacin used as positive controls were loaded on to each disc. DMSO was loaded as negative control. All plates were observed for a zone of inhibition at 35 °C after 16-18 hr. The activity has determined by measuring the diameter of the inhibition

zone in mm. Each inhibition zone was measured by using ruler. The samples were analyzed in triplicates.

### 3.6.2 Determination of the Minimum Inhibitory Concentration (MIC)

The MIC of the antibacterials was determined by the method involving micro dilution in culture broth, as indicated by the Clinical and Laboratory Standards Institute (CLSI) of the USA (CLSI, 2019). MIC is the lowest concentration of an antimicrobial that inhibit the visible growth of a microorganism after 24 hr incubation. Stock solutions of standard compound and isolated compounds were prepared in 2 mL of DMSO. This stock solution with different concentration such as 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 µg/mL were prepared. 0.2 µL culture bacterial strains standardized with 0.5 McFarland standards turbidity were added to each test tube nutrient agar which was autoclaved at 121 °C for 15 min then cooled to 45 °C and incubated for 24 hr at 37 °C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentrations (MIC) (Rahman, A.U., *et al.*, 2001).

### 3.6.3 Cytotoxicity Activity

In order to investigate the cytotoxic effect of the samples human breast cancer cell line MCF-7 obtained from National Centre for Cell Science (NCCS), Pune, India were used. The cells were grown in T25 culture flasks containing DMEM supplemented with 10% FBS and 1% antibiotics (100 U mL<sup>-1</sup> penicillin and 100 µg mL<sup>-1</sup> streptomycin). The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>. The anti-proliferative effect of the samples at low concentration (20 µg) was studied on human breast cancer cell lines (MCF-7). Upon reaching confluence, the cells were detached using Trypsin–EDTA solution and were subcultured at a density of 5000 cells per well. At 50% confluence, the culture medium was aspirated, and the cells were treated with 20 µg of plant extract in DMSO (20 µL) for 24 hr at 37 °C in the CO<sub>2</sub> incubator. Later cells were incubated with MTT (4 mg mL<sup>-1</sup>) for 3 hrs. The absorbance was measured at 540 nm with a standard microplate reader. The cell viability was expressed as the percent. The results are presented as the mean of triplicate experiments. The cell viability was calculated as the following equation :

$$\% \text{ Cell viability} = \frac{\text{Absorbance of treated}}{\text{Absorbance of control}} \times 100$$

The half maximal inhibitory concentration (IC<sub>50</sub>) values were determined from a concentration response curve of the percentage of cell viability (y-axis) versus log concentration (µg/mL) of plant extract (x-axis) by using GraphPad Prism Version 8.0.

### 3.6.4 Radical Scavenging Activity

The antioxidant activity cucurbitacin B (**24**), cucurbitacin D (**26**),  $\alpha$ -spinasterol (**58**), isocucurbitacin D (**100**), 23,24-dihydroisocucurbitacin D (**102**), lignoceric acid (**104**), eicosane (**105**), stearic acid (**106**), 12-hydroxyoctacosanoic acid (**107**), 13-hydroxyoctadecanoic acid (**108**), kuguaglycoside E-3-acetate (**109**), octacosane (**110**), docosane (**111**), 17-(-5-ethyl-2,6-dihydroxy-6-methylhept-3-en-2-yl)-9-(hydroxymethyl)-13-methyl-1H cyclopenta[a]phenanthren-3-ol (**112**), erythrodiol (**113**), (9,12)-propyl icosanoic acid (**114**), 2-heptylcyclohexanol (**115**), decanoic acids (**116**) and 1,3-dihydroxypropan-2-yl dodecanoate (**117**) (Figures 18, 19 and 20) were measured in terms of hydrogen-donating or radical scavenging ability using the stable radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams, W., *et al.*, 1995). The DPPH sample was prepared by dissolving 4 mg of DPPH in 100 mL of MeOH. Four different concentrations of the samples were mixed with DPPH solution to furnish 12.5, 25, 50 and 100 µg/mL. The mixture was shaken vigorously and incubated at 37 °C for 30 min. Then, absorbance was measured at 517 nm by using UV-Vis spectrophotometer. The half maximal inhibitory concentration (IC<sub>50</sub>) was calculated by the linear regression analysis and expressed as mean of determinations. Ascorbic acid was used as control. The % inhibition was calculated using the following formula from literature (Brand-Williams, W., *et al.*, 1995).

$$\% \text{ Inhibition of DPPH radical (scavenging activity)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where  $A_{\text{control}}$  is the absorbance of the control (without extract) and  $A_{\text{sample}}$  is the absorbance in the presence of the extract with standard. The IC<sub>50</sub> (concentration for 50% inhibition) values of all the methods were calculated by using linear regression analysis. Higher the IC<sub>50</sub> value, lower is the scavenging activity.

### 3.7 In silico Molecular Docking Study of Isolated Compounds

Molecular docking studies were performed in order to predict the interaction of isolated compounds with the binding sites of *E. coli* DNA gyrase B (PDB ID: 6F86), *S. aureus* PK

(PDB ID: 3T07), human topoisomerase II  $\beta$  (PDB ID 3QX3) and kinase suppressor ras (KSR) 2 (PDB ID: 7JUT) were used as target proteins.

### **Preparation of ligands**

The 2D chemical structures (molecules) of NMR identified compounds namely cucurbitacin B (**24**), cucurbitacin D (**26**),  $\alpha$ -spinasterol (**58**), isocucurbitacin D (**100**), 23,24-dihydroisocucurbitacin D (**102**), lignoceric acid (**104**), eicosane (**105**), stearic acid (**106**), 12-hydroxyoctacosanoic acid (**107**), 13-hydroxyoctadecanoic acid (**108**), kuguaglycoside E-3-acetate (**109**), octacosane (**110**), docosane (**111**), 17-(5-ethyl-2,6-dihydroxy-6-methylhept-3-en-2-yl)-9-(hydroxymethyl)-13-methyl-1H cyclopenta[a]phenanthren-3-ol (**112**), erythrodiol (**113**), (9,12)-propyl icosanoic acid (**114**), 2-heptylcyclohexanol (**115**), decanoic acids (**116**) and 1,3-dihydroxypropan-2-yl dodecanoate (**117**) (Figures 18, 19 and 20) were drawn using Chem Bio Draw tool (Chem Bio Office Ultra 16.0 suite) assigned with proper 2D orientation, and structure of each was checked for structural drawing error. Energy of each molecule was minimized using Chem Bio 3D. The 3D coordinates protein data bank (PDB) of each molecule were obtained through optimized structure. The energy minimized ligand molecules were then used as input for AutoDock vina, in order to carry out the docking simulation.

### **Preparation of macromolecules**

The protein data bank (PDB) used crystal structure of the enzyme as receptor molecule. All the water molecules were removed from the receptor. The crystal structure of receptor molecules *E. coli* Gyrase (PDB ID:6F86), *S. aureus* Gyrase (PDB ID: 2XCT), KSR2 (PDB ID: 7JUT) and human topoisomerase II $\beta$  (PDB ID: 3QX3) proteins were downloaded from protein data bank. The protein preparation was done using the reported standard protocol by removing the co-crystallized ligand, selected water molecules and cofactors, the target protein file was prepared by leaving the associated residue with protein by using Auto Preparation of target protein file Auto Dock 4.2.6 (MGL tools 1.5.6).

### **AutoDock Vina analysis**

The graphical user interface program molecular graphics AutoDock 4.2.6 was used to set the grid box for docking simulations. The grid was set so that it surrounds the region of interest in the macromolecule. The docking algorithm provided with AutoDock Vina was used to search for the best docked conformation between ligand and protein. During the docking process, a maximum of nine conformer were considered for each ligand. The conformations with the most favorable (least) free binding energy were selected for

analyzing the interactions between the target receptor and ligands by discovery studio visualiser and PyMOL. AutoDock Vina with standard protocol was used to dock the protein *E. coli* DNA gyrase(PDB ID: 6F86), *S. aureus* Gyrase (PDB ID : 2XCT), human topoisomerase II $\beta$  (PDB ID : 3QX3) and KSR2(PDB ID: 7JUT) proteins and isolated ligands cucurbitacin B (**24**), cucurbitacin D (**26**),  $\alpha$ -spinasterol (**58**), isocucurbitacin D (**100**), 23,24-dihydroisocucurbitacin D (**102**), lignoceric acid (**104**), eicosane (**105**), stearic acid (**106**), 12-hydroxyoctacos-9,13-dienoic acid (**107**), 13-hydroxyoctadeca-9,11-dienoic acid (**108**), kuguaglycoside E-3-acetate (**109**), octacosane (**110**), docosane (**111**), 17-(-5-ethyl-2,6-dihydroxy-6-methylhept-3-en-2-yl)-9-(hydroxymethyl)-13-methyl-1H cyclopenta[a]phenanthren-3-ol (**112**), erythrodiol (**113**), (9,12)-propyl icos-9,12-dienoate (**114**), 2-heptylcyclohexanol (**115**), decanoic acids (**116**) and 1,3-dihydroxypropan-2-yl dodecanoate (**117**) (Figures 18, 19 and 20) into the active site of proteins. The molecular docking studies were carried out using AutoDockTools (ADT) (Trott, O., *et al.*, 2010) which is a free graphic user interface (GUI) for the AutoDock Vina program. The grid box was constructed using 20x20x20, pointing in x, y, and z directions, respectively, with a grid point spacing of 0.375 Å. The center grid box is of 59x30x59 Å for 2XCT and of 65x40x65 Å for 3QX3. Nine different conformations were generated for each ligand scored using AutoDock Vina functions and were ranked according to their binding energies. The ligands are represented in different colour, H-bonds and the interacting residues are represented in stick model representation.

## 4. RESULTS AND DISCUSSION

### 4.1 Essential Oil Analysis of *C. ficifolius*, *C. dipsaceus* and *C. prophetarum*

#### 4.1.1 Essential Oil and Extract Yield

##### Essential Oil Yield

*C. prophetarum*, *C. ficifolius* and *C. dipsaceus* were subjected to hydro-distillation using Clevenger type apparatus to afford a pale-yellow oil with a pleasant smell. The essential oils were less dense and insoluble in water. But they were miscible in *n*-hexane and chloroform. The yields of the essential oils from *C. ficifolius*, *C. prophetarum*, and *C. dipsaceus* were calculated in relation to dry weight plant materials. The highest yield was obtained from *C. prophetarum* stems and leaves (0.25% w/w each), and lowest yield was obtained from *C. ficifolius* root (0.02% w/w). The results (yields) of essential oils obtained from different parts of *C. prophetarum*, *C. ficifolius* and *C. dipsaceus* are given below (Table 1).

**Table 1:** Yield of essential oil (% w/w) extracted using hydrodistillation.

| Botanical name        | Plant part | Yield (% w/w) |
|-----------------------|------------|---------------|
| <i>C. ficifolius</i>  | Leaves     | 0.16          |
|                       | Fruits     | 0.12          |
|                       | Stems      | 0.12          |
|                       | Roots      | 0.02          |
| <i>C. prophetarum</i> | Leaves     | 0.25          |
|                       | Stems      | 0.25          |
|                       | Roots      | 0.21          |
| <i>C.dipsaceus</i>    | Leaves     | 0.15          |
|                       | Fruits     | 0.20          |
|                       | Stems      | 0.12          |

#### 4.1.2 Chemical Composition of the Essential Oil

The essential oil composition of different parts of *C. ficifolius*, *C. prophetarum* and *C. dipsaceus* were analyzed using GC-MS. The results revealed the presence of 50, 21 and 19 components in various parts of *C. ficifolius*, *C. dipsaceus* and *C. prophetarum*, respectively.

##### 4.1.2.1 *Cucumis ficifolius*

The chemical composition of the essential oils in the leaves, stems, fruits and roots *C. ficifolius* were analyzed by GC-MS. The analysis allow identification of 50 compounds

(compound 118-166) (Table 2). The constituents were identified based on their retention indices (RI) and compared with the retention indices reported in the literature for the same compound. Except linoleic acid, decanoic acid and Methyl linoleate all the essential constituents are new to the genus *Cucumis*.

**Table 2:** Compounds identified from the essential oil of various parts of *C. ficifolius*.

| Compound No | Chemical composition                              | RT     | RI   | RI (Lit.)<br>(Babushok, V.I., et al, 2011) | % Peak area |       |        |       |
|-------------|---------------------------------------------------|--------|------|--------------------------------------------|-------------|-------|--------|-------|
|             |                                                   |        |      |                                            | Leaves      | Stems | Fruits | Roots |
| 118         | Morpholine <sup>2</sup>                           | 3.509  | 96   | -                                          | -           | 0.24  | 0.01   | 0.36  |
| 119         | 2-Heptanol <sup>2</sup>                           | 4.26   | 959  | 959.4                                      | -           | 0.10  | -      | 0.17  |
| 120         | Hexanoic acid <sup>2</sup>                        | 7.211  | 997  | 996.4                                      | -           | 0.21  | -      | 0.49  |
| 121         | Limonene <sup>2</sup>                             | 8.684  | 1029 | 1029.5                                     | 0.05        | 1.23  | -      | 1.34  |
| 122         | Pinene hydrate, trans- <sup>2</sup>               | 13.293 | 1121 | 1121.2                                     | 0.06        | 0.52  | -      | 1.26  |
| 123         | Citronellal <sup>2</sup>                          | 15.314 | 1154 | 1153.7                                     | 0.03        | 0.16  | -      | 0.74  |
| 124         | Linalool oxide<br>(pyranoid), trans- <sup>2</sup> | 16.319 | 1171 | 1171.0                                     | 0.39        | -     | -      | -     |
| 125         | Dihydrocarvone, trans- <sup>2</sup>               | 17.965 | 1199 | 1201.4                                     | -           | 0.29  | -      | 0.21  |
| 126         | Isobornyl acetate                                 | 23.595 | 1286 | 1285.9                                     | 0.59        | 0.81  | -      | 3.12  |
| 127         | Indole <sup>2</sup>                               | 24.439 | 1298 | 1298.4                                     | -           |       | -      | 0.81  |
| 128         | Carvacrol <sup>2</sup>                            | 24.445 | 1299 | 1300.4                                     | 0.04        | 0.88  | -      | -     |
| 129         | Undecanal <sup>2</sup>                            | 24.912 | 1306 | 1306.5                                     | 0.016       | 0.21  | -      | -     |
| 130         | Piperitenone <sup>2</sup>                         | 27.361 | 1343 | 1340.7                                     | 0.19        | -     | -      | -     |
| 131         | Cyperene <sup>2</sup>                             | 30.971 | 1398 | 1397.8                                     | -           | 0.19  | -      | -     |
| 132         | Thujopsene, cis- <sup>2</sup>                     | 33.293 | 1435 | 1432.0                                     | 0.058       | -     | -      | 2.35  |

|            |                                            |        |      |        |      |       |      |       |
|------------|--------------------------------------------|--------|------|--------|------|-------|------|-------|
| <b>133</b> | Phenylethyl 3-methylbutanoate <sup>2</sup> | 36.781 | 1490 | 1490.5 | 0.1  | -     | -    | -     |
| <b>134</b> | Dodecanoic acid <sup>2</sup>               | 41.632 | 1570 | 1569.2 | 0.11 | -     | -    | -     |
| <b>135</b> | Humulene epoxide II <sup>2</sup>           | 43.859 | 1607 | 1604.7 | 0.18 | -     | -    | 3.28  |
| <b>136</b> | Bicyclo [4.2.1] nonan-1-ol <sup>2</sup>    | 44.266 | 1614 | -      | 3.93 | -     | 0.03 | 2.30  |
| <b>137</b> | $\gamma$ -Eudesmol, 10-epi <sup>2</sup>    | 44.595 | 1620 | 1618.7 | 0.59 | -     | -    | -     |
| <b>138</b> | 1-Tetradecanol <sup>2</sup>                | 47.904 | 1677 | 1676.3 | 0.1  | -     | -    | -     |
| <b>139</b> | Calamenene, trans- <sup>2</sup>            | 55.590 | 1823 | 1823.0 | 0.12 | -     | -    | -     |
| <b>140</b> | $\beta$ -Calacorene <sup>2</sup>           | 59.744 | 1939 | 1940.3 | 0.11 | -     | -    | 13.2  |
| <b>141</b> | Isophytol <sup>2</sup>                     | 59.934 | 1945 | 1946.5 | 8.07 | -     | -    | 6.85  |
| <b>142</b> | Jasmone, (Z)- <sup>2</sup>                 | 60.258 | 1956 | 1955.2 | -    | -     | -    | 0.38  |
| <b>143</b> | henylethyl 3-methylbutanoate <sup>2</sup>  | 60.552 | 1967 | 1969.5 | 3.23 | -     | 0.02 | 9.83  |
| <b>144</b> | 4,5-Epoxy-2-decenal, (E)- <sup>2</sup>     | 61.494 | 1999 | 2000.7 | 0.05 | -     | -    | -     |
| <b>145</b> | Manool, 13-epi- <sup>2</sup>               | 61.777 | 2010 | 2010.2 | 1.59 | -     | -    | -     |
| <b>146</b> | $\gamma$ -Nonalactone. <sup>2</sup>        | 62.158 | 2026 | 2026.1 | 1.34 | -     | -    | 0.47  |
| <b>147</b> | Furaneol <sup>2</sup>                      | 62.291 | 2031 | 2031.1 | -    | 2.23  | -    | -     |
| <b>148</b> | Neophytadiene <sup>2</sup>                 | 62.672 | 2047 | -      | 0.41 | -     | -    | -     |
| <b>149</b> | Cubenol <sup>2</sup>                       | 63.209 | 2069 | 2067.9 | 4.48 | 54.90 | -    | -     |
| <b>150</b> | Methyl linoleate <sup>1</sup>              | 63.711 | 2090 | 2091.1 | 0.26 | -     | -    | 14.15 |
| <b>151</b> | Methyl oleate <sup>2</sup>                 | 63.810 | 2094 | 2096.1 | 0.31 | -     | -    | -     |
| <b>152</b> | 2-hexyldecan-1-ol <sup>2</sup>             | 63.948 | 2100 |        | 0.23 | -     | -    | -     |

|            |                                                                                   |        |      |        |       |       |       |      |
|------------|-----------------------------------------------------------------------------------|--------|------|--------|-------|-------|-------|------|
| <b>153</b> | Linoleic acid <sup>1</sup>                                                        | 64.572 | 2129 | 2128.9 | 0.5   | 4.56  | -     | -    |
| <b>154</b> | 1-Dotriacontanol <sup>2</sup>                                                     | 66.085 | 2199 |        | -     | -     | -     | 0.76 |
| <b>155</b> | (S)-Methyl 1-(2,5-dimethoxyphenyl)-1H-benzo[f]chromene-3-carboxylate <sup>2</sup> | 66.316 | 2212 | -      | 0.71  | -     | -     | -    |
| <b>156</b> | Carbonic acid, eicosyl vinyl ester <sup>2</sup>                                   | 66.767 | 2236 | -      | 6.18  | -     | -     | -    |
| <b>157</b> | Decanoic acid <sup>1</sup>                                                        | 67.414 | 2270 | 2273.7 | 57.08 | 1.35  | -     | -    |
| <b>158</b> | 2-hexyl-1-Decanol <sup>2</sup>                                                    | 68.003 | 2302 | -      | 0.48  | 2.95  | -     | -    |
| <b>159</b> | 1-Decanol, 2-hexyl-Pentacosane <sup>2</sup>                                       | 68.436 | 2331 | -      | 1.08  | -     |       | -    |
| <b>160</b> | Neophytadiene <sup>2</sup>                                                        | 68.591 | 2345 | -      | 12.42 | -     | -     | -    |
| <b>161</b> | 9-Eicosyne <sup>2</sup>                                                           | 68.794 | 2355 | -      | -     | 0.86  | -     | -    |
| <b>162</b> | 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- <sup>2</sup>           | 68.823 | 2357 | -      | 0.5   | -     | -     | -    |
| <b>163</b> | 7-Hexadecenal <sup>2</sup>                                                        | 68.990 | 2369 | -      | 1.51  | -     | -     | -    |
| <b>164</b> | Benzyl methyl ether <sup>2</sup>                                                  | 69.025 | 2371 | -      | -     | 5.64  |       | -    |
| <b>165</b> | (E)-14-methylhexadec-8-enal <sup>2</sup>                                          | 69.158 | 2380 | -      | 0.93  | -     | -     | -    |
| <b>105</b> | Eicosane <sup>2</sup>                                                             | 69.273 | 2388 | -      | 0.90  | 13.55 | -     | 37.8 |
| <b>166</b> | Hentriacontane <sup>2</sup>                                                       | 69.510 | 2399 | -      | 0.38  | 9.09  | 99.76 | -    |

<sup>1</sup>Compounds reported previously from the genus; <sup>2</sup>New to the genus

Most of the chemical constituents identified from the leaves are mainly hydrocarbons (27.5%), alcohols (25%), carboxylic acids (15%), aldehyde (12.5 %), esters (12.5%) and ketones (7.5%). The principal components of the essential oils of the leaves of *C. ficifolius*

were found to be 3,7,11,15-tetramethyl-1-hexadecanol (57.08%) (**157**), neophytadiene (12.42%) (**160**), isophytol (8.07%) (**141**), eicosyl vinyl ester carbonic acid (6.18%) (**156**), 2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R- [R\*, R\*-(E)]]- (4.48%) (**162**), bicyclo [4.2.1] nonan-1-ol (3.93%) (**136**) (Table 2).

As displayed in Table 2, the second principal component of the essential oil of the leaves of *C. ficifolius* is neophytadiene (**160**). Various scientific reports revealed that this compound had antipyretic, anti-inflammatory, anti-microbial and antioxidant activity (Carretero M.E. *et al.*, 2008). Therefore, its presence in the leaves of this plant is one positive attributes of *C. ficifolius*.

The stem of *C. ficifolius* showed the presence of 20 components with the major constituents found to be 3,7,11,15-tetramethyl-, [R- [R\*, R\*-(E)]]- 2-hexadecen-1-ol (54.90%) (**162**), eicosane (13.55%) (**105**), hentriacontane (9.09%) (**166**), Benzyl methyl ether (5.64%), (**164**), linoleic acid (4.56%) (**153**), and 2-hexyl-1-decanol (2.95%) (**152**) (Table 2).. The principal components of the stem of *C. ficifolius* are mainly categorized under alcohols and hydrocarbons. Compound **162** is a diterpene reported to have immense biological activity including anti-microbial, antioxidant, anti-tumor, anti-cancer, anti-arthritis, immunostimulatory, anti-diabetic, chemopreventive, pesticidal and diuretic agent and has sunscreen properties (Jayashree, E., *et al.*, 2015). Linoleic acid (**153**) was reported to be used as an emollient and thickening agent in cosmetics, antioxidant and an anti-inflammatory agent in the treatment of burns, cold sores and other minor wounds (Koroma, L., *et al.*, 2018).

The GC-MS analysis of the fruits of *C. ficifolius* revealed the presence of four components with the major constituent found to be the hydrocarbon hentriacontane (99.76%) (**166**). The roots of *C. ficifolius* showed the presence of 20 components with the major compounds found to be eicosane (37.8%) (**105**) abietadiene (14.15%) (**150**), methyl hexadecanoate (13.2%) (**140**), bis(2-methylpropyl) ester-1,2-benzenedicarboxylic acid (9.83%) (**143**), isophytol (6.85%) (**141**), humulene epoxide II (3.28%) (**142**), isobornyl acetate (3.12%) (**136**), and thujopsene, cis-(2.35%) (**132**). The major components in the roots of *C. ficifolius* are mainly hydrocarbons, alcohols and esters. The number of components detected in the leaves of the essential oil of *C. ficifolius* was superior to those detected from the stems, roots and fruits (Table 2). The least number of components was identified from the essential oil of the fruit of *C. ficifolius*. Literature report showed that the seeds of *Cucumis melo* had 40 components with the highest yield identified as methyl linoleate (**150**) (15.27%) (Siddieg,

A., *et al.*, 2010). This compound was also identified from the leaves and stems of *C. ficifolius* but the composition is inferior compared with the one reported from *C. melo*. The ester compound group had the largest content (31.20%) among the other groups based on their functional groups; however, hydrocarbons are the highest constituents in the leaves of *C. ficifolius*, according to our findings.

#### 4.1.2.2 *Cucumis dipsaceus*

The chemical composition of the essential oils of the leaves, stems and fruits of *C. dipsaceus* were analyzed by GC-MS which led to identification of 21 components (124-173) (Table 3). Except linoleic acid (153), the remaining compounds presented in Table 3 are new to the genus *Cucumis*.

**Table 3:** Compounds identified from the essential oils of different parts of *C. dipsaceus*

| Compounds<br>No | Chemical composition                              | RT     | RI   | RI (Lit.)<br>(Babush<br>ok, V.I.,<br><i>et al.</i> ,<br>2011) | Percent peak area |       |        |
|-----------------|---------------------------------------------------|--------|------|---------------------------------------------------------------|-------------------|-------|--------|
|                 |                                                   |        |      |                                                               | Leaves            | Stems | Fruits |
| 167             | Verbenol, cis- <sup>2</sup>                       | 14.73  | 1145 | -                                                             | -                 | -     | 0.1    |
| 124             | Linalool oxide<br>(pyranoid), trans- <sup>2</sup> | 16.301 | 1171 | 1171.0                                                        | 0.19              | -     | -      |
| 126             | Isobornyl acetate <sup>2</sup>                    | 23.63  | 1286 | 1285.9                                                        | 0.11              | 0.1   | 1.66   |
| 128             | Carvacrol <sup>2</sup>                            | 24.44  | 1299 | 1300.4                                                        | 0.009             | 0.02  | 0.1    |
| 129             | Undecanal <sup>2</sup>                            | 24.918 | 1306 | 1306.5                                                        | 0.005             | 0.03  | 0.1    |
| 168             | $\delta$ -Elemene <sup>2</sup>                    | 27.026 | 1337 | 1337.0                                                        | 0.1               | -     | -      |
| 132             | Thujopsene, cis- <sup>2</sup>                     | 33.293 | 1435 | 1432.0                                                        | 0.02              | 0.04  |        |
| 169             | Geranyl butanoate <sup>2</sup>                    | 41.037 | 1560 | 1559.5                                                        | -                 | -     | 0.11   |
| 135             | Humulene epoxide II <sup>2</sup>                  | 43.86  | 1607 | 1604.7                                                        | 0.03              | 0.16  | -      |

|            |                                                                      |        |      |        |       |       |       |
|------------|----------------------------------------------------------------------|--------|------|--------|-------|-------|-------|
| <b>136</b> | Bicyclo [4.2.1] nonan-1-ol <sup>2</sup>                              | 44.27  | 1614 | -      | 0.29  | -     | -     |
| <b>140</b> | Methyl hexadecanoate <sup>2</sup>                                    | 59.744 | 1939 | -      | 0.12  | 0.02  | -     |
| <b>141</b> | Isophytol <sup>2</sup>                                               | 59.934 | 1945 | 1946.5 | 1.15  | -     | -     |
| <b>143</b> | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester <sup>2</sup> | 60.55  | 1967 | -      | 0.36  | -     | -     |
| <b>170</b> | Treicosane <sup>2</sup>                                              | 59.23  | 2029 | -      | 33.40 | -     | -     |
| <b>150</b> | Methyl linoleate <sup>2</sup>                                        | 63.711 | 2090 | 2091.1 | 0.20  | -     | -     |
| <b>153</b> | Linoleic acid <sup>1</sup>                                           | 64.555 | 2129 | 2128.9 | -     | 0.15  | -     |
| <b>171</b> | Intermedeol <sup>2</sup>                                             | 66.959 | 2242 | 2243.0 | 0.17  | -     | 0.02  |
| <b>172</b> | Eugenol acetate <sup>2</sup>                                         | 67.37  | 2268 | 2266.3 | 32.49 | -     | -     |
| <b>173</b> | Octacosane <sup>2</sup>                                              | 67.604 | 2280 | -      | 0.1   | 98.93 | -     |
| <b>105</b> | Eicosane <sup>2</sup>                                                | 69.27  | 2388 | -      | 0.22  | -     | -     |
| <b>166</b> | Hentriacontane <sup>2</sup>                                          | 69.91  | 2399 | -      | 30.61 | 0.25  | 96.65 |

<sup>1</sup>Compounds reported previously from the genus; <sup>2</sup>New to the genus

Most of the chemical constituents identified from the leaves are mainly hydrocarbons including treicosane (33.40%) (**170**), tetracosane (32.49%) (**172**), hentriacontane (30.61%) (**166**) isophytol (1.15%) (**141**), and bis(2-methylpropyl) ester-1,2 benzenedicarboxylic acid (0.36%) (**143**) (Table 3).

The fruits of *C. dipsaceus* showed the presence of seven components with the major constituents found to be the hydrocarbon hentriacontane (96.65%) (**166**) and the ester isobornyl acetate (1.66%) (**126**). The oil from stem of this species showed the presence of nine components with the major constituents found to be the hydrocarbon octacosane (98.93%) (**173**). The constituents of the essential oils of the fruits of *C. dipsaceus* were found to be inferior to the number of components detected from the leaves and stems while the components in the leaves were superior to those identified from the stems and fruits.

#### 4.1.2.3 *C. prophetarum*

The essential oil constituents of *C. prophetarum* were analyzed with GC-MS which has led to the identification of nineteen (19) components (Table 4).

**Table 4:** Compounds identified from the essential oil of various parts of *C. prophetarum*.

| Compounds<br>No | Chemical composition                                                                  | RT    | RI   | RI (Lit.)<br>(Babushok<br>, V.I., <i>et al.</i> ,<br>2011) | Percent peak area |       |       |
|-----------------|---------------------------------------------------------------------------------------|-------|------|------------------------------------------------------------|-------------------|-------|-------|
|                 |                                                                                       |       |      |                                                            | Leaves            | Stems | Roots |
| 118             | Morpholine <sup>2</sup>                                                               | 3.51  | 96   | -                                                          | 0.01              | 0.01  | 0.59  |
| 174             | 1-(4-methylcyclohex-3-enyl) ethanol <sup>2</sup>                                      | 10.49 | 1067 | -                                                          | -                 | 0.13  | -     |
| 175             | 2-Phenylethyl acetate <sup>2</sup>                                                    | 21.81 | 1258 | 1258.8                                                     | -                 | 0.02  | 0.53  |
| 126             | Isobornyl acetate <sup>2</sup>                                                        | 23.59 | 1286 | 1285.9                                                     | -                 | 1.1   | -     |
| 132             | Thujopsene, cis- <sup>2</sup>                                                         | 33.28 | 1435 | 1432.0                                                     | -                 | -     | 1.57  |
| 135             | Humulene epoxide II <sup>2</sup>                                                      | 43.86 | 1607 | 1604.7                                                     | -                 | -     | 9.90  |
| 136             | Bicyclo [4.2.1] nonan-1-ol <sup>2</sup>                                               | 44.27 | 1614 | -                                                          | 0.1               | 0.12  | -     |
| 140             | Methyl hexadecanoate <sup>2</sup>                                                     | 59.73 | 1939 | -                                                          | -                 | -     | 4.93  |
| 141             | Isophytol <sup>2</sup>                                                                | 59.93 | 1945 | 1946.5                                                     | -                 | 0.38  | -     |
| 142             | 3-(Benzyloxycarbonyl)-1-(p-toluenesulfonyl)-5,6-dihydro-2(1H)-2-pyridone <sup>2</sup> | 60.25 | 1956 | -                                                          | -                 | 0.1   | -     |
| 143             | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester <sup>2</sup>                  | 60.55 | 1967 | -                                                          | 0.02              | 0.67  | 2.73  |
| 176             | Hexadecanoic acid, methyl ester <sup>1</sup>                                          | 59.15 | 2025 | -                                                          | -                 | 0.05  | 0.75  |

|            |                                          |       |      |        |       |       |       |
|------------|------------------------------------------|-------|------|--------|-------|-------|-------|
| <b>177</b> | Abietadiene <sup>2</sup>                 | 63.71 | 2090 | 2080.5 | -     | 0.03  | 8.22  |
| <b>166</b> | Hentriacontane <sup>2</sup>              | 65.58 | 2176 | -      | 99.75 | -     | 1.18  |
| <b>154</b> | 1-Dotriacontanol <sup>2</sup>            | 66.04 | 2199 | -      | -     | 0.01  | -     |
| <b>171</b> | Ethyl linoleate <sup>2</sup>             | 66.93 | 2242 | 2158.8 | -     | -     | 17.79 |
| <b>160</b> | Neophytadiene <sup>2</sup>               | 68.63 | 2345 | -      | -     | 96.31 | -     |
| <b>178</b> | (E)-14-methylhexadec-8-enal <sup>2</sup> | 69.15 | 2380 | -      | 0.01  | 0.1   | -     |
| <b>105</b> | Eicosane <sup>2</sup>                    | 69.27 | 2388 | -      | -     | -     | 51.79 |

<sup>1</sup>Compounds reported previously from the genus; <sup>2</sup>New to the genus

The GC-MS analysis of the leaves of *C. prophetarum* revealed the presence of five components with the major constituents found to be the hydrocarbon hentriacontane (99.75%) (**166**). 166, has been reported to have various pharmacological activities including anti-inflammatory, antitumor and antimicrobial activities (Khajuria, V., *et al.*, 2017). The presence of this compound is may be one positive attributes of this plant against various deleterious diseases. The stem of *C. prophetarum* showed the presence of 13 components with the major constituents found to be neophytadiene (96.31%) (**160**). This compound was also identified as a major constituent in the leaves of *C. ficifolius*. The second component detected from the stem of *C. prophetarum* was isobornyl acetate (1.1%) (**126**). Its aroma made it a common ingredient of perfumes used in air fresheners, cleaners, and personal care products. It is reported to have anti-inflammatory, analgesic, antibiotic, and sedative properties (Martino, L.D. *et al.*, 2009). The essential oils of the roots of *C. prophetarum* also showed the presence of 11 components with the major constituents found to be eicosane (51.79%) (**105**), ethyl linoleate (17.79%) (**171**), humulene epoxide II (9.90%) (**135**), abietadiene (8.22%) (**150**), methyl hexadecanoate (4.93%) (**140**) and bis(2-methylpropyl) ester-1,2-benzenedicarboxylic acid (2.73%) (**143**), thujopsene, cis-(1.57%) (**132**) and hentriacontane (1.18%) (**166**) (Figure 17). The structures of some of the major constituents of the three plants are depicted in Figure 17.

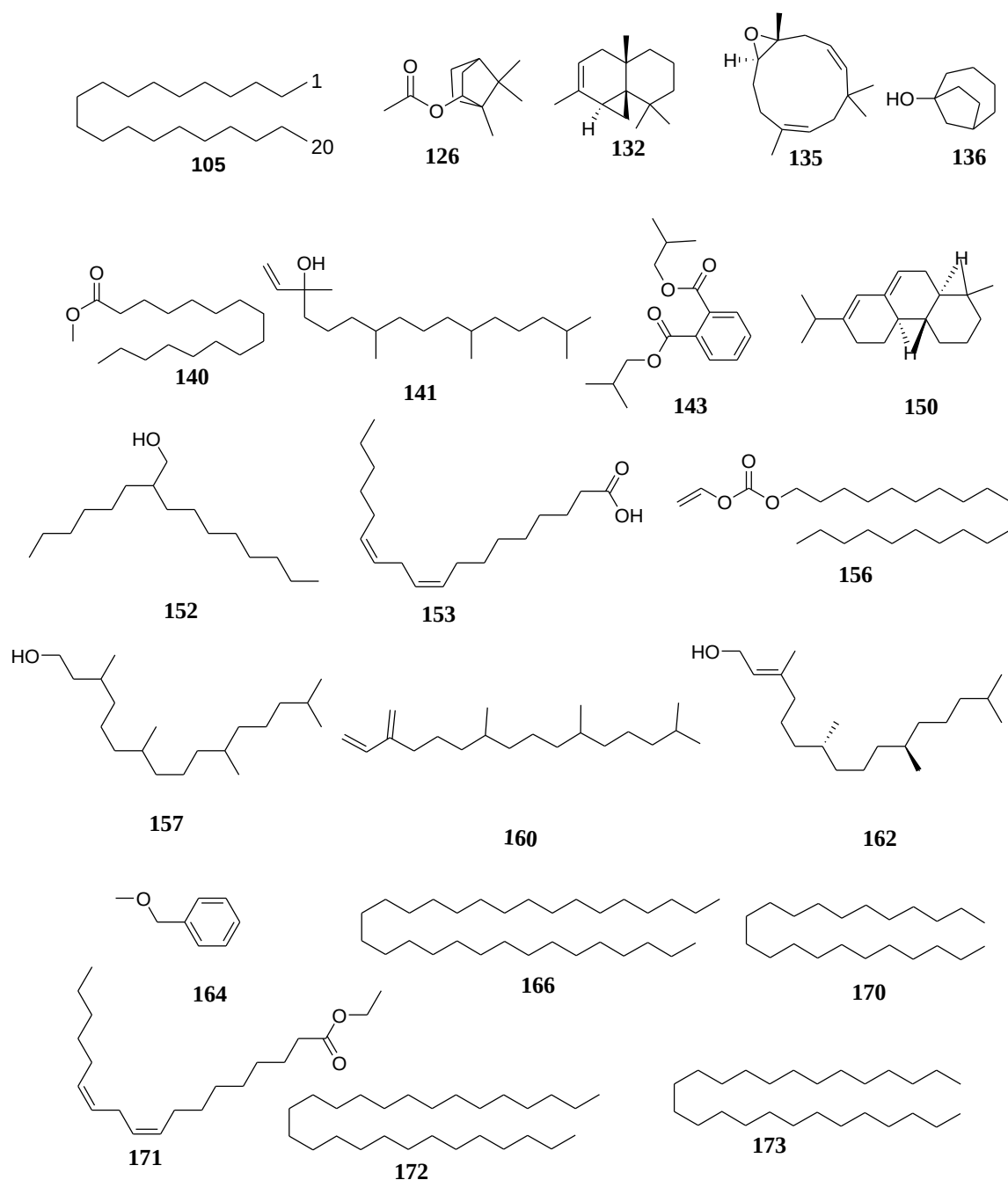


**Figure 17:** The structures of the major constituents of the essential oils of *C. ficifolius*, *C. dipsaceus* and *C. prophetarum*.

This study further found out that the essential oil compositions of the various parts of *C. ficifolius*, *C. dipsaceus* and *C. prophetarum* had some common compounds. Yet, variations in the chemical compositions of the three different species were also observed. This is in agreement with the report given by Lis-Balchin, M., while analyzing the chemical composition of the essential oils from different *Lavandula* species (Lis-Balchin, M., 2002). This study further demonstrated that the leaves, fruits, roots and stems of some species of

the plants of the genus *Cucumis* studied herein displayed different percentage of chemical compositions. The result is in good agreement with the study reported by Bouyahya, A., *et al.*, on leaves and fruits of *Pistacia lentiscus* which revealed differences among intra species (Bouyahya, A., *et al.*, 2019).

## 4.2 Phytochemical Analysis

In the course of this work, twenty-five compounds (Figure 18, 19 and 20) were isolated from the *n*-hexane/petroleum ether and ethyl acetate extracts of the stems, leaves, roots and fruits of *C. ficifolius*, fruits of *C. dipsaceus* and leaves of *C. prophetarum*. The chemical structures of the isolated compounds were determined using physical methods and NMR spectroscopy. In some cases, the NMR spectral data of the isolated compounds were compared with the data reported for the same compounds in the literature.

### Extracts Yield

Each extract was filtered and concentrated under reduced pressure at 40 °C using rotary evaporator using *n*-hexane/ petroleum ether, EtOAc and methanol respectively to obtain 2.9 g (0.58%), 5.9 g (1.17%) and 13.2 g (2.64%) from leaves, 2.8 g (0.93%), 5.3g (1.75%) and 9.3 g (3.10%) from stem, 1.2 g (0.48%), 1.7 g (0.68%) and 4.5 g (1.81%) from root, 7.6 g (1.52%), 12.0 g (2.4%), and 13.6 g (2.72%) from fruit. Likewise, the dried powdered fruits of *C. dipsaceus* (500 g) was successively extracted with each 2.5 L of petroleum ether, EtOAc and methanol on maceration for 72 hr to give 18.6 g (3.72%), 7.8g (1.57%) and 12.8 g (2.57%), respectively. The air dried and powdered leaves of *C. prophetarum* (700 g) was extracted with methanol for 72 hr, filtered and concentrated on rotatory evaporator to afford 28.4 g (4.05%) of petroleum ether, 1.44 g (0.20%) of chloroform and 2.63 g (0.375%) ethyl acetate extracts.

#### 4.2.1 Compounds Isolated from *C. ficifolius*

Eleven compounds were isolated and characterized from various parts of *C. ficifolius*. The detailed structure elucidations presented as follows

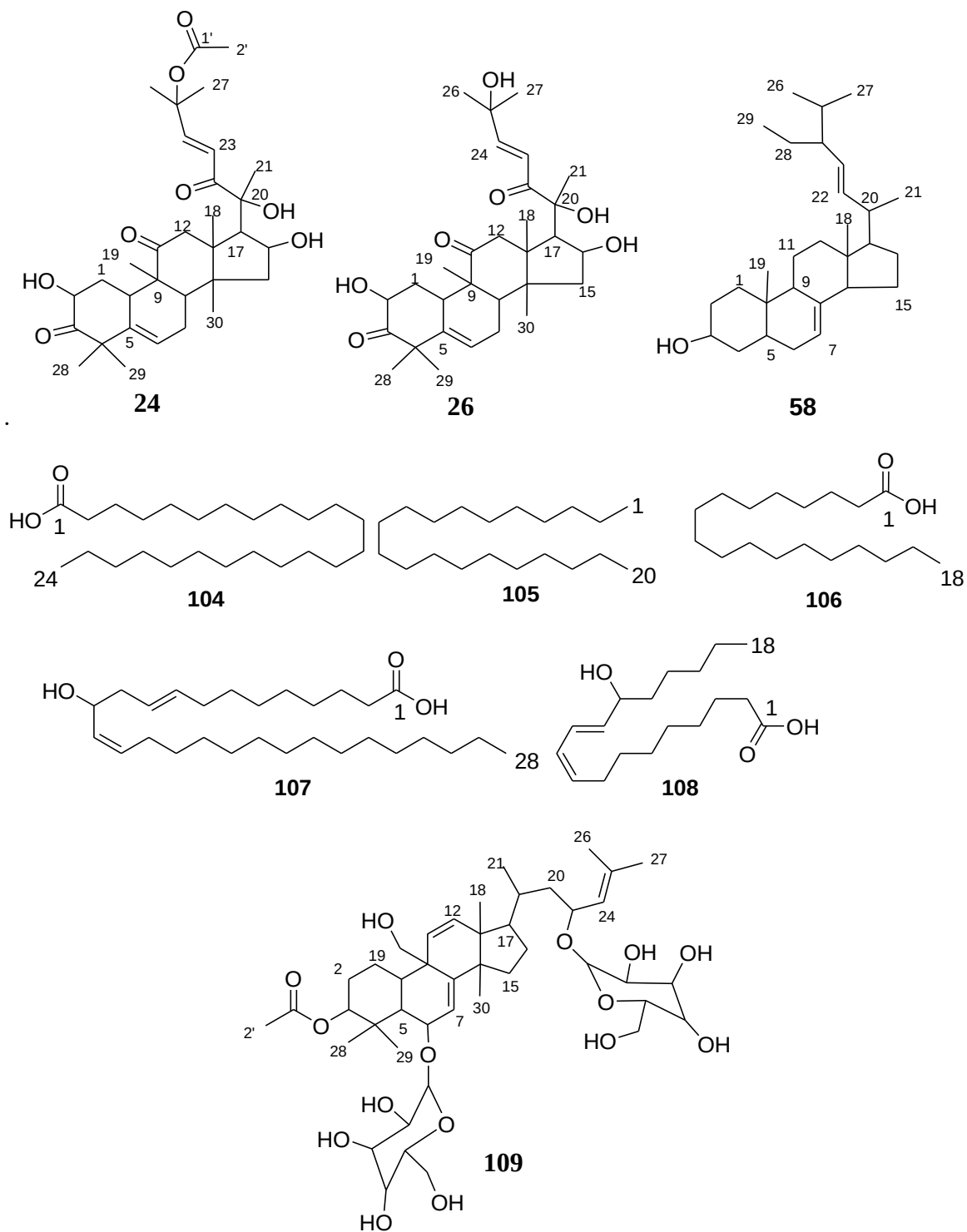


**Figure 18.** The structure of compounds isolated from *C. ficifolius*

Compound **24** (20 mg) was isolated from the roots of *C. ficifolius* as an orange solid. Its TLC showed a spot at  $R_f$  0.64 with *n*-hexane: EtOAc (7:3) as a mobile phase. The  $^1\text{H}$ -NMR spectrum of compound **24** revealed three olefinic protons at  $\delta_H$  7.07 (1H, d,  $J$  = 15.5 Hz), 6.49 (1H, d,  $J$  = 15.7 Hz) and 5.78 (1H, dd,  $J$  = 5.5, 5.5 Hz). The coupling constant for the protons at  $\delta_H$  7.07 (1H, d,  $J$  = 15.5 Hz) establishes the geometry of the olefin as *trans*. Two protons on oxygenated carbons were evident at the  $\delta_H$  4.35 (t,  $J$  = 7.6 Hz) and 4.21 (1H, m). The  $^{13}\text{C}$ -NMR spectrum deduced three carbonyl carbons of ketons at  $\delta_C$  213.0, 212.2 and 202.5 and one ester carbonyl at  $\delta_C$  170.2. Four olefinic carbons at  $\delta_C$  140.4, 120.3, 120.4 and 151.9 and four oxygenated aliphatic carbons at  $\delta_C$  71.6, 71.2, 78.2 and 79.3. The presences of four  $\text{CH}_2$  groups were evident at  $\delta_C$  36.0, 23.9, 48.6 and 45.3. The data generated for compound **24** is in good agreement with cucurbitacin B (Figure 18) (Seif-Eldin, N., *et al.*, 2011).

**Table 5:**  $^1\text{H}$  NMR (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **24** in  $\text{CDCl}_3$  and NMR data reported in the literature for cucurbitacin B.

| Position | NMR data of compound <b>24</b>                    | NMR data of Cucurbitacin B (Seif-Eldin, N., <i>et al.</i> , 2011). |                                 |
|----------|---------------------------------------------------|--------------------------------------------------------------------|---------------------------------|
|          | $\delta^1\text{H}$ -NMR (multiplicity, $J$ in Hz) | $\delta^{13}\text{C}$ / ppm                                        | $\delta^{13}\text{C}$ -NMR /ppm |
| 1        | 1.43 (2H, t, $J$ = 8.1 Hz)                        | 36.0                                                               | 35.8                            |
| 2        | 4.35 (1H, t, $J$ = 7.6 Hz)                        | 71.6                                                               | 71.5                            |
| 3        |                                                   | 213.0                                                              | 213.0                           |
| 4        |                                                   | 50.2                                                               | 50.1                            |
| 5        |                                                   | 140.4                                                              | 140.2                           |
| 6        | 5.78 (1H, dd, $J$ = 5.5, 5.5 Hz,)                 | 120.3                                                              | 120.3                           |
| 7        | 1.97 (1H, d, $J$ = 8.0 Hz)                        | 23.8                                                               | 23.7                            |
|          | 1.61 (1H, d, $J$ = 8.0 Hz)                        |                                                                    |                                 |
| 8        | 2.30(1H, m)                                       | 42.4                                                               | 42.2                            |

|     |                                |       |       |
|-----|--------------------------------|-------|-------|
| 9   |                                | 48.1  | 48.2  |
| 10  | 2.71 (1H, m)                   | 33.8  | 33.5  |
| 11  |                                | 212.2 | 212.2 |
| 12a | 3.25 (1H, d, $J = 14.0$ Hz)    | 48.4  | 48.4  |
| 12b | 2.50(1H, d, $J = 14.0$ Hz)     |       |       |
| 13  |                                | 50.6  | 50.5  |
| 14  |                                | 48.6  | 47.9  |
| 15  | 1.56 (2H, d, $J = 3.8$ Hz)     | 45.3  | 45.1  |
| 16  | 4.21 (1H, m)                   | 71.2  | 71.0  |
| 17  | 2.30 (1H, $m$ )                | 58.2  | 58.0  |
| 18  | 0.97 (3H, $s$ )                | 19.8  | 19.7  |
| 19  | 1.24 (3H, $s$ )                | 18.8  | 18.7  |
| 20  |                                | 78.2  | 79.2  |
| 21  | 1.43 (3H, $s$ )                | 23.9  | 23.6  |
| 22  |                                | 202.5 | 202.4 |
| 23  | 6.51 (1H, d, $J = 15.7$ Hz)    | 120.4 | 120.2 |
| 24  | 7.07 (1H, $d$ , $J = 15.5$ Hz) | 151.9 | 151.8 |
| 25  |                                | 79.3  | 78.1  |
| 26  | 1.33 (3H, $s$ )                | 25.9  | 25.8  |
| 27  | 1.34 (3H, $s$ )                | 26.4  | 26.2  |
| 28  | 1.24 (3H, $s$ )                | 29.6  | 29.2  |
| 29  | 1.24 (3H, $s$ )                | 21.2  | 21.1  |
| 30  | 1.06 (3H, $s$ )                | 20.0  | 19.8  |
| 1'  |                                | 170.2 | 170.2 |

2'                      1.27 (3H, s)                      21.9                      21.8

Cucurbitacin B (**24**) has shown to possess strong anticancer activity in a variety of cancers cell lines *in vitro* and *in vivo* including breast cancer, lung cancer, skin cancer, liver cancer, brain cancer, blood cancer, prostate cancer, gastric cancer, bone cancer and also other cell lines (Sukant, G., *et al.*, 2018). Potential of **24** against cardiac, liver, lung, neuronal and skin injuries, systemic infections, inflammation, sex-related behavior and adipocyte differentiation has also been explored. Cucurbitacins exhibit a broad range of pharmacological properties such as antioxidant, antiviral, antipyretic, analgesic and anti-malarial (Chen, J.C., *et al.*, 2005; Jayaprakasam, B., *et al.*, 2003; Miro, M., 1995).

Compound **26** (22 mg) was obtained from the roots of *C. ficifolius* as a colorless crystalline solid with a melting point of 151–152 °C. Its TLC showed spot at  $R_f$  0.76 (6:4) *n*-hexane:EtOAc. The  $^1\text{H}$  NMR spectrum of compound **26** revealed three olefinic protons at  $\delta_{\text{H}}$  6.68 (1H, *d*,  $J = 15.2$ ), 7.14 (1H, *d*,  $J = 15.2$ ) and 5.80 (1H, *t*,  $J = 4.8$ ), two carbinol protons at the  $\delta_{\text{H}}$  4.43 (1H, *m*) and 4.16 (1H, *m*). The  $^{13}\text{C}$ -NMR spectrum of compound **26** revealed three carbonyls' carbons of ketones  $\delta_{\text{C}}$  213.0, 212.2, and 202.8. Four olefinic signals at  $\delta_{\text{C}}$  140.4, 120.3, 119.0 and 155.7. The spectrum also demonstrated two carbinol carbons at  $\delta_{\text{C}}$  71.6 and 71.3. The  $^{13}\text{C}$  NMR data with the help of DEPT-135 showed the presence of ten quaternary carbons at the  $\delta_{\text{C}}$  213.0, 50.2, 140.4, 48.4, 212.2, 50.7, 48.2, 78.3, 202.5 and 79.2. In contrast to compound **24**, the signal due ester carbonyl is missing from compound **26**. Hence, compound **26** is identified as cucurbitacin D (Figure 18).

**Table 6:**  $^1\text{H}$  NMR (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **26** in  $\text{CDCl}_3$  and NMR data reported in the literature for cucurbitacin D (Abdulrhman, A., *et al.*, 2018).

| NMR data of compound <b>26</b> |                                                      |                             | NMR data of<br>cucurbitacin D    |
|--------------------------------|------------------------------------------------------|-----------------------------|----------------------------------|
| Position                       | $\delta^1\text{H}$ -NMR (multiplicity,<br>$J$ in Hz) | $\delta^{13}\text{C}$ / ppm | $\delta^{13}\text{C}$ -NMR data. |
| 1                              | 1.34 (2H, <i>m</i> )                                 | 36.0                        | 35.9                             |
| 2                              | 4.43 (1H, <i>m</i> )                                 | 71.6                        | 71.6                             |
| 3                              |                                                      | 213.0                       | 213.1                            |

|     |                                         |       |       |
|-----|-----------------------------------------|-------|-------|
| 4   |                                         | 50.2  | 50.2  |
| 5   |                                         | 140.4 | 140.3 |
| 6   | 5.80 (1H, <i>dd</i> , <i>J</i> = 4.8)   | 120.3 | 120.2 |
| 7a  | 2.58 (1H, <i>d</i> , <i>J</i> = 6.9 Hz) | 23.8  | 23.8  |
| 7b  | 2.61 (1H, <i>d</i> , <i>J</i> = 6.8 Hz) | 23.8  | 23.8  |
| 8   | 2.11 (1H, <i>s</i> )                    | 42.3  | 42.3  |
| 9   |                                         | 48.4  | 48.3  |
| 10  | 2.97 (1H, <i>m</i> )                    | 33.7  | 33.7  |
| 11  |                                         | 212.3 | 212.3 |
| 12a | 2.68 (1H, <i>d</i> , <i>J</i> = 5.2)    | 48.6  | 48.6  |
| 12b | 2.72 (1H, <i>d</i> , <i>J</i> = 5.1)    | 48.6  | 48.6  |
| 13  |                                         | 50.7  | 50.7  |
| 14  |                                         | 48.2  | 48.2  |
| 15a | 1.57 (1H, <i>d</i> , <i>J</i> = 8.1)    | 45.4  | 45.4  |
| 15b | 1.98 (1H, <i>d</i> , <i>J</i> = 8.3)    | 45.4  | 45.4  |
| 16  | 4.16 (1H, <i>m</i> )                    | 71.3  | 71.3  |
| 17  | 2.18 (1H, <i>s</i> )                    | 57.4  | 57.3  |
| 18  | 0.99 (3H, <i>s</i> )                    | 19.9  | 19.9  |
| 19  | 1.26 (3H, <i>s</i> )                    | 18.9  | 19.2  |
| 20  |                                         | 78.2  | 78.1  |
| 21  | 1.41 (3H, <i>s</i> )                    | 22.3  | 21.2  |
| 22  | .                                       | 202.8 | 202.6 |
| 23  | 6.68 (1H, <i>d</i> , <i>J</i> = 15.2)   | 120.3 | 118.9 |
| 24  | 7.14 (1H, <i>d</i> , <i>J</i> = 15.2)   | 155.7 | 155.7 |

|    |                      |      |      |
|----|----------------------|------|------|
| 25 |                      | 70.2 | 71.1 |
| 26 | 1.37 (3H, <i>s</i> ) | 28.7 | 28.9 |
| 27 | 1.26 (3H, <i>s</i> ) | 29.7 | 29.3 |
| 28 | 1.37 (3H, <i>s</i> ) | 24.7 | 29.5 |
| 29 | 1.37 (3H, <i>s</i> ) | 24.5 | 23.9 |
| 30 | 1.08 (3H, <i>s</i> ) | 20.0 | 20.1 |

Cucurbitacins D have biological activities, such as anti-inflammatory, hepatoprotective, antimicrobial, antioxidant, anthelmintic, antiviral, antihyperglycemic, and cardioprotective properties. Cucurbitacin D also possesses potent anticancer effects towards the various cancer cells (Ding, N., *et al.*, 2016).

Compound **58** was isolated as a white solid from the leaves, stems, fruits and roots of *C. ficifolius*. It melted at 171-172 °C [Lit. 169-171 °C] (Consolacion, Y.R and Kathleen, L., 2005). TLC showed a spot at  $R_f$  0.46 with *n*-hexane:EtOAc (7:3) as a mobile phase. The  $^1\text{H}$  NMR spectrum of compound **58** indicated three olefinic proton signals at  $\delta_H$  5.07 (1H, dd,  $J = 15.3, 8.8$  Hz), 5.19 (1H, d,  $J = 8.5$  Hz) and 5.17 (1H, d,  $J = 8.5$  Hz). The diagnostic signal due to oxymethine is evident at  $\delta_H$  3.62 (1H, m). Methyl proton signals were observed at  $\delta_H$  0.57 (3H, *t*), 0.81 (3H, bro *s*), 0.82 (3H, bro *s*), 0.83 (3H, bro *s*), 0.85 (3H, d,  $J = 1.8$  Hz) and 0.87 (3H, d,  $J = 1.5$  Hz). The spectrum also showed a proton signal resonated at  $\delta_H$  0.95 (2H, d,  $J = 6.6$  Hz), 1.05 (2H, d,  $J = 6.6$  Hz), 1.27 (6H, m), 1.31 (2H, m), 1.39 (2H, m), 1.42 (1H, m), 1.58 (4H, m), 1.79 (2H, m), 1.83 (2H, m), 2.01 (1H, m) and 2.05 (1H, m), which accounted for methylenes and methine protons. The  $^{13}\text{C}$ -NMR spectral data of compound **58** with the aid of DEPT-135 revealed the presence of well resolved resonances of twenty-nine carbons, of which four olefinic carbons signals were observed at  $\delta_C$  117.4, 129.4, 138.1 and 139.5. The signal due to carbon bearing oxygen appeared at  $\delta_C$  71.0. The  $^{13}\text{C}$ -NMR spectrum displayed signals due to methyls at  $\delta_C$  12.0, 12.2, 13.0, 19.0, 21.0 and 21.3. The remaining carbon signals appeared at  $\delta_C$  37.1, 31.4, 31.8, 37.9, 40.3, 29.7, 49.4, 34.2, 21.5, 39.5, 43.3, 55.9, 23.0, 29.6, 55.1, 40.8, 51.3, and 25.4. Based on the above data and comparison with literature value, compound **58** is in good agreement with  $\alpha$ -spinasterol (Figure 18) (Seif-Eldin, N., *et al.*, 2011).

**Table 7:**  $^1\text{H}$  NMR (400MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **58** in  $\text{CDCl}_3$  and NMR data reported for  $\alpha$ -spinasterol.

| Position | NMR data of compound <b>58</b>                    |                                  | NMR data reported for $\alpha$ -spinasterol (Seif-Eldin, N., <i>et al.</i> , 2011). |
|----------|---------------------------------------------------|----------------------------------|-------------------------------------------------------------------------------------|
|          | $\delta^1\text{H}$ -NMR (multiplicity, $J$ in Hz) | $\delta^{13}\text{C}$ -NMR / ppm | $\delta^{13}\text{C}$ /ppm                                                          |
| 1        | 1.39(2H, m)                                       | 37.1                             | 37.2                                                                                |
| 2        | 1.58 (1H, m)                                      | 31.4                             | 31.5                                                                                |
|          | 1.27 (1H, m)                                      |                                  |                                                                                     |
| 3        | 3.62 (1H, <i>m</i> )                              | 71.0                             | 71.1                                                                                |
| 4        | 1.58 (1H, m)                                      | 37.9                             | 38.0                                                                                |
|          | 1.27 (1H, m)                                      |                                  |                                                                                     |
| 5        | 1.27 (1H, <i>m</i> )                              | 40.2                             | 40.3                                                                                |
| 6        | 1.83(1H, m)                                       | 29.7                             | 29.7                                                                                |
|          | 1.58 (1H, <i>m</i> )                              |                                  |                                                                                     |
| 7        | 5.07 (1H, dd, $J = 15.3, 8.8$ Hz)                 | 117.4                            | 117.5                                                                               |
| 8        |                                                   | 139.5                            | 139.5                                                                               |
| 9        | 1.79(1H, m)                                       | 49.4                             | 49.5                                                                                |
| 10       |                                                   | 34.2                             | 34.2                                                                                |
| 11       | 1.05 (2H, d, $J = 6.6$ Hz)                        | 21.3                             | 21.6                                                                                |
| 12       | 0.95 (2H, d, $J = 6.6$ Hz)                        | 39.4                             | 39.6                                                                                |
| 13       |                                                   | 43.2                             | 43.3                                                                                |
| 14       | 2.01 (1H, m)                                      | 55.9                             | 55.1                                                                                |

|    |                                 |       |       |
|----|---------------------------------|-------|-------|
| 15 | 1.31 (2H, bro s)                | 23.0  | 23.0  |
| 16 | 1.58 (1H, m)                    | 29.6  | 28.5  |
|    | 1.27 (1H, bro s)                |       |       |
| 17 | 1.42 (1H, <i>m</i> )            | 55.1  | 55.1  |
| 18 | 0.81 (3H, bro s)                | 12.0  | 12.0  |
| 19 | 0.82 (3H, bro s)                | 13.0  | 13.0  |
| 20 | 2.04 (1H, <i>m</i> )            | 40.8  | 40.8  |
| 21 | 0.83 (3H, bro s)                | 21.3  | 21.4  |
| 22 | 5.19 (1H, d, <i>J</i> = 8.5 Hz) | 138.1 | 138.1 |
| 23 | 5.17 (1H, d, <i>J</i> = 8.5 Hz) | 129.4 | 129.4 |
| 24 | 1.83 (1H, <i>m</i> )            | 51.2  | 51.2  |
| 25 | 1.79 (1H, <i>m</i> )            | 31.8  | 31.9  |
| 26 | 0.85 (3H, d, <i>J</i> = 1.8 Hz) | 19.0  | 19.0  |
| 27 | 0.87 (3H, d, <i>J</i> = 1.5 Hz) | 21.0  | 21.1  |
| 28 | 1.27 (2H, bro s)                | 25.3  | 25.4  |
| 29 | 0.57 (3H, t)                    | 12.2  | 12.2  |

$\alpha$ -Spinasterol is a stigmastane-type phytosterol found in a variety of plant sources. It was first isolated from spinach leaves in 1932 (Hart & Heyl, 1932), from which it gets its name.  $\alpha$ -Spinasterol is a bioavailable sterol, which can cross the blood–brain barrier (Trevisan, G., *et al.*, 2012). The biological properties of  $\alpha$ -spinasterol include anti-diabetes, antiinflammatory, hypolipidemic, neuroprotective, antiulcer, antinociceptive, antitumour and antimicrobial activities (Muhammed, M., *et al.*, 2022).

Compound **104** (16 mg) was isolated as white solid from the extract of the stems of *C. ficifolius*. It melted at 84–85 °C. TLC displayed a spot at *R<sub>f</sub>* 0.80 (*n*-hexane:EtOAc, 9:1) as mobile phase. The <sup>1</sup>H NMR spectrum showed the presence of one terminal methyl protons at  $\delta_{\text{H}}$  0.95 (3H, *t*, *J* = 6.1 Hz). Methylene proton signals were evident at  $\delta_{\text{H}}$  2.37 (2H,

t,  $J = 7.5$  Hz) and 1.64 (2H, q,  $J = 7.4$  Hz). The earlier one is due to methylene attached to carbonyl carbon. The proton signals centered at  $\delta_H$  1.27 integrating for 40 protons were diagnostics for the presence of many overlapping methylene groups. The  $^{13}\text{C}$  NMR spectrum with the help of DEPT-135 revealed that the presence of one carbonyl ( $\delta_C$  179.5), one methyl and the remaining are methylene carbons. Hence, compound **104** is found to be lignoceric acid (Figure 18). This compound was reported for the first time from the genus *Cucumis*.

**Table 8:**  $^1\text{H}$  NMR (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **104** in  $\text{CDCl}_3$  and literature reported for lignoceric acid.

| Position     | NMR data of compound <b>104</b>                      |                                     |                                          | $^{13}\text{C}$ -NMR data reported<br>for lignoceric acid        |
|--------------|------------------------------------------------------|-------------------------------------|------------------------------------------|------------------------------------------------------------------|
|              | $\delta^1\text{H}$ -NMR<br>(multiplicity, $J$ in Hz) | $\delta^{13}\text{C}$ -NMR /<br>ppm | $\delta^{13}\text{C}$ DEPT-<br>135 / ppm | $\delta^{13}\text{C}$ -NMR (Yong-Hui Z.<br><i>et al.</i> , 2004) |
| <b>1</b>     |                                                      | 179.6                               |                                          | 176.0                                                            |
| <b>2</b>     | 2.37 (2H, $t$ , $J = 7.5$ Hz)                        | 34.1                                | 34.1 ( $\text{CH}_2$ )                   | 34.9                                                             |
| <b>3</b>     | 1.64 (2H, $q$ , $J = 7.4$ Hz)                        | 24.8                                | 24.8( $\text{CH}_2$ )                    | 25.7                                                             |
| <b>4</b>     | 1.27 (2H, $m$ )                                      | 29.2                                | 29.2( $\text{CH}_2$ )                    | 29.4                                                             |
| <b>5 –21</b> | 1.27 (34H, $m$ )                                     | 30.5                                | 30.5( $\text{CH}_2$ )                    | 30.0                                                             |
| <b>22</b>    | 1.27 (2H, $m$ )                                      | 32.0                                | 32.0( $\text{CH}_2$ )                    | 32.1                                                             |
| <b>23</b>    | 1.27 (2H, $m$ )                                      | 22.8                                | 22.8( $\text{CH}_2$ )                    | 23.0                                                             |
| <b>24</b>    | 0.95 (3H, $t$ , $J = 6.1$ Hz)                        | 14.3                                | 14.3( $\text{CH}_3$ )                    | 14.3                                                             |

Lignoceric acid was reported to have various pharmacological activities including anti-inflammatory, antitumor and antimicrobial activities (Yong-Hui, Z., *et al.*, 2004). That the compound isolated from stem of *C. ficifolius* and show good results in antioxidant and antibacterial activities.

The fruit extract of *C. ficifolius* has led to the isolation of five (5) compounds.

Compound **105** (50 mg) was isolated from the fruit extract of *C. ficifolius* as a white solid with melting point at  $37 - 39^\circ\text{C}$ . TLC showed a spot at  $R_f$  0.95 with  $n$ -hexane:EtOAc (9:1) as a mobile phase. The  $^1\text{H}$ -NMR spectrum showed the presence of terminal methyl protons

at  $\delta_H$  0.88 (6H, t). The proton signals centered at  $\delta_H$  1.25 integrating for 36 protons were diagnostics for the presence of 18 overlapping methylene groups. The  $^{13}\text{C}$ -NMR spectrum of compound **105** with the help of DEPT-135 revealed the presence of five well resolved carbon resonances, of these the signal at  $\delta_C$  14.1 is due to terminal methyl group. The other signals observed at  $\delta_C$  22.7, 29.3 and 31.9 are due to (C2, C19), (C4, C17) and (C3, C18), respectively. The signal due to overlapping methylenes was evident at  $\delta_C$  29.7. The spectral data generated for compound **105** is found to be eicosane (Figure 18).

**Table 9:**  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **105** in  $\text{CDCl}_3$  and NMR data reported in the literature for eicosane.

| position | NMR data of compound <b>105</b>      |                           |                                      | NMR data of eicosane (DBSWeb, 2014). |
|----------|--------------------------------------|---------------------------|--------------------------------------|--------------------------------------|
|          | $\delta^1\text{H}$ -NMR ( $J$ in Hz) | $^{13}\text{C}$ NMR / ppm | $\delta^{13}\text{C}$ DEPT-135 / ppm | $\delta^{13}\text{C}$ -NMR           |
| 1 / 20   | 0.88 (6H, t, $J = 6.9$ Hz)           | 14.2                      | 14.2( $\text{CH}_3$ )                | 14.1                                 |
| 2 / 19   | 1.25 (4H, bro s)                     | 22.8                      | 22.8( $\text{CH}_2$ )                | 22.8                                 |
| 3 / 18   | 1.25 (4H, bro s)                     | 32.1                      | 32.1( $\text{CH}_2$ )                | 32.0                                 |
| 4 / 17   | 1.25 (4H, bro s)                     | 29.5                      | 29.5( $\text{CH}_2$ )                | 29.5                                 |
| 5 - 16   | 1.25 (24H, bro s)                    | 29.8                      | 29.8( $\text{CH}_2$ )                | 29.8                                 |

Research shows that eicosane has strong anti-inflammatory, analgesic, and antipyretic effects (Patrick, N.O., 2020). The antibacterial and antifungal activity of eicosane was also documented in the literature (Boussaada, O., *et al.*, 2008).

Compound **106** (80 mg) was isolated as a white solid from the extract of the fruits of *C. ficifolius* with a melting point of 69-71  $^\circ\text{C}$  [lit. 70-72] (Ibrahim, A. and Yang, Cai-Xiab, 2019). TLC showed a spot at  $R_f$  0.60 with *n*-hexane:EtOAc (4:1) as mobile phase. The  $^1\text{H}$  NMR spectrum of compound **106** displayed proton signal for methyl group at  $\delta_H$  0.88 (3H, t,  $J = 7.0$  Hz). The spectrum also showed proton signals at  $\delta_H$  1.25 (24H, m), 1.33 (2H, m), 1.63 (2H, m) and 2.34 (2H, t,  $J = 7.6$  Hz) which are accounted for the presence of methylene groups. The  $^{13}\text{C}$ -NMR spectrum of compound **106** with the help of DEPT-135 revealed the presence of well resolved twelve carbon resonances of which the diagnostic signal due

carboxylic carbon (C-1) appeared at  $\delta_C$  180.3. The spectrum also displayed a signal due to methyl group at  $\delta_C$  14.2. Furthermore, signals due methylenes were evident at  $\delta_C$  22.8, 24.8, 29.2, 29.3, 29.5, 29.5, 29.7, 29.7, 29.7, 29.7, 29.8, 29.8, 29.8, 32.0 and 34.2, Based on the above spectral data, compound **106** is in good agreement with stearic acid (Figure 18) (Ibrahim, A. and Yang, Cai-Xiab, 2019).

**Table 10:**  $^1\text{H}$  NMR (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **106** in  $\text{CDCl}_3$  and NMR data reported for stearic acid.

| Position | NMR data of Compound <b>106</b>     |                                    |                                 | Reported NMR data for stearic acid   |
|----------|-------------------------------------|------------------------------------|---------------------------------|--------------------------------------|
|          | $\delta^1\text{H}$ NMR ( $J$ in Hz) | $\delta^{13}\text{C}$ -NMR/<br>ppm | DEPT-135<br>ppm                 | $\delta^{13}\text{C}$ /ppm from ref. |
| 1        |                                     | 180.4                              |                                 | 178.7                                |
| 2        | 2.34 (2H, $t$ , $J = 7.6$ Hz)       | 34.2                               | 34.2 ( $\text{CH}_2$ )          | 33.8                                 |
| 3        | 1.63 (2H, $m$ )                     | 24.8                               | 24.8( $\text{CH}_2$ )           | 24.5                                 |
| 4 – 15   | 1.25 (24H, bro s)                   | 29.2 –29.8                         | 29.2 –29.8<br>( $\text{CH}_2$ ) | 29.7–30.0                            |
| 16       | 1.25 (2H, bro s)                    | 32.0                               | 32.0( $\text{CH}_2$ )           | 31.9                                 |
| 17       | 1.33 (2H, $m$ )                     | 22.8                               | 22.8( $\text{CH}_2$ )           | 22.7                                 |
| 18       | 0.88 (3H, $t$ , $J = 7.0$ Hz)       | 14.2                               | 14.2( $\text{CH}_3$ )           | 14.1                                 |

**106** can protect brain slices (cortical and hippocampal) against injury induced by OGD or glutamate (Ze-jian, W., *et al.*, 2006). The **106** is widely applied in cosmetics technology and bio-medicals, as therapeutic adjuvant and drug delivery agents (Kelm, G.R., *et al.*, 2017). In addition, polyunsaturated fatty acids also have been implicated in the prevention of various human diseases, including obesity, diabetes, coronary artery disease and stroke, and inflammatory and neurological diseases (Hwang, D.H., 1989).

Compound **107** (35 mg) was isolated as a white crystal from the extract of the fruit of *C. ficifolius*. TLC revealed a spot at  $R_f$  0.54 using  $n$ -hexane:EtOAc (7:3) as mobile phase. The  $^1\text{H}$  NMR spectrum of compound **107** demonstrated the presence of four olefinic proton

signals at  $\delta_H$  5.37 (2H, m,) and 5.33 (2H, m). The oxymethine and methyl protons were observed at  $\delta_H$  4.13 (1H, m) and  $\delta_H$  0.88 (3H, t), respectively. The spectrum also showed methylene proton signals at  $\delta_H$  1.25 (20 H, m), 1.63 (4H, m), 2.05 (m, 2H) and 2.33 (2H, t,  $J=6.6$  Hz)). The  $^{13}\text{C}$ -NMR spectrum of compound **107** with the help of DEPT-135 revealed the presence of well resolved twenty carbon resonances of which four are due to olefinic carbons ( $\delta_C$  130.3, 130.0, 128.1 and 128.0). The diagnostic signal at  $\delta_C$  178.9 is due to carboxyl carbon. Signals due to oxymethine and methyl carbons were observed at  $\delta_C$  77.2 and 14.1, respectively. The carbon signals due to methylenes were evident at  $\delta_C$  32.0, 25.7, 29.1, 29.4, 29.8, 22.8, 43.8, 33.8, 31.5, 33.3 and 22.7. Hence, compound **107** is identified as 12-hydroxyoctacos-9,13-dienoic acid (Figure 18).

**Table 11:**  $^1\text{H}$  NMR (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **107** in  $\text{CDCl}_3$ .  
position

| NMR data of compound <b>107</b> |                                                                   |                                 |                                  |
|---------------------------------|-------------------------------------------------------------------|---------------------------------|----------------------------------|
|                                 | $\delta^1\text{H}$ NMR (no of H,<br><i>multiplicity, J</i> in Hz) | $\delta^{13}\text{C}$ NMR / ppm | DEPT-135 ( $\delta_C$ in<br>ppm) |
| 1                               |                                                                   | 178.9                           |                                  |
| 2                               | 2.33 (2H, t, $J=6.6$ Hz)                                          | 39.5                            | 39.5( $\text{CH}_2$ )            |
| 3                               | 1.61 (2H, <i>m</i> )                                              | 24.7                            | 24.7( $\text{CH}_2$ )            |
| 4                               | 1.30 (2H, <i>m</i> )                                              | 28.8                            | 28.8( $\text{CH}_2$ )            |
| 5                               | 1.30 (2H, <i>m</i> )                                              | 29.4                            | 29.4( $\text{CH}_2$ )            |
| 6                               | 1.30 (2H, <i>m</i> )                                              | 29.8                            | 29.8( $\text{CH}_2$ )            |
| 7                               | 1.25 (2H, <i>m</i> )                                              | 31.6                            | 31.6( $\text{CH}_2$ )            |
| 8                               | 2.05 (2H, <i>m</i> )                                              | 27.3                            | 27.3( $\text{CH}_2$ )            |
| 9                               | 5.33 (1H, <i>m</i> )                                              | 130.3                           | 130.3( $\text{CH}$ )             |
| 10                              | 5.37 (1H, <i>m</i> )                                              | 128.0                           | 128.0( $\text{CH}$ )             |
| 11                              | 2.33 (2H, <i>m</i> )                                              | 43.9                            | 43.9( $\text{CH}_2$ )            |
| 12                              | 4.13 (1H, <i>m</i> )                                              | 77.2                            | 77.2( $\text{CH}$ )              |

|       |                       |           |                             |
|-------|-----------------------|-----------|-----------------------------|
| 13    | 5.37 (1H, <i>m</i> )  | 128.1     | 128.1(CH)                   |
| 14    | 5.33 (1H, <i>m</i> )  | 130.1     | 130.1(CH)                   |
| 15    | 2.05 (2H, <i>m</i> )  | 34.0      | 34.0(CH <sub>2</sub> )      |
| 16    | 1.25 (2H, <i>m</i> )  | 31.6      | 31.6(CH <sub>2</sub> )      |
| 17–25 | 1.30 (18H, <i>m</i> ) | 29.1–29.8 | 29.1–29.8(CH <sub>2</sub> ) |
| 26    | 1.30 (2H, <i>m</i> )  | 32.0      | 32.0(CH <sub>2</sub> )      |
| 27    | 1.25 (2H, <i>m</i> )  | 22.70     | 22.70(CH <sub>2</sub> )     |
| 28    | 0.88 (3H, <i>t</i> )  | 14.1      | 14.1(CH <sub>3</sub> )      |

Compound **108** (30 mg) was isolated as a white crystal from the fruit extract of *C. ficifolius*. TLC showed a spot at  $R_f$  0.60 using *n*-hexane:EtOAc (3:2) as a mobile phase. The <sup>1</sup>H-NMR spectrum of compound **108** indicated four olefinic proton signals at  $\delta_H$  5.42 (1H, *m*), 5.63 (2H, *m*) and 5.98 (1H, *m*). Signal due to oxymethine and methyl protons were evident at  $\delta_H$  4.08 (1H, *m*) and 0.91 (3H, *t*,  $J = 8.0$  Hz), respectively. Signals due to methylenes were presented at  $\delta_H$  1.34 (12H, *m*), 1.60 (4H, *m*), 2.06 (2H) and 2.28 (2H, *t*). The <sup>13</sup>C-NMR spectrum data of compound **108**, with the help of DEPT-135, revealed the presence of well resolved eighteen carbon resonances of which four olefinic carbons signals were observed at  $\delta_C$  137.4, 126.3, 129.3 and 132.9. The signal due to carboxylic carbon appeared at  $\delta_C$  177.7. The signal due to carbon bearing oxygen appeared at  $\delta_C$  73.3. The carbon signal displayed at  $\delta_C$  14.4 was due to methyl carbon. The remaining carbon signals appeared at  $\delta_C$  34.9, 26.0, 30.1, 30.2, 30.6, 26.2, 28.5, 38.4, 30.0, 32.9 and 23.7. Thus, based on the above spectral data the compound **108** is suggested as 13-hydroxyoctadeca-9,11-dienoic acid (Figure 18) (Mariana, D.A.G., *et al.*, 2022).

**Table 12:**  $^1\text{H}$  NMR (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **108** in  $\text{CD}_3\text{OD}$  and NMR data reported for 13-hydroxyoctadeca-9,11-dienoic acid (Mariana, D.A.G., *et al.*, 2022).

| Position | NMR data of compound 108            |                             |                                                 | NMR data reported for<br>13-hydroxyoctadeca-9,11-<br>dienoic acid |
|----------|-------------------------------------|-----------------------------|-------------------------------------------------|-------------------------------------------------------------------|
|          | $\delta^1\text{H}$ NMR ( $J$ in Hz) | $\delta^{13}\text{C}$ / ppm | $\delta^{13}\text{C}$ from<br>DEPT-135 /<br>ppm | $\delta^{13}\text{C}$ /ppm                                        |
| 1        |                                     | 177.7                       |                                                 | 179.5                                                             |
| 2        | 2.28 (2H, m)                        | 34.9                        | 34.9(CH <sub>2</sub> )                          | 34.1                                                              |
| 3        | 1.63 (2H, <i>m</i> )                | 26.0                        | 26.0(CH <sub>2</sub> )                          | 24.8                                                              |
| 4        | 1.34 (2H, <i>m</i> )                | 30.1                        | 30.1 (CH <sub>2</sub> )                         | 29.0                                                              |
| 5        | 1.34 (2H, <i>m</i> )                | 30.2                        | 30.2 (CH <sub>2</sub> )                         | 29.0                                                              |
| 6        | 1.34 (2H, <i>m</i> )                | 30.6                        | 30.6 (CH <sub>2</sub> )                         | 29.5                                                              |
| 7        | 1.34 (2H, <i>m</i> )                | 26.2                        | 26.2 (CH <sub>2</sub> )                         | 25.2                                                              |
| 8        | 2.06 (2H, m)                        | 28.5                        | 28.5 (CH <sub>2</sub> )                         | 27.7                                                              |
| 9        | 5.63 (1H, <i>m</i> )                | 132.9                       | 132.9 (CH)                                      | 132.9                                                             |
| 10       | 5.42(1H, <i>m</i> )                 | 129.3                       | 129.3 (CH)                                      | 128.0                                                             |
| 11       | 5.63 (1H, <i>m</i> )                | 126.3                       | 126.3 (CH)                                      | 126.0                                                             |
| 12       | 5.98 (1H, <i>m</i> )                | 137.4                       | 137.4 (CH)                                      | 135.8                                                             |
| 13       | 4.08 (1H, <i>m</i> )                | 73.3                        | 73.3(CH)                                        | 73.1                                                              |
| 14       | 2.28 (2H, m)                        | 38.4                        | 38.4 (CH <sub>2</sub> )                         | 37.4                                                              |
| 15       | 2.06 (2H, <i>t</i> )                | 30.0                        | 30.0 (CH <sub>2</sub> )                         | 29.0                                                              |
| 16       | 1.34 (2H, <i>m</i> )                | 32.9                        | 32.9 (CH <sub>2</sub> )                         | 31.9                                                              |

|    |                                      |      |                         |      |
|----|--------------------------------------|------|-------------------------|------|
| 17 | 1.34 (2H, <i>m</i> )                 | 23.7 | 23.7 (CH <sub>2</sub> ) | 22.7 |
| 18 | 0.91 (3H, <i>t</i> , <i>J</i> = 8.0) | 14.4 | 14.4 (CH <sub>3</sub> ) | 14.2 |

Compound **109** (40 mg) was isolated as a white crystal from the fruit extract of *C. ficifolius*. The TLC profile showed a spot at *R<sub>f</sub>* 0.40 using methanol:EtOAc (3:7) as mobile phase. The <sup>1</sup>H-NMR spectrum of compound **109** indicated four olefinic proton signals at  $\delta_H$  5.13 (1H, d, *J* = 7.7 Hz), 5.38 (1H, d, *J* = 5.0 Hz), 5.45 (1H, d, *J* = 7.7 Hz) and 5.69 (1H, d, *J* = 5.0 Hz). The proton signal at  $\delta_H$  4.09 (1H, dd, *J* = 5.6, 3.4 Hz), 3.12 (1H, bro s), 3.12 (1H, bro s) are accounted for methine protons while 3.63 (1H, d, *J* = 4.0 Hz) and 3.60 (1H, d, *J* = 4.0 Hz) are for oxymethaylene protons. The proton signal at  $\delta_H$  4.36 (1H, d, *J* = 7.8 Hz), 4.26 (1H, d, *J* = 6.7 Hz), 3.77 (4H, bro s), 3.44 (2H, m) and 3.12 (4H, bro s) are accounted for protons on glucose moiety. The signals due to methyl protons were observed at  $\delta_H$  1.25 (6H, s) 1.21 (15H, s) for methyl protons and 2.05 (3H, s) for methyl protons next to ester carbonyl carbon. The spectrum also showed signals at  $\delta_H$  1.56 (1H, *m*), 2.00 (2H, q, *J* = 5.0 Hz), 2.24 (1H, t, *J* = 7.5 Hz), 1.77 (1H, *m*), 1.72 (1H, *m*), 1.90 (1H, *m*) and 1.37 (2H, *m*). The <sup>13</sup>C NMR spectrum of compound **109** with the help of DEPT-135 revealed the presence of well resolved forty four carbon resonances of which six olefinic carbons signals were observed at  $\delta_C$  139.6, 117.3, 134.0, 131.1, 129.1 and 138.2. The signal due to ester carbonyl appeared at  $\delta_C$  171.7. The signals due to oxymethine appeared at  $\delta_C$  78.9, 75.6 and 72.1, respectively. The signal at  $\delta_C$  101.0, 73.3, 77.0, 70.0, 76.3 and 61.6 are accounted for **6-O-Glc** carbons while signals at  $\delta_C$  102.9, 73.5, 77.3, 69.8, 76.0 and 63.2 are for **23-O-Glc**. The carbon signals at  $\delta_C$  18.8, 20.7, 22.9, 18.9, 23.0, 29.2, 19.7 and 21.4 were due to methyl carbons. The remaining carbon signals appeared at  $\delta_C$  22.7, 31.9, 43.4, 45.8, 39.5, 40.1, 48.7, 50.0, 33.9, 29.7, 56.0, 65.4, 36.5 and 37.0. Thus, based on the above spectral data compound **109** was found to be kuguaglycoside E-3-acetate (Figure 18).

**Table 13:**  $^1\text{H}$  NMR (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **109** in  $\text{CDCl}_3/\text{CD}_3\text{OD}$ ) and NMR data reported for kuguaglycoside E (Jian-Chao, Ch., *et al.*, 2008).

| Position | NMR data of compound 109            |                             |                                           | NMR data of kuguaglycoside E |
|----------|-------------------------------------|-----------------------------|-------------------------------------------|------------------------------|
|          | $\delta^1\text{H}$ NMR ( $J$ in Hz) | $\delta^{13}\text{C}$ / ppm | $\delta^{13}\text{C}$ from DEPT-135 / ppm | $\delta^{13}\text{C}$ / NMR  |
| 1        | 1.56 (1H, <i>m</i> )                | 22.7                        | 22.7 ( $\text{CH}_2$ )                    | 20.5                         |
| 2        | 2.00 (2H, q, $J = 5.0$ Hz)          | 31.9                        | 31.9 ( $\text{CH}_2$ )                    | 30.4                         |
| 3        | 4.09 (1H, dd, $J = 5.6, 3.4$ Hz)    | 78.9                        | 78.9 (CH)                                 | 76.1                         |
| 4        |                                     | 43.4                        |                                           | 42.1                         |
| 5        | 2.24 (1H, t, $J = 7.5$ Hz)          | 45.8                        | 45.8 (CH)                                 | 39.1                         |
| 6        | 3.12 (1H, bro s)                    | 75.6                        | 75.6 (CH)                                 | 75.7                         |
| 7        | 5.13 (1H, d, $J = 7.7$ Hz)          | 117.3                       | 117.3(CH)                                 | 121.0                        |
| 8        |                                     | 139.6                       |                                           | 148.9                        |
| 9        |                                     | 39.5                        |                                           | 39.2                         |
| 10       | 1.56 (1H, <i>m</i> )                | 40.1                        | 40.1 (CH)                                 | 39.0                         |
| 11       | 5.38 (1H, d, $J = 5.0$ Hz)          | 134.0                       | 134.0(CH)                                 | 27.0                         |
| 12       | 5.69 (1H, d, $J = 5.0$ Hz)          | 131.1                       | 131.1(CH)                                 | 28.3                         |
| 13       |                                     | 48.7                        |                                           | 46.3                         |
| 14       |                                     | 50.0                        |                                           | 47.8                         |
| 15       | 1.77 (2H, <i>m</i> )                | 33.9                        | 33.9 ( $\text{CH}_2$ )                    | 35.4                         |
| 16       | 1.72 (2H, <i>m</i> )                | 29.7                        | 29.7 ( $\text{CH}_2$ )                    | 30.5                         |
| 17       | 1.56 (1H, <i>m</i> )                | 56.0                        | 56.0(CH)                                  | 51.5                         |

|    |                                         |       |                         |       |
|----|-----------------------------------------|-------|-------------------------|-------|
| 18 | 1.21 (3H, <i>s</i> )                    | 20.7  | 20.7(CH <sub>3</sub> )  | 19.8  |
| 19 | 3.63 (1H, <i>d</i> , <i>J</i> = 4.0 Hz) | 65.4  | 65.4(CH <sub>2</sub> )  | 65.6  |
|    | 3.60 (1H, <i>d</i> , <i>J</i> = 4.0 Hz) |       |                         |       |
| 20 | 1.90 (1H, <i>m</i> )                    | 36.5  | 36.5 (CH)               | 32.9  |
| 21 | 1.21 (3H, <i>s</i> )                    | 18.9  | 18.9(CH <sub>3</sub> )  | 18.4  |
| 22 | 1.37 (2H, <i>m</i> )                    | 37.0  | 37.0 (CH <sub>2</sub> ) | 44.1  |
| 23 | 3.12(1H, bro <i>s</i> )                 | 72.1  | 72.1 (CH                | 72.8  |
| 24 | 5.37 (1H, <i>d</i> , <i>J</i> = 7.7 Hz) | 129.1 | 129.1(CH)               | 129.2 |
| 25 |                                         | 138.2 |                         | 132.1 |
| 26 | 1.25 (3H, <i>s</i> )                    | 19.7  | 19.7(CH <sub>3</sub> )  | 18.4  |
| 27 | 1.25 (3H, <i>s</i> )                    | 29.2  | 29.2 (CH <sub>3</sub> ) | 28.3  |
| 28 | 1.21 (3H, <i>s</i> )                    | 18.8  | 18.8 (CH <sub>3</sub> ) | 15.1  |
| 29 | 1.21 (3H, <i>s</i> )                    | 22.9  | 22.9 (CH <sub>3</sub> ) | 25.9  |
| 30 | 1.21 (3H, <i>s</i> )                    | 23.0  | 23.0 (CH <sub>3</sub> ) | 26.4  |
| 1' |                                         | 171.7 |                         |       |
| 2' | 2.05 (3H, <i>s</i> )                    | 21.4  | 21.4(CH <sub>3</sub> )  |       |

**6-O-Glc**

|   |                                         |       |                        |       |
|---|-----------------------------------------|-------|------------------------|-------|
| 1 | 4.36 (1H, <i>d</i> , <i>J</i> = 7.8 Hz) | 101.0 | 101.0(CH)              | 101.3 |
| 2 | 3.24 (1H, <i>m</i> )                    | 76.3  | 76.3(CH)               | 78.4  |
| 3 | 3.44 (1H, <i>m</i> )                    | 73.5  | 73.5(CH)               | 74.9  |
| 4 | 3.12(1H, bro <i>s</i> )                 | 70.0  | 70.0(CH)               | 71.8  |
| 5 | 3.12(1H, bro <i>s</i> )                 | 77.0  | 77.0(CH)               | 79.1  |
| 6 | 3.77 (2H, bro <i>s</i> )                | 61.6  | 61.6(CH <sub>2</sub> ) | 59.8  |

### 23-O-Glc

|   |                                         |       |                        |       |
|---|-----------------------------------------|-------|------------------------|-------|
| 1 | 4.26 (1H, <i>d</i> , <i>J</i> = 6.7 Hz) | 102.9 | 102.9(CH)              | 104.5 |
| 2 | 3.24 (1H, m)                            | 76.0  | 76.0(CH)               | 75.6  |
| 3 | 3.44 (1H, m)                            | 73.5  | 73.3(CH)               | 74.0  |
| 4 | 3.12(1H, bro s)                         | 69.8  | 69.8(CH)               | 71.8  |
| 5 | 3.12(1H, bro s)                         | 77.3  | 75.6(CH)               | 79.3  |
| 6 | 3.77 (2H, bro s)                        | 63.2  | 63.2(CH <sub>2</sub> ) | 63.0  |

The leaves, stems, fruits and roots of *C. ficifolius* also gave  $\alpha$ -spinasterol. The detailed structure elucidation of  $\alpha$ -spinasterol was presented in the page 54 table 7.

#### 4.2.2 Compounds Isolated from Fruit of *C. dipsaceus*

Silica gel column chromatography fractionation of the fruit extracts of *C. dipsaceus* has led to the isolation of ten (10) compounds (Figure 19). The structures of the isolated compounds were established using NMR and in some cases by comparing with literature reported for the same compounds (Wadah, O., *et al.*, 2019; MEI, W.L., *et al.*, 2012; Shi, Y.R., *et al.*, 1994).

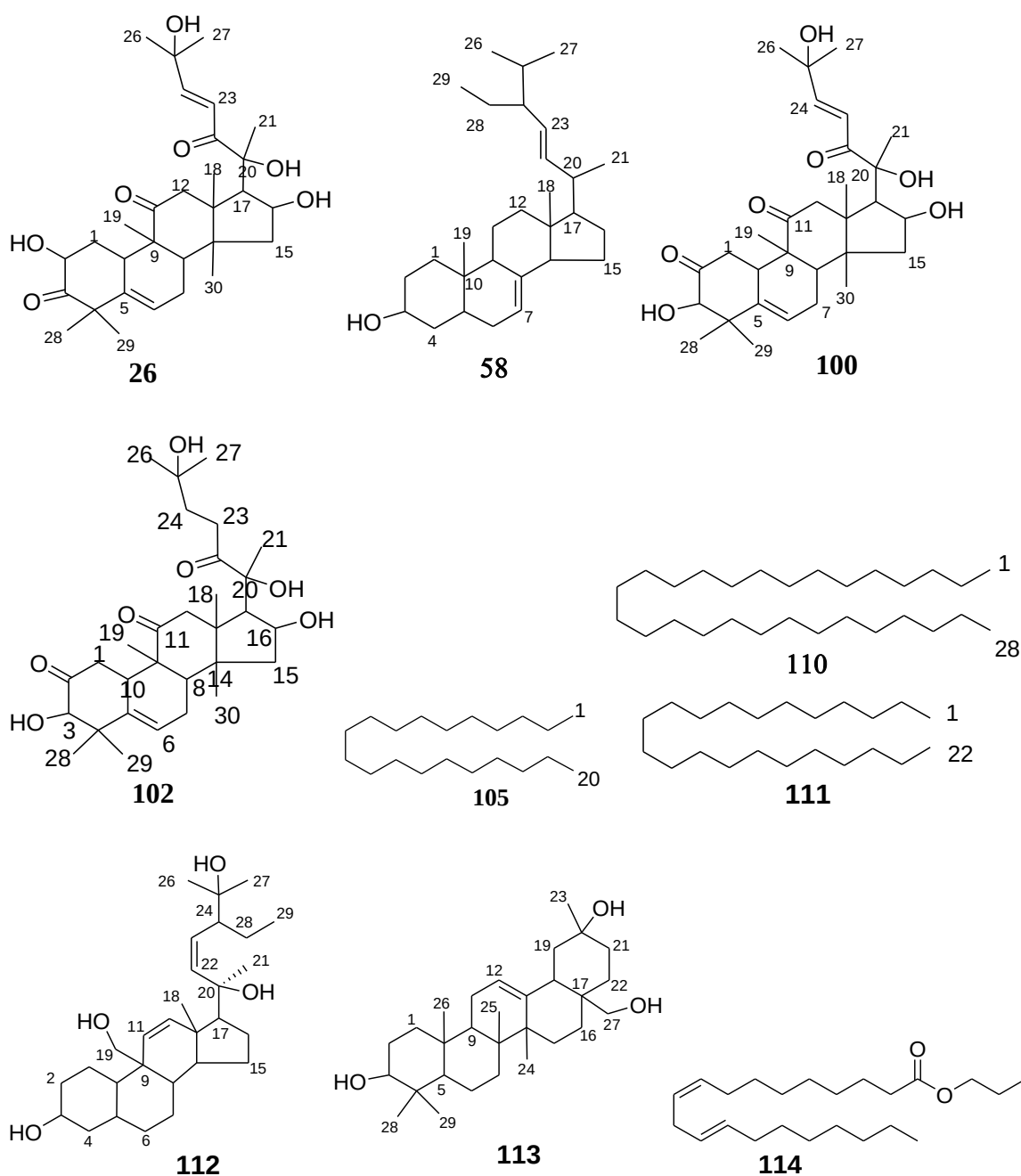


**Figure 19:** The structure of compounds isolated from fruit extract of *C. dipsaceus*

Compound **110** (28 mg) was isolated as a white solid from the petroleum ether extract of the fruits of *C. dipsaceus*. The TLC profile showed a spot at  $R_f$  0.95 using petroleum ether:EtOAc (9:1) as a mobile phase. The  $^1\text{H}$ -NMR spectrum showed signal due to terminal methyl protons at  $\delta_{\text{H}}$  0.81 (6H, t). The proton signals centered at  $\delta_{\text{H}}$  1.18 (52 H, bro s) could suggest diagnostics for the presence of overlapping methylene groups. The  $^{13}\text{C}$ -NMR spectrum of compound **110** with the help of DEPT-135 revealed the presence of five well resolved carbon resonances of 28 carbons. The terminal methyl group is evident at  $\delta_{\text{C}}$  14.1 while the signals due to methylenes were observed at  $\delta_{\text{C}}$  22.7, 29.3, 29.6 and 31.9. The

intense signal observed at  $\delta_C$  29.6 could suggest the presence of eighteen overlapping methylene groups. Based on the above spectral data, compound **110** is found to be identical to octacosane (Figure 19).

**Table 14:**  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **110** in  $\text{CDCl}_3$

| Position | $\delta_H$ (multiplicity, J in Hz) | $^{13}\text{C}$ NMR ( $\delta_C$ in ppm) | DEPT-135 ( $\delta_C$ in ppm) |
|----------|------------------------------------|------------------------------------------|-------------------------------|
| 1/28     | 0.81(6H, t)                        | 14.1                                     | 14.1( $\text{CH}_3$ )         |
| 2/27     | 1.18 (4H, bro s)                   | 22.7                                     | 22.7( $\text{CH}_2$ )         |
| 3/26     | 1.18 (4H, bro s)                   | 31.9                                     | 31.9( $\text{CH}_2$ )         |
| 4/25     | 1.18 (4H, bro s)                   | 29.3                                     | 29.3( $\text{CH}_2$ )         |
| 5-24     | 1.18 (40H, bro s)                  | 29.6                                     | 29.6( $\text{CH}_2$ )         |

Compound **111** (15 mg) was isolated as a white solid from the petroleum ether extract of the fruits of *C. dipsaceus*. The TLC profile showed a spot at  $R_f$  0.90 using petroleum ether:EtOAc (9:1) as a mobile phase. The  $^1\text{H}$ -NMR spectrum showed signal due to terminal methyl protons at  $\delta_H$  0.88 (6H, t). The proton signals centered at  $\delta_H$  1.26 (40H, m) is diagnostics for the presence of overlapping methylene groups. The  $^{13}\text{C}$ -NMR spectrum of compound **111** with the help of DEPT-135 revealed the presence of five well resolved carbon resonances. The terminal methyl group is evident at  $\delta_C$  14.1 while signals due to methylenes were observed at  $\delta_C$  22.6, 29.3, 29.7 and 31.9. The intense signal observed at  $\delta_C$  29.7 was likely due to the presence of many overlapping methylene groups. Based on the above spectral data, compound **111** is found to be identical to docosane (Figure 19).

Docosane was reported to have antibacterial activity (Togashi, N., *et al.* 2007).

**Table 15:**  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **111** in  $\text{CDCl}_3$

| Position | $\delta_H$ (multiplicity, J in Hz) | $^{13}\text{C}$ NMR ( $\delta_C$ in ppm) | DEPT-135 ( $\delta_C$ in ppm) |
|----------|------------------------------------|------------------------------------------|-------------------------------|
| 1/22     | 0.88(6H, t)                        | 14.2                                     | 14.2( $\text{CH}_3$ )         |
| 2/21     | 1.26 (4H, bro s)                   | 22.8                                     | 22.8( $\text{CH}_2$ )         |
| 3/20     | 1.26 (4H, bro s)                   | 32.0                                     | 32.0( $\text{CH}_2$ )         |
| 4/19     | 1.26 (4H, bro s)                   | 29.5                                     | 29.5( $\text{CH}_2$ )         |
| 5-18     | 1.26 (28H, bro s)                  | 29.8                                     | 29.8( $\text{CH}_2$ )         |

Compound **112** (35 mg) was isolated as a white solid from the petroleum ether extract of the fruits of *C. dipsaceus*. The TLC showed a spot at  $R_f$  0.60 with petroleum ether:EtOAc (4:1) as a mobile phase. The  $^1\text{H}$  NMR spectrum of compound **112** indicated the presence of four olefinic proton signals at  $\delta_H$  5.14 (1H, m), 5.10 (1H, m), 6.43 (1H, d,  $J = 8.5$  Hz) and 6.17 (1H, d,  $J = 8.5$  Hz). The diagnostic signal due to proton on C-3 is evident at  $\delta_H$  4.21 (1H, m) and that of C-19 at  $\delta_H$  4.08 (2H, m). Methyl proton signals were observed at  $\delta_H$  0.75 (3H, t), 0.81 (3H, bro s), 0.81 (3H, bro s), 1.18 (3H, bro s) and 1.24 (3H, bro s). The spectrum also showed a proton signal resonated at  $\delta_H$  1.24 (15H, bro s), 1.18 (8H, bro s) and 2.24 (5H, bro s) which accounted for methylenes and methine protons. The  $^{13}\text{C}$  NMR spectr of compound **112** with the aid of DEPT-135 revealed the presence of well resolved resonances of twenty-nine carbons, of which four olefinic carbons signals were observed at  $\delta_C$  135.5, 135.3, 130.8 and 132.4. The signal due to carbon bearing oxygen appeared at  $\delta_C$  66.6 (C-3), 62.2(C-19), 79.5(C-21) and 82.2(C-25). The  $^{13}\text{C}$ -NMR spectrum displayed signals at  $\delta_C$  18.3, 19.7, 20.0, 21.0 and 12.9 were due to methyls. The remaining carbon signals appeared at  $\delta_C$  23.4, 36.8, 39.3, 33.1, 34.6, 30.0, 39.8, 44.8, 42.8, 37.0, 51.8, 34.0, 24.8, 51.2, 56.3 and 20.7. Based on the above data, compound **112** is in agreement with 17-(-5-ethyl-2,6-dihydroxy-6-methylhept-3-en-2-yl)-9-(hydroxymethyl)-13-methyl-1H cyclopenta[ $\alpha$ ]phenanthren-3-ol (Figure 19).

**Table 16:**  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **112** in  $\text{CDCl}_3$

| Position | $\delta_H$ (multiplicity, J in Hz) | $^{13}\text{C}$ NMR ( $\delta_C$ in ppm) | DEPT-135 ( $\delta_C$ in ppm) |
|----------|------------------------------------|------------------------------------------|-------------------------------|
| 1        | 1.24 (1H, bro s), 1.18(1H, bro s)  | 23.4                                     | 23.4(CH <sub>2</sub> )        |
| 2        | 2.24 (2H, m)                       | 36.8                                     | 36.8(CH <sub>2</sub> )        |
| 3        | 4.21 (1H, m)                       | 66.6                                     | 66.6(CH)                      |
| 4        | 2.24 (2H, m)                       | 39.3                                     | 39.3 (CH <sub>2</sub> )       |
| 5        | 1.24(1H, bro s)                    | 33.1                                     | 33.1(CH)                      |
| 6        | 1.24 (1H, bro s), 1.18 (1H, bro s) | 34.6                                     | 34.6(CH <sub>2</sub> )        |
| 7        | 1.24 (1H, bro s), 1.18(1H, bro s)  | 30.0                                     | 30.0(CH <sub>2</sub> )        |
| 8        | 1.24(1H, bro s)                    | 39.8                                     | 39.8 (CH)                     |
| 9        |                                    | 44.8                                     |                               |
| 10       | 1.24 (1H, bro s)                   | 42.8                                     | 42.8 (CH)                     |
| 11       | 5.14 (1H, s)                       | 135.5                                    | 135.5 (CH)                    |

|    |                                  |       |                         |
|----|----------------------------------|-------|-------------------------|
| 12 | 5.10 (1H, s)                     | 135.3 | 135.3(CH)               |
| 13 |                                  | 37.0  |                         |
| 14 | 1.24 (1H, bro s)                 | 51.8  | 51.8(CH)                |
| 15 | 1.24(1H, bro s), 1.18(1H, bro s) | 34.0  | 34.0 (CH <sub>2</sub> ) |
| 16 | 1.24(1H, bro s), 1.18(1H, bro s) | 24.8  | 24.8 (CH <sub>2</sub> ) |
| 17 | 1.24(1H, bro s)                  | 51.2  | 51.2(CH)                |
| 18 | 1.18(3H, bro s)                  | 18.3  | 18.3(CH <sub>3</sub> )  |
| 19 | 4.08 (2H, m)                     | 62.2  | 62.2(CH <sub>2</sub> )  |
| 20 |                                  | 79.5  |                         |
| 21 | 1.24(3H, bro s)                  | 19.7  | 19.7(CH <sub>3</sub> )  |
| 22 | 6.43 (1H, d, $J = 8.5$ Hz)       | 130.8 | 130.8(CH)               |
| 23 | 6.17 (1H, d, $J = 8.5$ Hz)       | 132.4 | 132.4(CH)               |
| 24 | 2.24(1H, m)                      | 56.3  | 56.3(CH)                |
| 25 |                                  | 82.2  |                         |
| 26 | 0.83 (3H, bro s)                 | 20.0  | 20.0(CH <sub>3</sub> )  |
| 27 | 0.81(3H, bro s)                  | 21.0  | 21.0(CH <sub>3</sub> )  |
| 28 | 1.24(2H, bro s)                  | 20.7  | 20.7(CH <sub>2</sub> )  |
| 29 | 0.75 (3H, t)                     | 12.9  | 12.9(CH <sub>3</sub> )  |

Compound **113** (20 mg) was isolated as a yellow solid from the petroleum ether extract of the fruits of *C. dipsaceus*. The TLC showed a spot at  $R_f$  0.64 with petroleum ether:EtOAc (4:1) as a mobile phase. The <sup>1</sup>H NMR spectrum of compound **113** indicated the presence of one olefinic proton signals at  $\delta_H$  5.61 (1H, t,  $J = 6.1$  Hz). The diagnostic signal due to proton on C-3 is evident at  $\delta_H$  3.46 (1H, t,  $J = 3.0$  Hz) and C-27 at  $\delta_H$  4.36 (1H, m), 4.20 (1H, m). Methyl proton signals were observed at  $\delta_H$  1.31(6H, bro s), 1.21 (3H, s), 1.15 (3H, s), 1.07 (3H, s) and 0.91 (3H, s). The spectrum also showed a proton signal resonated at  $\delta_H$  1.36 (10H, bro s), 1.63 (6H, m), 2.35 (2H, t,  $J = 7.5$  Hz), 2.30 (1H, dd,  $J = 7.5, 2.5$  Hz) and 2.03 (2H, m) which accounted for methylenes and methine protons. The <sup>13</sup>C NMR spectrum of compound **113** with the aid of DEPT-135 revealed the presence of well resolved resonances of twenty-nine carbon, of which two olefinic carbons signals were observed at  $\delta_C$  143.9 and 121.3. The signal due to carbon bearing oxygen appeared at  $\delta_C$  77.0 (C-3),  $\delta_C$  70.6(C-20) and at  $\delta_C$  63.3 (C-27). The <sup>13</sup>C-NMR spectrum displayed signals due to methyl groups at  $\delta_C$  16.8, 19.0, 20.1, 29.9, 32.5 and 32.9. The remaining carbon signals appeared at  $\delta_C$  37.2, 31.3,

40.4, 51.6, 19.6, 35.9, 40.5, 44.5, 39.0, 24.6, 41.4, 29.3, 33.2, 31.5, 48.8, 39.9, 34.2, and 36.1. Based on the above data compound **113** is established as erythrodiol (Figure 19) (Wadah, O., *et al.*, 2019).

**Table 17:**  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **113** in  $\text{CDCl}_3$  and NMR data reported for erythrodiol.

| Position | $\delta\text{H}$ (multiplicity, J in Hz) | $^{13}\text{C}$ NMR<br>( $\delta\text{C}$ in ppm) | DEPT-135<br>( $\delta\text{C}$ in<br>ppm) | Erythrodiol<br>(Wadah, O., <i>et al.</i> , 2019). |
|----------|------------------------------------------|---------------------------------------------------|-------------------------------------------|---------------------------------------------------|
| 1        | 1.36 (2H, bro s)                         | 37.2                                              | 37.2 ( $\text{CH}_2$ )                    | 38.9                                              |
| 2        | 2.03 (2H, m)                             | 31.3                                              | 31.3 ( $\text{CH}_2$ )                    | 28.1                                              |
| 3        | 3.46 (1H, t, $J = 3.0$ Hz)               | 77.0                                              | 77.0 (CH)                                 | 78.3                                              |
| 4        |                                          | 40.4                                              |                                           | 39.7                                              |
| 5        | 1.36 (1H, bro s)                         | 51.6                                              | 51.6 (CH)                                 | 55.8                                              |
| 6        | 1.63 (2H, m)                             | 19.6                                              | 19.6 ( $\text{CH}_2$ )                    | 18.8                                              |
| 7        | 1.63 (2H, m)                             | 35.9                                              | 35.9 ( $\text{CH}_2$ )                    | 33.7                                              |
| 8        |                                          | 40.5                                              |                                           | 39.9                                              |
| 9        | 1.36 (1H, bro s)                         | 44.5                                              | 44.5 (CH)                                 | 48.2                                              |
| 10       |                                          | 39.0                                              |                                           | 37.5                                              |
| 11       | 2.35 (2H, t, $J = 7.5$ Hz)               | 24.6                                              | 24.6 ( $\text{CH}_2$ )                    | 23.8                                              |
| 12       | 5.61 (1H, t, $J = 6.1$ Hz)               | 121.3                                             | 121.3 (CH)                                | 125.2                                             |
| 13       |                                          | 143.9                                             |                                           | 139.5                                             |
| 14       |                                          | 41.4                                              |                                           | 42.7                                              |
| 15       | 1.36 (2H, bro s)                         | 29.3                                              | 29.3 ( $\text{CH}_2$ )                    | 27.2                                              |
| 16       | 1.36 (2H, bro s)                         | 33.2                                              | 33.2 ( $\text{CH}_2$ )                    | 29.0                                              |
| 17       |                                          | 31.5                                              |                                           | 31.1                                              |
| 18       | 2.30 (1H, dd, $J = 7.5, 2.5$ Hz)         | 48.8                                              | 48.8 (CH)                                 | 53.9                                              |
| 19       | 1.63 (2H, m)                             | 39.9                                              | 39.9 ( $\text{CH}_2$ )                    | 39.6                                              |
| 20       |                                          | 70.6                                              |                                           | 42.3                                              |
| 21       | 1.63 (2H, m)                             | 34.2                                              | 34.2 ( $\text{CH}_2$ )                    | 29.9                                              |
| 22       | 1.36 (2H, bro s)                         | 36.1                                              | 36.1 ( $\text{CH}_2$ )                    | 38.4                                              |
| 23       | 1.31(3H, bro s)                          | 16.8                                              | 16.8( $\text{CH}_3$ )                     | 15.9                                              |

|    |                            |      |                         |      |
|----|----------------------------|------|-------------------------|------|
| 24 | 1.31(3H, bro s)            | 20.1 | 20.1(CH <sub>3</sub> )  | 17.5 |
| 25 | 1.21 (3H, s)               | 29.9 | 29.9(CH <sub>3</sub> )  | 26.2 |
| 26 | 1.07 (3H, s)               | 32.5 | 32.5(CH <sub>3</sub> )  | 28.4 |
| 27 | 4.36 (1H, m), 4.20 (1H, m) | 63.3 | 63.3 (CH <sub>2</sub> ) | 61.6 |
| 28 | 0.91 (3H, s)               | 19.0 | 19.0(CH <sub>3</sub> )  | 17.4 |
| 29 | 1.15 (3H, s)               | 32.9 | 32.9(CH <sub>3</sub> )  | 28.3 |

Erythrodiol has been demonstrated to have antioxidant, antiproliferative, and proapoptotic effects on human breast cancer cells (Allouche, Y., *et al.*, 2011), HT-29 human adenocarcinoma cells (Juan, M.E., *et al.*, 2008), and astrocytoma cells (Martín, R., *et al.*, 2009). Moreover, erythrodiol has shown anti-inflammatory activity (De-la, P.R., *et al.*, 2000), vasorelaxant effects (Rodríguez, R., *et al.*, 2004), and the capacity to modulate cytokine secretion in human cells (Márquez-Martín, A., *et al.*, 2006).

Compound **114** (35 mg) was isolated as a white solid from the ethyl acetate extract of the fruits of *C. dipsaceus*. The TLC showed a spot at  $R_f$  0.76 with petroleum ether:EtOAc (9:1) as eluent. The <sup>1</sup>H-NMR spectrum of compound **114** indicated four olefinic proton signals at  $\delta_H$  5.31 (2H, m) and 5.28 (2H, m). The triplet proton signals at  $\delta_H$  3.97 (2H, t,  $J$  = 6.7 Hz),  $\delta_H$  0.89 (3H, t,  $J$  = 7.4 Hz) and 0.83 (3H, t,  $J$  = 7.4 Hz) suggest the presence of oxygenated methylene and methyl protons, respectively. The spectrum also showed a proton signal at  $\delta_H$  1.20 (4H, bro s), 1.26 (8H, bro s), 1.57 (8H, m), 1.59 (4H, m), 2.0 (4H, m), 2.24 (2H, m) and 2.71 (2H, t,  $J$  = 6.5 Hz) ascribed to the presence of methylenes. The <sup>13</sup>C NMR spectrum of compound **114** with the help of DEPT-135 revealed the presence of twenty-three well resolved carbon resonances of which four olefinic carbons were observed at  $\delta_C$  130.2, 130.0, 128.0 and 127.9. The diagnostic signal due to ester carbonyl was observed at  $\delta_C$  174.6. The signal due to oxygenated methylene carbon appeared at  $\delta_C$  66.0. The spectrum also displayed signals due to methyl carbons at  $\delta_C$  10.2 and 13.9. The remaining carbon signals appeared at  $\delta_C$  34.4, 27.1, 29.1, 29.3, 29.5, 29.6, 31.9, 34.4, 31.9, 29.6, 29.5, 29.3, 31.5, 22.5 and 21.9. The above spectral data revealed that compound **114** is (9,12)-propyl icoso-9,12-dienoate (Figure 19).

**Table 18:**  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **114** in  $\text{CDCl}_3$ 

| Position | $\delta_{\text{H}}$ (multiplicity, $J$ in Hz) | $^{13}\text{C}$ NMR ( $\delta_{\text{C}}$ in ppm) | DEPT-135 ( $\delta_{\text{C}}$ in ppm) |
|----------|-----------------------------------------------|---------------------------------------------------|----------------------------------------|
| 1        |                                               | 174.6                                             |                                        |
| 2        | 2.25 (2H, t, $J = 7.5$ Hz)                    | 34.4                                              | 34.4 (CH <sub>2</sub> )                |
| 3        | 1.59 (2H, m)                                  | 27.1                                              | 27.1 (CH <sub>2</sub> )                |
| 4        | 1.26 (2H, m)                                  | 29.1                                              | 29.1(CH <sub>2</sub> )                 |
| 5        | 1.20 (2H, m)                                  | 29.3                                              | 29.3(CH <sub>2</sub> )                 |
| 6        | 1.26 (2H, m)                                  | 29.5                                              | 29.5(CH <sub>2</sub> )                 |
| 7        | 1.57 (2H, m)                                  | 29.6                                              | 29.6(CH <sub>2</sub> )                 |
| 8        | 2.0 (2H, m)                                   | 31.9                                              | 31.9(CH <sub>2</sub> )                 |
| 9        | 5.28 (1H, m)                                  | 130.2                                             | 130.2(CH)                              |
| 10       | 5.31 (1H, m)                                  | 128.0                                             | 128.0(CH)                              |
| 11       | 2.71 (2H, t, $J = 6.5$ Hz)                    | 34.4                                              | 34.4(CH <sub>2</sub> )                 |
| 12       | 5.28 (1H, m)                                  | 127.9                                             | 127.84(CH)                             |
| 13       | 5.31 (1H, m)                                  | 130.0                                             | 130.14(CH)                             |
| 14       | 2.0 (2H, m)                                   | 31.9                                              | 31.9(CH <sub>2</sub> )                 |
| 15       | 1.57 (2H, m)                                  | 29.6                                              | 29.6(CH <sub>2</sub> )                 |
| 16       | 1.26 (2H, m)                                  | 29.5                                              | 29.5(CH <sub>2</sub> )                 |
| 17       | 1.20 (2H, m)                                  | 29.3                                              | 29.3(CH <sub>2</sub> )                 |
| 18       | 1.26 (2H, m)                                  | 31.5                                              | 31.5(CH <sub>2</sub> )                 |
| 19       | 1.57 (2H, m)                                  | 22.5                                              | 22.5 (CH <sub>2</sub> )                |
| 20       | 0.83 (3H, t, $J = 7.4$ Hz)                    | 13.9                                              | 13.9(CH <sub>3</sub> )                 |
| 1'       | 3.97 (2H, t, $J = 6.7$ Hz)                    | 66.0                                              | 66.0 (CH <sub>2</sub> )                |

|    |                            |      |                         |
|----|----------------------------|------|-------------------------|
| 2' | 1.59 (2H, m)               | 21.9 | 21.9 (CH <sub>2</sub> ) |
| 3' | 0.89 (3H, t, $J = 7.4$ Hz) | 10.2 | 10.2 (CH <sub>3</sub> ) |

Compound **26** (67.9 mg) was isolated as a solid from ethyl acetate extract of the fruits of *C. dipsaceus*. Its TLC showed a spot at  $R_f$  0.36 with petroleum ether:EtOAc (3:2) as a mobile phase. The <sup>1</sup>H-NMR spectrum of compound **26** revealed three olefinic protons at  $\delta_H$  7.02 (1H, d,  $J = 15.4$  Hz, H-24), 6.83 (1H, d,  $J = 15.5$  Hz, H-23) and 5.84 (1H, dd,  $J = 5.9, 2.4$  Hz, H-6). Two protons on oxygenated carbons were evident at the  $\delta_H$  4.62 (1H, dd,  $J = 12.8, 5.8$  Hz) and 3.45 (1H, dt,  $J = 4.1, 2.1$  Hz) and the remaining protons at  $\delta_H$  3.03 (2H, d,  $J = 13.3$  Hz), 2.03 (1H, t) and 2.63 (1H, d,  $J = 7.4$  Hz) are methine protons and 2.63 (2H, m), 2.63 (2H, d,  $J = 7.4$  Hz), 3.45 (2H, m) and 1.74 (2H, d,  $J = 8.4$  Hz) are methylene protons and also 1.42(6H, s), 1.30(6H, s), 1.21(3H, s), 1.30(3H, s), 1.32(3H, s), 1.07(3H, s) and 0.96 (3H, s) are methyls. The <sup>13</sup>C-NMR spectrum deduced three carbonyl carbons of ketones at  $\delta_C$  214.8, 212.6 and 203.5. Four olefinic carbons at  $\delta_C$  140.7, 119.8, 119.9 and 153.9 and four oxygenated aliphatic carbons at  $\delta_C$  70.1, 71.1, 71.4 and 78.5. The presences of four CH<sub>2</sub> groups were evident at  $\delta_C$  36.6, 48.8, 45.2 and 23.4. The data generated for compound **26** is in good agreement with cucurbitacin D but had compound **100** characterized as isocucurbitacin D as minor impurity (Figure 19) (MEI, W.L., *et al.*, 2012).

**Table 19:**  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **26** and **100** in  $\text{CD}_3\text{OD}$  and NMR data reported for Cucurbitacin D and isocucurbitacin D.

| Compound 26 |                                             |                                                   |                       | Compound 100                                |                                                   |                          |
|-------------|---------------------------------------------|---------------------------------------------------|-----------------------|---------------------------------------------|---------------------------------------------------|--------------------------|
| Position    | $\delta_{\text{H}}$ (multiplicity, J in Hz) | $^{13}\text{C}$ NMR ( $\delta_{\text{C}}$ in ppm) | Cucurbitacin D (Lit.) | $\delta_{\text{H}}$ (multiplicity, J in Hz) | $^{13}\text{C}$ NMR ( $\delta_{\text{C}}$ in ppm) | Isocucurbitacin D (Lit.) |
| 1           | 2.63 (2H, m)                                | 36.6                                              | 37.4                  | 2.63 (2H, m)                                | 36.73                                             | 40.4                     |
| 2           | 4.62 (1H, m)                                | 71.4                                              | 72.7                  |                                             | 215.8                                             | 213.6                    |
| 3           |                                             | 214.8                                             | 206.9                 | 4.62 (1H, m)                                | 78.50                                             | 81.7                     |
| 4           |                                             | 50.4                                              | 50.8                  |                                             | 49.9                                              | 47.9                     |
| 5           |                                             | 140.7                                             | 144.6                 |                                             | 140.6                                             | 140.6                    |
| 6           | 5.84 (1H, dd, $J = 5.9, 2.4$ )              | 119.8                                             | 120.7                 | 5.84 (1H, dd, $J = 5.9, 2.4$ )              | 119.7                                             | 123.2                    |
| 7           | 2.63 (2H, d, $J = 7.4$ Hz)                  | 23.4                                              | 23.9                  | 2.63 (2H, d, $J = 7.4$ Hz)                  | 23.5                                              | 22.4                     |
| 8           | 2.03 (1H, t)                                | 42.6                                              | 41.6                  | 2.03 (1H, t)                                | 42.6                                              | 44.8                     |
| 9           |                                             | 48.4                                              | 47.5                  |                                             | 50.4                                              | 50.0                     |
| 10          | 3.03 (1H, m)                                | 33.4                                              | 34.7                  | 3.03 (1H, m)                                | 33.4                                              | 37.6                     |

|    |                             |       |       |                            |       |       |
|----|-----------------------------|-------|-------|----------------------------|-------|-------|
| 11 |                             | 212.6 | 212.8 |                            | 215.2 | 211.8 |
| 12 | 3.45 (2H, m)                | 48.8  | 48.8  | 3.45 (2H, m)               | 48.8  | 50.5  |
| 13 |                             | 50.4  | 48.2  |                            | 51.9  | 52.3  |
| 14 |                             | 48.5  | 48.8  |                            | 49.9  | 49.8  |
| 15 | 1.74 (2H, d, $J = 8.4$ Hz)  | 45.2  | 45.6  | 1.74 (2H, d, $J = 8.4$ Hz) | 48.5  | 47.5  |
| 16 | 3.57 (1H, m)                | 71.1  | 71.4  | 3.57 (1H, m)               | 70.9  | 72.2  |
| 17 | 2.63 (1H, d, $J = 7.4$ Hz)  | 57.9  | 57.6  | 2.63 (1H, d, $J = 7.4$ Hz) | 58.10 | 59.6  |
| 18 | 1.42(3H, s)                 | 19.1  | 20.1  | 1.47(3H, s)                | 18.6  | 21.3  |
| 19 | 1.30(3H, s)                 | 20.5  | 20.2  | 1.30(3H, s)                | 18.7  | 21.6  |
| 20 |                             | 78.1  | 78.1  |                            | 84.1  | 80.2  |
| 21 | 1.21(3H, s)                 | 24.0  | 23.6  | 1.21(3H, s)                | 27.1  | 25.5  |
| 22 |                             | 203.5 | 203.0 |                            | 212.5 | 204.6 |
| 23 | 6.83 (1H, d, $J = 15.5$ Hz) | 119.9 | 119.1 | 6.83 (1H, d, $J = 15.5$ )  | 119.9 | 121.6 |
| 24 | 7.02 (1H, d, $J = 15.4$ Hz) | 153.9 | 155.5 | 7.02 (1H, d, $J = 15.4$ )  | 153.9 | 156.3 |
| 25 |                             | 70.1  | 71.2  |                            | 79.42 | 71.7  |

|    |             |      |      |             |      |      |
|----|-------------|------|------|-------------|------|------|
| 26 | 1.41(3H, s) | 28.4 | 31.9 | 1.41(3H, s) | 27.7 | 25.5 |
| 27 | 1.30(3H, s) | 28.0 | 30.9 | 1.30(3H, s) | 27.6 | 25.5 |
| 28 | 1.32(3H, s) | 27.8 | 29.3 | 1.32(3H, s) | 7.7  | 26.2 |
| 29 | 1.07(3H, s) | 23.3 | 22.7 | 1.05(3H, s) | 30.4 | 30.6 |
| 30 | 0.98(3H, s) | 18.8 | 19.9 | 0.95(3H, s) | 17.2 | 20.2 |

Compound **102** (20.1 mg) was isolated as a white solid from ethyl acetate extract of the fruits of *C. dipsaceus*. The  $R_f$  value of the compound was 0.22 using *n*-hexane:EtOAc (6:4) as a mobile phase. The  $^1\text{H}$ -NMR spectrum of compound **102** revealed the presence of one olefinic proton signal at  $\delta_H$  6.00 (1H, dd,  $J = 6.1, 2.4$  Hz). Two protons on oxygenated carbons were evident at the  $\delta_H$  3.97 (1H, s) and 3.86 (1H, m). The  $^{13}\text{C}$ -NMR spectrum deduced three carbonyl carbons of ketones at  $\delta_C$  215.8, 213.9 and 210.5. Two olefinic carbons at  $\delta_C$  138.5 and 121.2 and four oxygenated aliphatic carbons at  $\delta_C$  80.0, 71.2, 79.3 and 69.4. The presences of six methylenes carbons were evident at  $\delta_C$  38.8, 24.6, 48.4, 45.1, 29.3 and 33.59 (Table 20). The data generated for compound **102** is in good agreement with 23,24-dihydroisocucurbitacin D (Figure 19) (Shi, Y.R., *et al.*, 1994).

23, 24-dihydrocucurbitacin D has been reported anti-inflammatory effect (Yuan. G., *et al.*, 2006).

**Table 20:**  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **102** in  $\text{CD}_3\text{OD}$  and NMR data reported for 23,24-dihydroisocucurbitacin D.

| Position | $\delta_H$ (multiplicity, J in Hz) | $^{13}\text{C}$ NMR ( $\delta_C$ in ppm) | DEPT-135 ( $\delta_C$ in ppm) | NMR data of 23,24-dihydroisocucurbitacin D |
|----------|------------------------------------|------------------------------------------|-------------------------------|--------------------------------------------|
| 1        | 3.54 (2H, dd, $J = 4.6, 1.8$ Hz)   | 38.8                                     | 38.8 ( $\text{CH}_2$ )        | 38.8                                       |
| 2        |                                    | 213.9                                    |                               | 210.5                                      |
| 3        | 3.97 (1H, s)                       | 80.0                                     | 80.0 (CH)                     | 80.2                                       |
| 4        |                                    | 46.0                                     |                               | 46.7                                       |
| 5        |                                    | 138.5                                    |                               | 138.2                                      |
| 6        | 6.00 (1H, dd, $J = 6.1, 2.4$ Hz)   | 121.2                                    | 121.2(CH)                     | 121.9                                      |
| 7        | 2.58 (2H, dd, $J = 10.9, 3.7$ Hz)  | 24.6                                     | 24.6 ( $\text{CH}_2$ )        | 23.9                                       |
| 8        | 2.18 (1H, dd, $J = 3.4, 1.4$ Hz)   | 42.9                                     | 42.9 (CH)                     | 42.7                                       |
| 9        |                                    | 48.8                                     |                               | 48.3                                       |
| 10       | 3.45 (1H, t, $J = 1.8$ Hz)         | 35.9                                     | 35.9 (CH)                     | 36.2                                       |

|    |                                      |       |                         |       |
|----|--------------------------------------|-------|-------------------------|-------|
| 11 |                                      | 215.8 |                         | 211.9 |
| 12 | 3.45 (2H, s)                         | 48.4  | 48.4(CH <sub>2</sub> )  | 48.5  |
| 13 |                                      | 48.7  |                         | 48.1  |
| 14 |                                      | 50.2  |                         | 50.7  |
| 15 | 1.72 (2H, dd, $J = 6.7$ ,<br>2.2 Hz) | 45.1  | 45.1(CH <sub>2</sub> )  | 45.4  |
| 16 | 3.86 (1H, m)                         | 71.2  | 71.2 (CH)               | 71.3  |
| 17 | 2.32 (1H, d, $J = 7.6$<br>Hz)        | 57.8  | 57.8(CH)                | 57.4  |
| 18 | 1.14(3H, s)                          | 17.3  | 17.3 (CH <sub>3</sub> ) | 20.0  |
| 19 | 1.17(3H, s)                          | 17.2  | 17.2 (CH <sub>3</sub> ) | 19.0  |
| 20 |                                      | 79.3  |                         | 78.1  |
| 21 | 1.32(3H, s)                          | 23.2  | 23.2 (CH <sub>3</sub> ) | 23.8  |
| 22 |                                      | 210.5 |                         | 202.4 |
| 23 | 3.45 (2H, t, $J = 1.8$<br>Hz)        | 29.3  | 29.3 (CH <sub>2</sub> ) | 119.0 |
| 24 | 2.08 (2H, t, $J = 2.7$<br>Hz)        | 33.5  | 33.5 (CH <sub>2</sub> ) | 155.7 |
| 25 |                                      | 69.4  |                         | 71.1  |
| 26 | 1.20(3H, s)                          | 27.7  | 27.7(CH <sub>3</sub> )  | 29.0  |
| 27 | 1.20(3H, s)                          | 28.0  | 28.0 (CH <sub>3</sub> ) | 29.5  |
| 28 | 1.31(3H, s)                          | 20.7  | 20.7 (CH <sub>3</sub> ) | 20.9  |
| 29 | 1.31(3H, s)                          | 24.0  | 24.0 (CH <sub>3</sub> ) | 24.1  |
| 30 | 1.13(3H, s)                          | 19.1  | 19.1 (CH <sub>3</sub> ) | 20.1  |

Compound **58** (30 mg) and compound **105** (15 mg) identified as  $\alpha$ -spinasterol and eicosane were also isolated from ethyl acetate extract and petroleum ether extract of the fruit of *C. dipsaceus* respectively. The structure elucidations were respectively presented in Table 7 and Table 9.

#### 4.2.3 Compound Isolated from Leaves of *C. prophetarum*

Fractionation of the leaves extract of *C. prophetarum* furnished four compounds (Figure 20).

The detailed structure elucidation of the isolated compounds was presented as follows

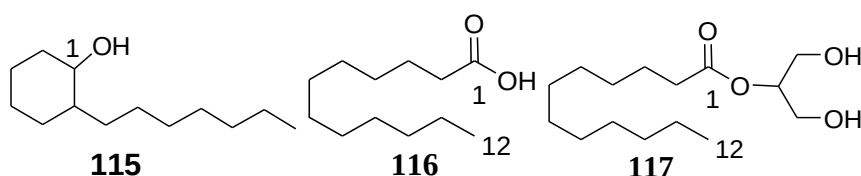


**Figure 20:** The structure of compounds isolated from leaves of *C. prophetarum*

Compound **115** (20mg) was isolated as a white solid from the petroleum ether and ethyl acetate extracts of the leaves of *C. prophetarum*. The TLC showed a spot at  $R_f$  0.9 using *n*-hexane:EtOAc (9:1) as mobile phase. The  $^1\text{H}$ -NMR spectrum of (Table 21) showed the presence of terminal methyl protons at  $\delta_H$  0.88 (3H, t). The diagnostic signal due to proton on C-1 is evident at  $\delta_H$  4.13 (1H, m) and C-6 at  $\delta_H$  2.35(1H, m). The proton signals centered at  $\delta_H$  1.31 integrating for 12 hydrogens were diagnostics for the presence of overlapping methylene groups and the others methylene at  $\delta_H$  1.38 (6H, m) and 1.25(2H, bro s). The  $^{13}\text{C}$  NMR spectral data of compound **115** with the aid of DEPT-135 revealed the presence of well resolved resonances of seven carbon, of which one signal due to carbon bearing oxygen C-1 appeared at  $\delta_C$  79.8, aliphatic methine carbon (C-6) at  $\delta_C$  47.1 and the signal at  $\delta_C$  14.0 is due to terminal methyl group in the compound. The remaining carbon signals appeared at  $\delta_C$  22.7, 29.3, 29.7 and 31.9. The spectral data generated for compound **115** is found to be identical to 2-heptylcyclohexanol (Figure 20).

**Table 21:**  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **115** in  $\text{CDCl}_3$

| Position | $\delta_H$ (multiplicity, J in Hz) | $^{13}\text{C}$ NMR ( $\delta_C$ in ppm) | DEPT-135 ( $\delta_C$ in ppm) |
|----------|------------------------------------|------------------------------------------|-------------------------------|
| 1        | 4.13 (1H, m)                       | 79.8                                     | 79.8 (CH)                     |
| 2        | 1.38 (2H, m)                       | 31.9                                     | 31.9(CH <sub>2</sub> )        |
| 3        | 1.31 (2H, bro s)                   | 22.7                                     | 22.7(CH <sub>2</sub> )        |
| 4        | 1.38 (2H, m)                       | 29.3                                     | 29.3(CH <sub>2</sub> )        |
| 5        | 1.31 (2H, bro s)                   | 29.7                                     | 29.7(CH <sub>2</sub> )        |
| 6        | 2.35(1H, m)                        | 47.1                                     | 47.1(CH)                      |
| 7        | 1.25(2H, bro s)                    | 29.7                                     | 29.7(CH <sub>2</sub> )        |
| 8        | 1.31(2H, bro s)                    | 29.3                                     | 29.3(CH <sub>2</sub> )        |
| 9        | 1.31(2H, bro s)                    | 29.7                                     | 29.7(CH <sub>2</sub> )        |

|    |                 |      |                        |
|----|-----------------|------|------------------------|
| 10 | 1.31(2H, bro s) | 29.7 | 29.7(CH <sub>2</sub> ) |
| 11 | 1.31(2H, bro s) | 31.9 | 31.9(CH <sub>2</sub> ) |
| 12 | 1.38(2H, bro s) | 22.7 | 22.7(CH <sub>2</sub> ) |
| 13 | 0.88 (3H, t)    | 14.0 | 14.0(CH <sub>3</sub> ) |

Compound **116** (30 mg) was isolated as a white solid from leaves of *C. prophetarum* extracts. TLC showed a spot at  $R_f$  0.61 with *n*-hexane:EtOAc (4:1) as mobile phase. The <sup>1</sup>H NMR spectrum of compound **116** displayed proton signal for méthyl group at  $\delta_H$  0.88 (3H, t,  $J = 7.0$  Hz). The spectrum also showed proton signals at  $\delta_H$  1.25 (14H, bro s), 1.33 (2H, bro s), 1.63 (2H, m) and 2.35 (2H, t,  $J = 7.6$  Hz) which are accounted for the presence of methylene groups. The <sup>13</sup>C NMR spectrum of compound **116** with the help of DEPT-135 revealed the presence of well resolved nine carbon resonances of which the diagnostic signal due carboxylic carbon (C-1) appeared at  $\delta_C$  178.5. The spectrum also displayed a signal due to methyl group at  $\delta_C$  14.0. Furthermore, signals due methylenes were evident at  $\delta_C$  33.8, 24.5, 28.7, 28.9, 29.7, 31.8, and 22.5. Based on the above spectral data, compound **116** is in good agreement with decanoic acid (Figure 20).

**Table 22:** <sup>1</sup>H (400 MHz) and <sup>13</sup>C NMR (100 MHz) data of compound **116** in CDCl<sub>3</sub>

| NMR data of Compound <b>116</b> |                                                               |                             |                               |
|---------------------------------|---------------------------------------------------------------|-----------------------------|-------------------------------|
| Position                        | $\delta^1\text{H}$ (no of H, <i>multiplicity</i> , $J$ in Hz) | $\delta^{13}\text{C}$ / ppm | DEPT-135 ( $\delta_C$ in ppm) |
| 1                               |                                                               | 178.5                       |                               |
| 2                               | 2.35 (2H, t, $J = 7.6$ Hz)                                    | 33.8                        | 33.8 (CH <sub>2</sub> )       |
| 3                               | 1.63 (2H, m)                                                  | 24.5                        | 24.5(CH <sub>2</sub> )        |
| 4                               | 1.25 (2H, bro S)                                              | 28.7                        | 28.7(CH <sub>2</sub> )        |
| 5/8                             | 1.25 (2H, bro S)                                              | 28.9                        | 28.9(CH <sub>2</sub> )        |
| 9                               | 1.25 (2H, bro S)                                              | 29.7                        | 29.7(CH <sub>2</sub> )        |
| 10                              | 1.25 (2H, bro S)                                              | 31.8                        | 31.8(CH <sub>2</sub> )        |
| 11                              | 1.33 (2H, bro S)                                              | 22.5                        | 22.5(CH <sub>2</sub> )        |
| 12                              | 0.88 (3H, t, $J = 7.0$ Hz)                                    | 14.0                        | 14.0(CH <sub>3</sub> )        |

Compound **117** (25mg) was isolated as a white solid from the petroleum ether and ethylacetate extract of the leaves of *C. prophetarum*. The TLC showed black spot at  $R_f$  0.3 *n*-hexane: EtOAc (9:1) as eluent. The  $^1\text{H}$ -NMR spectrum of showed the presence of terminal methyl protons at  $\delta_H$  0.88 (3H, t). The diagnostic signal due to proton on C-1'/3' is evident at  $\delta_H$  3.58 (4H, m) and proton on C-2' is evident at  $\delta_H$  4.09 (1H, m). The proton signals centered at  $\delta_H$  1.29 integrating for 16 hydrogens were diagnostics for the presence of eight overlapping methylene groups, The  $^{13}\text{C}$  NMR spectral data of compound **117** with the aid of DEPT-135 revealed the presence of well resolved resonances of nine carbon, of which two signal due to carbon bearing oxygen (C-1'/3' and C-2') appeared at  $\delta_C$  63.0 and 72.4 respectively the spectrum also displayed a signal due to methyl group at  $\delta_C$  13.0 (C-12) in the compound. The remaining carbon signals appeared at  $\delta_C$  31.6 (C-10), 25.8 (C-3), 29.3 (C-4 up to C-9), 36.7(C-2), and 22.3 (C-11). The spectral data generated for compound **117** is found to be identical to 1,3-dihydroxypropan-2-yl dodecanoate (Figure 20).

**Table 23:**  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of compound **117** in  $\text{CD}_3\text{OD}$

| position | $\delta_H$ (multiplicity, J in Hz) | $^{13}\text{C}$ NMR ( $\delta_C$ in ppm) | DEPT-135 ( $\delta_C$ in ppm) |
|----------|------------------------------------|------------------------------------------|-------------------------------|
| 1'/3'    | 3.58 (4H, m)                       | 63.0                                     | 63.0 ( $\text{CH}_2$ )        |
| 2'       | 4.09 (1H, m)                       | 72.4                                     | 72.4 (CH)                     |
| 1        |                                    | 180.5                                    |                               |
| 2        | 2.18 (2H, m)                       | 36.7                                     | 36.7 ( $\text{CH}_2$ )        |
| 3        | 1.29 (2H, bro s)                   | 25.8                                     | 25.8 ( $\text{CH}_2$ )        |
| 4-9      | 1.29 (2H, bro s)                   | 29.3                                     | 29.3 ( $\text{CH}_2$ )        |
| 10       | 1.29 (2H, bro s)                   | 31.6                                     | 31.6 ( $\text{CH}_2$ )        |
| 11       | 1.34 (2H, bro s)                   | 22.3                                     | 22.3 ( $\text{CH}_2$ )        |
| 12       | 0.90 (3H, t, $J = 6.9$ Hz)         | 13.0                                     | 13.0( $\text{CH}_3$ )         |

Additionally compound **58** (30 mg) identified as white crystal was also isolated from leaves of *C. prophetarum*. The detailed structure elucidation is given in Table 7 on page 54.

### 4.3 Biological Activity

#### 4.3.1 Antibacterial Activity

In this study, the bacterial activity of essential oil of the leaves, stems, fruits and roots of *C. ficifolius*, *C. dipsaceus* and *C. prophetarum* were evaluated using agar disc diffusion method against four bacterial strains (two Gram negative: *E. coli* and *P. aeruginosa* and two Gram positive: *S. aureus* and *S. pyogenes*) in Adama Science and Technology University laboratory. Table 24 show the antibacterial activity of the essential oils of the leaves, stem, fruit and root of the three plants against four pathogenic bacteria species. The essential oil of these three plant species showed varying degree of inhibitory activity against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes* at different concentrations.

**Table 24:** The effect of essential oils from *C. ficifolius*, *C. dipsaceus* and *C. prophetarum*.

| Sample               | Plant parts | Conc. $\mu\text{g/mL}$ | Inhibition zone in mm (Mean $\pm$ Standard Deviation) |                      |                  |                    |
|----------------------|-------------|------------------------|-------------------------------------------------------|----------------------|------------------|--------------------|
|                      |             |                        | <i>E. coli</i>                                        | <i>P. aeruginosa</i> | <i>S. aureus</i> | <i>S. pyogenes</i> |
| <i>C. ficifolius</i> | Leaves      | 200                    | 7.93 $\pm$ 0.31                                       | 6.60 $\pm$ 0.20      | 6.30 $\pm$ 0.15  | 7.13 $\pm$ 0.21    |
|                      |             | 100                    | 7.73 $\pm$ 0.31                                       | 6.27 $\pm$ 0.23      | 6.13 $\pm$ 0.12  | 6.60 $\pm$ 0.26    |
|                      |             | 50                     | 7.1 $\pm$ 0.95                                        | 6.27 $\pm$ 0.23      | 6.13 $\pm$ 0.12  | 6.50 $\pm$ 0.36    |
|                      | Stems       | 200                    | 6.90 $\pm$ 0.85                                       | 7.60 $\pm$ 1.40      | 7.20 $\pm$ 0.30  | 6.83 $\pm$ 0.67    |
|                      |             | 100                    | 7.20 $\pm$ 1.38                                       | 7.60 $\pm$ 0.56      | 6.37 $\pm$ 0.15  | 7.80 $\pm$ 0.96    |
|                      |             | 50                     | 6.63 $\pm$ 0.57                                       | 6.33 $\pm$ 0.31      | 6.10 $\pm$ 0.1   | 6.47 $\pm$ 0.32    |
|                      | Fruits      | 200                    | 7.00 $\pm$ 0.92                                       | 6.43 $\pm$ 0.35      | 7.07 $\pm$ 0.57  | 6.80 $\pm$ 0.44    |
|                      |             | 100                    | 6.13 $\pm$ 0.12                                       | 6.30 $\pm$ 0.10      | 6.50 $\pm$ 0.59  | 6.30 $\pm$ 0.2     |
|                      |             | 50                     | 6.07 $\pm$ 0.12                                       | 6.13 $\pm$ 0.12      | 6.27 $\pm$ 0.46  | 6.17 $\pm$ 0.15    |
|                      | Roots       | 200                    | 7.00 $\pm$ 0.92                                       | 6.63 $\pm$ 0.45      | 7.07 $\pm$ 0.57  | 6.80 $\pm$ 0.44    |
|                      |             | 100                    | 6.13 $\pm$ 0.12                                       | 6.40 $\pm$ 0.35      | 6.50 $\pm$ 0.59  | 6.30 $\pm$ 0.20    |
|                      |             | 50                     | 6.07 $\pm$ 0.12                                       | 6.20 $\pm$ 0.12      | 6.27 $\pm$ 0.46  | 6.17 $\pm$ 0.15    |
| <i>C. dipsaceus</i>  | leaves      | 200                    | 11.93 $\pm$ 0.67                                      | 9.00 $\pm$ 4.1       | 6.43 $\pm$ 0.75  | 8.27 $\pm$ 2.56    |
|                      |             | 100                    | 11.27 $\pm$ 0.42                                      | 8.5 $\pm$ 0.18       | 6.27 $\pm$ 0.21  | 7.20 $\pm$ 0.99    |

|                       |               |     |              |              |              |              |
|-----------------------|---------------|-----|--------------|--------------|--------------|--------------|
| <i>C. prophetarum</i> |               | 50  | 10.00±0.3    | 7.5 ± 0.95   | 6.17 ± 0.38  | 7.00 ± 0.56  |
|                       | Stems         | 200 | 7.53 ± 0.58  | 7.93 ± 0.40  | 8.33 ± 0.80  | 8.50 ± 0.56  |
|                       |               | 100 | 6.93 ± 0.12  | 7.53 ± 0.31  | 7.40 ± 0.15  | 7.60 ± 0.72  |
|                       |               | 50  | 6.93 ± 0.38  | 6.83 ± 0.29  | 7.20 ± 0.8   | 8.13 ± 0.10  |
|                       | Fruits        | 200 | 11.03± 0.70  | NA           | NA           | 6.03 ± 0.06  |
|                       |               | 100 | 10.53±0.35   | NA           | NA           | 6.07 ± 0.12  |
|                       |               | 50  | 8.63± 0.55   | NA           | NA           | 6.03 ± 0.06  |
|                       | Leaves        | 400 | 9.20 ± 0.29  | 9.40 ± 0.63  | 7.43 ± 1.0   | 6.20 ± 0.10  |
|                       |               | 200 | 8.50 ± 0.90  | 8.20± 0.12   | 6.50 ± 0.44  | 6.10 ± 0.10  |
|                       |               | 100 | 8.23 ± 0.53  | 7.77 ± 0.72  | 6.23 ± 0.32  | NA           |
|                       | Stems         | 200 | 7.03 ± 0.50  | 6.67 ± 0.35  | 7.70 ± 0.4   | 7.40 ± 0.96  |
|                       |               | 100 | 7.13 ± 0.15  | 6.77 ± 0.38  | 7.07 ± 0.38  | 7.40 ± 0.72  |
|                       |               | 50  | 7.17 ± 0.57  | 6.33 ± 0.41  | 6.03 ± 0.06  | 6.13 ± 0.23  |
|                       | Roots         | 200 | 11.75 ± 0.13 | 7.15 ± 0.49  | 7.35 ± 0.21  | 7.55 ± 0.21  |
|                       |               | 100 | 11.0± 0.07   | 6.70 ± 0.57  | 6.80 ± 0.56  | 6.05 ± 0.07  |
|                       |               | 50  | 10.15±0.92   | 6.40 ± 0.56  | 6.20 ± 0.286 | NA           |
|                       | Ciprofloxacin | 200 | 16.97±1.56   | 16.67 ± 0.40 | 16.90 ± 0.65 | 17.50 ± 0.29 |
|                       |               | 100 | 14.33±0.65   | 15.43 ± 0.55 | 14.80 ± 0.15 | 14.70 ± 0.27 |
|                       |               | 50  | 13.53±0.71   | 14.13 ± 0.31 | 13.23±0.29   | 12.93 ± 0.36 |

Results are Mean ± Standard Deviation of triplicates; Ciprofloxacin was used as positive control.

Among the essential oil of the three plants, *C. ficifolius* leaves exhibited the highest inhibition zone against *E. coli* (11.93± 0.67mm) at concentration of 200 µg/mL and 100 µg/mL (11.27±0.42mm). Whereas essential oil from root *C. prophetarum* was the second with inhibition zone of 11.75 ± 0.13 mm against *E. coli* at 200 µg/mL and 11.0± 0.07mm at concentration 100 µg/mL. The least inhibition zone was observed with the essential oils of

fruits of *C. dipsaceus* against *S. pyogenes* with inhibition diameter of  $6.03 \pm 0.06$  and some are NA. It was found out that the activity of the essential oil in most cases inhibit the bacterial pathogens in a dose dependent manner. The essential oil of fruits of *C. dipsaceus* was ineffective against *S. aureus*, *P. aeruginosa* and *S. pyogenes*. *E. coli* was the most susceptible test organism for all essential oil from all plants. *S. aureus* was relatively the least susceptible test organisms displaying least inhibition zones by the essential oil of *C. ficifolius* stems, *C. dipsaceus* leaves, and *C. prophetarum* stems and roots. The *in vitro* antibacterial activity evaluation of the essential oil of *C. ficifolius*, *C. dipsaceus* and *C. prophetarum* were compared with the essential oils of *C. melo*. Results showed that the essential oil of *C. melo* showed comparable antibacterial activity with the essential oil of *C. ficifolius*, *C. dipsaceus* and *C. prophetarum* against three strains of Gram-positive and Gram-negative bacteria (Siddeeg, A. *et al.*, 2010).

The activity displayed herein by the essential oils of the three species of *Cucumis* is superior in Gram negative bacteria (*E. coli*) than Gram positive bacteria. This is against findings reported by Bouyahya, A., *et al.*, 2019 where Gram-negative bacteria were turned out to be resistant to the essential oils of *Centaureum erythraea* (Bouyahya, A., *et al.*, 2019). However, our finding agreed with the data reported by the same author on the essential oils of *O. compactum* which was found to have significant antibacterial activity against *E. coli* (Bouyahya, A., *et al.*, 2019). Since, the essential oils of *C. ficifolius*, *C. dipsaceus* and *C. prophetarum* had several kinds of chemical constituents; it seems very difficult to attribute the activity to one component alone. Some of the chemical constituents responsible for the antibacterial activity of the essential oil of *C. erythraea* and *O. compactum* including hentriacontane (Bouyahya, A., *et al.*, 2019) were detected in the essential oils of *C. ficifolius*, *C. dipsaceus* and *C. prophetarum*.

### **Antibacterial activity of extracts of *C. ficifolius***

The *in vitro* antibacterial activity of the extracts was assessed using agar disc diffusion method against four bacterial pathogens including *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes*. The findings were presented in Table 25.

**Table 25:** The antibacterial activity of the extracts of the leaves, stems, fruits and roots of *C. ficifolius*.

| Plant Name           | Parts  | Extracts                 | Conc.<br>( $\mu\text{g/mL}$ ) | Zone of inhibition in mm |                      |                  |                    |
|----------------------|--------|--------------------------|-------------------------------|--------------------------|----------------------|------------------|--------------------|
|                      |        |                          |                               | <i>E. coli</i>           | <i>P. aeruginosa</i> | <i>S. aureus</i> | <i>S. pyogenes</i> |
| <i>C. ficifolius</i> | Leaves | <i>n</i> -hexane extract | 400                           | 7.00 $\pm$ 0.00          | 8.16 $\pm$ 0.50      | 7.83 $\pm$ 0.29  | 7.83 $\pm$ 0.28    |
|                      |        |                          | 200                           | 6.83 $\pm$ 0.29          | 7.83 $\pm$ 0.29      | 6.83 $\pm$ 0.29  | 6.66 $\pm$ 0.29    |
|                      |        |                          | 100                           | 6.50 $\pm$ 0.00          | 7.10 $\pm$ 0.79      | 6.56 $\pm$ 0.40  | 7.00 $\pm$ 0.50    |
|                      |        |                          | 50                            | 7.00 $\pm$ 0.86          | 6.4 $\pm$ 0.17       | 6.76 $\pm$ 0.25  | 6.50 $\pm$ 0.00    |
|                      |        | EtOAc extract            | 400                           | 7.86 $\pm$ 0.11          | 8.96 $\pm$ 0.24      | 8.76 $\pm$ 0.05  | 7.83 $\pm$ 0.85    |
|                      |        |                          | 200                           | 8.50 $\pm$ 0.92          | 8.70 $\pm$ 0.30      | 8.06 $\pm$ 0.05  | 8.73 $\pm$ 0.53    |
|                      |        |                          | 100                           | 8.13 $\pm$ 0.13          | 8.50 $\pm$ 0.95      | 7.86 $\pm$ 0.64  | 7.33 $\pm$ 0.25    |
|                      |        |                          | 50                            | 8.00 $\pm$ 0.54          | 7.86 $\pm$ 0.35      | 7.66 $\pm$ 0.35  | 7.60 $\pm$ 0.35    |
|                      | Stem   | MeOH extract             | 400                           | 7.30 $\pm$ 0.30          | 7.80 $\pm$ 0.20      | 6.80 $\pm$ 0.30  | 6.60 $\pm$ 0.40    |
|                      |        |                          | 200                           | 6.70 $\pm$ 0.40          | 7.20 $\pm$ 0.70      | 6.60 $\pm$ 0.40  | 7.20 $\pm$ 0.50    |
|                      |        |                          | 100                           | 7.30 $\pm$ 0.40          | 6.50 $\pm$ 0.40      | 6.90 $\pm$ 0.50  | 6.80 $\pm$ 0.50    |
|                      |        |                          | 50                            | 6.50 $\pm$ 0.60          | 6.20 $\pm$ 0.10      | 6.20 $\pm$ 0.20  | 6.80 $\pm$ 0.50    |
|                      |        | <i>n</i> -hexane extract | 400                           | 13.00 $\pm$ 0.50         | 14.0 $\pm$ 0.20      | 10.17 $\pm$ 0.76 | 13.50 $\pm$ 0.50   |
|                      |        |                          | 200                           | 10.16 $\pm$ 0.04         | 11.10 $\pm$ 0.11     | 9.17 $\pm$ 0.76  | 11.70 $\pm$ 0.53   |
|                      |        |                          | 100                           | 10.90 $\pm$ 0.15         | 9.83 $\pm$ 0.02      | 8.67 $\pm$ 0.52  | 11.17 $\pm$ 0.14   |
|                      |        |                          | 50                            | 9.17 $\pm$ 0.05          | 9.33 $\pm$ 0.76      | 10.33 $\pm$ .57  | 10.67 $\pm$ 0.52   |
|                      |        | EtOAc extract            | 400                           | 13.00 $\pm$ 0.20         | 12.00 $\pm$ 0.10     | 11.22 $\pm$ 0.60 | 12.60 $\pm$ 0.15   |
|                      |        |                          | 200                           | 10.9 $\pm$ 0.15          | 10.80 $\pm$ 2.02     | 10.17 $\pm$ 0.95 | 10.70 $\pm$ 0.53   |
|                      |        |                          | 100                           | 9.16 $\pm$ 0.76          | 11.33 $\pm$ 0.76     | 9.67 $\pm$ 0.52  | 9.70 $\pm$ 0.14    |
|                      |        |                          | 50                            | 8.17 $\pm$ 0.25          | 9.16 $\pm$ 0.08      | 11.33 $\pm$ 0.70 | 8.60 $\pm$ 0.82    |

|       |                      |     |                  |                  |                  |                  |
|-------|----------------------|-----|------------------|------------------|------------------|------------------|
| Fruit | MeOH<br>extract      | 400 | $8.83 \pm 0.15$  | $9.67 \pm 0.58$  | $8.4 \pm 0.52$   | $9.17 \pm 0.76$  |
|       |                      | 200 | $8.16 \pm 0.76$  | $9.33 \pm 0.58$  | $7.83 \pm 0.28$  | $8.50 \pm 0.54$  |
|       |                      | 100 | $7.50 \pm 0.50$  | $8.67 \pm 0.58$  | $8.17 \pm 0.76$  | $7.50 \pm 0.58$  |
|       |                      | 50  | $7.16 \pm 0.28$  | $7.50 \pm 0.50$  | $8.00 \pm 0.5$   | $7.20 \pm 0.55$  |
|       | <i>n</i> -<br>hexane | 400 | $11.00 \pm 0.20$ | $12.33 \pm 0.52$ | $10.67 \pm 0.58$ | $13.00 \pm 0.73$ |
|       |                      | 200 | $10.67 \pm 0.58$ | $9.83 \pm 0.56$  | $10.00 \pm 0.2$  | $12.00 \pm 0.20$ |
|       |                      | 100 | $9.67 \pm 0.76$  | $10.67 \pm 0.31$ | $9.83 \pm 1.04$  | $11.17 \pm 0.61$ |
|       |                      | 50  | $9.00 \pm 0.00$  | $7.67 \pm 0.15$  | $9.50 \pm 0.5$   | $10.50 \pm 0.50$ |
|       | EtOAc<br>extract     | 400 | $12.53 \pm 0.58$ | $11.93 \pm 0.40$ | $12.40 \pm 0.15$ | $9.50 \pm 0.56$  |
|       |                      | 200 | $11.93 \pm 0.12$ | $10.83 \pm 0.29$ | $11.20 \pm 0.8$  | $9.13 \pm 0.10$  |
|       |                      | 100 | $9.93 \pm 0.38$  | $9.93 \pm 0.40$  | $10.33 \pm 1.80$ | $8.60 \pm 0.72$  |
|       |                      | 50  | $8.53 \pm 0.58$  | $9.53 \pm 0.31$  | $10.40 \pm 1.15$ | $7.50 \pm 0.56$  |
|       | MeOH<br>extract      | 400 | $10.24 \pm 0.29$ | $11.8 \pm 0.63$  | $10.32 \pm 0.44$ | $10.56 \pm 0.10$ |
|       |                      | 200 | $9.50 \pm 0.90$  | $8.90 \pm 0.12$  | $9.81 \pm 0.20$  | $8.93 \pm 0.10$  |
|       |                      | 100 | $8.23 \pm 0.53$  | $10.50 \pm 0.72$ | $8.40 \pm 0.32$  | $9.72 \pm 0.5$   |
|       |                      | 50  | $7.03 \pm 0.50$  | $8.78 \pm 0.35$  | $8.56 \pm 0.40$  | $8.59 \pm 0.96$  |
| Roots | <i>n</i> -<br>hexane | 400 | $9.67 \pm 0.08$  | $14.00 \pm 0.10$ | $13.67 \pm 0.53$ | $11.33 \pm 0.15$ |
|       |                      | 200 | $8.50 \pm 0.18$  | $13.33 \pm 0.53$ | $12.33 \pm 0.53$ | $9.25 \pm 0.76$  |
|       |                      | 100 | $7.50 \pm 0.50$  | $12.00 \pm 0.50$ | $11.00 \pm 0.64$ | $8.50 \pm 0.32$  |
|       |                      | 50  | $6.67 \pm 0.29$  | $9.00 \pm 0.50$  | $10.00 \pm 0.50$ | $7.33 \pm 0.57$  |
|       | EtOAc<br>extract     | 400 | $10.83 \pm 0.93$ | $12.17 \pm 0.76$ | $12.67 \pm 0.08$ | $11.00 \pm 0.73$ |
|       |                      | 200 | $9.67 \pm 0.36$  | $11.33 \pm 0.53$ | $10.67 \pm 0.52$ | $10.33 \pm 0.53$ |
|       |                      | 100 | $10.17 \pm 0.75$ | $11.00 \pm 1.00$ | $9.33 \pm 0.53$  | $9.50 \pm 0.50$  |
|       |                      | 50  | $10.00 \pm 0.50$ | $10.33 \pm 0.53$ | $9.17 \pm 0.76$  | $8.17 \pm 0.61$  |

|               |     |              |             |             |             |
|---------------|-----|--------------|-------------|-------------|-------------|
| MeOH          | 400 | 10.00 ± 0.10 | 8.50 ± 0.29 | 7.17 ± 0.76 | 6.43 ± 0.40 |
| extract       | 200 | 8.67 ± 0.15  | 7.50 ± 0.32 | 6.83 ± 0.29 | 6.27 ± 0.21 |
|               | 100 | 7.33 ± 0.58  | 6.13 ± 0.23 | 6.67 ± 0.29 | 6.50 ± 0.50 |
|               | 50  | 6.67 ± 0.29  | 6.03±0.057  | 6.07 ± 0.11 | NA          |
| Ciprofloxacin | 50  | 15.63 ± 0.55 | 14.8 ± 0.15 | 14.65±0.27  | 15.33±0.65  |

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The experiments were done in triplicates and results were presented as mean ±SD; NA not active

As revealed from Table 25, the extracts displayed broad range of antibacterial activity against all the pathogens used in the experiment in a dose-dependent manner. The *n*-hexane extract of the stem of *C. ficifolius* exhibited the highest inhibition zone against *P. aeruginosa* (14.00±0.20 mm) at concentration of 400 µg/mL. Against the same bacterial strain, the *n*-hexane extract of the root showed bacterial inhibition zone of 14.0±0.10 and 13.33 ±0.53 at concentration of 400 µg/mL and 200 µg/mL, respectively. It was also noted that the *n*-hexane extract of the roots displayed an inhibition zone of 13.67±0.53 mm against *S. aureus* at 400 µg/mL. At the same concentration, the EtOAc extract of the stem showed an inhibition zone of 13.00 ± 0.20 against *E. coli*. Therefore, bioactive compounds reside in the *n*-hexane /petroleum ether and ethyl acetate extracts of *C. ficifolius*.

#### **Antibacterial activity of isolated compounds from *C. ficifolius***

The *in vitro* antibacterial activity of isolated compounds was assessed using the agar disc diffusion method against four bacterial pathogens (*E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes*). The results are presented below (Table 26).

**Table 26:** The antibacterial activities of compounds isolated from various parts of *C. ficifolius*.

| Plant Name           | Compounds  | Conc.<br>( $\mu\text{g/mL}$ ) | Zone of inhibition in mm |                      |                  |                    |
|----------------------|------------|-------------------------------|--------------------------|----------------------|------------------|--------------------|
|                      |            |                               | <i>E. coli</i>           | <i>p. aeruginosa</i> | <i>S. aureus</i> | <i>S. pyogenes</i> |
| <i>C. ficifolius</i> | <b>24</b>  | 100                           | $11.90 \pm 0.71$         | $12.60 \pm 0.82$     | $6.13 \pm 0.05$  | $8.33 \pm 0.52$    |
|                      |            | 50                            | $10.50 \pm 0.05$         | $11.50 \pm 0.03$     | $6.10 \pm 0.00$  | $6.10 \pm 0.10$    |
|                      |            | 25                            | $9.70 \pm 0.80$          | $8.50 \pm 0.45$      | $6.10 \pm 0.00$  | $6.57 \pm 0.40$    |
|                      |            | 12.5                          | $8.20 \pm 0.80$          | $8.00 \pm 0.45$      | NA               | $6.33 \pm 0.20$    |
|                      | <b>26</b>  | 100                           | $10.30 \pm 0.90$         | $11.50 \pm 0.70$     | NA               | $6.37 \pm 0.15$    |
|                      |            | 50                            | $8.50 \pm 0.32$          | $10.40 \pm 0.26$     | NA               | $6.57 \pm 0.40$    |
|                      |            | 25                            | $7.50 \pm 0.50$          | $9.60 \pm 0.30$      | NA               | $6.33 \pm 0.10$    |
|                      |            | 12.5                          | $7.20 \pm 0.13$          | $7.80 \pm 0.24$      | NA               | NA                 |
|                      | <b>58</b>  | 100                           | $13.67 \pm 0.57$         | $15.00 \pm 0.10$     | $7.40 \pm 0.10$  | $13.33 \pm 0.50$   |
|                      |            | 50                            | $11.67 \pm 0.55$         | $14.33 \pm 0.52$     | $7.23 \pm 0.65$  | $12.67 \pm 0.57$   |
|                      |            | 25                            | $11.33 \pm 0.34$         | $12.0 \pm 0.25$      | $7.03 \pm 0.15$  | $11.00 \pm 0.50$   |
|                      |            | 12.5                          | $10.67 \pm 0.57$         | $11.0 \pm 0.10$      | $6.50 \pm 0.05$  | $10.50 \pm 0.50$   |
|                      | <b>104</b> | 100                           | $10.50 \pm 0.50$         | $6.27 \pm 0.05$      | NA               | $13.0 \pm 2.64$    |
|                      |            | 50                            | $10.05 \pm 0.32$         | $6.20 \pm 0.00$      | NA               | $11.33 \pm 0.57$   |
|                      |            | 25                            | $9.50 \pm 0.50$          | $6.10 \pm 0.00$      | NA               | $11.2 \pm 0.60$    |
|                      |            | 12.5                          | $9.30 \pm 0.58$          | NA                   | NA               | $10.0 \pm 0.10$    |
|                      | <b>105</b> | 100                           | $6.77 \pm 0.25$          | $6.97 \pm 0.15$      | $6.37 \pm 0.15$  | $6.2 \pm 0.22$     |
|                      |            | 50                            | $6.37 \pm 0.15$          | $6.33 \pm 0.20$      | $6.13 \pm 0.11$  | $6.07 \pm 0.15$    |
|                      |            | 25                            | $6.50 \pm 0.30$          | $6.23 \pm 0.25$      | NA               | $6.03 \pm 0.15$    |
|                      |            | 12.5                          | NA                       | NA                   | NA               | NA                 |
|                      | <b>106</b> | 100                           | $8.50 \pm 0.20$          | $10.67 \pm 0.52$     | NA               | $10.33 \pm 0.22$   |

|               |      |              |             |            |            |
|---------------|------|--------------|-------------|------------|------------|
|               | 50   | 8.00±0.30    | 9.67±0.57   | NA         | 9.33±0.51  |
|               | 25   | 7.50±0.10    | 8.00±0.20   | NA         | 8.17±0.14  |
|               | 12.5 | 7.00±0.30    | 7.00±0.10   | NA         | 7.00±0.10  |
| <b>107</b>    | 100  | 14.00±0.20   | 14.50±0.20  | 7.17±0.76  | 6.10±0.00  |
|               | 50   | 12.00±0.10   | 14.0±0.20   | 6.60±0.36  | NA         |
|               | 25   | 11.17±0.76   | 12.33±0.55  | 6.30±0.17  | NA         |
|               | 12.5 | 7.17±0.76    | 10.33±0.52  | NA         | NA         |
| <b>108</b>    | 100  | 6.10±0.00    | NA          | NA         | NA         |
|               | 50   | NA           | NA          | NA         | NA         |
|               | 25   | NA           | NA          | NA         | NA         |
|               | 12.5 | NA           | NA          | NA         | NA         |
| <b>109</b>    | 100  | 6.50±0.10    | 6.40±0.00   | NA         | NA         |
|               | 50   | 6.40±0.00    | 6.10±0.00   | NA         | NA         |
|               | 25   | 6.20±0.00    | 6.10±0.00   | NA         | NA         |
|               | 12.5 | NA           | NA          | NA         | NA         |
| Ciprofloxacin | 25   | 16.63 ± 0.55 | 15.8 ± 0.15 | 15.65±0.27 | 16.33±0.65 |

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The experiments were done in triplicates and results were presented as mean ±SD

As displayed above (Table 26), all compounds displayed a wide range of antibacterial activity with inhibition zones ranging from NA to 15.0 ±0.10 mm. Among the compounds assessed in the present work, compound **58** displayed inhibition zone of 13.67±0.57, 15.0 ±0.10 and 13.33±0.50 mm against *E. coli*, *P. aeruginosa* and *S. pyogenes*, respectively. The activity is significant compared with ciprofloxacin. At the same concentration, compound **107** exhibited inhibition zones of 14.00±0.20 and 14.50±0.20 mm against *E. coli* and *P. aeruginosa*, respectively. Hence, the activity displayed by the fruit extract of *C. ficifolius* could be attributed to presence of compound **58** and **107**. It was also noted that the inhibition zone displayed by the *n*-hexane extract of the stem of *C. ficifolius* was superior to the isolated compounds. The

better activity of the extract could be due to the synergistic interactions of several phytochemicals present in the *n*-hexane extract, which cannot be the case when pure compounds are evaluated alone. As observed in the extracts, it was also found out that the inhibition zones of the compounds increase in a dose dependent manner.

#### Antibacterial activity of extracts and isolated compounds from fruit of *C. dipsaceus*

The *in vitro* antibacterial activity of the isolated compounds was assessed using the agar disc diffusion method against four bacterial pathogens (*E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes*). The results are presented below (Table 27).

**Table 27:** The antibacterial activity of fruit extracts and isolated compounds of *C. dipsaceus*

| Plant name                   | Extracts/Compounds      | Conc. (µg/mL) | Inhibition Zone Diameter (mm) |                      |                  |                    |
|------------------------------|-------------------------|---------------|-------------------------------|----------------------|------------------|--------------------|
|                              |                         |               | <i>E. coli</i>                | <i>P. aeruginosa</i> | <i>S. aureus</i> | <i>S. pyogenes</i> |
| Fruit of <i>C. dipsaceus</i> | petroleum ether extract | 400           | 11.00 ± 0.20                  | 12.33±0.52           | 10.67 ± 0.58     | 13.00±0.20         |
|                              |                         | 200           | 10.67 ± 0.58                  | 10.67±0.31           | 10.00± 0.2       | 12.00±0.73         |
|                              |                         | 100           | 9.67 ± 0.76                   | 9.83 ± 0.56          | 9.83 ± 1.04      | 11.17±0.61         |
|                              |                         | 50            | 9.00± 0.00                    | 7.67 ± 0.15          | 9.50± 0.50       | 10.50±0.50         |
|                              | EtOAc extract           | 400           | 12.53± 0.58                   | 11.93±0.40           | 12.40±1.15       | 9.50±0.56          |
|                              |                         | 200           | 11.93 ± 0.12                  | 10.53±1.30           | 11.20±0.80       | 9.13±0.10          |
|                              |                         | 100           | 9.93 ± 0.38                   | 9.83±0.29            | 10.33±0.80       | 8.60±0.72          |
|                              |                         | 50            | 8.53 ± 0.58                   | 9.57 ± 0.40          | 10.20±0.15       | 7.50 ±0.56         |
|                              | MeOH extract            | 400           | 8.33±0.57                     | 8.00±1.0             | 8.16±0.28        | 7.10±0.10          |
|                              |                         | 200           | 7.40±0.52                     | 7.33±0.61            | 7.13±0.11        | 6.66±0.28          |
|                              |                         | 100           | 7.33±0.14                     | 6.83±0.28            | 7.00±0.0         | 6.83±0.28          |
|                              |                         | 50            | 6.33±0.15                     | 6.40±0.17            | 6.66±0.28        | 6.43±0.11          |
|                              | 58                      | 100           | 13.67±0.57                    | 15.0 ±0.10           | 7.40±0.10        | 13.33±0.50         |
|                              |                         | 50            | 11.67±0.55                    | 14.33±0.52           | 7.23±0.65        | 12.67±0.57         |
|                              |                         | 25            | 11.33±0.34                    | 12.00±0.25           | 7.03±0.15        | 11.00±0.50         |

|                   |      |              |              |           |            |
|-------------------|------|--------------|--------------|-----------|------------|
|                   | 12.5 | 10.67±0.57   | 11.00±1.0    | 6.50±0.05 | 10.50±0.50 |
| <b>26 and 100</b> | 100  | 10.50±0.32   | 9.6±0.52     | 7.23±0.15 | 7.17±0.76  |
|                   | 50   | 9.26±0.64    | 8.5 ± 0.23   | 7.15±0.10 | 6.57±0.40  |
|                   | 25   | 8.00±0.50    | 7.50±0.65    | 7.10±0.05 | 6.37±0.15  |
|                   | 12.5 | 7.50±0.50    | 7.00± 0.45   | 7.00±0.10 | NA         |
| <b>102</b>        | 100  | 11.50 ± 0.71 | 12.60±0.42   | 6.13±0.05 | 8.33±0.52  |
|                   | 50   | 10.50± 0.05  | 10.5± 0.03   | 6.10±0.00 | 6.10±0.10  |
|                   | 25   | 9.70 ± 0.80  | 8.50±0.45    | 6.10±0.00 | 6.57±0.40  |
|                   | 12.5 | 8.20 ± 0.80  | 7.00± 0.45   | NA        | 6.33±0.20  |
| <b>110</b>        | 100  | 8.50±0.10    | 7.00±1.00    | 6.13±0.05 | 7.00±0.10  |
|                   | 50   | 8.00±0.10    | 6.57±0.4     | 6.10±0.0  | 6.90±0.65  |
|                   | 25   | 7.80±0.57    | 6.33±0.20    | NA        | 6.57±0.40  |
|                   | 12.5 | 6.67±1.00    | 6.10± 0      | NA        | 6.33±0.20  |
| <b>111</b>        | 100  | 10.40± 0.17  | 9.83±0.28    | NA        | 6.46±0.05  |
|                   | 50   | 9.33±0.11    | 9.33±0.11    | NA        | 6.83±0.28  |
|                   | 25   | 8.27± 0.05   | 8.16± 0.06   | NA        | 6.43±0.35  |
|                   | 12.5 | 7.10±0.00    | 7.00± 0.50   | NA        | 6.20±0.10  |
| <b>112</b>        | 100  | 11.67±0.70   | 11.60±0.82   | 6.40±0.10 | 13.33±0.57 |
|                   | 50   | 10.50±0.05   | 10.40 ± 0.03 | 6.23±0.05 | 12.67±0.57 |
|                   | 25   | 9.70±0.80    | 8.50±0.45    | 6.03±0.05 | 11.00±0.10 |
|                   | 12.5 | 7.20±0.80    | 7.00± 0.45   | NA        | 10.50±0.50 |
| <b>113</b>        | 100  | 9.30 ± 0.99  | 10.50±0.70   | NA        | 6.37±0.15  |
|                   | 50   | 8.50±0.32    | 9.60± 0.90   | NA        | 6.57±0.40  |
|                   | 25   | 7.50±0.50    | 9.40±0.06    | NA        | 6.33±1.00  |

|               |      |              |             |             |             |
|---------------|------|--------------|-------------|-------------|-------------|
|               | 12.5 | 7.20 ± 0.03  | 7.80±0.24   | NA          | NA          |
| <b>114</b>    | 100  | 9.00±0.5     | 8.00±0.10   | 6.20±0.00   | 12.33±0.52  |
|               | 50   | 8.83±0.76    | 7.83±0.52   | 6.10±0.00   | 11.33±0.52  |
|               | 25   | 7.50±0.50    | 7.33±0.22   | NA          | 10.00±0.10  |
|               | 12.5 | 7.00±0.10    | 7.00±0.60   | NA          | 9.00±0.10   |
| Ciprofloxacin | 25   | 16.63±0.55   | 15.8± 0.15  | 15.65±0.27  | 16.33±0.65  |
|               | 50   | 18.63 ± 0.55 | 17.8 ± 0.15 | 17.65± 0.27 | 18.33± 0.65 |

The experiments were done in triplicates and results were presented as mean ±SD; NA, not active.

As revealed above (Table 27), the extracts of the fruit of *C. dipsaceus* displayed a broad range of antibacterial activity against the tested pathogens in dose-dependent manner. The petroleum ether extract exhibited the higher inhibition zone against *P. aeruginosa* and *S. pyogenes* with IZD of 12.33±0.52mm and 13.00±0.20mm at 400 µg/mL and the of EtOAc extract against *E. coli* and *S. aureus* with IZD of 12.53 ± 0.58 mm and 12.4 ± 0.15 mm at 400 µg/mL, respectively. It was also revealed that the inhibition zones of the isolated compounds increase in dose-dependent manner. All compounds displayed a wide range of antibacterial activity with inhibition zones ranging from NA to 15.0 ±0.10 mm. Among the compounds evaluated for their antibacterial activity, compound **58** displayed IZD of 13.67±0.57, 15.00±1.00 and 12.67±0.57 mm against *E. coli*, *P. aeruginosa* and *S. pyogenes* at 100 µg/mL, respectively. The activity is significant compared with ciprofloxacin. At the same concentration, compound **112** exhibited IZDs of 11.67±0.70, 11.60±0.82 and 13.33±0.57 mm against *E. coli*, *P. aeruginosa* and *S. pyogenes*, respectively. Compound **102** had IZD of 11.50 ± 0.71 and 12.60±0.42 mm at 100 µg/mL against *E. coli* and *P. aeruginosa*, respectively (Table28).

#### **Antibacterial activity of isolated compounds from leaves of *C. prophetarum***

The antibacterial activity of the extracts and isolated compounds from *C. prophetarum* were assessed using disc diffusion assay with the finding presented below (Table 28).

**Table 28:** The antibacterial activity of extracts and isolated compounds of *C. prophetarum*

| Plant name                      | Extracts/Compound       | Conc. (µg/mL) | Inhibition Zone Diameter (mm) |                      |                  |                    |
|---------------------------------|-------------------------|---------------|-------------------------------|----------------------|------------------|--------------------|
|                                 |                         |               | <i>E. coli</i>                | <i>p. aeruginosa</i> | <i>S. aureus</i> | <i>S. pyogenes</i> |
| Leaves of <i>C. prophetarum</i> | petroleum ether extract | 400           | 8.0 0± 0.10                   | 8.16 ± 0.23          | 7.83 ± 0.11      | 7.50 ± 0.28        |
|                                 |                         | 200           | 7.83±0.20                     | 8.10± 0.40           | 7.83 ± 0.24      | 7.16 ± 0.29        |
|                                 |                         | 100           | 7.50 ± 0.30                   | 7.4 ± 0.27           | 7.56 ± 0.46      | 7.00 ± 0.12        |
|                                 |                         | 50            | 6.40±0.50                     | 6.83± 0.19           | 6.76 ± 0.65      | 6.50 ± 0.45        |
|                                 | EtOAc extract           | 400           | 10.86± 0.11                   | 8.96±0.24            | 8.06 ± 0.25      | 8.83 ± 0.85        |
|                                 |                         | 200           | 9.50 ± 0.92                   | 8.70 ± 0.30          | 8.76 ± 0.15      | 7.73 ± 0.53        |
|                                 |                         | 100           | 9.40±0.13                     | 8.50 ± 0.95          | 7.88± 0.65       | 7.63 ± 0.25        |
|                                 |                         | 50            | 9.13 ± 0.54                   | 7.86± 0.35           | 7.66± 0.85       | 7.30 ± 0.35        |
|                                 | MeOH extract            | 400           | 8.30 ± 0.30                   | 7.80 ± 0.20          | 6.90 ± 0.10      | 7.60 ± 0.40        |
|                                 |                         | 200           | 7.70 ± 0.40                   | 7.20 ± 0.10          | 6.70 ± 0.40      | 7.20± 0.20         |
|                                 |                         | 100           | 7.30 ± 0.46                   | 6.70 ± 0.70          | 6.60 ± 0.20      | 6.80 ± 0.50        |
|                                 |                         | 50            | 6.50 ± 0.60                   | 6.50 ± 0.40          | 6.20 ± 0.25      | 6.40 ± 0.30        |
|                                 | <b>115</b>              | 100           | 9.40± 0.17                    | 8.83±0.28            | 8.40±0.40        | 8.46±0.20          |
|                                 |                         | 50            | 8.33±0.11                     | 8.33±0.11            | 8.23±0.35        | 7.83±0.42          |
|                                 |                         | 25            | 7.27± 0.05                    | 7.16± 0.06           | 7.03±0.55        | 7.43±0.23          |
|                                 |                         | 12.5          | 7.10±0.00                     | 6.50±0.30            | 6.50±0.15        | 7.20±0.18          |
|                                 | <b>116</b>              | 100           | 11.67±0.57                    | 12.00 ±0.10          | 7.40±0.10        | 10.33±0.50         |
|                                 |                         | 50            | 10.17±0.55                    | 11.33±0.52           | 7.23±0.65        | 9.67±0.57          |
|                                 |                         | 25            | 9.33±0.34                     | 10.00±0.25           | 7.03±0.15        | 9.00±0.50          |
|                                 |                         | 12.5          | 8.67±0.57                     | 19.00±1.0            | 6.50±0.05        | 8.50±0.50          |
|                                 |                         | 100           | 10.00±0.5                     | 9.00±0.60            | 6.30±0.0         | 10.43±0.52         |

|            |      |           |           |          |           |
|------------|------|-----------|-----------|----------|-----------|
|            | 50   | 9.83±0.26 | 8.33±0.22 | 6.20±0.0 | 9.63±0.32 |
| <b>117</b> | 25   | 8.50±0.40 | 7.33±0.52 | NA       | 8.00±0.30 |
|            | 12.5 | 7.00±0.70 | 6.70±0.50 | NA       | 7.20±0.45 |

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The EtOAc extract of the leaves of *C. prophetarum* exhibited better inhibition zone against *E. coli* and *S. aureus* with IZD of 10.86± 0.11 mm and 8.96±0.24 mm at 400 µg/mL, respectively (Table 28). The inhibition zones of the isolated compounds displayed a wide range of antibacterial activity with inhibition zones ranging from NA to 12.00 ±0.10 mm. Among the compounds evaluated for their antibacterial activity, compound **116** displayed IZD of 11.67±0.57, 12.00 ±0.10 and 10.33±0.50 mm against *E. coli*, *P. aeruginosa* and *S. pyogenes* at 100 µg/mL, respectively (Table 28). At the same concentration, compound **117** exhibited IZDs of 10.00±0.5, 9.00±0.60 and 10.43±0.52 mm against *E. coli*, *P. aeruginosa* and *S. pyogenes*, respectively.

The comparison of antibacterial activities of the extracts and isolated compounds of the three plants were done with related work from literature. One of the reported antibacterial activities of the ethyl acetate extracts of the flower of *Cucumis sativus* against *S. typhi* and *E. coli* exhibited zone inhibition diameter of 0 mm, 7 mm for 10 mg/mL and 10 mm, 11 mm for 30 mg/mL respectively using disc diffusion method (Muruganantham, N., *et al.*, 2016). While our work result showed zone inhibition diameter of the range 0-15 mm for concentration range 12.5-400 µg/mL, using disc diffusion method. This clarified that the inhibition zone displayed by the extracts and isolated compounds in the present work was superior compared with the antibacterial activity reported in the literature for sister species, named as *Cucumis sativus* (Muruganantham, *et al.*, 2016). Further comparison was also made with the seed extract of *Cucumis melo* at different concentrations (10 mg, 20 mg and 30 mg /mL) against pathogenic bacterial strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* (Gnanasingh, D., *et al.*, 2017). The literature report revealed zone inhibition diameter of 0 mm for 10 mg/mL and 12 mm, 11 mm, 10 mm for 20 mg/mL against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* respectively. This work showed zone of inhibition diameter of the range 13.67±0.57, 15.0 ±0.10 and 13.33±0.50 mm against *E. coli*, *P. aeruginosa* and *S. pyogenes*, respectively for some the compounds (**58**) for concentration range 12.5-100 µg/mL. Interestingly better antibacterial activity was also

obtained in this work even at lower concentration against similar bacterial strain compared with the extract of *Cucumis melo*.

#### 4.3.2 Determination of the Minimum Inhibitory Concentration (MIC)

In the present study, the MIC of the isolated compounds was determined by using serial dilution technique. The results obtained revealed that compound **58** showed highest activity against tested microorganisms (Table 29). This demonstrated that the *in vitro* antibacterial activity and the MIC are in good agreement.

**Table 29:** The MIC ( $\mu\text{g/mL}$ ) values of isolated compounds

| Compounds     | Bacterial species |                      |                  |                    |
|---------------|-------------------|----------------------|------------------|--------------------|
|               | <i>E. coli</i>    | <i>P. aeruginosa</i> | <i>S. aureus</i> | <i>S. pyogenes</i> |
| <b>24</b>     | 3.12              | 6.12                 | 25               | 6.25               |
| <b>26</b>     | 3.12              | 3.12                 | —                | 25.0               |
| <b>58</b>     | 3.12              | 3.12                 | 6.25             | 3.12               |
| <b>102</b>    | 3.12              | 3.12                 | 25.0             | 12.5               |
| <b>104</b>    | 6.5               | 25                   | —                | 6.25               |
| <b>105</b>    | 25                | 25                   | 50               | 25                 |
| <b>106</b>    | 6.25              | 6.25                 | —                | 6.25               |
| <b>107</b>    | 12.5              | 12.5                 | 25               | 100                |
| <b>108</b>    | 100               | —                    | —                | —                  |
| <b>109</b>    | 25                | 25                   | —                | —                  |
| <b>110</b>    | 6.25              | 12.5                 | 50.0             | 12.5               |
| <b>111</b>    | 12.5              | 12.5                 | —                | 12.5               |
| <b>112</b>    | 3.12              | 3.12                 | 50.0             | 3.12               |
| <b>113</b>    | 12.5              | 12.5                 | —                | 25.0               |
| <b>114</b>    | 6.25              | 6.25                 | —                | 12.5               |
| <b>115</b>    | 6.25              | 12.5                 | 12.5             | 6.25               |
| <b>116</b>    | 6.25              | 6.25                 | 12.5             | 6.25               |
| <b>117</b>    | 6.25              | 6.25                 | 50               | 12.5               |
| Ciprofloxacin | 0.78              | 0.78                 | 0.78             | 0.78               |

The findings presented as minimum inhibition concentrations (MIC) indicated that the isolated compounds possess varying antimicrobial potencies (Tables 29). The lowest MIC value of

3.12 µg/mL was recorded with compound **24**, **26**, **58**, **102** and **112** against *E. coli*, *P. aeruginosa* and *S. pyogenes*. According to Rios and Recio (2005), plant extracts that are recommended in the drug discovery initiatives are those with MIC values less than 1 mg/mL (Rios, J., and Recio, M., 2005). Thus, our MIC value shows better results that is in good agreement with *in vitro* antibacterial activity and molecular docking result and recommended in the drug discovery initiatives as the MIC values is less than 1 mg/mL for more investigation.

#### 4.3.3 Cytotoxicity Analysis.

To investigate cytotoxicity activity, MCF-7 breast cancer cell lines were treated with the *n*-hexane, EtOAc and methanol extracts of the leaves, stems and roots of *C. ficifolius* and fruit and stems of *C. dipsaceus* at concentrations 20 µg/mL, and their viability was evaluated by MTT assay. The results revealed that the methanol and *n*-hexane extracts of leaves of *C. ficifolius* and the methanol extracts of fruit of *C. dipsaceus* induced a significant reduction of cell growth in breast cancer cell lines (Table 30). The EtOAc extract of the stem of *C. dipsaceus* gives the highest potency relative to the other samples at low micromolar concentrations (IC<sub>50</sub> = 10.41 µg/mL) and the methanol extract of the leaves of *C. ficifolius* is the second with IC<sub>50</sub> value of 10.48 µg/mL compared to the other samples.

**Table 30:** Cytotoxicity of extracts of some *Cucumis* species against breast cancer cell lines MCF-7 (IC<sub>50</sub> in µg/mL)

| Plant name           | parts  | Extract          | Conc.<br>(µg/mL) | % Cell viability | IC <sub>50</sub><br>µg/mL | in |
|----------------------|--------|------------------|------------------|------------------|---------------------------|----|
| <i>C. ficifolius</i> | Stems  | <i>n</i> -hexane | 20               | 75.58±4.41       | 13.64                     |    |
|                      |        | EtOAc extract    | 20               | 79.25±2.69       | 19.18                     |    |
|                      |        | Methanol         | 20               | 91.36±2.55       | 13.75                     |    |
|                      | Leaves | <i>n</i> -hexane | 20               | 71.73±3.32       | 15.1                      |    |
|                      |        | Methanol         | 20               | 68.32±2.73       | 10.48                     |    |
|                      | Roots  | <i>n</i> -hexane | 20               | 83.83±3.29       | 13.40                     |    |
|                      |        | EtOAc extract    | 20               | 94.14±3.49       | 16.22                     |    |
|                      |        | Methanol         | 20               | 75.06±2.81       | 12.93                     |    |

|                     |             |                 |    |            |       |
|---------------------|-------------|-----------------|----|------------|-------|
| <i>C. dipsaceus</i> | Fruits      | Petroleum ether | 20 | 81.86±3.04 | 17.48 |
|                     |             | EtOAc           | 20 | 59.12±3.70 | 16.05 |
|                     |             | Methanol        | 20 | 48.36±2.48 | 16.81 |
|                     | Stems       | Petroleum ether | 20 | 38.20±2.27 | 11.84 |
|                     |             | EtOAc           | 20 | 34.91±2.25 | 10.41 |
|                     |             | Methanol        | 20 | 37.55±2.83 | 11.35 |
|                     | Doxorubicin |                 | 20 | 15.12±2.8  | 7.06  |

#### 4.3.4 Radical Scavenging Activity

DPPH is a simple method used to test the antioxidant activity of extracts and isolated compounds by measuring the reduction of absorbance at 517 nm. Extracts and compounds having radical scavenging activity reduce absorbance at 517 nm which is in fact due to the disappearance of the radical. Once the radical is scavenged by the antioxidants, the absorbance at 517 nm is reduced. Hence, in the present study, the radical scavenging activity of the extracts and isolated compounds were assessed using DPPH. The results are given below (Table 31).

**Table 31:** Radical scavenging effect of the extracts and isolated compounds of *C. ficifolius*.

| Plant name           | Parts  | Extract or compound | Conc. (µg/mL) | % Inhibition | IC <sub>50</sub> in µg/mL |
|----------------------|--------|---------------------|---------------|--------------|---------------------------|
| <i>C. ficifolius</i> | Leaves | EtOAc extract       | 100           | 85.24        | 13.4                      |
|                      |        |                     | 50            | 63.66        |                           |
|                      |        |                     | 25            | 59.38        |                           |
|                      |        |                     | 12.5          | 46.34        |                           |
|                      |        | Methanol Extract    | 100           | 72.76        | 22.95                     |
|                      |        |                     | 50            | 61.24        |                           |
|                      |        |                     | 25            | 50.10        |                           |
|                      |        |                     | 12.5          | 45.59        |                           |

|  |       |                         |      |       |       |
|--|-------|-------------------------|------|-------|-------|
|  | Stems | Petroleum ether extract | 100  | 72.41 | 24.16 |
|  |       |                         | 50   | 62.41 |       |
|  |       |                         | 25   | 54.48 |       |
|  |       |                         | 12.5 | 40.31 |       |
|  |       | EtOAc extract           | 100  | 79.66 | 26.65 |
|  |       |                         | 50   | 63.93 |       |
|  |       |                         | 25   | 54.07 |       |
|  |       |                         | 12.5 | 37.58 |       |
|  |       | Methanol Extract        | 100  | 77.52 | 15.78 |
|  |       |                         | 50   | 67.34 |       |
|  |       |                         | 25   | 59.31 |       |
|  |       |                         | 12.5 | 40.86 |       |
|  | Fruit | Petroleum ether extract | 100  | 75.72 | 10.19 |
|  |       |                         | 50   | 65.03 |       |
|  |       |                         | 25   | 56.66 |       |
|  |       |                         | 12.5 | 47.17 |       |
|  |       | EtOAc extract           | 100  | 73.52 | 18.74 |
|  |       |                         | 50   | 65.31 |       |
|  |       |                         | 25   | 55.86 |       |
|  |       |                         | 12.5 | 41.72 |       |
|  |       | Methanol                | 100  | 75.38 | 18.12 |
|  |       |                         | 50   | 59.00 |       |
|  |       |                         | 25   | 51.79 |       |

|  |               |                                         |      |       |       |
|--|---------------|-----------------------------------------|------|-------|-------|
|  |               |                                         | 12.5 | 48.97 |       |
|  | Roots         | Petroleum ether<br>and EtOAc<br>extract | 100  | 81.86 | 10.32 |
|  |               |                                         | 50   | 70.14 |       |
|  |               |                                         | 25   | 58.72 |       |
|  |               |                                         | 12.5 | 45.38 |       |
|  |               | Methanol                                | 100  | 83.10 | 16.17 |
|  |               |                                         | 50   | 70.07 |       |
|  |               |                                         | 25   | 55.97 |       |
|  |               |                                         | 12.5 | 43.34 |       |
|  | Roots         | <b>24</b>                               | 100  | 81.1  | 7.24  |
|  |               |                                         | 50   | 71.76 |       |
|  |               |                                         | 25   | 63.17 |       |
|  |               |                                         | 12.5 | 43    |       |
|  |               | <b>26</b>                               | 100  | 80.86 | 16.16 |
|  |               |                                         | 50   | 67.79 |       |
|  |               |                                         | 25   | 52.83 |       |
|  |               |                                         | 12.5 | 46.48 |       |
|  | Stems, Leaves | <b>58</b>                               | 100  | 71.52 | 11.30 |
|  | Root & Fruits |                                         | 50   | 59.55 |       |
|  |               |                                         | 25   | 56.48 |       |
|  |               |                                         | 12.5 | 48.97 |       |
|  | Stems         |                                         | 100  | 69.72 | 39.91 |
|  |               |                                         | 50   | 56.41 |       |

|               |     |      |       |       |       |       |
|---------------|-----|------|-------|-------|-------|-------|
|               |     | 104  | 25    | 46.0  |       |       |
|               |     |      | 12.5  | 37.66 |       |       |
|               |     | 105  | 100   | 64.14 | 47.66 |       |
|               |     |      | 50    | 54.21 |       |       |
|               |     |      | 25    | 45.55 |       |       |
|               |     |      | 12.5  | 35.14 |       |       |
|               |     |      | 106   | 100   | 76.28 | 27.31 |
|               |     |      |       | 50    | 65.66 |       |
|               |     |      |       | 25    | 50.07 |       |
|               |     |      |       | 12.5  | 39.45 |       |
|               |     | 107  | 100   | 73.24 | 25.34 |       |
|               |     |      | 50    | 59.38 |       |       |
|               |     |      | 25    | 50.14 |       |       |
|               |     |      | 12.5  | 44.83 |       |       |
|               |     | 108  | 100   | 75.38 | 24.47 |       |
|               |     |      | 50    | 62.28 |       |       |
|               |     |      | 25    | 54.00 |       |       |
|               |     |      | 12.5  | 40.66 |       |       |
|               | 109 | 100  | 85.52 | 4.83  |       |       |
|               |     | 50   | 73.86 |       |       |       |
|               |     | 25   | 62.79 |       |       |       |
|               |     | 12.5 | 45.97 |       |       |       |
| Ascorbic acid |     |      | 12.5  | 98%   | 2.31  |       |

Results give above revealed (Table 31) the radical scavenging activity of the extracts and isolated compounds to increase with increasing concentration in a dose dependent manner. The EtOAc extract of the leaves, Petroleum ether extract of fruit and Petroleum ether and EtOAc extract of roots of *C. ficifolius* inhibited the DPPH radical by 85.24%, 75.72% and 81.86% with IC<sub>50</sub> values of 13.4, 10.19 and 10.32 µg/mL, respectively. Compound **109** had radical scavenging activity and IC<sub>50</sub> value of 85.52% and 4.83 µg/mL, respectively. The activity displayed by compound **109** might be due to the presence of hydroxy groups of the glycans. Hence, the activity displayed by the extracts of the fruit of *C. ficifolius* might be due to compound **109**.

**Table 32:** Radical scavenging effect of the extracts and isolated compounds from fruit extract of *C. dipsaceus*

| Plant name and its part      | Extract or compound     | Conc. (µg/mL) | % Inhibition | IC <sub>50</sub> (µg/mL) |
|------------------------------|-------------------------|---------------|--------------|--------------------------|
| Fruit of <i>C. dipsaceus</i> | Petroleum ether extract | 100           | 60.59        | 18.28                    |
|                              |                         | 50            | 57.31        |                          |
|                              |                         | 25            | 50.82        |                          |
|                              |                         | 12.5          | 47.72        |                          |
|                              | EtOAc extract           | 100           | 74.41        | 9.41                     |
|                              |                         | 50            | 66.59        |                          |
|                              |                         | 25            | 58.82        |                          |
|                              |                         | 12.5          | 44.75        |                          |
|                              | Methanol extract        | 100           | 78.37        | 7.03                     |
|                              |                         | 50            | 68.24        |                          |
|                              |                         | 25            | 59.96        |                          |
|                              |                         | 12.5          | 46.03        |                          |
|                              |                         | 100           | 67.52        |                          |

|  |                   |      |       |       |
|--|-------------------|------|-------|-------|
|  | <b>26 and 100</b> | 50   | 60.07 | 8.84  |
|  |                   | 25   | 57.93 |       |
|  |                   | 12.5 | 45.93 |       |
|  | <b>102</b>        | 100  | 70.27 | 8.95  |
|  |                   | 50   | 65.38 |       |
|  |                   | 25   | 53.65 |       |
|  |                   | 12.5 | 48.14 |       |
|  | <b>110</b>        | 100  | 60.95 | 48.97 |
|  |                   | 50   | 52.7  |       |
|  |                   | 25   | 46.09 |       |
|  |                   | 12.5 | 38.24 |       |
|  | <b>111</b>        | 100  | 61.55 | 45.43 |
|  |                   | 50   | 54.55 |       |
|  |                   | 25   | 46.83 |       |
|  |                   | 12.5 | 38.48 |       |
|  | <b>112</b>        | 100  | 68.14 | 7.99  |
|  |                   | 50   | 61.1  |       |
|  |                   | 25   | 55.1  |       |
|  |                   | 12.5 | 48.34 |       |
|  | <b>113</b>        | 100  | 78.72 | 7.09  |
|  |                   | 50   | 69.06 |       |
|  |                   | 25   | 60.58 |       |
|  |                   | 12.5 | 45.31 |       |

|               |     |      |       |       |
|---------------|-----|------|-------|-------|
|               | 114 | 100  | 68.23 | 37.31 |
|               |     | 50   | 56.71 |       |
|               |     | 25   | 48.23 |       |
|               |     | 12.5 | 38.85 |       |
| Ascorbic acid |     | 12.5 | 98%   | 2.31  |

Results depicted in Table 32 demonstrated that the radical scavenging activity of the extracts and isolated compounds increases in a dose dependent manner. The methanol extract of fruits inhibited the DPPH radical by 78.37 % with IC<sub>50</sub> values of 7.03 µg/mL. Compound **26**, **100**, **102**, **112** and **113** had IC<sub>50</sub> value of 8.84, 8.95, 7.99 and 7.09 µg/mL, respectively.

#### 4.4 *In silico* Molecular Docking Studies of Isolated Compounds

Molecular docking studies were performed in order to predict the interaction of isolated compounds with DNA-gyrase, which is required for maintenance of proper DNA topology during transcription and replication (Trott, O., *et al.*, 2010). The interactions of the isolated compounds were also studied against KSR2 (PDB ID:7JUT) and human topoisomerase IIβ. Results obtained from the molecular docking study demonstrated that more compounds had shown good binding affinity against *E. coli* DNA Gyrase (PDB ID:6F86), *S. aureus* DNA Gyrase (PDB ID:2XCT), KSR2 (PDB ID:7JUT) and human topoisomerase IIβ (PDB ID:3QX3) proteins.

*E. coli* DNA gyrase is an enzyme that is required for bacterial DNA replication and transcription to maintain topology and integrity (Janupally, R., *et al.*, 2015). Currently, DNA gyrase is considered as one of the primary targets and has been clinically validated in most pathogenic bacteria (Narramore, S., *et al.*, 2019). In this study, *E. coli* DNA gyrase (PDB ID:6F86) was used as a target and ciprofloxacin was used as a reference drug for docking. Binding affinity and interactions with of the isolated compounds against *E. coli* DNA gyrase were presented in Table 33. The 3D and 2D binding interactions were depicted in Figures 21.

**Table 33:** Binding affinity and interaction of isolated compounds with *E. coli* DNA gyrase

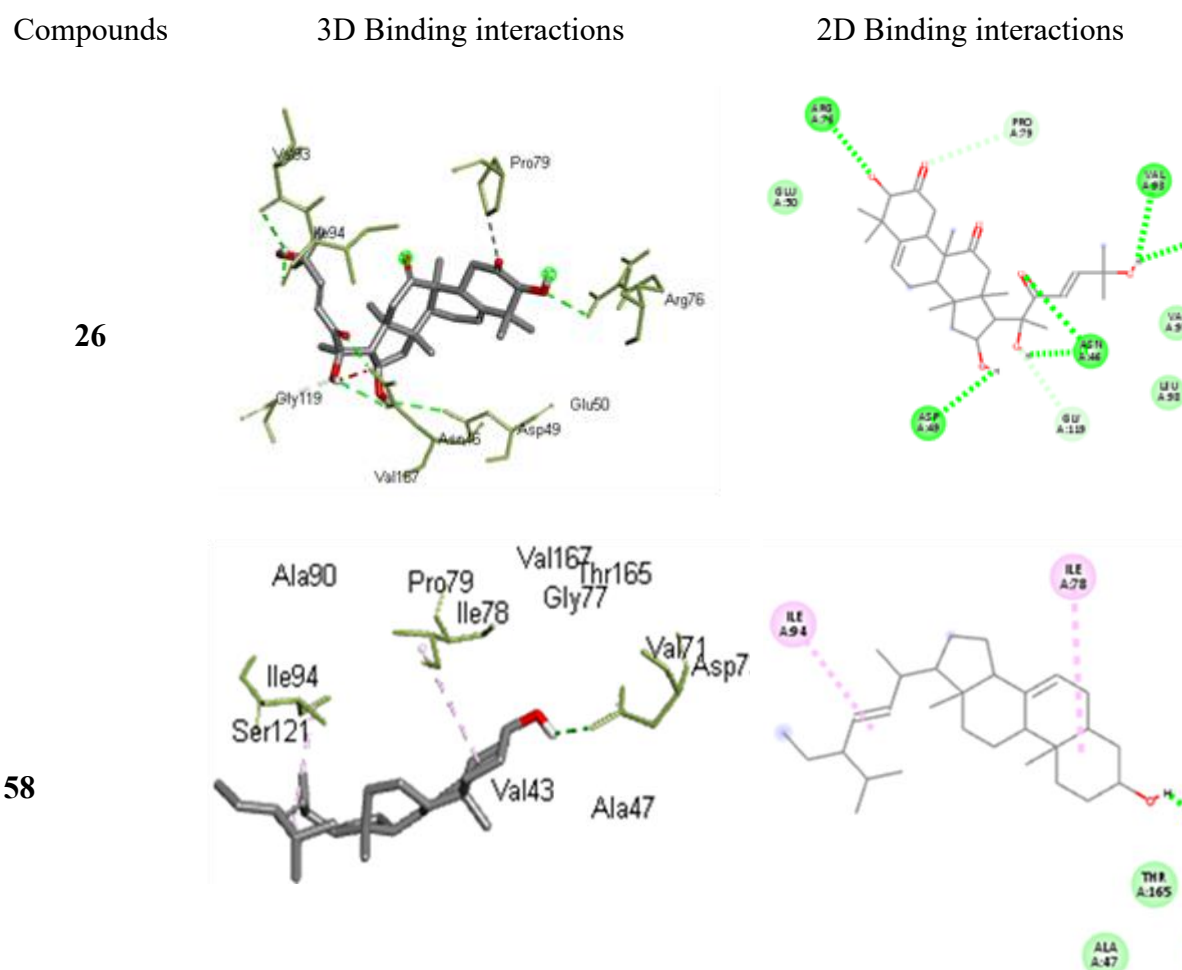
| Compound      | $\Delta G_{\text{binding}}$<br>(kcal/mol) | H-bond                                                    | Residual Amino acid Interactions                      |                                                 |
|---------------|-------------------------------------------|-----------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------|
|               |                                           |                                                           | Hydrophobic/Pi-Cation/Pi-Anion/ Pi-Alkyl interactions | Van-der Walls interactions                      |
| <b>26</b>     | -7.5                                      | Asn-46, Asp-49, Arg-76, Val-93, Ile-94, Gly-119*, Pro-79* | -                                                     | Glu-50, Leu-98, Val-97                          |
| <b>58</b>     | -8.0                                      | Asp-73                                                    | Ile-94, Ile-78                                        | Gly-77, Ala-47, Thr-165                         |
| <b>100</b>    | -7.0                                      | Asn-46, Val-120, Ser-121, Leu-98                          | -                                                     | Gly-77, Glu-50, Arg-76, Ile-94, Gly-119, Leu-98 |
| <b>102</b>    | -7.0                                      | Asn-46, Arg-76, Arg-136                                   | -                                                     | Glu-50, Ile-94, Pro-79, Ile-78                  |
| <b>110</b>    | -4.8                                      | -                                                         | -                                                     | -                                               |
| <b>111</b>    | -4.7                                      | -                                                         | -                                                     | -                                               |
| <b>112</b>    | -7.6                                      | Asn-46, Asp-73, Gly-117, Thr-165                          | Ile-78                                                | Gly-77, Glu-50, Arg-76, Pro-79, Ile-94          |
| <b>113</b>    | -7.1                                      | Asn-46                                                    | Ile-94                                                | Glu-50, Pro-79, Arg-136, Ile-78                 |
| <b>114</b>    | -5.1                                      | -                                                         | -                                                     | -                                               |
| Ciprofloxacin | -7.3                                      | Asn-46, Asp-73, Asp-49*                                   | Pro-79, Asn-46, Asp-49                                | Ala-47, Gly-77, Glu-50, Arg-76, Ile-78          |

\*Carbon Hydrogen bond

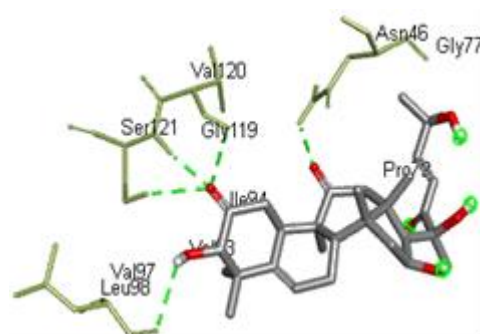
Among the tested compounds, compounds **58** (-8.0 kcal/mole), **112** (-7.6 kcal/mole) and **26** (-7.5 kcal/mole) showed higher binding affinity compared to ciprofloxacin (-7.3 kcal/mole). Ciprofloxacin formed hydrogen bonding interactions with Asn-46 and Asp-73. Similarly, compound **100** showed H-bonding interactions with these two amino acids along with Gly-117 and Thr-165. Compounds **26**, **100** and **102** showed H-bonding interactions with Asn-46 whereas, compounds **112** and **58** interacts only with Asn-46 and Asp-73, respectively. Most of

the compounds exhibited Van der Waals interactions with Glu-50 and Gly-77. Since compounds **110**, **111** and **114** showed less binding affinity ( $< -5.2$  kcal/mol), their interactions are not presented.

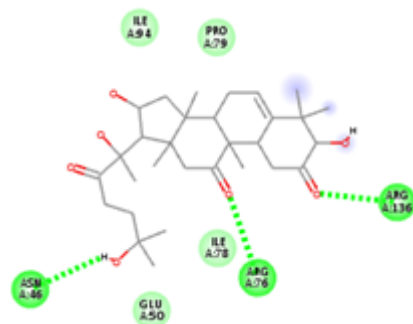
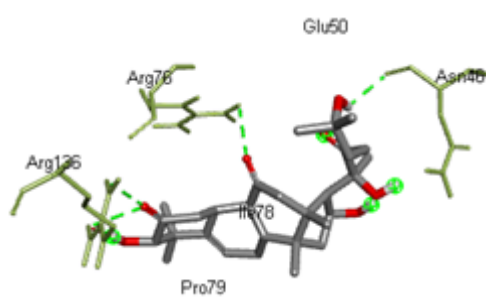
The 3D and 2D binding interactions of the isolated compounds against *E. coli* DNA gyrase were depicted in Figures 21. Hydrogen bond between compound and amino acids are shown as green dash lines, hydrophobic interactions are shown as pink lines. Electrostatic interaction is shown as orange line.



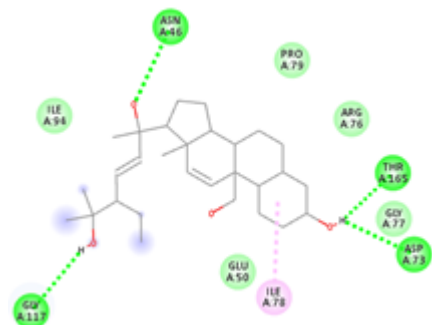
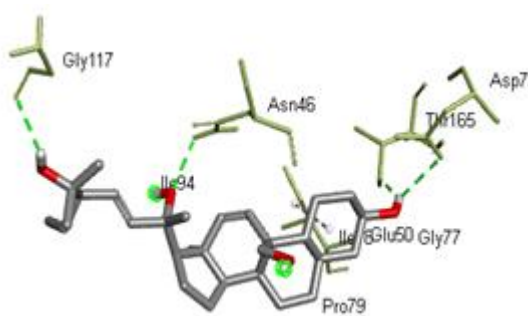
100



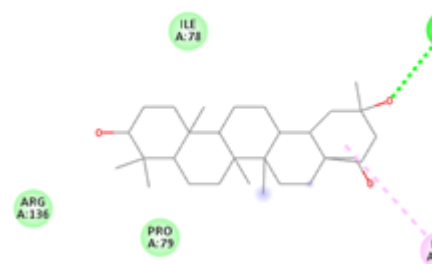
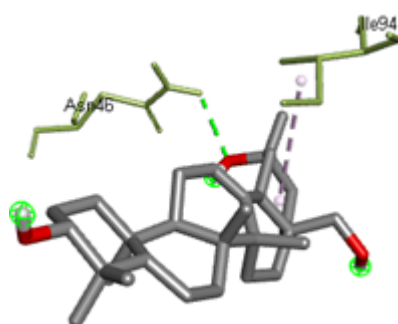
102



112



113



Ciprofloxacin

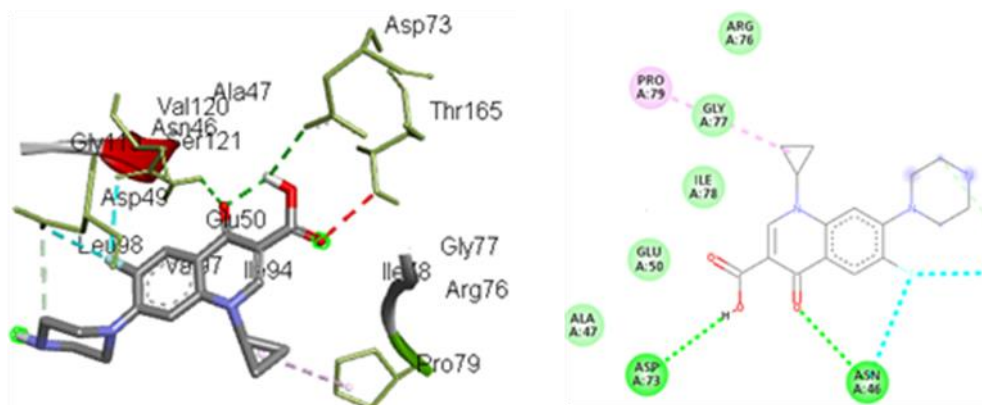


**Figure 21:** The binding interactions (2D & 3D) interactions of compounds and ciprofloxacin with *E. coli* DNA gyrase (PDB ID: 6F86).

Pyruvate kinase (PK) has been identified as a crucial enzyme in staphylococci and regulates their growth, antibiotic resistance, and biofilm formation (Vasu, D., *et al.*, 2015). It was also found to be structurally distinct from human homologs, and hence it provides a promising target for novel antimicrobial agents (Akunuri, R. *et al.*, 2002). For the docking studies, *S. aureus* PK was used as a target and ciprofloxacin was used as a reference control. Binding affinity and interactions with different amino acids are presented in Table 34, and 3D and 2D binding interactions are depicted in Figure 22.

**Table 34:** Molecular docking results of isolated compounds against *S. aureus* DNA gyrase B

| SNo.       | Binding Affinity (kcalmol <sup>-1</sup> ) | H-bond                                                                                                                                                                     | Residual interactions                                                                       |               |                                                                                   |
|------------|-------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|---------------|-----------------------------------------------------------------------------------|
|            |                                           |                                                                                                                                                                            | Hydrophobic, and others                                                                     | Electrostatic | Van der Waals                                                                     |
| <b>24</b>  | - 9.1                                     | Arg-1122, DA-11, DT-10, DC-12 (Dist. 2.70382 Å), DC-12 (Dist. 2.93235 Å)                                                                                                   | —                                                                                           |               | Glu-1088, Tyr-1093, Ser-1084, DG-9, DT-10, Asp-1083, Arg-1122, Ala-1120, Met-1121 |
| <b>26</b>  | - 9.8                                     | DA-11, DC-12, Ser-1084 (Dist. 2.14101 Å), Ser-1084 (Dist. 2.076 Å), DG-9 (Dist. 2.3551 Å), DG-9 (Dist. 2.79485 Å), DT-10 (Dist. 2.66453 Å), DT-10 (Dist. 3.6341 Å), UNK1:O | —                                                                                           |               | Glu-1088, Ala-1120, Arg-1122, Met-1121, DT-10, Tyr-1093, Mn-2000, Asp-1083        |
| <b>58</b>  | - 8.4                                     | Ala-1118                                                                                                                                                                   | (Hydrophobic-Pi-Alkyl-) Ala-1120 (Dist. 3.83568 Å)<br>Met-1121(Dist. 3.72231 Å)<br>Tyr-1093 |               | Ala-1119, Ser-438, DG-9, DT-10, Arg-1122, Ser-1084, Asp-1083                      |
| <b>100</b> | -5.5                                      | Ser-362, Thr-366, His-365*, Thr-353*                                                                                                                                       | -                                                                                           |               | -                                                                                 |

|            |       |                                                                                                       |                                                                                                                                                                                                                                             |                                                                              |
|------------|-------|-------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------|
| <b>102</b> | -5.0  | Ser-362, Thr-366, Thr-353, Ser-354                                                                    | -                                                                                                                                                                                                                                           | -                                                                            |
| <b>104</b> | - 5.6 | Arg-1122 (Dist. 2.23926 Å), Arg-1122 (Dist. 2.30393 Å), DG-9 (Dist. 3.0202 Å), DG-9 (Dist. 3.57409 Å) | Hydrophobic-Alkyl- Ala-1120(Dist. 4.59496 Å)<br>Hydrophobic-Alkyl- Ala-1120(Dist. 4.72431 Å)<br>Hydrophobic-Alkyl- Ala-1120(Dist. 4.40521 Å)<br>Hydrophobic-Alkyl- Met-1121<br>Hydrophobic-Pi-Alkyl- Tyr-1093<br>Hydrophobic-Pi-Alkyl- DG-9 | Asp-1083, Met-1121, Arg-1122, DT-10, Ser-1084, Glu-1088                      |
| <b>105</b> | - 5.5 | —                                                                                                     | Hydrophobic-Pi-Sigma- DT-8<br>Hydrophobic-Alkyl- Arg-458(Dist. 4.03348 Å)<br>Hydrophobic-Pi-Alkyl- DA-13<br>Hydrophobic-Pi-Alkyl- Phe-1123(Dist. 4.01906 Å)<br>Hydrophobic-Pi-Alkyl- DT-8(Dist. 5.33207 Å)                                  | DC-12, Ser-438, Ala-439, Gly-584, Gly-436, Arg-1122, Asp-437, Gly-459, DT-10 |

|            |       |                                                                               |                                                                                                                                                                                                                                                  |                                                                                                           |
|------------|-------|-------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|
|            |       |                                                                               | Hydrophobic-Pi-Alkyl- DG-9(Dist. 5.2356 Å)                                                                                                                                                                                                       |                                                                                                           |
| <b>106</b> | – 6.3 | DG-9 (Dist. 2.54359 Å), DG-9 (Dist. 2.34835 Å)                                | Hydrophobic-Alkyl- Ala-439;<br>Hydrophobic-Alkyl- Arg-458;<br>Hydrophobic-Pi-Alkyl- DG-9;<br>Hydrophobic-Pi-Alkyl- Phe 1123(Dist. 4.2691 Å);<br>Hydrophobic-Pi-Alkyl- Phe-1123(Dist. 4.15503 Å);<br>Hydrophobic-Pi-Alkyl- DT-8(Dist. 4.01375 Å); | Ser-438, Gly-436,<br>Asp-437, Gly-584,<br>Arg-1122, Gly-459,<br>DT-10, Gly-582, Glu-585, Mn-2492, Asp-508 |
| <b>107</b> | – 6.6 | Arg-1122, DA-11, DC-12 (Dist. 3.03043 Å), DC-12 (Dist. 2.80861 Å)             | ; Hydrophobic-Alkyl- Ala-1120; Hydrophobic-Alkyl- Met-1121(Dist. 4.99344 Å)                                                                                                                                                                      | DT-10, Asp-1083,<br>Ser-438, Phe-1123,<br>Asp-437, Ser-1084                                               |
| <b>108</b> | – 6.5 | Gln-480, Pro-456, DC-12, Arg-458 (Dist. 1.81966 Å), Arg-458 (Dist. 2.48834 Å) | Hydrophobic-Pi-Sigma- DT-8;<br>Hydrophobic-Alkyl- Lys-417;<br>Pro-456, Arg-458(Dist. 4.11469 Å); Hydrophobic-Pi-Alkyl- DA-13                                                                                                                     | Gly-459, Leu-455,<br>DT-10, Asp-437, DA-11                                                                |
| <b>109</b> | – 8.4 | DC-5, DC-14, DG-4 (Dist. 2.37329 Å), DG-4 (Dist. 2.29755 Å), DG-              | Hydrophobic-Pi-Alkyl- DG-14                                                                                                                                                                                                                      | DC-15, DC-12, DT-3,<br>DA-13, Arg-1272,<br>Arg-1092, DG-6,                                                |

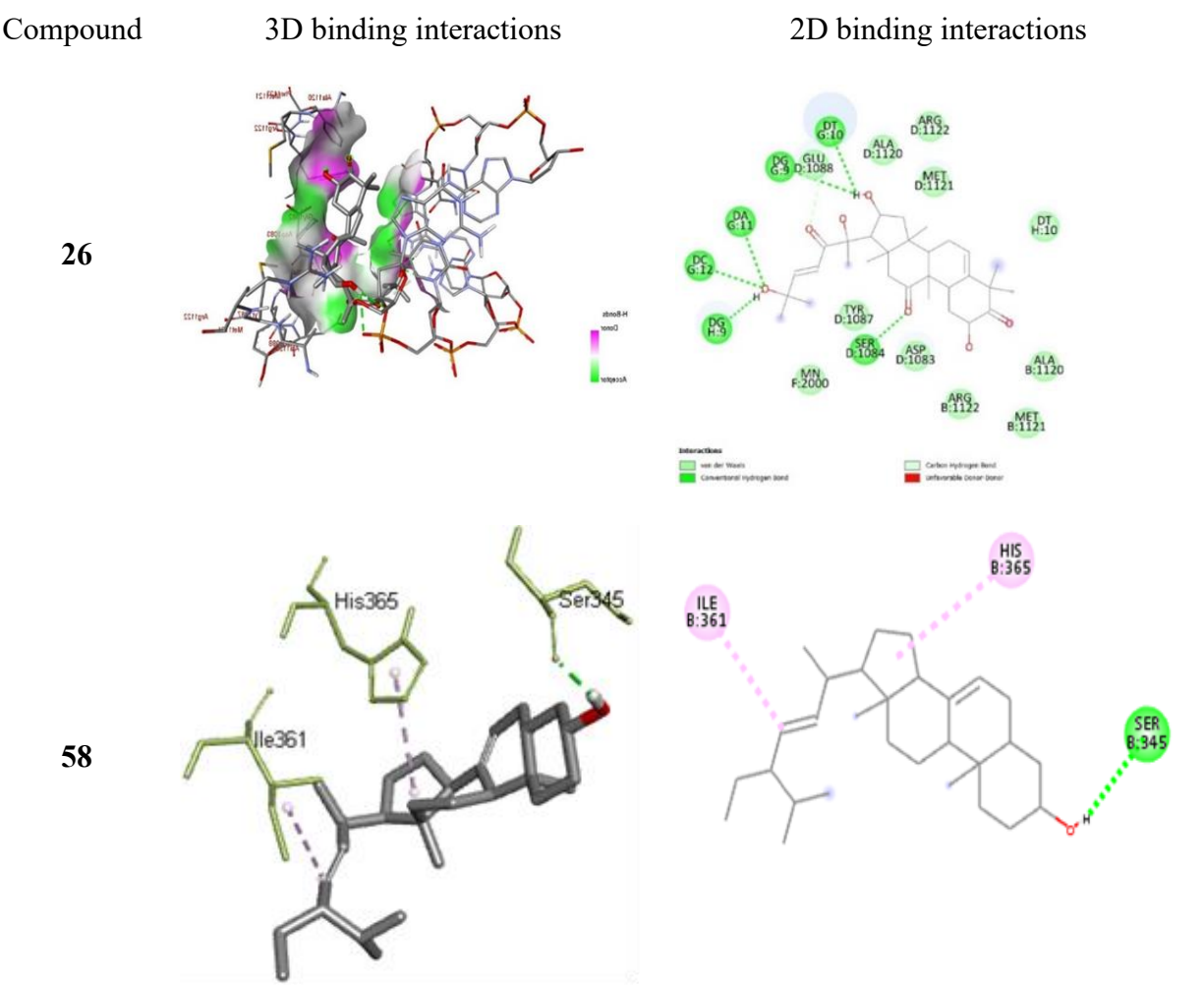
|               |       |                                                                                                                                             |                                                                                              |                                                                 |
|---------------|-------|---------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|-----------------------------------------------------------------|
|               |       | 16, DG-7, DG-9, Ser-1084, UNK1:0                                                                                                            |                                                                                              | Glu-1088, Ser-1085, DT-8                                        |
| <b>110</b>    | -2.5  | -                                                                                                                                           | -                                                                                            | -                                                               |
| <b>111</b>    | -2.8  | -                                                                                                                                           | -                                                                                            | -                                                               |
| <b>112</b>    | -5.0  | Ser-362, Asn-369                                                                                                                            | Leu-370                                                                                      | -                                                               |
| <b>113</b>    | -5.2  | Ser-362                                                                                                                                     | Ile-361, Ala-358                                                                             |                                                                 |
| <b>114</b>    | -3.3  | -                                                                                                                                           | -                                                                                            | -                                                               |
| ciprofloxacin | -4.9  | Ser-359, Thr-366, Asn-369                                                                                                                   | Ile-361                                                                                      | His-365                                                         |
| doxycycline   | -13.0 | Ser-438, Ser-1084: HN, Ser-1084: HG, Ser-1084: CB, Arg-1122: HH12 (Dist. 2.84177 Å), Arg-1122: HH12 (Dist. 2.50931 Å), Arg-1122: HH22, DG-9 | Electrostatic-Attractive Charge-Asp-1083<br>Hydrophobic-Pi-Alkyl-) Ala-1120(Dist. 4.00436 Å) | Arg-1122, Met-1121, Phe-1123, Asp-437, DG-9, DT: G-10, DT: H-10 |

DA = deoxyadenosine; DG = deoxyguanosine; DT = deoxythymidine; DC = Deoxycytidine.

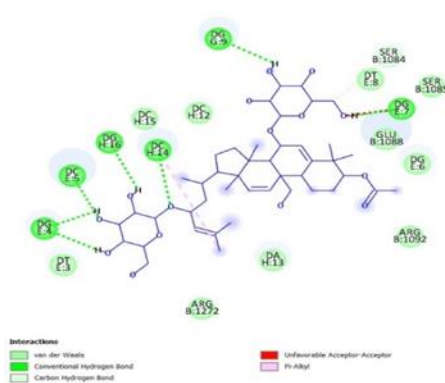
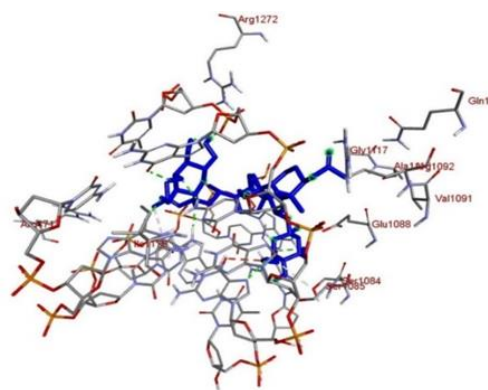
The isolated compounds exhibited binding affinities ranging from - 2.5 to - 9.8 kcal/mol against *S. aureus* DNA gyrase B. Lignoceric acid (**104**) showed hydrogen bonding interaction with Ala-1120, Met-1121, Tyr-1093 while  $\alpha$ -spinasterol (**58**) showed hydrogen bonding interaction with Ala-1118. Cucurbitacin B (**24**) showed hydrogen bonding with Arg-1122, cucurbitacin D (**26**) with Ser-1084, 12-hydroxyoctacosanoic acid (**100**) with Arg-1122, 13-hydroxyoctadecanoic acid (**101**) with Gln-480, Pro-456, Arg-458 and compound **109** with Ser-1084. Lignoceric acid showed hydrophobic interactions with Asp-1083, Met-1121, Arg-1122, Ser-1084

and Glu-1088 while  $\alpha$ -spinasterol (**58**) showed interactions with Ala-1120, Met-1121, Tyr-1093, eicosane (**105**) with Arg-458, Phe-1123; Stearic acid (**106**) with- Ala-439, Arg-458, Phe-1123, 12-hydroxyoctacos-9,13-dienoic acid (**107**) with Ala-1120, Ala-1120, Met-1121, Tyr-1093, 13-hydroxyoctadeca-9,11-dienoic acid (**108**) with Lys-417, Pro-456 suggesting the compounds may act as DNA gyrase B inhibitors. From the docked ligands,  $\alpha$ -spinasterol (**58**), compound **109**, cucurbitacin B (**24**) and cucurbitacin D (**26**) showed comparable binding affinity and amino acid interactions (-8.4, -8.4, -9.1, and -9.8 kcal/mol, respectively) with doxycycline (-13.0 kcal/mol).

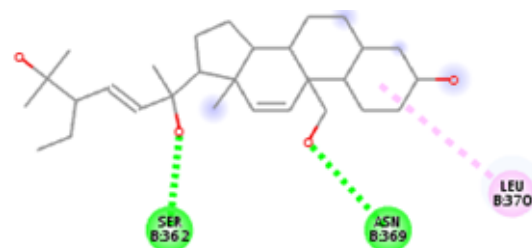
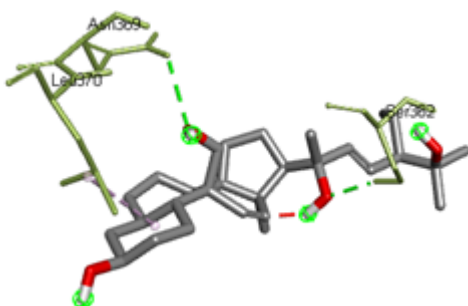
The 2D and 3D binding interactions of the isolated compounds, ciprofloxacin and doxycycline against *S. aureus* Gyrase B were depicted in Figure 22. Hydrogen bonding interactions between the isolated compounds and amino acids of the target proteins were shown as green dash lines, hydrophobic interactions are shown as pink lines. Electrostatic interaction is shown as orange line.



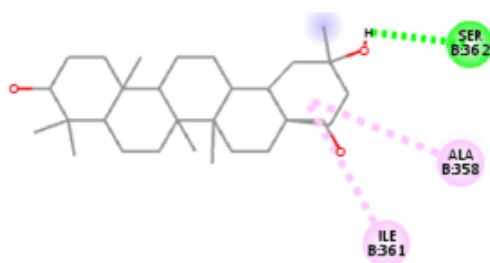
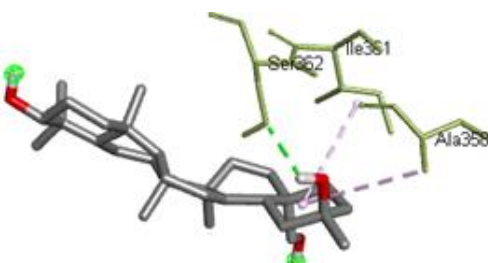
109



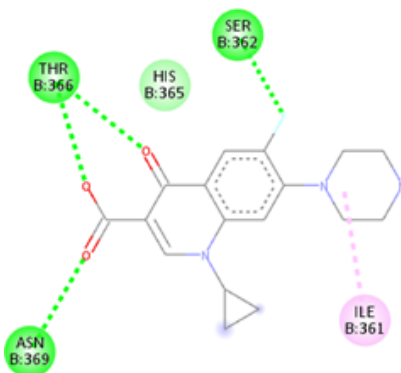
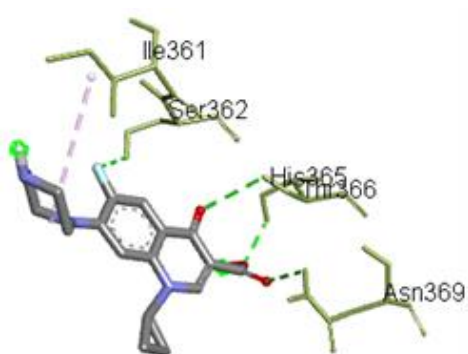
112



113



Ciprofloxacin



## Doxycycline

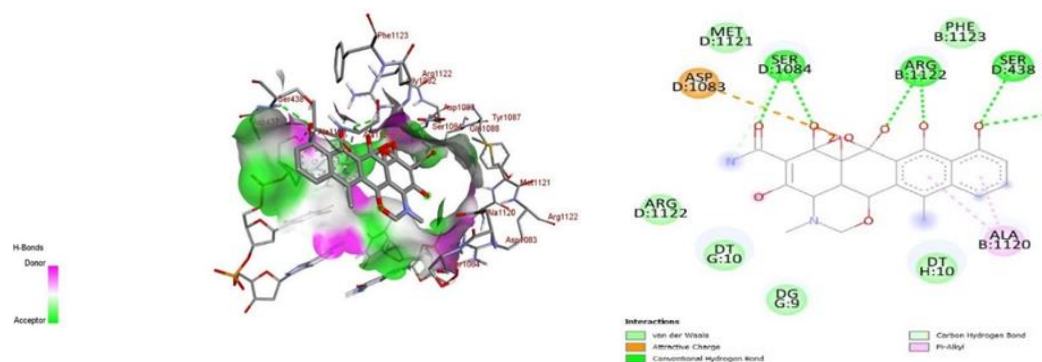


**Figure 22:** The 3D and 2D binding interactions of the isolated compounds, ciprofloxacin and doxycycline against *S. aureus* Gyrase B

The kinase suppressor of ras (KSR) proteins is molecular scaffolds that coordinate Raf/MEK/extracellular signal-regulated kinase (ERK) cascade signaling (Dougherty, M.K., 2009). In *C. elegans*, KSR2 is required for ras-mediated signaling during germ line meiotic progression and functions redundantly with KSR1 during development of the excretory system, hermaphrodite vulva, and male spicules (Ohmachi M., *et al.*, 2002). KSR1 is an essential component for ras signaling, as loss-of-function alleles restore the hyperactive ras phenotype in *Drosophila melanogaster* and *Caenorhabditis elegans* (Sundaram M, and Han M., 1995). Subsequent studies in mammalian systems showed that KSR1 and KSR2 interact with Raf, MEK, and ERK to coordinate the intensity and duration of ERK signaling (Ohmachi, M., *et al.*, 2002). Studies with mammalian KSR2 showed that it uniquely coordinates calcium-mediated ras to-ERK signaling (Ohmachi, M., *et al.*, 2002). Disruption of KSR2 in mice led to reduced expression of genes responsible for oxidative phosphorylation (OXPHOS) and deregulated 5-AMP-activated protein kinase (AMPK)-mediated processes, which led to spontaneous obesity and insulin resistance. KSR2 interacts with all AMPK subunits and promotes AMPK phosphorylation *in vitro* and *in vivo* (Costanzo, G. *et al.*, 2009).

The molecular docking study demonstrated that the isolated compounds had shown good binding affinity against the target KSR2 proteins when compared with Selumetinib and Gefitinib. The isolated compounds exhibited binding affinities ranging from - 5.6 to – 10.2 kcal/mol against the target KSR2 proteins (Figure 23 and Table 35). Cucurbitacin B (**24**) showed hydrogen bonding with amino acid residue with Arg-818, Ser-194, Lys-821, cucurbitacin D (**26**) with Arg-692, Arg-692, Asp-803, Gly-673, Ile-672;  $\alpha$ -spinasterol (**58**) showed hydrogen bonding interaction with

Phe-209; lignoceric acid (**104**) showed hydrogen bonding interaction with Phe-677, and Asp-803, stearic acid (**106**) with Arg-692; 12-hydroxyoctacos-9,13-dienoic acid (**100**) with Arg-692, Asn-791, Asp-803, Ser-749; 13-hydroxyoctadec-9,11-dienoic acid (**108**) with Arg-676, Lys-788, Gln-825, Asp-803 and kuguaglycoside E-3-acetate (**109**) with Arg-818, Lys-82, Gln-153, Thr-226, Arg-227 and Leu-74, Ser-194.

Lignoceric acid (**104**) had hydrophobic interactions with Val-680, Cys-742, Lys-790, Ile-672, Ala-690, Leu-741, Phe-793 while  $\alpha$ -spinasterol (**58**) exhibited interactions with Pro-938, Leu-215, Met-230. Stearic acid (**106**) with- Arg-692; 12-hydroxyoctacos-9,13-dienoic acid (**107**) with Val-680, Cys-742, Leu-741, Lys-790, Ile-672, Phe-793; 13-hydroxyoctadec-9,11-dienoic acid (**108**) with Val-680, Val-812, Leu-806, Lys-790, Phe-677 suggesting these compounds may act as KSR2 proteins suppressor. Cucurbitacin B (**24**), cucurbitacin D (**26**) and compound **109** does not have hydrophobic interactions with amino acid residues with target. From the docked ligands cucurbitacin B (**24**), cucurbitacin D (**26**) (Figure 23),  $\alpha$ -spinasterol (**58**) and compound **109** showed binding affinity of -10.2, -9.7, -8.4, and -8.4 kcal/mol, respectively. Especially noteworthy is the binding affinity of compounds **24** (-10.2 kcal/mol) and **26** (-9.7 kcal/mol) which showed comparable results with selumetinib (-7.7 kcal/mol) and gefitinib (-7.7 kcal/mol) suggesting the potential of these compounds for further research.

**Table 35:** Molecular docking results of isolated compounds against KSR2:MEK1

| Compounds | Binding Affinity (kcal/mol) | H-bond                                                                          | Residual interactions                                                              |                                                                                                                              |
|-----------|-----------------------------|---------------------------------------------------------------------------------|------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|
|           |                             |                                                                                 | Hydrophobic and Electrostatic                                                      | Van der Waals                                                                                                                |
| <b>24</b> | – 10.2                      | Arg-818, Ser-194, Lys-821 (Dist. 2.84075 Å), Lys-821 (Dist. 2.59188 Å), Lys-821 | —                                                                                  | Leu-74, Val-82, Val-81, Lys-192, Leu-98, Phe-223, Met-219, Ile-99, His-100                                                   |
| <b>26</b> | – 9.7                       | Arg-692 (Dist. 2.13915 Å), Arg-692 (Dist. 2.249 Å), Asp-803, Gly-673, Ile-672   | —                                                                                  | Val-680, Phe-793, Arg-745, Thr-746, Lys-790, Gly-675, Arg-676, Asn-791, Lys-788, Val-812, Asp-786, Lys-674, Phe-677, Ser-810 |
| <b>58</b> | – 8.4                       | Phe-209                                                                         | Hydrophobic-Alkyl- Pro-938, Hydrophobic-Alkyl- Leu-215, Hydrophobic-Alkyl- Met-230 | Ala-939, Ser-222, Arg-234, Glu-880, Ile-216, Gly-210, Val-211, Asp-208, Met-219, Arg-195,                                    |

|            |       |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                                   |
|------------|-------|------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
|            |       |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                       | Tyr-229, Lys-192, Asp-190, Ser-228                                                                |
| <b>104</b> | – 5.7 | Phe-677, Asp-803 | Hydrophobic-Alkyl- Val-680(Dist. 4.80542 Å),<br>Hydrophobic-Alkyl- Val-680(Dist. 5.06748 Å)<br>Hydrophobic-Alkyl- Cys-742(Dist. 5.19315 Å)<br>Hydrophobic-Alkyl- Cys-742(Dist. 5.45045 Å)<br>Hydrophobic-Alkyl- Lys-790(Dist. 4.83019 Å)<br>Hydrophobic-Alkyl- Lys-790(Dist. 4.71037 Å)<br>Hydrophobic-Alkyl- Ile-672(Dist. 5.01133 Å)<br>Hydrophobic-Alkyl- Ile-672(Dist. 4.52346 Å)                                                 | Arg-676, Gly-675, Thr-802, Lys-788, Asn-791, Thr-746, Gly-673, Gly-744, Arg-745                   |
| <b>105</b> | – 5.7 | —                | Hydrophobic-Alkyl- Ala-690, Hydrophobic-Alkyl- Leu-806, Hydrophobic-Alkyl- Ile-672,<br>Hydrophobic-Alkyl- Val-680(Dist. 4.17549 Å),<br>Hydrophobic-Alkyl- Val-680(Dist. 4.55834 Å),<br>Hydrophobic-Alkyl- Val-680(Dist. 4.54836 Å),<br>Hydrophobic-Alkyl- Val-812(Dist. 4.71743 Å),<br>Hydrophobic-Alkyl- Val-812(Dist. 3.74594 Å),<br>Hydrophobic-Pi-Alkyl- Phe-793(Dist. 5.27388 Å), Hydrophobic-Pi-Alkyl- Phe-793Dist. 5.31973 Å), | Gly-675, Thr-802, Leu-741, Asp-803, Arg-692, Phe-677, Arg-676, Asn-791, Ser-810, Asp-786, Lys-788 |

|            |       |                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                     |
|------------|-------|------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|
| <b>106</b> | – 5.6 | Arg-692 (Dist. 2.23814 Å), Arg-692 (Dist. 2.11661 Å) | Hydrophobic-Alkyl- Ala-690, Hydrophobic-Alkyl- Lys-790, Hydrophobic-Alkyl- Val-680(Dist. 5.21116 Å), Hydrophobic-Alkyl- Val-680(Dist. 4.3179 Å), Hydrophobic-Alkyl- Val-680(Dist. 5.34575 Å), Hydrophobic-Alkyl- Ile-672(Dist. 4.39653 Å), Hydrophobic-Alkyl- Ile-672(Dist. 3.99384 Å), Hydrophobic-Alkyl- Leu-741(Dist. 5.46476 Å), Hydrophobic-Alkyl- Leu-741(Dist. 4.49417 Å), Hydrophobic-Pi-Alkyl- Phe-793(Dist. 5.38238 Å), Hydrophobic-Pi-Alkyl- Phe-793(Dist. 4.9036 Å) | Thr-802, Thr-746, Asp-803, Lys-788, Cys-742, Gly-675, Phe-677                                                       |
| <b>107</b> | – 6.3 | Arg-692, Asn-791, Asp-803, Ser-749                   | Hydrophobic-Alkyl- Val-680, Hydrophobic-Alkyl- Cys-742, Hydrophobic-Alkyl- Leu-741, Hydrophobic-Alkyl- Lys-790, Hydrophobic-Alkyl- Ile-672(Dist. 5.03277 Å), Hydrophobic-Alkyl- Ile-672(Dist. 4.42833 Å), Hydrophobic-Pi-Alkyl- Phe-793(Dist. 4.96855 Å), Hydrophobic-Pi-Alkyl- Phe-793(Dist. 5.11679 Å), Hydrophobic-Pi-Alkyl- Phe-793(Dist. 4.68493 Å)                                                                                                                        | Ala-690, Thr-802, Gly-673, Thr-746, Tyr-748, Val-812, Lys-788, Asp-786, Lys-674, Phe-677, Gly-675, Arg-676, Leu-806 |

|                 |       |                                                                                                                                                                  |                                                                                                                                                                                       |                                                                                                                      |
|-----------------|-------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| <b>108</b>      | – 6.2 | Arg-676 (Dist. 2.24745 Å), Arg-676 (Dist. 1.91558 Å), Lys-788                                                                                                    | , Hydrophobic-Alkyl- Val-680, Hydrophobic-Alkyl- Val-812, Hydrophobic-Alkyl- Leu-806                                                                                                  | Lys-674, Gly-675, Ser-810, Arg-692, Asn-791, Phe-807                                                                 |
| <b>109</b>      | – 8.4 | Arg-818, Lys-821 (Dist. 2.46106 Å), Lys-821 (Dist. 2.79526 Å), Lys-821 (Dist. 2.93967 Å), Gln-153 (Dist. 3.02459 Å), Gln-153 (Dist. 2.72253 Å), Thr-226, Arg-227 | —                                                                                                                                                                                     | Lys-156, Ser-150, Val-82, Asn-195, Asp-208, Val-81, Glu-819, Lys-192, Asp-820, Gly-225, Tyr-229                      |
| Selumetini<br>b | – 7.7 | HOH-1104, Arg-692, Asn-791, Asp-803, Asp-786, Gly-675                                                                                                            | Hydrophobic-Pi-Sigma- Val-680, Hydrophobic-Alkyl- Ala-690, Hydrophobic-Pi-Alkyl- Tyr-748, Hydrophobic-Pi-Alkyl- Val-680, Hydrophobic-Pi-Alkyl- Ala-690, Hydrophobic-Pi-Alkyl- Lys-790 | Arg-676, Phe-677, Thr-802, HOH-1103, Thr-739, Lys-788, Lys-674, Thr-746, Cys-742, Phe-793, Leu-741, Gly-673, Ser-749 |

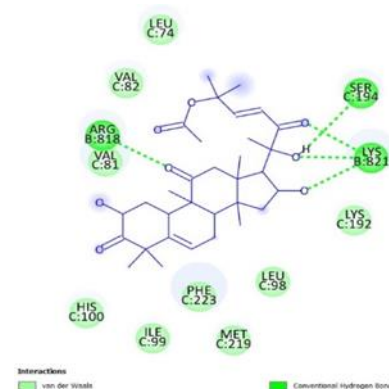
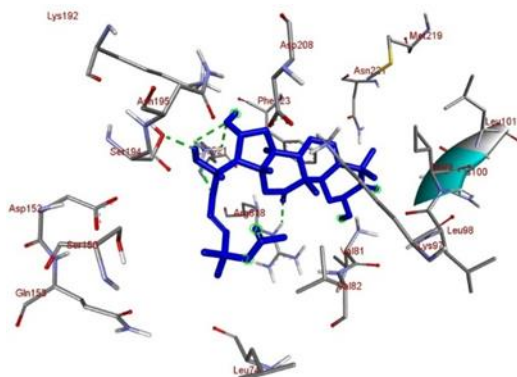
|          |       |                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                                               |
|----------|-------|---------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Geftinib | - 7.7 | Arg-676, Phe-677,<br>Arg-692, UNKO: N | Electrostatic-Pi-Cation- Arg-692, Electrostatic-<br>Pi-Anion- Asp-803, Hydrophobic-Pi-Sigma-<br>Ile-672, Hydrophobic-Pi-Sigma- Val-680,<br>Hydrophobic-Pi-Sigma- Thr-746,<br>Hydrophobic-Alkyl- Ile-672, Hydrophobic-<br>Alkyl- Leu-741, Hydrophobic-Alkyl- Lys-788,<br>Hydrophobic-Pi-Pi-T-Shaped- Phe-793,<br>Hydrophobic-Pi-Alkyl- His-682, Hydrophobic-<br>Pi-Alkyl- Val-680, Hydrophobic-Pi-Alkyl- Ala-<br>690, Hydrophobic-Pi-Alkyl- Lys-790 | Ser-749, Cys-742, Tyr-<br>748, HOH-1103, Thr-<br>802, Gly-673, HOH-<br>1104, Asn-791, Gly-675 |
|----------|-------|---------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|

The 2D and 3D binding interactions of compounds **24**, **58** and Gefitinib against KSR2:MEK1 were shown in Figure 23. Hydrogen bonding between the isolated compounds and amino acid residues of the target protein was shown as a green dash line while hydrophobic interactions were shown as pink line

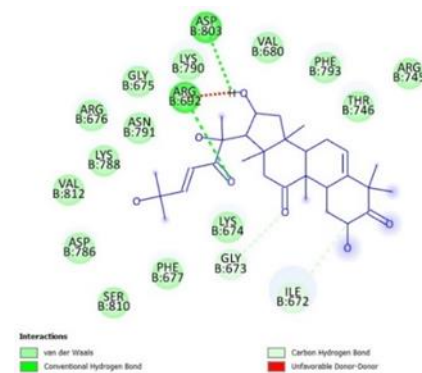
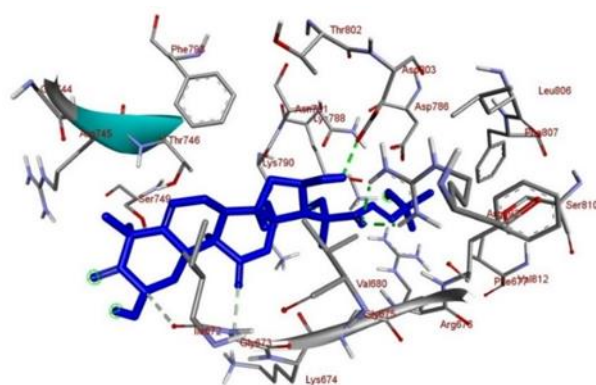
Compound      The 2D binding interactions

The 3D binding interactions

**24**



**26**



Gefitinib

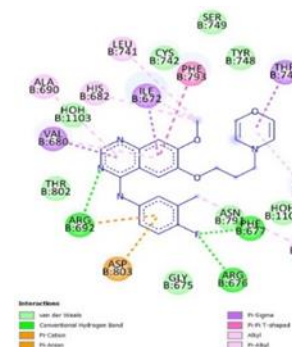
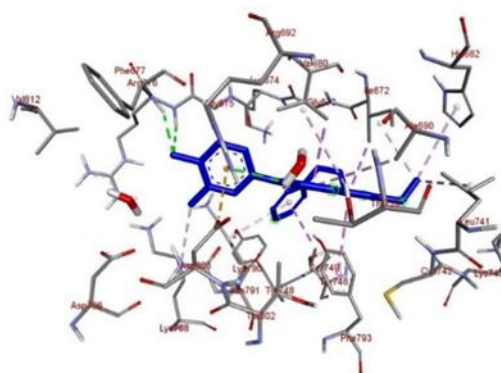


**Figure 23:** The 2D and 3D binding interactions of compounds **24**, **26** and reference drug Gefitinib against KSR2:MEK1

Topoisomerase II $\beta$  (TOP2) is a nuclear enzyme that catalyses the relaxing and unwinding of double-stranded DNA, which is essential for DNA operations like replication, transcription, and repair (Delgado, U.L.D., *et al.*, 2018). Recent studies showed that TOP2 $\beta$  is an important target for many anticancer agents including etoposide (EVP) (Farouka, F., *et al.*, 2023). In this regard, the binding affinities of the isolated compounds were done against human topoisomerase and the results were presented in Table 36, and 3D and 2D binding interactions were depicted in Figure 24. Among the test compounds, compound **24** (-9.0 kcal/mole), **26** (-9.4 kcal/mole), **58** (-9.6 kcal/mole) and **109** (-9.4 kcal/mole) showed higher binding affinity than EVP (-7.5 kcal/mole). Compounds **113**, **100** and **102** showed similar H-bonding interactions with two of these amino acids (His-775, Asp-561) along with other amino acids, whereas compounds **113** and **26** with His-775, and compound **58** interacts with Asp-561. Compound **58** showed residual interactions with Arg-503, which is similar to EVP. On the other hand, all compounds displayed a binding affinity for topoisomerase II $\beta$  ranging from -5.5 to -9.8 kcal/mol (Table 37) whereas abiraterone showed -11.8 kcal/mol. The binding affinity, H-bond and residual interaction of compounds and abiraterone were summarized in Table 36.

Compounds **109**, **26** and **58** showed binding affinity of -9.4, -9.4 and -9.6 kcal/mol, respectively (Figure 24). The compounds showed almost similar residual and DNA interaction profile with amino acid residues compared to abiraterone (-11.8 kcal/mol). Compound **26** showed binding affinity of -5.9 kcal/mole followed by compound **100** (-5.5 kcal/mole), compound **113** (-5.2 kcal/mole), **113** (-5.0 kcal/mole) and **102** (-5.0 kcal/mole), while the other test compounds (**103**, **111** and **115**) exhibited less binding affinity than the standard. Compounds **112**, **113**, **26**, **100** and **102** showed H-bonding interactions with one or two of these amino acids, whereas, compound **26** showed interactions with a different amino acid, Ser-345. Only compounds **113** and **58** showed residual amino acid interactions with Ile-361 (Table 36).

**Table 36:** Molecular docking results of isolated compounds against human topoisomerase II  $\beta$  (PDB ID 3QX3)

| S. No.     | Binding Affinity (kcal/mol) | H-bond                                                           | Residual interactions                                                                                        |                                                                                                         |
|------------|-----------------------------|------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|
|            |                             |                                                                  | Hydrophobic and Electrostatic                                                                                | Van der Waals                                                                                           |
| <b>24</b>  | - 9.0                       | Arg-820, DC-8, DG-13, DG-7, Lys-814, DC-11 (Dist. 2.23188 Å),    | —                                                                                                            | Ala-816, Met-782, DG-10, Pro-819, Ser-818, DT-9, DA-12, Gln-778, DC-14                                  |
| <b>26</b>  | - 9.4                       | DG-10, Ser-480 (Dist. 2.01136 Å), DT-9 (Dist. 2.85503 Å), UNK1:O | Hydrophobic-Pi-Sigma- Tyr-821                                                                                | Tyr-773, Glu-777, Lys-759, His-774, Asp-557, Ala-481, Asp-559, Gly-776, Glu-477, Gly-478, Asp-479, DC-8 |
| <b>58</b>  | - 9.6                       | DG-13                                                            | Hydrophobic-Pi-Sigma- DA-12<br>(Hydrophobic-Alkyl-) Lys-456, Arg-503, Ala-521<br>Hydrophobic-Pi-Alkyl- DG-13 | Pro-501, Phe-500, Glu-522, Asn-525, DG-10, Pro-455, DT-9, DC-8, Gly-504                                 |
| <b>100</b> | -7.2                        | His-775, Asp-561, Gly-776, Leu-502                               | -                                                                                                            | -                                                                                                       |
| <b>102</b> | -6.4                        | His-775, Asp-561, Gly-776, Asp-479, Ser-480                      | -                                                                                                            | -                                                                                                       |

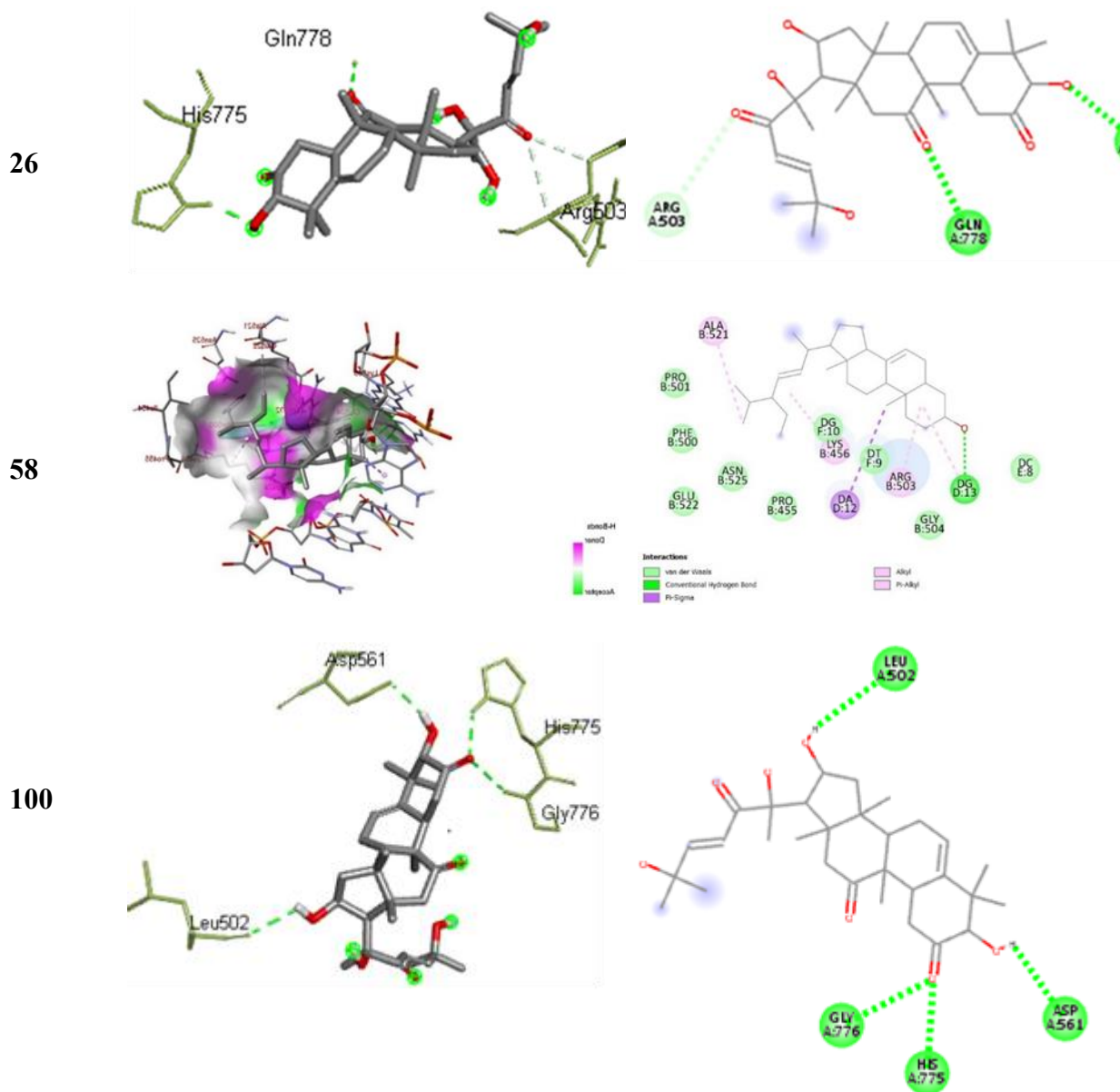
|            |       |                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                            |
|------------|-------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------|
| <b>104</b> | – 6.4 | DG-10 (Dist. 2.41937 Å),<br>DG-10 (Dist. 2.34042 Å),<br>DG-10 (Dist. 2.24169 Å) | Hydrophobic-Pi-Sigma- DG-13,<br>Hydrophobic-Pi-Sigma- DT-9, Hydrophobic-<br>Alkyl- Arg-503(Dist. 4.49965 Å),<br>Hydrophobic-Alkyl- Arg-503(Dist. 4.44711<br>Å), Hydrophobic-Pi-Alkyl- DA-12,<br>Hydrophobic-Pi-Alkyl- DG-13, Hydrophobic-<br>Pi-Alkyl- DC-8(Dist. 4.09218 Å),<br>Hydrophobic-Pi-Alkyl- DC-8(Dist. 5.19932<br>Å), Hydrophobic-Pi-Alkyl- DT-9(Dist.<br>5.49992 Å), Hydrophobic-Pi-Alkyl- DT-<br>9(Dist. 5.4579 Å) | Gln-778, Ala-779,<br>Met-782, Gly-504,<br>Gly-478, Lys-456 |
| <b>105</b> | – 5.9 | —                                                                               | Hydrophobic-Pi-Sigma- DG-13;<br>Hydrophobic-Alkyl- Arg-503<br>(Dist. 4.3344 Å); Hydrophobic-Pi-Alkyl- DA-<br>12(Dist. 3.7859 Å); Hydrophobic-Pi-Alkyl-<br>DG-13(Dist. 4.86663 Å); Hydrophobic-Pi-<br>Alkyl- DC-8(Dist. 3.72851 Å); Hydrophobic-<br>Pi-Alkyl- DT-9(Dist. 4.31634 Å)                                                                                                                                              | Gly-504, Gln-778                                           |
| <b>106</b> | – 6.1 | Asp-479, Ser-480, Ala-481,<br>DG-10, Ser-480                                    | Hydrophobic-Pi-Sigma- DC-8; Hydrophobic-<br>Alkyl- Arg-503; Hydrophobic-Pi-Alkyl- DA-<br>12; Hydrophobic-Pi-Alkyl- DG-13;<br>Hydrophobic-Pi-Alkyl- DT-9(Dist. 4.76523                                                                                                                                                                                                                                                           | Gly-478, Glu-477,<br>Gln-778                               |

|            |       |                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                        |
|------------|-------|---------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------|
|            |       |                                                                                                                                 | Å); Hydrophobic-Pi-Alkyl- DT-9(Dist. 3.69921 Å)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                        |
| <b>107</b> | – 6.9 | Lys-456 (Dist. 2.57188 Å),<br>Lys-456 (Dist. 2.69663 Å),<br>Asp-479 (Dist. 2.76945 Å),<br>Asp-479 (Dist. 2.63932 Å),<br>Gly-478 | Hydrophobic-Alkyl- Arg-503(Dist. 3.84251 Å); Hydrophobic-Alkyl- Arg-503(Dist. 4.59667 Å); Hydrophobic-Alkyl- Met-782(Dist. 5.2198 Å); Hydrophobic-Alkyl- Met-782(Dist. 4.41203 Å); Hydrophobic-Pi-Alkyl- DA-12(Dist. 5.20378 Å); Hydrophobic-Pi-Alkyl- DA-12(Dist. 4.79059 Å); Hydrophobic-Pi-Alkyl- DA-12(Dist. 4.91234 Å); Hydrophobic-Pi-Alkyl- DG-13(Dist. 4.97328 Å); Hydrophobic-Pi-Alkyl- DG-13(Dist. 5.31093 Å); Hydrophobic-Pi-Alkyl- DC-8(Dist. 3.95749 Å); Hydrophobic-Pi-Alkyl- DC-8(Dist. 4.84314 Å); Hydrophobic-Pi-Alkyl- DT-9(Dist. 4.39513 Å); Hydrophobic-Pi-Alkyl- DT-9(Dist. 5.49525 Å) | Gly-504, Gln-778,<br>Leu-502, DC-11                    |
| <b>108</b> | – 6.6 | Arg-820 (Dist. 2.83695 Å),<br>Arg-820 (Dist. 2.56388 Å),<br>Arg-820 (Dist. 2.51388 Å),                                          | Hydrophobic-Pi-Sigma-DG-13;<br>Hydrophobic-Alkyl- Arg-503(Dist. 4.38599 Å); Hydrophobic-Pi-Alkyl- DG-13;<br>Hydrophobic-Pi-Alkyl- DT-9(Dist. 4.23158 Å)                                                                                                                                                                                                                                                                                                                                                                                                                                                     | DG-10, Ser-818, Pro-819, Gln-778, DA-12, DC-8, Gly-504 |

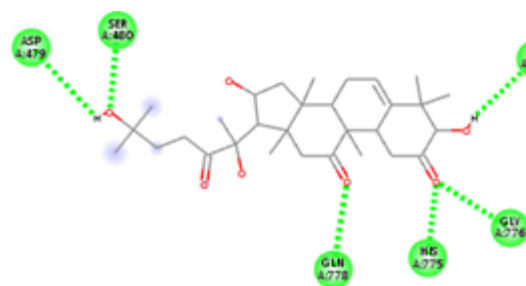
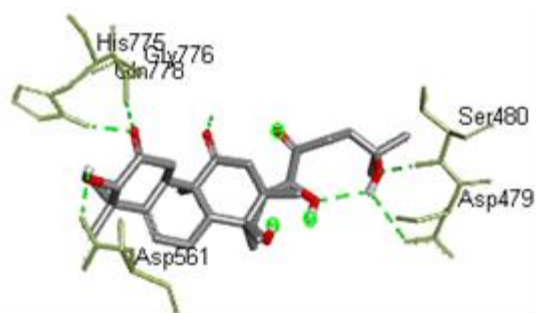
|            |       |                                                                                                                                                                                  |                             |                                                                          |
|------------|-------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|--------------------------------------------------------------------------|
|            |       | DG-13 (Dist. 2.8059 Å),<br>DG-13 (Dist. 2.92282 Å)                                                                                                                               |                             |                                                                          |
| <b>109</b> | – 9.4 | DC-11, DA-6 (Dist. 3.00543 Å), DA-6 (Dist. 2.80034 Å),<br>DC-8, DG-5 (Dist. 2.28319 Å), DG-5 (Dist. 2.49208 Å),<br>DA-12 (Dist. 2.12364 Å),<br>DA-12 (Dist. 3.98863 Å),<br>DG-13 | Hydrophobic-Pi-Sigma- DA-12 | DC-4, DC-16, Lys-814, Met-782, DC-14, DC-3, DG-2, DT-9, Gln-778, Ala-779 |
| <b>110</b> | -3.1  | -                                                                                                                                                                                | -                           | -                                                                        |
| <b>111</b> | -2.9  | -                                                                                                                                                                                | -                           | -                                                                        |
| <b>112</b> | -5.0  | Ser-362, Asn-369                                                                                                                                                                 | Leu-370                     | -                                                                        |
| <b>113</b> | -5.2  | Ser-362                                                                                                                                                                          | Ile-361, Ala-358            | -                                                                        |
| <b>114</b> | -3.3  | -                                                                                                                                                                                | -                           | -                                                                        |
| etoposide  | -7.5  | His-775, Asp-561, Lys-505, Arg-503                                                                                                                                               | Arg-503, His-775, Glu-522   | Gly-504, Gly-776, Ala-779, Arg-729, Asp-559, His-774, Glu-477,           |

DA = deoxyadenosine; DG = deoxyguanosine; DT = deoxythymidine; DC = Deoxycytidine.

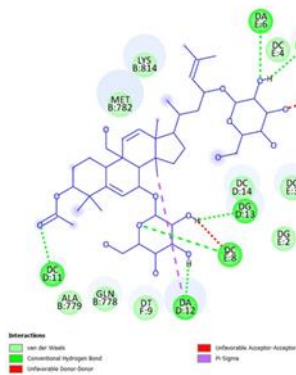
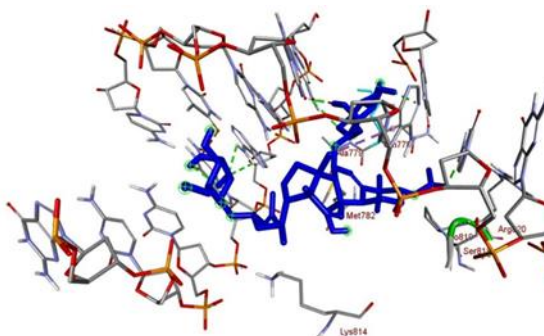
The 2D and 3D binding interactions of compounds and abiraterone against human topoisomerase II $\beta$  (PDB ID: 3QX3) are depicted in Figure 24. Hydrogen bond between the compound and amino acids is shown as a green dash line. Hydrophobic interactions are shown as pink lines.



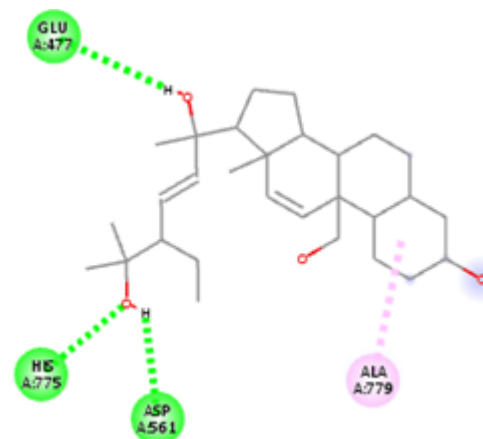
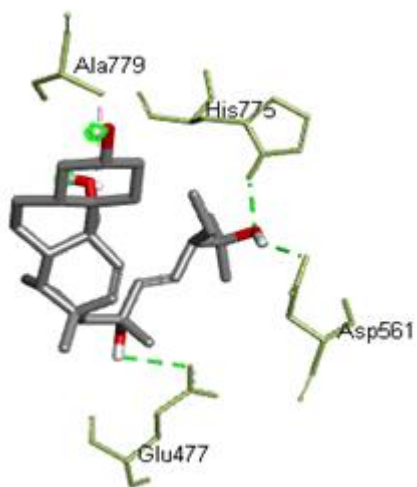
102



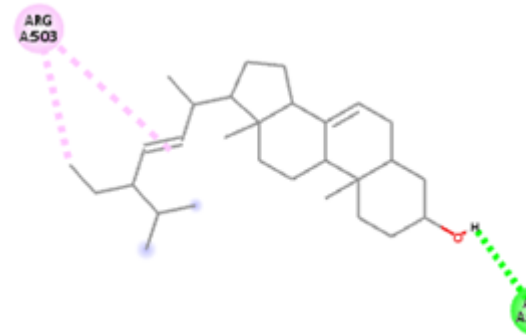
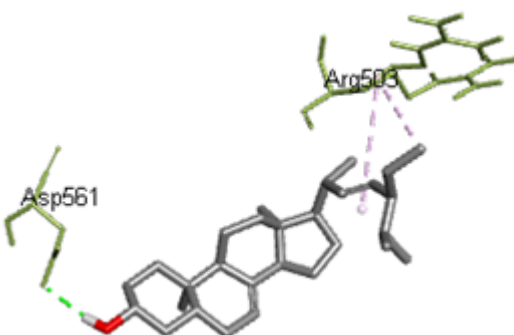
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113



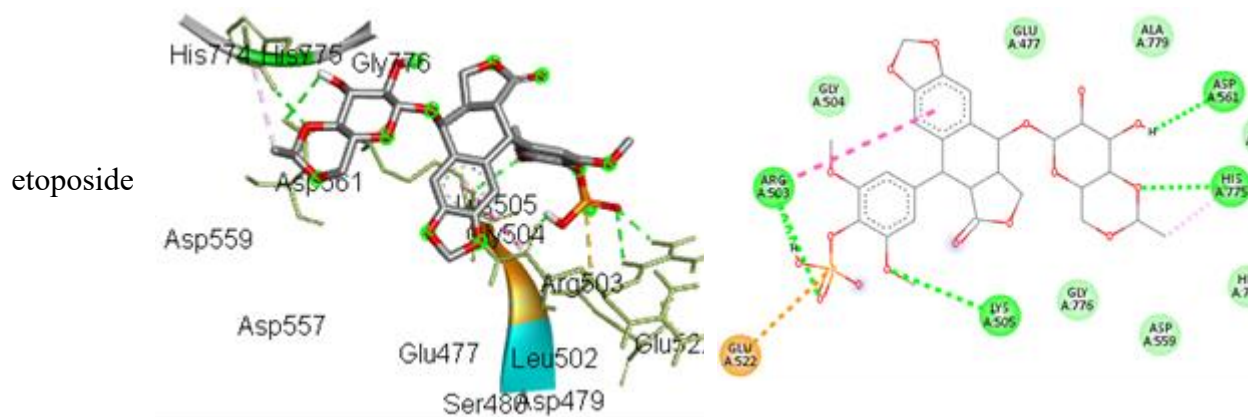


**Figure 24:** The 2D and 3D binding interactions of compounds and abiraterone against human topoisomerase II $\beta$ .

All compounds displayed a binding affinity for target protein topoisomerase II  $\beta$  (Figure 24) ranging from -3.1 to -9.6 kcal/mol (Table 36) where as etoposide showed -7.5 kcal/mol. The binding affinity, H-bond and residual interaction of compounds and etoposide are summarized in (Table 36). Compounds **58**, **26** and **109** showed binding affinity of -9.6, -9.4 and -9.4 kcal/mol, respectively. The compounds showed alsomst similar residual and DNA interaction profile with amino acid residues Lys-456, Arg-503, Gly-504, Gly-478, Asp-479, Pro-501, Phe-500, Glu-522, Asn-525, Pro-455, Gly-504, Arg-820, Lys-814, Ala-816, Met-782, Pro-819, Ser-818, Gln-778, Ser-480, Tyr-773, Glu-777, Lys-759, His-774, Asp-557, Ala-481, Asp-559, Gly-776, Glu-477, Asp-47, Ala-817, Lys-814, Met-782, and nucleic acid residues DG-10, DG-13, DT-9, DT-9, DC-8, DC-11, DA-12, DC-14, DC-8, DC-4, DG-5, DA-6, and DG-7 compared to etoposide (-7.5 kcal/mol) (Table 36)..

#### 4.5 *In silico* Pharmacokinetics (Drug-Likeness) and Toxicity Analysis of the Isolated Compounds

In association with *in vitro* antimicrobial activity, it is useful to carry out *in silico* studies to predict the orientation and binding affinity at the active site of the receptor. The molecular docking of NMR identified ligand molecules cucurbitacin B (**24**), cucurbitacin D (**26**),  $\alpha$ -spinasterol (**58**), Isocucurbitacin D (**100**), and 23,24-dihydroisocucurbitacin D (**102**), lignoceric acid (**104**), eicosane (**105**), stearic acid (**106**), 12-hydroxyoctacosanoic acid (**107**), 13-hydroxyoctadecanoic acid (**108**), Kuguaglycoside E-3-acetate (**109**), Octacosane (**110**),

Docosane (**111**), 17-(-5-ethyl-2,6-dihydroxy-6-methylhept-3-en-2-yl)-9-(hydroxymethyl)-13-methyl-1H cyclopenta[a]phenanthren-3-ol (**112**), Erythrodiol (**113**), (9,12)-propyl icos-9,12-dienoate (**114**), 2-heptylcyclohexanol (**115**), decanoic acids (**116**) and 1,3-dihydroxypropan-2-yl dodecanoate (**117**) (Figure 18, 19 and 20) with bacterial enzyme DNA gyrase.

The chemical structures of the isolated compound were converted to their canonical simplified molecular-input line-entry system (SMILE) and submitted to the Swiss ADME tool to estimate *in silico* pharmacokinetic parameters according to 'Lipinski's rule of five'. It denotes that the drugs and/or candidates should obey the rule of five parameters such as hydrogen-bond donors (HBDs) < 5, hydrogen-bond acceptors (HBAs) < 10, a molecular mass < 500 Da, log P not > 5, and total polar surface area (TPSA) should not be > 140 Å. Drug-likeness is a prediction that screens whether a particular organic molecule has properties consistent with being an orally active drug (Lipinski, C.A., *et al.*, 1997). In the present studies, the Swiss ADME prediction revealed that most all compounds obey Lipinski's rule of five with one violation (Table 37). The hydrogen bonding potential and bioavailability of molecules are closely correlated to the TPSA value. Thus, the TPSA value of the studied compounds was noticed in the range of 20.23–138.20°A and is well below the limit of 140 °A. The calculated number of rotatable bonds (NRB) values for the isolated compounds **24**, **26**, **58**, **111**, **112** and **113** are less than 10 which indicated the compounds are conformationally stable (Das, P., *et al.*, 2020).

**Table 37:** Drug-likeness predictions of compounds, computed by Swiss ADME

|             | Mol.Wt.<br>(gmol <sup>-1</sup> ) | NHD | NHA | NRB | TPSA<br>(Å <sup>2</sup> ) | Log <i>P</i><br>(iLOGP)<br>Lipophilicity | Log <i>P</i><br>(MLOGP)<br>Lipophilicity | Log <i>S</i><br>(ESOL)<br>Water Solubility | Lipinski's rule<br>of five with zero<br>violations |
|-------------|----------------------------------|-----|-----|-----|---------------------------|------------------------------------------|------------------------------------------|--------------------------------------------|----------------------------------------------------|
| <b>24</b>   | 558.7                            | 3   | 8   | 6   | 138.20                    | 3.45                                     | 1.76                                     | -4.57                                      | 1                                                  |
| <b>26</b>   | 516.67                           | 4   | 7   | 4   | 132.13                    | 2.35                                     | 1.44                                     | -4.08                                      | 1                                                  |
| <b>58</b>   | 412.69                           | 1   | 1   | 5   | 20.23                     | 4.85                                     | 6.59                                     | -7.30                                      | 1                                                  |
| <b>100</b>  | 296.44                           | 2   | 3   | 14  | 57.53                     | 3.86                                     | 3.59                                     | -4.12                                      | 0                                                  |
| <b>102</b>  | 839.02                           | 9   | 15  | 13  | 245.29                    | 3.92                                     | -0.46                                    | -5.17                                      | 3                                                  |
| <b>104</b>  | 368.37                           | 1   | 2   | 12  | 37.30                     | 3.32                                     | 3.69                                     | -4.31                                      | 0                                                  |
| <b>105</b>  | 282.55                           | 0   | 0   | 17  | 0.00                      | 5.64                                     | 7.38                                     | -7.05                                      | 1                                                  |
| <b>106</b>  | 284.46                           | 1   | 2   | 15  | 37.30                     | 4.01                                     | 4.57                                     | -5.41                                      | 1                                                  |
| <b>107</b>  | 436.44                           | 2   | 3   | 14  | 57.53                     | 3.93                                     | 3.59                                     | -3.94                                      | 0                                                  |
| <b>108</b>  | 296.44                           | 2   | 3   | 14  | 57.53                     | 3.86                                     | 3.59                                     | -4.12                                      | 0                                                  |
| <b>109</b>  | 839.02                           | 9   | 15  | 13  | 245.29                    | 3.92                                     | -0.46                                    | -5.17                                      | 3                                                  |
| <b>110</b>  | 228.37                           | 1   | 2   | 12  | 37.30                     | 3.32                                     | 3.69                                     | -4.31                                      | 0                                                  |
| <b>111</b>  | 412.69                           | 1   | 1   | 5   | 20.23                     | 4.85                                     | 6.59                                     | -7.30                                      | 1                                                  |
| <b>112</b>  | 558.7                            | 3   | 8   | 6   | 138.20                    | 3.45                                     | 1.76                                     | -4.57                                      | 1                                                  |
| <b>113</b>  | 516.67                           | 4   | 7   | 4   | 132.13                    | 2.35                                     | 1.44                                     | -4.08                                      | 1                                                  |
| <b>114</b>  | 282.55                           | 0   | 0   | 17  | 0.00                      | 5.64                                     | 7.38                                     | -7.05                                      | 1                                                  |
| Abiraterone | 349.51                           | 1   | 2   | 1   | 33.12                     | 3.42                                     | 4.42                                     | -5.03                                      | 1                                                  |
| Doxycycline | 444.43                           | 6   | 9   | 2   | 181.59                    | 1.11                                     | -2.08                                    | -2.59                                      | 1                                                  |

NHD = number of hydrogen donors, NHA = number of hydrogen acceptors, NRB = number of rotatable bonds, and TPSA = total polar surface area.

#### 4.6 BOILED-Egg model

The cLogP and TPSA values of the compounds were plotted to predict human intestinal absorption (HIA) and blood-brain barrier (BBB) access (Figure. 25). The egg-shaped plot is divided into 3 parts including a white area (HIA), a yellow area (BBB access) and a grey area (no HIA or BBB access) (Daina, A.; Zoete, V., 2016). In this prediction, compounds **58**, **111** and **114** were in the grey area, indicating no HIA or BBB access, whereas compounds **26**, **100**, **102**, **112** and **113** were in the white area, indicating that these compounds may be absorbed through the intestine (Figure. 25). None of the test compounds was in the yellow area, indicating that the test compounds might not have good BBB access. This model also predicted whether those compounds are substrates of P-gp (PGP) or not. Blue dots (PGP+) represent compounds that are substrates of PGP CNS efflux transporter and could be effluxed from the CNS, while, red dots (PGP-) represent compounds that are not substrates of the PGP and could pass through and act on CNS (Daina, A.; Zoete, V., 2016). In this study, compounds **58**, **111** and **114** showed red dots, while others showed blue dots and suggesting that only these compounds are not PGP substrates and eventually act on the CNS.

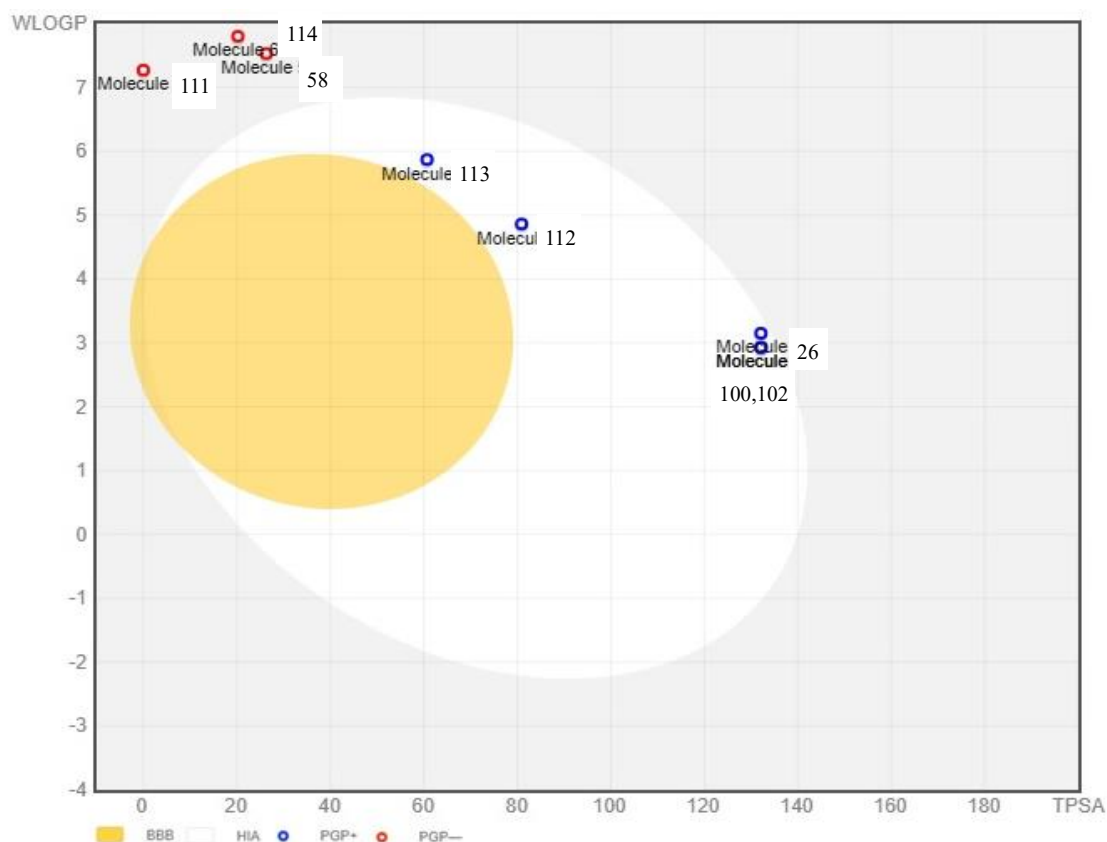


**Figure 25:** BOILED-Egg model for predicting gastrointestinal absorption and brain access.

#### 4.7 ADMET Properties of Isolated Compounds

The drug absorption, distribution, metabolism, excretion and toxicity (ADMET) analysis of isolated compounds were predicted by using Swiss ADMET. The skin permeability value (Kp) in cm/s denotes the skin absorption of molecules. Kp's skin permeability, values of all molecules ranged from -0.60 to -10.31 cm/s indicating low skin permeability and are with respect to Doxycycline (-9.03 cm/s) and under the clinical trial anticancer agent abiraterone (-5.14 cm/s). Additionally, gastrointestinal (GI) and blood-brain barrier (BBB) permeation indicates the absorption and distribution of drug molecules (Bouyahya, A., *et al.*, 2019; Abdelrheem, D.A., *et al.*, 2021). The *in-silico* prediction results of absorption, distribution, metabolism, and excretion (ADME) of the compounds studied are presented in Table 37.

The Swiss ADME prediction parameters have shown the compounds to have high gastrointestinal (GI) absorption and blood-brain barrier (BBB) permeation. Whereas, a range of cytochromes (CYP's) regulates the drug metabolism, in which CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 are vital for the biotransformation of drug molecules (Eswaramoorthy, R., *et al.*, 2021). About 60% of prescribed drugs are metabolized by CYP enzymes, with CYP3A4 accounting for about half of this metabolism, followed by CYP2D6, CYP2C9, and CYP2C19 (Esteves *et al.* 2021). Swiss ADME prediction has shown that compounds **104**, **105**, **106**, **107**, **108**, **111** and **115** inhibited CYP1A2 cytochromes, compounds **106**, **107** and **108** inhibited CYP2C9 cytochromes, compounds **107** and **108** inhibited CYP2D6 cytochromes, while compounds **24**, **26**, **100** and **102** inhibited CYP3A4 and substrate of permeability glycoprotein (P-gp). However, compounds **58** and **109** showed no action to all cytochromes and compound **58** does not inhibit substrate of permeability glycoprotein (P-gp) (Table 40).

Organic cation transporter 2 (OCT2) is the key transporter for cation influx in the renal epithelial cells (Wright, S.H., 2019). None of the test compounds and ciprofloxacin displayed OCT2 substrate (Table 40), suggesting that OCT2 may not be involved in the excretion of these compounds. Acute toxicity prediction results, such as toxicity class classification and LD<sub>50</sub> values, predict that none of the compound has acute toxicity. The toxicological prediction gives results of end points such as hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity. The studied compounds were predicted to be non-hepatotoxic, non-mutageni, non-cytotoxicity and non-irritant. However, compounds **24** and **26** have shown mild carcinogenicity and compounds

**58, 24, 26** and **109** are immunotoxic. ProTox-II and OSIRIS property explorer prediction analyses were shown in Table 38.

**Table 38:** ADME predictions of compounds, computed by SwissADME and PreADMET.

| S. No.      | Skin Permeation Value (Log $K_p$ ) $\text{cm s}^{-1}$ | GI Absorption | BBB Permeability | Inhibitor Interaction |                  |                   |                  |                  |                  |
|-------------|-------------------------------------------------------|---------------|------------------|-----------------------|------------------|-------------------|------------------|------------------|------------------|
|             |                                                       |               |                  | Pgp substrate         | CYP1A2 inhibitor | CYP2C19 inhibitor | CYP2C9 inhibitor | CYP2D6 inhibitor | CYP3A4 inhibitor |
| 24          | -7.83                                                 | Low           | No               | Yes                   | No               | No                | No               | No               | Yes              |
| 26          | -7.99                                                 | High          | No               | Yes                   | No               | No                | No               | No               | Yes              |
| 58          | -2.92                                                 | Low           | No               | No                    | No               | No                | No               | No               | No               |
| 100         | -4.31                                                 | High          | Yes              | No                    | Yes              | No                | Yes              | Yes              | No               |
| 102         | -10.31                                                | Low           | No               | Yes                   | No               | No                | No               | No               | No               |
| 104         | -3.35                                                 | High          | Yes              | No                    | Yes              | No                | No               | No               | No               |
| 105         | -0.60                                                 | Low           | No               | No                    | Yes              | No                | No               | No               | No               |
| 106         | -2.60                                                 | High          | No               | No                    | Yes              | No                | Yes              | No               | No               |
| 107         | -4.52                                                 | High          | Yes              | No                    | Yes              | No                | Yes              | Yes              | No               |
| 108         | -4.31                                                 | High          | Yes              | No                    | Yes              | No                | Yes              | Yes              | No               |
| 109         | -10.31                                                | Low           | No               | Yes                   | No               | No                | No               | No               | No               |
| 110         | -3.35                                                 | High          | Yes              | No                    | Yes              | No                | No               | No               | No               |
| 111         | -2.92                                                 | Low           | No               | No                    | No               | No                | No               | No               | No               |
| 112         | -7.83                                                 | Low           | No               | Yes                   | No               | No                | No               | No               | Yes              |
| 113         | -7.99                                                 | High          | No               | Yes                   | No               | No                | No               | No               | Yes              |
| 114         | -0.60                                                 | Low           | No               | No                    | Yes              | No                | No               | No               | No               |
| Abiraterone | -5.14                                                 | High          | Yes              | No                    | Yes              | No                | No               | No               | No               |
| Doxycycline | -9.03                                                 | Low           | No               | Yes                   | No               | No                | No               | No               | No               |

GI = gastrointestinal, BBB = blood brain barrier, P-gp = P-glycoprotein, and CYP = cytochrome-P

**Table 39:** Toxicity prediction of compounds, computed by ProTox-II and OSIRIS property explorer.

| S. No.      | LD <sub>50</sub><br>(mg/kg) | Toxicity<br>Class | Organ Toxicity |                 |                |              |              |          |
|-------------|-----------------------------|-------------------|----------------|-----------------|----------------|--------------|--------------|----------|
|             |                             |                   | Hepatotoxicity | Carcinogenicity | Immunotoxicity | Mutagenicity | Cytotoxicity | Irritant |
| <b>24</b>   | 14                          | 2                 | Inactive       | Mild active     | Active         | Inactive     | Inactive     | No       |
| <b>26</b>   | 5                           | 1                 | Inactive       | Mild active     | Active         | Inactive     | Inactive     | No       |
| <b>58</b>   | 2000                        | 4                 | Inactive       | Inactive        | Active         | Inactive     | Inactive     | No       |
| <b>100</b>  | 3200                        | 5                 | Inactive       | Inactive        | Inactive       | Inactive     | Inactive     | No       |
| <b>102</b>  | 100                         | 3                 | Inactive       | Inactive        | Active         | Inactive     | Inactive     | No       |
| <b>104</b>  | 900                         | 4                 | Inactive       | Inactive        | Inactive       | Inactive     | Inactive     | No       |
| <b>105</b>  | 750                         | 3                 | Inactive       | Inactive        | Inactive       | Inactive     | Inactive     | No       |
| <b>106</b>  | 48                          | 2                 | Inactive       | Inactive        | Inactive       | Inactive     | Inactive     | No       |
| <b>107</b>  | 2000                        | 6                 | Inactive       | Inactive        | Inactive       | Inactive     | Inactive     | No       |
| <b>108</b>  | 3200                        | 5                 | Inactive       | Inactive        | Inactive       | Inactive     | Inactive     | No       |
| <b>109</b>  | 100                         | 3                 | Inactive       | Inactive        | Active         | Inactive     | Inactive     | No       |
| <b>110</b>  | 900                         | 4                 | Inactive       | Inactive        | Inactive       | Inactive     | Inactive     | No       |
| <b>111</b>  | 2000                        | 4                 | Inactive       | Inactive        | Active         | Inactive     | Inactive     | No       |
| <b>112</b>  | 14                          | 2                 | Inactive       | Mild active     | Active         | Inactive     | Inactive     | No       |
| <b>113</b>  | 5                           | 1                 | Inactive       | Mild active     | Active         | Inactive     | Inactive     | No       |
| <b>114</b>  | 750                         | 3                 | Inactive       | Inactive        | Inactive       | Inactive     | Inactive     | No       |
| Abiraterone | 830                         | 4                 | Inactive       | Inactive        | Active         | Inactive     | Inactive     | No       |
| Doxycycline | 1007                        | 4                 | Active         | Inactive        | Active         | Inactive     | Inactive     | No       |

## 5. CONCLUSION AND RECOMMENDATIONS

### CONCLUSION

The extracts of the leaves, stems, fruits and roots of *C. ficifolius*, fruits of *C. dipsaceus* and leaves of *C. prophetarum* after silica gel column chromatography furnished 25 compounds identified as cucurbitacin B (**24**), cucurbitacin D (**26**),  $\alpha$ -spinasterol (**58**), isocucurbitacin D (**100**), and 23,24-dihydroisocucurbitacin D (**102**), lignoceric acid (**104**), eicosane (**105**), stearic acid (**106**), 12-hydroxyoctacos-9,13-dienoic acid (**107**), 13-hydroxyoctadeca-9,11-dienoic acid (**108**), kuguaglycoside E (**109**), hexacosane (**110**), docosane (**111**), 17-(-5-ethyl-2,6-dihydroxy-6-methylhept-3-en-2-yl)-9-(hydroxymethyl)-13-methyl-1H cyclopenta[ $\alpha$ ]phenanthren-3-ol (**112**), erythrodol (**113**), (9,12)-propyl icos-9,12-dienoate (**114**), 2-heptylcyclohexanol (**115**), decanoic acids (**116**) and 1,3-dihydroxypropan-2-yl dodecanoate (**117**). All Compounds are new to the genus except compound **24**, **26**, **58**, **100**, **102** and **116**. Different parts of *C. ficifolius*, *C. dipsaceus* and *C. prophetarum* were extracted by hydrodistillation to furnish different percentage of essential oil. Compounds 3,7,11,15-tetramethyl-1-hexadecanol, neophytadiene, and isophytol were obtained from the GC-MS analysis of the essential oil of the leaves while phytol and eicosane were obtained from the stems. The major constituent of the fruits of *C. ficifolius* was hentriacontane whereas eicosane, abietadiene were obtained as a major constituent from roots. Hydrocarbons including eicosane, treicosane, tetracosane, hentriacontane and hexacosane were the principal components of various parts of *C. dipsaceus*. While leaves, stems and roots of *C. prophetarum* contain hentriacontane, neophytadiene and eicosane, respectively. All most all the compounds in essential oils are new to the genus as no more research explored on the compound constituent of essential oils of this genus.

The oils, extract and isolated compound were evaluated for their *in vitro* antibacterial activity using disc diffusion method against four bacterial pathogens including *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes*. All bacterial strains were sensitive to the essential oils and may be good for drug discovery initiatives. The essential oil from the leaves of *C. dipsaceus* exhibited the highest inhibition zone against *E. coli* ( $11.93 \pm 0.67$  mm) while the other displayed modest activity compared with the positive control, ciprofloxacin. The *n*-hexane extract of the stem of *C. ficifolius* exhibited highest inhibition zone ( $14.00 \pm 0.20$  mm) against *P. aeruginosa* at 400  $\mu\text{g/mL}$ . At 100  $\mu\text{g/mL}$ , compound **58** displayed inhibition zone of  $13.67 \pm 0.57$ ,  $15.0 \pm 0.10$  and

13.33±0.50 mm against *E. coli*, *P. aeruginosa* and *S. pyogenes*, respectively. At the same concentration, compound **107** exhibited inhibition zones of 14.00±1.00 and 14.50±0.20 mm against *E. coli* and *P. aeruginosa*, respectively. That makes the plant may be good source of new compound for drug discovery.

The MTT assay result demonstrated that the EtOAc extract of the stem of *C. dipsaceus* (IC<sub>50</sub> = 10.41 µg/mL) and methanol extract of the leaves of *C. ficifolius* (IC<sub>50</sub> = 10.48 µg/mL) showed better cytotoxicity relative to the other samples at low micromolar concentrations and comparable to standard drug (IC<sub>50</sub> = 7.96 µg/mL) suggesting that these samples might serve as a source of bioactive compounds against cancer and therefore farther activity may be recommended. The petroleum ether and EtOAc extract of the roots, stems and fruits of *C. ficifolius* had better DPPH radical scavenging activity with IC<sub>50</sub> values of 4.48, 4.51 and 5.63 µg/mL, respectively and methanol extract of fruit of *C. dipsaceus* displayed IC<sub>50</sub> values of 7.03 µg/mL. Compounds **109** and **113** has better IC<sub>50</sub> value of 4.83 and 7.09 µg/mL than other. Hence, the extracts and compounds may be used against radicals. The *in silico* molecular docking analysis of compounds all were done using AutoDock vina against DNA Gyrase, protein kinas and human topoisomerase IIβ. Compounds **24** and **26** displayed binding affinity of -9.1 and -9.8 kcal/mol against *E. coli* DNA gyrase, respectively. Compounds **102**, (**58**, **111**), (**26**, **109**, **113**) and (**24**, **113**) showed better binding affinity against human topoisomerase IIβ with binding energy of -9.8, -9.6, -9.4 and -9.0 kcal/mol, respectively. Most of the compounds obeyed Lipinski's rule of five. Therefore, the antibacterial, cytotoxic and radical scavenging activities displayed by the constituents of *C. ficifolius*, *C. dipsaceus* and *C. propheterum* corroborate the traditional use of this plant against bacteria and cancer.

### Recommendation

Although, the compounds isolated are not the only compounds present in the plant material as evidenced from TLC analysis future work should aim at isolating other minor compounds not isolated in this study and determine their biological activities. Compounds from *C. ficifolius*, *C. dipsaceus* and *C. propheterum* showed broad range of biological activities and hence more work should be carried on pharmacological activities. It is recommended that further comprehensive evaluations, including *in vivo* activity and cytotoxicity tests could be done for

conclusive decision on the potential candidacy of the three plants for further formulation and medicinal uses. This plant has many active compounds like compound **58** that should be taken for further research to translate this knowledge into potential therapeutic drugs. All the three plants are in high demand owing so farming of medicinal plants must be prompted for assuring the future sustainability.

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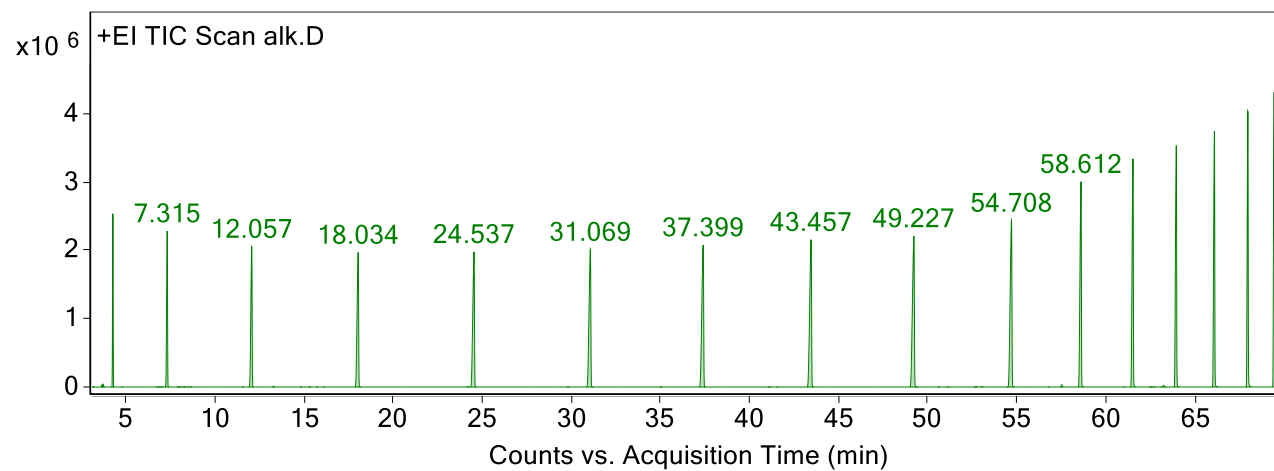
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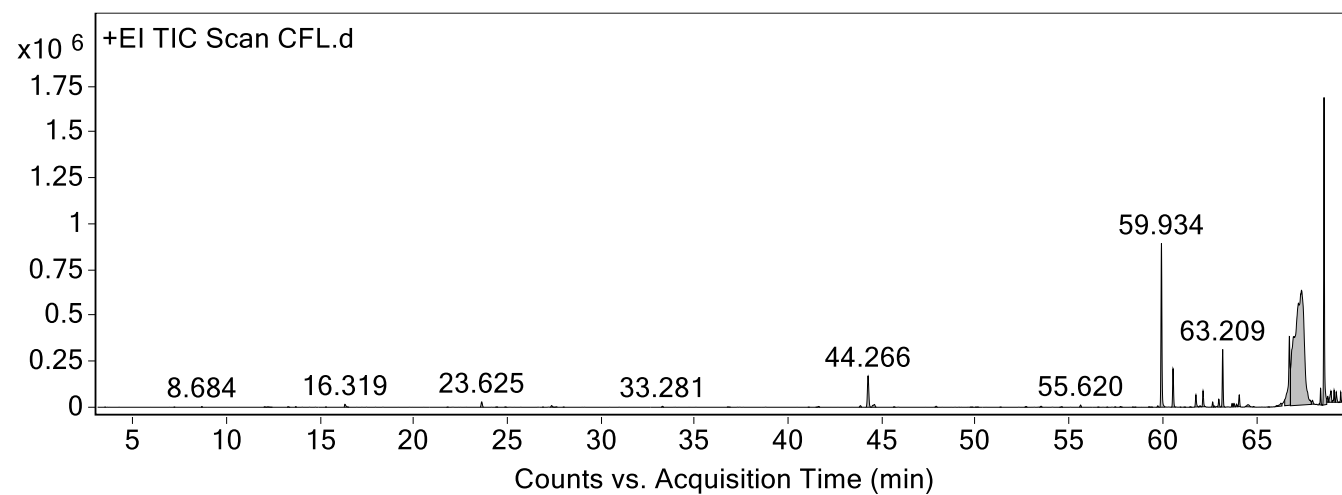
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## APPENDIXES

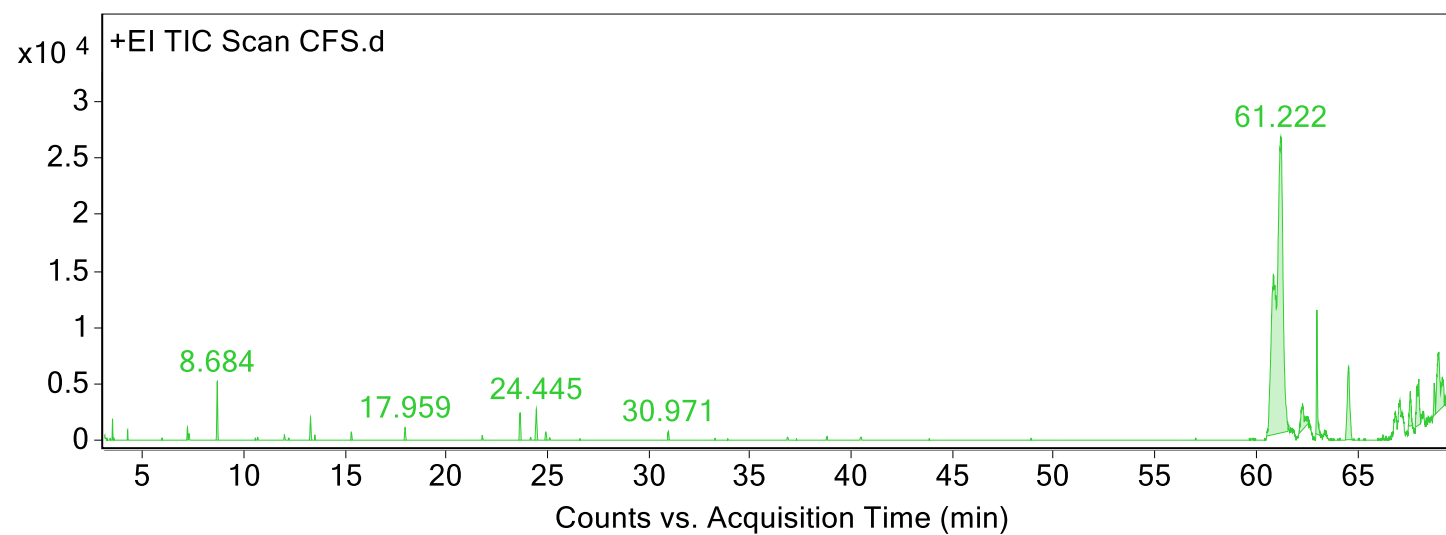
**Appendix 1:** The GC-MS chromatogram of standard n-alkane



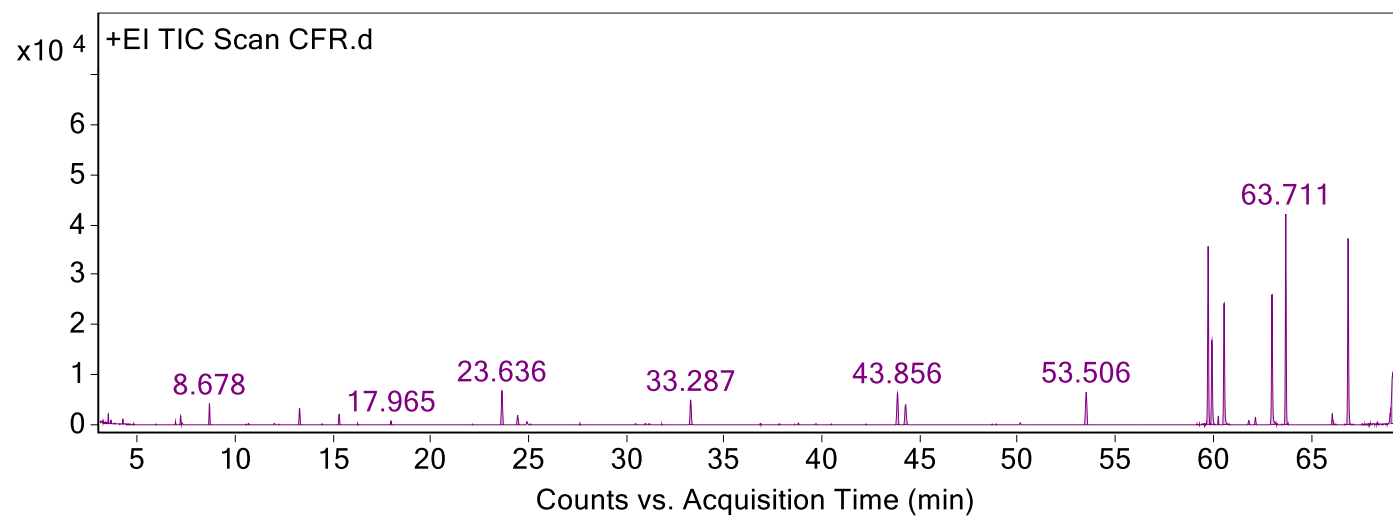
**Appendix 2:** The GC-MS chromatogram of leaves of *C. ficifolius*



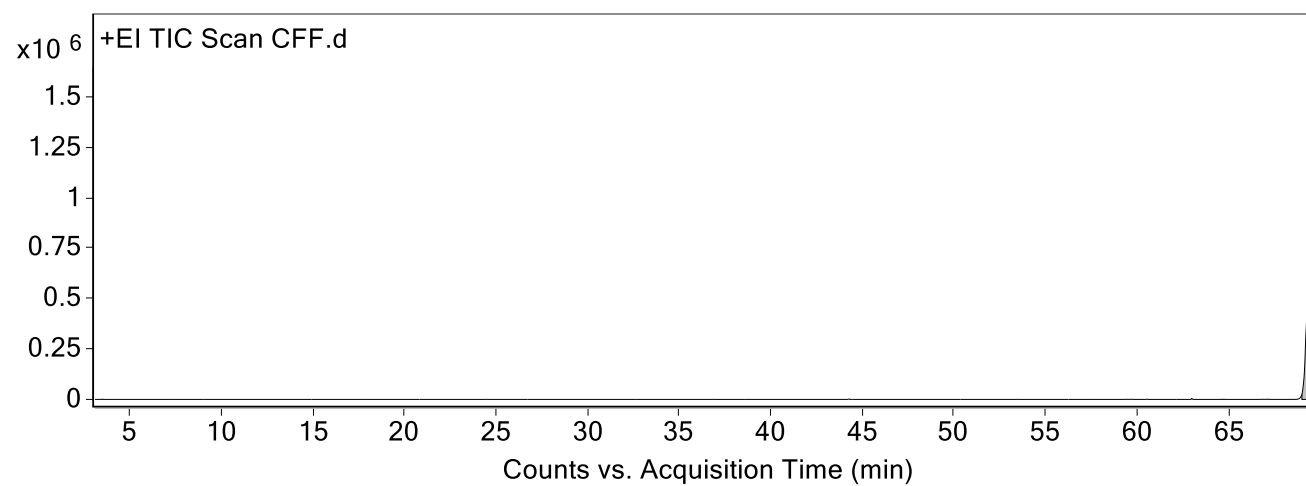
**Appendix 3:** The GC-MS chromatogram of stems of *C. ficifolius*



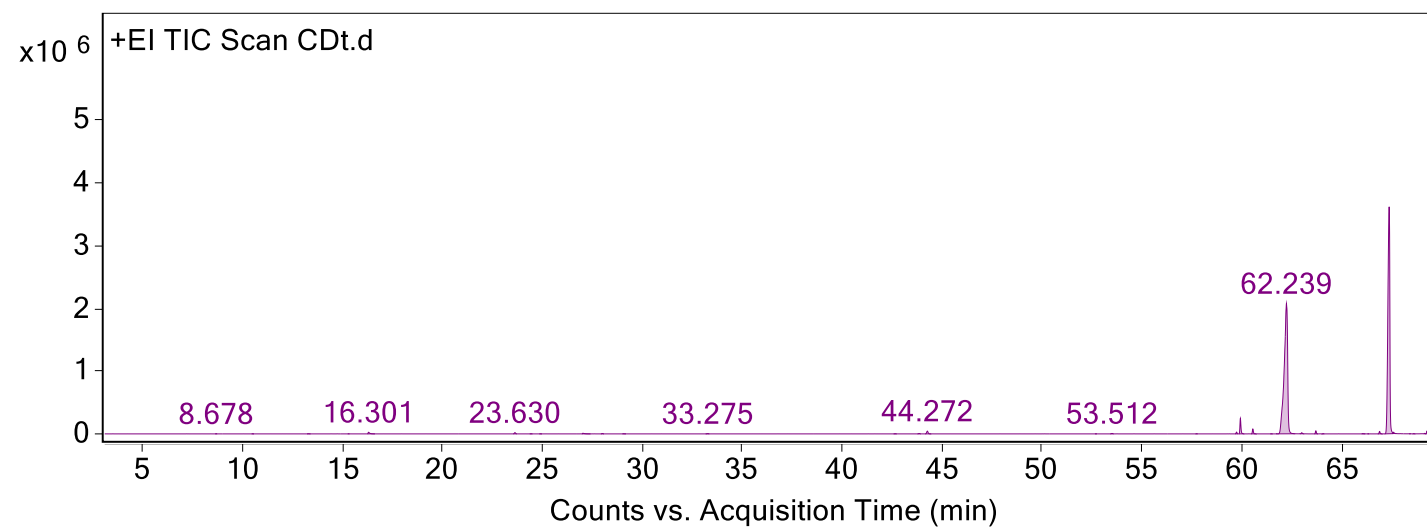
**Appendix 4:** The GC-MS chromatogram of root of *C. ficifolius*



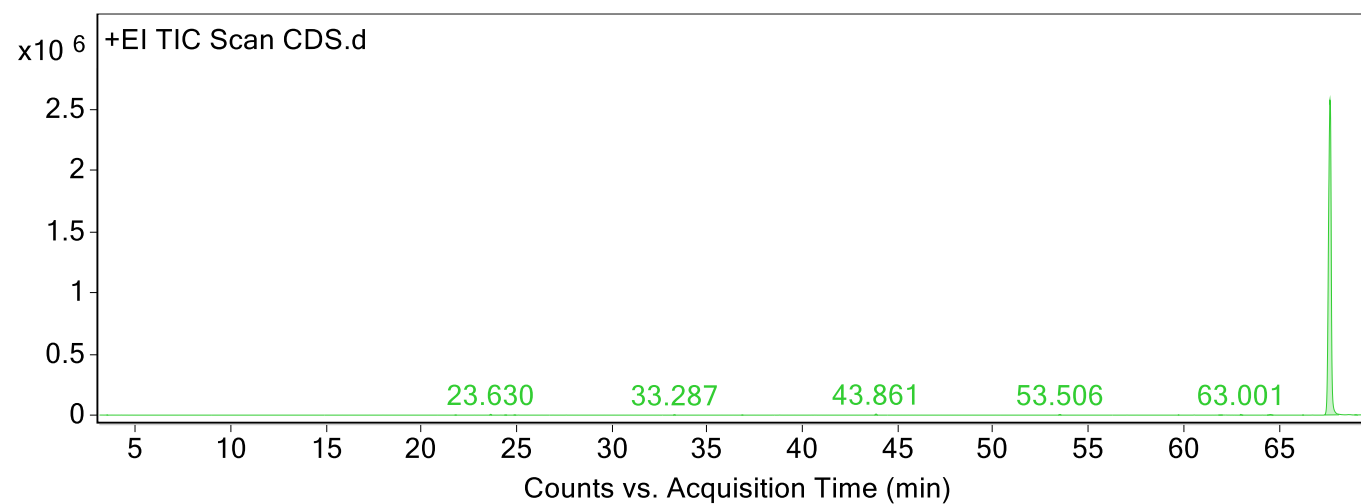
**Appendix 5:** The GC-MS chromatogram of fruit of *C. ficifolius*



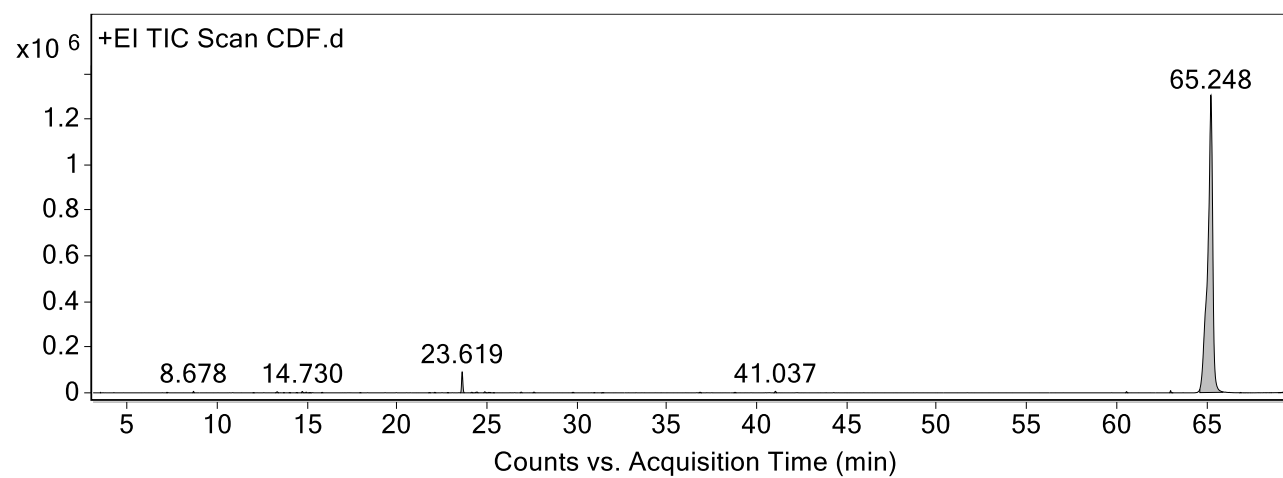
**Appendix 6:** The GC-MS chromatogram of leaves of *C. dipsaceus*



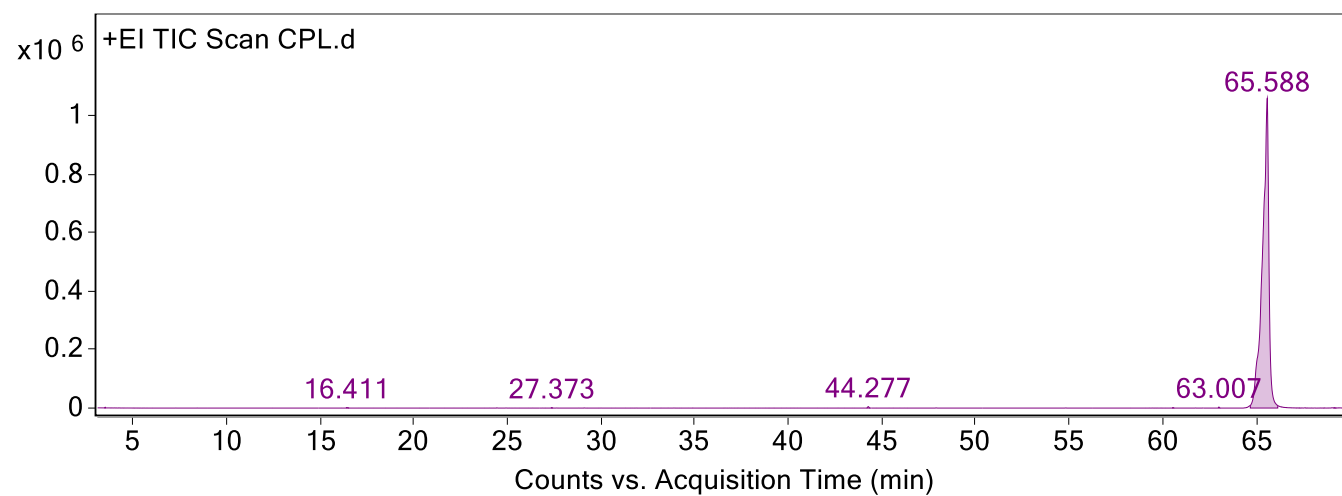
**Appendix 7:** The GC-MS chromatogram of stems of *C. dipsaceus*



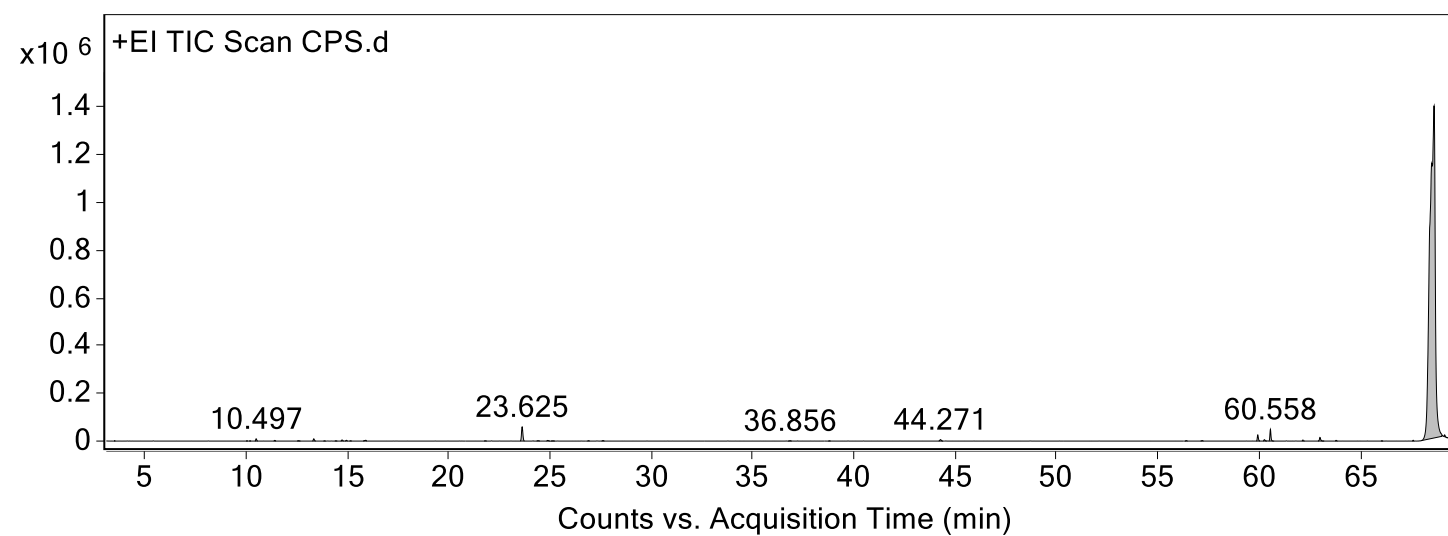
**Appendix 8:** The GC-MS chromatogram of fruit of *C. dipsaceus*



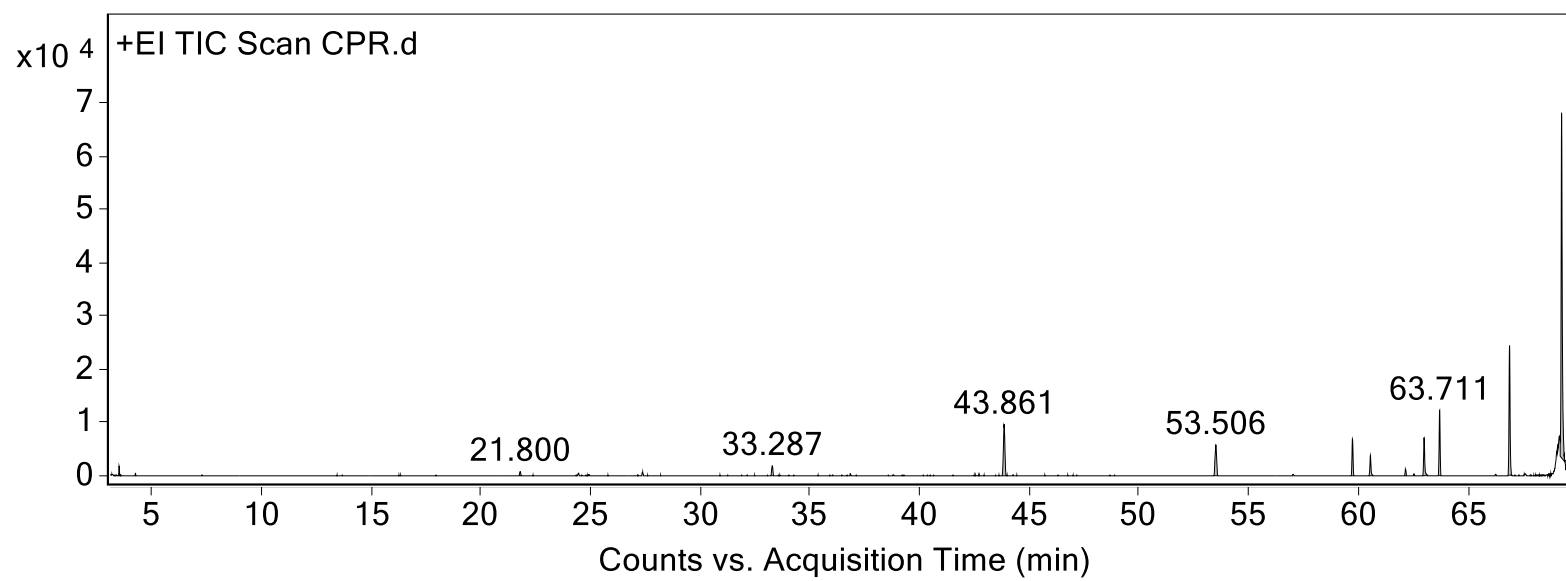
**Appendix 9:** The GC-MS chromatogram of leaves of *C. prophetarum*



**Appendix 10:** The GC-MS chromatogram of stems of *C. prophetarum*



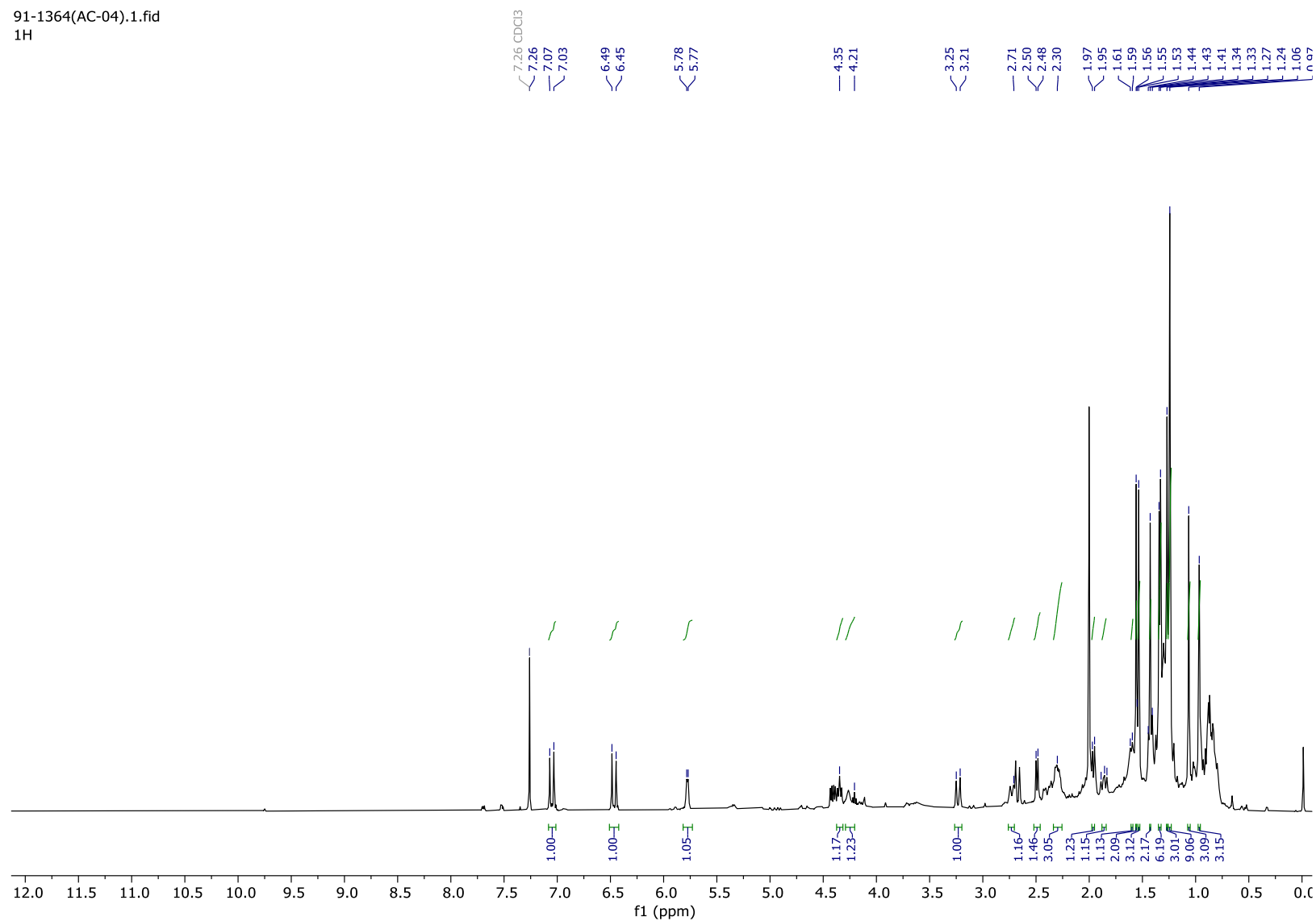
**Appendix 11:** The GC-MS chromatogram of root of *C. prophetarum*



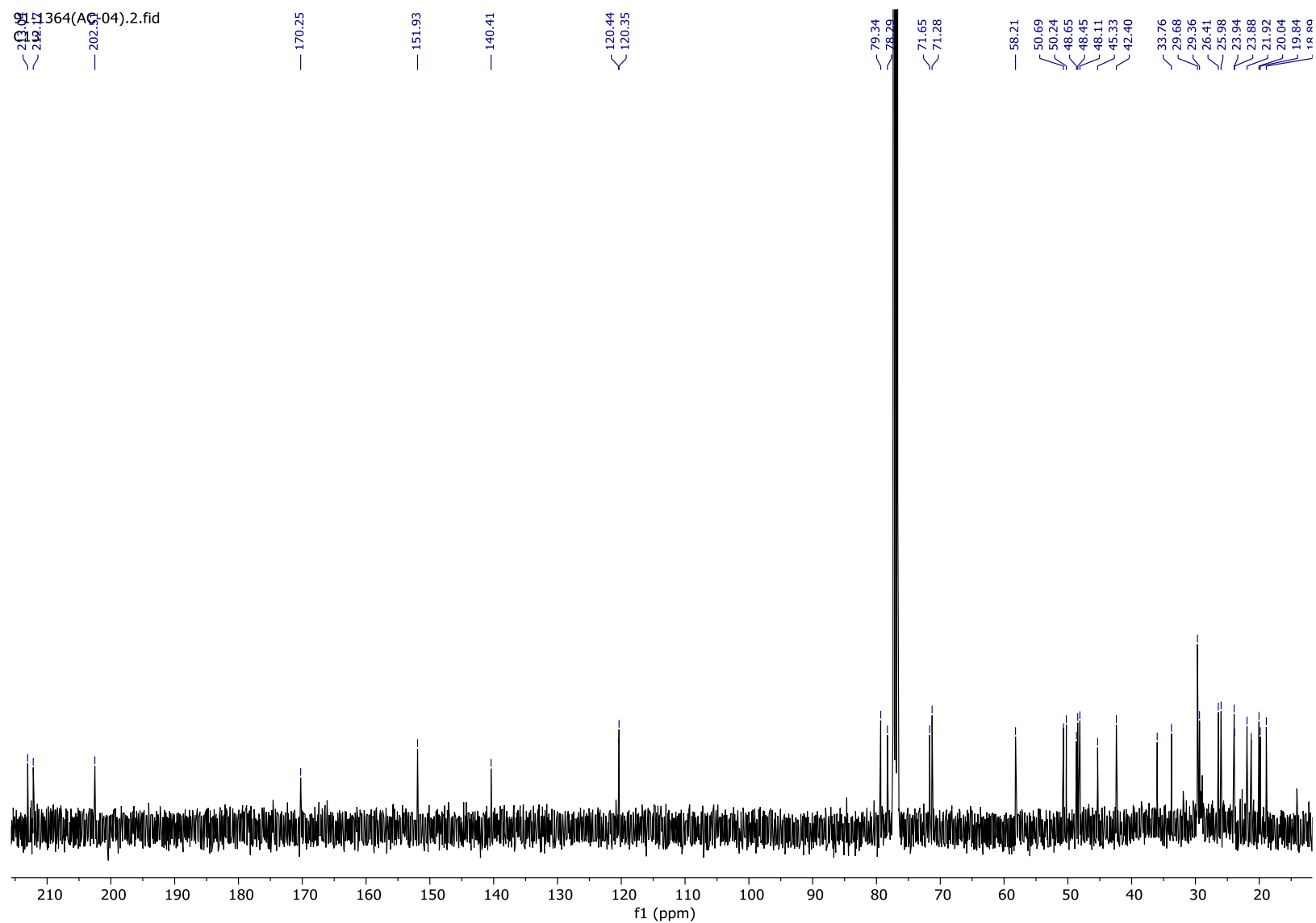
**Appendix 12a:**  $^1\text{H}$  NMR spectrum of compound **24** (400 MHz,  $\text{CDCl}_3$ )

91-1364(AC-04).1.fid

$^1\text{H}$

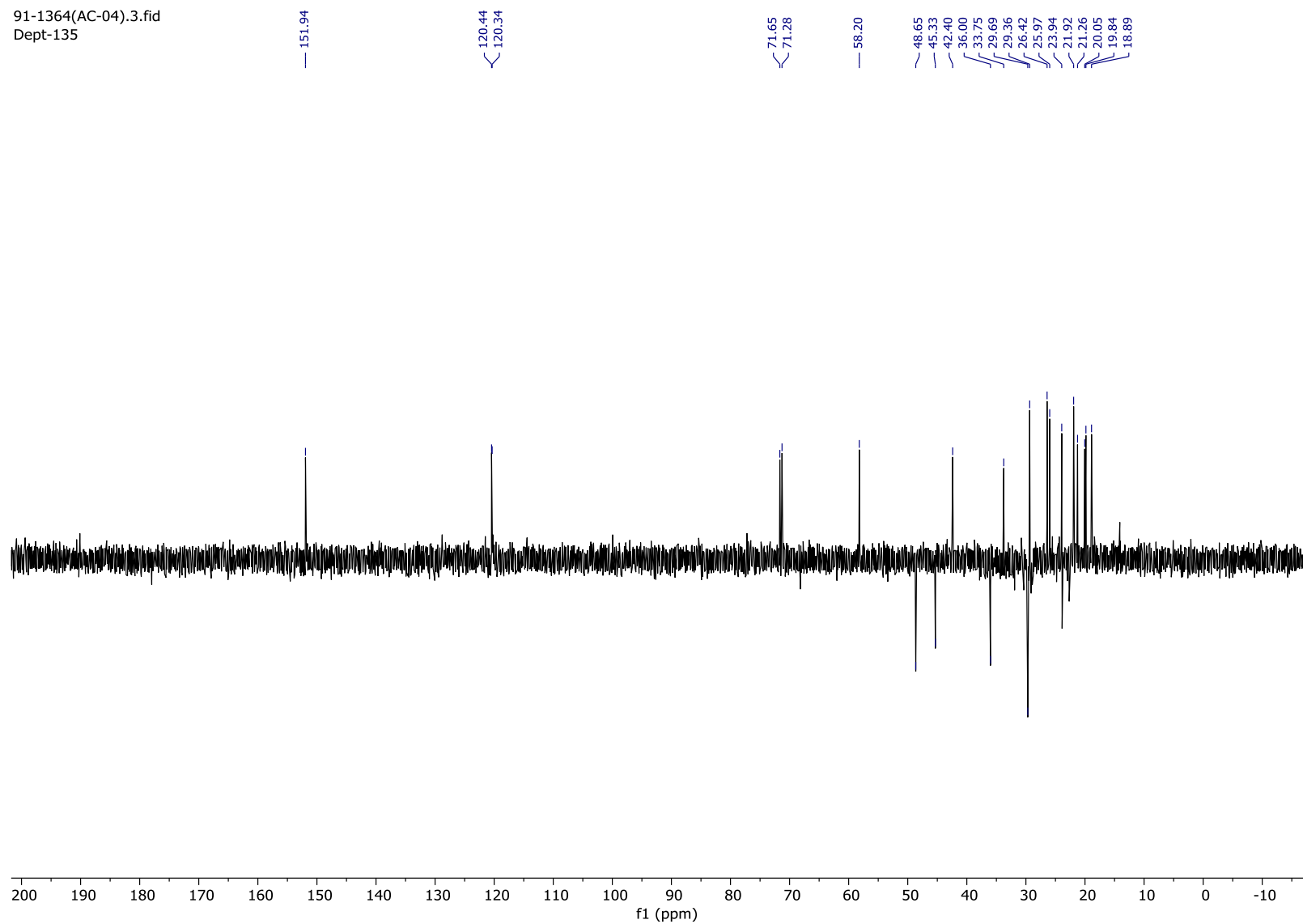


**Appendix 12b:**  $^{13}\text{C}$  NMR spectrum of compound **24** (100 MHz,  $\text{CDCl}_3$ )

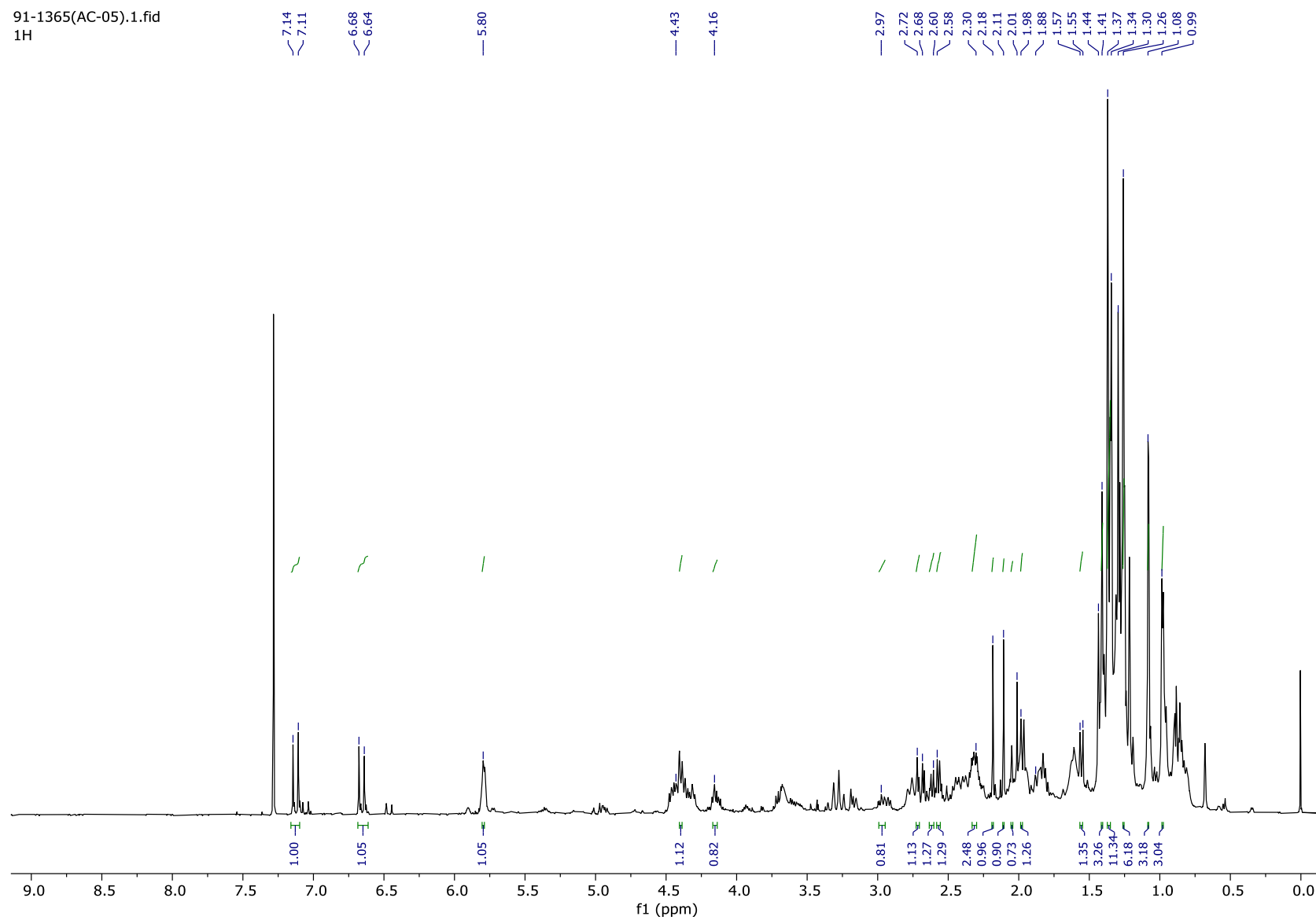


**Appendix 12c:** DEPT-135 NMR spectrum of compound **24** (100 MHz, CDCl<sub>3</sub>)

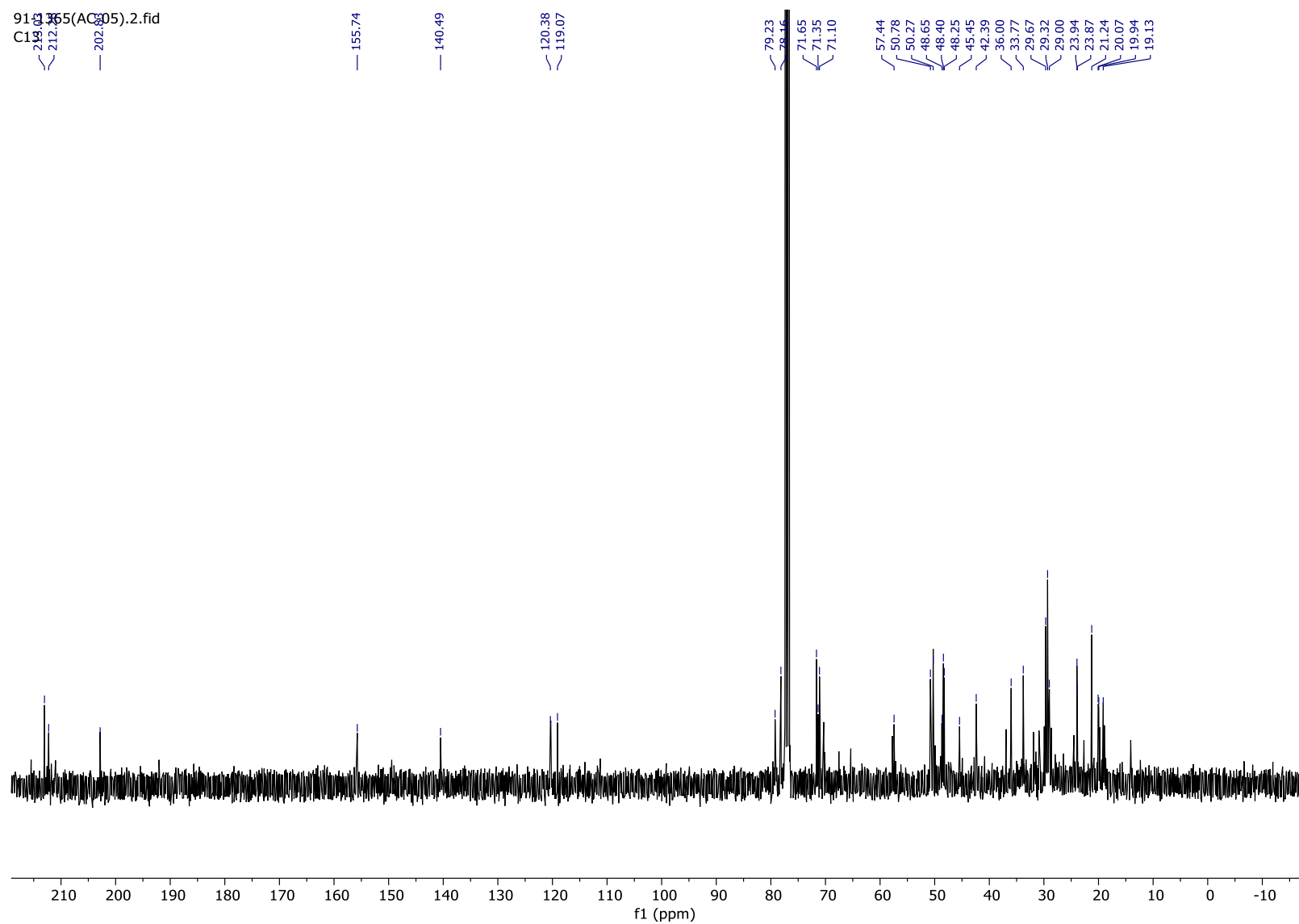
91-1364(AC-04).3.fid  
Dept-135



**Appendix 13a:**  $^1\text{H}$  NMR spectrum of compound **26** (400 MHz,  $\text{CDCl}_3$ )

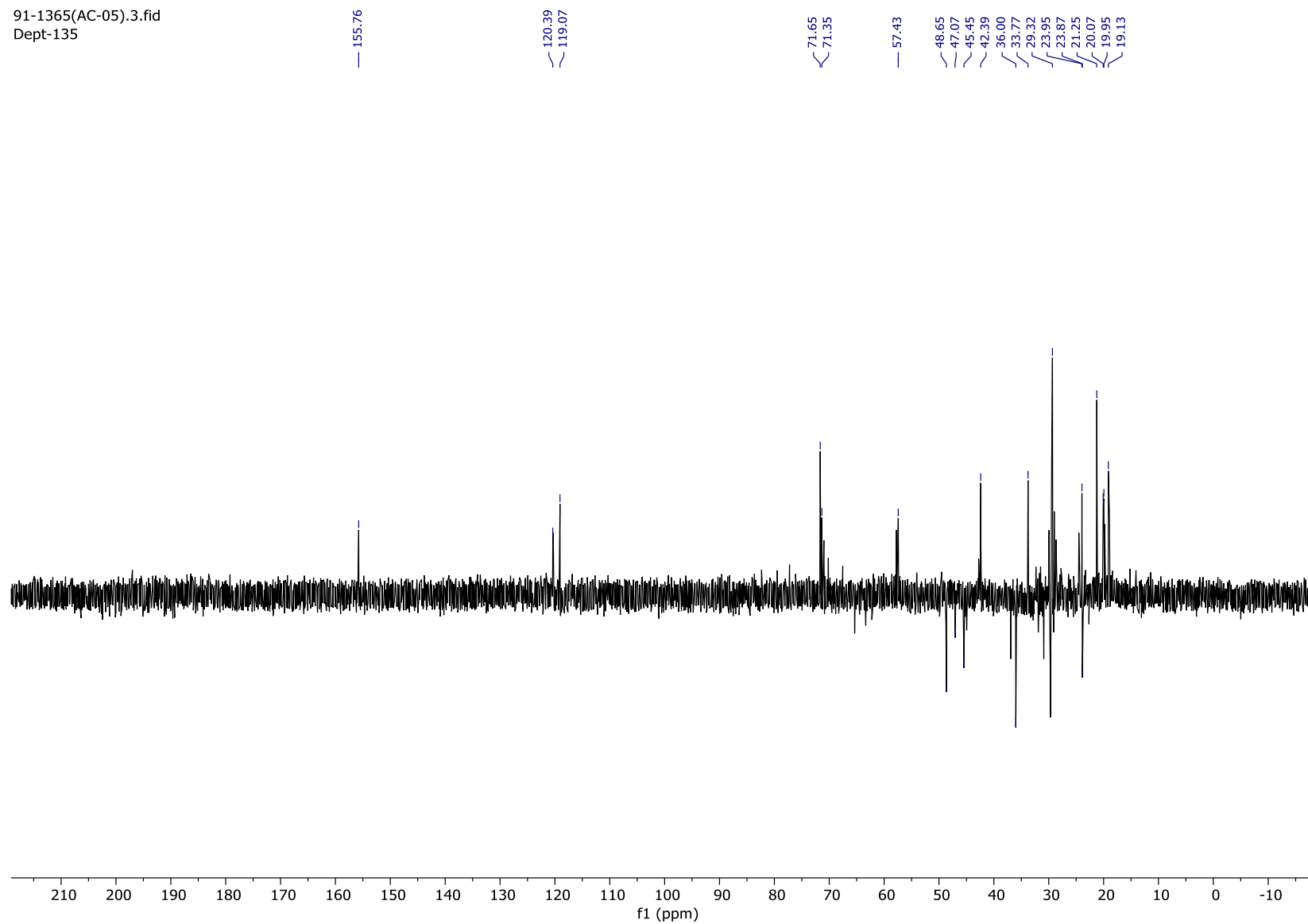


**Appendix 13b:**  $^{13}\text{C}$  NMR spectrum of compound **26** (100 MHz,  $\text{CDCl}_3$ )



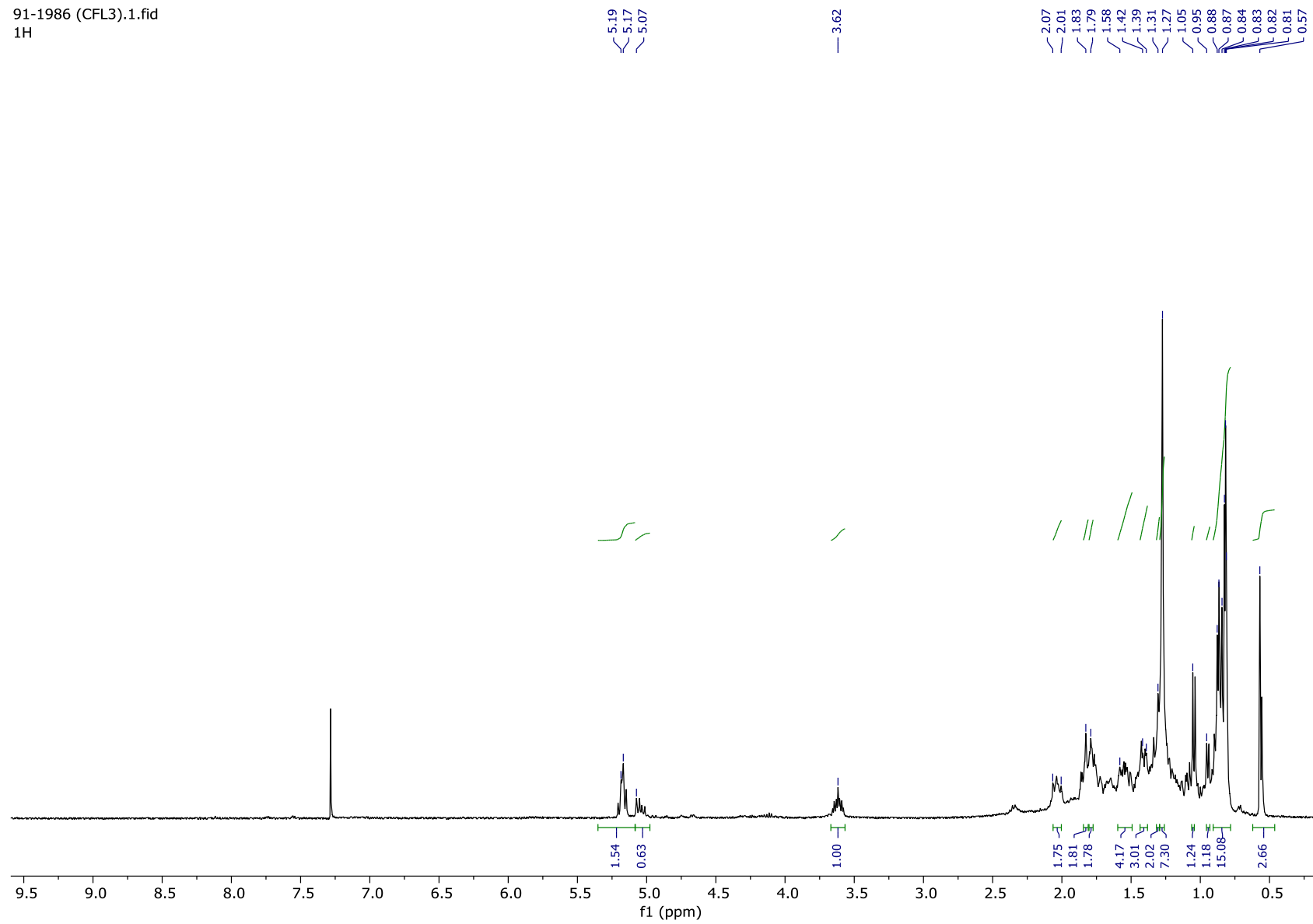
**Appendix 13c:** DEPT-135 NMR spectrum of compound **26** (100 MHz, CDCl<sub>3</sub>)

91-1365(AC-05).3.fid  
Dept-135



**Appendix 14a:**  $^1\text{H}$  NMR spectrum of compound **58** (400 MHz,  $\text{CDCl}_3$ )

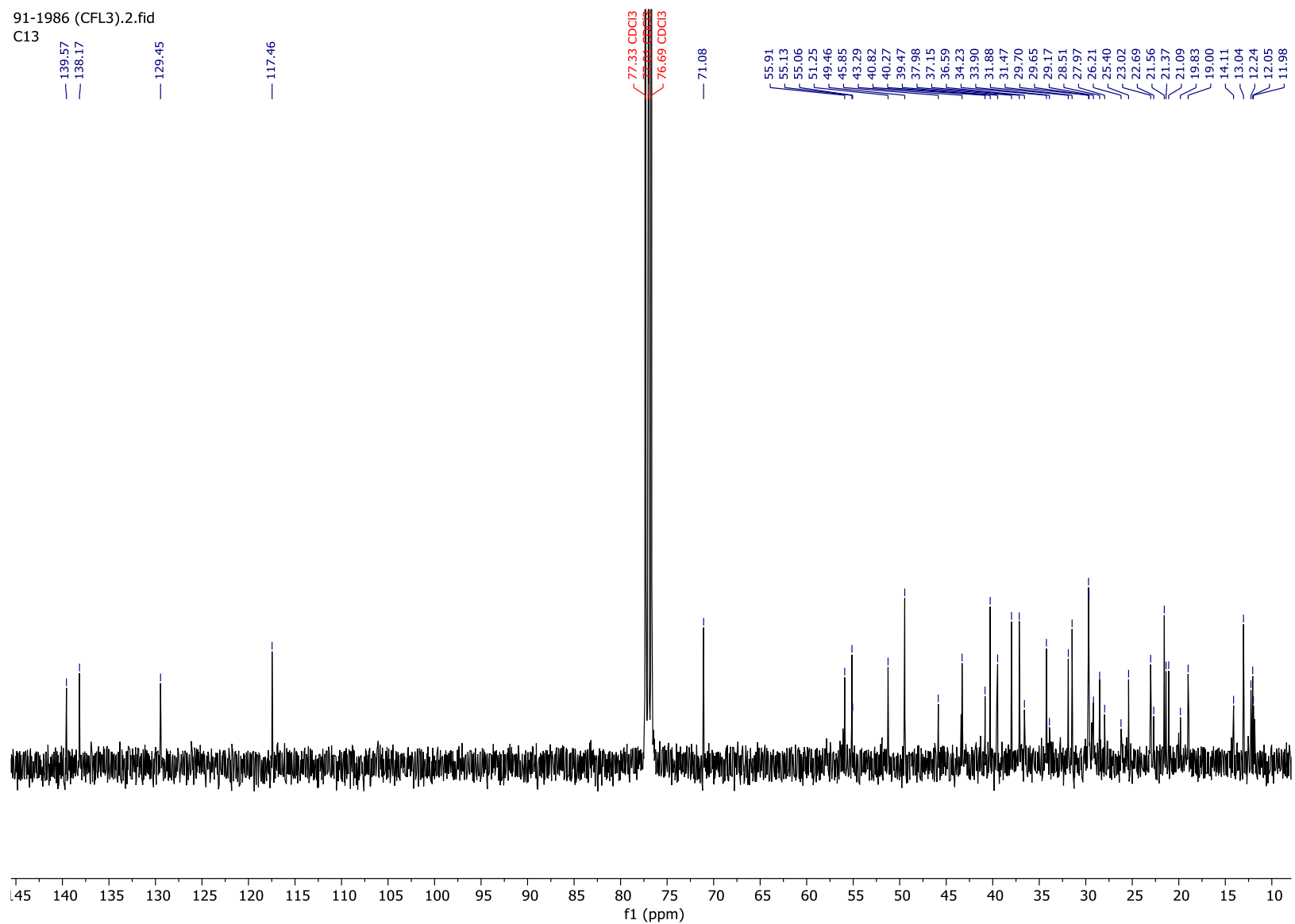
91-1986 (CFL3).1.fid  
 $^1\text{H}$



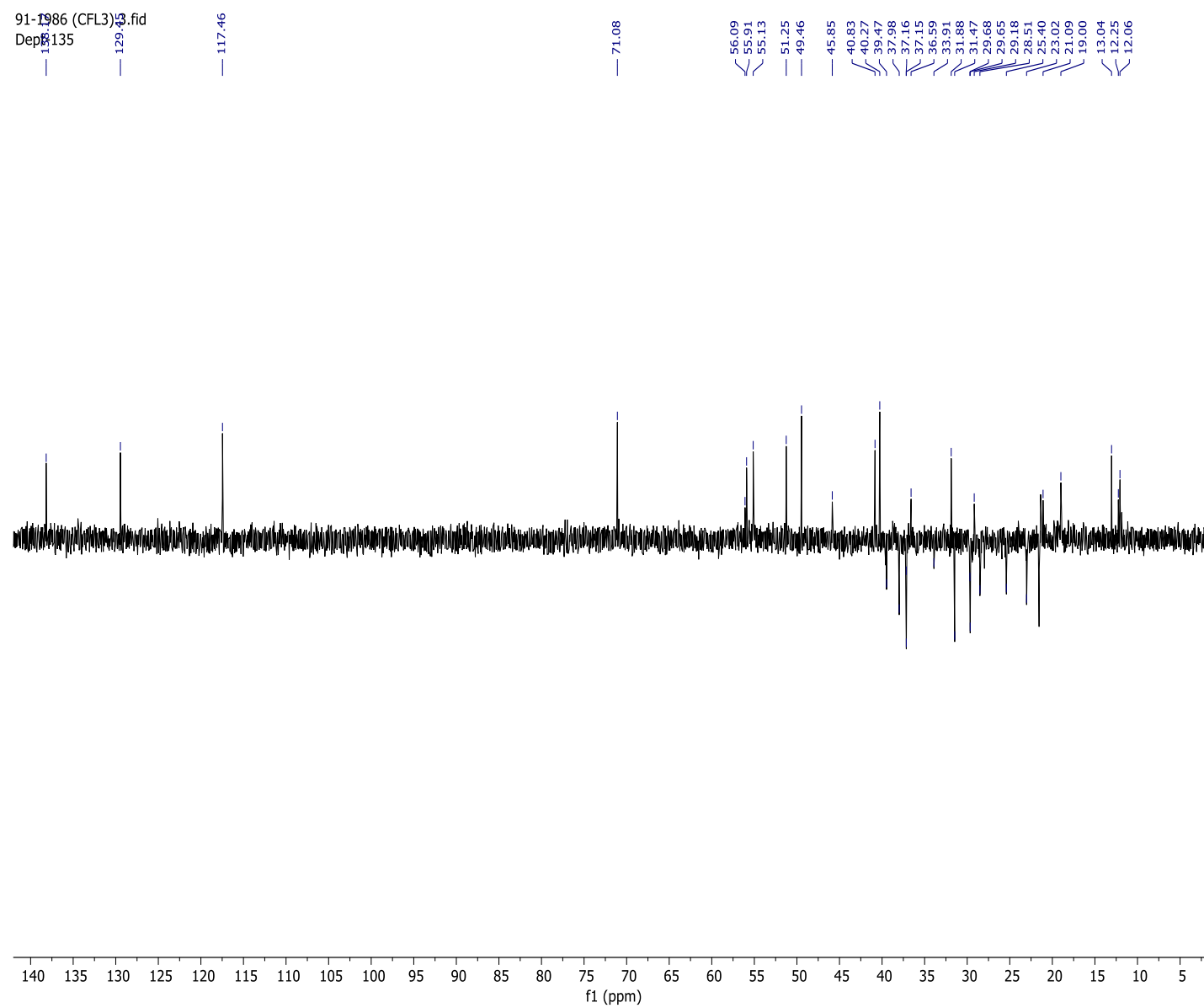
**Appendix 14b:**  $^{13}\text{C}$  NMR spectrum of compound **58** (100 MHz,  $\text{CDCl}_3$ )

91-1986 (CFL3).2.fid

C13

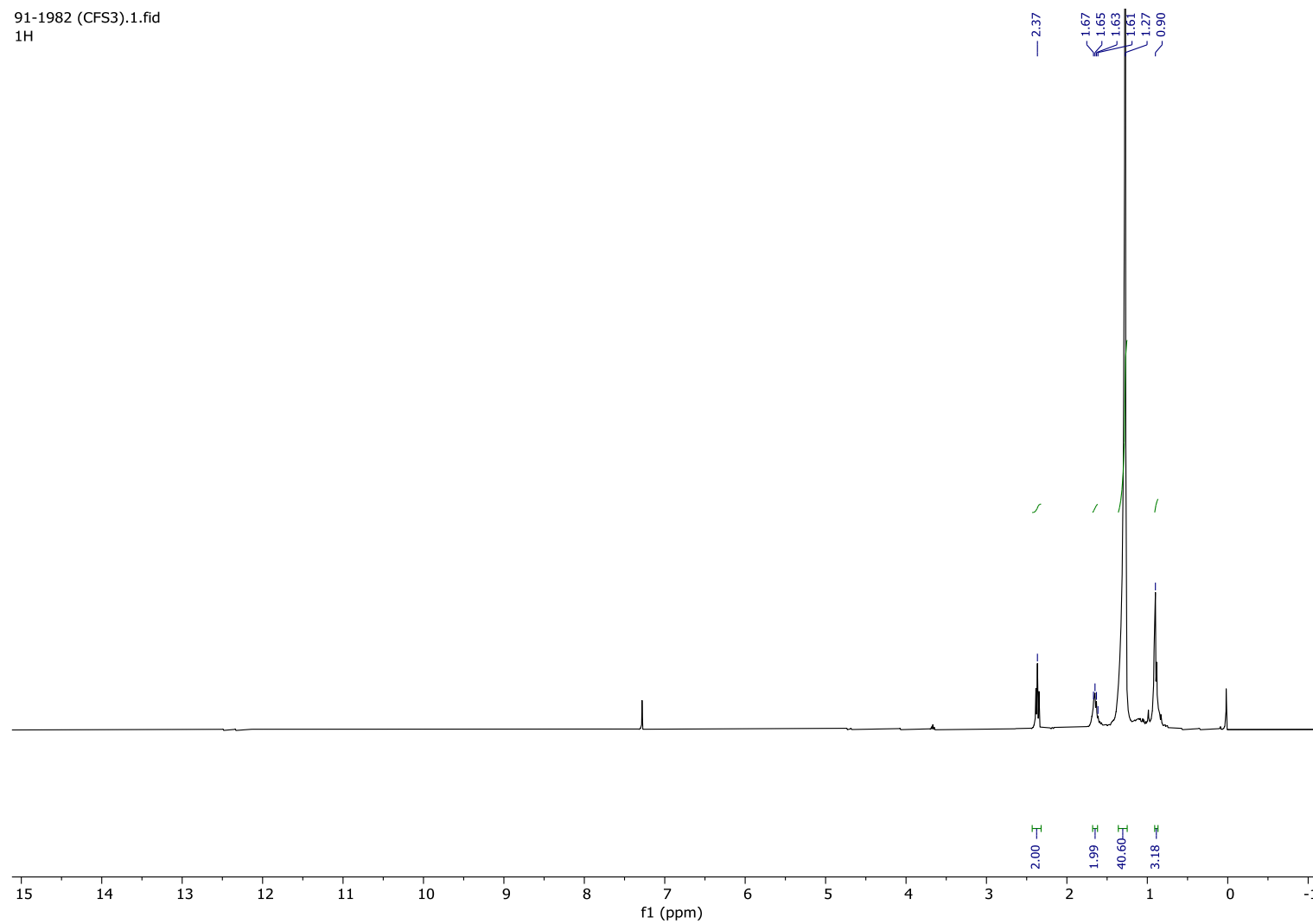


**Appendix 14c:** DEPT-135 NMR spectrum of compound **58** (100 MHz, CDCl<sub>3</sub>)



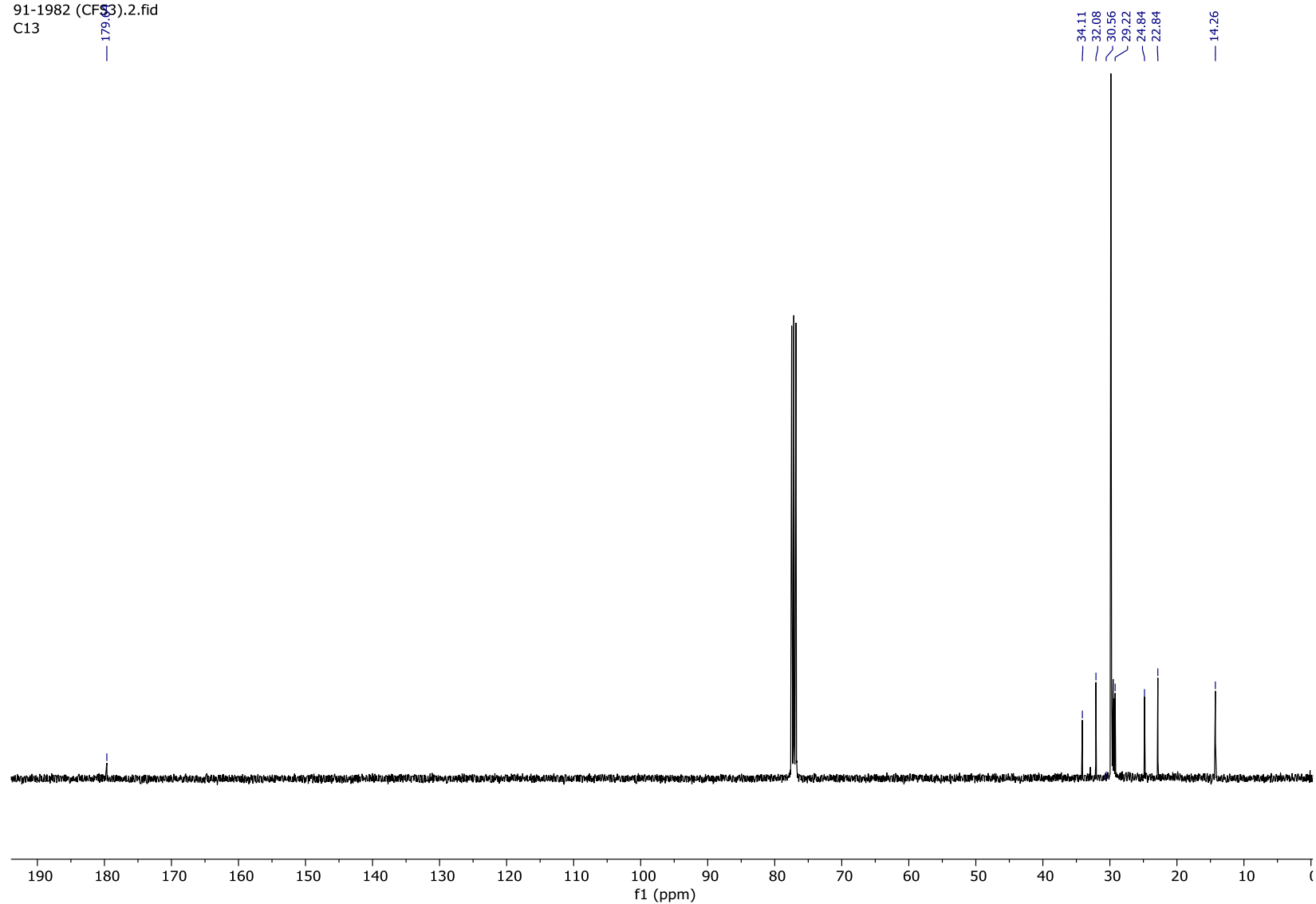
Appendix 15a:  $^1\text{H}$  NMR spectrum of compound **104** (400 MHz,  $\text{CDCl}_3$ )

91-1982 (CFS3).1.fid  
 $^1\text{H}$



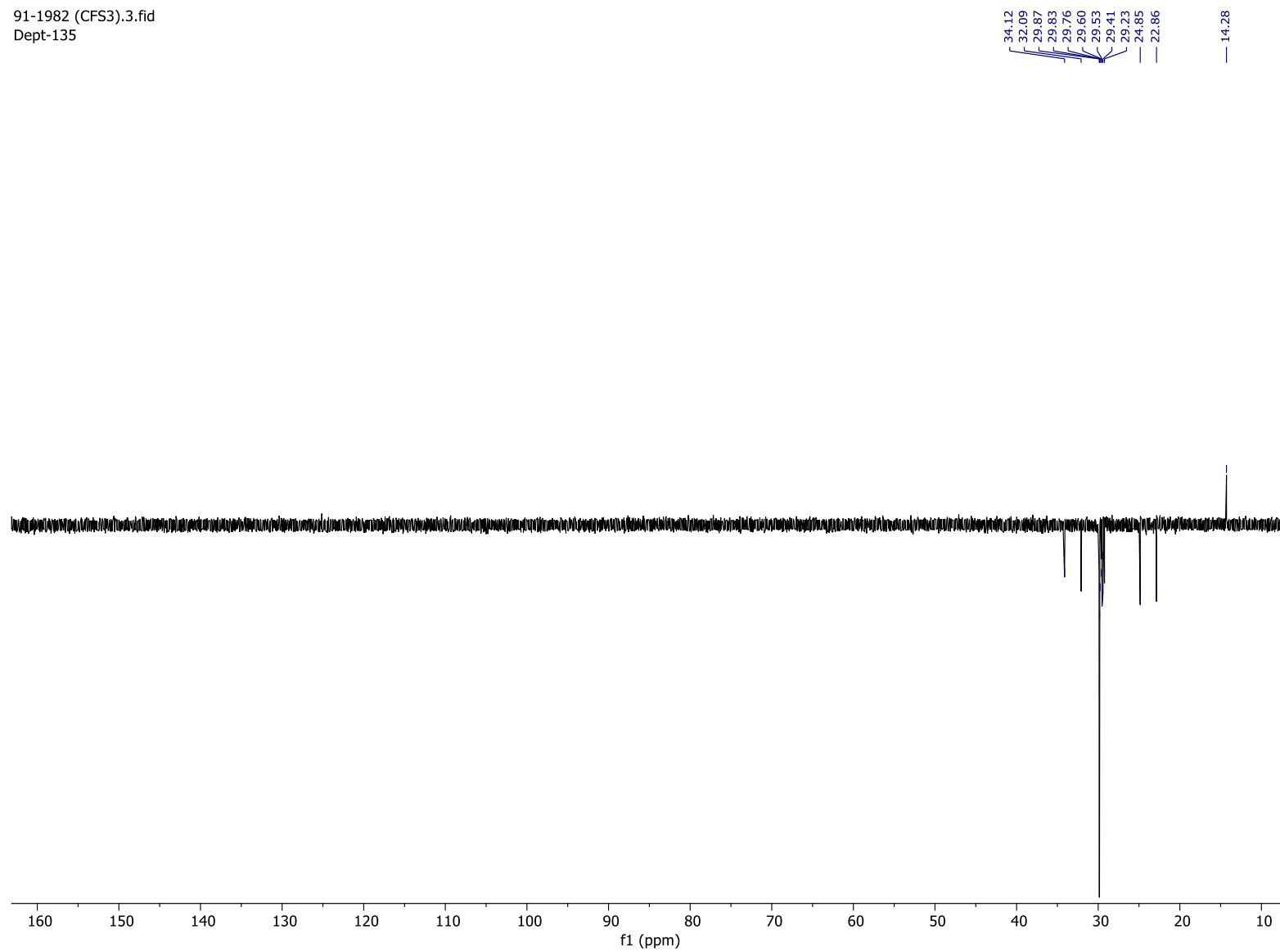
**Appendix 15b:**  $^{13}\text{C}$  NMR spectrum of compound **104** (100 MHz,  $\text{CDCl}_3$ )

91-1982 (CF53).2.fid  
C13



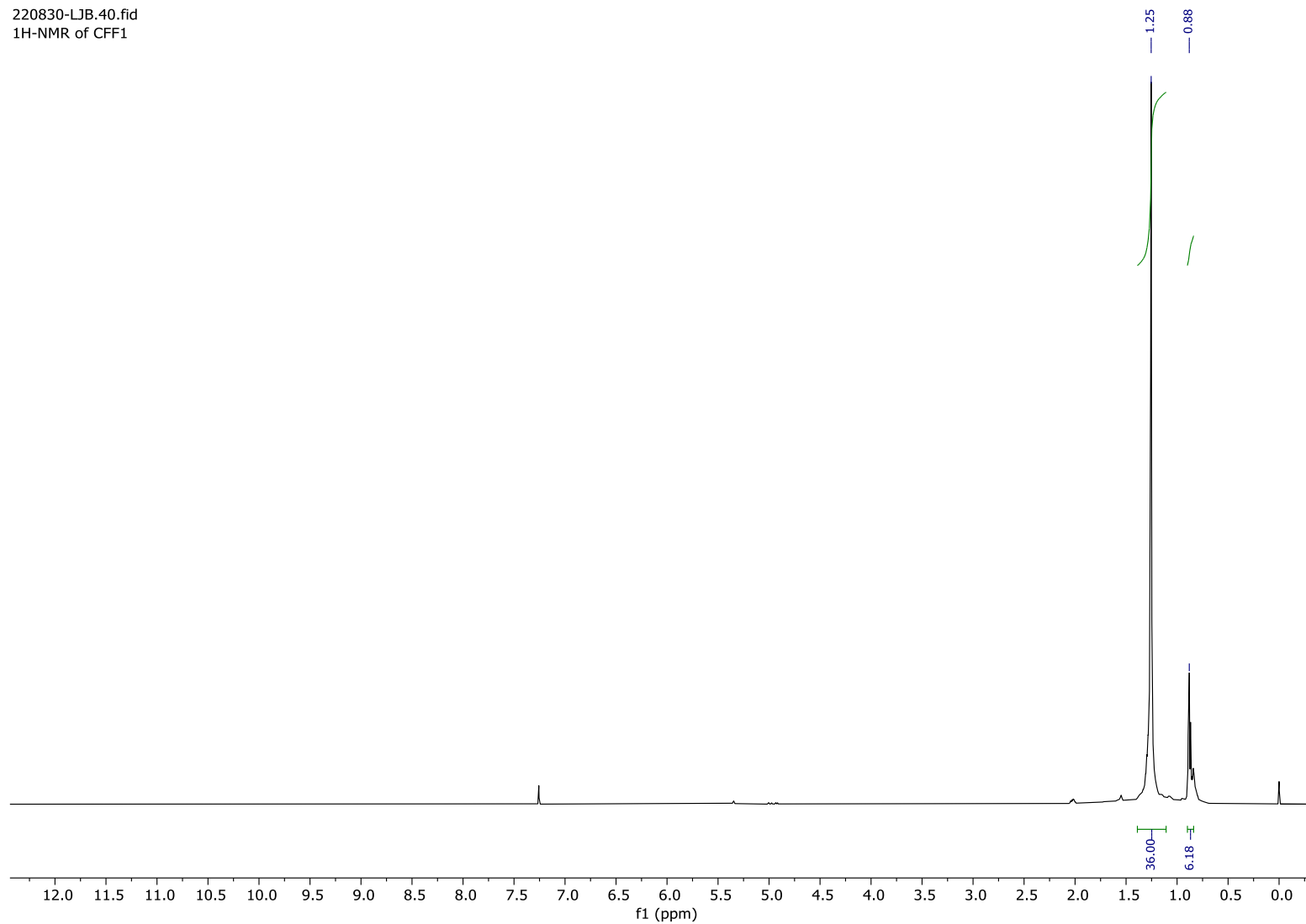
**Appendix 15c:** DEPT-135 NMR spectrum of compound **104** (100 MHz, CDCl<sub>3</sub>)

91-1982 (CFS3).3.fid  
Dept-135



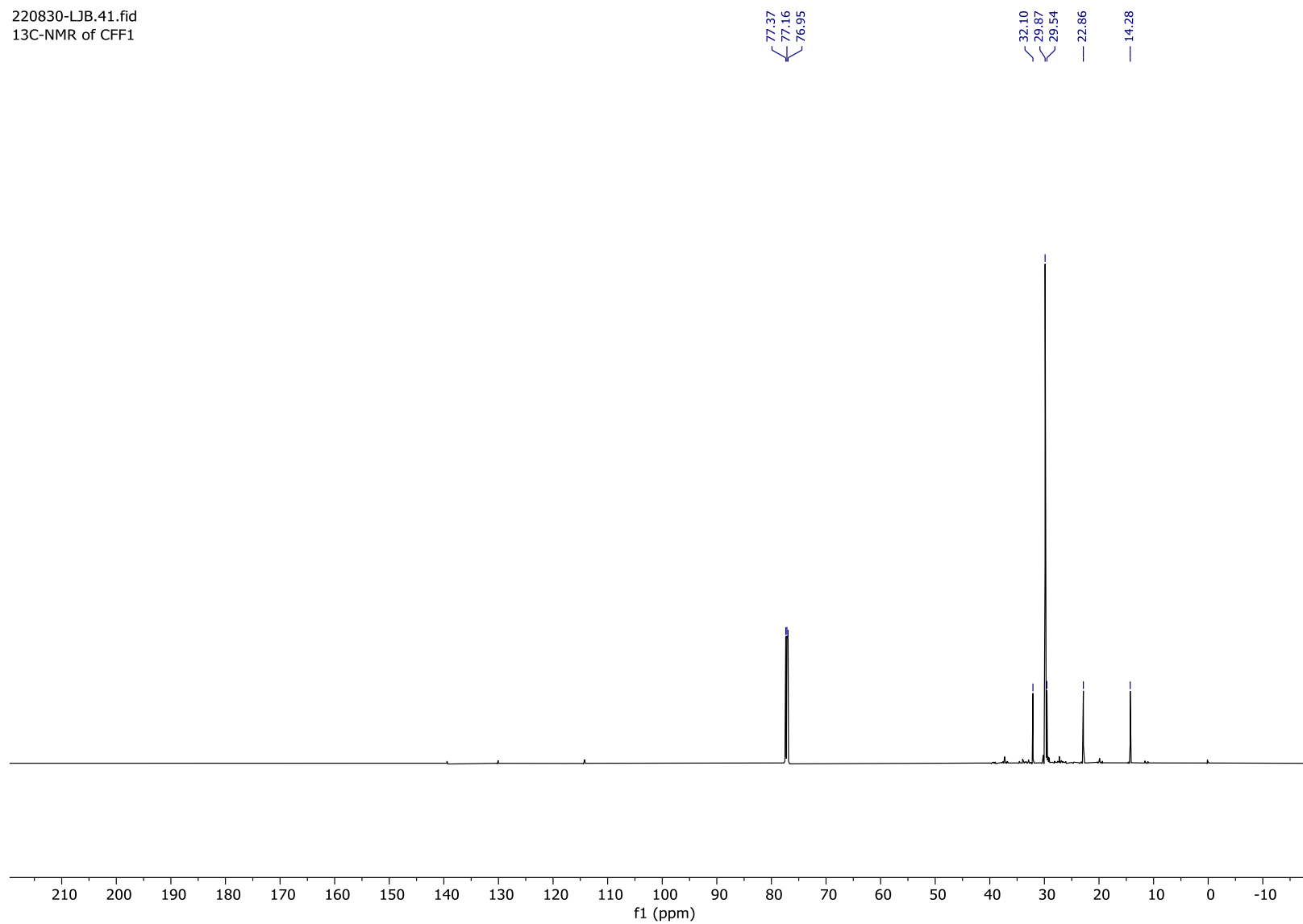
**Appendix 16a:**  $^1\text{H}$  NMR spectrum of compound **105** (400 MHz,  $\text{CDCl}_3$ )

220830-LJB.40.fid  
1H-NMR of CFF1



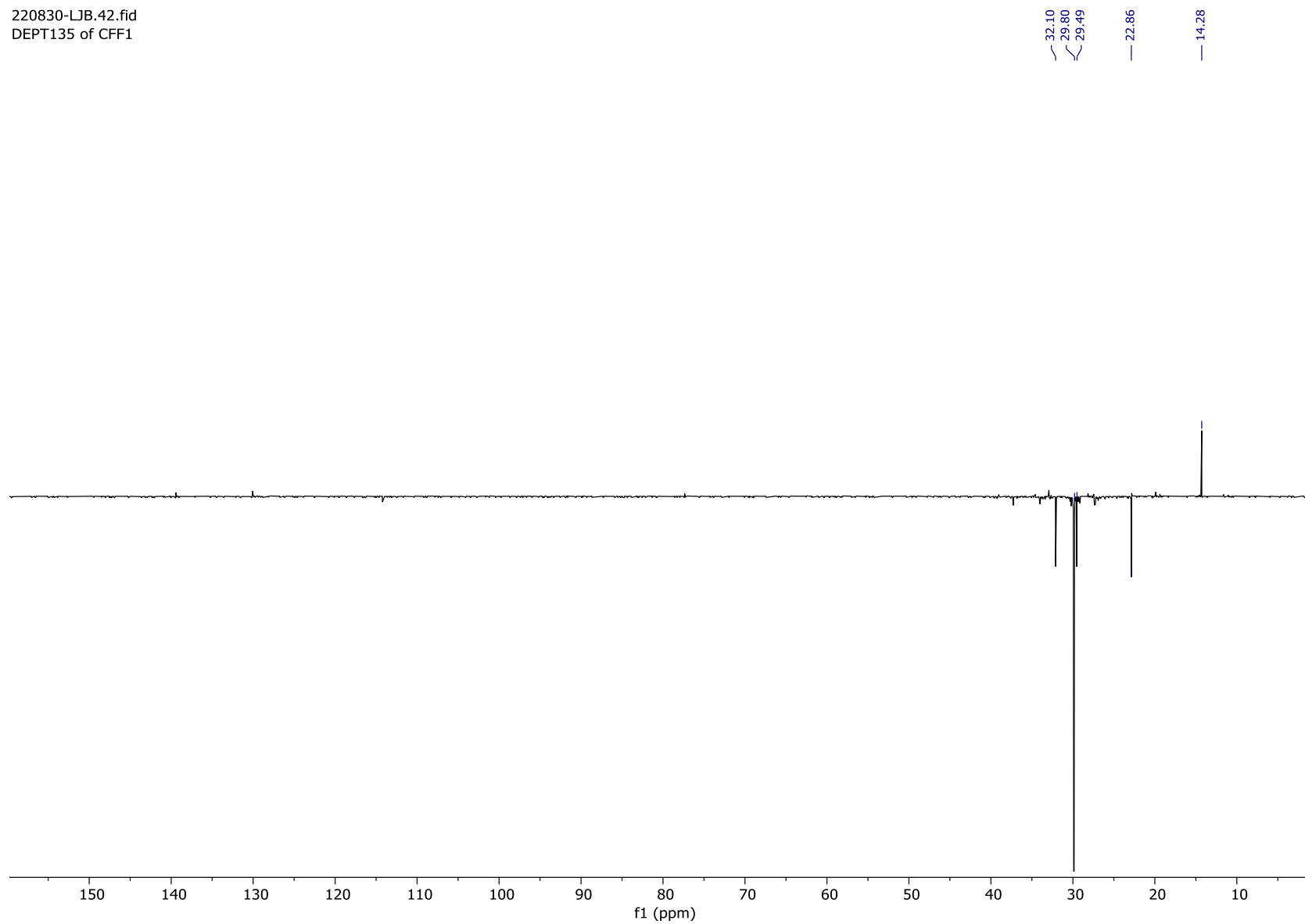
**Appendix 16b:**  $^{13}\text{C}$  NMR spectrum of compound **105** (400 MHz,  $\text{CDCl}_3$ )

220830-LJB.41.fid  
13C-NMR of CFF1



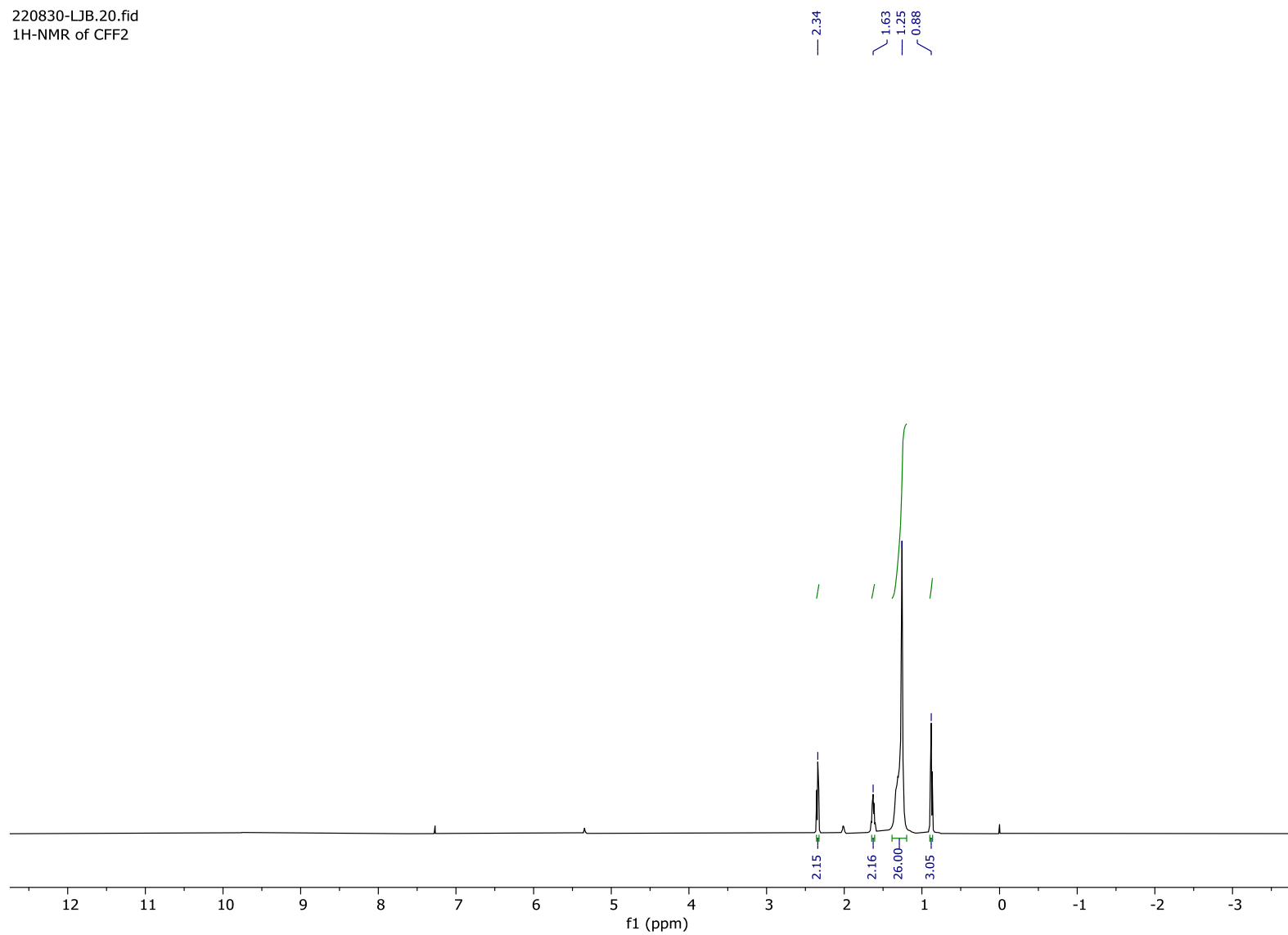
**Appendix 16c:** DEPT-135 NMR spectrum of compound **105** (100 MHz, CDCl<sub>3</sub>)

220830-LJB.42.fid  
DEPT135 of CFF1



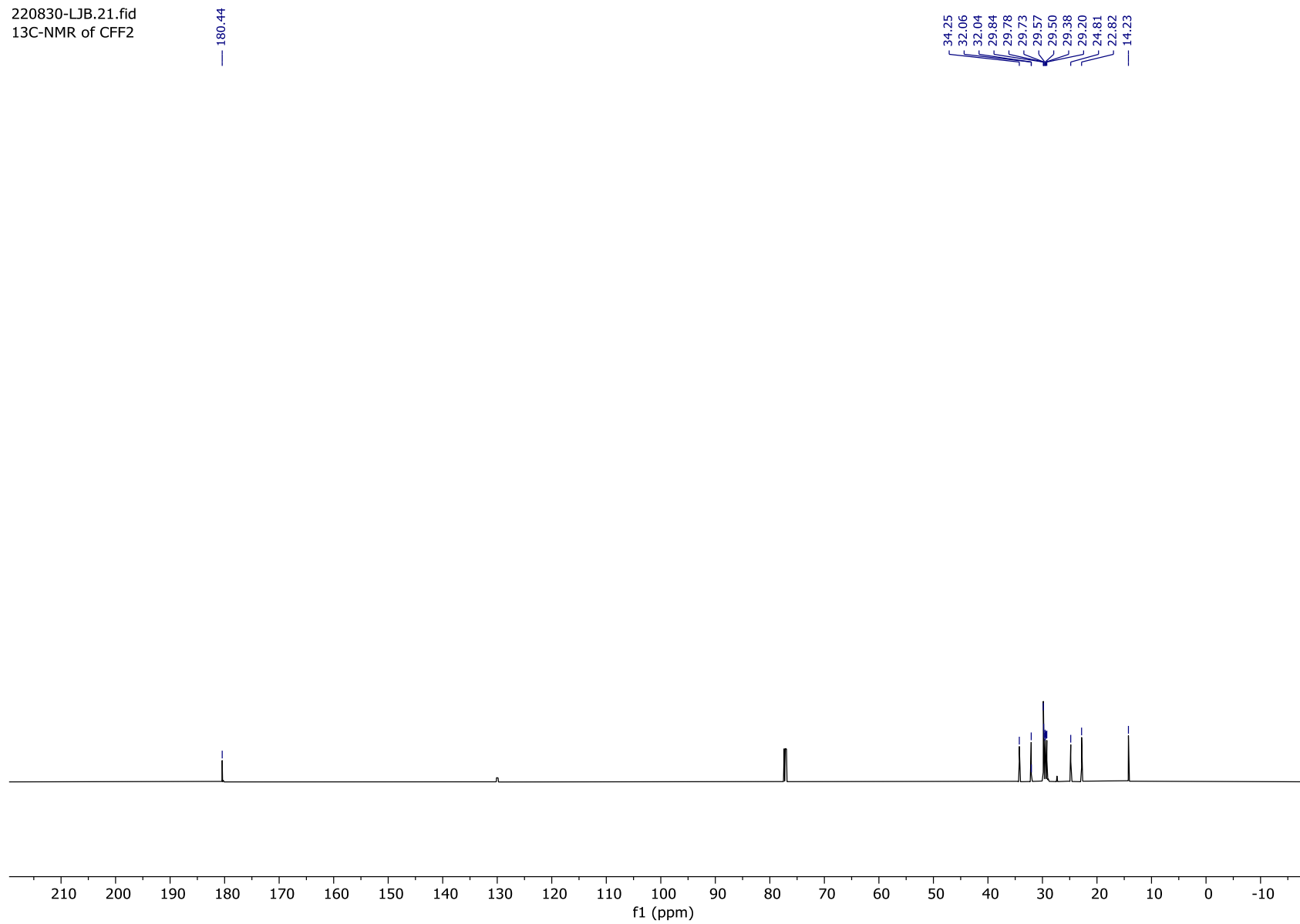
**Appendix 17a:**  $^1\text{H}$  NMR spectrum of compound **106** (400 MHz,  $\text{CDCl}_3$ )

220830-LJB.20.fid  
1H-NMR of CFF2



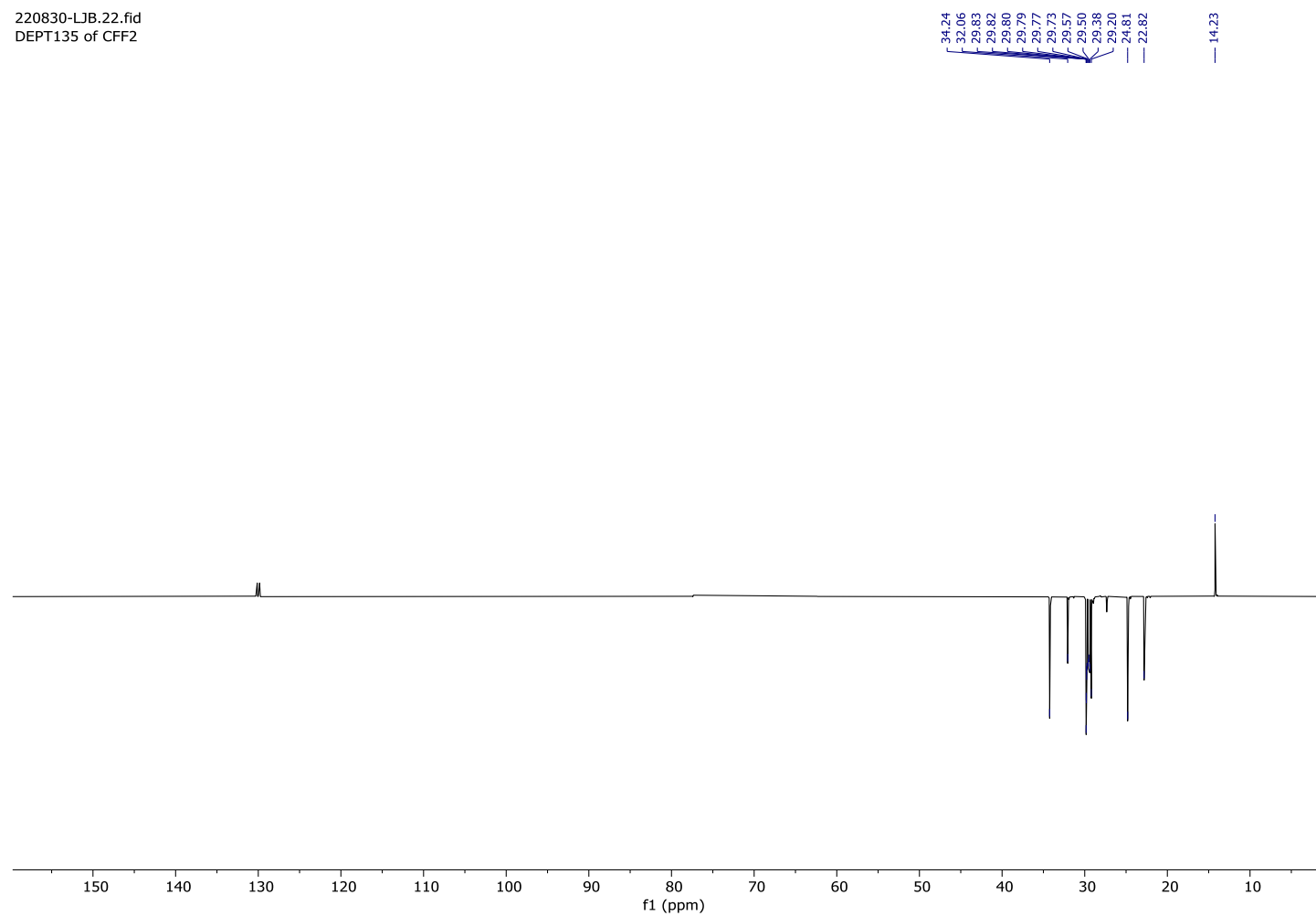
**Appendix 17b:**  $^{13}\text{C}$  NMR spectrum of compound **106** (400 MHz,  $\text{CDCl}_3$ )

220830-LJB.21.fid  
13C-NMR of CFF2



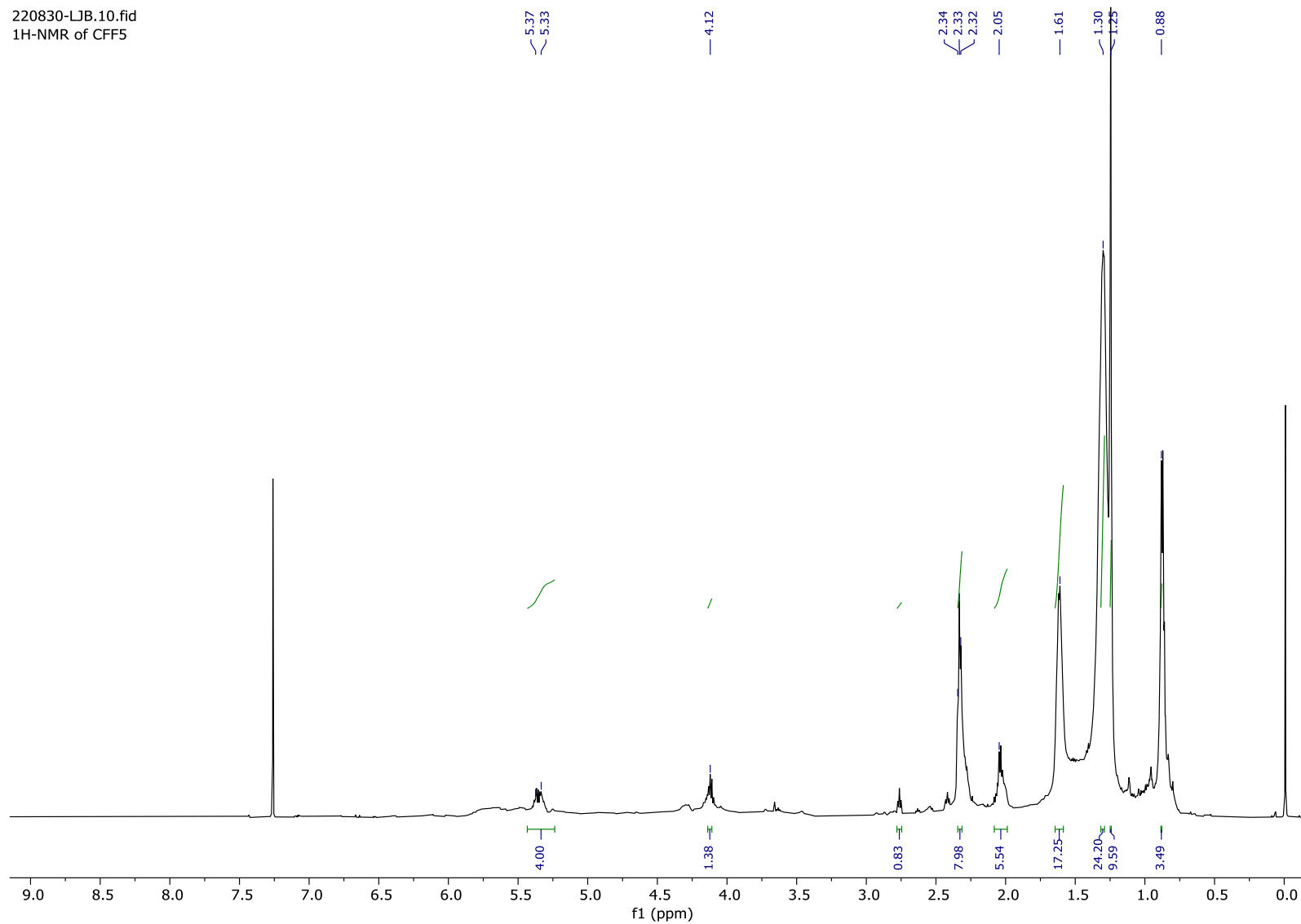
**Appendix 17c:** DEPT-135 NMR spectrum of compound **106** (100 MHz, CDCl<sub>3</sub>)

220830-LJB.22.fid  
DEPT135 of CFF2



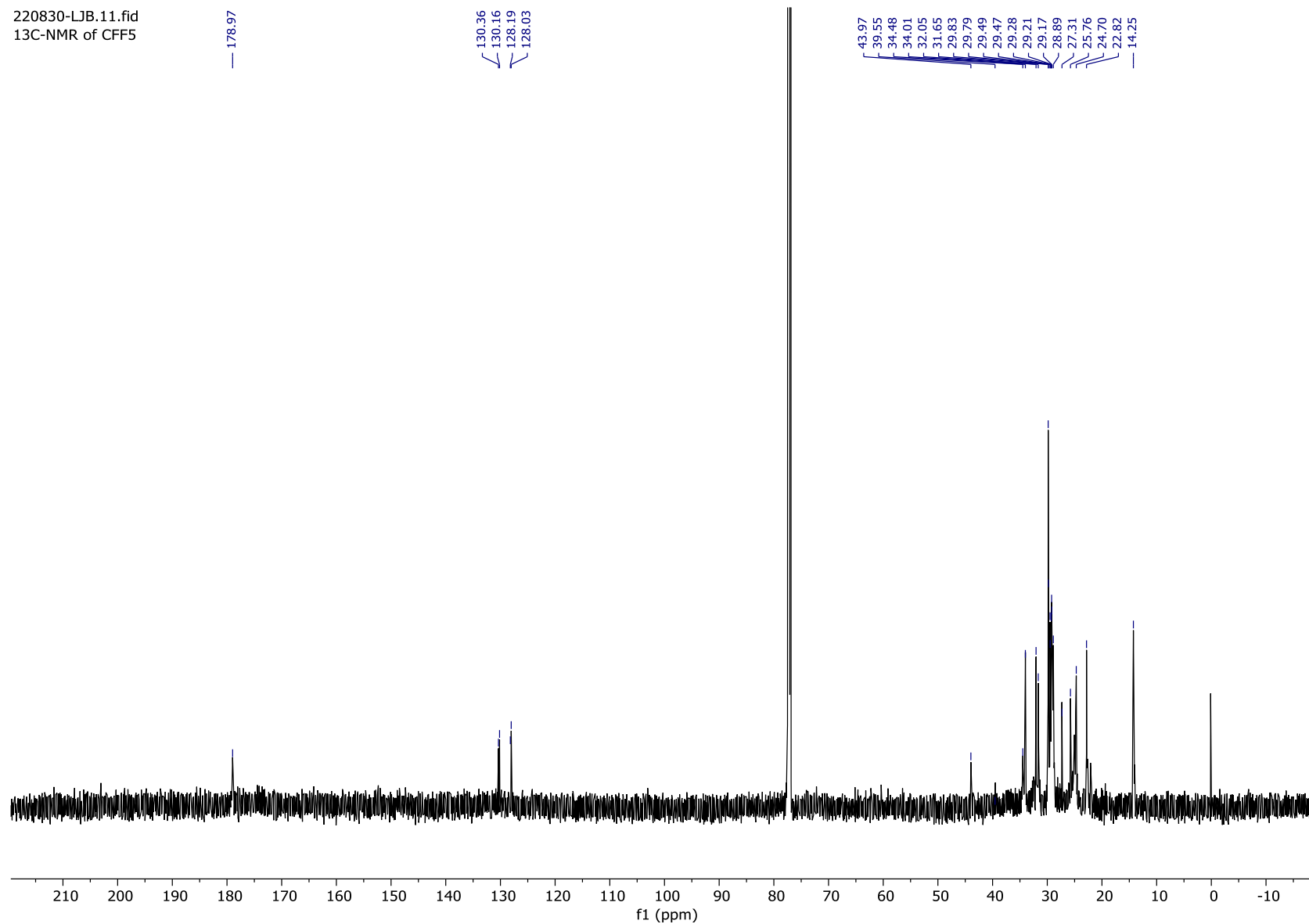
**Appendix 18a:**  $^1\text{H}$  NMR spectrum of compound **107** (400 MHz,  $\text{CDCl}_3$ )

220830-LJB.10.fid  
1H-NMR of CFF5



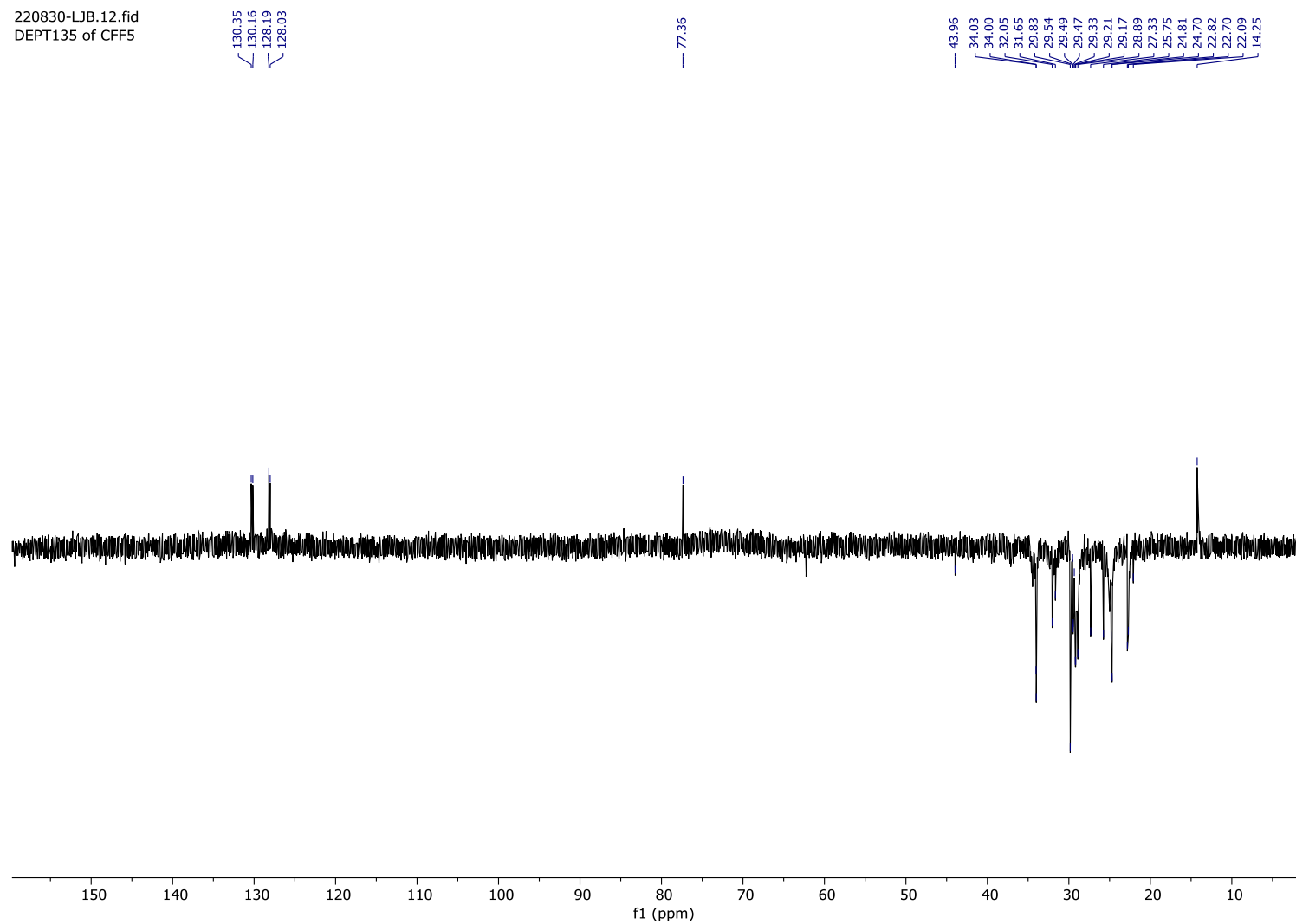
**Appendix 18b:**  $^{13}\text{C}$  NMR spectrum of compound **107** (400 MHz,  $\text{CDCl}_3$ )

220830-LJB.11.fid  
13C-NMR of CFF5



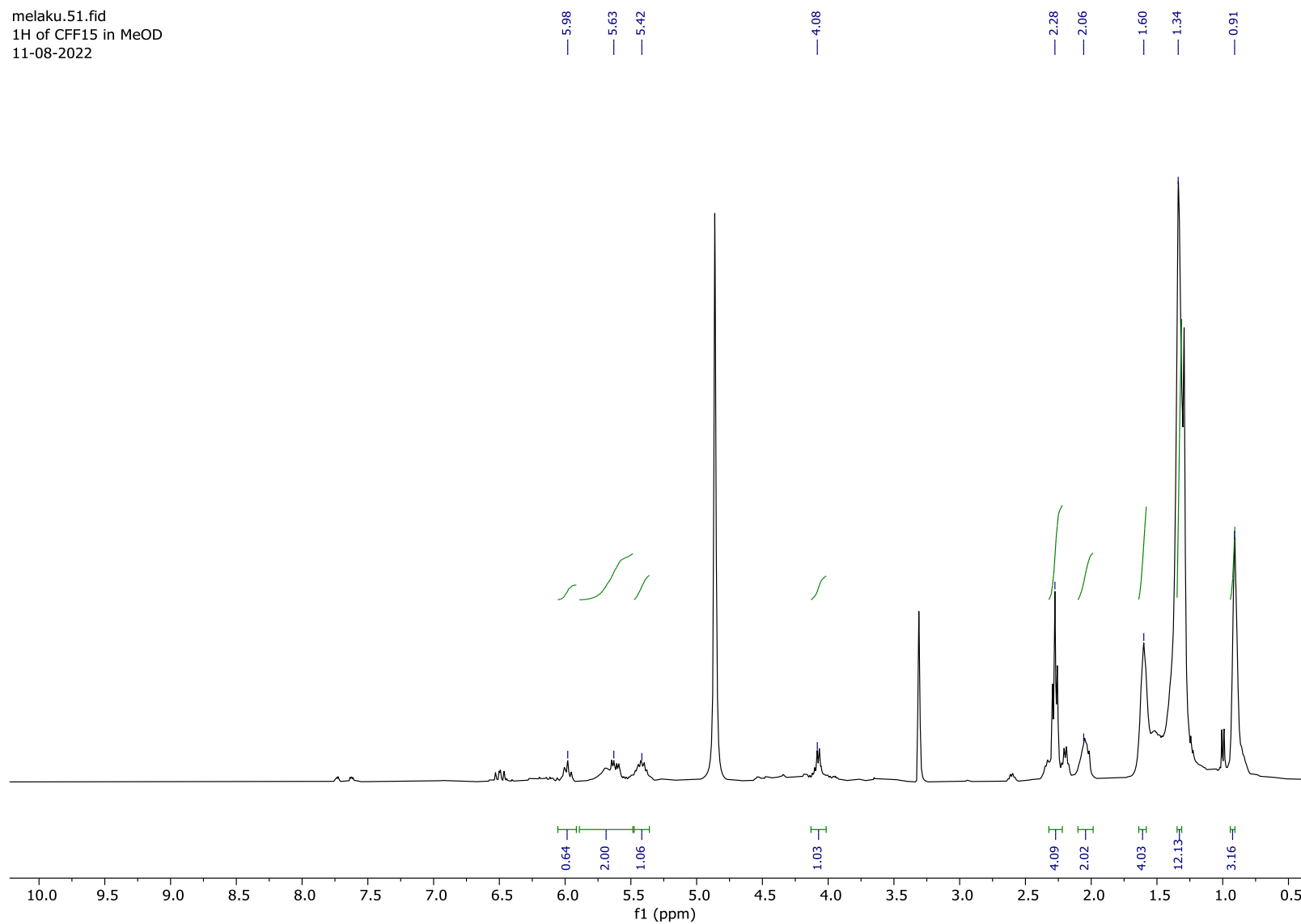
**Appendix 18c:** DEPT-135 NMR spectrum of compound **107** (100 MHz, CDCl<sub>3</sub>)

220830-LJB.12.fid  
DEPT135 of CFF5



**Appendix 19a:**  $^1\text{H}$  NMR spectrum of compound **108** (400 MHz,  $\text{CD}_3\text{OD}$ )

melaku.51.fid  
1H of CFF15 in MeOD  
11-08-2022

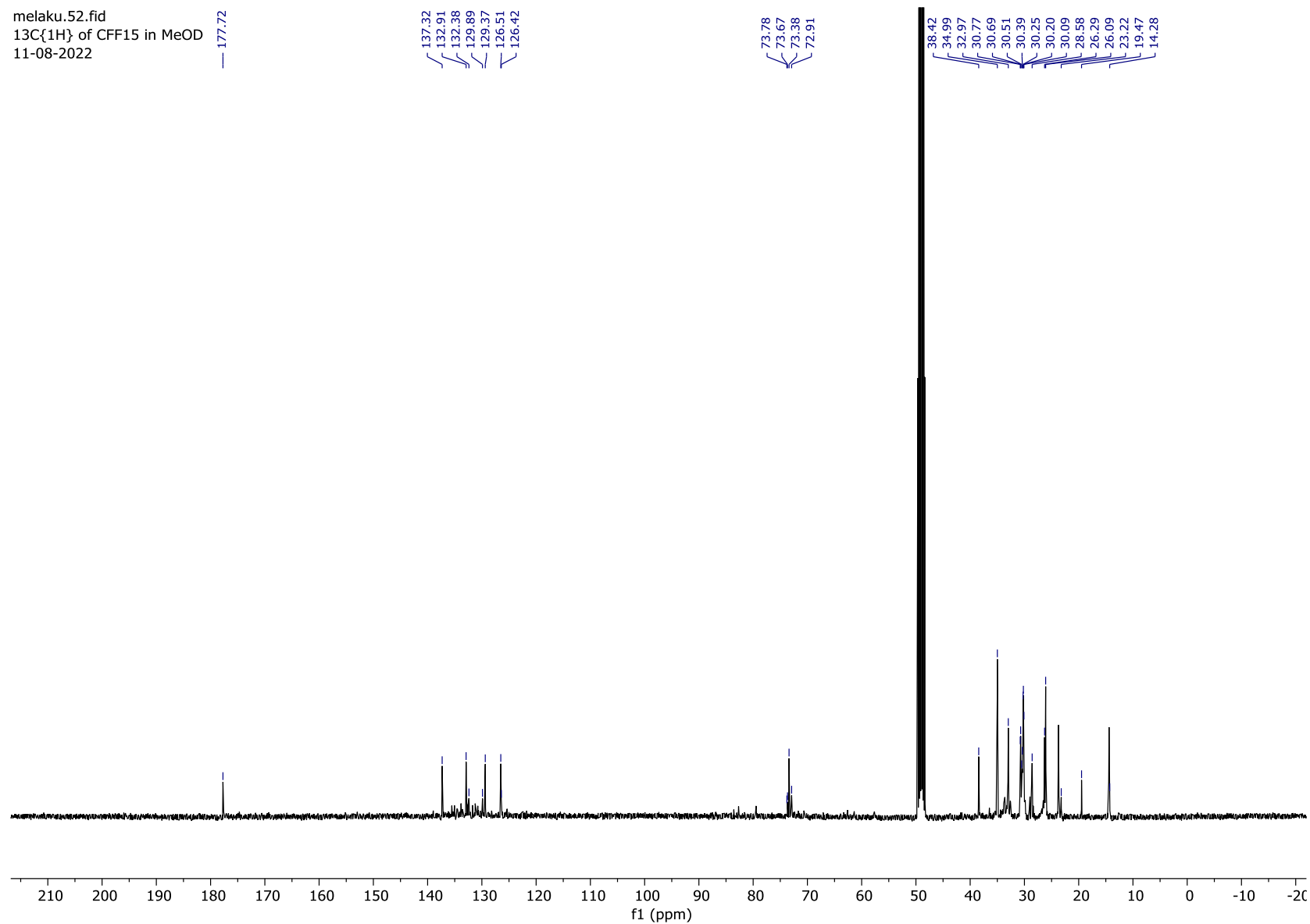


**Appendix 19b:**  $^{13}\text{C}$  NMR spectrum of compound **108** (400 MHz,  $\text{CD}_3\text{OD}$ )

melaku.52.fid

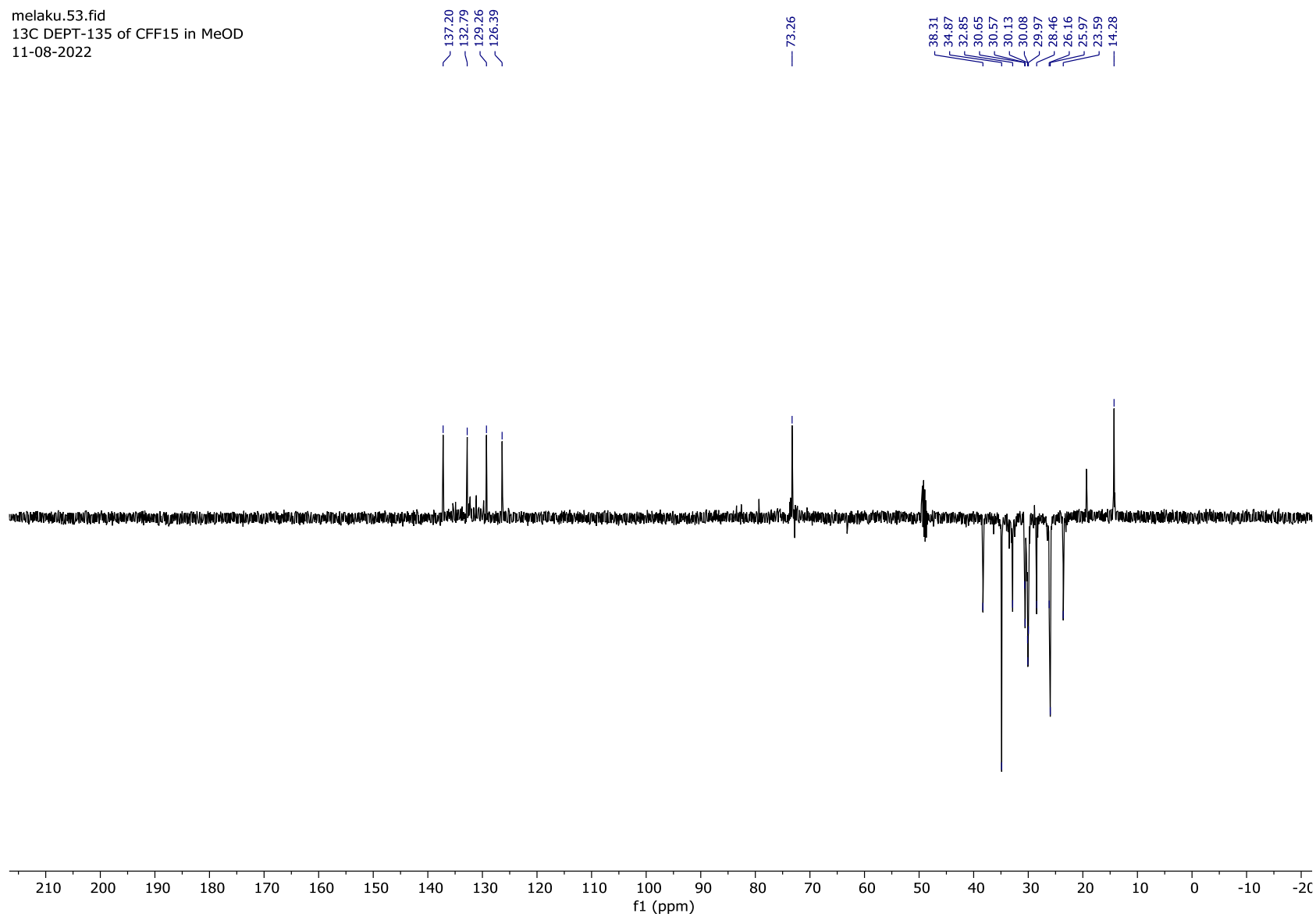
$^{13}\text{C}\{^1\text{H}\}$  of CFF15 in MeOD

11-08-2022



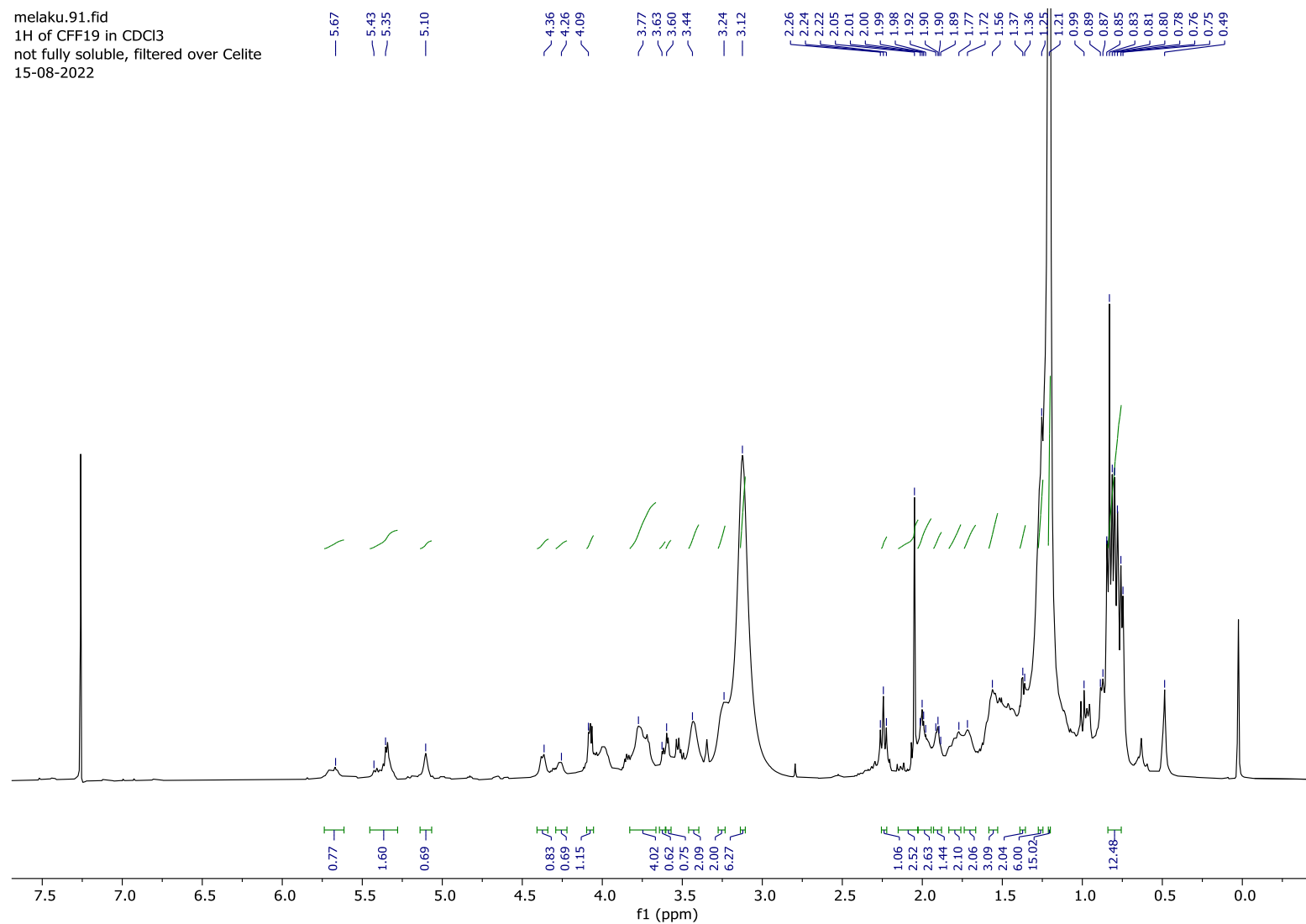
**Appendix 19c:** DEPT-135 NMR spectrum of compound **109** (100 MHz, CD<sub>3</sub>OD)

melaku.53.fid  
13C DEPT-135 of CFF15 in MeOD  
11-08-2022



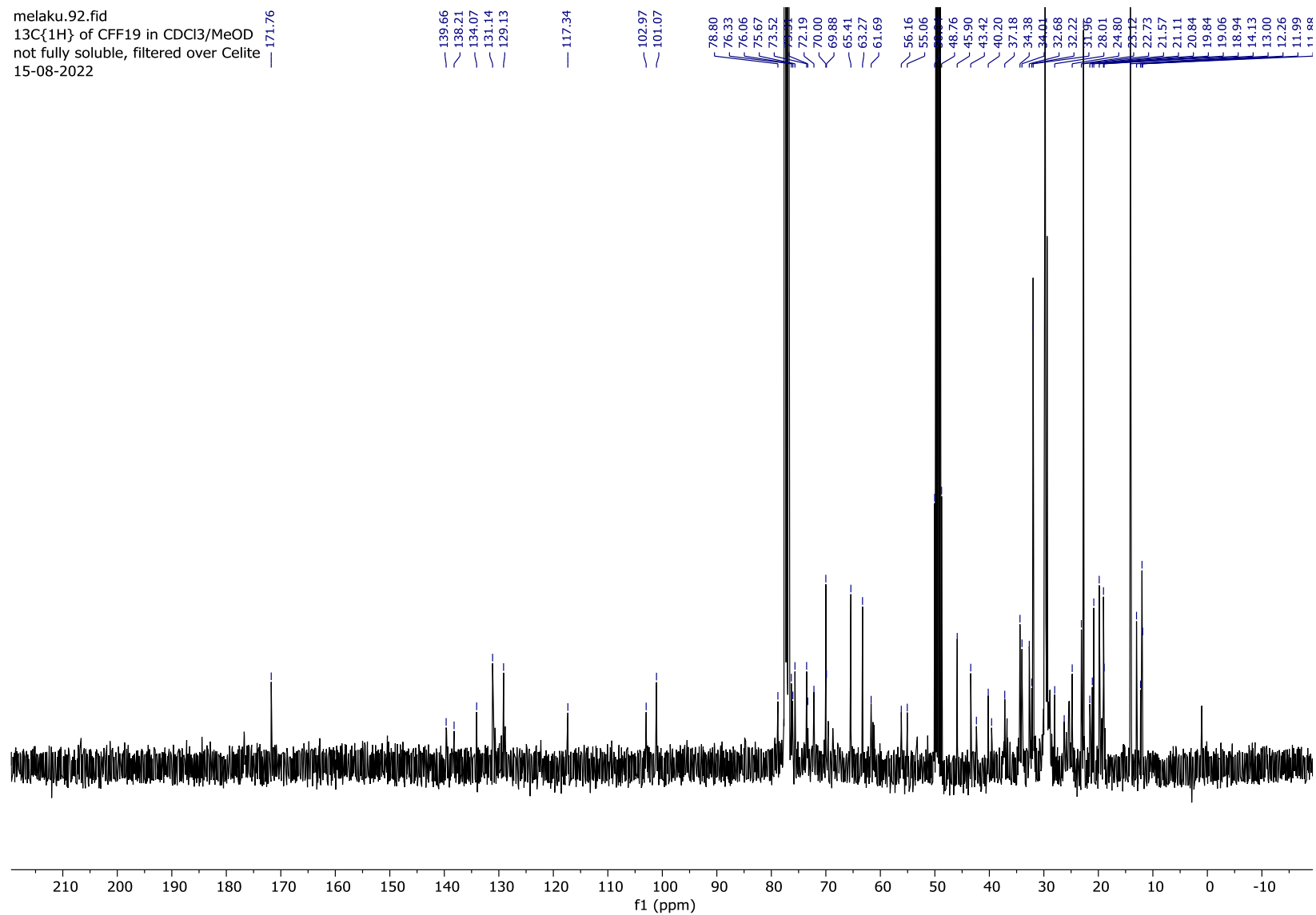
**Appendix 20:**  $^1\text{H}$  NMR spectrum of compound **109** (400 MHz,  $\text{CDCl}_3/\text{CD}_3\text{OD}$ )

melaku.91.fid  
1H of CFF19 in  $\text{CDCl}_3$   
not fully soluble, filtered over Celite  
15-08-2022



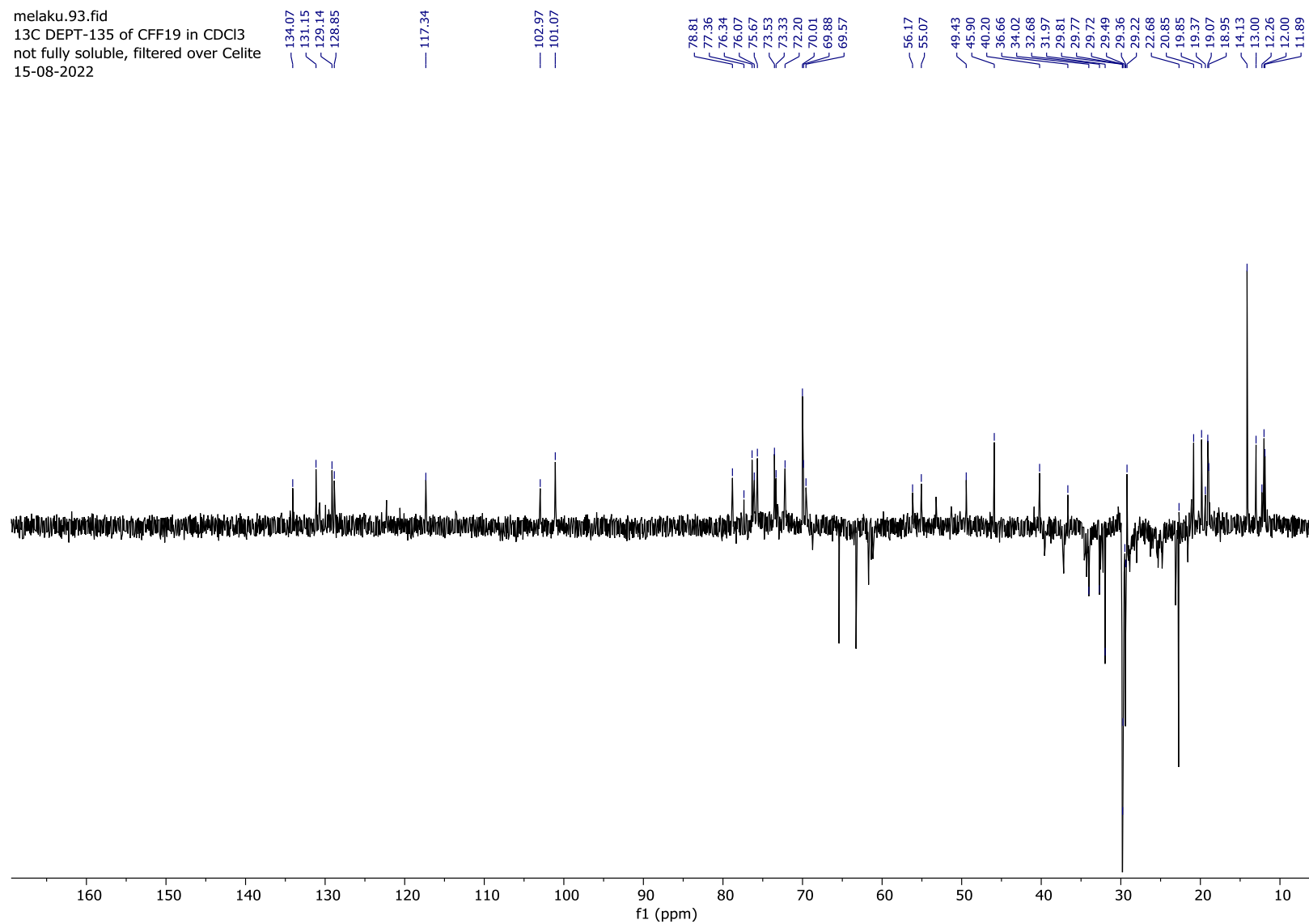
**Appendix 20b:**  $^{13}\text{C}$  NMR spectrum of compound **109** (400 MHz,  $\text{CDCl}_3/\text{CD}_3\text{OD}$ )

melaku.92.fid  
 $^{13}\text{C}\{^1\text{H}\}$  of CFF19 in  $\text{CDCl}_3/\text{MeOD}$   
not fully soluble, filtered over Celite  
15-08-2022



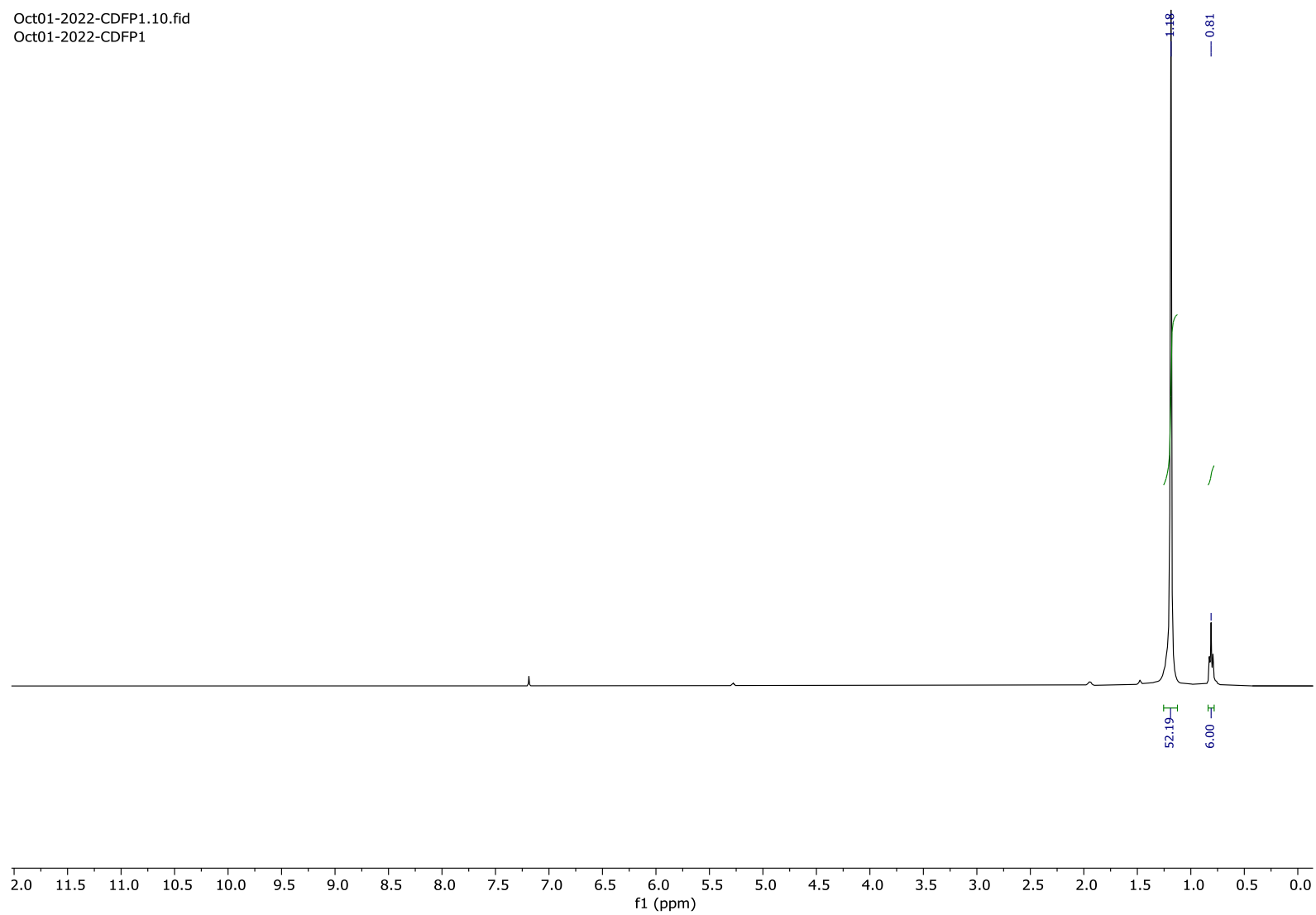
**Appendix 20c:** DEPT-135 NMR spectrum of compound **109** (100 MHz, CDCl<sub>3</sub>/ CD<sub>3</sub>OD)

melaku.93.fid  
13C DEPT-135 of CFF19 in CDCl<sub>3</sub>  
not fully soluble, filtered over Celite  
15-08-2022



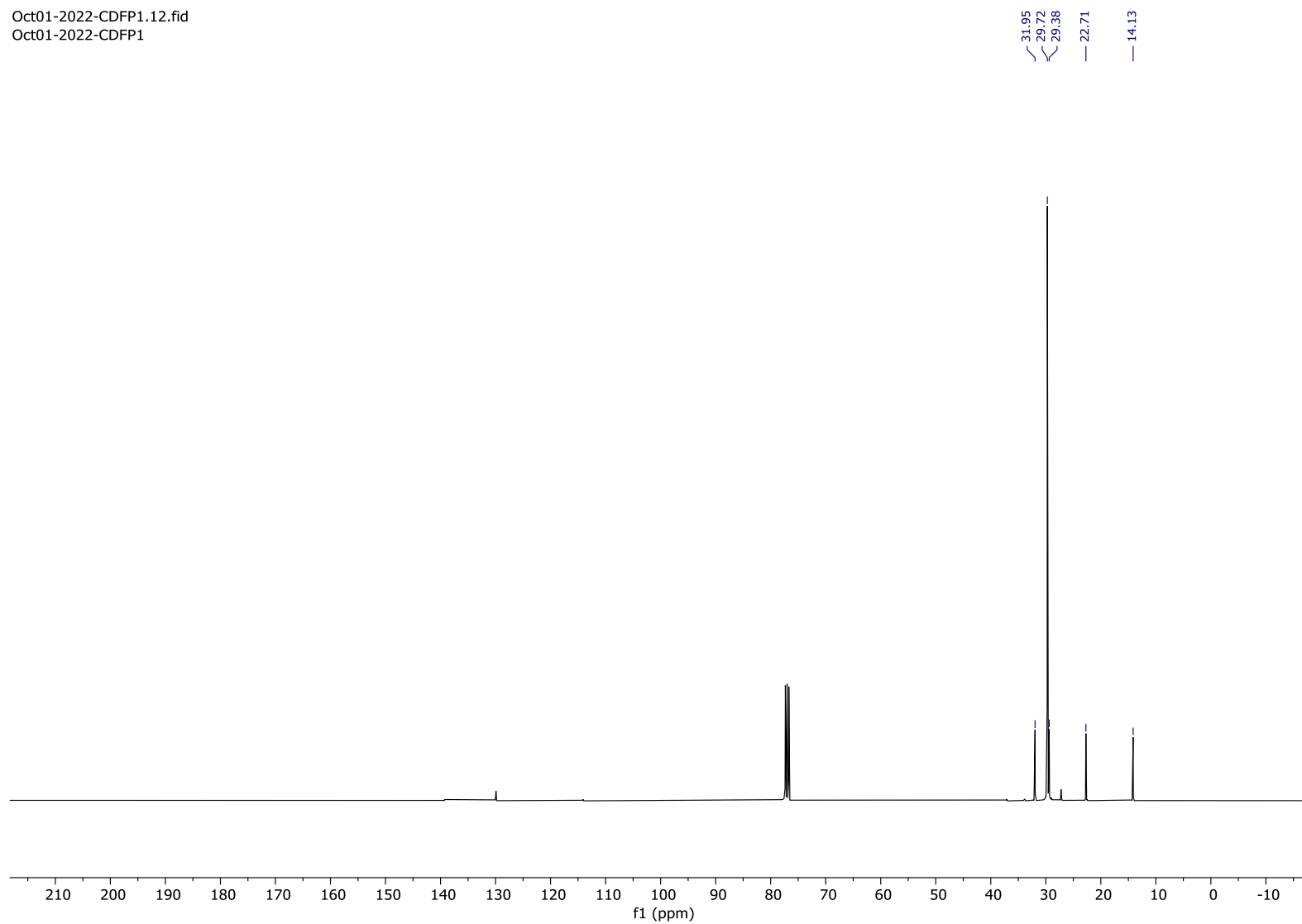
**Appendix 21a:**  $^1\text{H}$  NMR spectrum of compound **110** (400 MHz,  $\text{CDCl}_3$ )

Oct01-2022-CDFP1.10.fid  
Oct01-2022-CDFP1

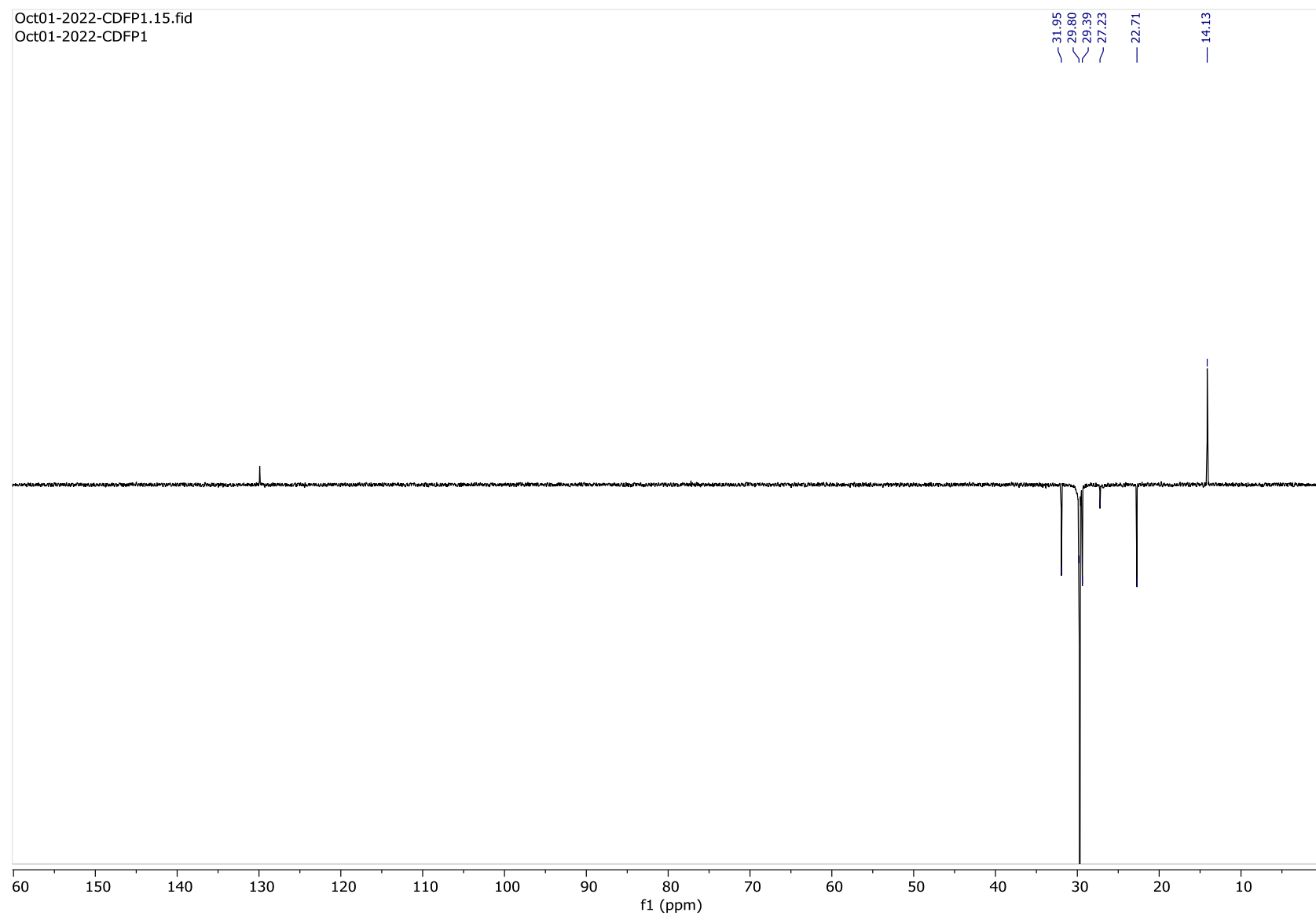


**Appendix 21:**  $^{13}\text{C}$  NMR spectrum of compound **110** (400 MHz,  $\text{CDCl}_3$ )

Oct01-2022-CDFP1.12.fid  
Oct01-2022-CDFP1

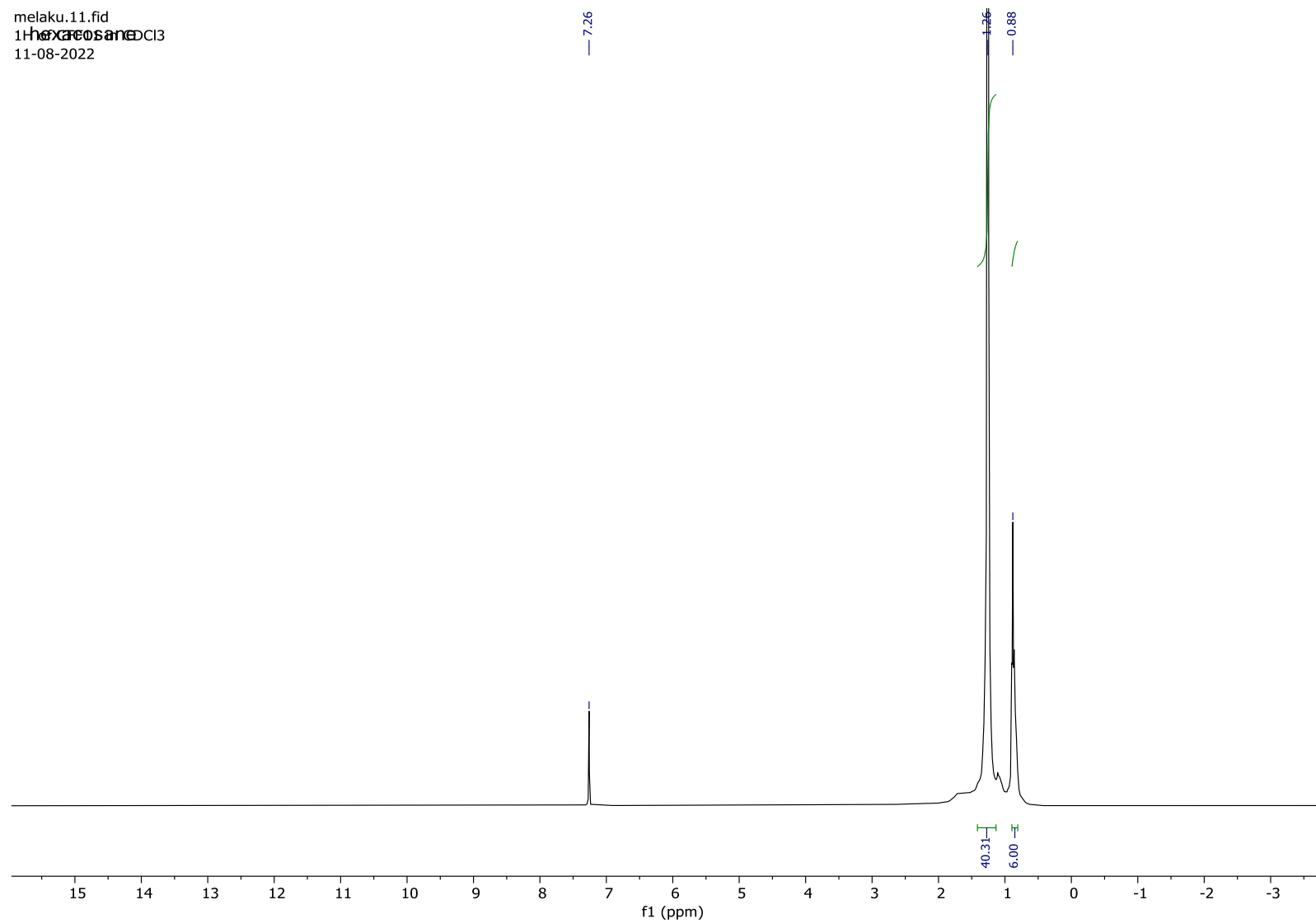


**Appendix 21c:** DEPT-135 NMR spectrum of compound **110** (100 MHz, CDCl<sub>3</sub>)



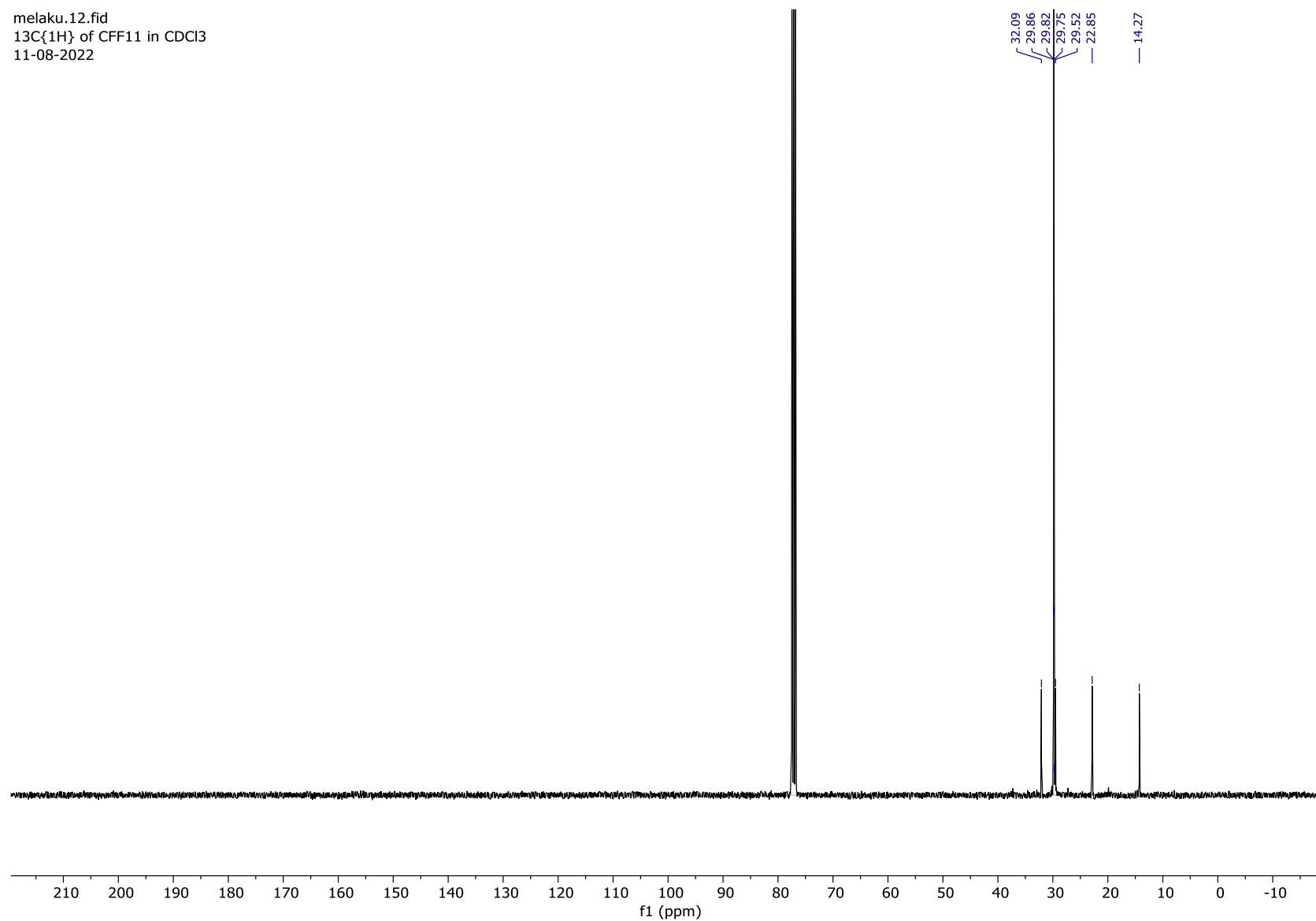
**Appendix 22:**  $^1\text{H}$  NMR spectrum of compound **111** (400 MHz,  $\text{CDCl}_3$ )

melaku.11.fid  
1H NMR of compound  
11-08-2022



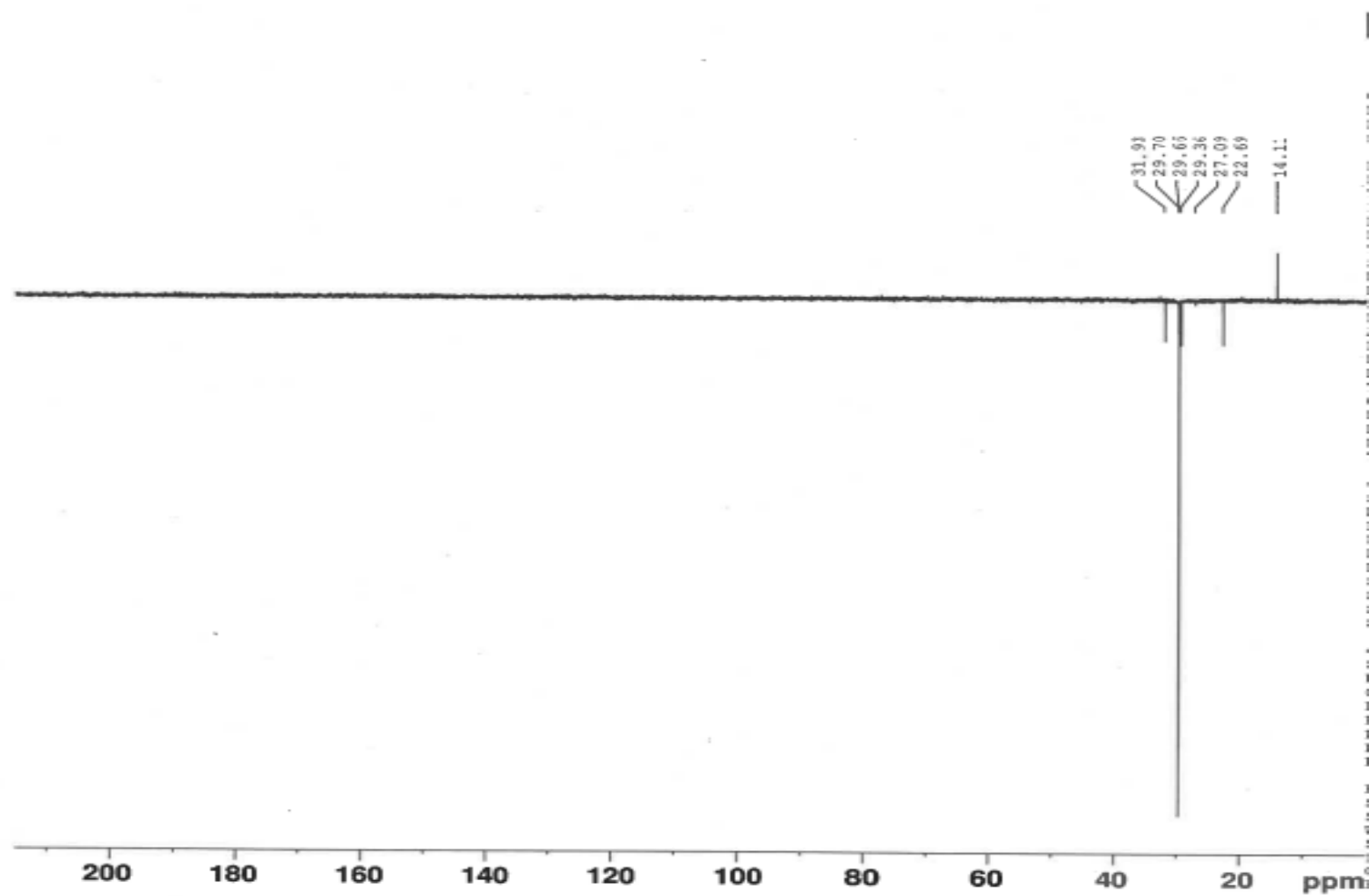
**Appendix 22b:**  $^{13}\text{C}$  NMR spectrum of compound **111** (400 MHz,  $\text{CDCl}_3$ )

melaku.12.fid  
 $^{13}\text{C}\{^1\text{H}\}$  of CFF11 in  $\text{CDCl}_3$   
11-08-2022



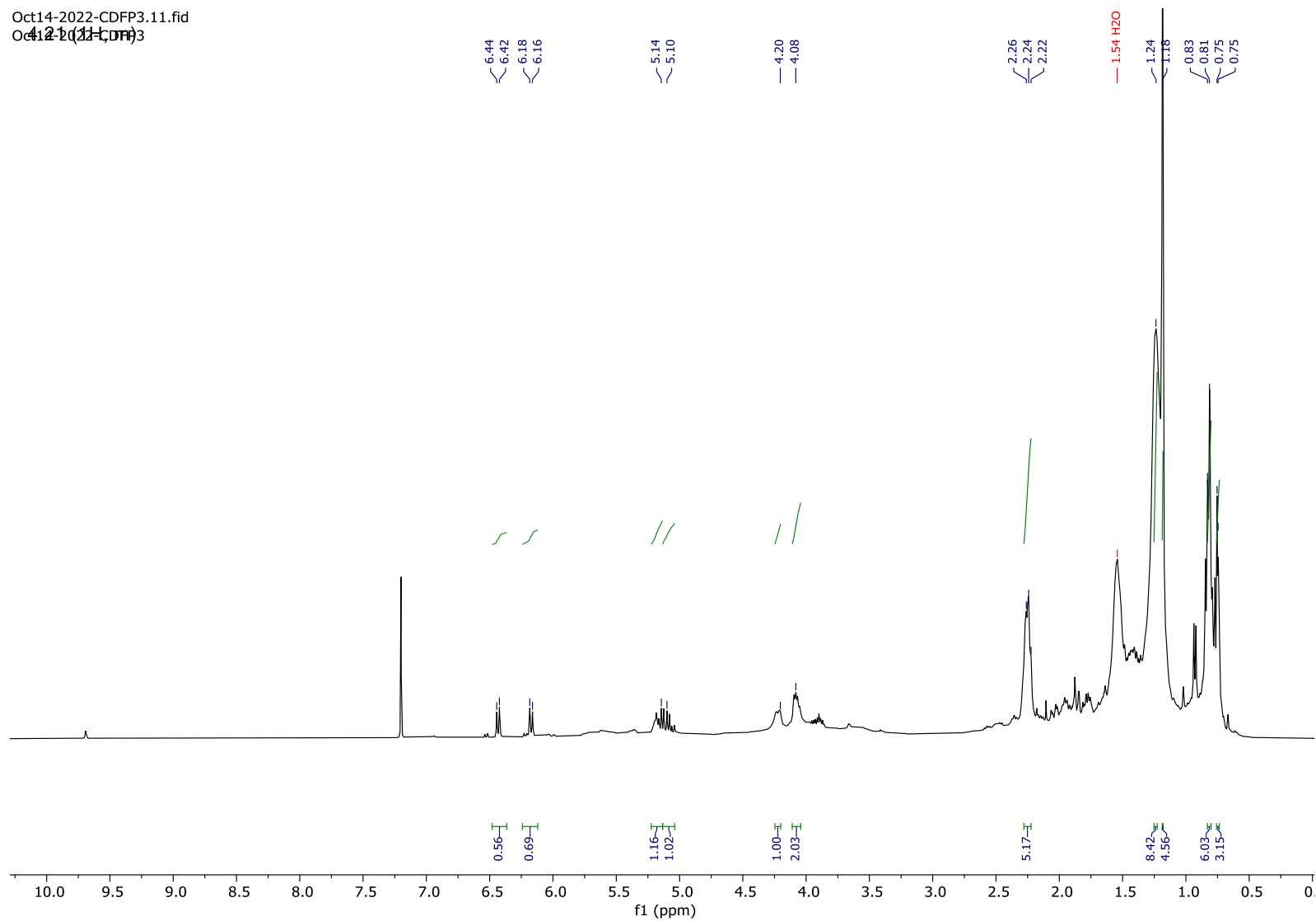
**Appendix 22c:** DEPT-135 NMR spectrum of compound **111** (100 MHz, CDCl<sub>3</sub>)

<sup>13</sup>C DEPT-135 of CFF11 in CDCl<sub>3</sub>  
11-08-2022



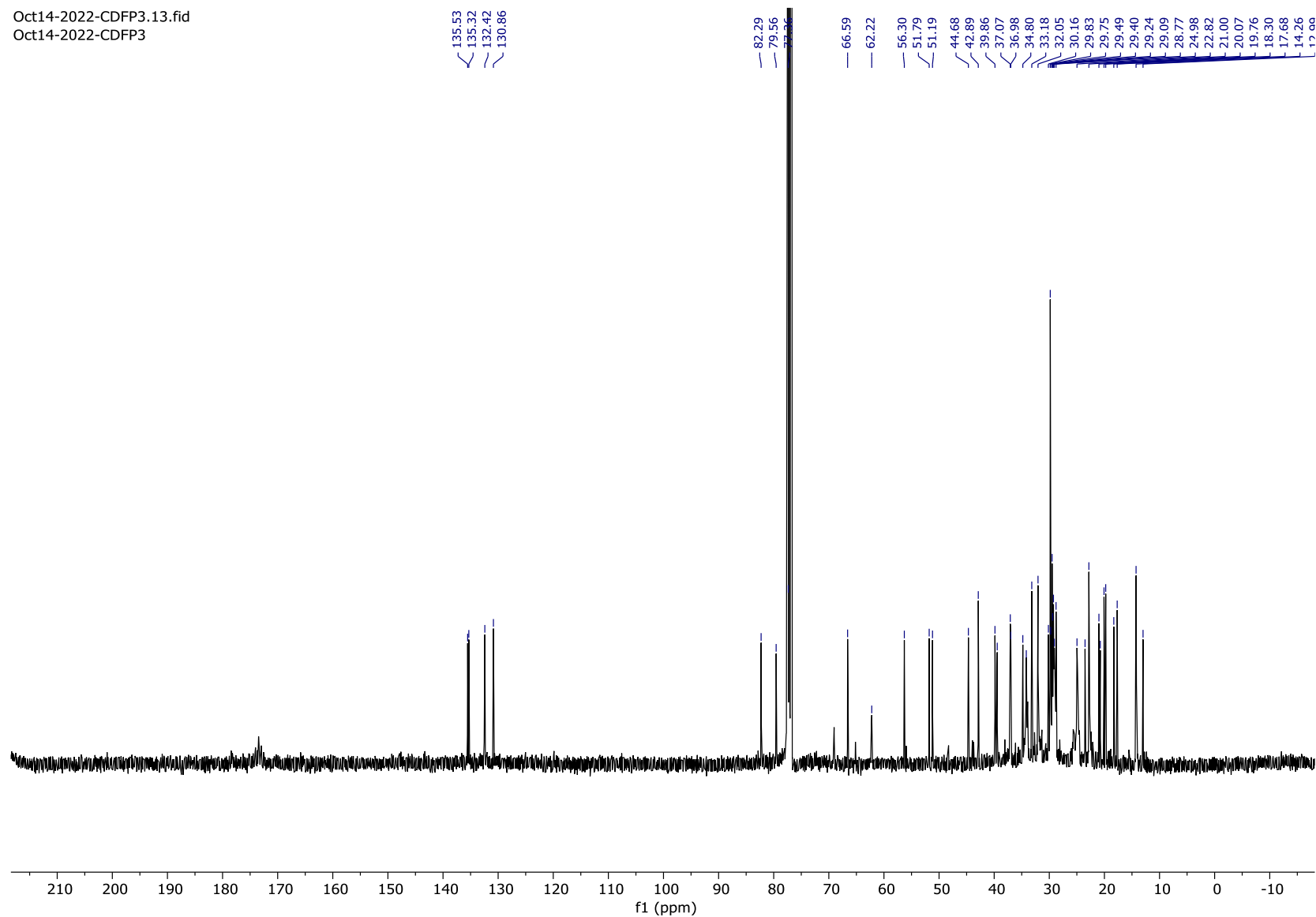
**Appendix 23a:**  $^1\text{H}$  NMR spectrum of compound **112** (400 MHz,  $\text{CDCl}_3$ )

Oct14-2022-CDFP3.11.fid  
 412-1011-003



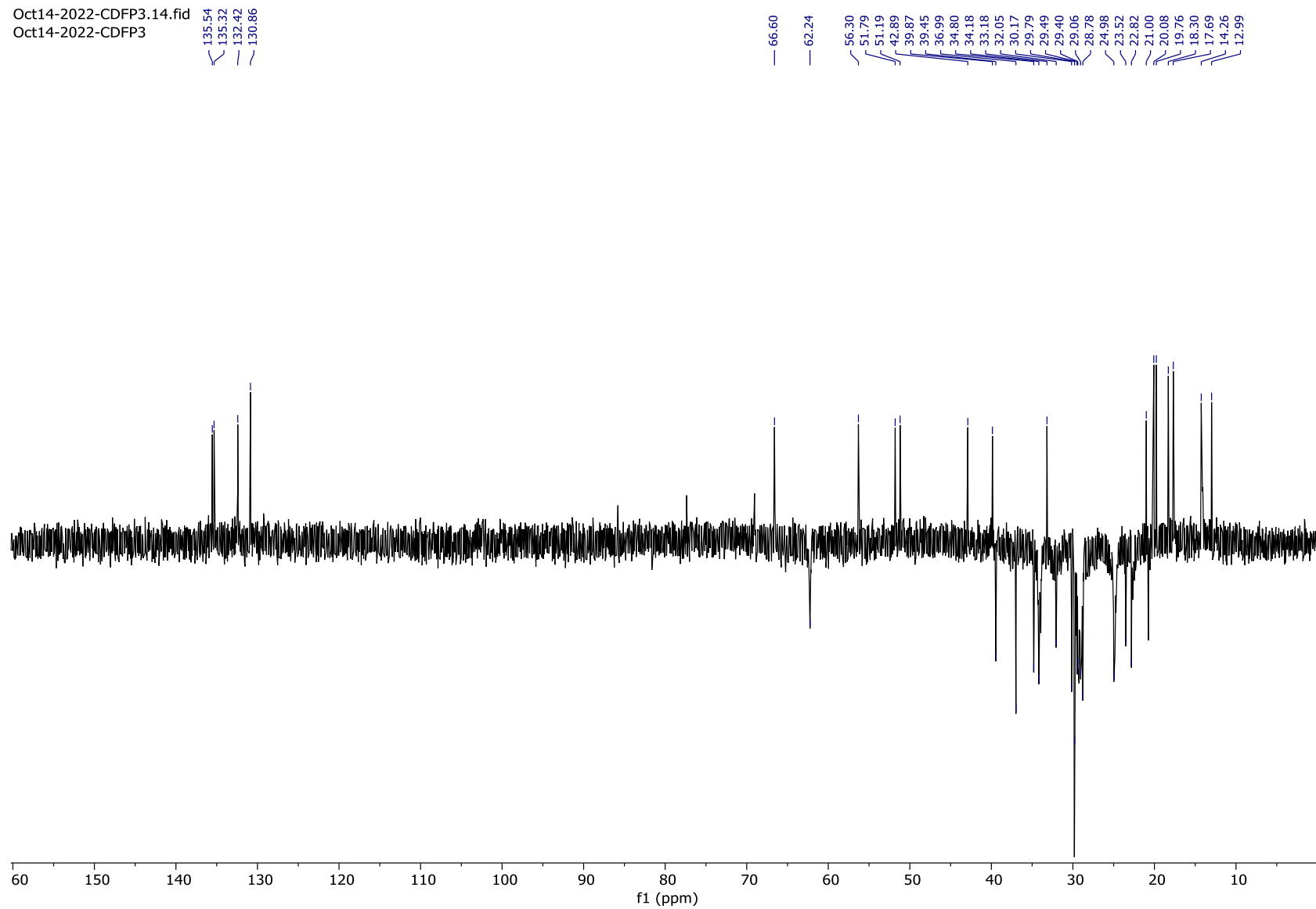
**Appendix 23b:**  $^{13}\text{C}$  NMR spectrum of compound **112** (400 MHz,  $\text{CDCl}_3$ )

Oct14-2022-CDFP3.13.fid  
Oct14-2022-CDFP3

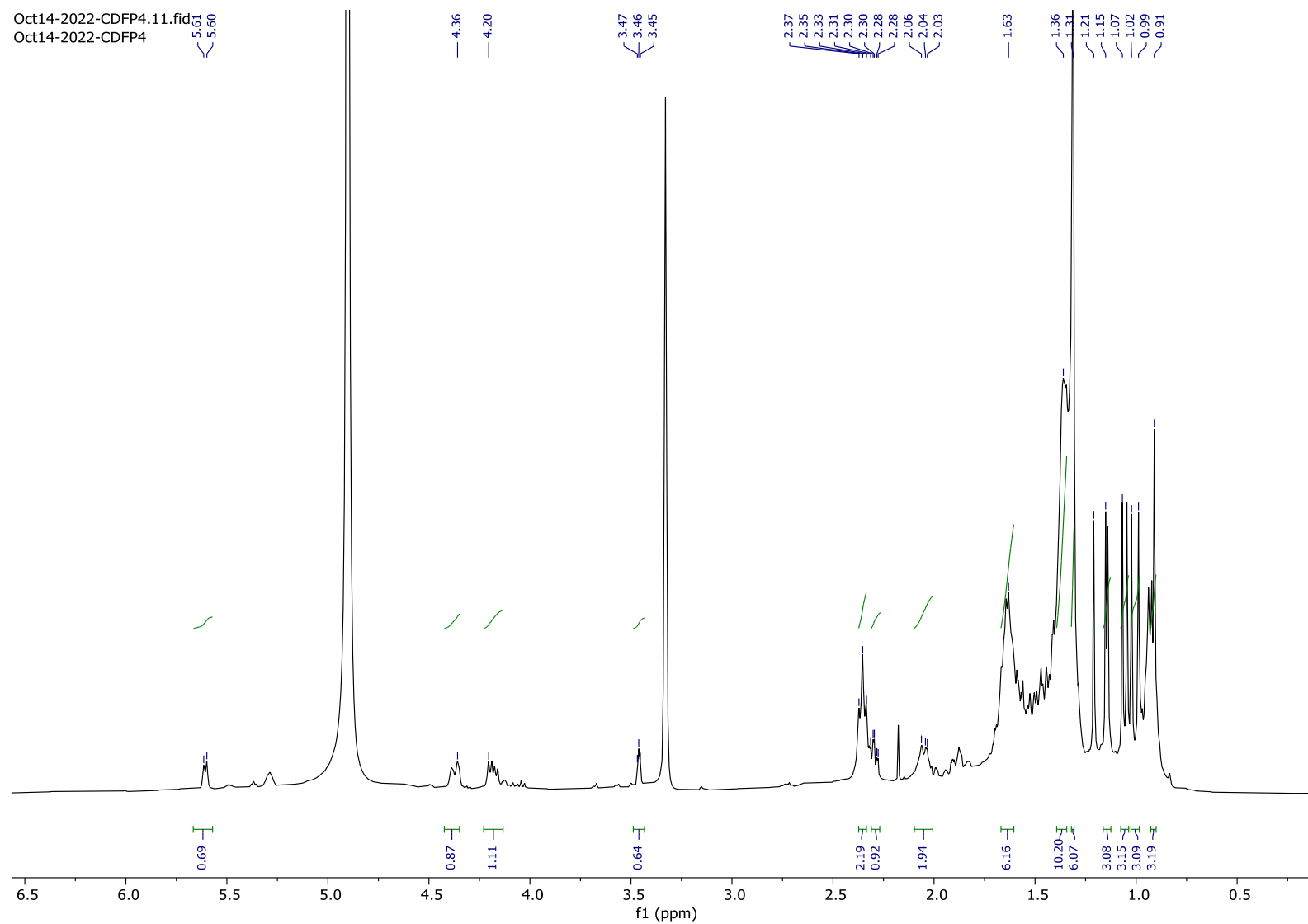


**Appendix 23c:** DEPT-135 NMR spectrum of compound **112** (100 MHz, CDCl<sub>3</sub>)

Oct14-2022-CDFP3.14.fid  
Oct14-2022-CDFP3

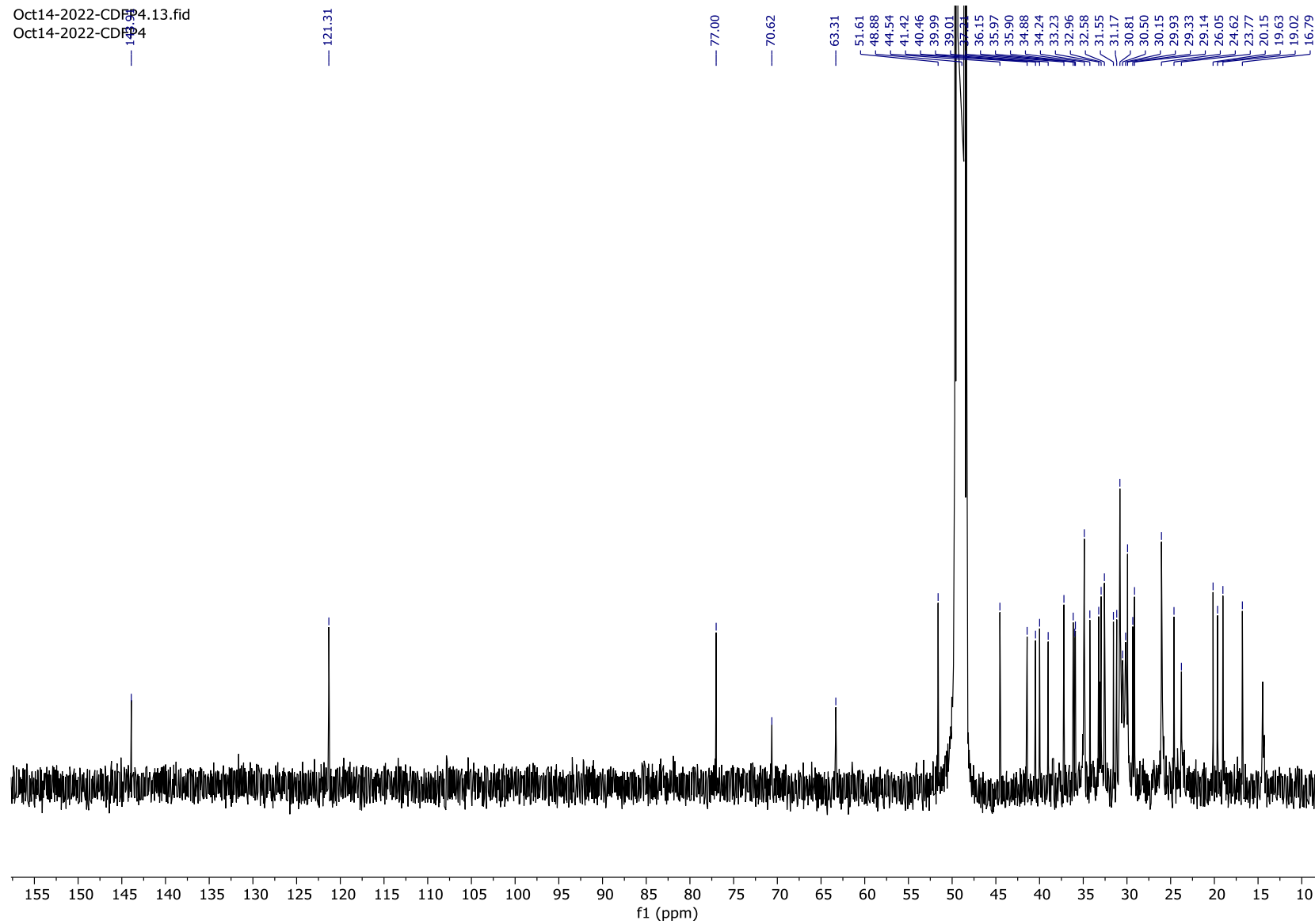


**Appendix 24a:**  $^1\text{H}$  NMR spectrum of compound **113** (400 MHz,  $\text{CDCl}_3$ )



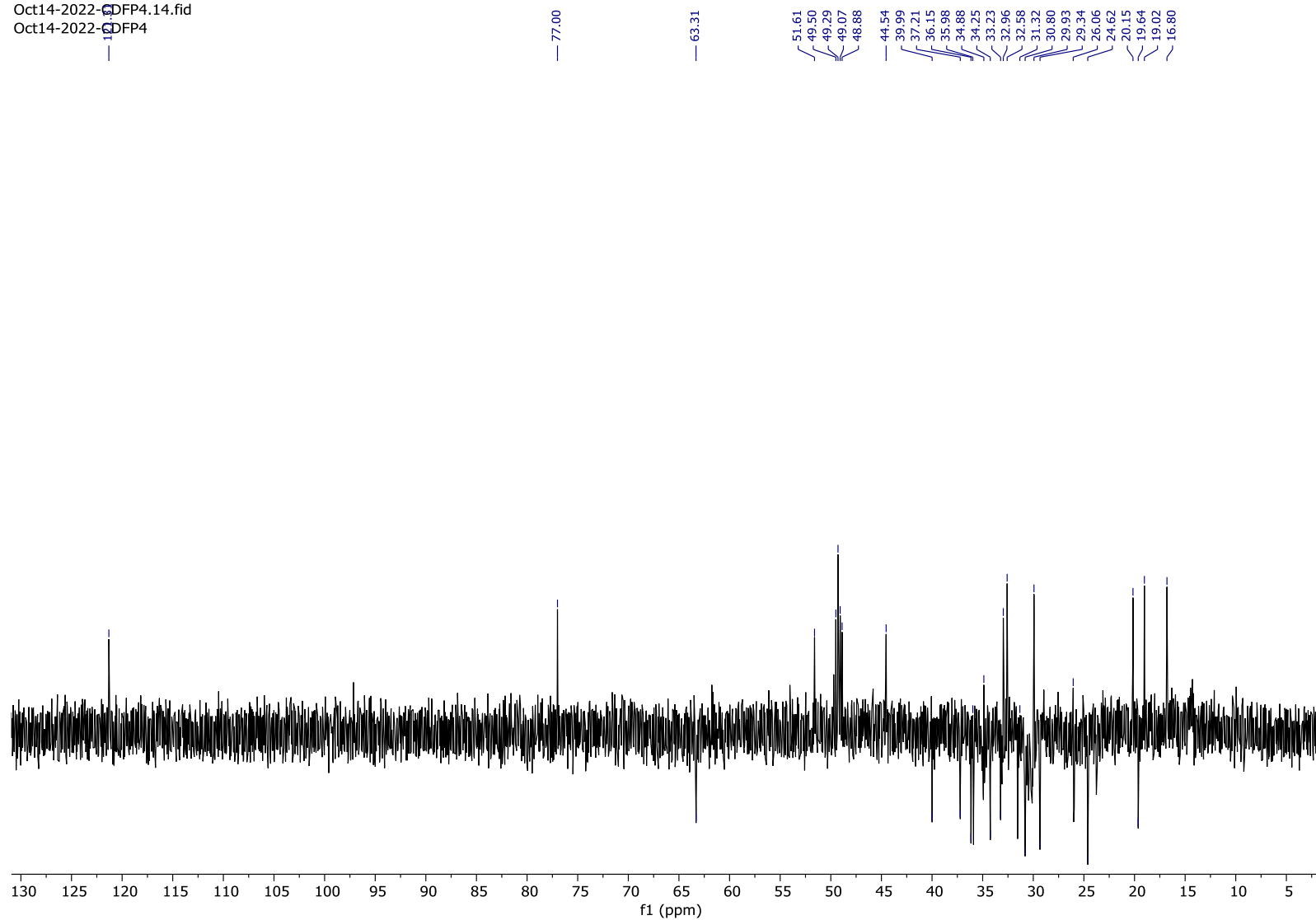
**Appendix 24b:**  $^{13}\text{C}$  NMR spectrum of compound **113** (400 MHz,  $\text{CDCl}_3$ )

Oct14-2022-CDP4.13.fid  
Oct14-2022-CDP4



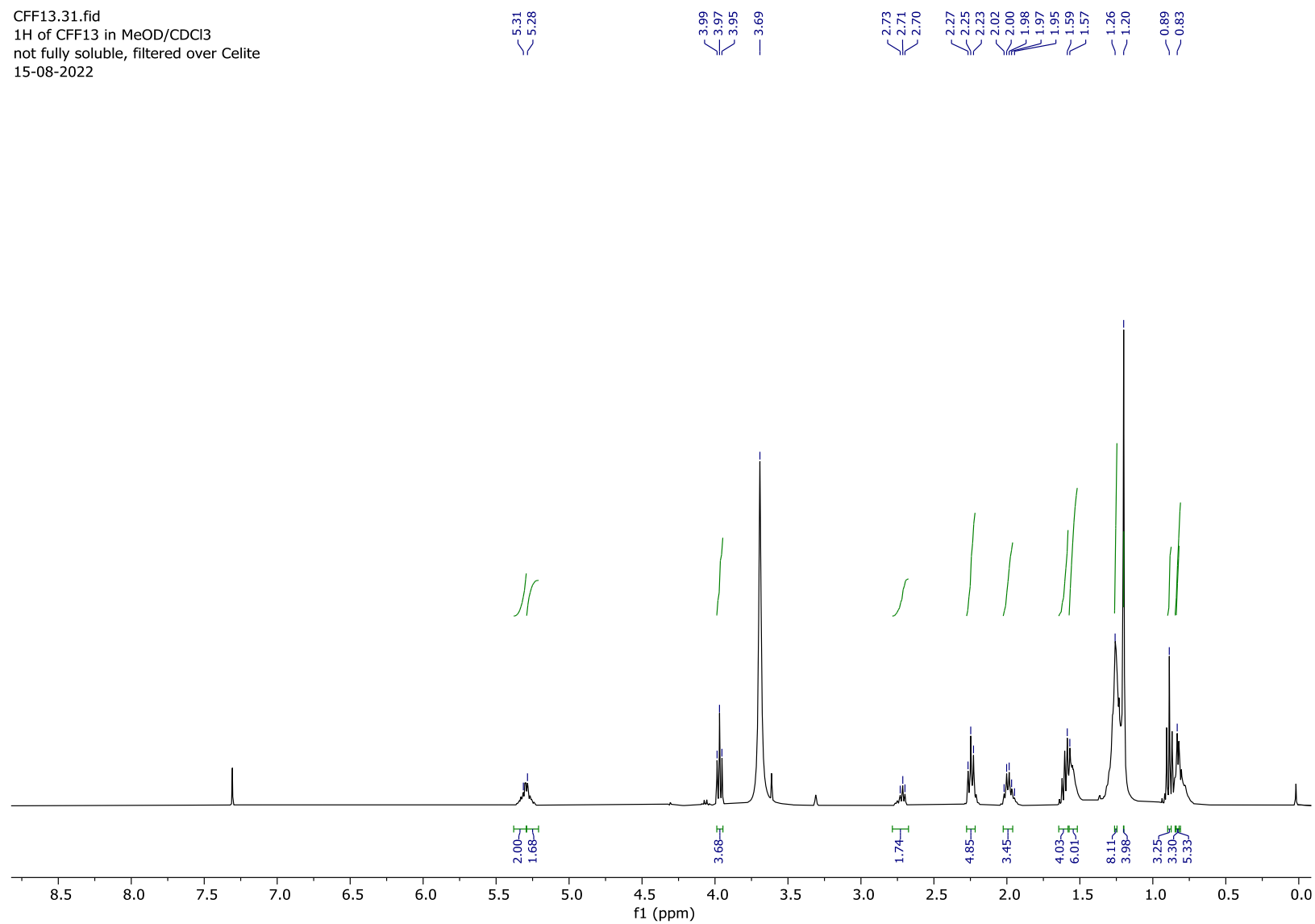
**Appendix 24c:** DEPT-135 NMR spectrum of compound **113** (100 MHz, CDCl<sub>3</sub>)

Oct14-2022-CDP4.14.fid  
Oct14-2022-CDP4



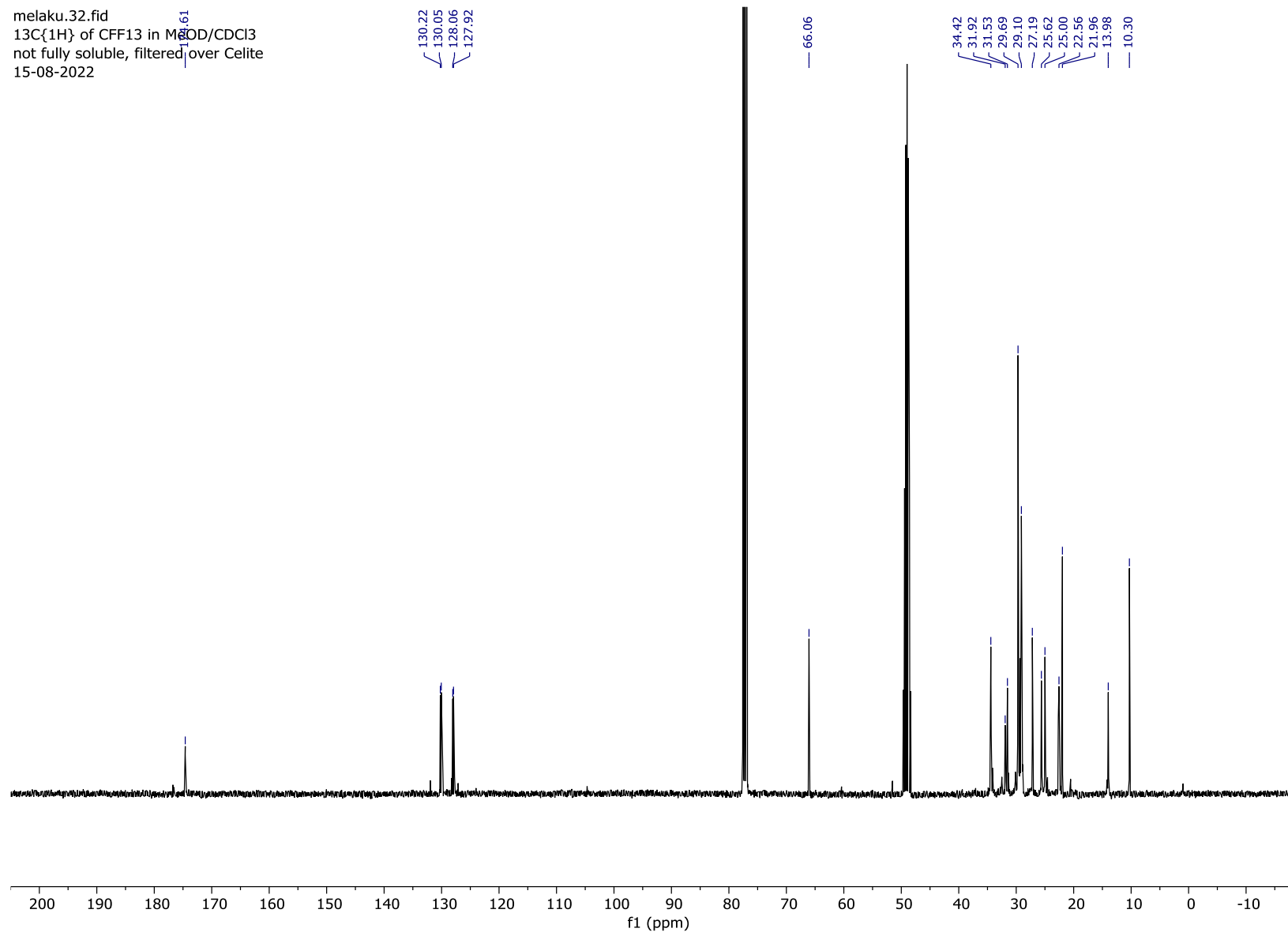
**Appendix 25a:**  $^1\text{H}$  NMR spectrum of compound **114** (400 MHz, MeOD/ $\text{CDCl}_3$ )

CFF13.31.fid  
1H of CFF13 in MeOD/ $\text{CDCl}_3$   
not fully soluble, filtered over Celite  
15-08-2022



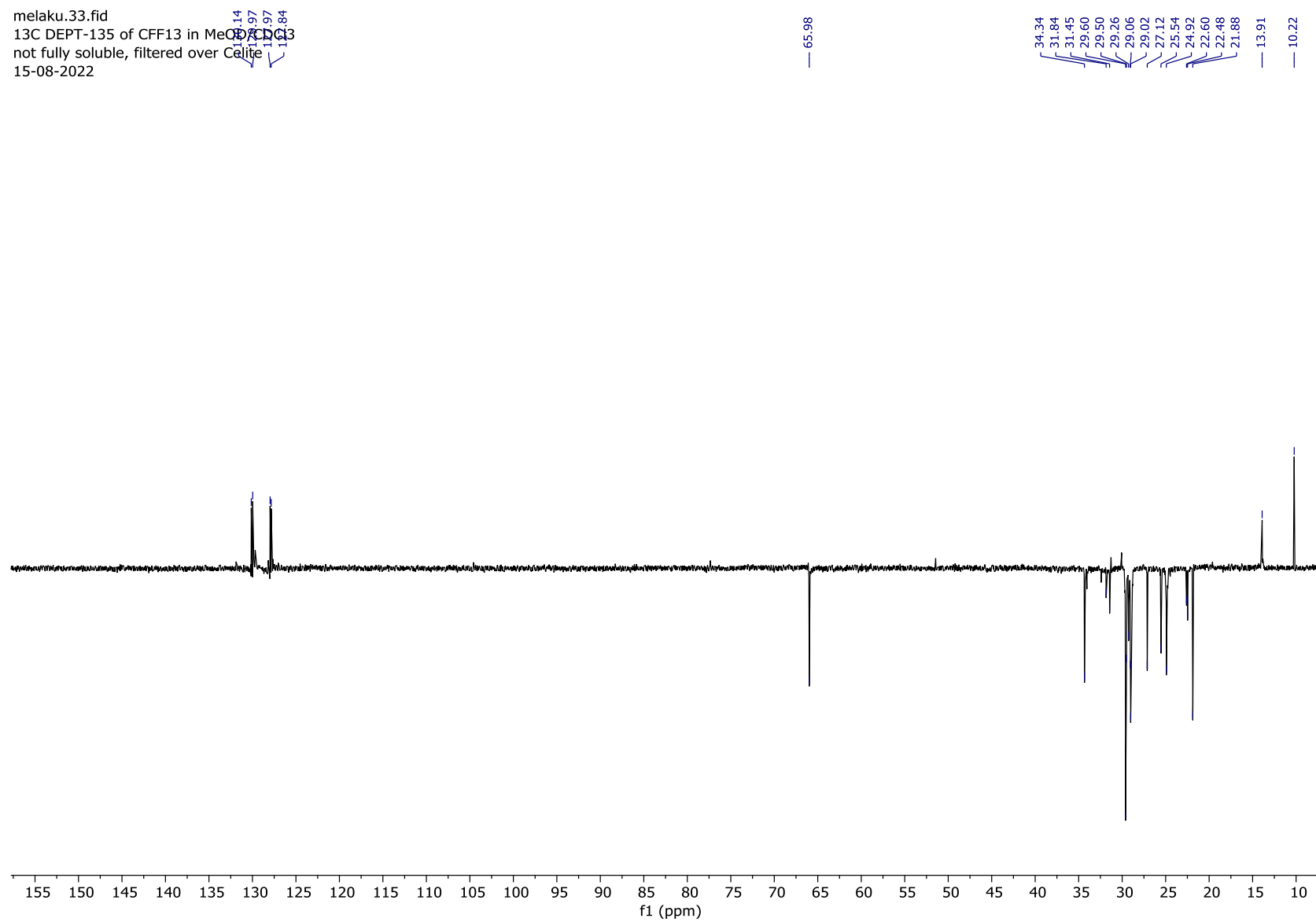
**Appendix 25b:**  $^{13}\text{C}$  NMR spectrum of compound **114** (400 MHz, MeOD/ $\text{CDCl}_3$ )

melaku.32.fid  
 $^{13}\text{C}\{^1\text{H}\}$  of CFF13 in MeOD/ $\text{CDCl}_3$   
not fully soluble, filtered over Celite  
15-08-2022



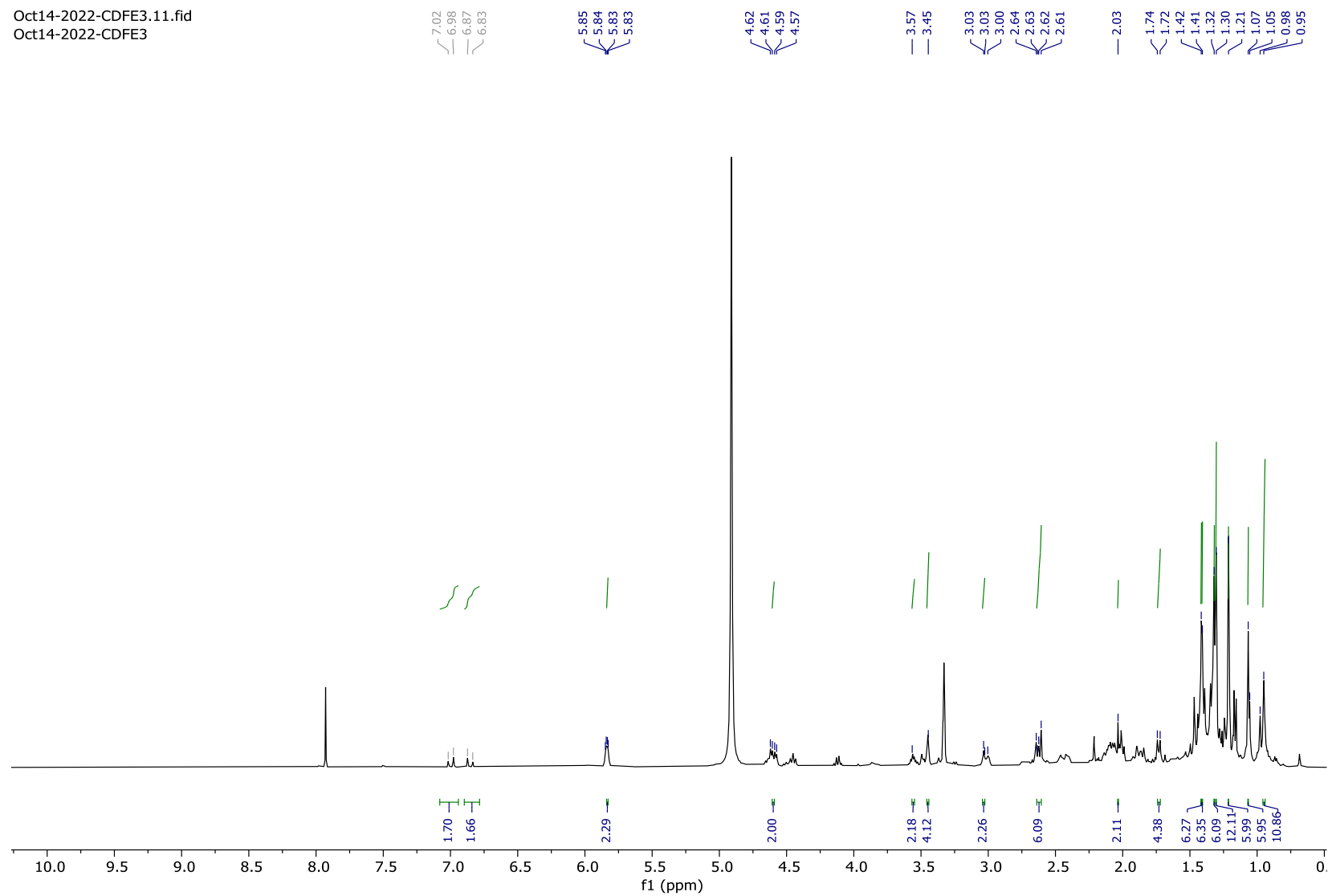
**Appendix 25c:** DEPT-135 NMR spectrum of compound **114** (100 MHz, MeOD/CDCl<sub>3</sub>)

melaku.33.fid  
13C DEPT-135 of CFF13 in MeOD/CDCl<sub>3</sub>  
not fully soluble, filtered over Celite  
15-08-2022

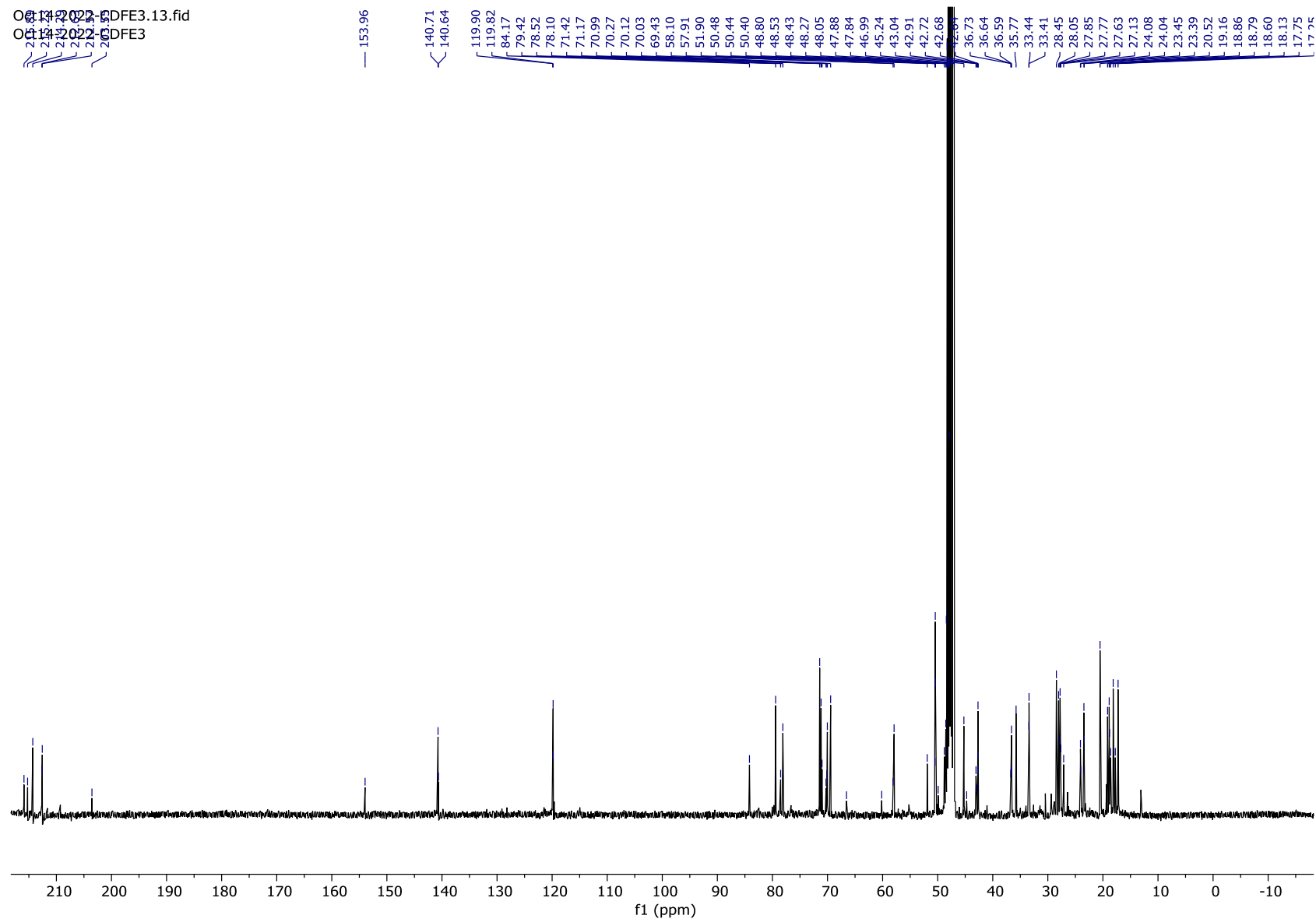


**Appendix 26a:**  $^1\text{H}$  NMR spectrum of compound **26 and 100** (400 MHz,  $\text{CDCl}_3$ )

Oct14-2022-CDFE3.11.fid  
Oct14-2022-CDFE3

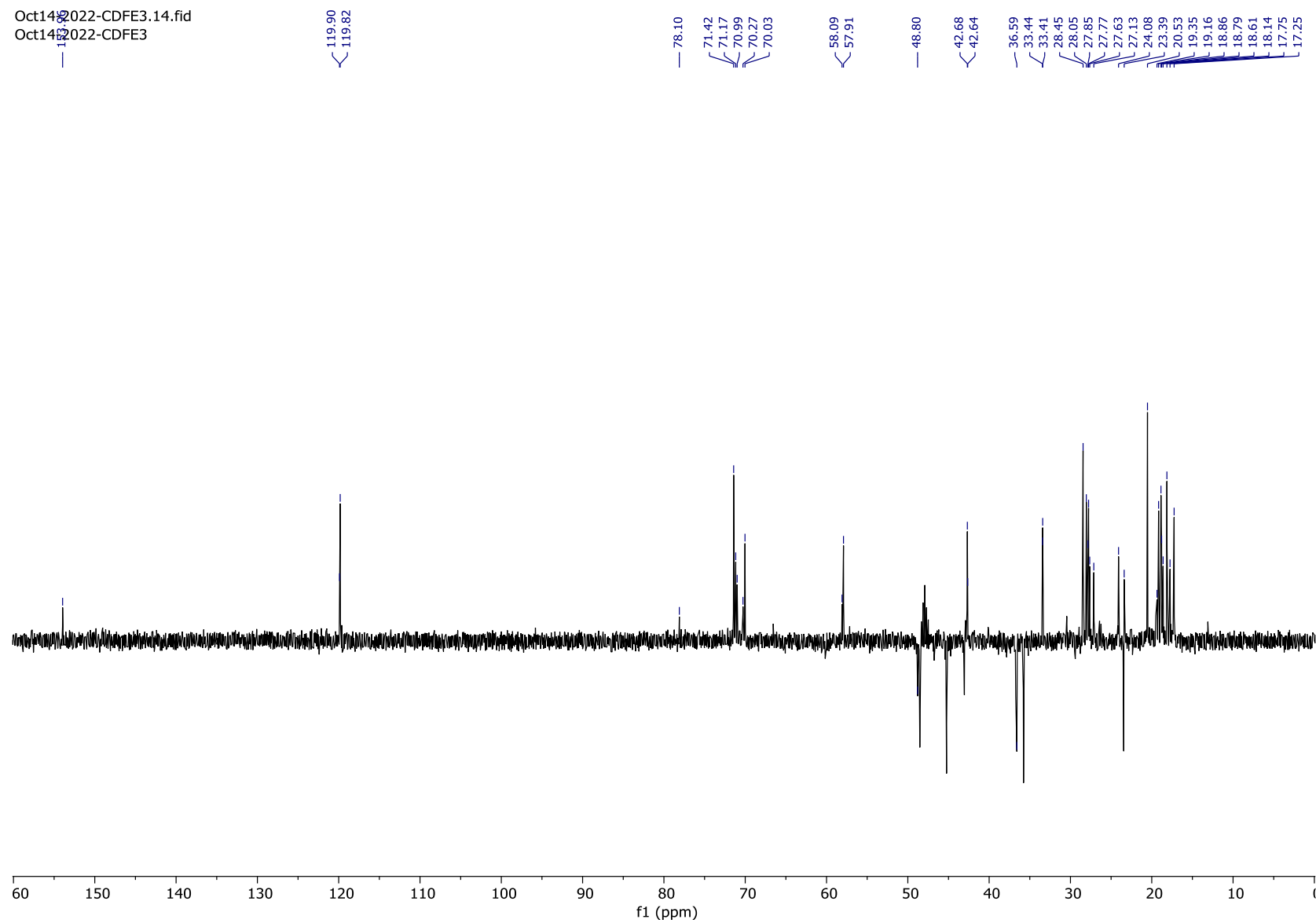


**Appendix 26b:**  $^{13}\text{C}$  NMR spectrum of compound **26** and **100** (400 MHz,  $\text{CDCl}_3$ )



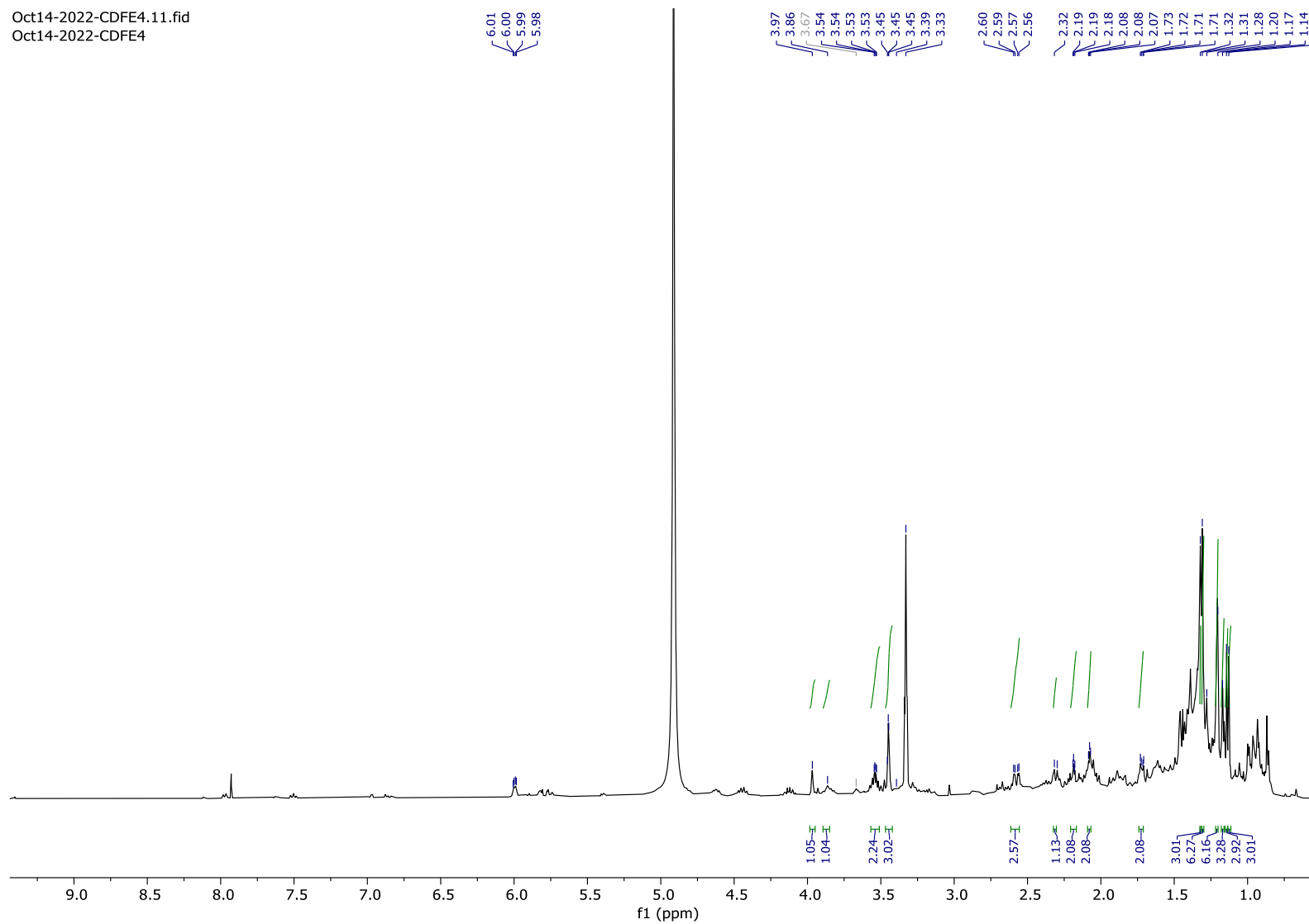
**Appendix 26c:** DEPT-135 NMR spectrum of compound **26** and **100** (100 MHz, CDCl<sub>3</sub>)

Oct14/2022-CDFE3.14.fid  
Oct14/2022-CDFE3



**Appendix 27a:**  $^1\text{H}$  NMR spectrum of compound **102** (400 MHz,  $\text{CDCl}_3$ )

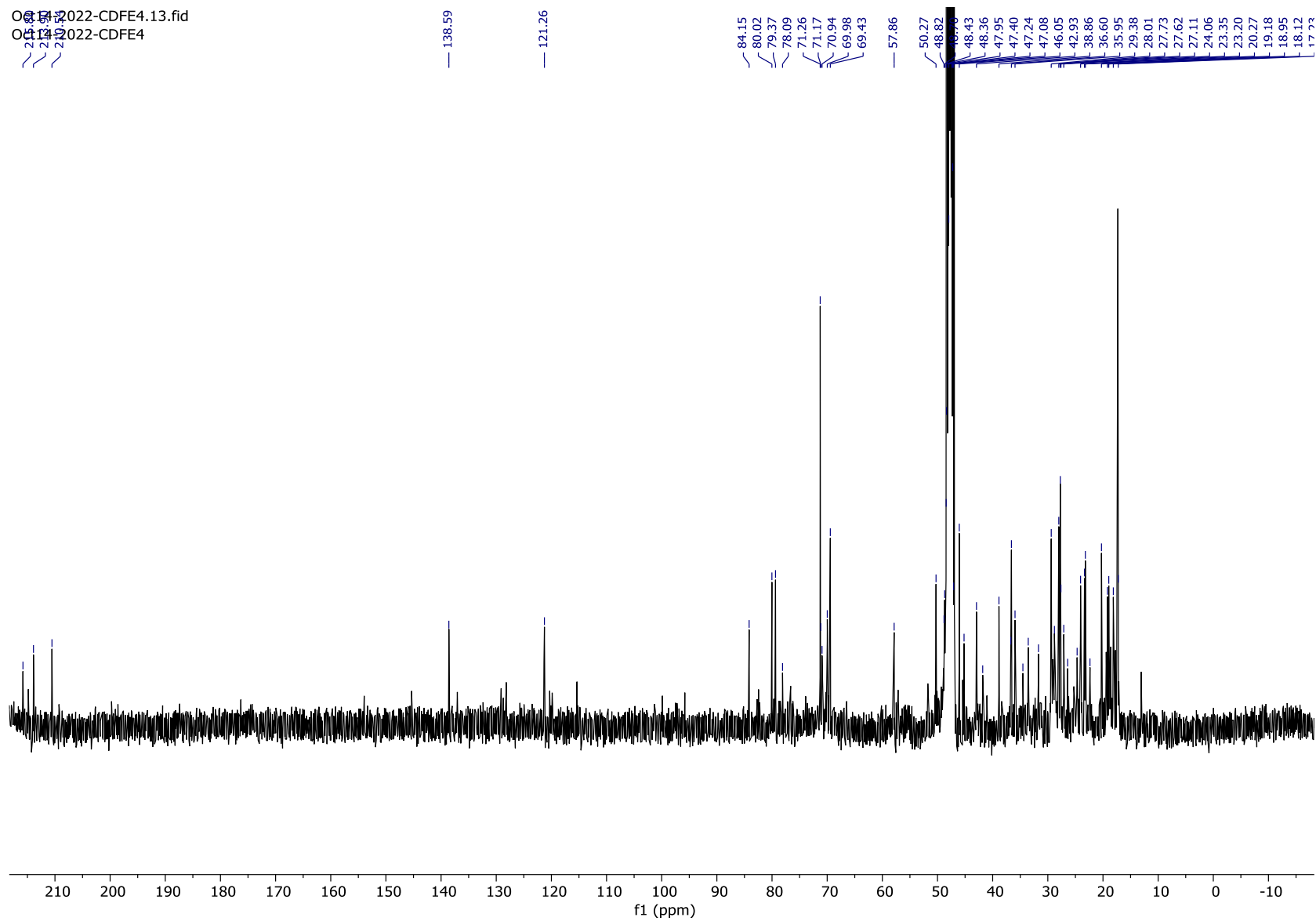
Oct14-2022-CDFE4.11.fid  
Oct14-2022-CDFE4



**Appendix 27b:**  $^{13}\text{C}$  NMR spectrum of compound **102** (400 MHz,  $\text{CDCl}_3$ )

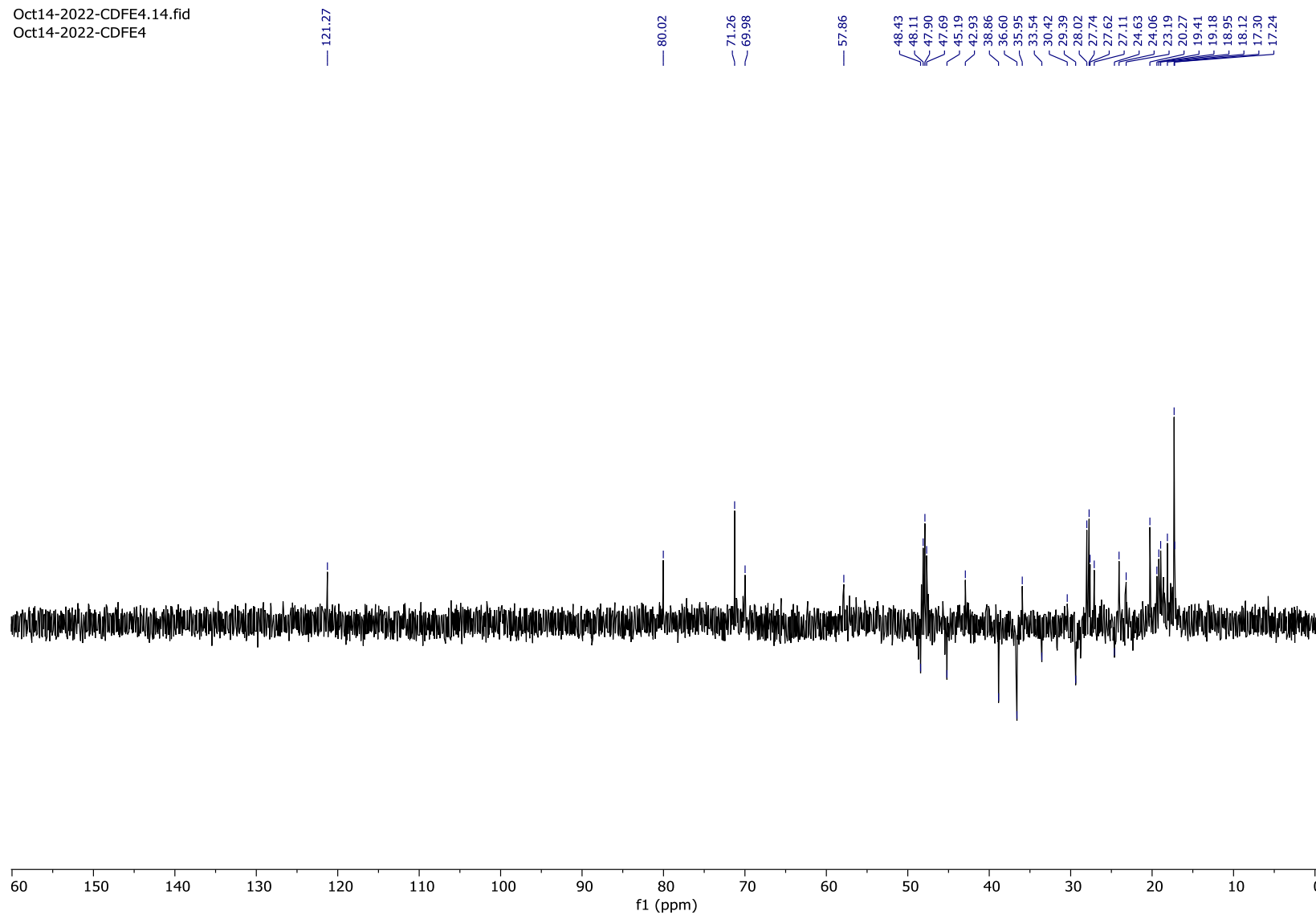
06192022-CDFE4.13.fid

06192022-CDFE4



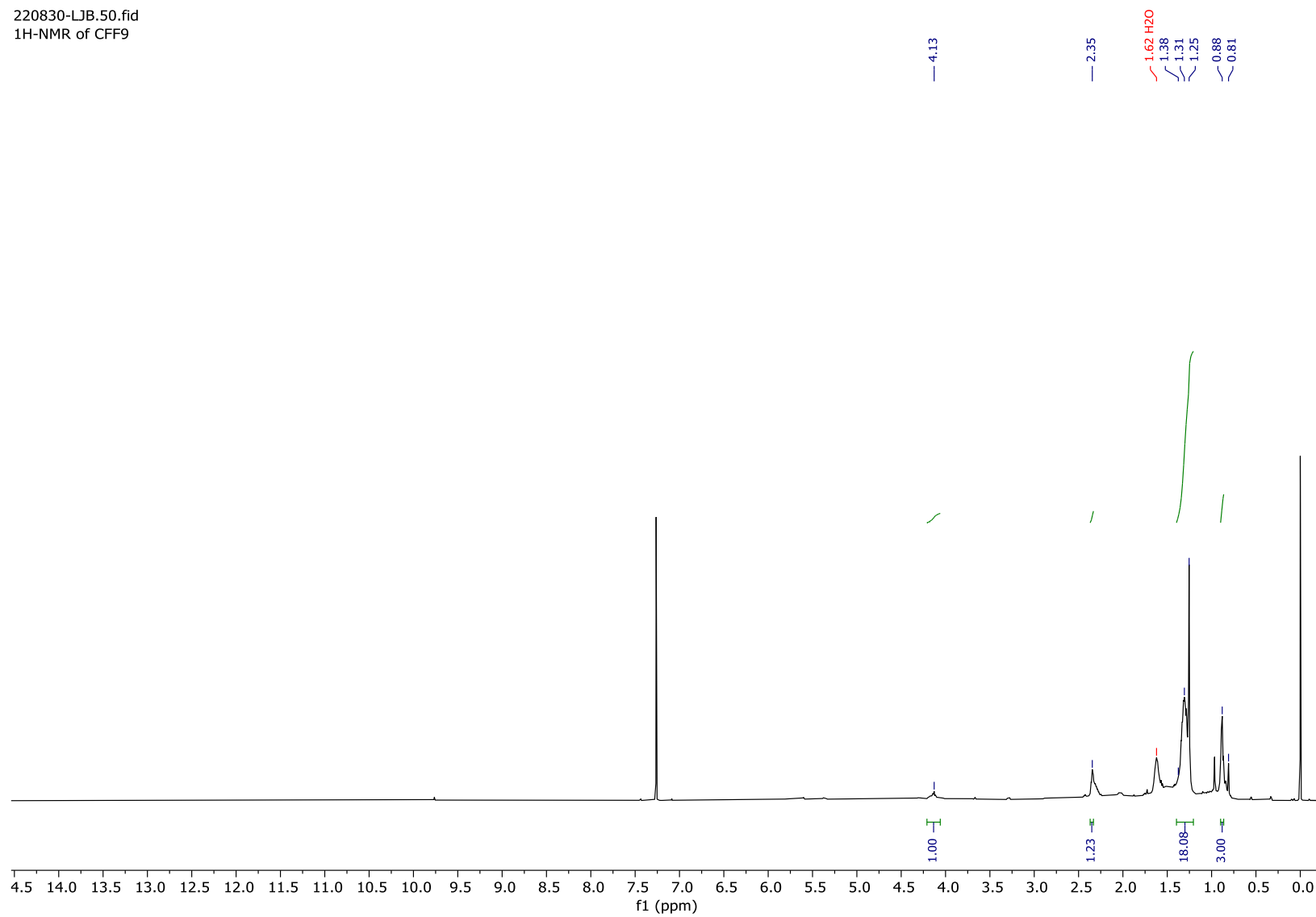
**Appendix 27c:** DEPT-135 NMR spectrum of compound **102** (100 MHz, CDCl<sub>3</sub>)

Oct14-2022-CDFE4.14.fid  
Oct14-2022-CDFE4



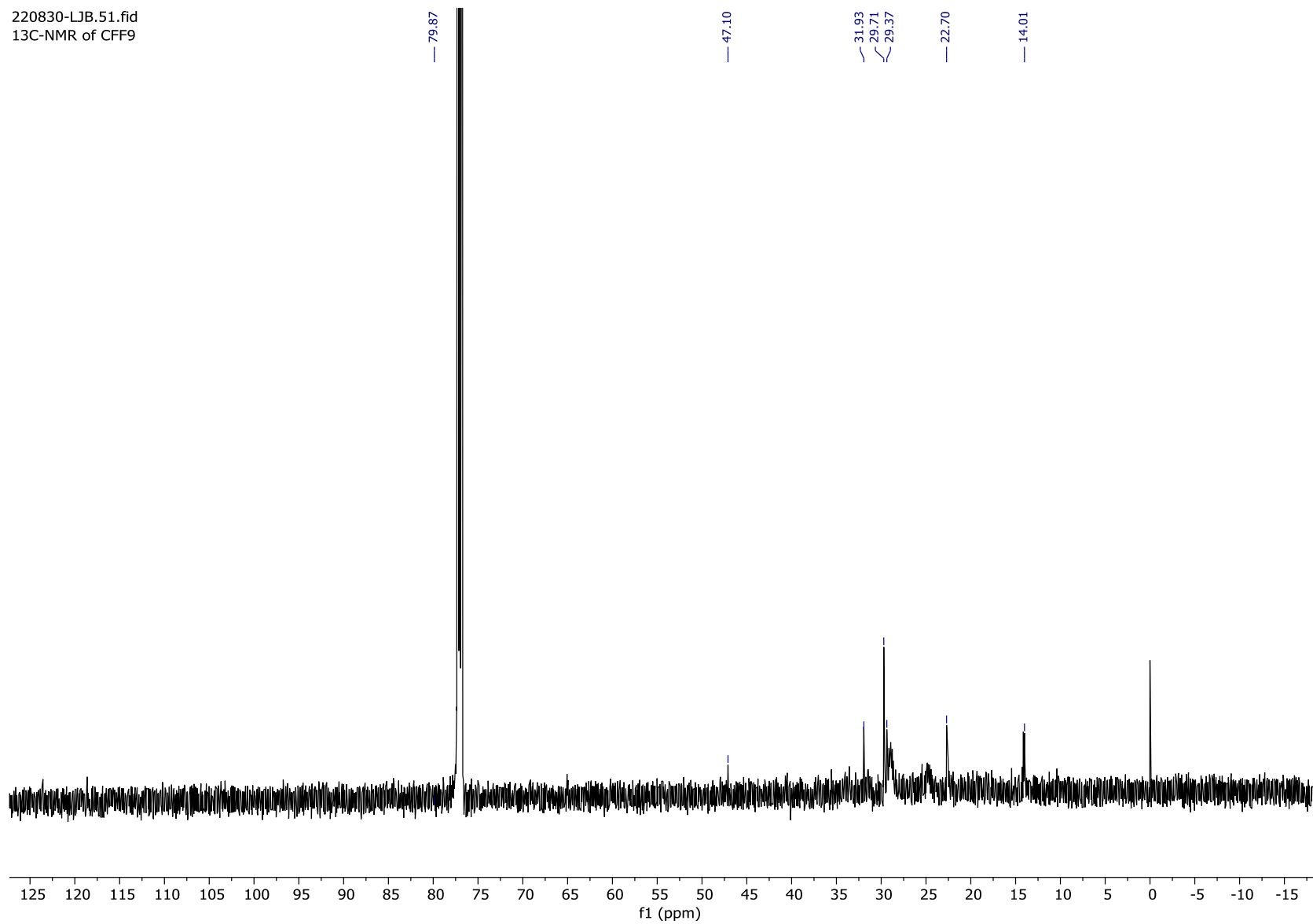
**Appendix 28a:**  $^1\text{H}$  NMR spectrum of compound **115** (400 MHz, MeOD)

220830-LJB.50.fid  
1H-NMR of CFF9



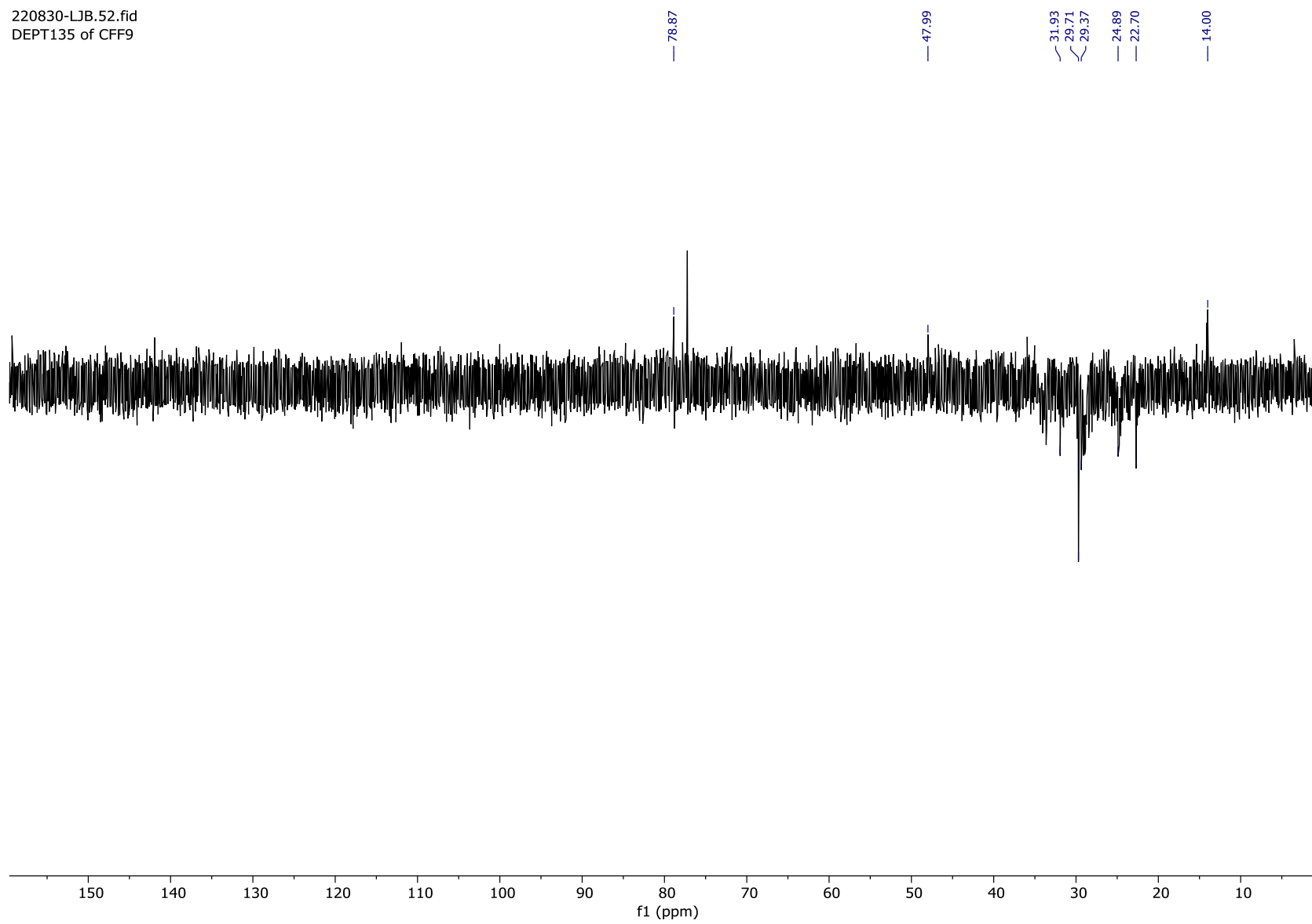
**Appendix 28b**  $^{13}\text{C}$  NMR spectrum of compound **115** (100 MHz, MeOD)

220830-LJB.51.fid  
13C-NMR of CFF9



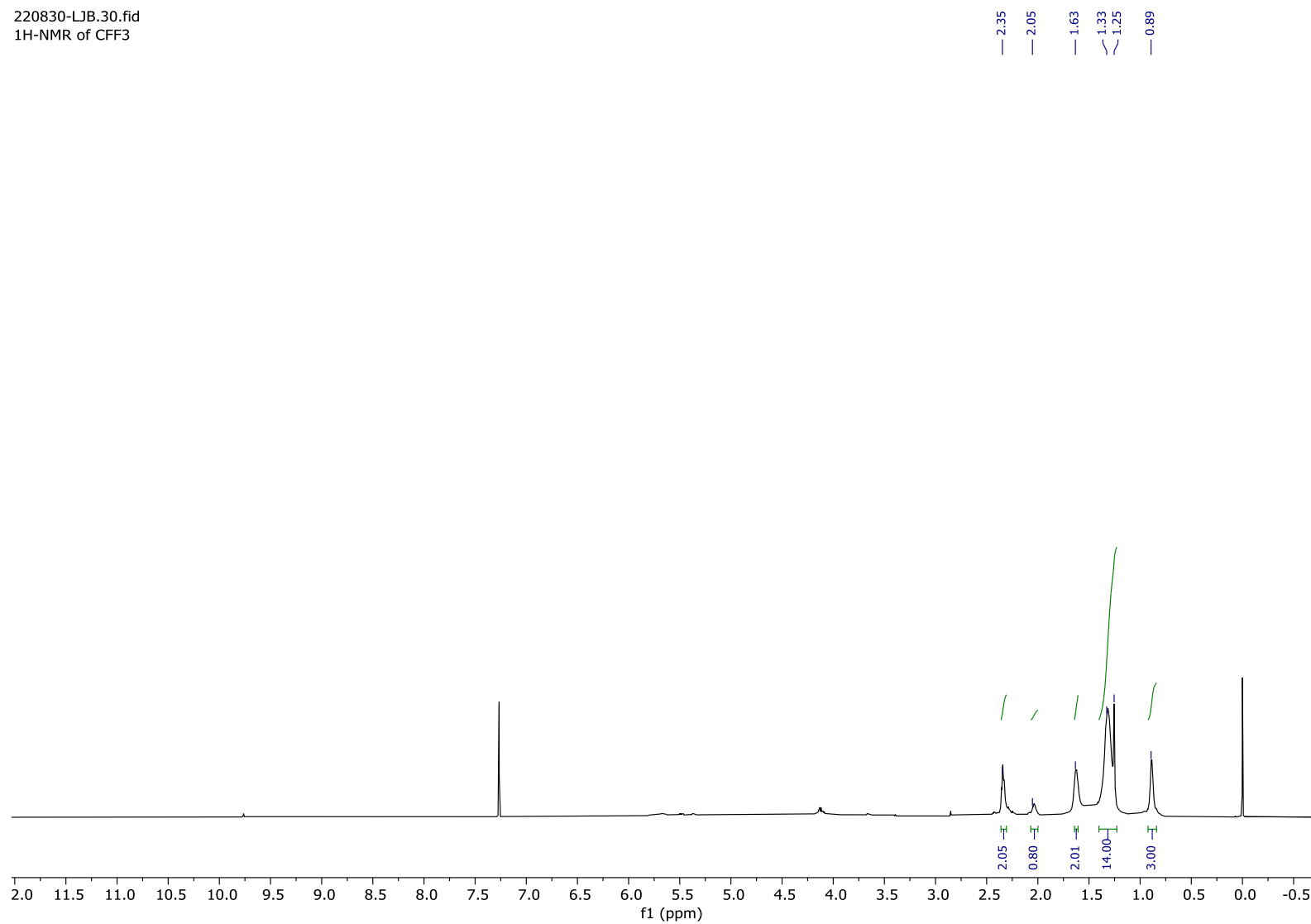
**Appendix 28c:** DEPT-135 NMR spectrum of compound **115** (100 MHz, MeOD)

220830-LJB.52.fid  
DEPT135 of CFF9



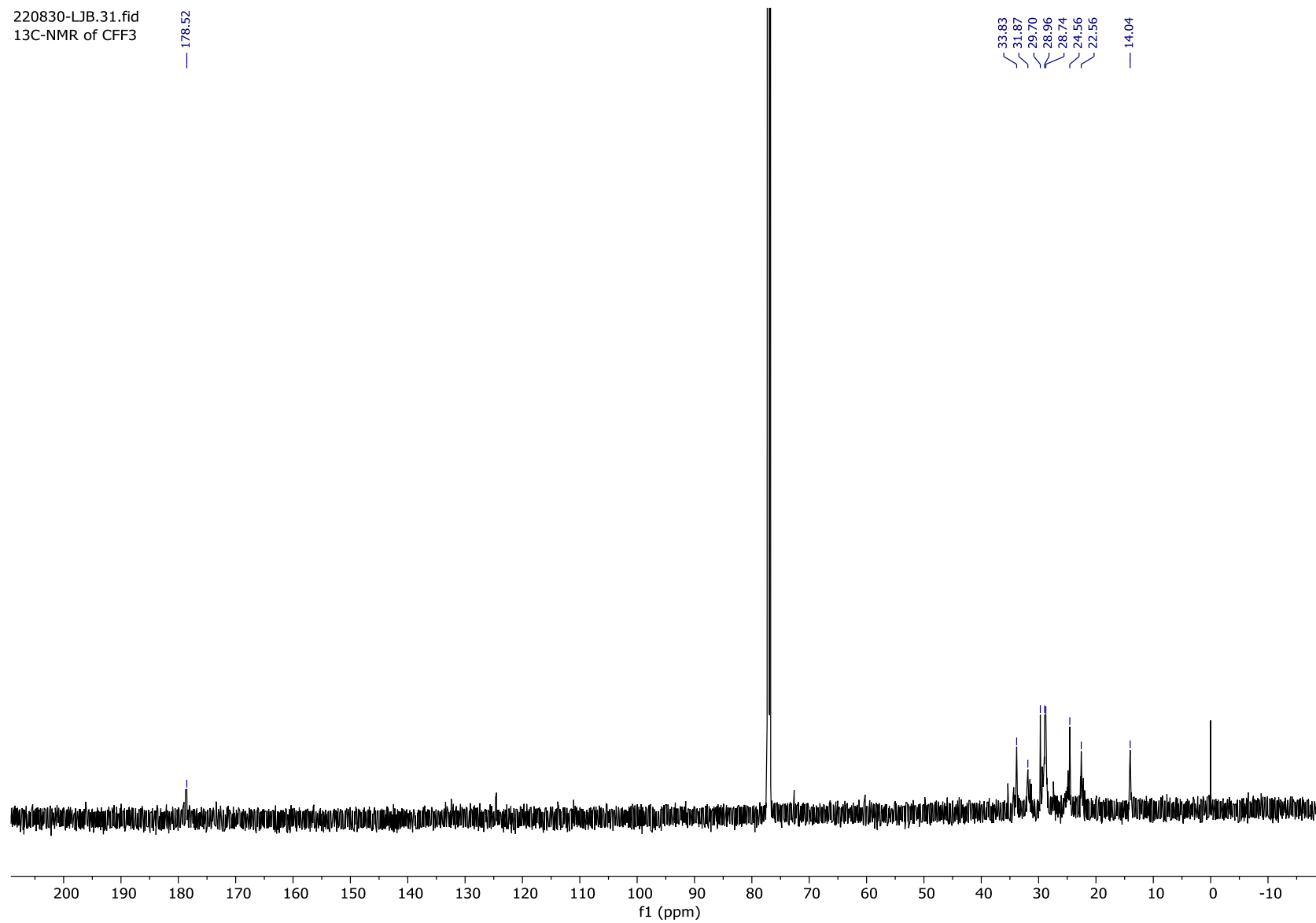
**Appendix 29a:**  $^1\text{H}$  NMR spectrum of compound **116** (400 MHz,  $\text{CDCl}_3$ )

220830-LJB,30.fid  
1H-NMR of CFF3



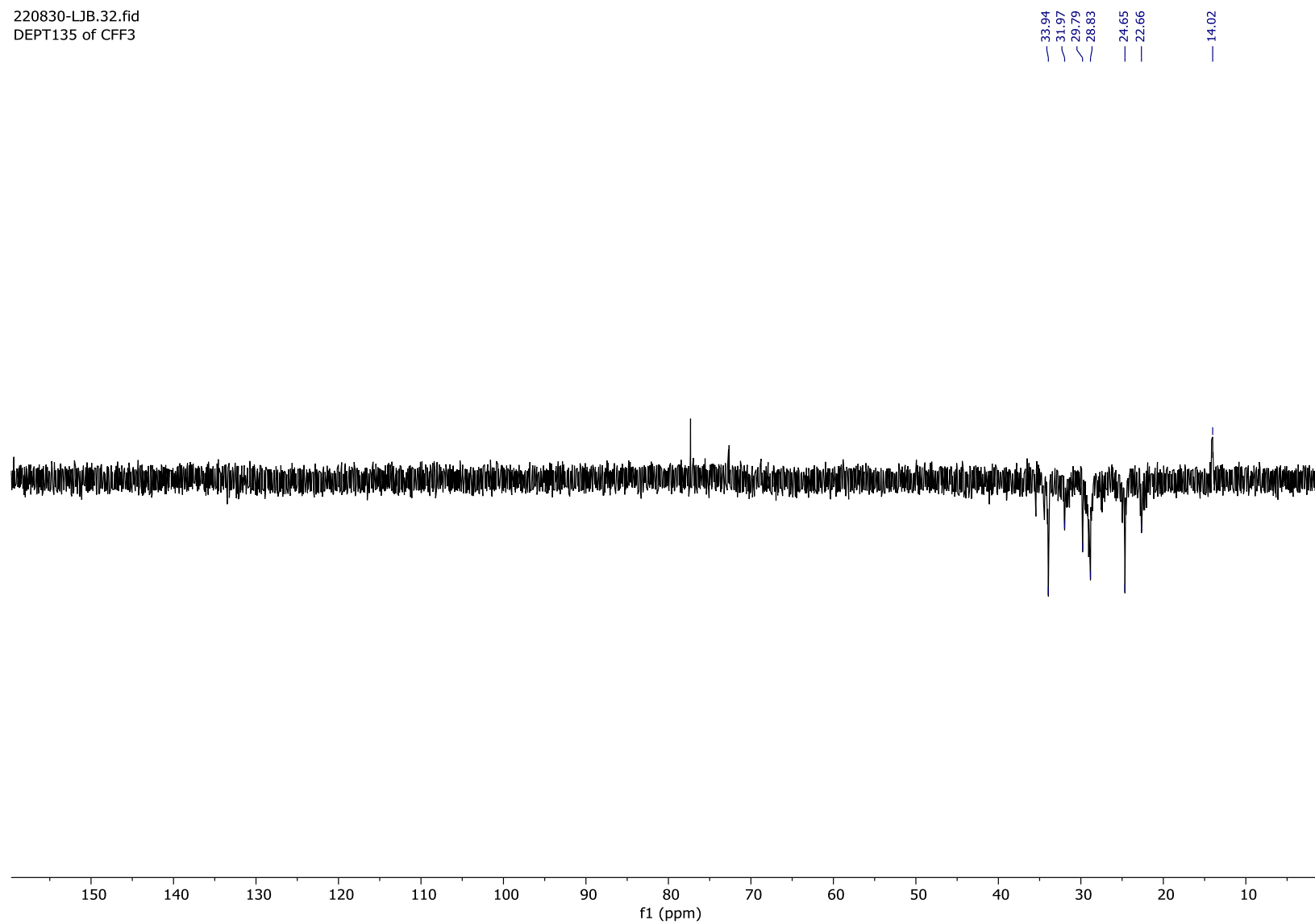
**Appendix 29b:**  $^{13}\text{C}$  NMR spectrum of compound **116** (400 MHz,  $\text{CDCl}_3$ )

220830-LJB.31.fid  
13C-NMR of CFF3



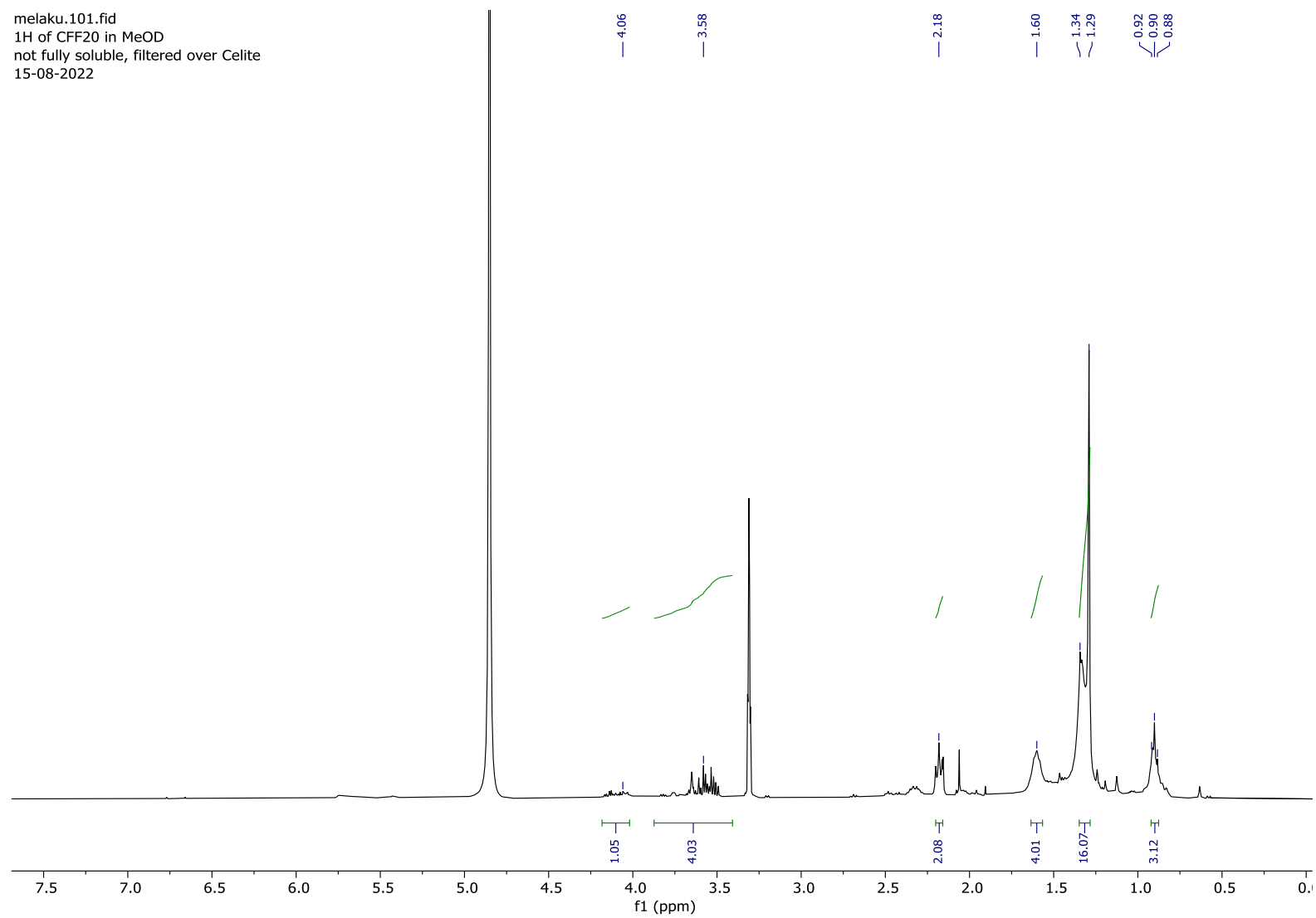
**Appendix 29c:** DEPT-135 NMR spectrum of compound **116** (100 MHz, CDCl<sub>3</sub>)

220830-LJB.32.fid  
DEPT135 of CFF3



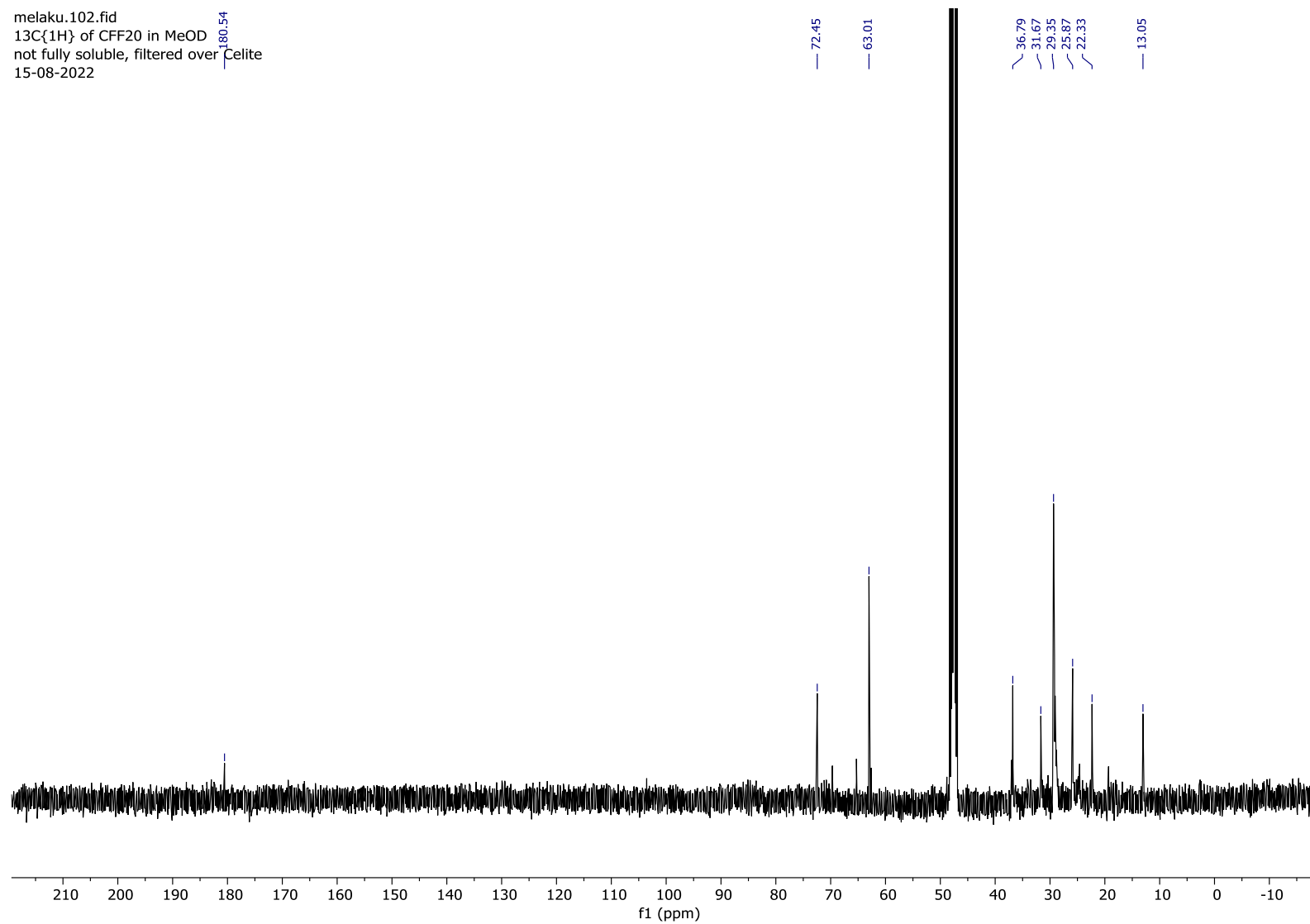
**Appendix 30a:**  $^1\text{H}$  NMR spectrum of compound **117** (400 MHz, MeOD)

melaku.101.fid  
1H of CFF20 in MeOD  
not fully soluble, filtered over Celite  
15-08-2022



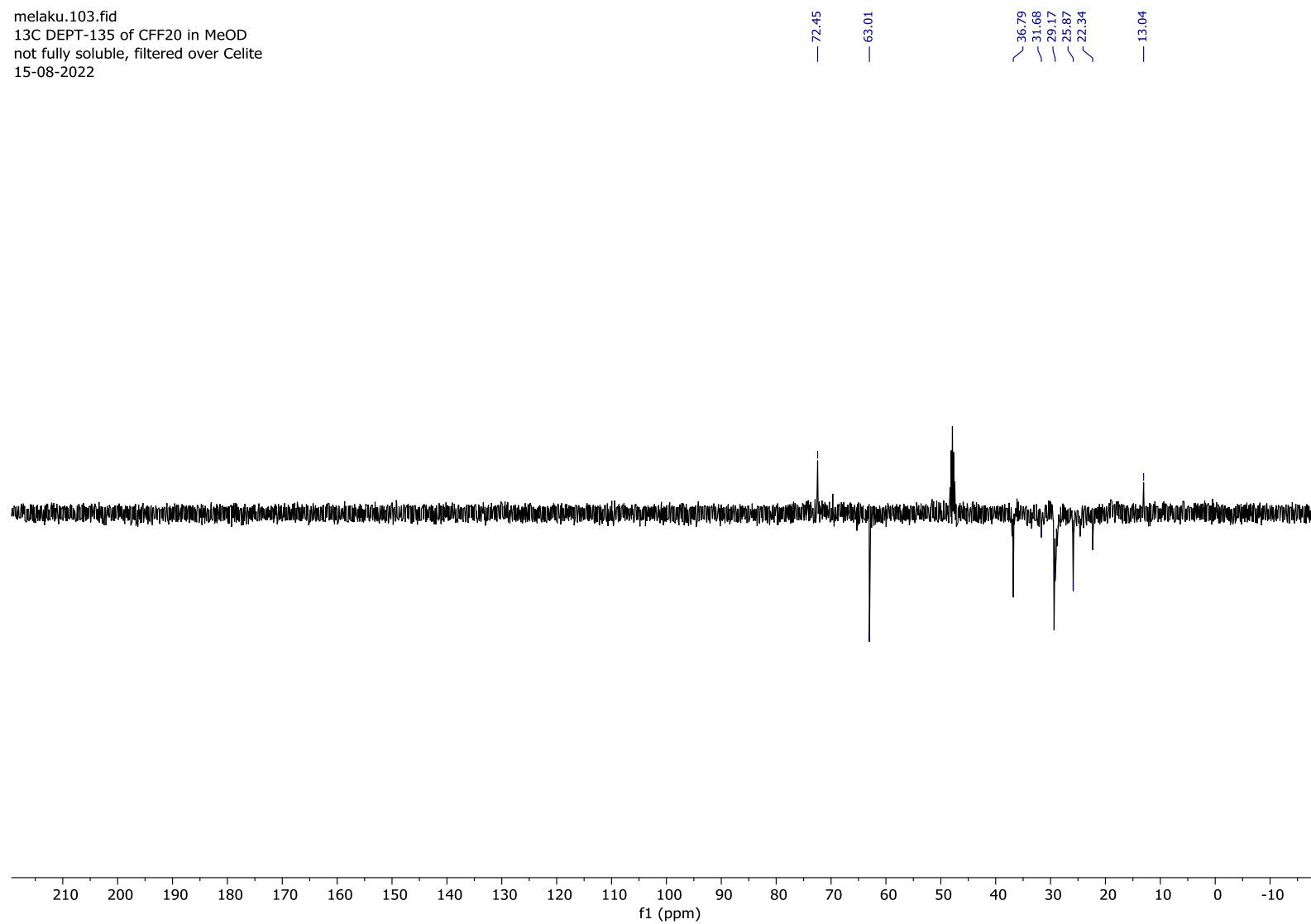
**Appendix 30b:**  $^{13}\text{C}$  NMR spectrum of compound **117** (100 MHz, MeOD)

melaku.102.fid  
13C{1H} of CFF20 in MeOD  
not fully soluble, filtered over  
15-08-2022



**Appendix 30c:** DEPT-135 NMR spectrum of compound **117** (100 MHz, MeOD)

melaku.103.fid  
13C DEPT-135 of CFF20 in MeOD  
not fully soluble, filtered over Celite  
15-08-2022



### List of Publications from this Work

- Teshale A., Hailemichael T., Venkatesha P.R., Leta G., Taye B.D., Japheth O.O., Rajalakshmanan E., and Yadessa M. (2024) in silico molecular docking analysis, cytotoxicity, and antibacterial activities of constituents of fruits of *cucumis dipsaceus*, *ACS Omega*, 9(1), 1945–1955
- Teshale A., Hailemichaele T., Eticha A., Leta G. and Yadessa M. (2023). chemical composition and antibacterial activity of essential oils from selected species of the genus *cucumis* in ethiopia, *Bull. Chem. Soc. Ethiop.*, 37(3),703-715.
- Teshale A., Hailemichael T., Awoke A., Lemma T.T., Ankita G., Leta G., Daniel R., Rajalakshmanan E., and Yadessa M. (2023), Antibacterial, Cytotoxic and Radical Scavenging Activities of Constituents of *Cucumis ficifolius*: A combined Experimental and Computational Study, *Journal of the Turkish Chemical Society Section A: Chemistry (Underreview)*
- Teshale A., Hailemichael T., Ankita G., Leta G. and Yadessa M. (2024), Antibacterial, Cytotoxic and Radical Scavenging Activities of Constituents of *Cucumis Prophetarum* and molecular docking analysis. Manuscript prepared.