Adama Science and Technology University

School of Applied Science

Department of Applied Chemistry

Final report for project entitled

"Isolation and Characterization of Anticancer, Radical Scavenging and Antimicrobial Natural Products Derived from Selected Medicinal Plants of Ethiopia"

By

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List of Abbreviations

ANOVA	Analysis of Variance
COSY	Correlation Spectroscopy
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethyl sulfoxide
DPPH	1, 1-diphenyl-2-picrylhydrazyl
HMBC	Heteronuclear Multiple Bond Correlation (^{2}JCH ,^{3}JCH)
HMQC	Heteronuclear Multiple Quantum Coherence (^{1}JCH)
IC_{50}	50 % Inhibition Concentration
IR	Infrared
MS	Mass Spectroscopy
NMR	Nuclear Magnetic Resonance
PDA	Potato Dextrose Agar
PTLC	Preparative Thin Layer Chromatography
TLC	Thin Layer Chromatography
UV	Ultra Violet
MP	Melting Point

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Summary

The increase in antibiotic resistance by microorganisms and the often lethal diseases caused by free radicals is posing serious ramifications to the lives and health of humans. Thus, there is a need to identify and process naturally occurring bioactive compounds which possess anticancer, antimicrobial and antioxidant activities. Based on the indigenous knowledge, literature reports and survey of ethno-botanical use in traditional medicine; this study have been designed to investigate anticancer, antioxidant, antifungal and antibacterial properties of four indigenous medicinal plants (*Brucea antidysenterica*, *Erythrina abyssinica*, *Bersama abyssinica and Embelia schimperi*) and to further isolate and characterize bioactive compounds that relates to the above biological properties. Sequential method of extraction with varying solvents polarities viz. *n*-hexane, dichloromethane: methanol (1:1) and methanol were used to extract the bioactive compounds from the ground powdered plant materials. The isolation and purification of bioactive compounds was done using chromatography techniques (CC and TLC). The root barks of *Embelia schimperi*, resulted in isolation and characterization of two flavans (**1**-**2**) whereas roots of *Brucea antidysentrica*, *Bersama abyssinica* and *Erythrina abyssinia* afforded compounds indole alkaloids (**3**-**6**), steroids (**7**-**9**) and flavanoids (**10**-**12**), respectively. Details of isolation, spectroscopic (UV-Vis, IR, NMR; 1D and 2D) characterization and antibacterial activity of the isolated compounds is included in this report. The antibacterial activity the crude extracts as well as isolated compounds were conducted using agar disk diffusion method. Molecular docking analysis was done for indole alkaloids (**3**-**6**) against DNA gyrase and aromatase enzymes in order to understand molecular mechanisms of antibacterial and anticancer activity of these alkaloids.

Students/researchers who are involved so far in the project directly

1. Tewabech Alemu, completed her study in MSc Medicinal Chemistry. She worked on *Brucea antidysenterica* as part of her MSc thesis work.
2. Fitsum Lemilemu 4th year BSc Applied Chemistry student, he worked on *Bersama abyssinica* as part of his BSc research work.
3. Mohammad Yasin, Mesfin Yonas and Gudata Gidi (completed their BSc in Applied Chemistry). They worked on *Embelia schimperi* as part of their BSc project work.

4. Kebede Shenkute, ARA at ASTU, he worked on *Erythrina abyssinica.*

1. Introduction

1.1. Background

Plants are the basis of traditional medicine in Africa and have been used for thousands of years. Plants often exhibit a wide range of biological and pharmacological activities, such as anti-inflammatory, anti-bacterial and anti-fungal properties (Ajayi *et al*., 2011). Due to the need for development of new drugs with better pharmacological activities, dependence on plants grew increasingly as scientists continuously exploited them for isolation of bioactive compounds (Ejele, 2010; Ugbogu *et al*., 2010).Natural products have been a source of medicinal agents since thousands of year and remarkable numbers of modern drugs have been derived from natural sources, predominantly based on their knowledge available in traditional medicine. Phytochemicals are natural products derived from plant sources, whose isolation and modification have led to production of numerous useful drugs (Okwu, 2005).

Traditional plants play an important role in medical system in Ethiopia and remain an important resource to combat serious diseases in the world. Pharmacognostic investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agents (Phillipson, 1991). Due to limited availability and/or affordability of pharmaceutical medicines in many tropical countries, the majority of the populations depend on traditional medical remedies derived mainly from medicinal plants (WHO, 2002; Zirihi, 2005).

Previous biological studies such as anti-tumor, antioxidant, antibacterial and antifungal activities were reported from the stem bark, roots and leaves of *Bersama abyssinica* fersen sub sp. *abyssinica* belonging to the family of Melianthaceae. Various class of secondary metabolites including xanthone glycosides, phenols, coumarins, flavonoids, triterpenenes, alkaloids and anthraquinones have been reported (Mbaveng *et al*., 2011; Zekeya *et al*., 2014). *Brucea antidysenterica* (Simareoubaceae) has been used for healing various diseases traditionally and various research reports revealed that quassinoids, alkaloids, triterpenes, steroids, coumarins, anthraquinones, flavonoids and other metabolites are available in various parts of the plant

(Barbosa *et al*., 2011) of which quassinoids are the most abundant in this plant and hence considered as a taxonomic marker for the plant and other species within the Simaroubaceae family (Saraiva *et al*., 2006; Almeida *et al*., 2007). *Erythrina abyssinica* (Leguminosae) is distributed in warm regions of southern Africa and the savannah of eastern Africa. Previous phytochemical studies on *Erythrina abyssinica* revealed that the plant elaborates alkaloids, flavanones, pterocarpans, chalcones and isoflavonoids (Machumi *et al*., 2006; Cui *et al*., 2008). The major groups of compounds in this plant are flavonoids, especially prenylated ones and these compounds are prevalent in the stem and root bark. The extracts from this plant and its species have been used in traditional medicine and have also shown anti-plasmodial, anti-microbial, anti-viral, anti-cancer, radical scavenging and cytotoxic activities (El Masry *et al*., 2000; El-Masry *et al*., 2002).

Embelia schimperi is one of the five known species of the family Myrsinaceae found in Kenya (Beerntje, 1994). The plant is also found in Ethiopia around Oromia region, Gamo Gofa and Amahara region. The fruit, seeds, and roots of the plant are used by the Maasai (Kenya) as an antibacterial and anthelminthic remedy, especially against tapeworm, (Kokwaro, 1993) diarrhea, fevers and chest and skin diseases (Cheikhyoussef *et al*.,2011; Lulekal *et al*., 2014) and these biological activities have been supported by systematic studies (Bogh *et al*., 1996). In Ethiopia, the bark or fruits from *E. schimperi* are combined with other species, such as *Albizia anthelmintica*, *Guizotia abyssinica*, *Glinus lotoides*, and *Hagenia abyssinica*, mixed with water and taken as a taenicide or used as a disinfectant (Avigdor *et al.,* 2014).

1.2. Statement of the problem

In Ethiopia up to 80% of the population uses traditional medicine due to the cultural acceptability of healers and local pharmacopeias, relatively low cost of traditional medicine and difficult access to modern health facilities. Ethiopia is one of the hotspot nations in biodiversity and more than 6500 medicinal plant species are identified and available in all corner of the country (Tewelde, 1991). However; detail information of the type of chemical constituents and responsible bioactive natural products derived from those species remains unexplored. The increase in antibiotic drug resistance by microorganisms and the often lethal diseases caused by

free radicals, fungi and bacteria to the lives and health of humans' raise a concern and urgent need to search for new bioactive compounds which possess antimicrobial, anticancer, and antioxidant activities. The current research project have been designed to work on four indigenous medicinal plants (*Brucea antidysenterica*, *Erythrina abyssinica*, *Bersama abyssinica* and *Embelia schimperi*) based on the indigenous knowledge, literature reports and survey of their ethno-botanical uses which have already been shown to have antioxidant, anticancer, anti-bacterial, and anti-fungal activities. Thus, the project is expected to isolate, characterize bioactive secondary metabolites derived from the aforementioned medicinal plants and screen the crude as well as isolated compounds against selected strains of bacteria, fungus and cancer cells.

To the best of our knowledge, the work done on isolation, characterization, antioxidant, anticancer, antifungal, and antibacterial activity of aforementioned four medicinal plants from Ethiopian flora is not exhaustive and hence this project is intended to fill this gap.

1.3. Significance and beneficiaries

Medicinal plants have been the focus of many anti-infective drugs and alternative sources of synthetic agents in various parts of the world since long time. The isolation of plant derived bioactive compounds still holds important significances in drug design and discovery; it can also allow rational planning of the new drugs as well as biomimetic synthesis development and discovery of new biological activity not yet related to the known compounds. Hence, this study will be used as input for validation and standardization of the traditional medicine in scientific way on four selected potential medicinal plants (*Brucea antidysenterica, Erythrina abyssinica, Bersama abyssinica* and *Embelia Schimperi*) in terms of antifungal, antibacterial, antioxidant and anticancer activities.The significance of this study also to investigate bioactive phytochemicals from the selected medicinal plant used in folklore medicine to act as the lead compound and will be used also as starting point in the development of new drug and it may be also as industrial raw materials for different applications.Researchers, students, research institutions, academia, pharmaceutical industries and local communities will be the main beneficiaries of the current study.

1.4. Objectives of the study

1.4.1. General objective

- To study the antimicrobial, anticancer and radical scavenging activities of the chemical constituents of *Brucea antidysenterica, Erythrina abyssinica, Bersama abyssinica* and *Embelia schimperi.*

1.4.2. Specific objectives

- To extract stem, root and root barks of the four medicinal plants with increasing polarity of organic solvents (*n*-hexane, dichloromethane: methanol (1:1) and methanol).
- To conduct preliminary screening on the crude extracts using standard procedures.
- To isolate bioactive compounds from the crude extracts using silica gel column chromatography (CC).
- To elucidate the structures of the isolated compounds using spectroscopic techniques (UV, IR, NMR and MS).
- To investigate *in vitro* antibacterial activity of crude extract and isolated pure compounds.
- To evaluate radical scavenging activity of crude extract and isolated pure compounds.
- To investigate molecular docking analysis against aromatase inhibitors of crude extract and isolated pure compounds so as to predict anticancer activity.

2. Literature review

2.1. Medicinal plants

Herbal medicines are plant-derived remedies that are used for their therapeutic properties and they have been an important tradition of many cultures and beliefs of African people (Fennell *et al*., 2004). Plants have long provided mankind with herbal remedies for many infectious diseases and they continue to play a major role in primary health care as therapeutic remedies in developing countries (Sokmen *et al*., 1999).Phytochemicals are natural products derived from medicinal plant, whose isolation and modification have led to production of numerous useful drugs. Plants are complex chemical storehouses of undiscovered biodynamic compounds with unrealized potential for use in modern medicine (Wang *et al*., 2002).

Traditionally used medicinal plants produce a variety of compounds for the treatment of various human ailments. These medicinal herbs constitute indispensable components of the traditional medicine practiced worldwide due to the low cost, easy access, and ancestral experience; and they are considered as candidates for developing new antimicrobial drugs (Abdalla *et al*., 2013). Ethnobotanical studies revealed that wider range of Ethiopian medicinal plants are being used in treatment of many diseases in the traditional health care system of the country (Giday *et al*., 2007; Teklehaymanot *et al*., 2007). Crude extracts of some Ethiopian plants are known to possess strong antimicrobial activity indicating that these plants can serve as sources of effective drugs against certain microbial agents (Mancini *et al*., 2015).

Plant species still serve as a rich source of many novel biologically active compounds. Very few plant species have been thoroughly investigated for their medicinal properties (Esmaeili *et al*., 2010; Heinrich and Gibbons, 2001). Plants are often the only available means of treating such infections since there is an increasing resistance to antibiotics by many pathogenic and opportunistic bacteria, plant extracts and plant-derived compounds have emerged as potential and promising antioxidant and antimicrobial agents (Weckesser *et al*., 2007).

2.2. Botanical description and Ethno-botanical uses

2.2.1 Botanical description of selected medicinal plants

2.2.1.1 Botanical description of *Bersama abyssinica*

Bersama abyssinica (synonyms: *Bersama engleriana*) is under family of Melianthaceae and genus of *Bersama* which comprises four more related species namely; *Bersama engleriana, Bersama swynnertonii, Bersama swinnyi* and *Bersama yangambiensis* (Djemgou *et al*., 2010). It grows in lowland bush savanna, gallery forests and montane forests, from sea-level up to 2700 m altitude. In East Africa mainly in Ethiopia, there are two subspecies of *Bersama abyssinica* namely; *Bersama abyssinica* Fresen. subssp.*abyssinica* and *Bersama abyssinica* subsp. paullinioides (Mikkelsen and Seberg, 2001). It is distributed in Democratic Republic of Congo, Tanzania, Mozambique, Zimbabwe, Angola, Nigeria, Ethiopia, Kenya, Sudan and Uganda, is used by local communities for the treatment of microbial infections (Lather *et al*., 2010).

2.2.1.2 Botanical description of *Brucea antidysenterica*

The Simarubaceae family is botanically related to the Rutaceae, Meliaceae and Burseraceae families, though, in this group, it is more related to the first one in terms of chemical composition, wood anatomy, lack of resin ducts in the bark and in the free stamens. It differs from the others by its absence of secretory cavities containing aromatic oils in leaves and floral parts and by the presence of quassinoids, exclusive of Simaroubaceae (Thomas, 1990). The genus *Brucea* consists of *Brucea antidysenterica*, *Brucea amarissima*, *Brucea mollis, Brucea javanica* and *Brucea sumatrana*, mainly found in tropical eastern hemisphere areas. *Brucea mollis* and *Brucea javanica*, the only two species in China, are used as traditional herbal medicines because of their antitumor and antimalarial activities (Kitagawa *et al*., 1994).

Brucea antidysenterica (Synonyms: *Brucea ferruginea*) is under the family of Simaroubaceae (Stannard, 1989). The plant is indigenous to Ethiopia locally named Abalo or Waginos in Amharic, Tollo in Oromeffa and Hadawi in Somaligna (Reinhard and Admasu, 1994). The specific epithet "*Brucea*" is named after James Bruce (1730-1794), a Scottish traveler in Ethiopia

(from 1768-1773), who brought seeds of the plant to Europe (Jansen, 1981). The plant is evergreen Shrub or small tree up to 15 m and it is usually found in forests, deforested areas and montane grassland of Ethiopia at altitudes of 1650-2800 m. This species is widespread in most tropical Africa. *Brucea antidysenterica* steam bark and root bark along with milk, honey or butter and other minerals is used in traditional medicine for treating a number of diseases including diarrhoea, skin problems, leprosy, cancerous tumors, wounds, rabies, syphilis, abdominal pains and asthma (Jansen, 1981; Stannard, 1989).

2.2.1.3 Botanical description of *Erythrina abyssinica*

The genus *Erythrina*, a member of the family Fabaceae and subfamily Papilionideae, comprises of over 110 species of trees, shrubs and herbicaceous plants that are widely distributed through the tropical warm regions of the world. Seven species of *Erythrina* are found in eastern and southern Africa, that is, *Erythrina caffra, Erythrina decora, Erythrina humeana, Erythrina livingstoniana, Erythrina lystemon, Erythrina abyssinica* and *Erythrina latissima* (Majinda *et al*., 2001). *Erythrina abyssinica* (synonyms: *Chirocalyx abyssinica; Erythrina tomentosa*) is indigenous to Ethiopia locally named in amahric Korch or Quara (Stannard, 1989). The plant is Shrub up to 15 m tall occurs in grassland, woodland, forest edges, rocky places of Ethiopia at the altitude of 1300-2400 m. The plant also found in eastern tropical Africa south to Zimbabwe, Botswana, Mozambique, and Angola.

2.2.2 Ethnobotanical uses of selected medicinal plants

2.2.2.1 Ethnobotanical uses of *Bersama abyssinica*

Bersama abyssinica is an indigenous plant to Ethiopia where it is known locally as Azamer or Afajeshgn (Amharic) and Lolchissa (Afan Oromo) and it distributed in most Ethiopian regions (Bernard Verdcourt, 1989). Ethno medicinal information conveying this genus reveals that the plant species are used for various medicinal purposes. *Bersama abyssinica* is an ever green shrub to small tree up to 18 m tall and its bark, leaf and root decoctions are widely taken as a purgative to treat a range of stomach disorders, such as abdominal pain, colic, diarrhea, cholera, intestinal

worms, dysentery, and also for the treatment of rabies, tumour, syphilis, gonorrhea, malaria, rheumatism, aphrodisiac and snake bites (Kuete *et al*., 2008; Lather *et al*., 2010).

2.2.2.2 Ethnobotanical uses of *Brucea antidysenterica*

Brucea antidysenterica is used in traditional medicine for multiple purposes. Different parts of the plant are widely used against malaria, helminthic infections, fever, dysentery and other disorders. Despite its wide application in the traditional healthcare domain only few organized and thorough scientific investigations have been undertaken to evaluate the safety and efficacy of Ethiopian traditional medicinal plants including *Brucea antidysenterica* (Dawit *et al*., 2003; Grace *et al*., 2008).

2.2.2.3 Ethnobotanical uses of *Erythrina abyssinica*

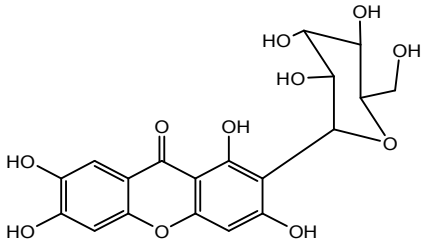
Erythrina abyssinica is mainly used in Ethiopia as a laxative (Fekadu, 2001) and the roots and bark are used to treat *Tenia versicolor* ("Madyat" in Amharic languadge) in Amharic, a disease causing dark patches on the face, more common among women (Gelahun and Debdabe, 1989). *Erythrina abyssinica* is a common plant in sub-Saharan Africa where it is used to treat inflammation, gonorrhoea, wounds, stomach problems, diarrhoea and viral infections (Bekalo *et al*., 2009).

2.3. Chemical constituents of selected plants

2.3.1 Chemical constituents of *Bersama abyssinica*

Xanthone glucosides including mangiferin (**1**) and isomangiferin (**2**) have been reported from *Bersama abyssinica* and the works revealed that Mangiferin (**1**) has antidiabetic, anticancer, anti-inflammatory and antioxidant properties (Zheng and Lu, 1990; Ojewole, 2005).The phytochemical study indicates the presence of flavonoids, phenols, triterpenes, and anthraquinones in methanol extracts (Lather *et al*., 2010). From the leaf, stem bark and root bark extract of *Bersama abyssinica* different bioactive compounds has been reported to possess a varied range of therapeutical and pharmacological applications due to presence of xanthone **(3),** hellebrigenin 3-acetate (**4**), bufadienolide-o-acetate **(5),** gallic acid **(6),** 2,3-dimethylfumaric acid (**7**), benzene-1,2,3-triol (**8**), 2-furancarboxaldehyde (**9**), 4-pyridinecarboxylic acid (**10**), D-

Melezitose (**11**), 4-pyridinecarboxylic acid, ethyl ester (**12**), 9,19-Cyclolanostane-3,7-diol (**13**), 7,8-Epoxylanostan-11-ol,3-acetoxy (**14**), 4-hydroxy-3-methoxybenzohydrazide (**15**), flavonol glycosides (**16**) (Kuete *et al*., 2008; Djemgou *et al*., 2010; Zekeya *et al*., 2014).



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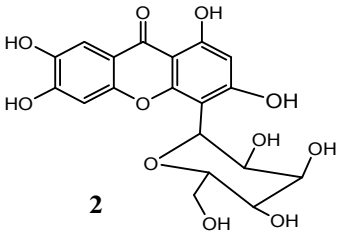

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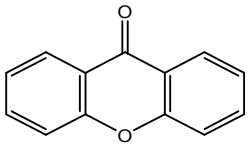

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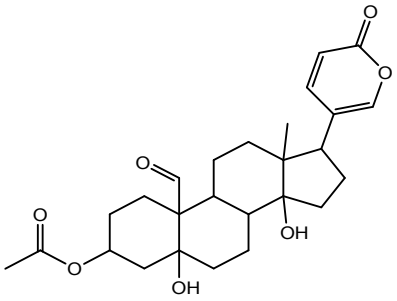

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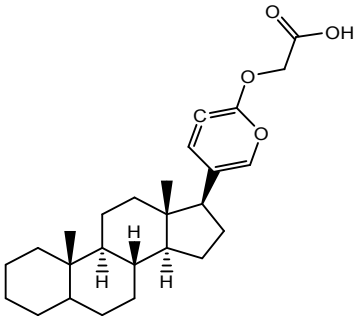

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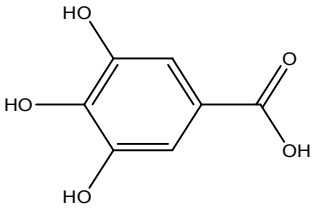

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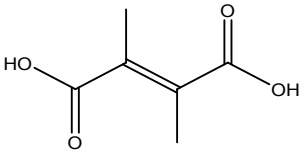

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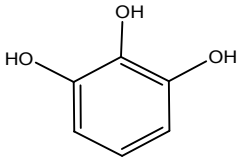

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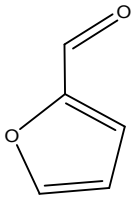

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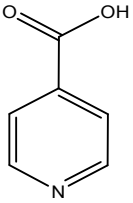

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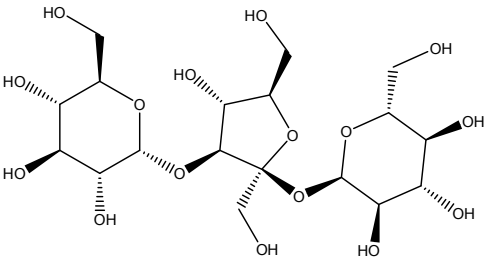

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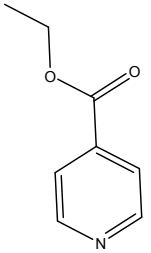

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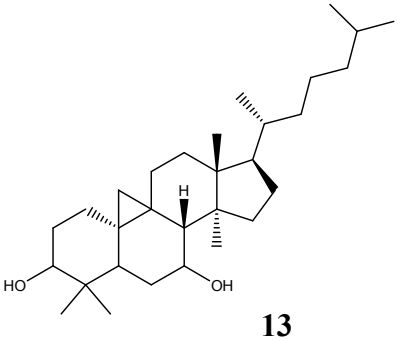

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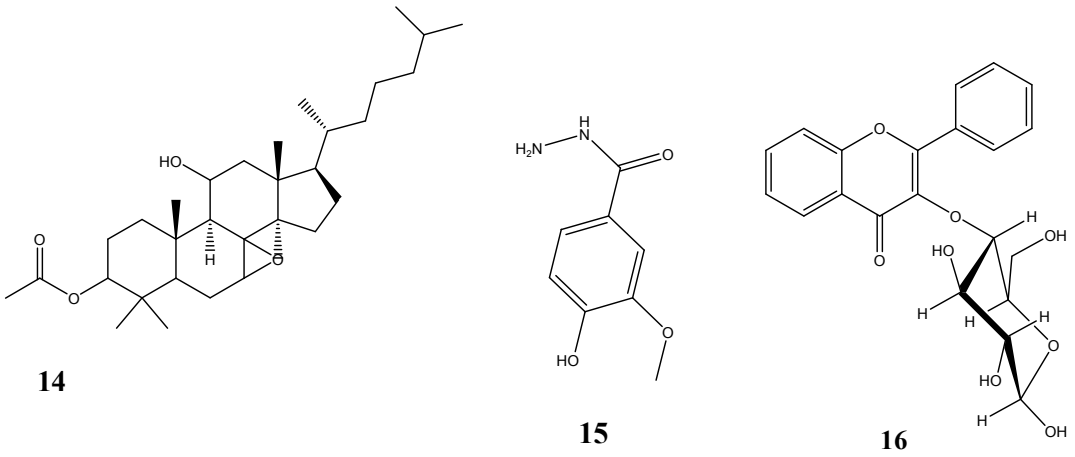


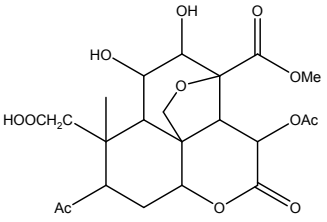
Figure 1. The structure of compounds reported from *Bersama abyssinica*

2.3.2 Chemical constituents of *Brucea antidysenterica*

Recently, much attention has been paid to the genus *Brucea* and its chemical constituents because of the multifaceted activities. Extensive studies of the genus *Brucea* have led to the identification of many compounds, such as nigakilactones, alkaloids, triterpenoids, fatty acids and flavonoids (Kitagawa *et al*., 1994).According to the previous reports some bioactive secondary metabolites were isolated from *Brucea antidysenterica* stem and root bark: Bruceaketolic acid (**17**), Bruceanic acid A (**17a**), Bruceanic acid B (**18**), Bruceanic acid C (**19**), Bruceanic acid D (**20**), Bruceantinoside A (**21**), Bruceantinoside B (**22**), Yadanzioside D (**23**), Yadanzioside E (**24**), Yadanzioside H (**25**), Bruceolline A (**26**), Bruceolline B (**27**), Luteolin-7-O-β-D-glucoside (**28**), Quercetin-3-O-β-D-galactoside (**29**)and Quassinoid derivatives of Ailantinol E (**31**), Ailantinol F (**30**), Brusatol, (**32**), Bruceantin, (**33**), and Bruceantinol (**34**), (Toyota *et al*., 1990; Ouyang *et al*., 1994; Subeki *et al*., 2007).

Due to the chemical diversity previously described for many species of Simaroubaceae family, it is worth noting that it can be characterized as a promising source of bioactive molecules with remarkable research potential. An example of this is that since 1961, when the first quassinoide structurewas elucidated, the growing interest on various species of Simaroubaceae family resulted in the isolation and identification of the more than 200 currently-known quassinoids (Vieira and Braz-Filho, 2006). Nevertheless, many of its species have not been studied or remain unexplored.Many genera from the Simaroubaceae family have been reported to express

quassinoids. These consist of triterpene degradation products, derived from the euphol/tirucalol series, highly oxygenated and structurally complex. Most of the isolated quassinoids have a twenty carbon skeleton (Vieira and Braz-Filho, 2006; Guo *et al*., 2009).Among the alkaloids isolated from the different genera of the Simaroubaceae family, the canthines derivatives deserve special attention such as Canthin-6- ones (**36**), canthin-2,6-dione (**38**), 5-hidroxycanthin-6-one (**39**), 1-vinyl-4,8-dimethoxy-β-carbolin (**37**) and 9-methoxycanthin-6-one (**35**) (Rivero-Cruz *et al*., 2005; Saraiva *et al*., 2006).



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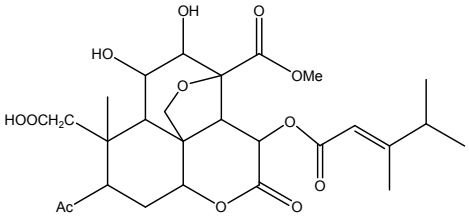
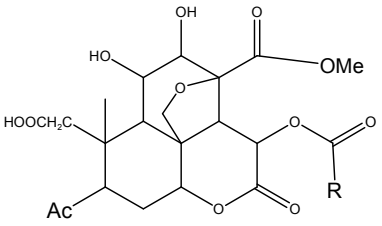
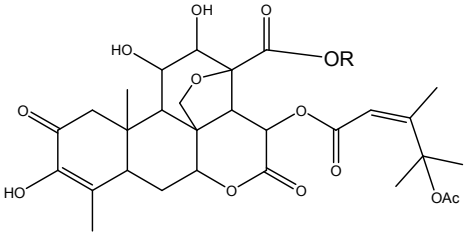


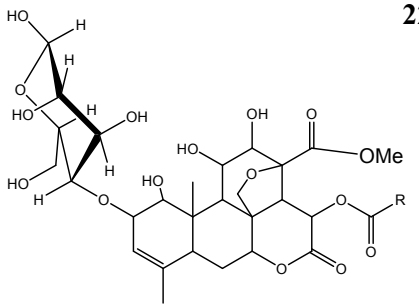
17a



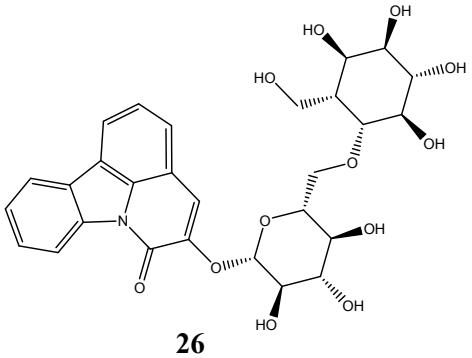
18 R= Ph
19 R = $AcOC(Me)_2C(Me)=CH$
20 R = $HOC(Me)_2C(Me)=CH$



21 R= H
22 R = Me



23 R= Me
24 R = $Me_2C=CH$
25R = i-Bu



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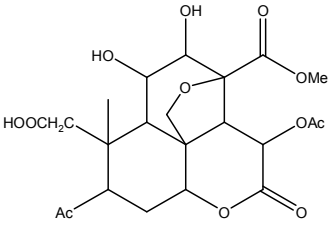

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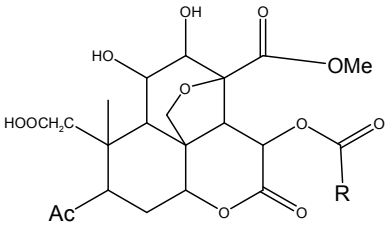
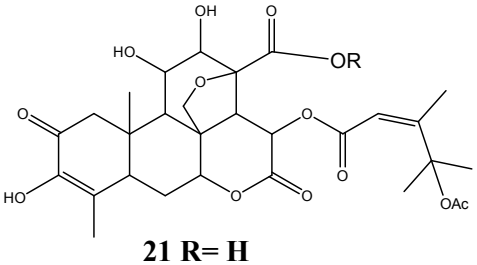
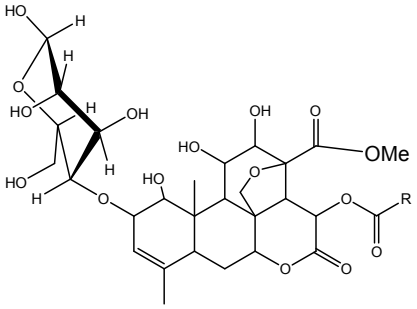


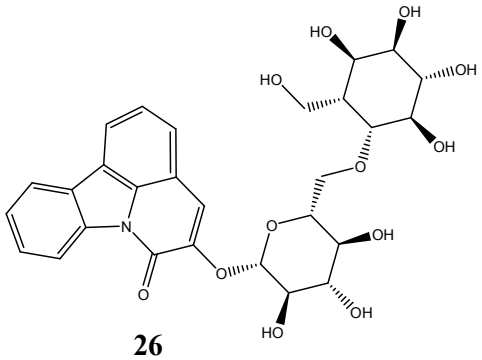
18 R= Ph
19 R = $AcOC(Me)_2C(Me)=CH$
20 R = $HOC(Me)_2C(Me)=CH$



21 R= H
22 R = Me



23 R= Me
24 R = $Me_2C=CH$
25R = i-Bu



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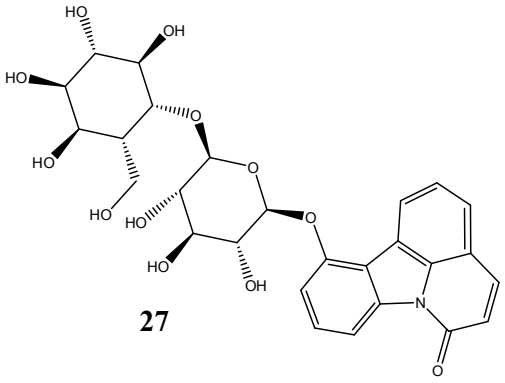

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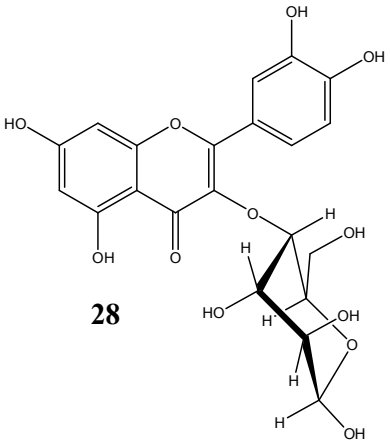

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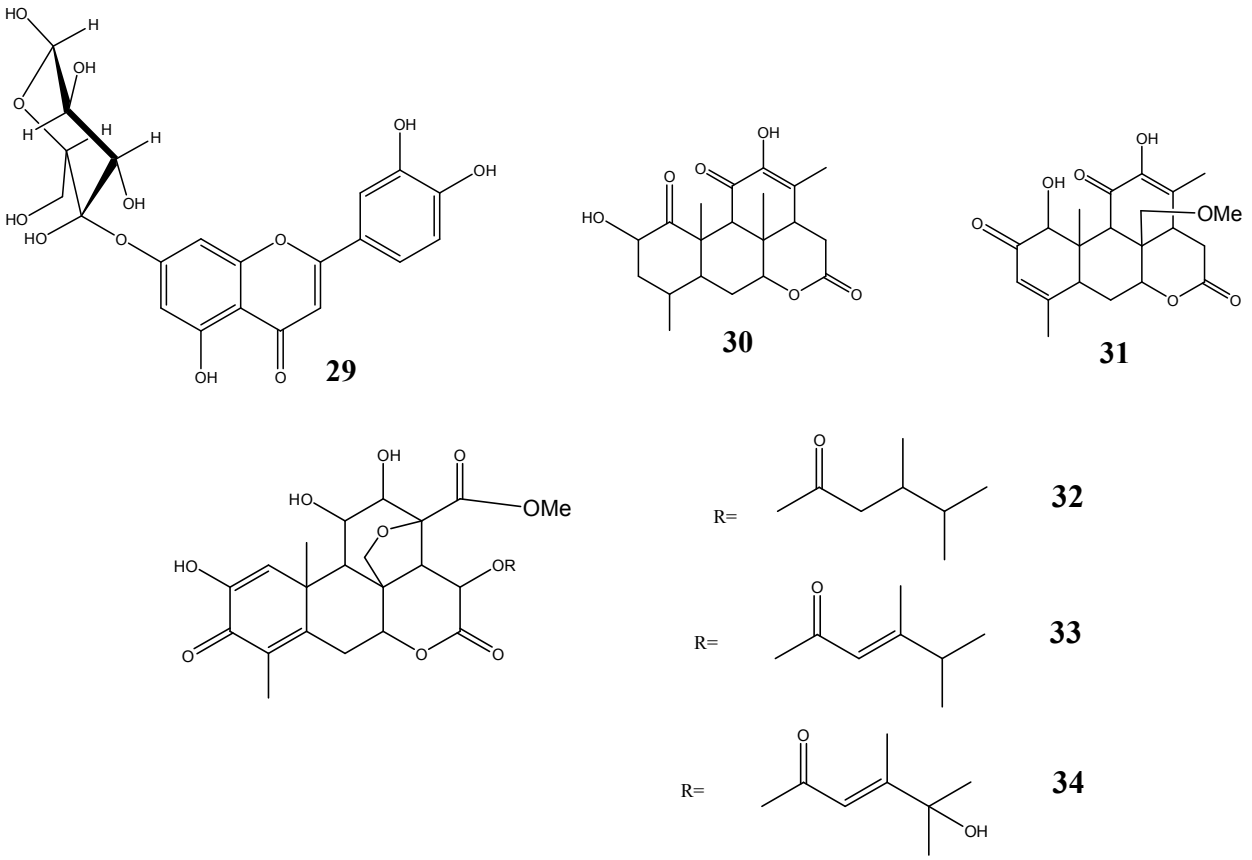


Figure 2. Structures of compounds reported from *Brucea antidysenterica*

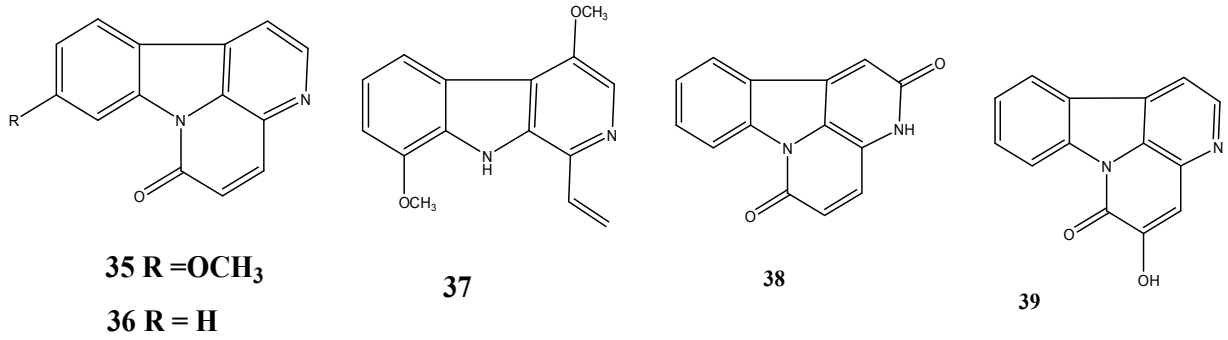
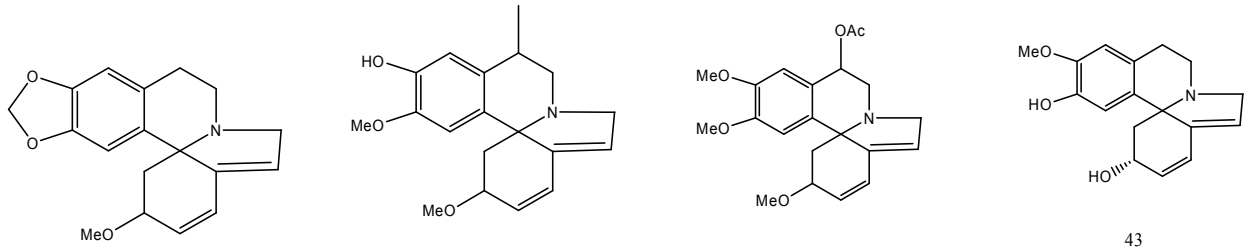


Figure 3. Structures of canthine alkaloids reported from the genus *Brucea*

2.3.3 Chemical constituents of *Erythrina abyssinica*

Previous studies show the genus *Erythrina* is a rich source of bioactive alkaloids terpenoids, saponins and flavonoids, especially, isoflavones, pterocarpans and flavanones. The alkaloids produced are of the *erythrina* type, some of which has been shown to have curare-like activity on the central nervous system. The bark of *Erythrina lattisima* is burnt and used as dressing for open wounds (Bojase *et al*., 2001).Previous Works on *Erythrina latissima* concentrated mainly on the seeds and a number of erythrina-type alkaloids were isolated. These were erythraline (**40**), 11-hydroxysodine (**41**), erythracine (**42**) and erythravine (**43**) (Bojase *et al*., 2001).



40 41 42 43

Figure 4. Structures of alkaloids from *Erythrina latissima*

Some flavonoids have been isolated from the roots of *Erythrina abyssinica*; abyssinone (**44**), cristacarpin (**45**), erythrabyssin II (**46**), phaseollidin (**47**) and phaseollin (**48**) (Bisby *et al*., 1994). From leaves the plant following erythrina-type alkaloids have been identified erythristemine (**49**), isoboldine (**50**), and orietaline (**51**) have been reported (Bisby *et al*., 1994; Yenesew *et al*., 2003).The acetone extract of the roots of *Erythrina abyssinica* were showed new pterocarpene [3-hydroxy-9- methoxy-10-(3,3-dimethyallyl)pterocarpene (**52**) and a new isoflav-3-ene [7,4′-dihydroxy-2′,5′-dimethoxyisoflav-3-ene] (**53**) and from ethyl acetate extract, a new chalcone, 2′,3,4,4′-tetrahydroxy-5-prenylchalcone (trivial name 5-prenylbutein) (**54**) and a new flavanone, 4′,7-dihydroxy-3′-methoxy-5-prenylflavanone (trivial name, 5-deoxyabyssinin II (**55**) have been isolated (Derese *et al*., 2003; Yenesew *et al*., 2004).

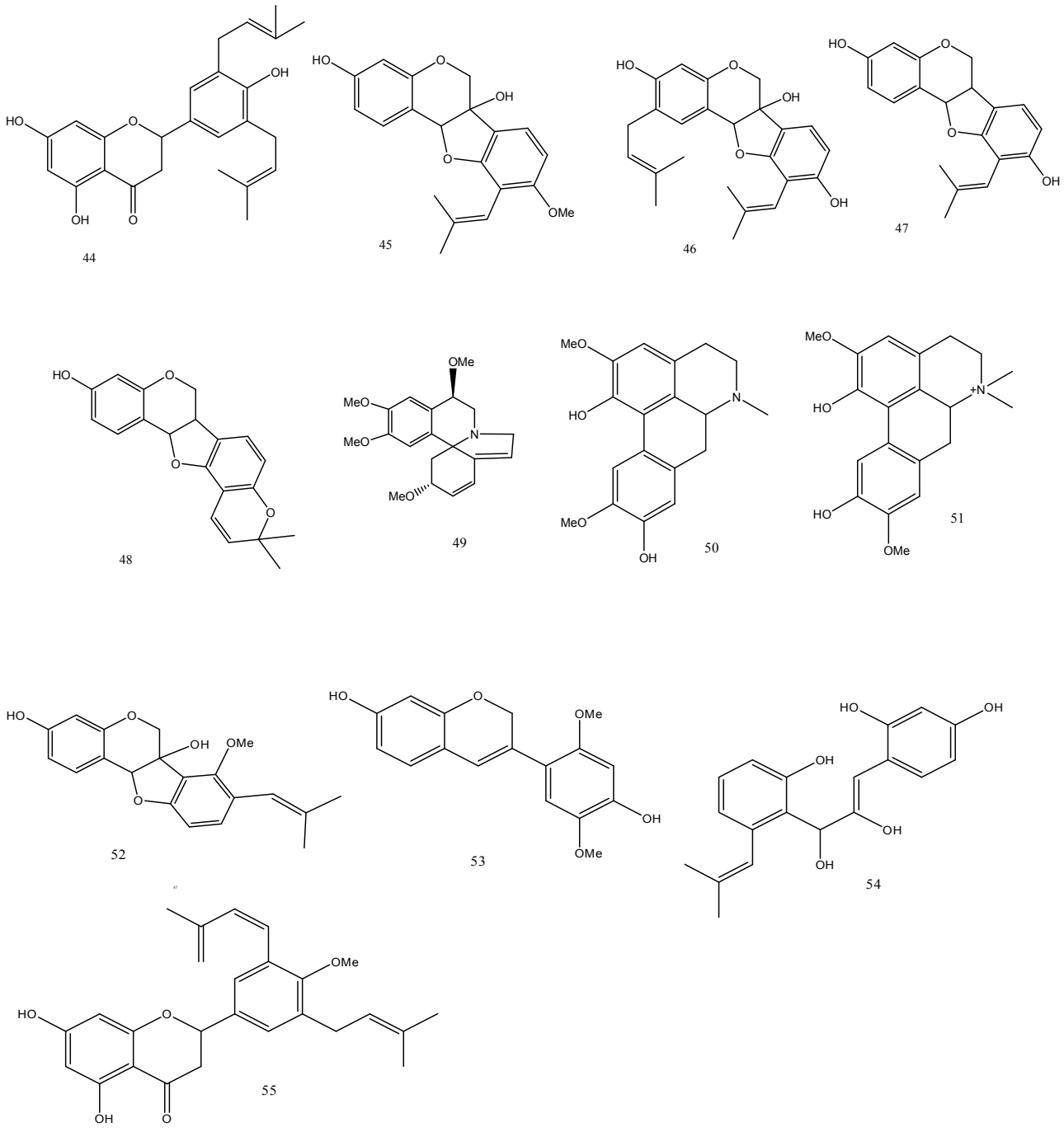


Figure 5. Structures of compounds reported from *Erythrina abyssinica*

2.3.4 Chemical constituents of *Embelia schimperi*

Previous phytochemical studies on *E. schimperi* reported the isolation of long alkyl chain substituted benzoquinones (Midiwo et al., 1988), pentacyclic triterpenoids (Machocho et al., 2003; Manguro et al., 2006), anthraquinones (Midiwo and Manguro, 2006), and flavonoids (Manguro and Williams, 1997). It is likely that any therapeutic activity in the herb is associated with polyvalent pharmacological effects brought on by the synergistic combination of several constituents rather than any single isolated one (Babe et al., 2018; Wagner, 2005).

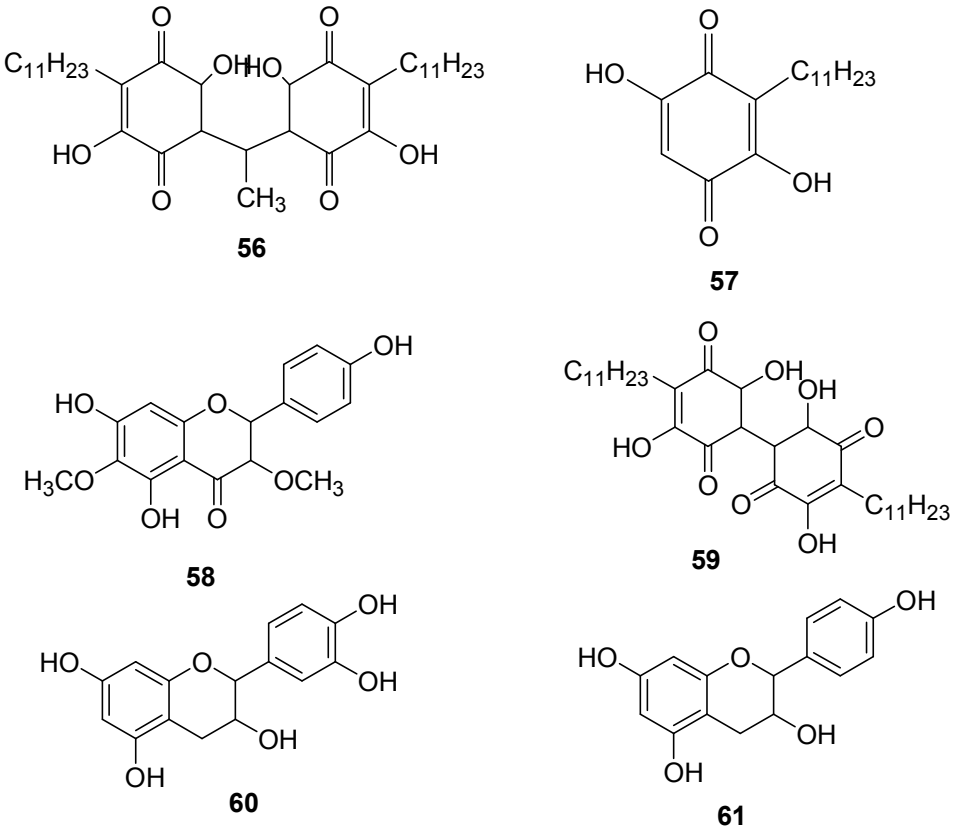


Figure 6. Structures of compounds reported from *E. schimperi*

2.4. Pharmacological activities of plant species

Ethno medicinal information conveying this genus reveals that the plant species are used for various medicinal purposes. Previous biological analysis of *Bersama abyssinica* revealed the presence of antimicrobial secondary metabolites. The phytochemical study of the composition of the leaves, stem bark and root bark of *Bersama abyssinica* revealed the presence of bioactive chemical compounds in the stem bark of which 1, 2, 3-benzenetriol (**8**) and 2, 3-dimethylfumaric

acid (**7**) which possessed antibacterial activities This would justify the antibacterial activity found on multidrug resistant *S. aureus* strains (Zekeya *et al*., 2014). Despite the activity displayed by *Bersama abyssinica*, only *Bersama engleriana* has been phytochemically studied where xanthone glycosides, terpenoids and anthraquinones with anti-tumour, antibacterial and antifungal activities were reported from the stem bark, roots and leaves of *Bersama engleriana* (Mbaveng *et al*., 2011).

Mangiferin (**1**) is a xanthone glycoside derivative widely distributed in higher plants especially *Bersama abyssinica* and *Bersama engleriana* contribute important role which is exhibiting antidepressant, antileukaemic, antitumor, antitubercular, choleretic, diuretic, antimicrobial, antifungal, antioxidant, anti-inflammatory, antiviral, hypoglycaemic and other biological activities (Neerja *et al*., 2000). Mangiferin and its derivatives enhance tumor cell cytotoxicity of lymphocytes and macrophages and antagonize *in vitro* the cytopathic effect of HIV (Peng *et al*., 2004). These activities are slightly similar to other chemopreventive agents such as 1-acetoxy chavicol acetate. Hence, mangiferin was studied both *in vitro* and *in vivo* for its possible anticancer effects. The effective bioavailability of mangiferin, iso mangiferin and Xanthone derivative makes them suiTable antioxidants with potential use in atherosclerosis susceptible conditions (Pardo-Andreu *et al*., 2008).

Bersama abyssinica Fres. spp., *abyssinica* researches on the ethnobotanical information on the plants show that it is used for gastrointestinal conditions especially in Ivory Coast where reasonable efficacy against Gram negative and Gram positive bacteria were realized (Bolou *et al*., 2011). There were also evidences that the bark and leaves of the plant has antimalarial activities (Zirihi *et al*., 2010). Methanol and ethyl acetate leaves extract *Bersama abyssinica* exhibited high antibacterial against bacteria which implies that polar secondary metabolites are responsible for the activity. Previous report on the leaves of *Bersama abyssinica* has established the presence of flavonol glycosides (**16**), xanthone (**3**) and mangiferin derivatives (**1, 2**) (Asres *et al*., 2006). Flavonol glycosides have been reported to possess high antimicrobial sensitivity to some bacterial strains than gentamycin reference drug (Fazilatun *et al*., 2012).

Simaroubaceous plants contain many quassinoids with various biological activities, such as antitumor, antimalarial, antifeedant, insecticidal, antiinflammatory, amoebicidal, and herbicidal

effects. From *Brucea antidysenterica* and *Brucea javanica* species ailantinol E (**31**) and ailantinol F (**30**) quassinoid derivatives are isolated and their biologically activity had been investigated (Daido *et al*., 1995; Fukamiya *et al*., 1992). Bruceantin (**32**) and analogues compounds are triterpenes of the quassinoid type isolated from the bark of the Ethiopian tree *Brucea antidysenterica*. They were capable of inducing an array of biological responses including anti-inflammatory effect with murine models (Kupchan *et al*., 1973). The alcoholic extract of *Brucea antidysenterica* showed significant inhibitory activity *in vitro* against cells derived from human carcinoma of the nasopharynx (KB), Walker 256 intramuscular carcinosarcoma in the rat, and P-388 lymphocytic leukemia in the mouse (Kupchan *et al*., 1975).

In vitro evaluation of anti-tuberculosis activity was conducted for different quassinoids such as bruceantinol (**34**) (Narihiko *et al*., 1997). Canthin-6-ones (**36**), canthin-2,6-dione (**38**), 5-hidroxycanthin-6-one (**39**), 1-vinyl-4,8-dimethoxy-β-carbolin (**37**) and 9-methoxycanthin-6-one (**35**) have been reported to have a large array of activities, such as antiviral, cytotoxic, antiparasitic, antibacterial, high pro-inflammatory cytokines reducer, among others (Showalter, 2013).

Species from the Simaroubaceae family, known for their medicinal properties, are used traditionally for the treatment of malaria, and also as anthelminthic, antitumor, antiinflammatory, antiviral, anorectic, tonic, insecticide and amebicide (Saraiva *et al*., 2006; Silva *et al*., 2010). There are reports of the use multi biological properties of *Brucea antidysenterica* in Africa, *Brucea javanica* and *Ailanthus altissima* in China, *Simaba guianensis*, *Quassia amara* and *Simarouba versicolor* in Brazil, *Castela texana* in Mexico (Mendes and Carlini, 2007; Silva *et al*., 2010) and *Quassia amara* in French Guyana (Cachet *et al*., 2009). The vast range of biological activities of the different species of Simaroubaceae are given, mainly, due to the quassinoids, for which were attributed antitumor, antimalarial, antiviral, anorectic, insecticide, amebicide, antiparasitic and herbicide activities (Bhattacharjee *et al*., 2008).

Previously in *Erythrina abyssinica* species have been studied by Yenesew and his coworkers (2004) and found that the ethyl acetate extract of the bark possesses, antiplasmodial activity against the chloroquine sensible (D6) and chloroquineresistant (W-2) strains in *Plasmodium falciparum* with values of IC_{50} of 7.9 ± 1.1 and 5.3 I 0.7 mg/ mL, respectively (Yenesew *et al*.,

2004). Several flavonoids, isoflavonoids and pterocarpanes have been reported as antimicrobial and antibacterial agents (Redko, *et al*., 2007; Chukwujekwu, *et al*., 2011). The chloroform extract of the stem bark of *Erythrina burttii* which is closely resembled to *Erythrina abyssinica* species showed antifungal and antibacterial activities using the disk diffusion method. Flavonoids were identified as the active principles and were observed against fungi and Gram (+) bacteria, and Gram (-) bacteria (Yenesew *et al.,* 2005). Previous studies of Leguminosae family particularly *Erythrina lysistemon, Erythrina abyssinica*, and *Erythrina burttii* have revealed erythrinaline alkaloids, some of which are distributed in several parts of this plant. The other major group of compounds is the flavonoids, especially prenylated ones and these compounds are prevalent in the stem and root bark (Masry *et al*., 2000). The extracts from this plant have been used in traditional medicine and have also shown antiviral, radical scavenging ability, antimicrobial, anticancer and cytotoxic activities (El-Masry*et al*., 2002).

Study conducted on the ethyl acetate extract of the stem bark of *Erythrina abyssinica* greatly inhibited PTP1B activity (>80% inhibition at 30 µg/mL). The *Erythrina* plants are widely distributed in tropical and subtropical regions, with some species in use as indigenous medicines. A number of chemical constituents including alkaloids, pterocarpans, flavonoids, and benzofurans have been isolated from this genus, some of which exhibited antimicrobial, antioxidative, and estrogen-like activities. Despite several studies on the chemical constituents and biological activities of the genus *Erythrina*, few limited phytochemical investigations are focused on *Erythrina abyssinica* (Yenesew *et al*., 2003).

The extracts of *Embelia schimperi* showed various degree of antibacterial activity against Gram negative bacterial (Elibariki *et al*., *2016*). Methyleneoxy bridged-oleanane type pentacyclic triterpenoids with antibacterial activity against *Escherichia coli*, *Pseudomonas putida*, and *Bacillus subtilis* were reported from the *E. schimperi* stem bark in Kenya (Machocho *et al*., 2003) which suggests that antibacterial activity against Gram negative bacteria reported might due to the presence of oleanane triterpenes. Another antibacterial investigation conducted by disc diffusion using pure compounds and crude extracts from *E. schimperi* stem ethyl acetate grown in Kenya demonstrated that the crude extract was inactive while 2,5-dihydroxy-3-methyl-1,4-benzoquinone showed significant activities against Gram-negative *Salmonella* spp*., Proteus* spp*.,*

P. aeruginosa, K. pneumoniae, and Gram-positive *Shigella dysentriae* and *Staphylococcus aureus (*Awino *et al*., 2008). However, another antibacterial investigation from Kenya conducted by using Embelin from *E. schimperi* fruit ethyl acetate extract and *Embelin* synthetic derivative indicated they were inactive against *P. aeruginosa* and *E. coli* (Chepkwony *et al*., 2011).

Most of commercially available anti-inflammatory drugs are steroids in chemical nature, and the adverse side effects are well known. *Embelin* finds use as anti-inflammatory drug in traditional medicine (Kapoor *et al.,* 1983) *Embelin* and its 2, 5-isobutylmine salts have been reported to possess anti- inflammatory activity in carrageenan-induced paw edema and cotton pellet granuloma formation in rats (Handa *et al*.,1992).

3. Materials and methods

3.1. Chemicals and reagents

The following chemicals and reagents were used for this experiment: chloroform, ethanol, n-hexane, ethyl acetate, dichloromethane, acetic acid, DMSO, methanol, anhydrous sodium sulphate, sulphuric acid, Silica gel (60-120 mesh), hydrochloric acid, lead acetate, ferric chloride, acetic anhydride, L-Ascorbic acid, DPPH, PDA and MHA. All the aforementioned chemicals and reagents had analytical and HPLC grade.

3.2. Apparatus and instruments

The following Apparatus and Instruments were used for this experiment: column, separatory funnel, oven, TLC plate, Whatman filter paper No.1, mortar, pipettes, water bath, UV lamp, beakers, UV light cabinet, electronic balance, conical flask, measuring cylinder, Rota vapor, chromatographic chamber, PTLC, FT-IR Spectrometer (400-4000 cm^{-1}) in KBr, and 400 MHz Bruker avance NMR Spectrometer.

3.3. Collection and identification of plant materials

Root bark, stem bark, root and leaves part of *Bersama abyssinica, Embelia schimperi* and *Erythrina abyssinica* were collected from Asela, Oromia regional state, Ethiopia on December, 2018. Root bark and root of *Brucea antidysenterica* was collected from Bahrdar, Amahara regional state, Ethiopia on December, 2019.Botanical specimens of the plants was collected and submitted to the National Herbarium, Department of Biology, Addis Ababa University for identification.

3.4. Preparation of plant materials

After collection, all the samples was washed with distilled water repetitively and air-dried at shade. The leaves, stem barks, root and root barks of the selected medicinal plants were ground using electrical grinder. The resulting powder was packed in polyethylene bag to avoid contamination until the experiment was conducted.

3.5. Extraction of plant materials

3.5.1. Solvent extraction of *Embelia schimperi*

Air-dried root bark powder was weighed (300 g) and extracted exhaustively with *n*-hexane for 48 hr at room temperature. The marc left was extracted with 1.5 L dichloromethane/methanol (1:1) soaked for 48 h at room temperature. The marc left was further extracted with 1.2 L methanol soaked for 48 h at room temperature. The mixture was filtered, and the filtrate was concentrated under reduced pressure and temperature of 40°C using rotary evaporator and afforded (60.5 g) (20.2%) crude extract.

3.5.2. Isolation of compounds from *Embelia schimperi*

Silica gel column chromatography was conducted for separation of chemical constituents using increasing gradient of ethyl acetate in *n*-hexane as eluent. A silica gel (150.0g) was mixed with *n*-hexane and the slurry was used to pack the column. The crude MeOH extract (6.0g) was adsorbed on 6 g silica gel and applied on column after drying. Elution was carried out with increasing gradient of ethyl acetate in *n*-hexane followed by increasing gradient of methanol in dichloromethane. Fraction that showed the same R_f value and the similar characteristics color on TLC were combined and further purified. The column was then eluted with increasing polarity of solvent system. A total of 88 fractions each with 100 mL were collected. Fractions 54-58 (60% ethyl acetate in *n*-hexane eluent) showed one spot. After drying, it was continuously washed with n-hexane to give compound **62** (26 mg). Fractions 72-80 (15% methanol in dichloromethane) showed one major spot. The fractions were combined and repurified by small silica gel column (60:40 ratio of ethyl acetate in *n*-hexane, isocratic mode) to give compound **63** (20 mg).

3.5.3. Solvent extraction of *Brucea antidysenterica*

Air-dried root powder was weighed (350 g) and extracted exhaustively with n-hexane for 72 h at room temperature. The marc left was extracted with 2.1 L dichloromethane: methanol (1:1) soaked for 72 h at room temperature. The mixture was filtered, and the filtrate was concentrated under reduced pressure and temperature of 40°C using rotary evaporator and afforded (12.77 g) (3.65%) crude extract.

The marc left was further extracted with 2.1 L methanol soaked for 72 hr at room temperature. The mixture was filtered, and the filtrate was concentrated under reduced pressure and temperature of 40°C using rotary evaporator and afforded (9.4 g) (2.68%) brownish crude extract. The dried crude extracts were kept at deep freezer for further experiments.

3.5.4. Acid-Base extraction of alkaloid constituents of *Brucea antidysenterica*

Alkaloids constituents were selectively extracted in acid-base extraction approach. Air dried powder of roots (350 g) of *Brucea antidysenterica*in *n*-hexane separately, filter the solution, and the residue was extracted in ethanol at room temperature for 72 hr. Then solvent was evaporated by using a rotary evaporator to yield crude alkaloids mixture. The defatted ethanolic crude extract was suspended in 5 % HCl at pH 5. Then pre-saturate with water and partition by adding chloroform, the extract was separated in to two layers, aqua solution layer and organic solution layer. Then aqua and organic layers were separated by using separatory funnel and repeat the procedure for three times. The acidic aqueous phases were basifed by adding 5 % NH_3 at pH 11, and then partitioned by chloroform. Then chloroform extracts were combined, and concentrated the solvent under vacuum rotary evaporator to obtain 5.1 g (1.45 %) of brownish crude alkaloid extract. According Mayer's reagent test a yellowish precipitate was observed which confirms for the presence of alkaloid constituents in the acid base crude extract. The acid base crude extract was collected in labeled sterile containers and kept in deep freezer for further experiments.

3.5.5. Isolation of compounds from *Brucea antidysenterica*

The crude extracts of dichloromethane/methanol (1:1) and methanol showed very close TLC profile and hence mixed together and dried. The crude extract (10 g) was adsorbed on 10 g of silica gel (mesh size 60-120) and subjected to silica gel column chromatography (170 g of silica gel) using increasing gradient of ethyl acetate in *n*-hexane and methanol in dichloromethane as eluent. A total of 145 fractions were collected and concentrated at 40 ^{o}C under reduced pressure. Fractions that showed similar R_f values and the same characteristic color on TLC were combined. Fractions from 29-31 afforded compound **64** with single spot on TLC (EtOAc/*n*-hexane, 1:1, R_f value of 0.44 (30 mg).

Similarly, acid-base crude extract (5 g) was adsorbed with 5 g of silica gel mesh size (60-120) and subjected to silica gel column chromatography (150 g of silica gel) using increasing gradient of ethyl acetate in *n*-hexane and methanol in dichloromethane as eluent. A total of 221 fractions (Table 3) were collected and concentrated at 40 ^{o}C under reduced pressure. Fractions that showed similar R_f values and the same characteristic color on TLC were combined. Fractions from 52-54 afforded single spot with similar R_f values (compound **65**) on TLC (EtOAc/*n*-hexane, 9:1, R_f value of 0.57 (25 mg). Fraction 84-87 afforded single spot with similar R_f values (compound **66**) on TLC (EtOAc/*n*-hexane, 8:2, R_f value of 0.41 (30 mg).

3.5.6. Solvent extraction of *Bersama abyssinica*

The fresh collected roots of *Bersama abyssinica* were washed and then dried under shade at room temperature and grounded into powder by grinding mills. Air-dried root powder was weighed (300 g) and extracted exhaustively with *n*-hexane for 48 hr at room temperature. The marc left was extracted with 1.5 L dichloromethane: methanol (1:1) soaked for 48 hr at room temperature. The mixture was filtered, and the filtrate was concentrated under reduced pressure at temperature of 40°C using rotary evaporator and afforded (25.68 g) (8.5%) brownish color crude extract. The marc left was further extracted with 1.5 L methanol soaked for 48 hr at room temperature. The mixture was filtered, and the filtrate was concentrated under reduced pressure a temperature of 40°C using rotary evaporator and afforded (30.8 g) (8.8%) brownish color crude extract.

3.5.7. Extraction and isolation of compounds from *Bersama abyssinica* crude extract

Air-dried root powder (300 g) was extracted exhaustively with dichloromethane/methanol (1:1) for 72 hr at room temperature. The marc left was further extracted with methanol (2 L) soaked for 72 hr at room temperature. The extracts were evaporated under reduced pressure at 40º C using Rotary evaporator to afford 23.68 g (7.89%) and 30.08 g (10.03%) crude extracts, respectively. The dichloromethane/methanol (1:1) crude extract (15 g) was adsorbed on 15 g silica gel and subjected to silica gel (160 g) column chromatography separation using increasing gradient of ethyl acetate in *n*-hexane followed by increasing gradient of methanol in dichloromethane as eluent. A total of 145 fractions were collected each concentrated under

reduced pressure to dryness. Fractions that showed similar R_f values and the same characteristic color on TLC were combined. Fraction 33-38 afforded single spot (*β*-sitosterol (**67,** 10.8 mg) derivative of *β*-stigmasterol (EtOAc/*n*-hexane 1:1 as eluent on TLC with R_f value of 0.64). Fraction 22-26 afforded single spot (7-hydroxy-*β*-sitosterol (**68,** 9.3 mg, EtOAc/*n*-hexane 3:7 as eluent on TLC with R_f value 0.85). Fractions 42-79 afforded 2-methyamino-lbutyric acid (**69**, 26.5 mg, EtOAc/*n*-hexane 8:2 as eluent on TLC with R_f value of 0.6) and Fractions 103-105 afforded compound **70** (16 mg, EtOAc/*n*-hexane 6:4 as eluent on TLC with R_f value of 0.54).

3.5.8. Solvent extraction of *Erythrina abyssinica*

The fresh collected root, stem and root bark of *Erythrina abyssinica* was washed and then dried under shade at room temperature and grounded into powder by grinding mills. Air-dried root powder was weighed (500 g) and extracted exhaustively with *n*-hexane for 72 hr at room temperature. The marc left was extracted with chloroform soaked for 72 hr at room temperature. The mixture was filtered, and concentrated under reduced pressure at temperature of 40 °C using rotary evaporator and afforded brown color crude extract. The marc left was further extracted with 2.5 L methanol soaked for 72 hr at room temperature. The mixture was filtered, and concentrated under reduced pressure at temperature of 40 °C using rotary evaporator and afforded brown color crude extract. Using the same solvent extraction procedure and methanol crude extract yield were obtained for roots and root bark, respectively.

3.5.9. Isolation of compounds from *Erythrina abyssinica* crude extract

Silica gel column chromatography isolation of *Erythrina abyssinica* crude extract using increasing gradient elution of ethyl acetate in n-hexane and dichloromethane in methanol as eluent is under progress. The crude dichloromethane/methanol (1:1) crude extract (10 g) was adsorbed on 10 g silica gel and subjected to silica gel (150 g) column chromatography separation. Elution was carried out with increasing gradient of ethyl acetate in *n*-hexane followed by increasing gradient of methanol in dichloromethane. A total of 100 fractions were collected each concentrated under reduced pressure to dryness. Fractions that showed similar R_f values and the same characteristic color on TLC were combined. Fraction 23-28 afforded single spot (compound **11**, 8 mg) (EtOAc/*n*-hexane 1:1 as eluent on TLC with R_f value of 0.54). Fraction 42-46 afforded single spot (compound **12**, 9.3 mg, EtOAc/*n*-hexane 3:7 as eluent on TLC with R_f

value 0.65). Fractions 72-78 afforded a single spot (compound **12**, 10 mg, EtOAc/*n*-hexane 6:4 as eluent with R_f value of 0.5).

3.6. Preliminary phytochemical screening of crude extracts

Phytochemical screening tests were done following standard procedures (Dyana and Kanchana, 2012; Iqbal *et al*., 2011).The crude extracts of the medicinal plants were screened to identify the presence or absence of secondary metabolites such as alkaloids, flavonoids, steroids, saponins, phytosterols, terpenoids, glycosides, tannins, phenols, anthraquinones and coumarins.

Detection of alkaloids

Extracts (0.2 g) dissolved individually in dilute Hydrochloric acid and filtered.

Dragendroff's test: Filtrate was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate confirms the presence of alkaloids.

Detection of glycosides

The extract (0.2 g) was hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test: The extracts (0.2 g) was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer confirms the presence of anthranol glycosides.

Detection of cardiac glycosides

Legal's Test

The extract (0.1 g) was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour confirms the presence of cardiac glycosides.

Detection of saponins

Froth Test: The extracts (0.1 g) were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam confirms the presence of saponins.

Detection of phytosterols

Salkowski's Test: The extracts (0. 1 g) was treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Detection of phenols

Ferric Chloride Test: The extracts (0.1 g) were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour confirms the presence of phenols.

Detection of tannins

Gelatin Test: To the extract (0.1 g), 1 % gelatin solution containing sodium chloride was added. Formation of white precipitate confirms the presence of tannins.

Detection of flavonoids

Alkaline Reagent Test: The extract (0.1 g) was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute acid, confirms the presence of flavonoids.

Detection of diterpenes

Copper acetate Test: The extract (0.1 g) was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour confirms the presence of diterpenes.

3.7. Structure elucidation of isolated compounds

The molecular structure elucidation of two isolated compounds from *E. schimperi* and three isolated compounds from *B. antidysenterica*, four compounds from *B. abyssinica* and two

compounds from *E. abyssinica* were fully characterized using spectroscopic techniques (UV-Vis, IR and NMR).

3.8. Bioassay analysis

Crude extracts and isolated compounds from four medicinal plants (*B. abyssinica, E. schimperi*, *E. abyssinica* and *B. antidysenterica*) were investigated for *in vitro* antibacterial assay using paper disc diffusion method against two Gram positive bacteria strains (*Staphylococcus aureus* and *Bacillus subtilis*), and two Gram negative bacteria (*Escherichia coli* and *Salmonella typhi*). The antioxidant assay of crude extracts and isolated compounds from *B. antidysenterica* were evaluated using DPPH radical scavenging assay. Molecular docking analysis of isolated compounds from *B. antidysenterica* was examined using autodoc vina version 4.2 on aromatase inhibitor protein domain target.

3.9. Data analysis

The NMR and FTIR data was analyzed using Mnova and origin software. The comparison of standard drugs and plant extract controls was analyzed by one-way ANOVA followed to analyze the effectiveness of the plant extracts against bacterial growth with different dosses.

3.10 Molecular docking analysis

Molecular docking studies were performed in order to predict the interaction of synthesized compounds with the binding sites of DNA-gyrase. Topoisomerases are interesting antibacterial drug targets, since bacteria express two isoforms, DNA gyrase and topoisomerase IV, which were both required for maintenance of proper DNA topology during transcription and replication (Duraipandiyan et al. 2014).

In this project, isolated compounds were subjected to molecular docking studies using the ADT version 1.5.2 and AutoDock version 4.2 docking program to investigate the potential binding mode. The crystal structure of the enzyme (PDB code 1KZN) with resolution 2.3 Å was chosen as the protein model. The structures of ligands were optimized using the HyperChem 7.0 software. Auto Dock version 4.2 was used to prepare the molecules and parameters before submitting it for docking analysis with Auto Dock. Polar hydrogen atoms were added while non-

polar hydrogen atoms will be merged and then, Gasteiger partial atomic charges were assigned to the ligands. All rotaTable bonds of ligands, defined by default of the program, was allowed to rotate during the automated docking process and then prepared protein and ligand structures were saved in the PDBQT format suiTable for calculating energy grid maps. A grid box size of 46×46×46 Å points with a grid spacing of 0.375 Å was considered. Lamarckian genetic algorithm (LGA) program with an adaptive whole method search in the Auto Dock was chosen to calculate the different ligand conformers. After 200 independent docking runs for each ligand, a cluster analysis was done. In according to the root mean square deviation (RMSD) tolerance of 2.0 Å conformations were clustered and ranked by energy of which the conformation with the best scored pose with the lowest binding energy will be selected for these ligands (Mansourian et al., 2015; Morris et al., 1998).

4. Results and discussion

4.1. Yield of crude extracts

The four medicinal plants crude extracts yield is reported in Table 1.

Table 1: Yield of crude extracts

S.No	medicinal plant	part of the plant	system of Extractions	sample taken in (gm)	actual yield in(gm)	percentage yield (%)
1	*E. schimperi*	Root bark	MeOH	300	60.5	20.2
2	*B. antidysenterica*	Root	DCM:MeOH (1:1)	350	12.77	3.65
	B. antidysenterica	Root	MeOH	350	9.4	2.68
	B. antidysenterica	Root	Acid-Base	350	5.1	1.46
3	*B. abyssinica*	Root bark	DCM:MeOH (1:1)	300	25.68	8.57
	B. abyssinica	Root bark	MeOH	300	30.1	8.56
4	*E.abyssinica*	Stem bark	MeOH	500	10.77	2.2
		Root bark	MeOH	500	8.5	1.7
		Root	MeOH	500	6.1	1.2

As per the results found, methanol extracts of *E. schimperi* and *B. abyssinica* afforded better yields (20.2 and 8.56 %, respectively) whereas DCM/MeOH (1:1) extracts of *B. antidysenterica, B. abyssinica* and *E. abyssinica* afforded better yields (3.65, 8.57 and 2.2%, respectively).

4.2. Phytochemical screening test

The phytochemical screening results showed absence of terpenoids, saponins, and phytosterols in methanol root bark and stem bark extracts of *E. abyssinica* and *E. schimperi*, respectively, whereas flavonoids, phenols, alkaloids, tannins, steroids and glycosides are present in all crude extracts (Table 2).

Table 2: phytochemical screening result of the crude extracts

Secondary metabolites	**Test**	***B. abyssinica* Root bark**		***B. antidysenterica* root**		***E.schimperia* root bark**	***E.abyssinica* Stem bark**	
		DCM:MeOH (1:1)	MeOH	DCM:MeOH (1:1)	MeOH	MeOH	$CHCl_3$	MeOH
Flavonoids	Alkaline	+	+	+	+	+	+	+
Saponnins	Froth	+	-	+	+	+	+	+
Phenols	Ferric chloride	+	+	+	+	+	+	+
Tannins	Gelatin	+	+	+	+	+	+	+
Terpenoids	Salkowski's	+	+	+	+	-	-	-
Steroids		+	+	+	+	+	+	+
Phytosterols	Salkowski's	+	+	+	+	_	_	_
Glycosides	Modified Borntrager's	+	+	+	+	+	+	+
Anthraquinones Glycosides	Borntrager's	+	+			+	+	+
Alkaloids	Mayer's test	+	+	+	+	+	+	+

+ indicates presence; − indicates absence.

4.3. Characterization of Isolated Compounds from *E. schimperi*

4.3.1. Characterization of compound 62

Compound **62** was obtained as a white powder isolated from MeOH extract with R_f value of 0.83 in dichloromethane/methanol (7:3). The IR (KBr disk) spectrum showed broad vibration at 3434 cm^{-1}attributed to hydroxyl moiety (OH), sharp absorption at 1631 cm^{-1} attributed to aromatic benzene ring, strong absorption band at 2900 cm^{-1}due to C-H stretching of saturated moiety, and absorption band at 1145 cm^{-1} due to C-O stretching.

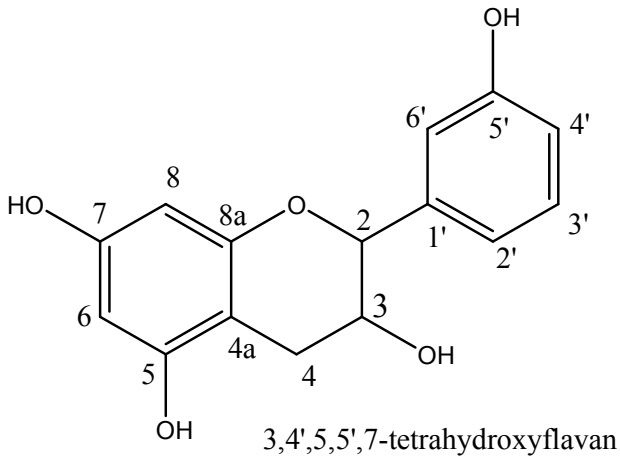
The ^{1}H-NMR (400 MHz, DMSO-d_6, Table 3) spectrum revealed the presence of proton signals at δ 5.91 (1H, d, J= 1.2 1Hz, H-6) and 5.88 (1H, d, J=2 1 Hz,) suggest the presence of two meta coupled aromatic protons that belong to a tetrasubstituted phenyl ring A. The presence of signals with ABX multiplicity pattern at δ 6.92 (1H, d, J=1.2Hz, H-2',6′) and δ 6.74 (1H, dd, J= 2.1, 8.1 Hz, H =3′,5′) suggest a trisubstituted phenyl ring B. Signals at δ 4.76 (1H,m,H 2) suggest the presence of proton and 4.12 (2H, d, J=2.1 Hz, H-8) suggest the presence of one oxygenated methine whereas signals a peak at δ 2.76 (2H, d, J= 16.5, 4.5 Hz, H-4) suggest the presence of diasterotopic methylene protons at C-4. The above ^{1}H-NMR pattern suggests that the compound has flavan skeleton with two aromatic protons (H-6 and 8) on ring A and four aromatic protons (H-2′,3'5′, and 6′) on ring B and devoid of the hydroxyl group at C-4 of ring C.

In agreement with the ^{1}H-NMR, the ^{13}C-NMR spectrum (Table 6) revealed a total of fifteen carbon signals. The presence of one oxygenated sp2 quaternary carbons was observed at δ 144.5 (C-4′), suggesting the vicinal substitution pattern on ring C, in agreement with the ABX multiplicity pattern, whereas the methines appear at δ 118.4 (C-6′) and δ 114.9 (C-3' 5′) and δ 113.7 (C-2'). The presence of two sp^2 oxygenated quaternary carbons at δ 156.4 (C-5) and δ 156 (C-7) along with two unfiled carbons chemical shifts at δ 95.6 (C-6) and δ 95 (C-8) suggest that ring A has 5, 7-dioxygenated substitution pattern. The following quaternary carbons are also clearly evident from ^{13}C-NMR spectrum: δ 98.7 (C-4a), δ 130.4 (C-1′), and 155.7 (C-8a). Signals at δ 78.3 (C-2) and δ 66.2 (C-3) are clearly evident due to the presence of sp3 oxygenated methines C-2 and C-3 of ring C. Moreover, the presence of one methylene (also supported by

DEPT-135 pointing down) observed at δ 27.8 (C-4) and is in good agreement with spectral data, and the structure of the compound has flavan skeleton.

Table 3: ^{1}H-NMR, ^{13}CNMR and DEPT-135 spectral data of compound **62** in $CDCl_3$ and DMSO-*d*6

Position	δ_H (ppm)	δ_C (ppm)	DEPT-135	^{13}C-NMR Babe et al., 2018
2	4.76 (1H,m, H-2)	78.3	CH	78.5
3	4.12 (2H,d,j=2.1Hz, H-8)	66.2	CH	65.4
4	2.76 (2H, dJ= 16.5, 4.5 Hz, H-4)	27.8	CH_2	28.7
4a	-	98.7	CH	99.0
5	-	156.4	CH	156.9
6	5.91 (1H, d, J= 2.1 Hz)	95.6	CH	95.6
7	-	156.0	quaternary	156.7
8	5.88 (1H, d, J= 2.1 Hz)	95	CH	94.6
8a	-	155.7	quaternary	156.2
1’	-	130.4	quaternary	131.1
2’	6.92 (d, J= 1.2, Hz, H-2’, 6’)	114.9	CH	-
3’	6.74 (2H, dd, J=8.1 Hz, H-3’,5’)	118.4	CH	118.4
4’	6.74 (2H, dd, J= 8.1, Hz, H-3’)	113.7	CH	-
5’	-	144.5	Quaternary	144.7
6’	6.92 (d, J=1.2 Hz, H-2’, 6’)	113.7	CH	-



3,4',5,5',7-tetrahydroxyflavan

Figure 7: Proposed structure of compound **62**

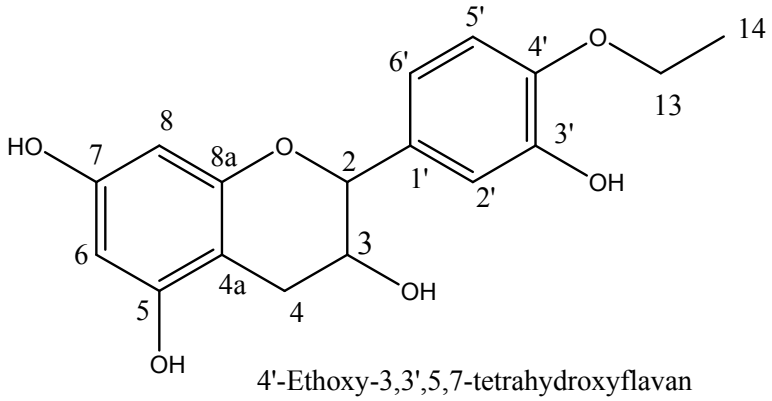
4.3.2. Characterization of compound 63

Compound **63** was obtained as a yellow powered isolated from MeOH extract with R_f value of 0.75 in ethylacetate/methanol (9:1).The IR (KBr disk) spectrum showed broad vibration at 3425 cm^{-1} attributed to hydroxyl moiety (OH), sharp absorption at 1630 cm^{-1}attributed to aromatic benzene ring, strong absorption band at 2926 cm^{-1}due to C-H stretching of saturated moiety, and absorption band at 1148 cm^{-1} due to C-O stretching.

The ^{1}H NMR (400 MHz, DMSO-d_6) spectrum (Table 4) revealed the presence of proton signals at δ 5.95 (1H, d, *J* = 2.1Hz, H-6) and 5.96 (1H, d, *J* = 2.1 Hz, H-8) suggest the presence of two meta coupled aromatic protons that belong to a tetra substituted phenyl ring. The presence of signals with ABX multiplicity pattern at δ 6.8 (d, *J* = 8.0, Hz, H-6') and 6.98 (dd, *J* = 2.1, 8.0 Hz, H-2', 5') suggest a tri substituted phenyl ring. Signals at δ 4.82 (1H, m, H-2,) and 4.28 (2H, d, J=2.1 Hz, H-3) suggest the presence of two oxygenated methine. Whereas signals peak at δ 2.77 (2H, d, *J* = 16.5, 4.5 Hz, H-4) suggest the presence of diasterotopic methylene protons. The above ^{1}H NMR pattern suggest that the compound have flavan skeleton with two aromatic protons (H-6, 8) on ring A and three aromatic protons (H-2', 5', 6') in ring B and devoid of carbonyl group at C-4 of ring C (also supported by ^{13}C NMR).The ^{13}C NMR spectrum revealed a total of seventeen carbon signals. Two oxygenated sp^2 quaternary carbons at δ 144.3 (C-3') and 144.4 (C-4') suggesting vicinal substitution pattern in agreement with the ABX multiplicity pattern of ring C where the methines appear at δ 118.4 (C-6') and δ 115.0 (C-2', 5'). In addition, the presence of two sp^2 oxygenated quaternary carbons at δ 156.4 (C-5) and δ 156.0 (C-7) along with two unfiled carbons chemical shifts i.e. δ 95.6 (C-6) and δ 95.0 (C-8) suggest that ring A have 5,7-dioxygenated substitution pattern. The following quaternary carbons are also clearly evident at δ 98.7 (C-4a), δ 130.6 (C-1') and δ 155.7 (8a). Signals at δ 78.4 (C-2) and δ 66.2 (C-3) are clearly evident due to the presence of sp^3 oxygenated methines C-2 and C-3 of ring C. Moreover, the presence of one methylene (also supported by DEPT-135 pointing down) at δ 27.9 (C-4) is in good agreement with spectral data of the compound with flavan skeleton.

Table 4: ^{1}H NMR, ^{13}C NMR and DEPT-135 spectral data of compound **63** in $CDCl_3$ and DMSO-d_6

Position	δ_H (ppm)	δ_C (ppm)	DEPT- 135	δ_C (ppm) Babe et al., 2018
1’	--	130.6	quaternary	131.1
2’	6.98(dd, J=8.0, 2.1Hz)	113.8	-	115.2
3’	-	144.3	CH	144.6
4’	-	144.4	quaternary	144.7
5’	6.79 (d, J=8.0Hz)	115	CH	115.4
6’	6.8(d, J=8.0Hz)	118.4	CH	118.4
2	4.82 (1H, m, H-2)	78.4	CH	78.5
3	4.28 (2H, d, J=2.1Hz,H-8)	66.2	CH	65.4
4	2.77 (2H, d, j=16.5,4.5,Hz,H-4	27.9	CH_2	28.7
5	-	156.4	quaternary	156.9
6	5.95 (1H,d, J=2.1Hz)	95.6	CH	95.6
7	-	156	quaternary	156.7
8	5.96 (1H, d, J=2.1Hz)	95	CH	94.6
4a	-	98.7	-	99.0
8a	-	155.7	Quaternary	156.2
13	3.4 (2H, q)	71.9	CH_2	-
14	1.24 (3H, t)	18.9	CH_3	-



4'-Ethoxy-3,3',5,7-tetrahydroxyflavan

Figure 8: The proposed structure of compound **63**

4.3.4. Characterization of isolated compounds from *B. antidysenterica*

4.3.4.1. Phytochemical Screening. Phytochemical screening test of dichloromethane/methanol (1: 1) and methanol roots extracts revealed the presence of alkaloids, flavonoids, phytosterols, phenols, steroids, tannins, terpenoids, glycosides and saponins (Table 5).

Table 5. Phytochemical screening tests of crude extracts

Phytochemical screening	Test	DCM: MeOH (1:1)	MeOH 100%
Flavonoids	Alkaline test	+	+
Saponins	Froth test	+	+
Phenols	Ferric chloride test	+	+
Tannins	Gelatin test	+	+
Terpenoids	Salkowski's test	+	+
Steroids		+	+
Phytosterols	Salkowski's test	+	+
Glycosides	Modified Borntrager'stest	+	+
Alkaloids		+	+

4.3.4.2. Characterization of compounds

Compound **64** was obtained as a brown solid (mp: 155°C) with an R_f value of 0.44 (*n*-hexane/EtOAc (1:1) as eluent). A positive mayer's reagent was observed suggesting that the compound was an alkaloid. The IR (KBr disk) spectrum showed broad and sharp vibrations at 3441.92 and 1744 cm^{-1} attributed to hydroxyl (OH) and carbonyl moiety, respectively. The absorption bands at 1637, 1462 and 1230.05 cm^{-1} suggest the presence of aromatic system (C=C), C=N and C-O stretching vibrations, respectively. The presence of symmetric and asymmetric C-H stretching vibrations are clearly evident from vibrations at 2854.95 and 2927.92 cm^{-1}, respectively.

The ^{1}H NMR (400 MHz, $CDCl_3$, Table 6) spectrum revealed the presence of proton signals at δ_H 8.82 (*d*, H-13, *d, J*=12), 7.98 (*d,* H-1, *J*=4), 8.66 (*d*, H-4, *J*= 12), 7.00(*d*, H-4' *J*=8), 8.09(*d*, H-3'), 7.55 (H-6, *t*) and 7.72 (H-7, *t*). The presence of doublet of doublet peak was observed at δ_H 8.09 (H-3') suggest allylic and ortho coupled with δ_H 7.98 (H-1) and δ_H 7.00 (H-4'). The ^{13}C NMR and DEPT-135 spectrum revealed a total of 20 well resolved carbon peaks. The presence of eleven aromatic carbon groups, one methine group (C-6'), one methyl and six quaternary carbon atoms (of which four of them are aromatic carbon) are all evident. The existence of carboxylic acid carbonyl carbon at δc173.5 (C-9) and sp^2 oxygenated quaternary carbon at δc 159.5 (C-2') are clearly marked (Table 6). The peaks at δc 67.8 and 62.3 suggest oxygenated methylene and methyl signals forming CH_2-O-CH_3 moiety, also supported by DEPT-135

spectrum, where the former peak is pointing down whereas the later pointing up. Peaks at δc 139.2 and 145.5 suggest olefinic methine near to heteroatom, preferably next to nitrogen, supported by DEPT-135 spectrum. The above spectral data and comparison with literature the compound was found to be an indole alkaloid with trivial name of flazin methyl ether derivative (**64**, fig 9) previously isolated from juice of *Ribes nigrum* L.(Blech and Budzikiewicz, 1994). However, this is the first report from roots of *B. antidysentrica*.

Table 6. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectral data of flazin methyl ether derivative (**64**) in $CDCl_3$.

Position	Compound **64**			Flazin methyl ether (Blech and Budzikiewicz, 1994)	
	^{1}H	**^{13}C**	**DEPT-135**	**^{1}H**	**^{13}C**
1	7.98 (1H, *d*, *J*=4)	145.5	145.5	-	137.1
3	-	130.7	-	-	132.2
4	8.67 (1H, *d*, *J*= 4)	117.3	117.34	8.85 (1H, *s*)	115.9
5	7.98 (1H, *d, J*=8)	127.9	127.9	8.41 (1H, *d, J*=7.8)	122.0
6	7.55(*t*)	128.1	128.1	7.34 (1H,*dd,J*=7.8/8.2)	120.5
7	7.72 (*t*)	129.1	129.1	7.65 (1H,*dd,J*=7.8/8.2)	128.9
8	7.00 (1H,*d*, *J*=8)	116.5	116.5	7.82 (1H, *d, J*=8.2)	112.9
10		139.6	-	-	141.5
11		124.3	-	-	120.9
12		127.10	-	-	129.9
13	8.82 (1H, *d, J*=4)	139.2	139.2	-	132.0
2’		159.5	-	-	151.7
3’	8.09 (1H, *dd, J*=8, 4)	116.5	116.5	7.45 (1H, *d, J*=3.4)	111.1
4’	7.00 (1H, *d, J*=8)	116.5	116.5	6.77 (1H,*dd, J*=3.4)	111.9
5’	-	159.5	-		153
CO	-	173.5	-		166.5
CH_2	3.96 (2H, *s*)	68.7	68.7	4.64 (2H, *s*)	65.5
CH_3	3.31 (3H,*s*)	62.3	62.3	3.34 (3H, *s*)	57.2

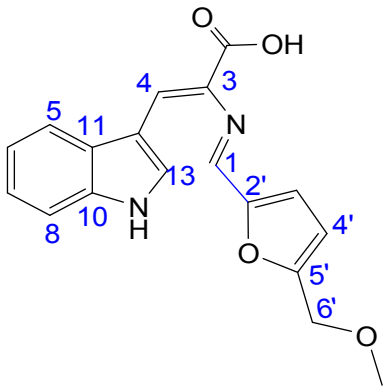


Fig. 9. Structure of derivative of flazin methyl ether (**64**)

Compound **65** was isolated as yellowish amorphous solid with melting point of 149 ^{o}C and R_f value of 0.57 (80% EtOAc in *n*-hexane as eluent). A positive mayer's reagent was observed suggesting that the compound was an alkaloid. The IR (KBR) spectrum of the compound showed a broad vibration at 3451.87 cm^{-1}, sharp vibrations at 2924 cm^{-1} and 2854.54 cm^{-1}, 1637 cm^{-1}, and 1400 cm^{-1} attributed to amine group (N-H), sp^3 H-C, sp^2 C-H, imine bond C=N and C=C bonds, respectively.

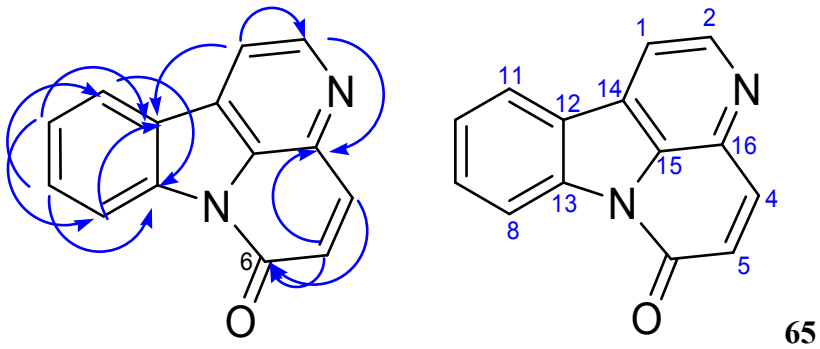
The ^{1}H NMR spectrum showed peaks at δ 8.07 (H-4, *d*, *J*=7.8), 7.54 (H-10, *t*, *J*=8), 7.72 (H-9, *t*, *J*=8) and 8.82 (H-2, *d*, *J*=8), 7.98 (H-1, *d*, *J*=8), 7.00 (H-5, *d*, *J*=8) and 8.11 (H-11, *d*, *J*=8). The ^{13}C NMR and DEPT-135 spectrum of showed the presence of fourteen carbon signals including aromatic peaks between δ_C 116.5-159.5, of which six of them are sp^2 quaternary at δ 159.5 (C-6), 139.5 (C-13), 136,09 (C-16), 132.06 (C-15) , 130.5 (C-14) and 124.36 (C-12). Upfeild chemical shift of carbonyl carbon at δ 159.5 (C-6) suggest that the carbonyl group is directly linked to nitrogen of indole moiety and it is also α,β-conjugated (δ 139.4 (C-4) and 129.0, C-5) suggesting that the compound have β-carboline alkaloid canthine-6-one skeleton.

The COSY spectrum supported correlations between H1↔H2, H4↔H5, H8↔H9, H9↔H10 and H10↔H11 (Table 7). The HSQC spectrum suggested direct connectivity between H1→C1, H2→C2, H4→C4, H5→C5, H8→C8, H9→C9, H10→C10, and H11→C11 (Table 7). The HMBC spectrum showed correlations between H1→C2,9,12; H2→C1,15,16; H4→C6,9;

H5→C-6,16; H8→C12; H9→C11,13; H10→C8,12 and H11→C-3,15 (Table 7) in good agreement with canthine-6-one alkaloids. Thus, based on the above spectral data and comparison with literature (Ouyang et al., 1994), compound **65** was suggested to be identical with canthin-6-one (fig 10) previously isolated from roots of *Brucea mollis* (Ouyang et al., 1994).

Table 7. ^{1}H, ^{13}C, DEPT-135, COSY, HSQC and HMBC spectral data of canthin-6-one (**65**, Fig 10)

Position	**65**						Ouyang et al., 1994	
	^{1}H	^{13}C	DEPT-135	COSY	HSQC	HMBC	^{1}H	^{13}C
1	7.98 (1H, *d, J*=8)	116.4	116.5	$H_1 \leftrightarrow H_2$	$H_1 \rightarrow C_1$	$H_1 \rightarrow C_{2,12,15}$	7.59(1H, *d, J*=5)	115.4
2	8.82 (1H, *d, J*=5)	145.7	145. 7		$H_2 \rightarrow C_2$	$H_2 \rightarrow C_{1,14,16}$	8.58(1H, *d, J*=5)	144.8
4	8.07 (1H, *d, J*=8)	139.4	139.4	$H_4 \leftrightarrow H_5$	$H_4 \rightarrow C_4$	$H_4 \rightarrow C_{6,15,16}$	7.77(1H, *d, J*=9.7)	138.6
5	7.00 (1H, *d, J*=8)	129.0	129.0		$H_5 \rightarrow C_5$	$H_5 \rightarrow C_{6,16}$	6.75(1H, *d, J*=9.7)	127.9
6	-	159.5						158.2
8	8.69 (1H, *dd, J*=8,4)	117.3	117.3	$H_8 \leftrightarrow H_9$	$H_8 \rightarrow C_8$	$H_8 \rightarrow C_{12}$	8.28(1H, *d, J*=7.7)	116.3
9	7.72 (1H, *t*)	130.9	130.9	$H_9 \leftrightarrow H_{10}$	$H_9 \rightarrow C_9$	$H_9 \rightarrow C_{13}$	7.45(1H, *t*)	129.8
10	7.54 (1H, *t*)	125.7	125.7	$H_{10} \leftrightarrow H_{11}$	$H_{10} \rightarrow C_{10}$	$H_{10} \rightarrow C_{8,12}$	7.28(lH, *t*)	124.7
11	8.11 (1H, *dd, J*=8,4)	122.7	122.7		$H_{11} \rightarrow C_{11}$	$H_{11} \rightarrow C_{13,14}$	7.73(1H, *d, J*=7.7)	121.6
12	-	124.4						123.3
13	-	139.5						138.2
14	-	130.5						128.9
15	-	132.1						130.9
16	-	136.1						135.2



65

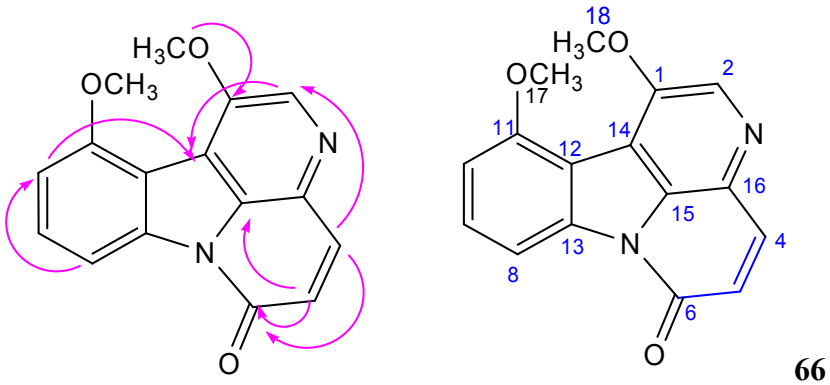
Figure 10. Structure of canthine-6-one (**65**)

Compound **66** was isolated as yellowish amorphous solid (mp 180-182 ^{o}C) and R_f value of 0.41 (20 % ethyl acetate in *n*-hexane as eluent). The IR spectrum of compound showed the presence of amine group (N-H) at 3400 cm^{-1}, C-H band at 2924 cm^{-1}, C=O band at 1744 cm^{1}, C=C and C=N at 1644 cm^{-1} and C-N at 1398 cm^{-1}. The ^{1}H NMR spectrum (Table 8) revealed the presence of two three-proton singlet at δ 4.08 (3H) and 4.23 (3H) attributed to methoxy protons, the presence of aromatic protons were observed at δ 8.5 (*s*, H-1), 8.38 (*d*, J=8, H-8), 7.97 (*d*, *J*=8, H-4), 7.65 (*t*, *J*=8, H-9), 7.01 (*d*, *J*=8, H-5) and 6.86 (*d*, *J*=12, H-10). The ^{13}C NMR and DEPT-135 spectrum (Table 8) showed two methoxy carbon atom signals appearing at δ 57.7 and 56.2. The presences of eleven aromatic peaks were observed at δ 160.4, 155.9, 151.3, 140.1, 139.2, 132.1, 132.9, 131.6, 130.5, 113.1, 109.8 and 107.9.

In agreement with the spectral data of canthine-6-one (**65**), upfeild chemical shift of carbonyl carbon at δ_C 160.4 (C-6) suggest that the carbonyl group is directly linked to nitrogen of indole moiety and it is also α,β-conjugated (δ 139.15 (C-4) and 125.1 C-5) suggesting that the compound have β-carboline alkaloid canthine-6-one skeleton. Close inspection of the 2D NMR spectra (Table 8) showed the following correlations. COSY spectrum showed correlations between aromatic protons appearing at δ 7.97 (H-4) and δ 6.86 (H-5), δ 8.38 (H-8) and δ 7.65 (H-9), δ 7.65 (H-9) and δ 7.01 (H-10). HSQC spectrum showed ^{1}J correlations between aromatic protons and their respective carbons H2→C2, H4→C4, H5→C5, H8→C8, H9→C9, H10→C10, and methoxy groups (H17→C17 and H18→C18). HMBC spectrum (Table 8) showed correlations between H2→C1,11,14; H5→C6,9,16; H8→C10,12; H10→C11,8,12. The HMBC spectrum also indicated correlations of the methoxy protons at δ 4.23 (H-17) and 4.08 (H-18) with C-11 and C-1, respectively, confirming their location at C-1, 11 of the canthine-6-one skeleton. Thus, based on the above spectral data and extensive comparison with literature, the compound was identified as 1,11-dimethoxycanthin-6-one (**66**, Fig 11), previously reported from stem of *B. antidysentrica* (Narihiko *et al*., 1986).

Table 8. ^{1}H, ^{13}C, DEPT-135, COSY, HSQC and HMBC NMR spectral data of 1,11-dimethoxychan tin-6-one (**66**).

Position	compound **66**			COSY	HSQC	HMBC	1,11-dimethoxychan tin-6-one (Narihiko *et al*., 1986)	
	^{1}H	^{13}C	DEPT-135				^{1}H	^{13}C
1	-	155.9	-				-	155.6
2	8.5 (1H, *s*)	132.1	132.05		$H_2 \rightarrow C_2$	$H_2 \rightarrow C_{1,9,11,14}$	8.41 (1H, *s*)	131.3
4	7.97 (1H,*d*,*J*=8)	139.15	-	$H_4 \leftrightarrow H_5$	$H_4 \rightarrow C_4$		7.91 (1H, *d*, *J*=10)	139.0
5	6.86 (1H,*d*,*J*=8)	131.6	131.60		$H_5 \rightarrow C_5$	$H_5 \rightarrow C_{6,16}$	6.79 (1H,*d*,*J*=10)	131.8
6	-	160.4	-				-	160.2
8	8.38 (1H,*dd*,*J*=8,4)	109.8	109.78	$H_8 \leftrightarrow H_9$	$H_8 \rightarrow C_8$	$H_8 \rightarrow C_{10,12}$	8.29 (1H,*d*,*J*=8)	109.5
9	7.65 (1H,*t*, *J*=8)	125.1	125.06	$H_9 \leftrightarrow H_{10}$	$H_9 \rightarrow C_9$	$H_9 \rightarrow C_{11,13}$	7.54 (1H,*t*, *J*=8)	124.8
10	7.01 (1H,*dd*, *J*=8,4)	107.9	107.86		$H_{10} \rightarrow C_{10}$	$H_{10} \rightarrow C_{12,8}$	6.86 (1H,*d*,*J*=8)	107.5
11	-	151.3	-				-	151.0
12	-	113.1	-				-	116.6
13	-	132.9					-	132.6
14	-	113.1	-				-	112.8
15	-	130.5	-				-	130.2
16	-	140.1			$H_2 \rightarrow C_2$	$H_2 \rightarrow C_{1,14,16}$	-	139.8
OCH_3	4.23 (3H,s)	57.7	57.69			$H_{17} \rightarrow C_{11}$	4.16 (3H,s)	57.4
OCH_3	4.08 (3H,s)	56.2	56.22			$H_{18} \rightarrow C_1$	4.0 (3H,s)	56.0



66

Figure 11. Structure of 1,11-dimethoxycanthin-6-one (**66**)

4.3.4.3. Antibacterial activity

The antibacterial activity of the crude extracts and alkaloids (**3-5**) were examined at a concentration of 0.5 mg/mL against four pathogenic bacterial strains two Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) and two Gram-negative (*Escherichia coli* and *Salmonella thphimurium*). Antibacterial potential of crude extracts and alkaloids (**64-66**) were assessed in terms of zone of inhibition of bacterial growth (Table 9). The results revealed that the isolated compounds showed promising antibacterial activity against *S. aureus, B. subtilis, E. coli* and *S. thphimurium.* Canthine-6-one (**64**) showed moderate activity (12.66±0.6 and 12.33±2.31) against *E. coli* and *S. thphimurium*, respectively, compared to ciprofloxacin (27.3±2.52 and 29±1.00). 1,11-dimethoxycanthin-6-one (**65**) showed moderate antibacterial activity (12.5±0.87 and 12.3±1.65 mm) against *S. thphimurium* and *B. subtilis* compared to ciprofloxacin (29±1.00 and 33.3±3.22 mm). The DCM:MeOH (1:1) crude, acid-base crude extract and derivative of flazin methyl ether (**64**) showed moderate antibacterial activity against *S. aureus*, *E. coil, S. thphimurium* and *B. subtilis*, respectively.

Table 9. Zone of bacteria growth inhibition diameter (mm)

Sample (0.5mg/mL)	Inhibition diameter (mm) ± SD			
	E. coli	*S. thphimurium*	*B. subtilis*	*S. aureus*
Methanol/dichloromethane (1:1) extract	11.6±1.53	10.66± 1.15	11.33±1.15	9.33±0.58
Acid-base extract	11.33±2.08	11.0±1.73	12.0±3.74	11.33±0.58
Derivative of flazin methyl ether (**64**)	11.33±0.58	11.66±3.06	11.33±1.53	11.0±1.00
Canthin-6-one (**65**)	12.66±0.60	12.33±2.31	11.0±0.707	9.6±1.16
1,11-dimethoxycanthin-6-one (**66**)	11.3±0.58	12.5±0.87	12.3±1.65	10±1.00
Ciprofloxacin	27.3±2.52	29±1.00	33.6±3.22	23.5±0.58

SD- Standard deviation

4.3.4.4 Antioxidant activity

In the DPPH scavenging assay, crude extracts and alkaloids (**64-66**) of *B. antidysentrica* were investigated through the free radical scavenging activity via their reaction with the sTable DPPH radicals. The reduction of the DPPH was followed via the decrease in absorbance at 517 nm. The DPPH radical scavenging activities (in %) of extracts and isolated compounds were found to be 87.5 (canthine-6-one, **64**), 85.3 (flazin methyl ether derivative, **65**), 83.3 (DCM: methanol (1:1) crude extract), 80.00 (acid-base crude extract), 78.4 (1,11-dimethoxycanthin-6-one, **66**)

respectively at 100µg/ml (Table 10). It was observed that the DPPH scavenging activity increased with increasing concentration of the samples. For the various concentrations, canthine-6-one (**65**) exhibited the highest percent inhibition of the DPPH compared to ascorbic acid which showed maximum scavenging effect at very low concentration (Figure 12).

Table 10. % of scavenging activity of the extracts and isolated compounds of *B. antidysentrica*

C (µg/mL)	Samples									
	DCM:MeOH(1:1)extract		Acid-base extract		**64**		**65**		**66**	
	A	% scavenging activity	A	% scavenging activity	A	% scavenging activity	A	% scavenging activity	A	% scavenging activity
100	0.2	83.3	0.26	80	0.15	85.3	0.1	87.5	0.28	78.4
50	0.3	75	0.27	79.2	0.23	77.4	0.2	83.3	0.31	76.1
25	0.35	70.8	0.3	76.9	0.3	70.5	0.3	75	0.34	73.8
12	0.4	66.6	0.4	69.2	0.4	60.7	0.38	68.3	0.45	65.4

A= absorbance N.B: the experiment was done in triplicate

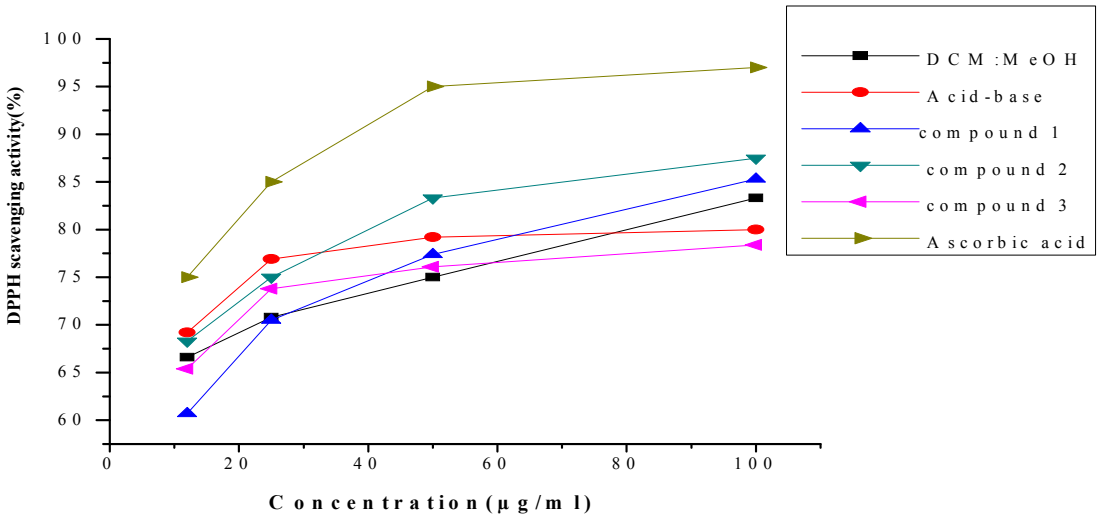


Figure 12. DPPH scavenging activity (%) of *B. antidysentrica* extracts, isolated compounds and positive reference (L-Ascorbic acid)

4.3.4.5 Molecular docking analysis

In association with *in vitro* antibacterial activity, molecular docking studies were performed for three alkaloids in order to predict the orientation and binding affinity of ligand molecules at the active site of receptor with bacterial enzyme DNA gyrase. In docking study, all the compounds

were found to have minimum binding energy ranging from -5.8 to -4.8 KJ/mol (Table 11). On comparative basis alkaloids (**64-66**) revealed minimum binding energy of -5.8, -5.7 and -5.7 kcal/mol, respectively (Table 11). These molecules were unwrapped by active site amino acid residues at the active site pocket region (as shown in Figure 13a-c). The protein (DNA-gyrase) comprises of fourteen active site residues, which are promiscuous to the ligands. Out of which only two (PHE198 and ALA 51) residue is directly interacting with the ligands. The other residues are in close proximity to the inhibitor are hydrophobic in nature. Derivative of flazin methyl ether (**64**) was found to show hydrogen bond interaction with active site amino acid residue PHE196 at a distance of (1.5A^{o}) and 1,11-dimethoxycanthin-6-one (**66**) was found to show hydrogen bond interaction with active site amino acid residue ALA 51 at a distance of (1.5A^{o}), respectively. From the Figure yellow dashed line indicates a possible hydrogen bond formed between the connections residues (Figure 13a-c) and red color also indicated the hydrogen bond interaction (Figure 14a-c). Canthin-6-one (**65**) had no hydrogen bond interaction with the protein. Derivative of flazin methyl ether (**64**) showed two hydrophobic interactions with TRY 218 and GLU 219, Canthin-6-one (**65**) showed six hydrophobic interactions with VAL201, LEU 52 LYS57, GLY200 ASN198 and ALA52 and 1,11-dimethoxycanthin-6-one (**66**) showed four hydrophobic interactions with ASN 198, LEU52, VAL201 and LYS57. All ligands displayed no pi to pi and pi to cation interactions with the protein. DNA gyrase is an essential bacterial enzyme that catalyzes the introduction of negative (-) supercoils into chromosomal and plasmid DNA. Gyrase was discovered soon after it was clear that in vitro recombination of bacteriophage λ required a negatively supercoiled DNA substrate. DNA gyrase cleave and relegate DNA to regulate DNA topology and are a major class of antibacterial drug targets [21]. The docking of alkaloids (**64-66**) with DNA gyrase showed higher binding affinity as well as hydrogen bonding and good hydrophobic interaction with the receptor. Thus, from this study it can be concluded that the compounds **64-66** may act as potential inhibitors by hindering the function of DNA gyrase enzyme.

Table 11. Docking score of DNA gyrase protein and alkaloids (**64-66**)

Mode	Derivative of flazin methyl ether (**64**)			Canthin-6-one (**65**)			1,11-dimethoxycanthin-6-one (**66**)		
	Affinity (Kcal/mol)	dist from best mode		Affinity (Kcal/mol)	dist from best mode		Affinity (Kcal/mol)	dist from best mode	
		rmsd i.b.	rmsd u.b.		rmsd i.b.	rmsd u.b.		rmsd i.b.	rmsd u.b.

1	-5.8	0.000	0.000	-5.7	0.000	0.000	-5.7	0.000	0.000
2	-5.5	8.225	10.733	-5.7	22.422	25.040	-5.3	1.873	4.233
3	-5.5	1.802	5.669	-5.6	1.130	3.936	5.2	2.391	4.508
4	-5.4	8.135	11.181	-5.5	12.175	13.368	-5.0	15.021	16.977
5	-5.3	2.110	3.485	-5.3	22.536	25.215	-5.0	15.786	17.759
6	-5.3	7.920	11.682	-5.2	14.292	15.937	-4.9	16.498	18.132
7	-5.3	23.777	26.168	-5.2	14.977	16.780	-4.8	16.396	18.263
8	-5.1	7.742	10.427	-5.2	14.559	0.000	-4.8	13.336	15.474
9	-5.1	8.762	11.879	-5.1	14.289	25.040	-4.8	14.126	16.244

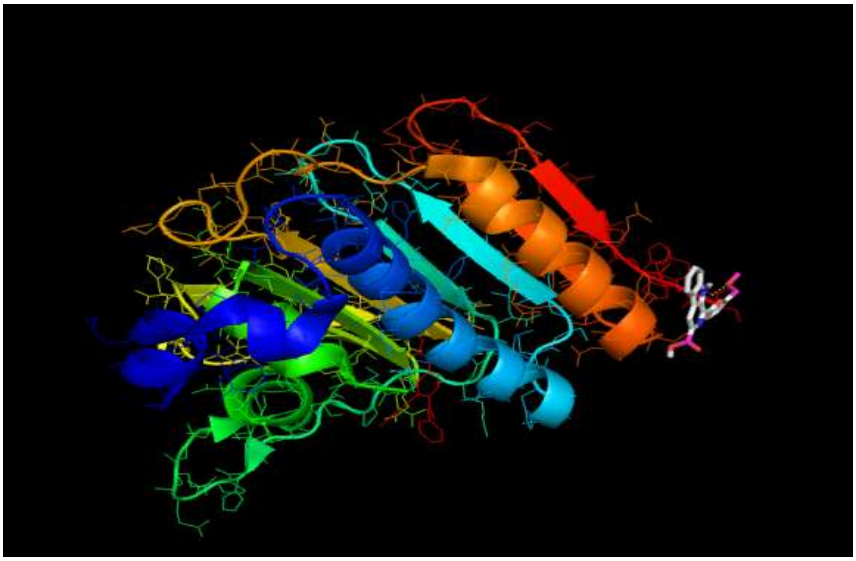


Figure 13a. Molecular docking of derivative of flazin methyl ether (**64**) with DNA gyrase receptor

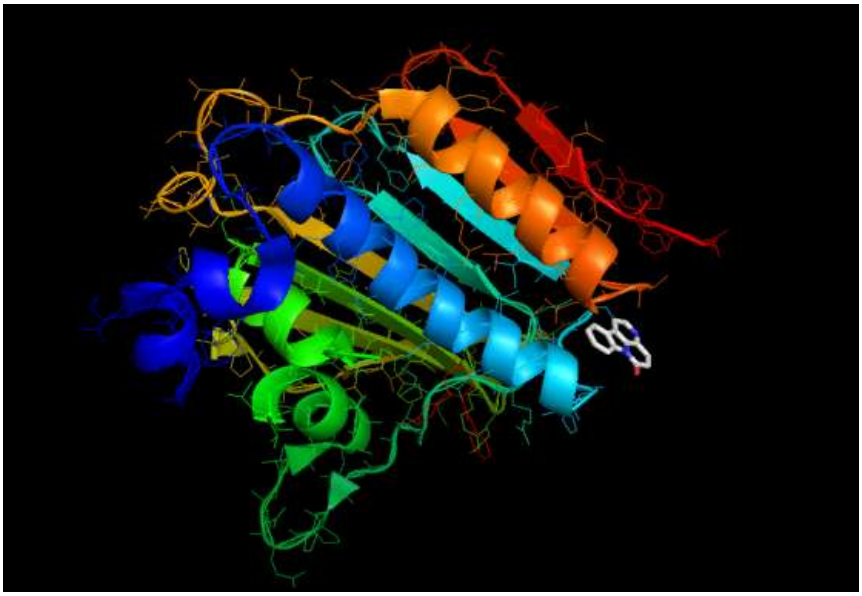


Figure 13b. Molecular docking of canthin-6-one (**65**) with DNA gyrase receptor

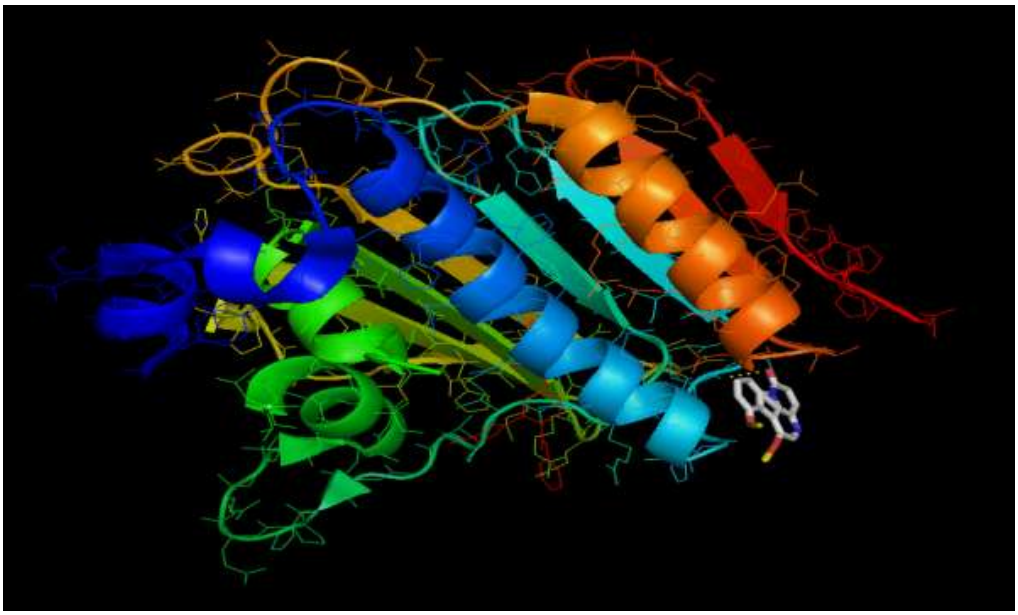


Figure 13c. Molecular docking of 1,11-dimethoxycanthin-6-one (**66**) with DNA gyrase receptor

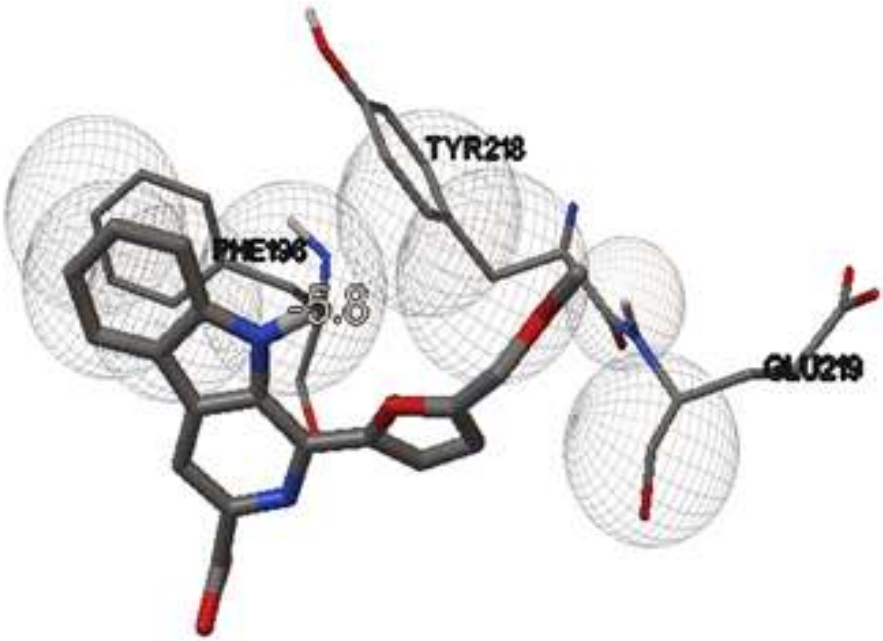


Figure 14a. Molecular docking of flazin methyl ether (**64**) with hydrophobic and hydrogen bond interactions

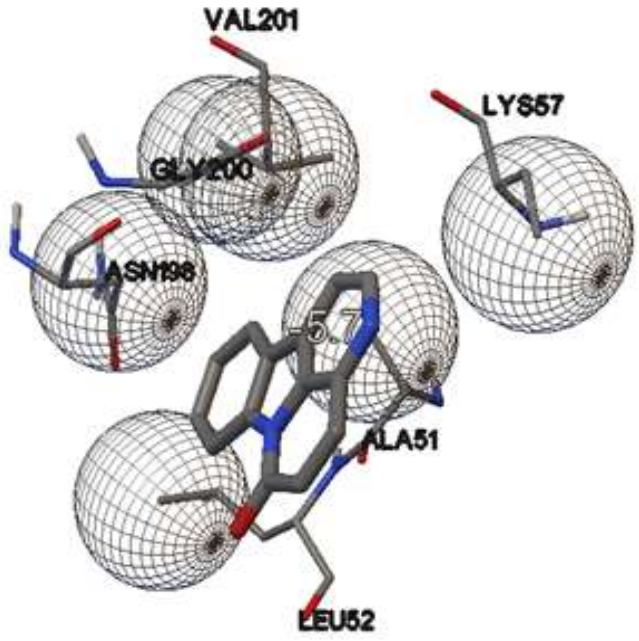


Figure 14b. Molecular docking of canthin-6-one (**65**) with hydrophobic and hydrogen bond interactions

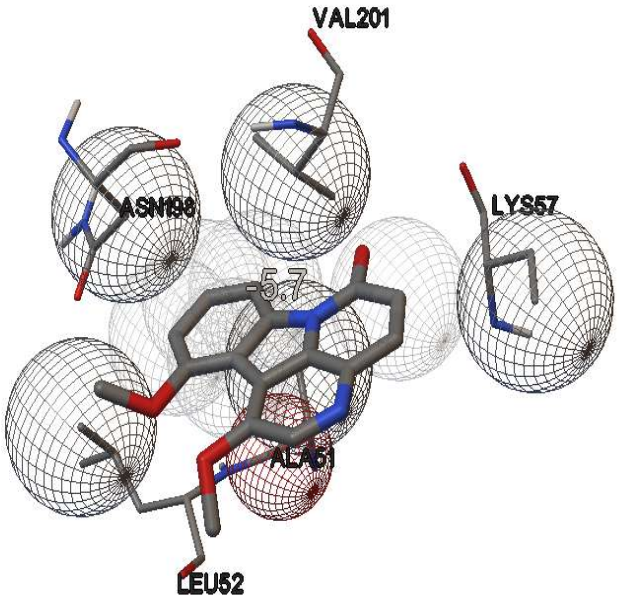


Figure 14c. Molecular docking of 1,11-dimethoxycanthin-6-one (**66**) with hydrophobic and hydrogen bond interactions

Molecular docking against protein aromatase

Breast cancer is one of the leading causes of death noticed in women across the world. Recent years, the most successful treatments rendered are the use of aromatase inhibitors (AIs). Aromatase enzyme plays a very crucial role in the oestrogen positive breast cancers. Oestrogen receptor positive breast cancer is resistant to tamoxifen and oestrogen receptor positive signaling was assumed to play a paramount role in this. Therefore, the drugs involved in treating the oestrogen positive breast cancers act either by interfering with oestrogen production or by action. Here in, we studied the molecular docking of newly isolated alkaloid compounds against Aromatase enzymes (PDB ID: 3S7S) shown better anti cancer potency and were displayed better docking score within binding pocket. Binding mode of active compounds **64-66** within the binding region, have better to moderate docked score (− 6.6 to -5.3 kcal/mol). The docking analysis revealed that Compound A has higher binding affinity (− 6.6 kcal/mol) than compound B (− 5.5 kcal/mol) and compound C (− 5.3 kcal/mol) for Aromatase based on the docking energy and number of H-bonds. Compound **64** shown strong interaction with Aromatase through H-bond (SER90), Pi-Pi interaction (PHE116), Pi-cation interaction (LYS376) (Table 1) and hydrophobic interactions with the amino acid residues ASN136, ARG115, GLU92, and GLY117. Compounds **65** binds to aromatase through Pi-Pi interaction (TYR52 and hydrophobic interaction with amino acid resides TRP88, and PRO58. Compound **66** binds to the protein through one hydrogen bond (LYS376) and hydrophobic interaction with amino acid residues LYS119, GLU92, and GLY117 (Figure 15a-c, and Figure 16a-c). All details of the atoms involved in bonding with ligands, bond lengths, docking energies, and RMSD values are given in Table 12.

Table 12: The binding interactions of aromatase

Ligand	Affinity (kcal/mol)	Hydrogen bonding	Interacting Amino acids
64	-6.6	SER90	LEU125, GLY 123, GLN45, ARG122, ASP43, LYS42
65	-5.5	--	HIS119 (H), GLY 123, ALA121, GLY12, ARG122, LYS42, ASP43, LEU125
66	-5.3	LYS376	LEU125, HIS119, ASP43, ARG122, LYS42

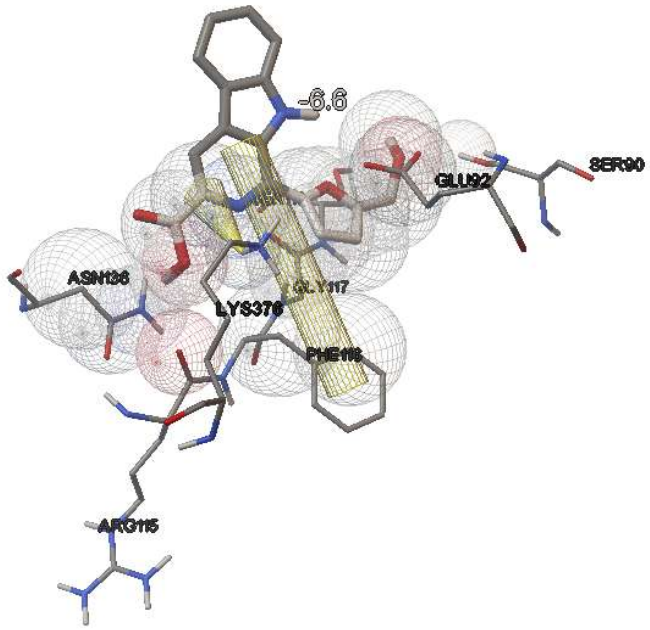


Figure 15a. Molecular docking of compound **64** with aromatase receptor

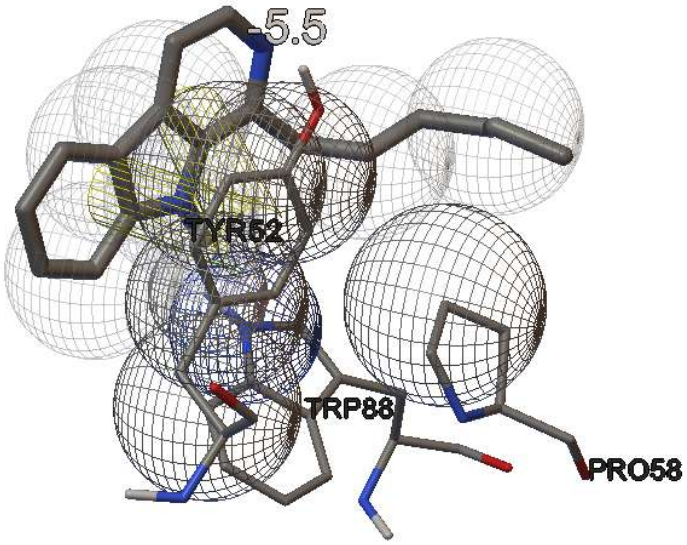


Figure 15b. Molecular docking of compound **65** with Aromatase receptor

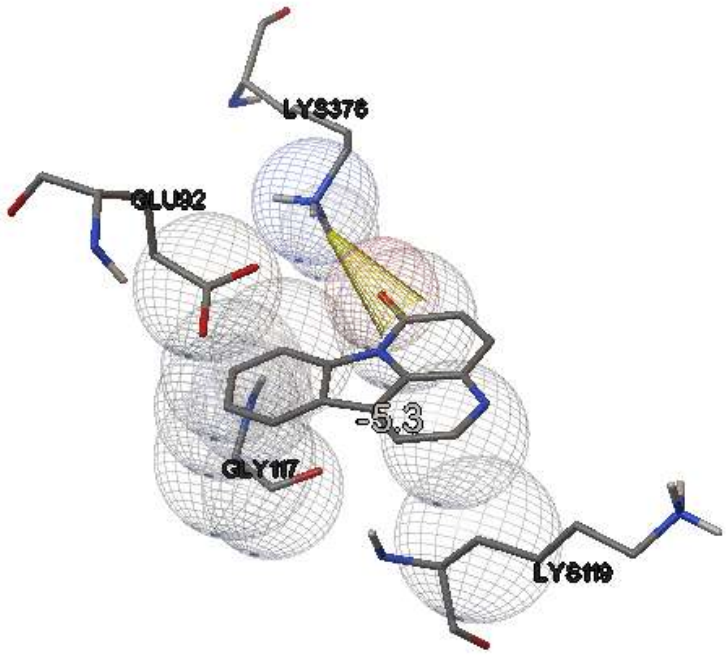


Figure 15c. Molecular docking of Compound **66** with Aromatase receptor

Figure 16a. Molecular docking of compound **64** with hydrophobic, and hydrogen bond interactions

Figure 16b. Molecular docking of compound **65** with hydrophobic and Pi-Pi interactions

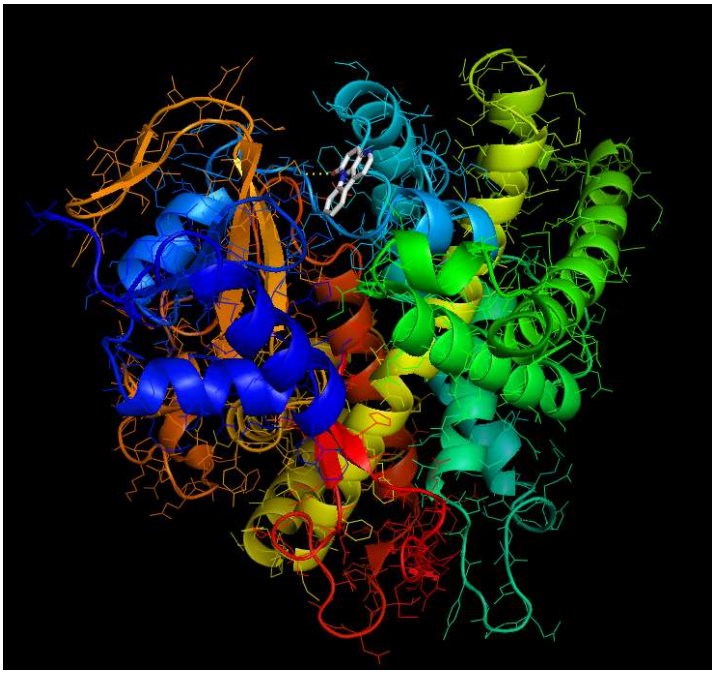


Figure 16c. Molecular docking of compound **66** with hydrophobic and hydrogen bond interactions

4.3.5. Results of the phytochemical investigation of roots of *Bersama abysinica*

4.3.5.1 Phytochemical screening results of roots of *Bersama abysinica*

Phytochemical screening test of dichloromethane/methanol (1:1) and methanol roots extracts revealed the presence of alkaloids, flavonoids, phytosterols, phenols, steroids, tannins, terpenoids, coumarins, anthraquinones, terpenes and saponins whereas saponins were found to be absent in methanol extract (Table 13).

Table 13:Phytochemical screening tests of crude extracts of DCM:MeOH (1:1) and MeOH (100%).

Phytochemical screening	Test	DCM:MeOH(1:1)	MeOH(100%)
Alkaloids	Wagner's test	+	+
Flavonoids		+	+
Phytosterols	Salkowski's test	+	+
Steroids	Salkowski's test	+	+
Phenols	Ferric Chloride test	+	+
Tannins	Gelatin Test	+	+
Terpenoids	*Salkowski's test*	+	+

Coumarins		+	+
Anthraquinones		+	+
Terpenes	Salkowski test	+	+
Saponins	Froth test	+	-

Key: DCM=Dichoromethane and MeOH=Methanol

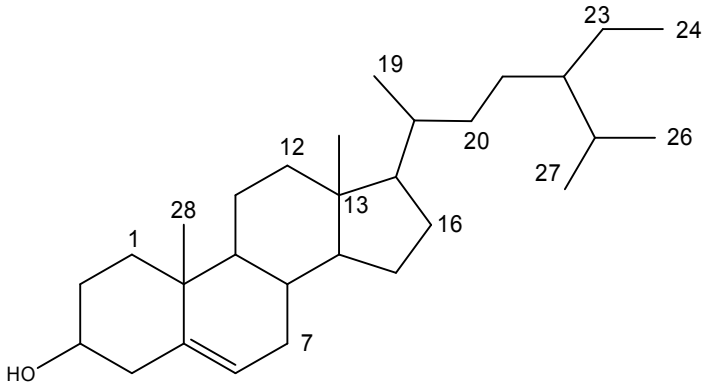
4.3.5.2 Characterization of compounds from roots of *B. abysinica*

Compound **67** was isolated as white solid with melting point 134-136°C and R_f value of 0.64 (50% EtOAc in *n*-hexane as eluent). The ^{1}H NMR spectrum (Table 14) showed a series of proton signal at δ 1.0-1.8 due to overlapping of methylenes and methines, a characteristic frame work of steroid. Oxygenated sp^3 methine proton was observed at δ 3.68 (m, 1H, H-3) which is a characteristic of steroids with hydroxyl group at C-3 position. Olefinic proton was observed at δ 5.3 suggesting that the proton is next to methylene. The presence of six methyl groups at δ 0.68, 0.93, 0.83, 0.81, 0.84 and 1.01 is also in agreement with the steroidal nucleus. The ^{13}C NMR spectrum (Table 14) revealed the presence of twenty carbon signals which is a characteristic feature of triterpenes. The ^{13}C NMR and DEPT-135 spectra displayed the presence of seven methyl carbons which resonated at δ 14.6, 16.0, 15.4, 18.0, 16.2, 19.4 and 28.0. Eleven methylene carbon signals were observed resonating at δ_C 18.4, 21.0, 25.2, 27.5, 29.7, 29.9, 34.3, 35.6, 38.8 and 40.0 in (Table 2). Presence of five methine carbons (δ_C 48.0, 48.30, 50.5, 55.3 and 38.1), two olefinic carbons (δ 143.8 and 114.2), of which one is quaternary, and additional quaternary carbon peaks (at δ 38.9, 37.2, 40.8, 42.8 and 43.0) were also confirmed. Oxygenated sp^3methine at C-3 was observed at δ 76.8. Thus, based on the above spectral data and comparison with literature, the structure of the compound was identified as *β*-sitosterol (**67**) (Chaturvedula and Prakash, 2012).

Table 14: ^{1}H ($CDCl_3$, 400MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectral data of β-sitosterol (**6**) in $CDCl_3$.

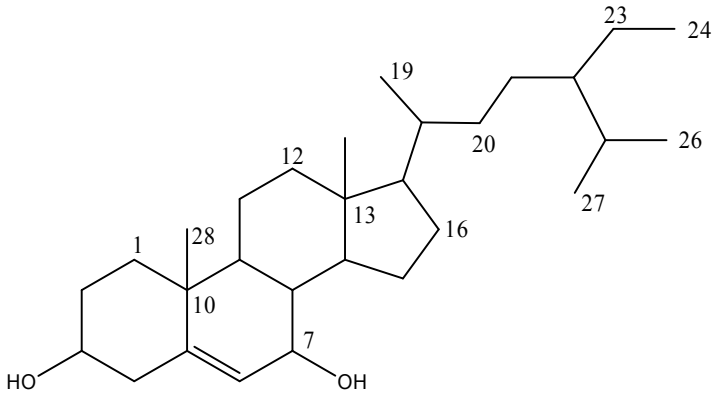
Position	δ_H (δ in ppm)	δ_C	Chaturvedula and Prakash, 2012)	
1		37.3		37.2
2		31.9		31.6
3	3.18 (1H, t)	71.4	3.53 (tdd, 1H, *J* 4.5, 4.2, 3.8 Hz)	71.7
4		42.3		42.2
5		143.8	5.36 (t, 1H, *J* = 6.4 Hz)	140.9
6	5.38 (1H, t)	121.2		121.9
7		32.0		32.1

8		33.1		32.1
9		50.4		50.3
10		36.2		36.7
11		21.1		21.3
12		39.8		39.9
13		42.8		42.6
14		56.8		56.9
15		25.9		26.3
16		28.3		28.5
17		56.1		56.3
18		36.2		36.3
19		19.4		19.2
20		35.5	0.93 (d, 3H, *J* = 6.5 Hz)	34.2
22		45.9		46.1
23		23.1		23.3
24	1.85	11.9	0.84 (t, 3H, *J* = 7.2 Hz)	12.2
25	2.06	29.2		29.4
26	0.96	19.8	0.83 (d, 3H, *J* = 6.4 Hz)	20.1
27	1.65	19.2	0.81 (d, 3H, *J* = 6.4 Hz)	19.6
28		18.8	0.68 (s, 3H)	19.0
29		11.9	1.01 (s, 3H)	12.0



67

Compound **68** was isolated as white solid with melting point of 135-137°Cand R_f value of 0.6 (50% EtOAc in *n*-hexane as eluent). The ^{1}H NMR spectrum and ^{13}C NMR spectrum showed comparable spectral feature to that of β-sitosterol except the peak observed at δ 79.1 suggesting the presence of additional sp^3 oxygenated methine. Comparison with literatures reported and with NMR features of β-sitosterol observed, 7-hydroxy-β-sitosterol (**68**) was suggested.

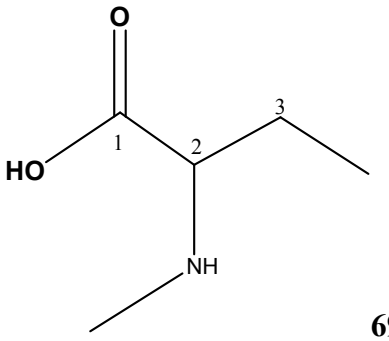


68

Compound **69** was isolated as a white solid with R_f value of 0.7 (*n*-hexane/EtOAc (8/2) as eluent. The ^{1}H-NMR spectrum showed (Table 15) the presence of one terminal methyl protons at δ 1.27 (3H, t) suggesting it is next to methylene. The spectrum also displayed a multiplet methine signal at δ 3.5 (q, 1H), methylene at 2.36 (2H, t) and methyl at 2.8 (3H, m) where the former suggests a methine protons α to a carbonyl of carboxylic acid and also connected to hetroatom whereas the later suggests methyl attached to hetroatom. The ^{13}C NMR spectrum with the help of DEPT-135 (Table 3) revealed the presence of five well resolved carbon resonances of which one carbonyl carbon (δ 179.2), methyl (δ 17.6), methine (δ 48.7), one methylene (δ 29.0), and methyl (δ 30.7) supported by DEPT-135. Thus, based on the above spectral data the compound was found to be 2-methylamino-butyric acid (**69**) isolated for the first time from a natural source.

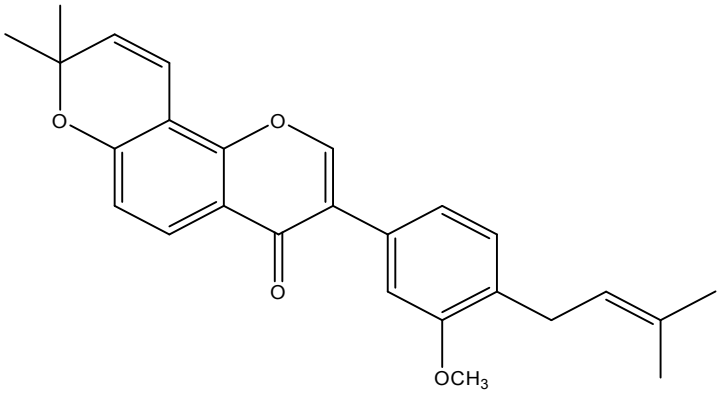
Table 15: ^{1}H and ^{13}C NMR spectral data of compound **69**

Position	δ_H (multiplicity)	^{13}C NMR (δ_C in ppm)	DEPT-135 (δ_C in ppm)
1		175.1	-
2	3.5 (1H, m)	49.4	49.4
3	2.3 (2H, m).	29.0	29.0
4	1.27 (3H, t).	17.6	17.6
5	2.8 (3H, s)	30.7	30.7



69

Compound **70** was isolated as deep yellow solid with R_f value of 0.6 in *n*-hexane/ethyl acetate (7:3). Its ^{1}H-NMR (400MHz, DMSO-d_6) (Appendix 42) spectrum revealed a pair of two ortho couples doublets at δ 7.5 (*d*, *J*=8) and 6.78 (*d*, *J*=8) suggesting ortho coupled aromatic protons (as suggested in ring A) and δ 7.4 (*d*, *J*= 7.8) and 6.2 (*d*, *J*= 7.8) suggesting pyran ring ortho coupled olefinic protons. Methoxy peak was also observed at δ 3.8 (3H, s). Two ortho coupled aromatic protons observed at δ 6.70 (1H, *d*, *J*= 8, 1.2) and 6.76 (1H, *d*, *J*= 8) coupled with a meta coupled aromatic peak observed at δ 5.8 (1H, *d*, *J*= 1.2) suggest ABX spin pattern aromatic ring (as suggested in ring B). A singlet aromatic proton observed at δ 8.3 (1H, s) suggest a proton at C-2 position of an isoflavone. The downfield chemical shift of this proton suggests that the proton is at β position of the carbonyl carbon, in agreement with the isoflavone skeleton. The presence of prenyl moiety was also confirmed on the basis of peaks observed at δ 1.7 (3H, s), δ 2.1 (3H, s), and δ 5.2 (1H, t). Thus, based on the above spectral data and comparison with the NMR features of isoflavanoids the following structure (compound **70**) was proposed. ^{13}C NMR of this compound was run for longer time but due to small peak of the isolated sample the spectrum couldn't be generated.



70

4.3.5.3 Antibacterial activity of *Bersama abysinica*

The antibacterial activity of the crude extracts of DCM:MeOH(1:1), MeOH and isolated compound were examined at a concentration of 0.5mg/mL against four pathogenic bacterial strains. Promising antibacterial activity was observed for DCM:MeOH (1:1) and methanol extracts against *E. coli*, *S. thyphimerium, S. aureus* and *B. subtlis* with zone of inhibition of 13±0, 11.6±0.48, 13±2, and 12.3±1.25, respectively, for DCM: MeOH extract and 13.6±0.55, 12±0, 12±2 and 11.6 ±0.48, respectively, for methanol extract. 7-hydroxy-β-sitosterol (**7**) showed promising antibacterial activity against *E. coli* and *S.aureus* with zone of inhibition of 12.6±0.48 and 12.5±0.5, respectively, compared to ciprofloxacin 28.6±1.25 and 26±5.1 (Table 16).

Table 16: Zone of bacterial growth inhibition diameter (mm)

Sample name	Zone of inhibition (mm) Mean ± standard deviation			
	E. coli	*S. thyphimerium*	*S.aureus*	*B. subtlis*
DCM: MetOH extracts	13±0	11.6±0.48	13±2	12.3±1.25
MetOH extracts	13.6±0.55	12±0	12±2	11.6±0.48
β-sitosterol (**67**)	11.6±0.55	11.6±0.48	12.5±0.5	11±0
Hydroxy-β-sitosterol (**68**)	12.5±0.48	10.6±0.48	12.5±0.5	11±0.82
2-methylamino-butyric acid (**69**)	11.3±0.48	11± 0.82	11.5±0.5	11±0.1
Ciprofloxacin	28.6±1.25	28.6±0.94	26±5.1	34.3±0.94

4.3.6. Results of the phytochemical investigation of roots of *Erythrina abysinica*

4.3.6.1 Phytochemical screening results of roots of *Erythrina abysinica*

Phytochemical screening test of dichloromethane/methanol (1: 1) and methanol roots extracts revealed the presence of alkaloids, flavonoids, phytosterols, phenols, steroids, tannins, terpenoids, coumarins, anthraquinones whereas saponins were found to be absent in both DCM:MeOH and methanol extracts (Table 17).

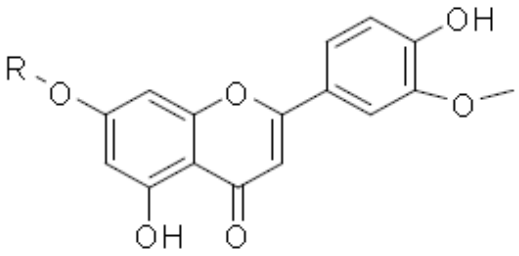
Table 17:Phytochemical screening tests of crude extracts of DCM:MeOH (1:1) and MeOH (100%).

Phytochemical screening	Test	DCM:MeOH(1:1)	MeOH(100%)
Alkaloids	Wagner's test	+	+
Flavonoids		+	+
Phytosterols	Salkowski's test	+	+
Steroids	Salkowski's test	+	+
Phenols	Ferric Chloride test	+	+
Tannins	Gelatin Test	+	+
Terpenoids	*Salkowski's test*	+	+
Coumarins		+	+
Anthraquinones		+	+
Terpenes	Salkowski test	+	+
Saponins	Froth test	-	-

Key: DCM=Dichoromethane and MeOH=Methanol

4.3.6.2 Characterization of compounds from roots of *E. abysinica*

Compound **71** was isolated as yellow solid with R_f value of 0.65 in 40% ethylacetate in *n*-hexane. Its ^{1}H NMR spectrum (appendix 43) revealed singlet peak at δ 8.22 (1H, s), three pairs of doublet of doublet at δ 7.52 (*dd*, *J*= 8,2), 7.08 (*dd*, *J*= 8,2), and 6.97 (*dd*, *J*= 8,2) suggesting an ABX multiplicity pattern. Singlet aromatic peaks were observed at δ 6.49 and 6.6 suggesting a meta substituted aromatic ring. Methoxy and anomeric proton peaks appeared at δ 4.0 and δ 5.1, respectively. Its ^{13} NMR spectrum (appendix 44,45) showed methyl peak at δ 16.2, methoxy peak at δ 60.1, sugar peaks δ 60-80, two anomeric carbon peaks at δ 98.1 and 100.1, and peaks attributed to flavanoid skeleton δ 102.1 (C-3), 105.4 (C-10), 109.0 (C-2'), 115.8 (C-5'), 120.1 (C-1'), 120.8 (C-6'), 148.7 (C-3'), 160.2 (C-5), 162.8 (C-72), 165.5 (C-2), and 182.5 (C-4). Based on the above spectral data and comparison with literature (Cimanadgda et al.,1995) the compound was found to be identical with chrysoeriol-7-O-α-L-rhamnosyl-(1→6)-β-D-glucoside (**71**).



R = apiosyl-glucoside

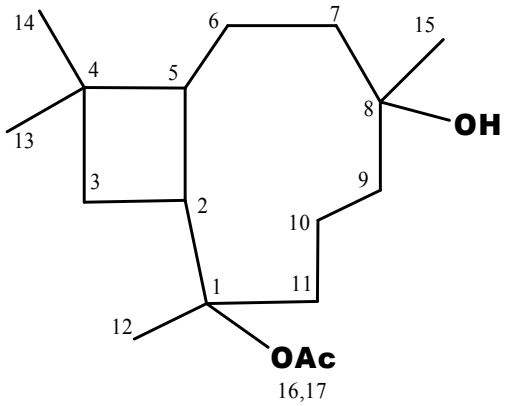
71

Compound **72** was isolated as a colorless solid with R_f value of 0.7 in 30% ethylacetate in *n*-hexane. Its ^{1}H NMR spectrum (appendix 46) revealed four methyl protons between δ 0.8-1.5 and one methyl proton at δ 2.0. The later suggests this methyl is connected to sp^2 carbon. Its ^{13}C NMR spectrum (appendix 47) revealed seventeen carbons of which five methyls at δ 7.5, 13.5, 14.2, 17.1, and 20.1, six methylenes at δ 17.0, 20.6, 28.9, 29.2, 40.9, and 59.8, methine carbon at δ 33.8, carbonyl carbon peak at δ 170.3, and two sp^3 oxygenated quaternary carbons at δ 83.3 and δ 80.8. The latter two sp^3 oxygenated quaternary carbons that appeared at δ 83.3 and 80.8 in ^{13}C NMR spectrum disappeared in DEPT-135 spectrum (appendix 48) along with the ester carbonyl carbon δ 170.3 confirming that these peaks belong to quaternary carbons. Based on the above spectral data five methyls, six methylenes, two methines, two sp^3 oxygenated quaternary carbons (C-1,8), and one methyl ester are clearly evident. Thus, based on the above spectral data and comparison with literature (Henry et al., 1994) a caryophyllane sesequiterpene skeleton was suggested with trivial name 1-acetoxy-8-hydroxy-caryophyllane type sesequiterpene (**72**, Table 18). Closely related compounds have been previously reported from *Sindora sumatrana* (Henry et al., 1994) and 3,9-caryolanediol was isolated from *Erythrina abyssinica* (Machumi et al., 2006).

Table 18: Spectral data of compound **72**

Position	**^{1}H NMR**	**^{13}C NMR**
1.		83.3
2.		33.8
3.		40.9
4.		39.3
5.		31.7
6.		29.2
7.		28.9

8.		80.8
9.		29.2
10.	2.0 (2H, m)	40.9
11.	2.2 (2H, m)	59.8
12.	1.3 (s, 3H)	17.1
13.	1.2 (s, 3H)	14.2
14.	1.1 (s, 3H)	13.5
15.	0.8 (s, 3H)	7.5
16.		170.3
17.	2.0 (s, 3H)	20.1



72

4.3.6.3 Antibacterial activity of *E. abysinica*

The antibacterial activity of the crude extracts of DCM/MeOH(1:1) and MeOH were examined at a concentration of 20 µg/mL against four pathogenic bacterial strains. Promising antibacterial activity was observed for DCM/MeOH (1:1) and methanol extracts against *E. coli*, and *S. aureus* with zone of inhibition of 11±0.2 and 12 ±0.3, 13±0.3 and 13±1.5, respectively, compared to ciprofloxacin 22±1.5 and 21±0.2 (Table 19).

Table 19: Zone of bacterial growth inhibition diameter (mm)

Sample name	**Zone of inhibition (mm) Mean ± standard deviation**			
	E. coli	*S. thyphimerium*	*S.aureus*	*B. subtlis*
DCM: MetOH extracts	11±0.2	9±0.4	12±0.3	10±1.5
MetOH extracts	13±0.3	11±0	13±1.5	10±0.4
Ciprofloxacin	22±1.5	24±0.4	21±0.2	26±0.4

5. Conclusion and recommendation

For decades traditional medicines have been used and continue to be an alternative approach on treatment for various diseases. Due to the growing interest of consumers in substances of natural origin coupled with the increasing apprehension surrounding potentially harmful infectious disease, our group did a comprehensive phytochemical analysis on the roots of *B. antidysentrica*. Silica gel column chromatographic separation of the crude extracts led to isolation of derivative flazin methyl ether (**64**), canthin-6-one (**65**), and 1,11-dimethoxycanthin-6-one (**66**) all of which are identified for the first time from the species. In addition, derivative flazin methyl ether (**64**) was isolated for the first time from the genus. The antibacterial test results revealed that the isolated compounds showed promising antibacterial activity against *S. aureus*, *E. coli*, *S. thphimurium,* and *B. subtilis*. Derivative flazin methyl ether (**64**) and canthin-6-one (**65**) exhibited comparable (12.66±0.60 and 12.5±0.87 mm zone of inhibition) antibacterial activity against *E. coli* and *S. thphimurium* compared to that of ciprofloxacin (27.3±2.52 and 29±1.00 mm zone of inhibition). Canthin-6-one (**65**) had promising scavenging of DPPH free radical (87.5% at 100 µg/mL) compared to ascorbic acid. Molecular docking was found that among isolated compounds best scoring pose (lowest energy) was -5.8 Kcal/mole, -5.7 Kcal/mol and -5.7 Kcal/mol for compound **64-66**, respectively. The finding of these pharmacologically important secondary metabolites, the observed biological activity supported by molecular docking analysis and free radical activity support the traditional use of the plant to treat various infectious diseases.

B. abyssinica is one of these medicinal plants used traditionally to heal various infectious diseases. The phytochemical screening tests showed that crude extracts of root barks *B. abyssinica* plants are rich in alkaloids, flavonoids, saponins, phenols, tannins, terpenoids, steroids, phytosterols and glycosides. Silica gel column chromatography separation of the DCM:MeOH (1:1) crude extract furnished two triterpenoids named β-sitosterol (**67**), Hydroxy-β-sitosterol **(68)** and 2-methylaminobutyric acid (**69**). The latter was isolated for the first time from natural source. The extracts and isolated compounds were evaluated *in vitro* for antibacterial activities using the disc diffusion method against *E coli, S. aureus, S. thphimurium* and *B. subtilis*. Promising antibacterial activity was observed for DCM: MeOH and methanol extracts against *E. coli, S. thyphimerium, S. aureus and B. subtlis* with zone of inhibition of 13± 0,

11.6±0.48, 13±2, and 12.3±1.25, respectively, for DCM: MeOH (1:1) extract and 13.6±0.55, 12±0, 12±2 and 11.6±0.48, respectively, for methanol extract. 7-Hydroxy-β-sitosterol (**68**) showed promising antibacterial activity against *E. coli* and *S.aureus* with zone of inhibition of 12.6±0.48 and 12.5±0.5, respectively, compared to ciprofloxacin 28.6±1.25 and 26±5.1.

The roots of *E. abyssinica* afforded chrysoeriol-7-O-α-L-rhamnosyl-(1→6)-β-D-glucoside (**71**) and caryophyllane sesquiterpene (**72**). The latter was isolated for the first time from the genus. Antibacterial screening of the roots of *E. abyssinica* revealed promising antibacterial activity of the methanol extract of the roots of *E. abyssinica* against *E. coli* and *S.aureus* with zone of inhibition of 13±0.3 and 13±1.5, respectively, compared to positive control ciprofloxacin with zone of inhibition of 22±1.5 and 21±0.2, respectively.

The findings of this project suggest that the traditional uses of the plants for infectious disease have been supported with detailed scientific study and in some cases active ingredients and cruds extracts have been identified. These findings promote further research work on formulations of crudes extracts such as methanol extracts of *Bersama abysinica,* against *E. coli* and promising activity of canthine-6-one (**63**) (12.66±0.6 and 12.33±2.31 mm) against *E. coli* and *S. thphimurium*, need further analysis and formulations in various doses and examine in treating infections caused by *E. coli*, and *S. thphimurium*. DPPH radical scavenging activities (in %) of extracts and isolated compounds of *Brucea antydycentrica* were found to be 87.5 (canthine-6-one, **63**), 85.3 (flazin methyl ether derivative, **3**), 83.3 (Dichloromethane/methanol (1:1) crude extract), 80.00 (acid-base crude extract), and 78.4 (1,11-dimethoxycanthin-6-one, **64**) respectively at 100 µg/mL suggesting that the plant can be used as antioxidant food supplement. It was observed that the DPPH scavenging activity increased with after a comprehensive analysis of cytotoxicity assay. More work is also recommended to examine structure activity relationship of the crude extracts and isolated compounds in various doses and examine cytotoxicity assay so as to use them as a remedy to treat infections originated from aforementioned strains. Last but not least, anticancer and cytoxicity assay work is also recommended for the crude extracts and isolated compounds.

6. Budget utilization

Summary of the budget utilized is presented here below (Table 20).

Table 20. Budget utilization summary

S.No	Items	Budget allocated	Budget utilized	Percentage
1.	Transport cost for sample collection, identification, chemical purchasing and sample analysis	30,000	30,000	100%
2.	Chemicals and materials purchase by the University	228,197	130,000	56.96%
3.	Project administrative, instrumental analysis, and bioassay budget	261, 680	212,000	84.5%
	Total	519,877	372,000	
	Contingency 10%	52,393	-	
	Grand total	**572,270.00**	**372,000**	**65.00%**

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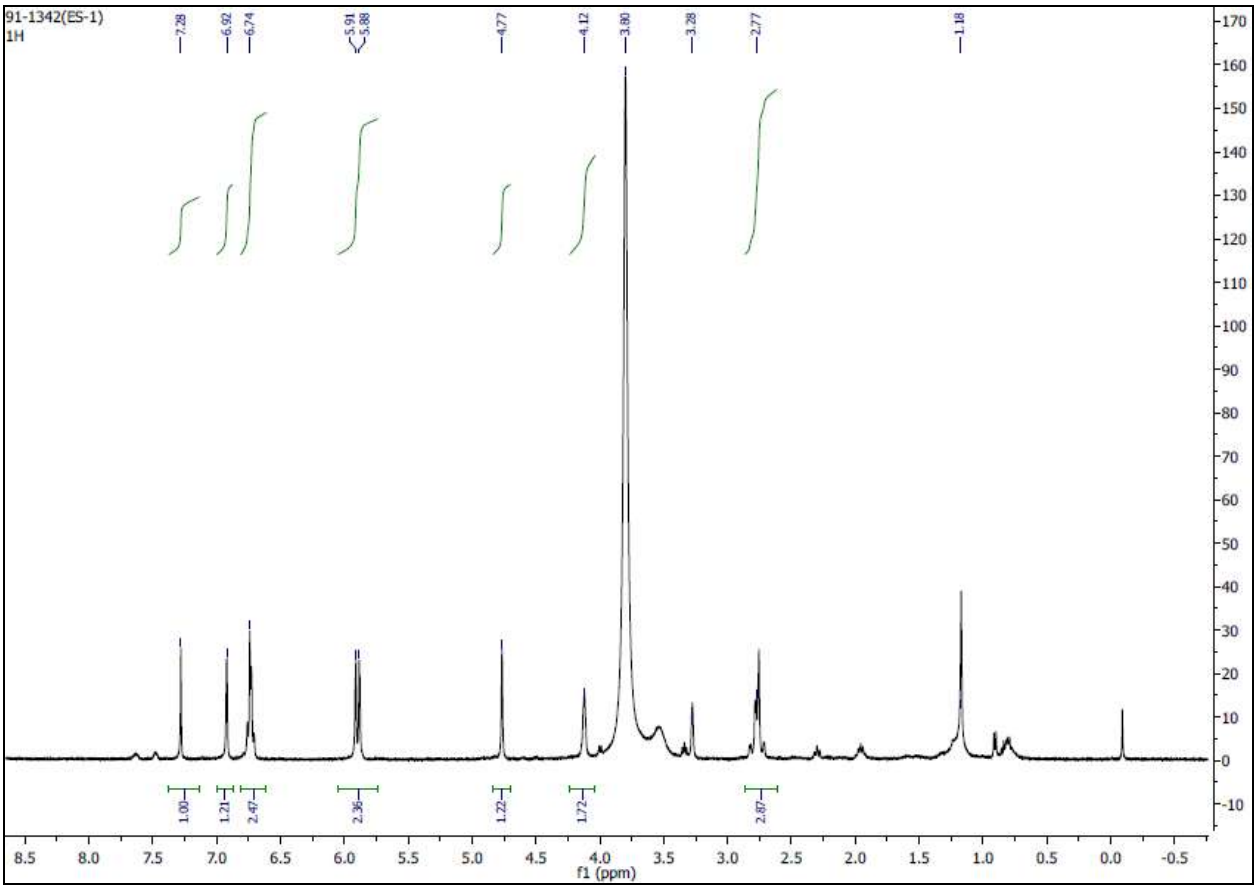
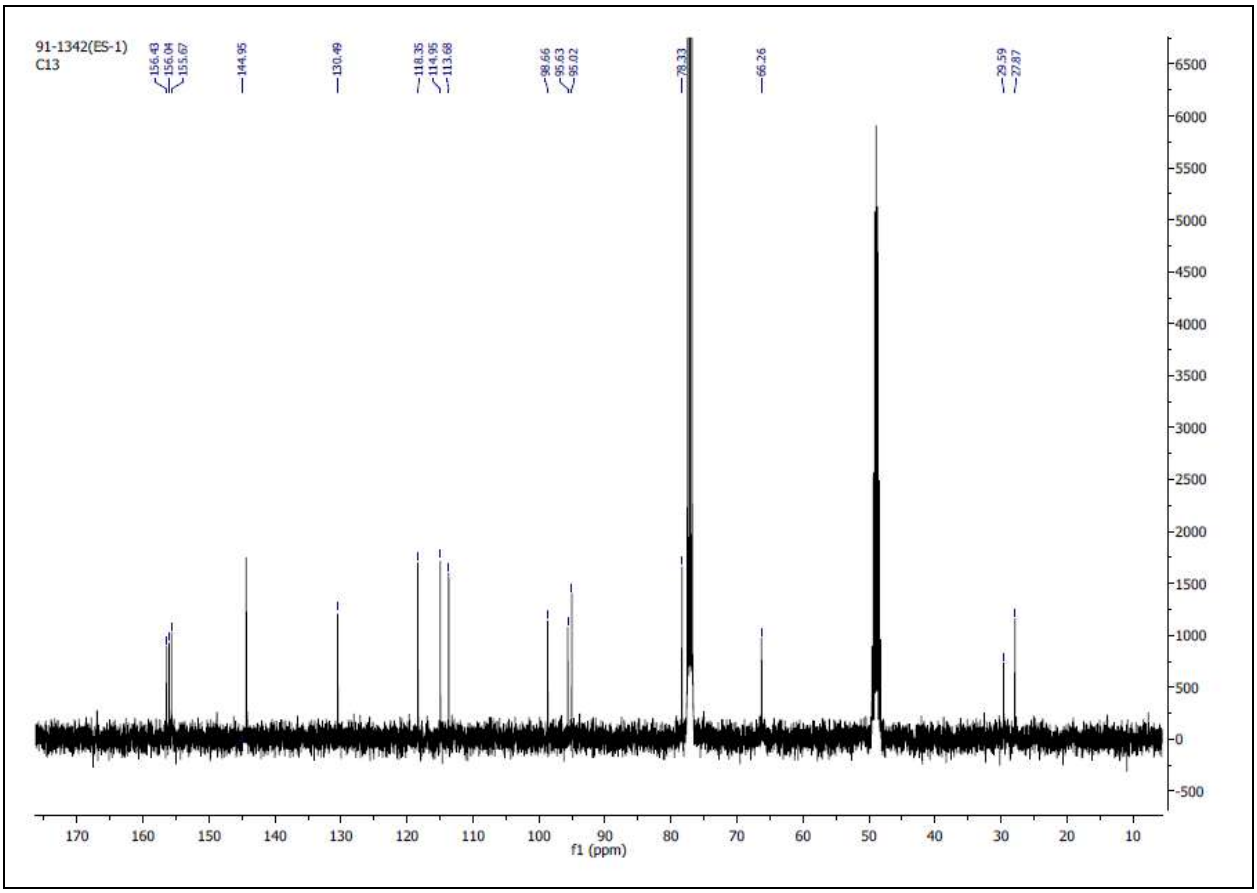
8. Appendix

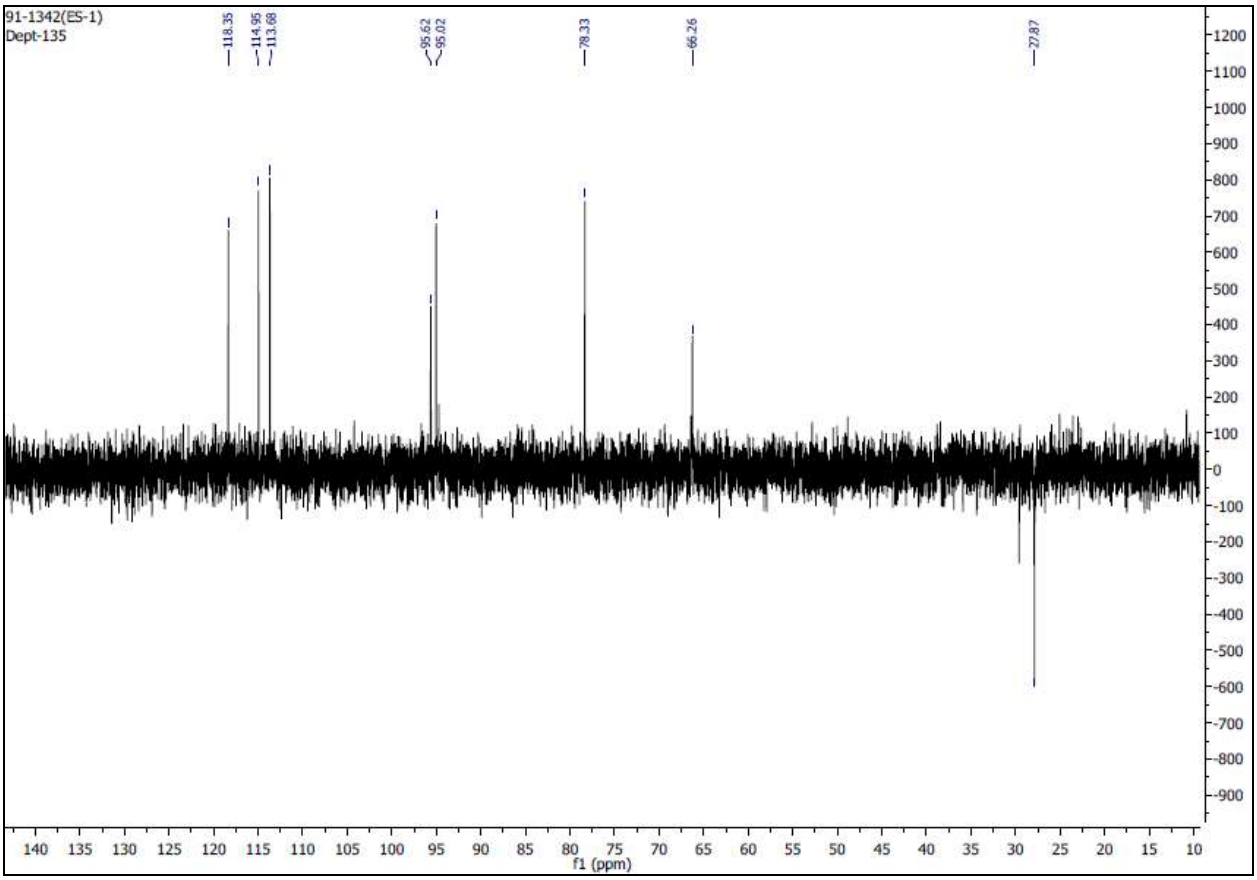
Appendix 1: ^{1}H-NMR spectrum of compound **1**



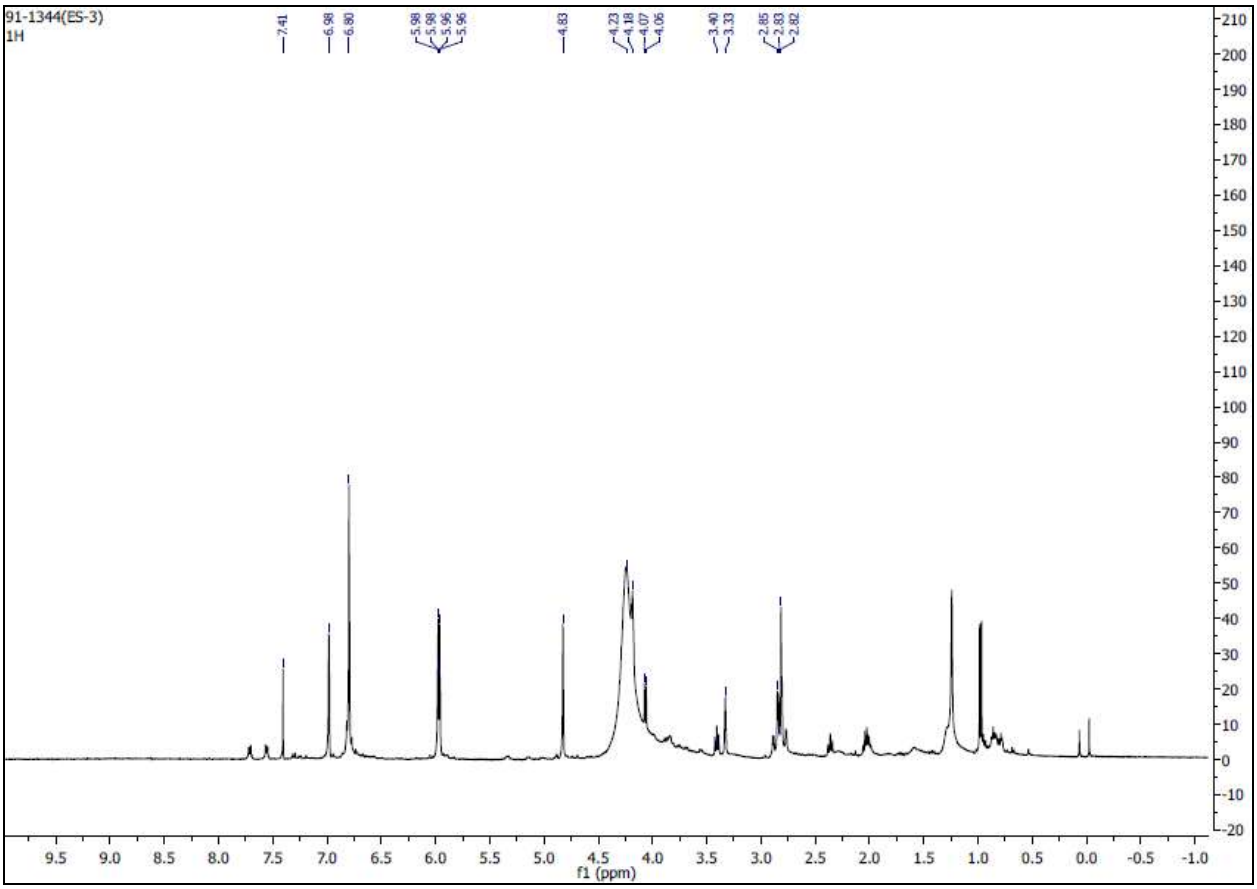
Appendix 2: ^{13}C-NMR spectrum of compound **1**



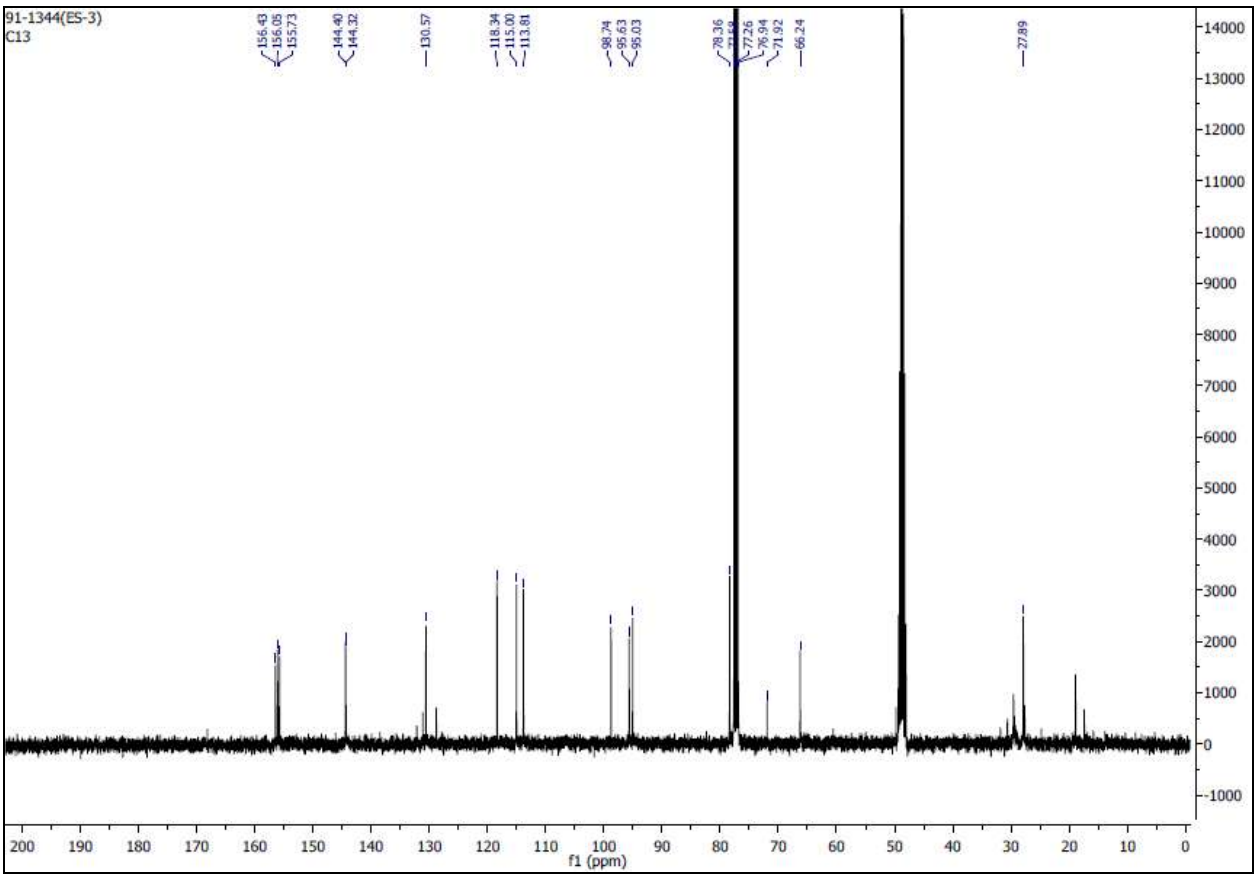
Appendix 3: DEPT-135 NMR spectrum of compound **1**



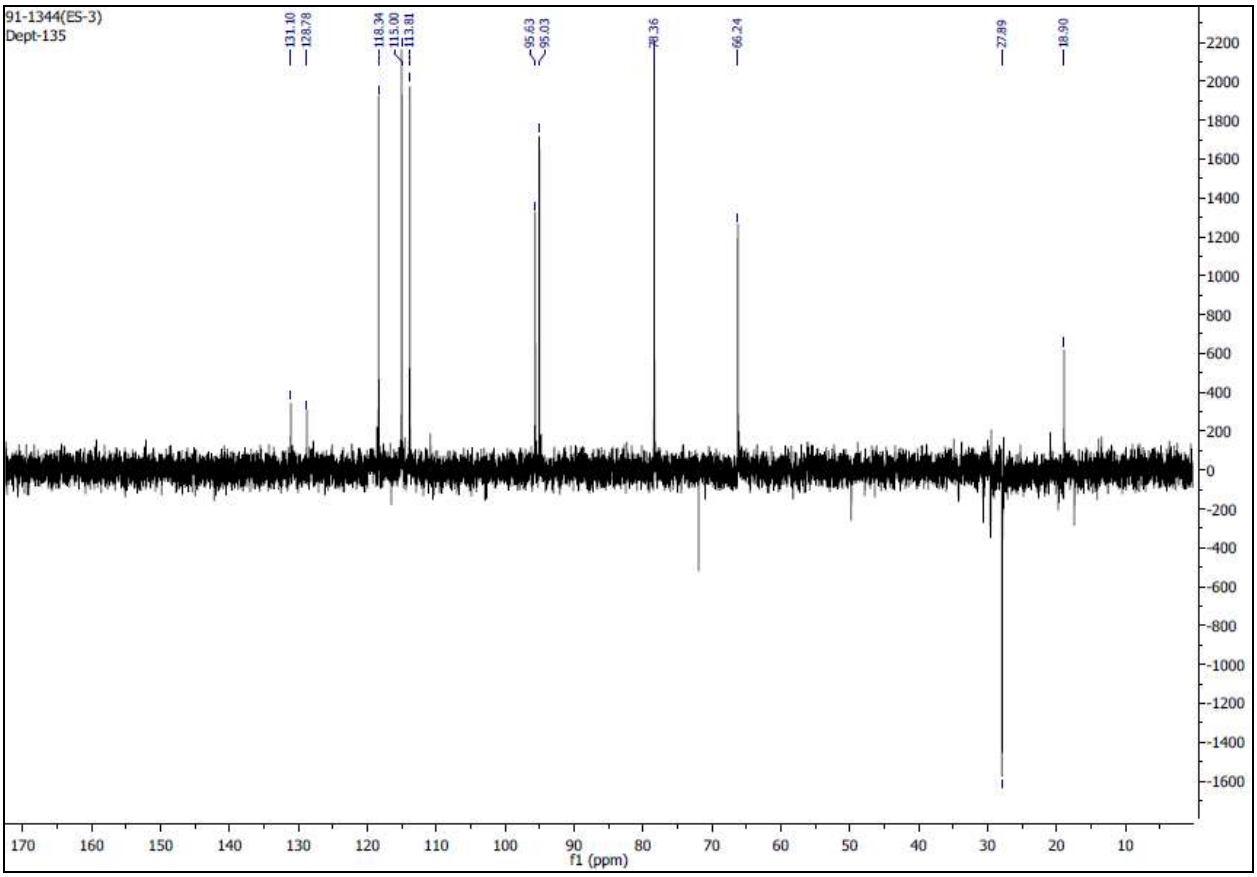
Appendix 4: ^{1}H-NMR spectrum of compound **2**



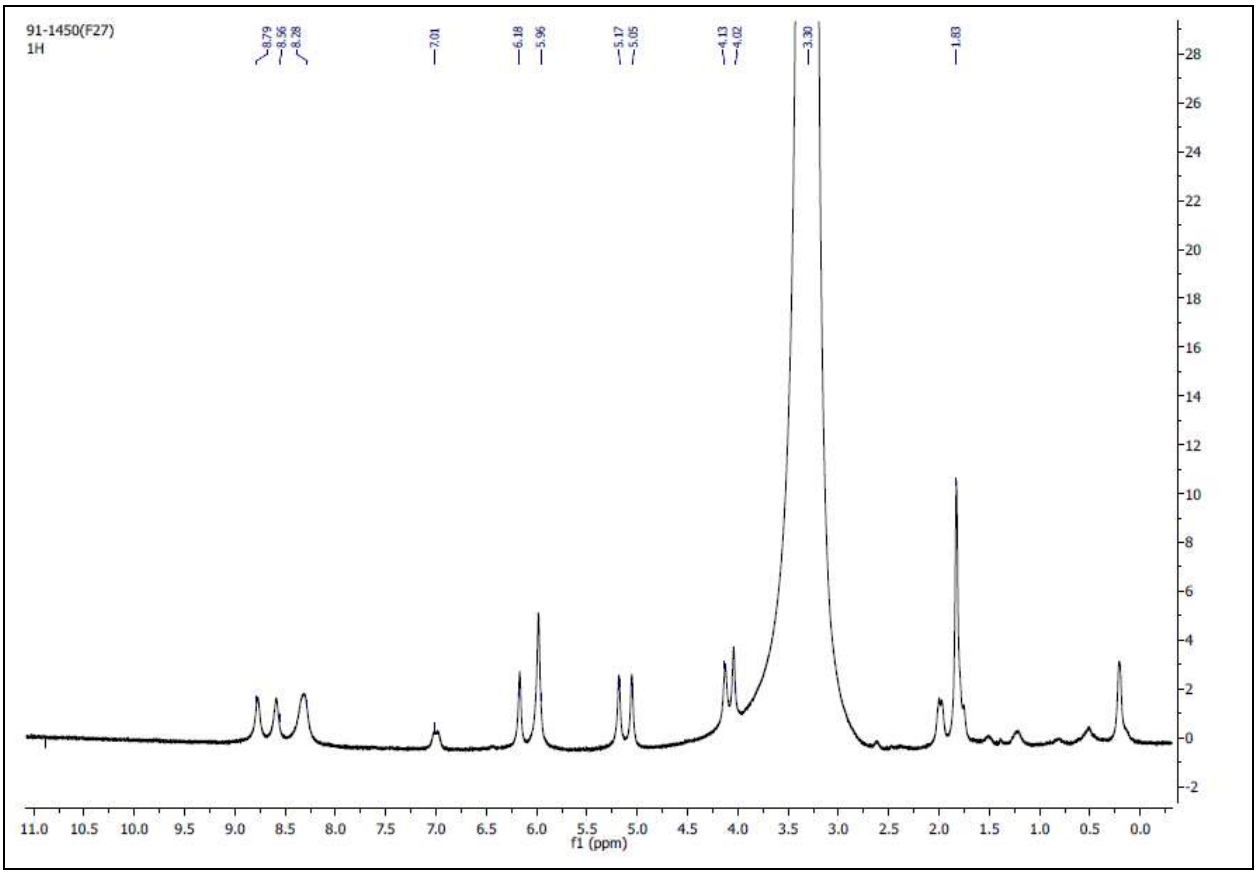
Appendix 5: ^{13}C-NMR spectrum of compound **2**



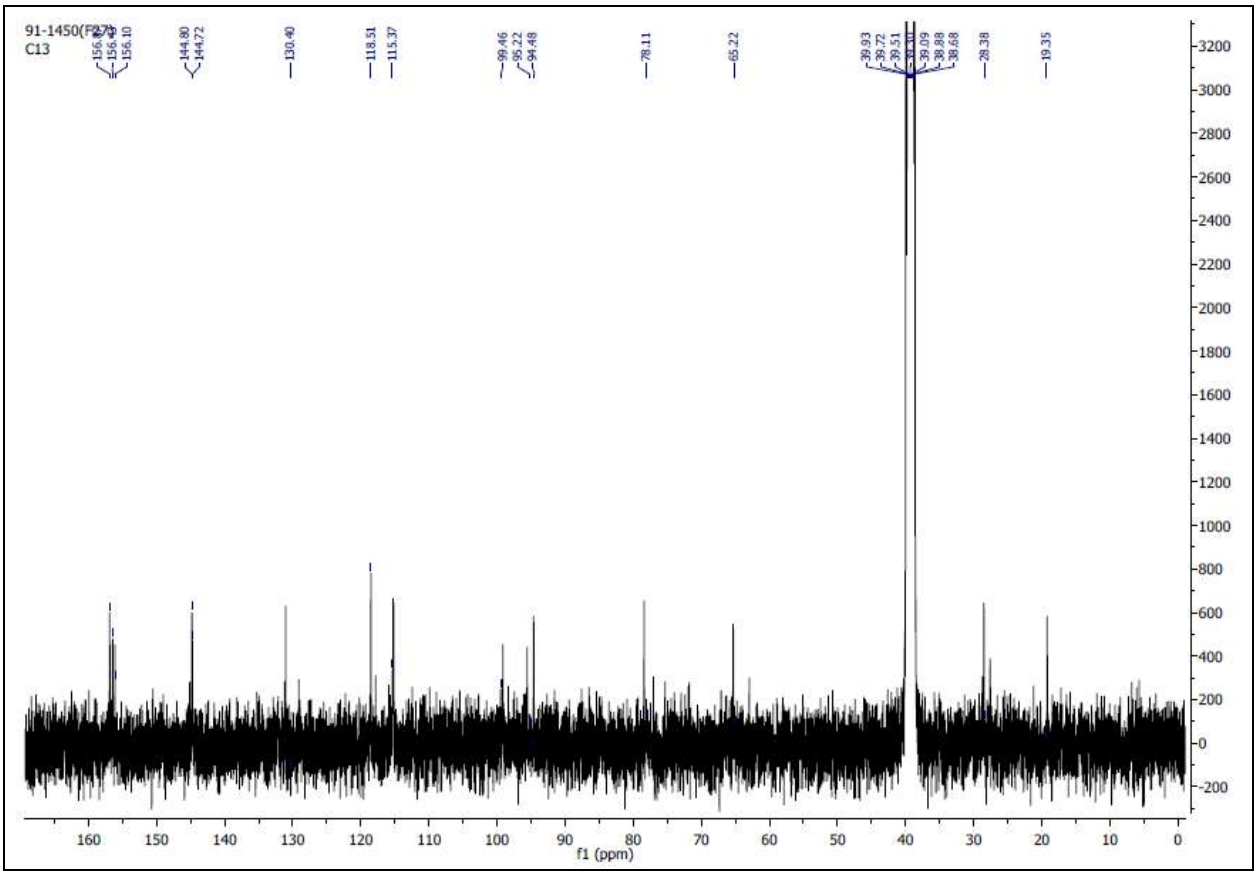
Appendix 6: DEPT-135 NMR spectrum of compound **2**



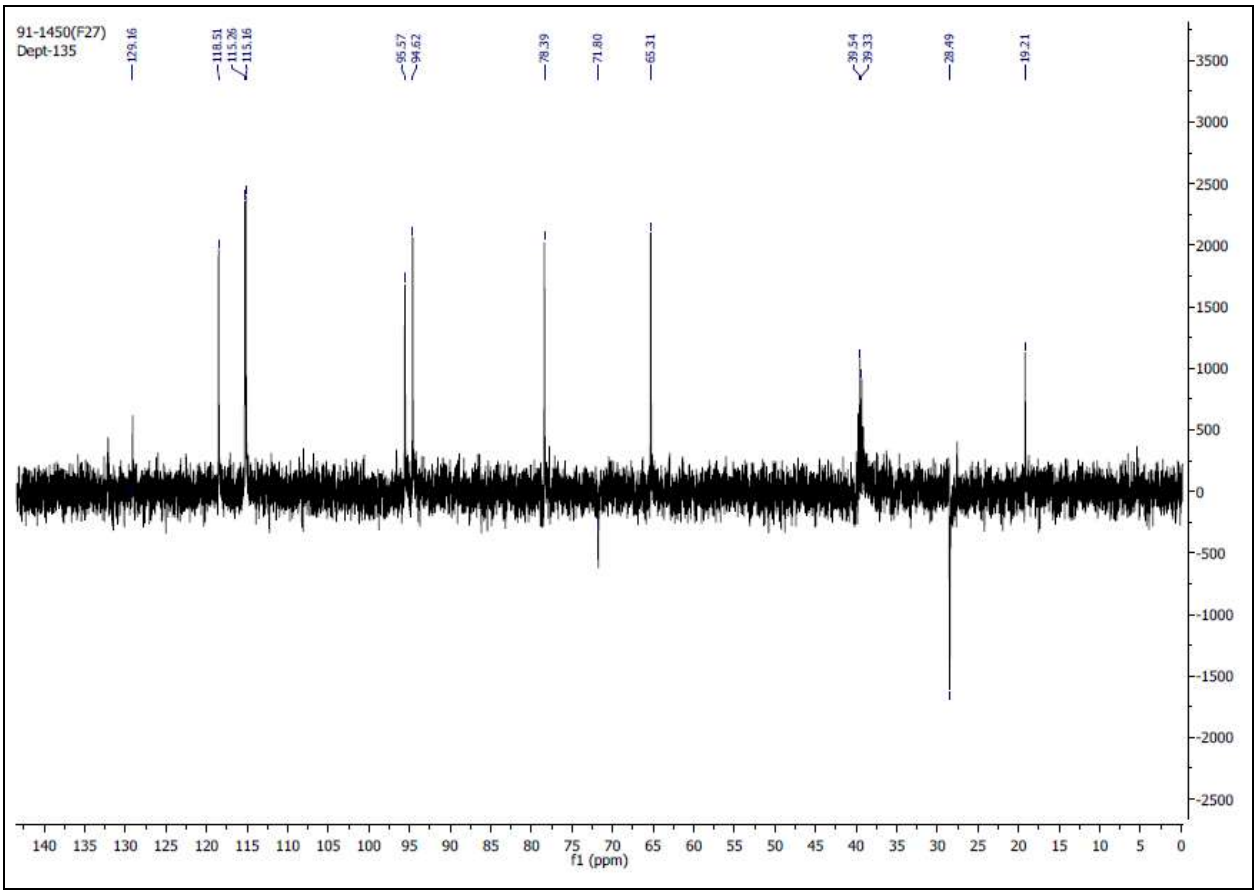
Appendix 7: ^{1}H -NMR spectrum of compound **2** related compound



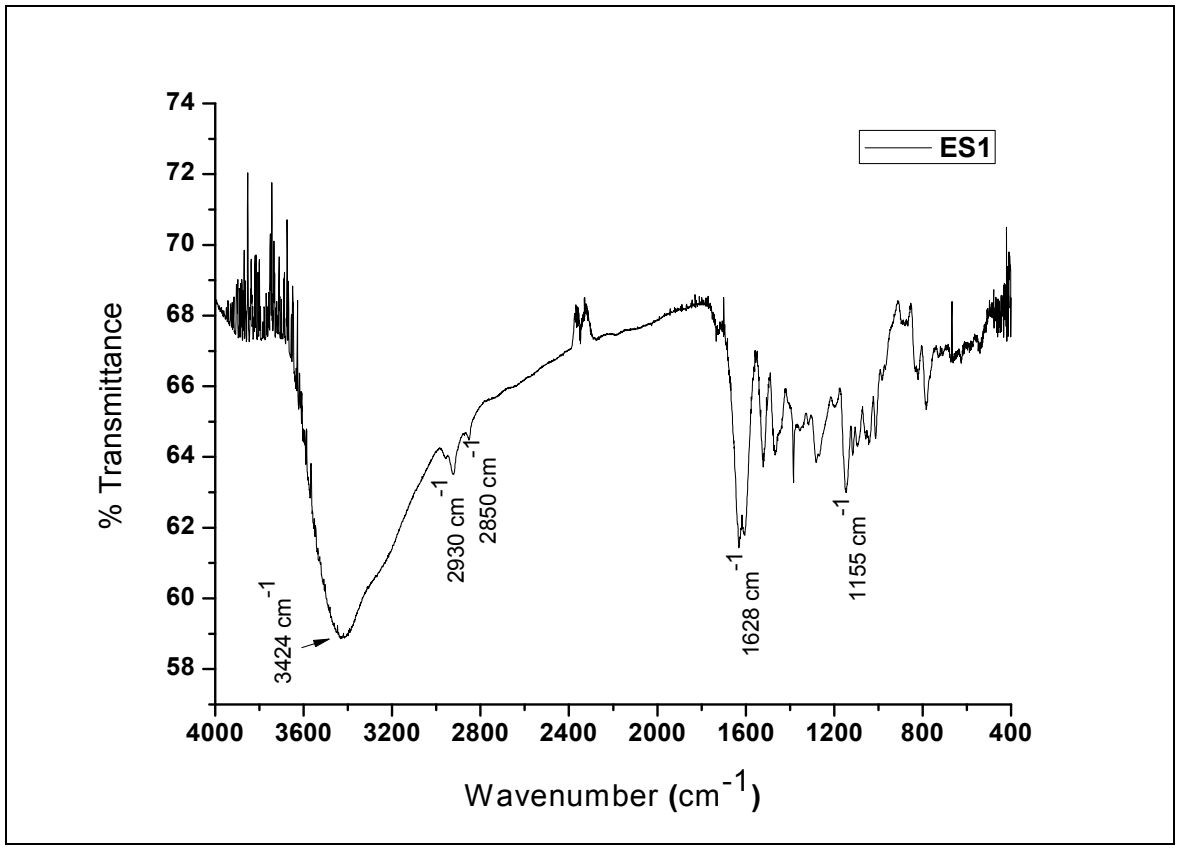
Appendix 8: ^{13}C -NMR spectrum of ES4



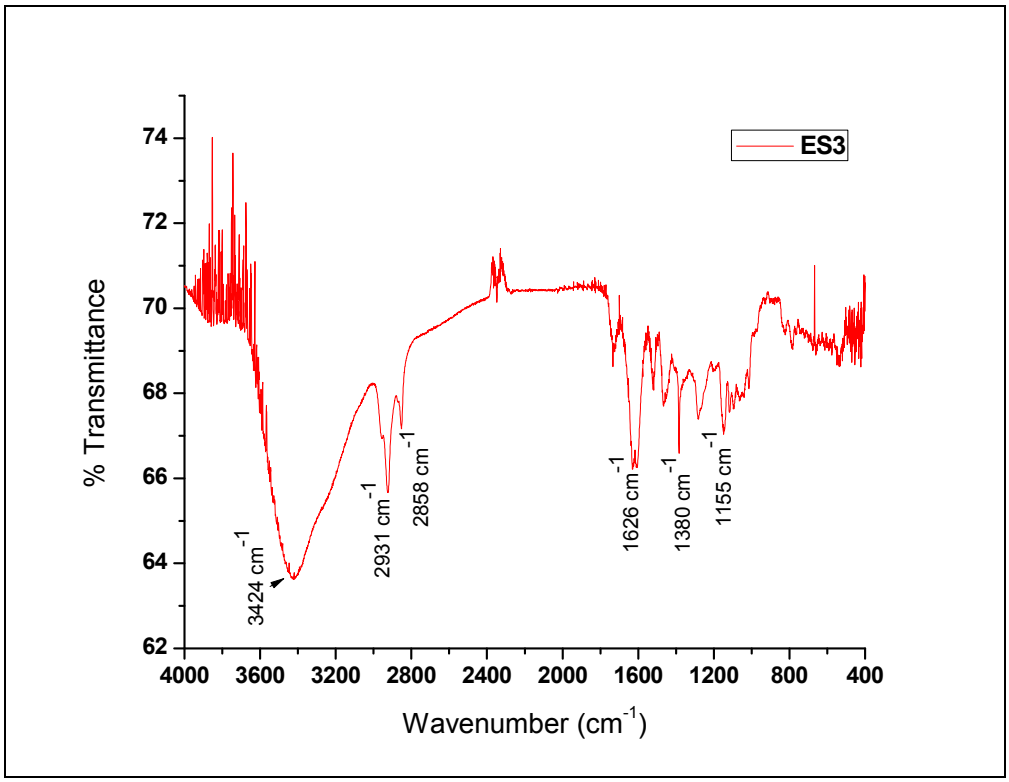
Appendix 9: DEPT 135-NMR spectrum of ES4



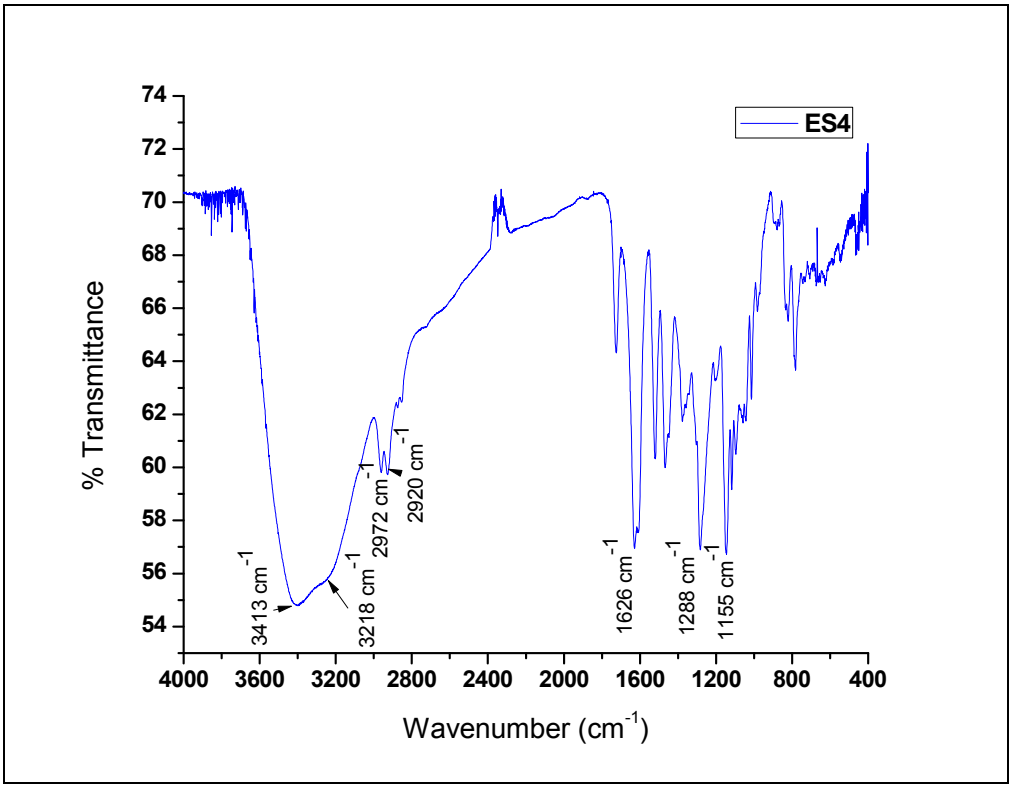
Appendix 10: FT-IR spectrum of ES1



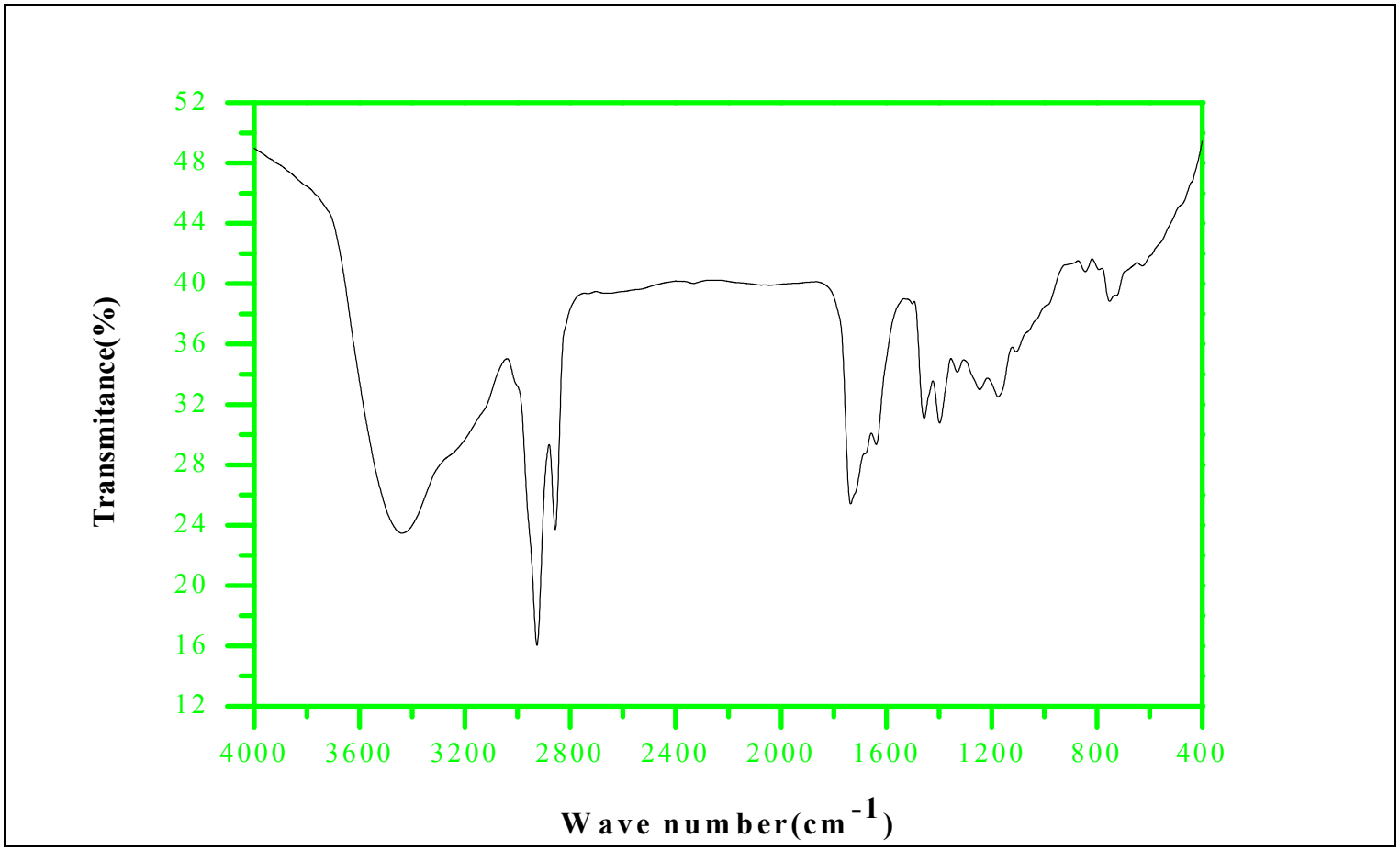
Appendix 11: FT-IR spectrum of compound **2**



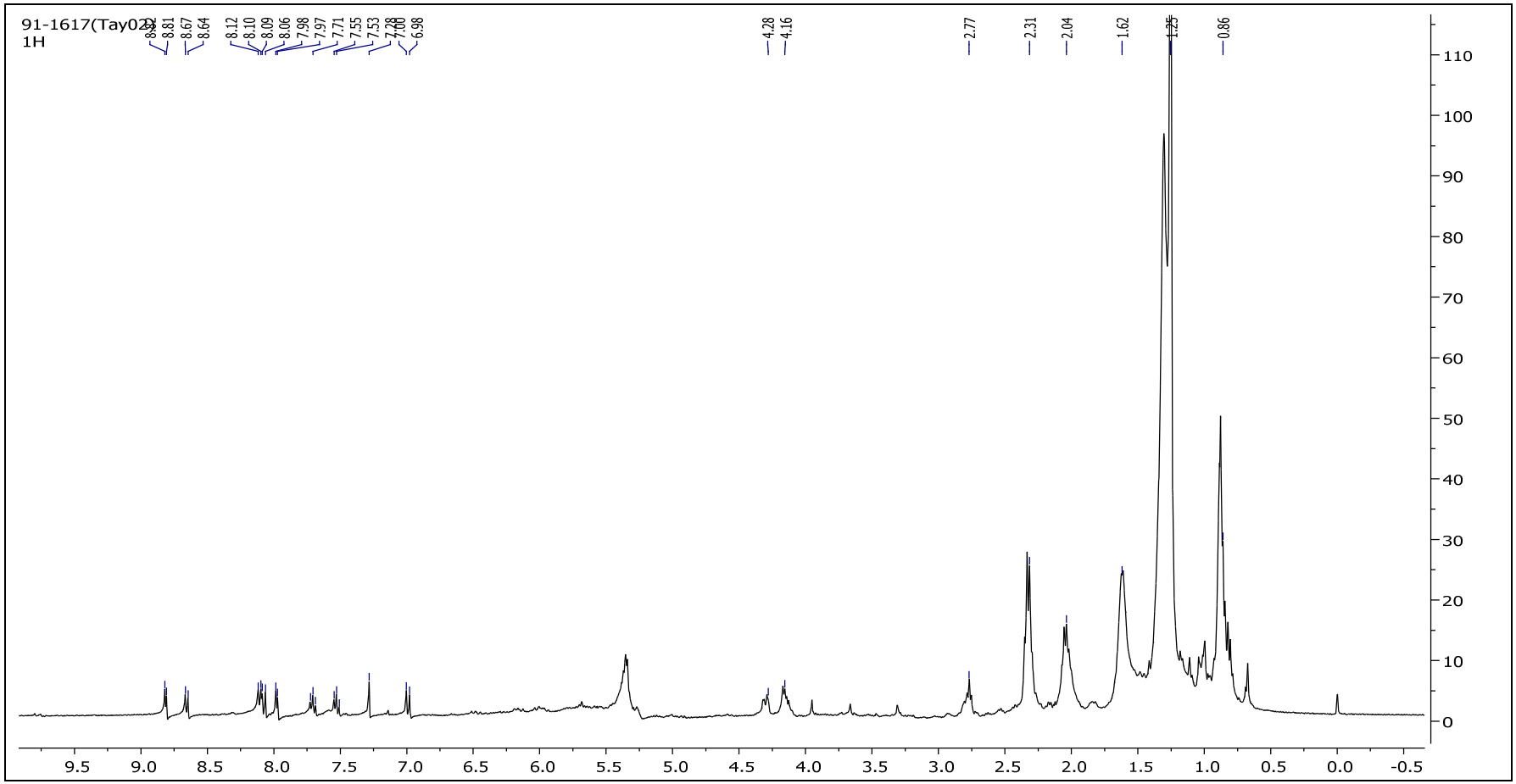
Appendix 12: FT-IR spectrum of compound **2**


ES4
% Transmittance
Wavenumber (cm-1)
3413 cm-1
3218 cm-1
2972 cm-1
2920 cm-1
1626 cm-1
1288 cm-1
1155 cm-1
74
72
70
68
66
64
62
60
58
56
54
4000
3600
3200
2800
2400
2000
1600
1200
800
400

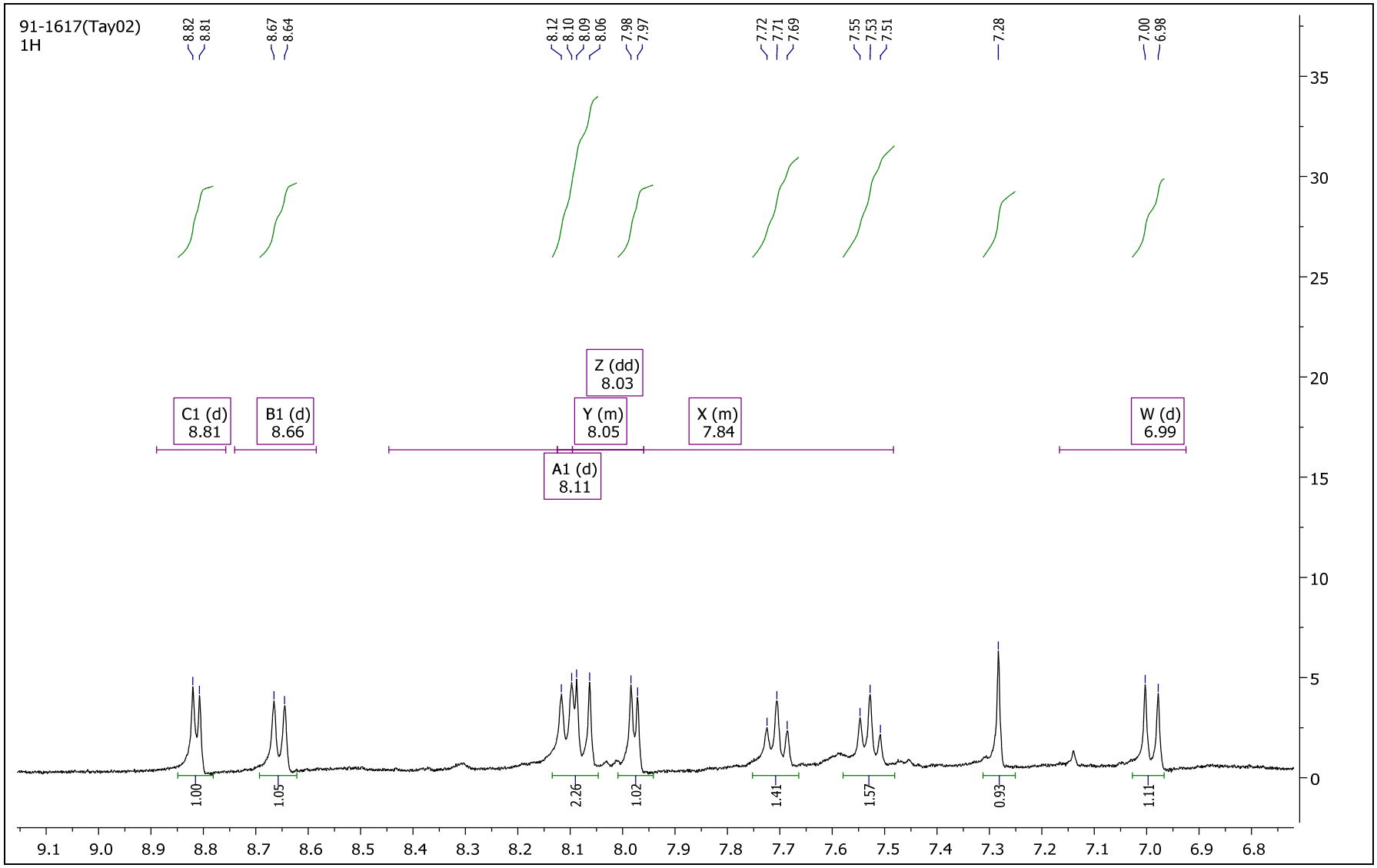
Appendix 13: IR spectrum of derivative of flazin methyl ether **(3)**



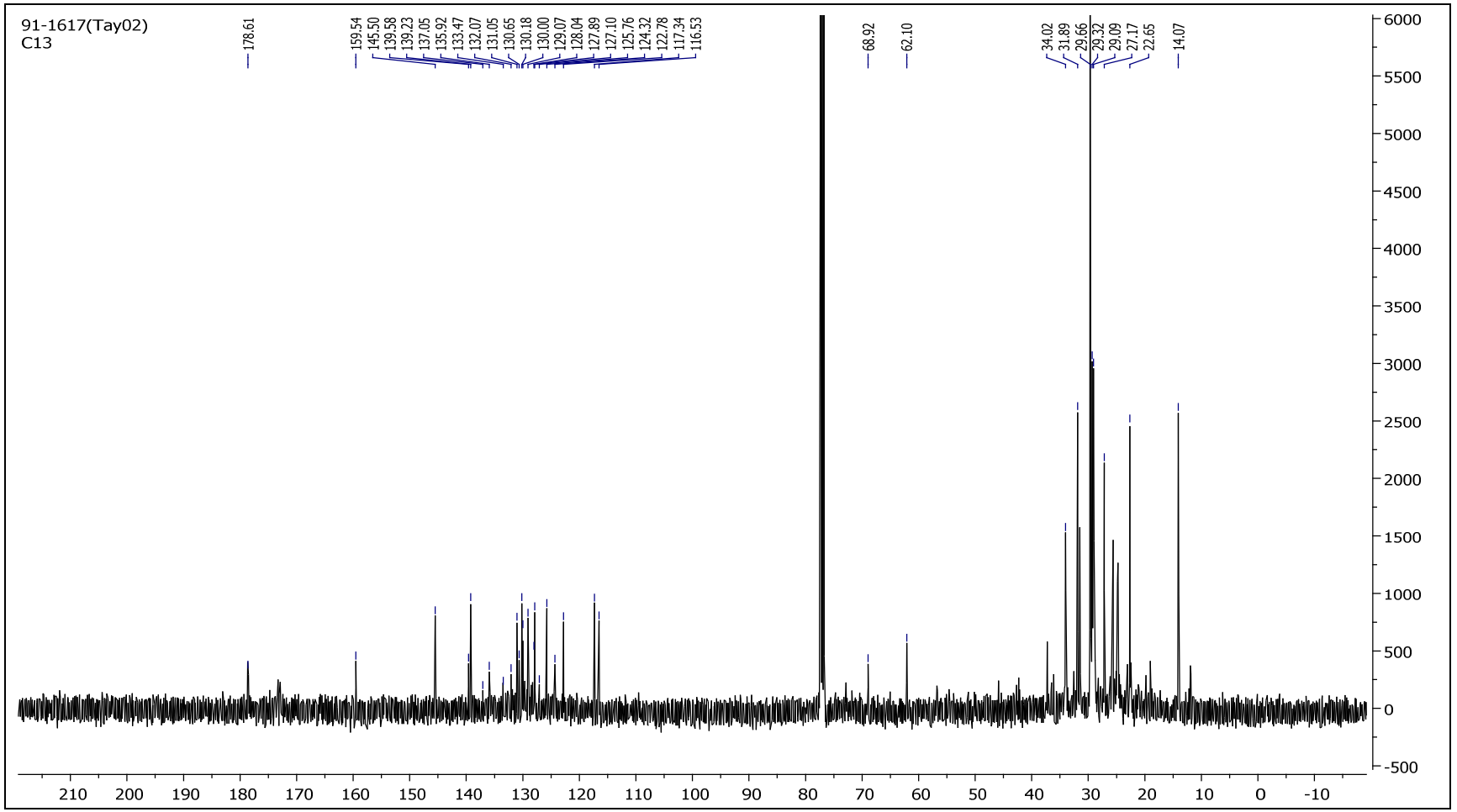
Appendix 14: ^{1}H NMR spectrum of derivative of flazin methyl ether **(3)**



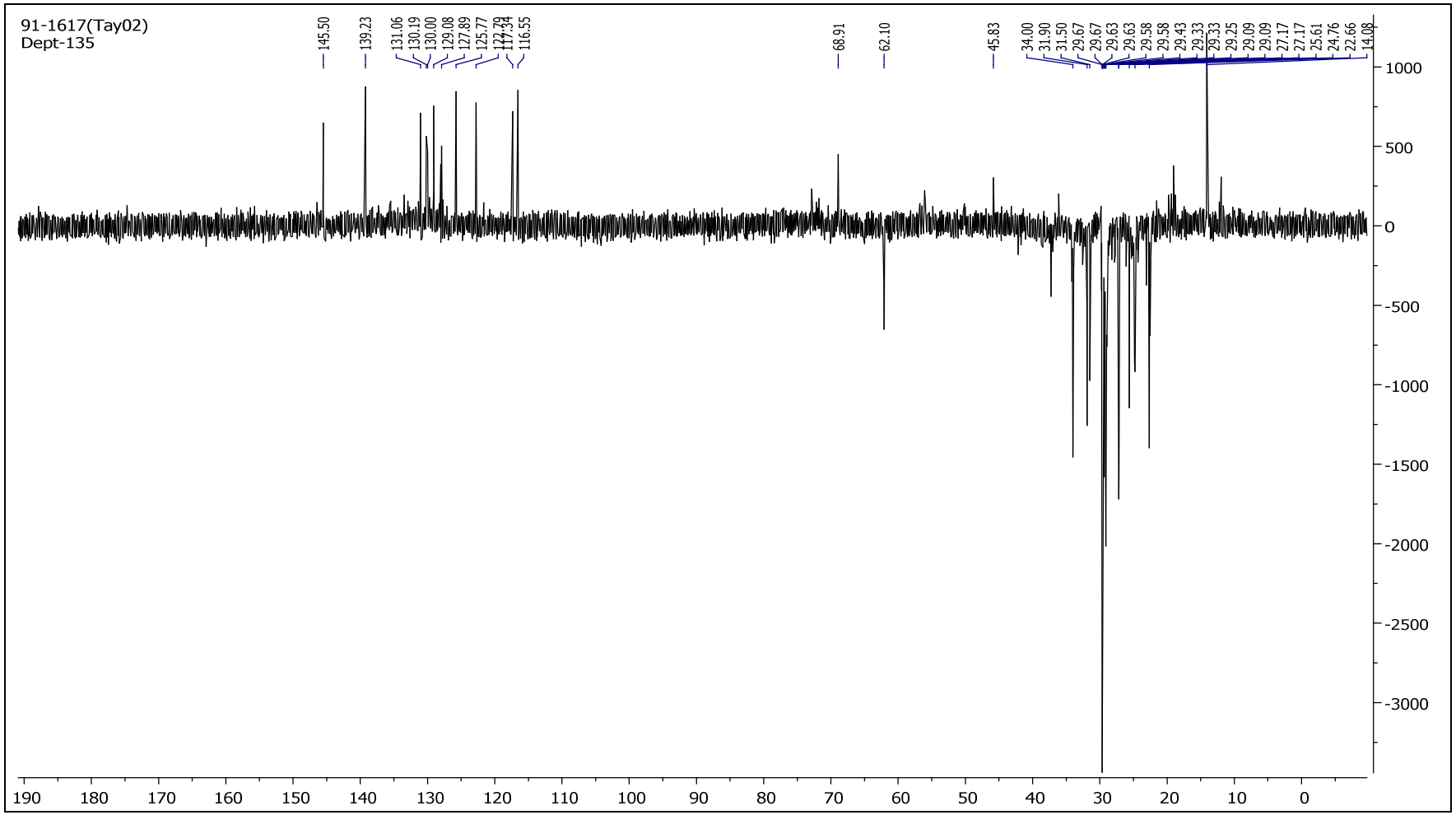
Appendix 15: ^{1}H NMR (from 6-9 ppm) spectrum of derivative of flazine methyl ether **(3)**



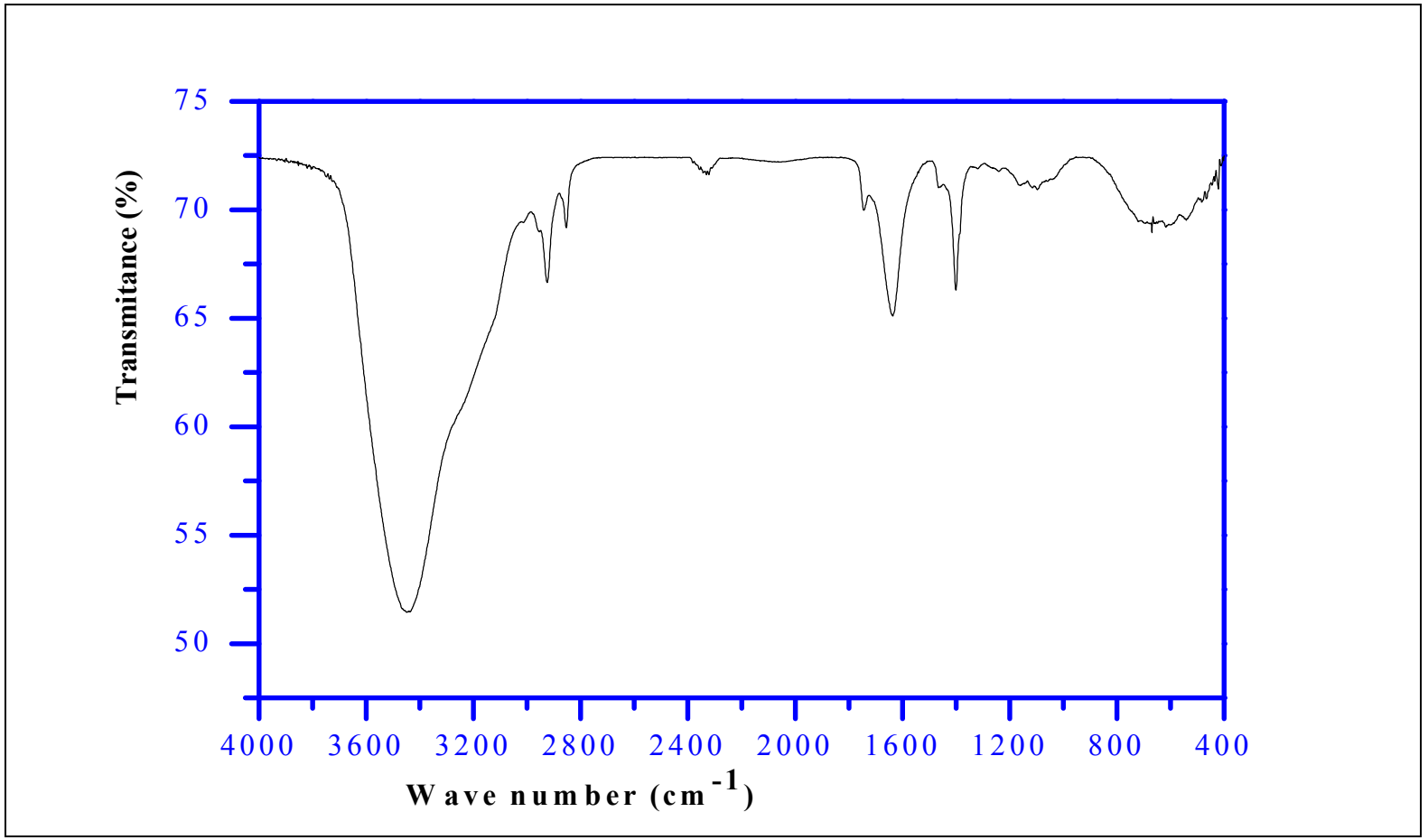
Appendix 16: ^{13}C NMR spectrum of derivative of flazin methyl ether **(3)**



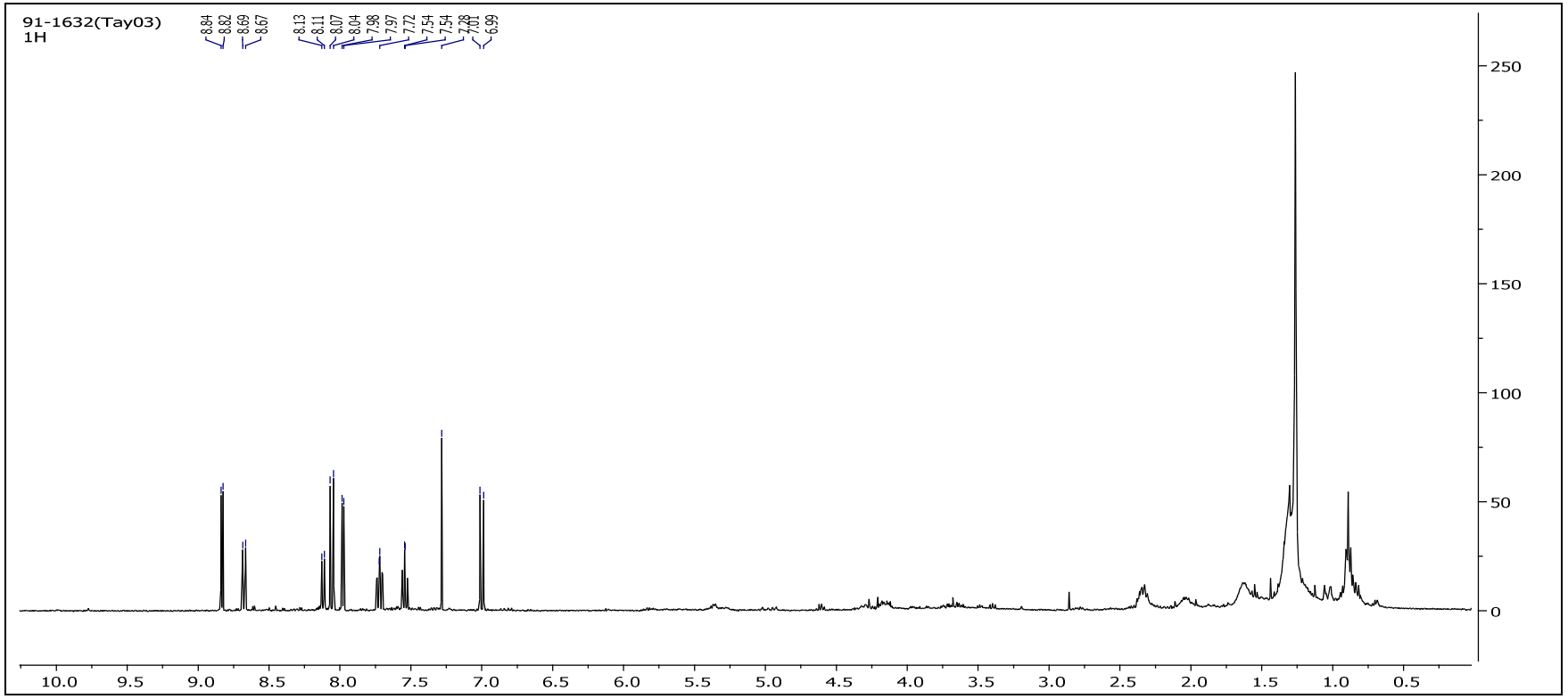
Appendix 17: DEPT-135 spectrum of derivative of flazin methyl ether **(3)**



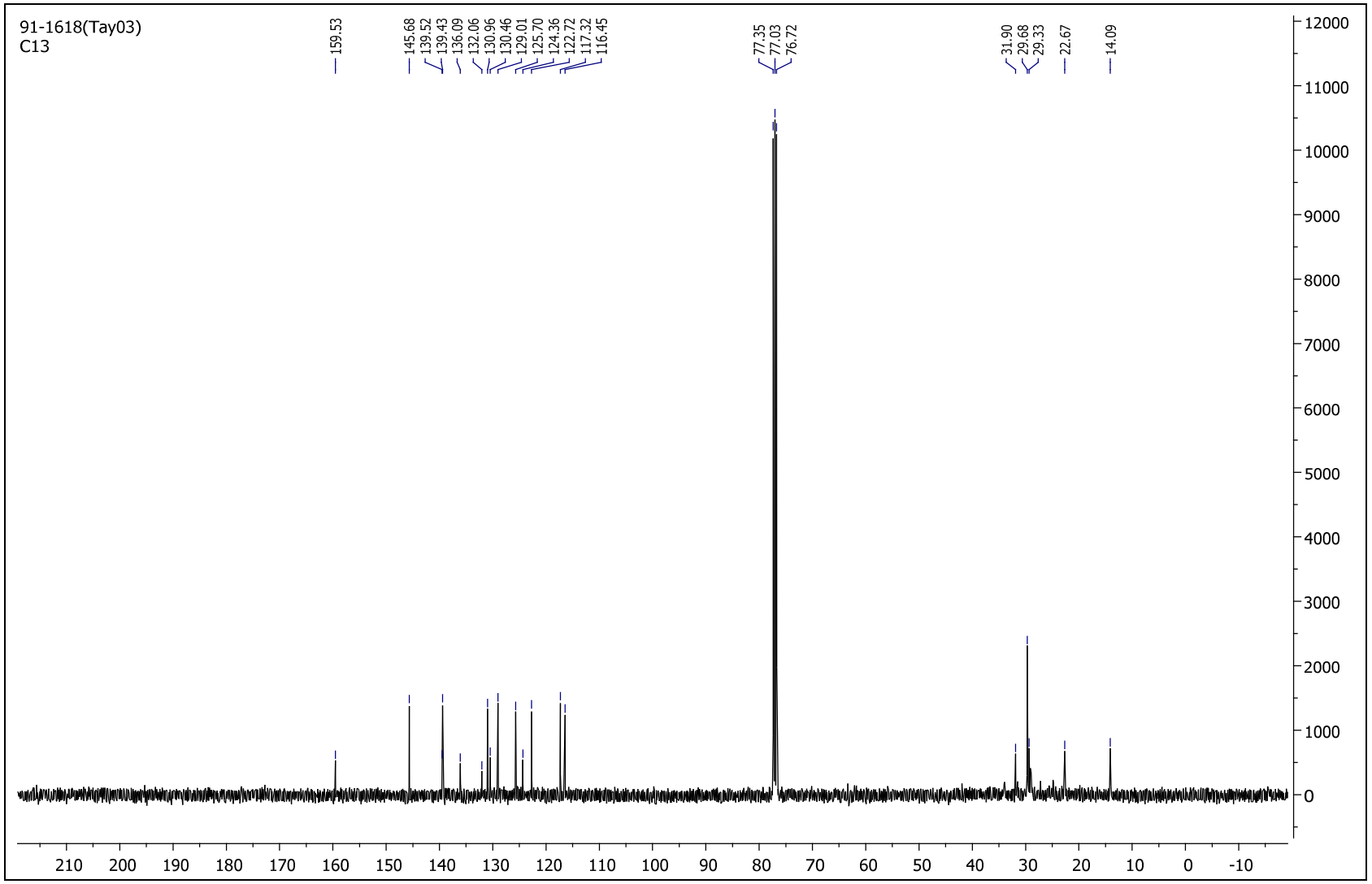
Appendix 18: IR spectrum of canthine-6-one **(4)**



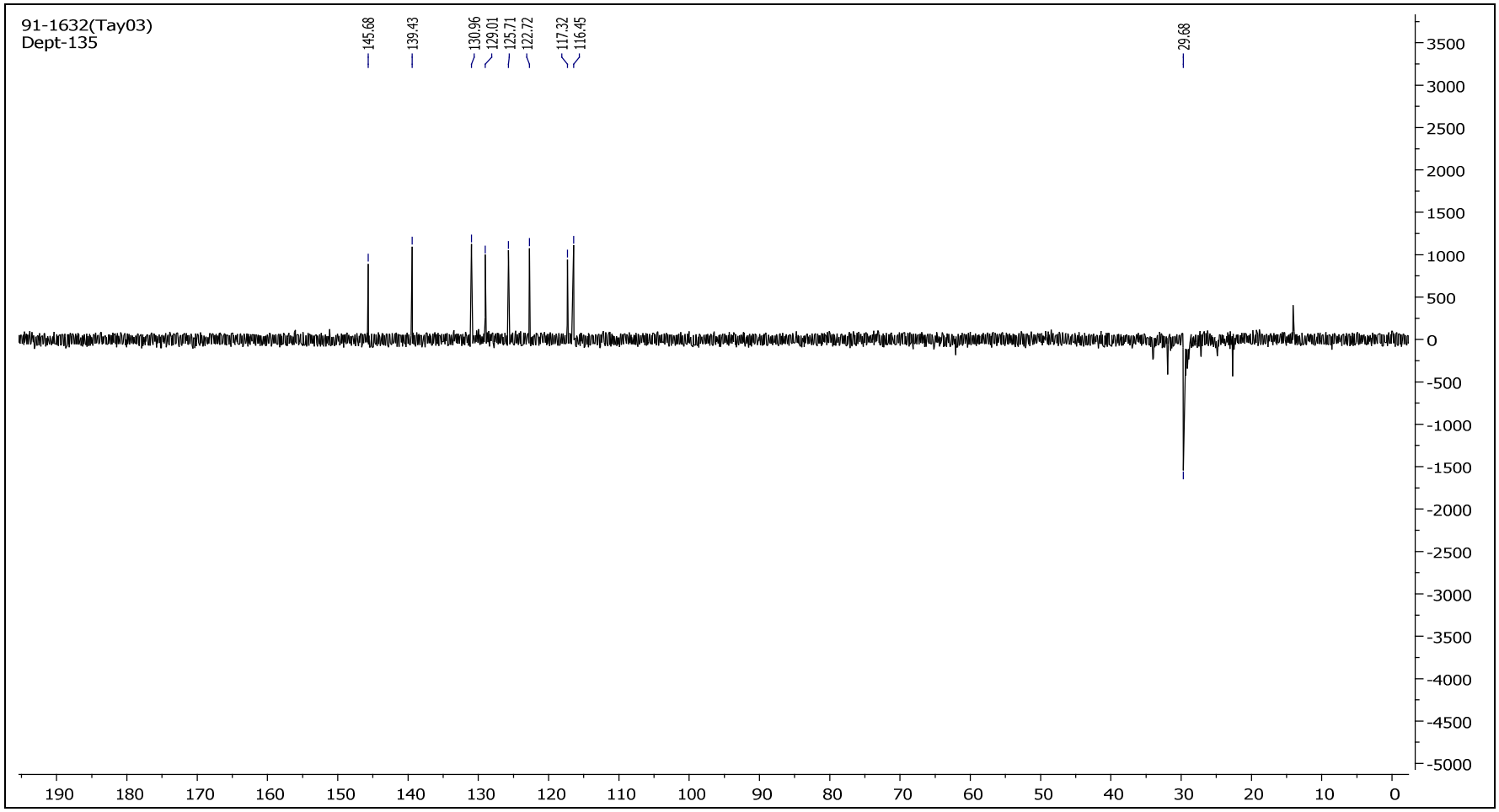
Appendix 19: ^{1}H NMR spectrum of canthine-6-one **(4)**



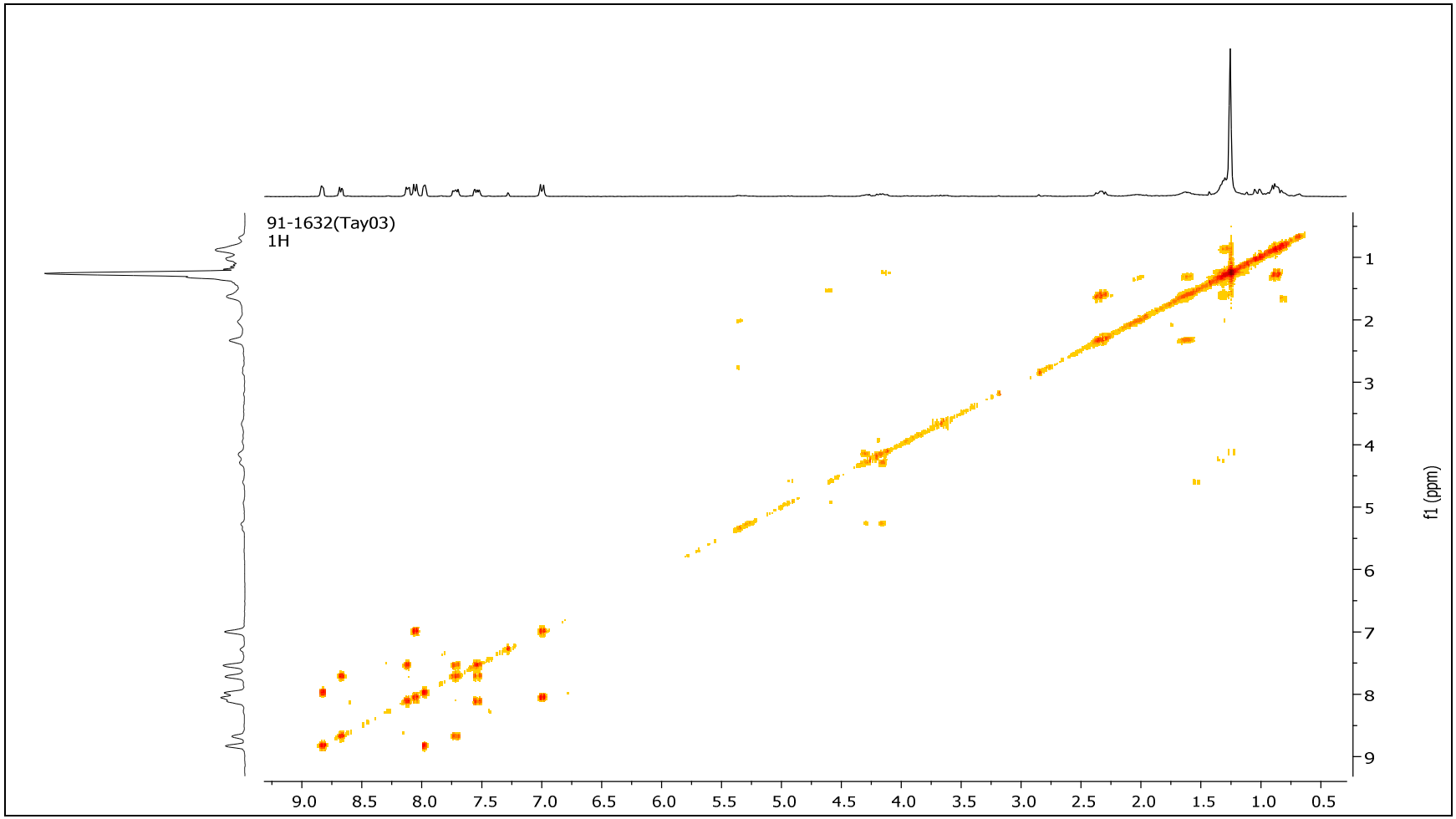
Appendix 20: ^{13}C NMR spectrum of canthine-6-one **(4)**



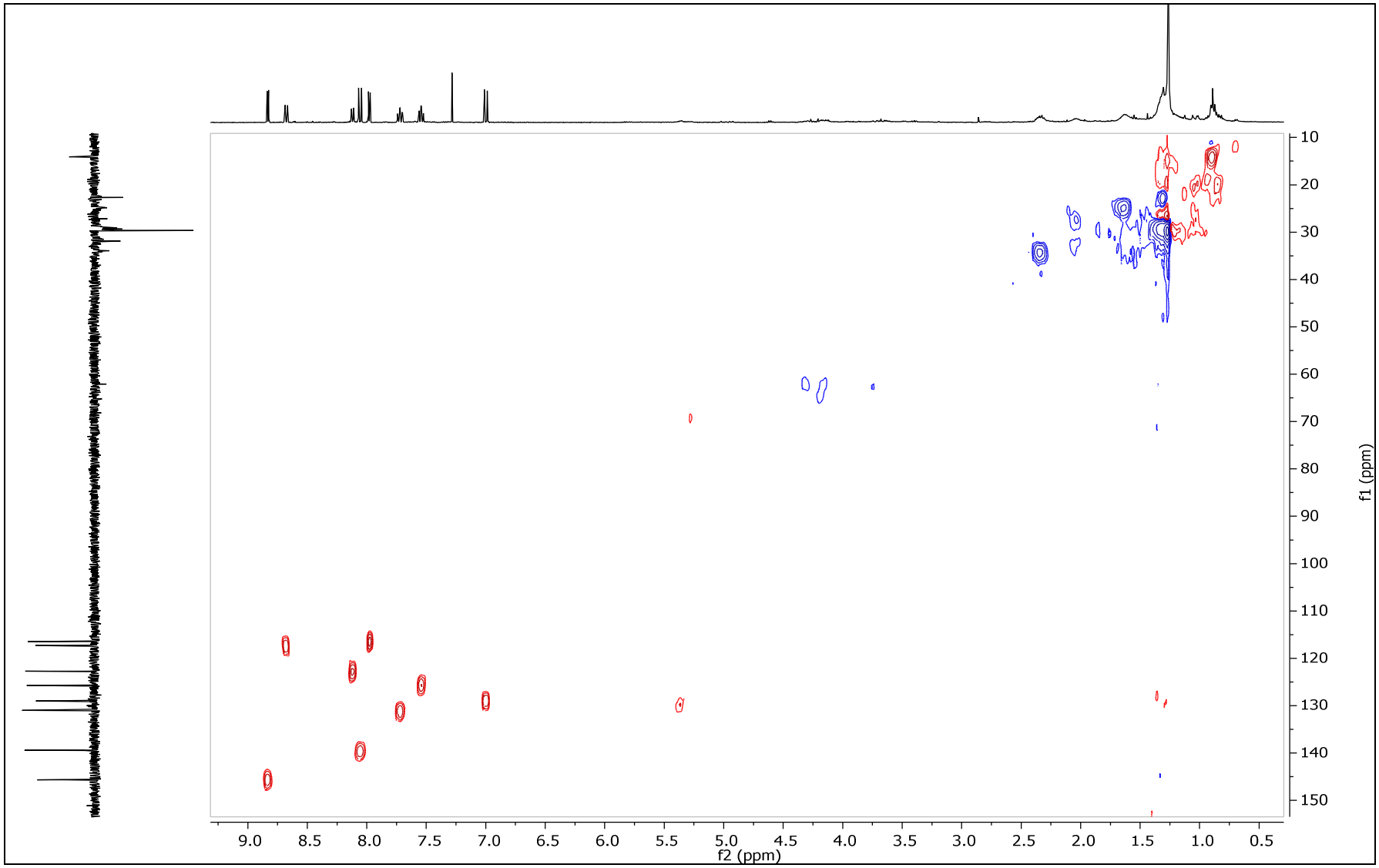
Appendix 21: DEPT-135 spectrum of canthine-6-one **(4)**



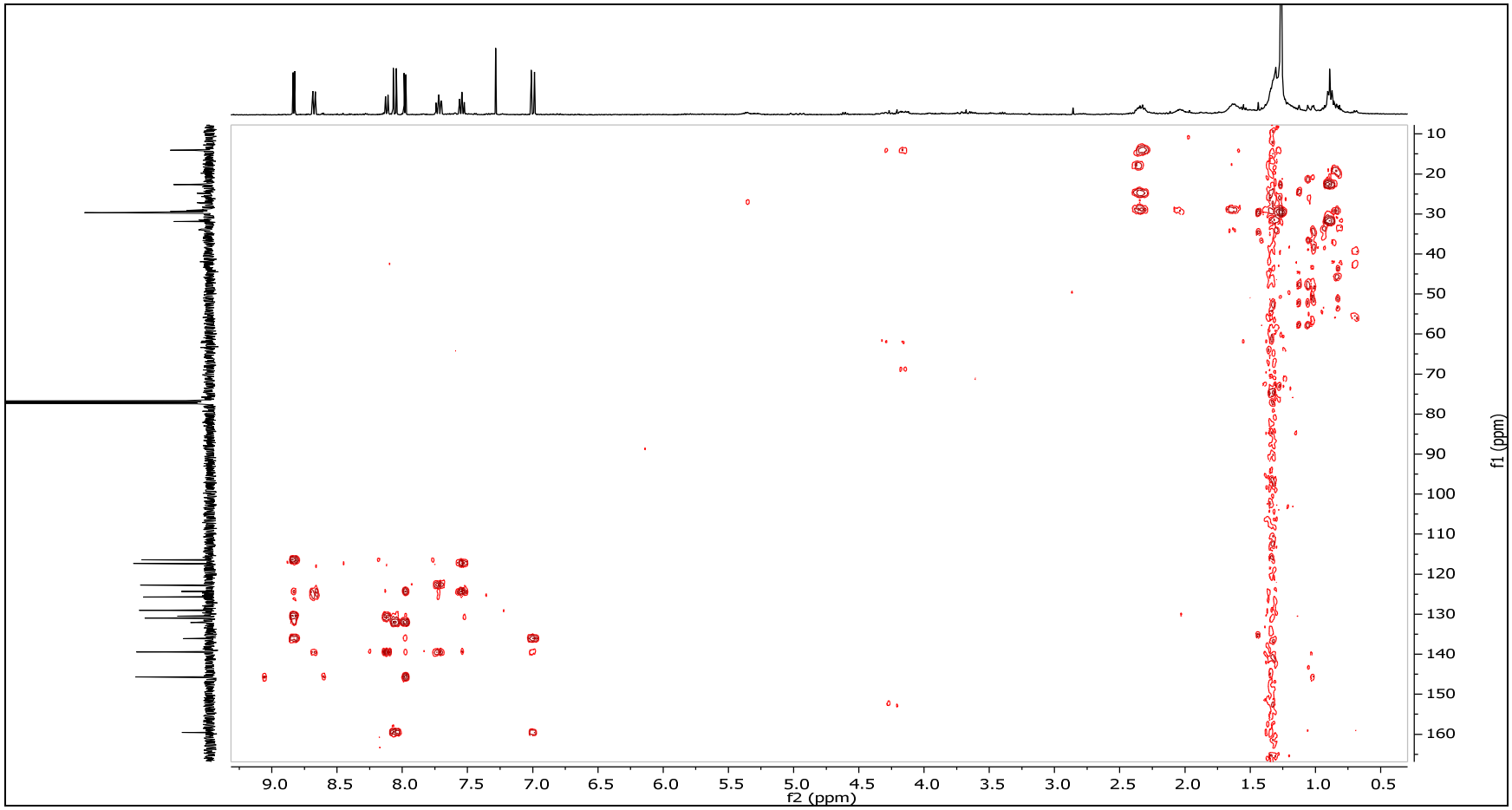
Appendix 22:: COSY spectrum of canthine-6-one **(4)**



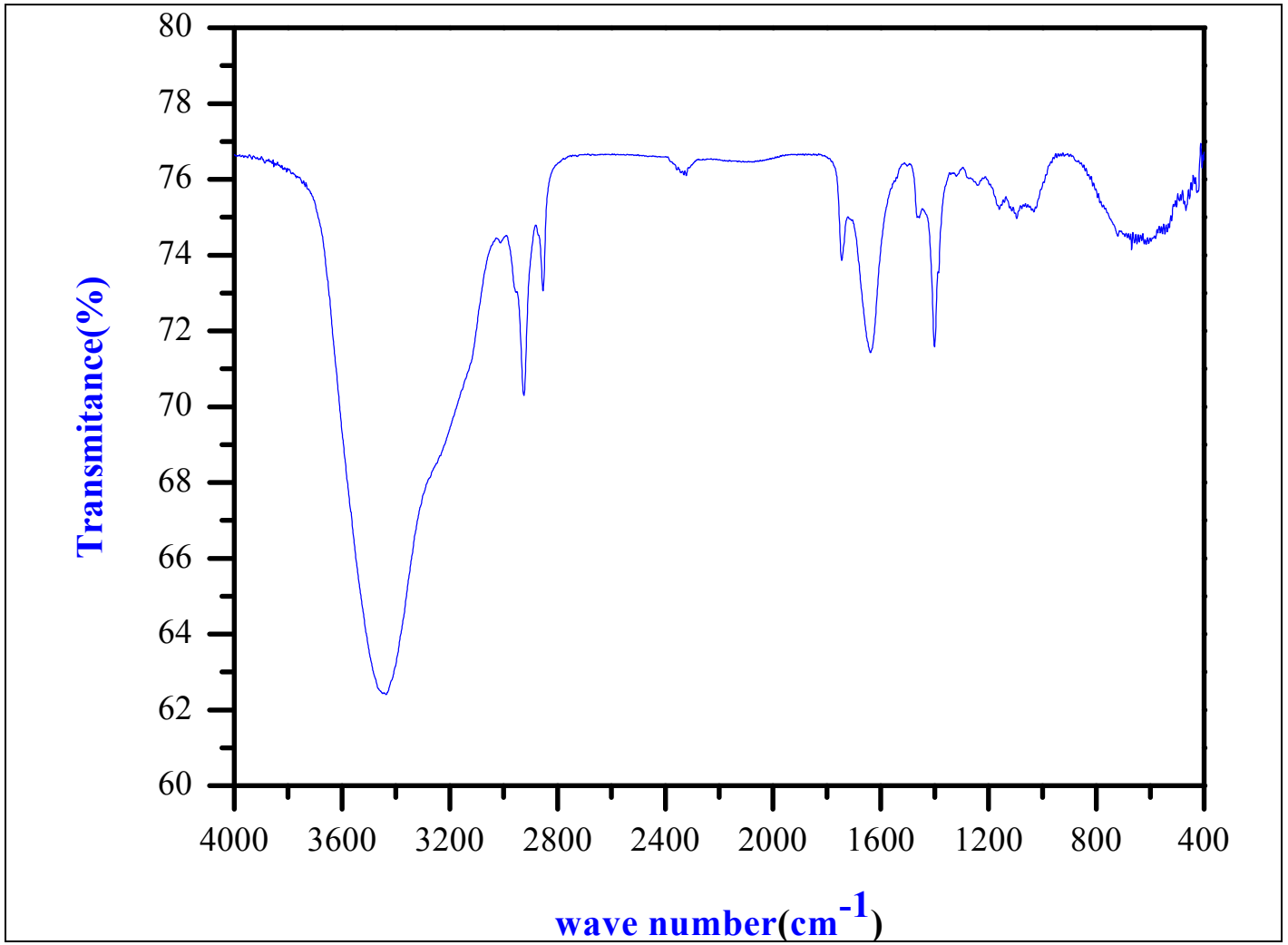
Appendix 23: HSQC spectrum of canthine-6-one **(4)**



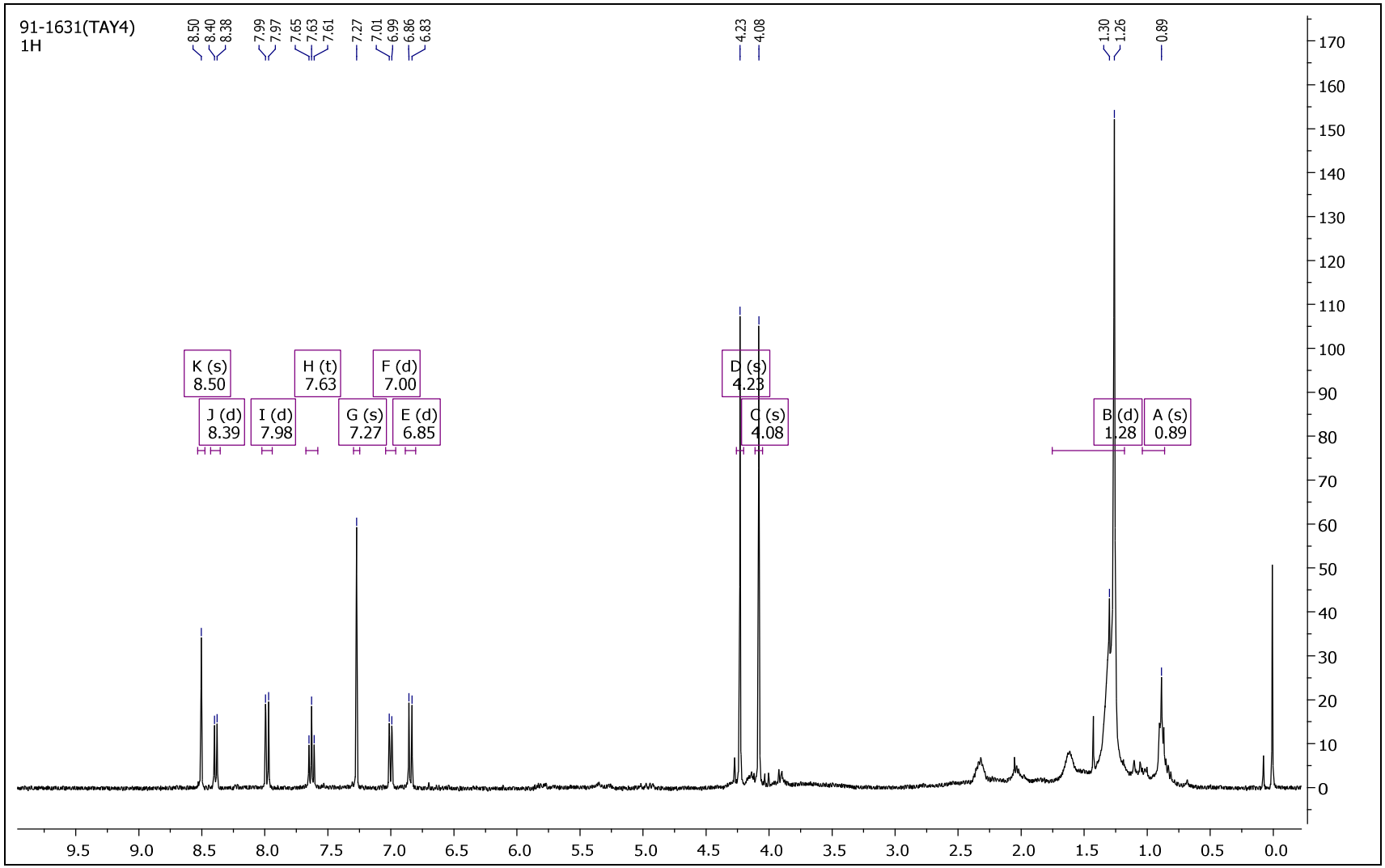
Appendix 24: HMBC spectrum of canthine-6-one **(4)**



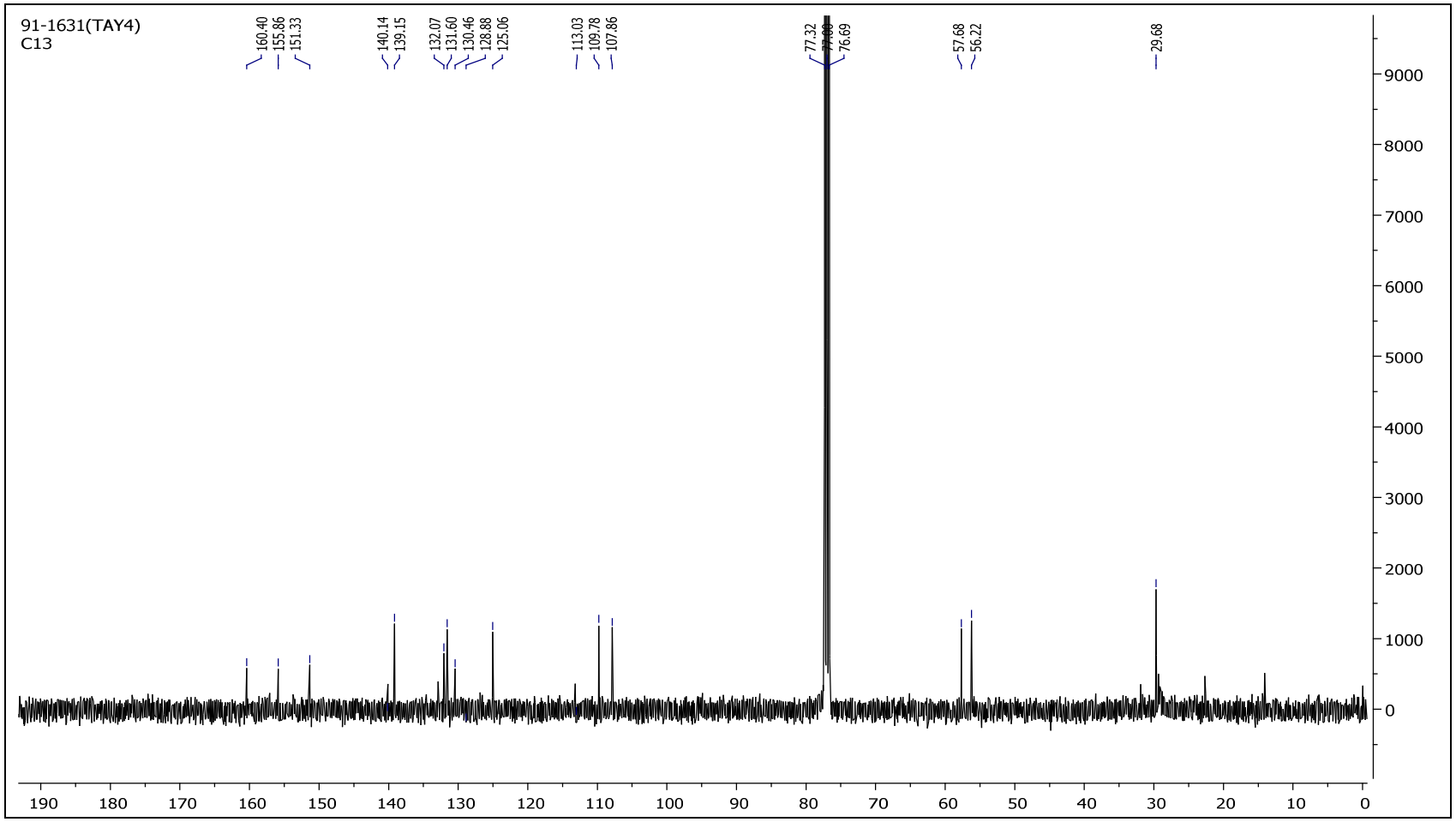
Appendix 25: IR spectrum of 1,11-dimethoxycanthin-6-one **(5)**



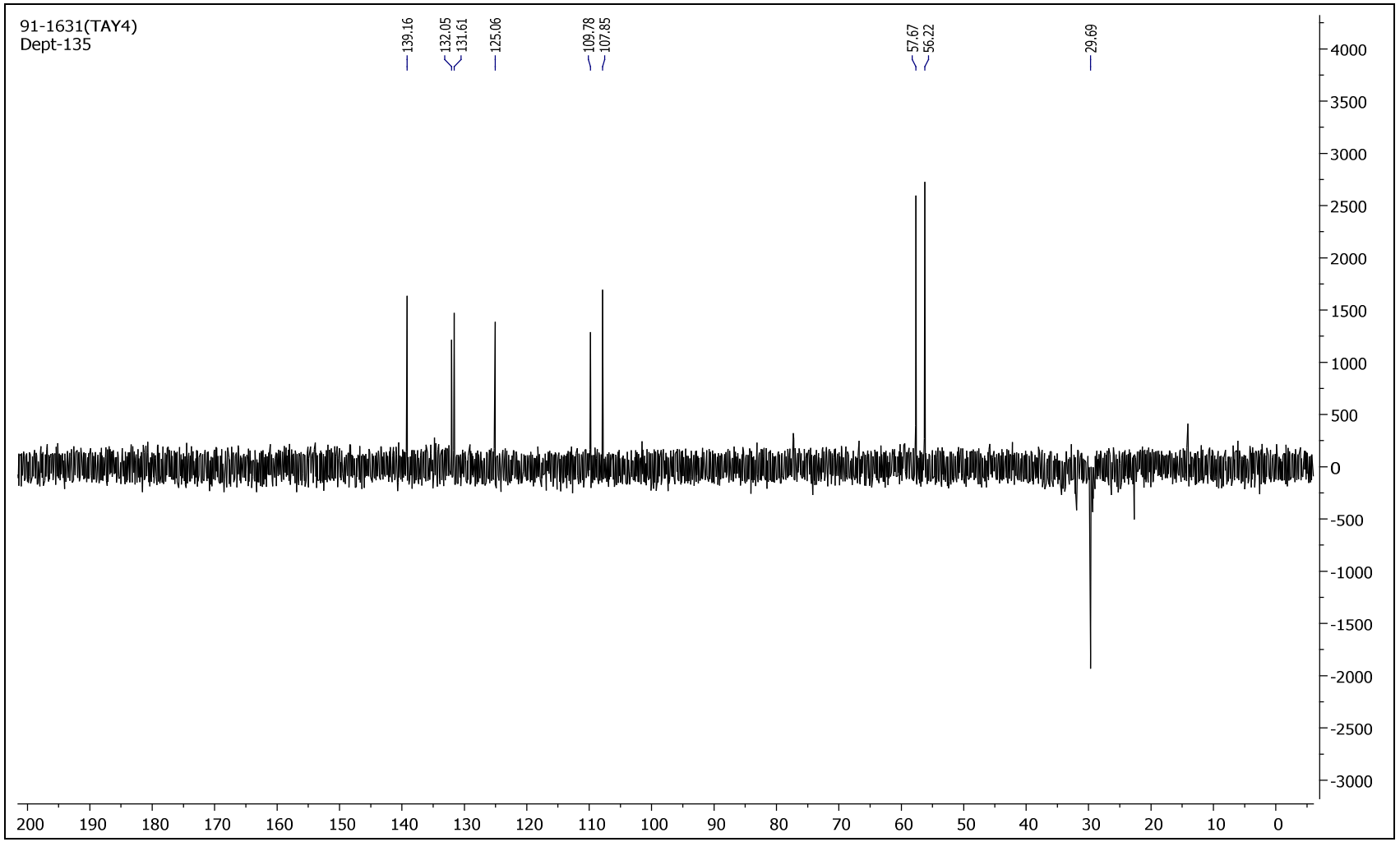
Appendix 26: ^{1}H NMR spectrum of 1,11-dimethoxycanthin-6-one **(5)**



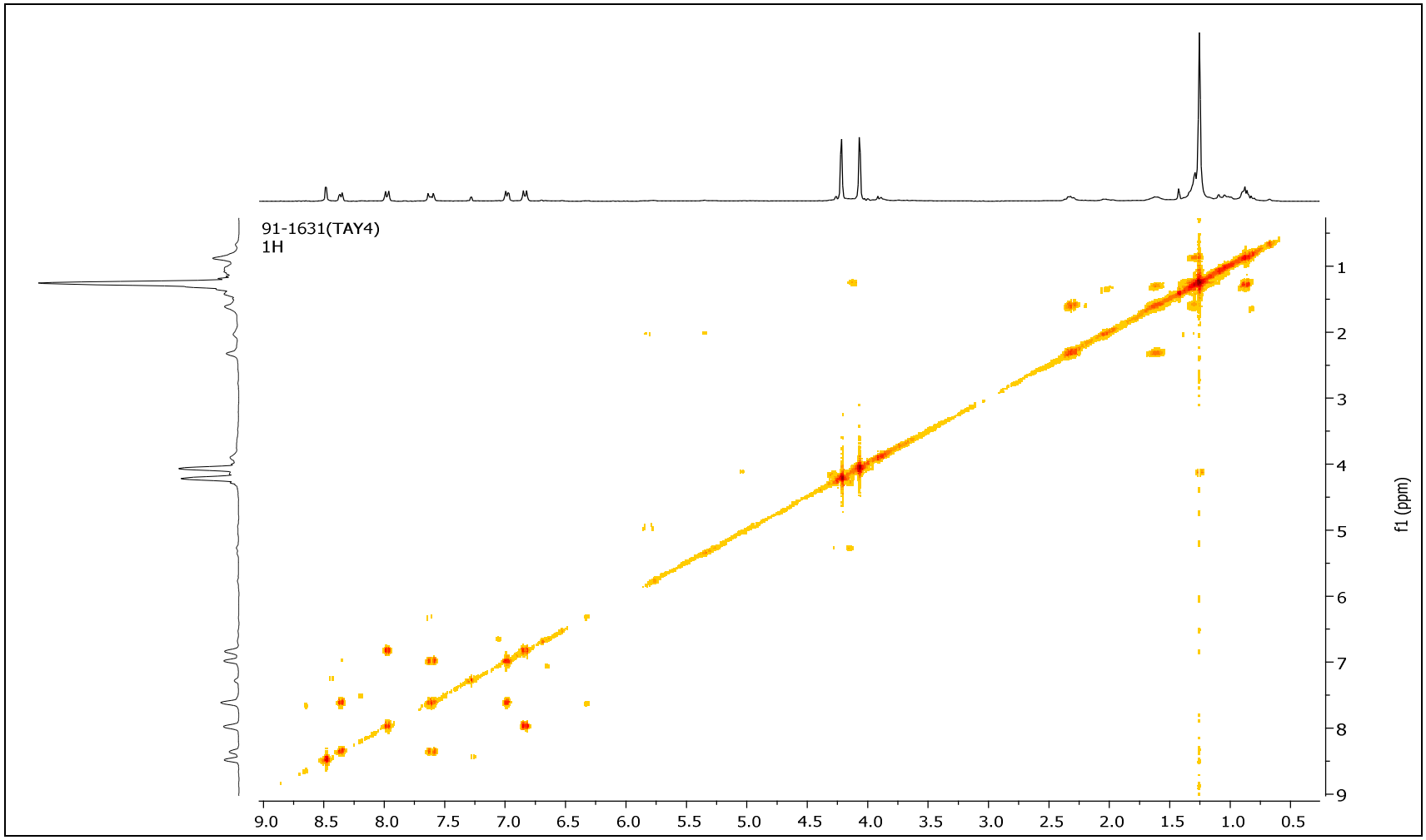
Appendix 27: ^{13} C NMR spectrum of 1,11-dimethoxycanthin-6-one **(5)**



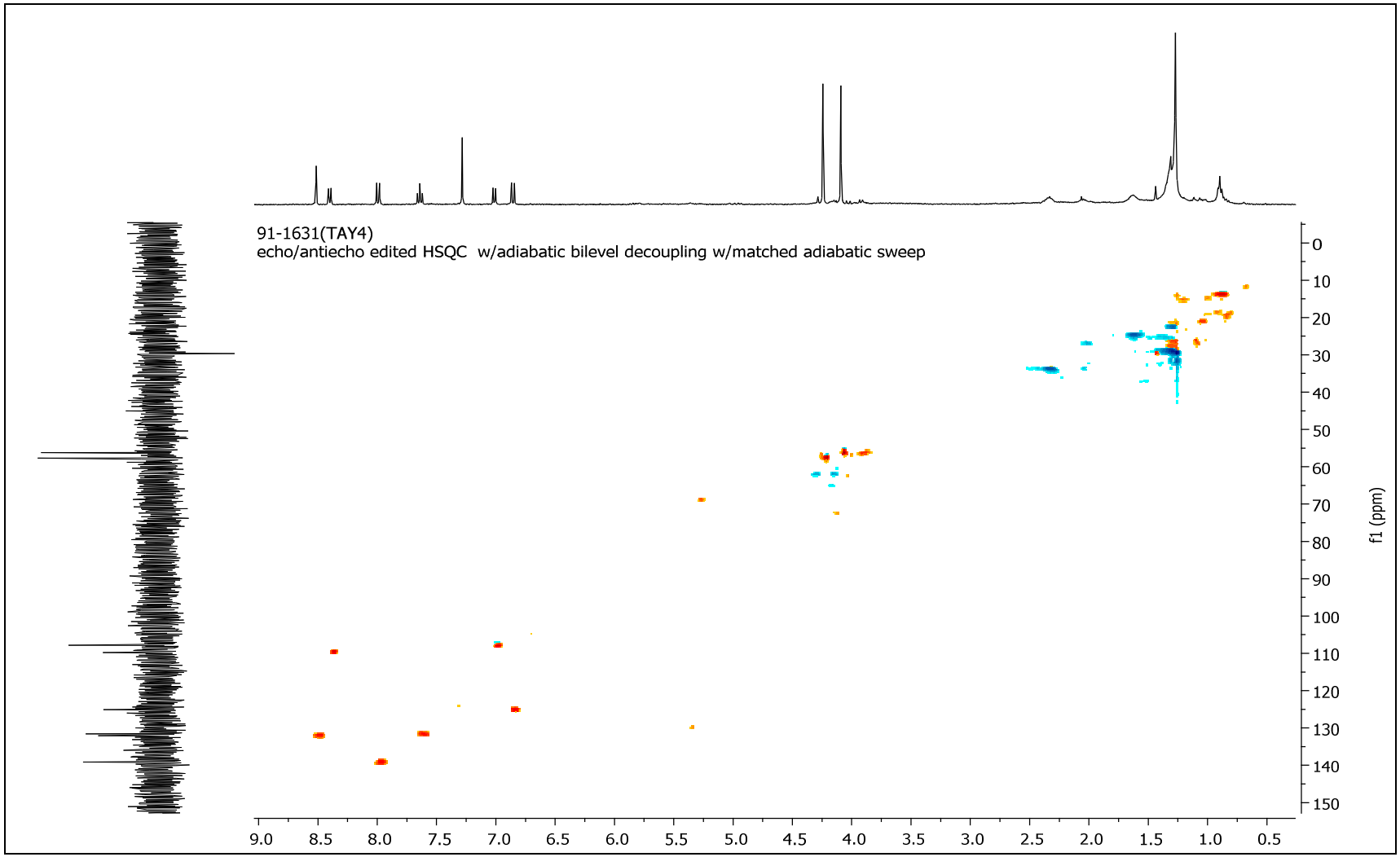
Appendix 28: DEPT-135 spectrum of 1,11-dimethoxycanthin-6-one **(5)**



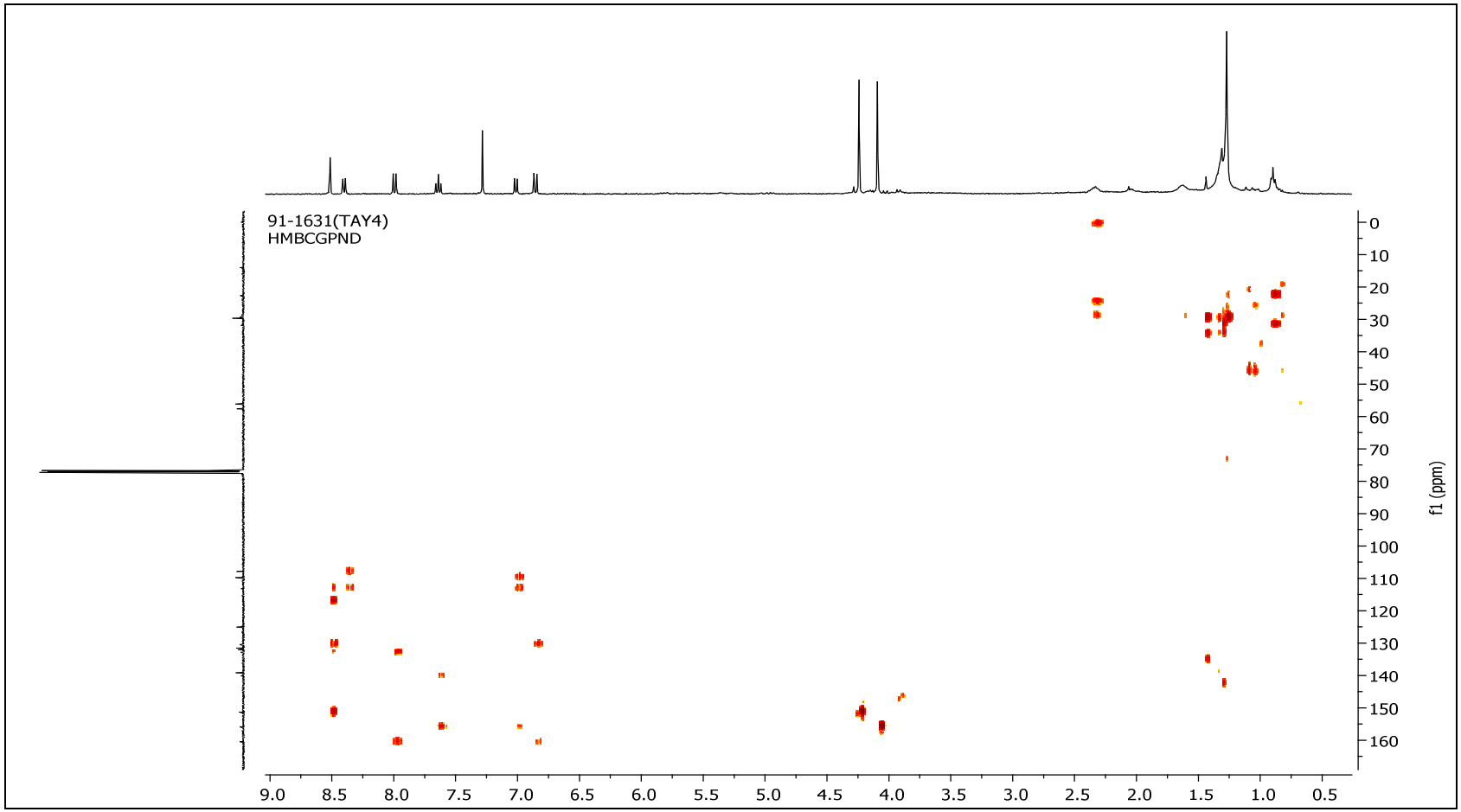
Appendix 29: COSY spectrum of 1,11-dimethoxycanthin-6-one **(5)**



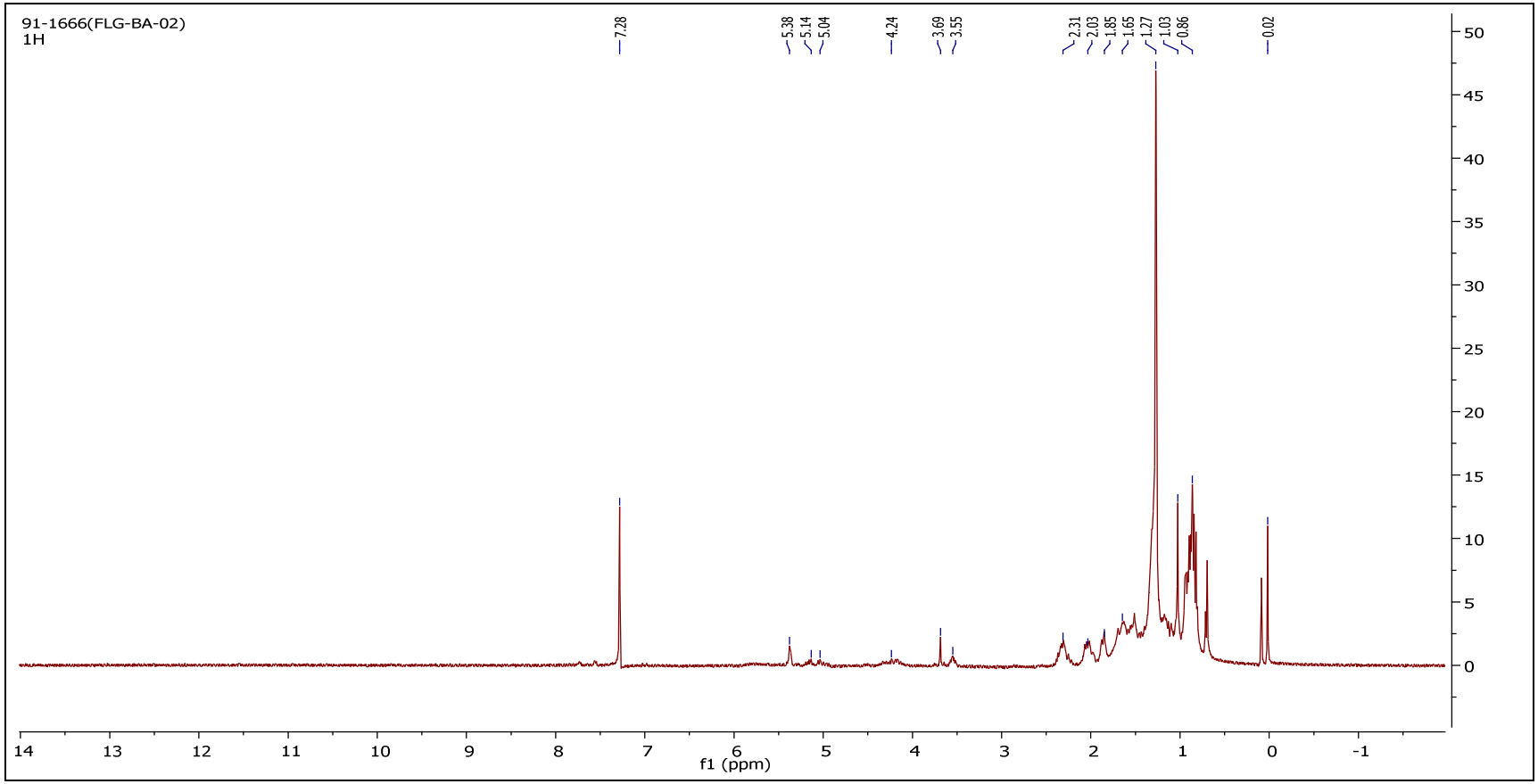
Appendix 30: HSQC spectrum of 1,11-dimethoxycanthin-6-one **(5)**



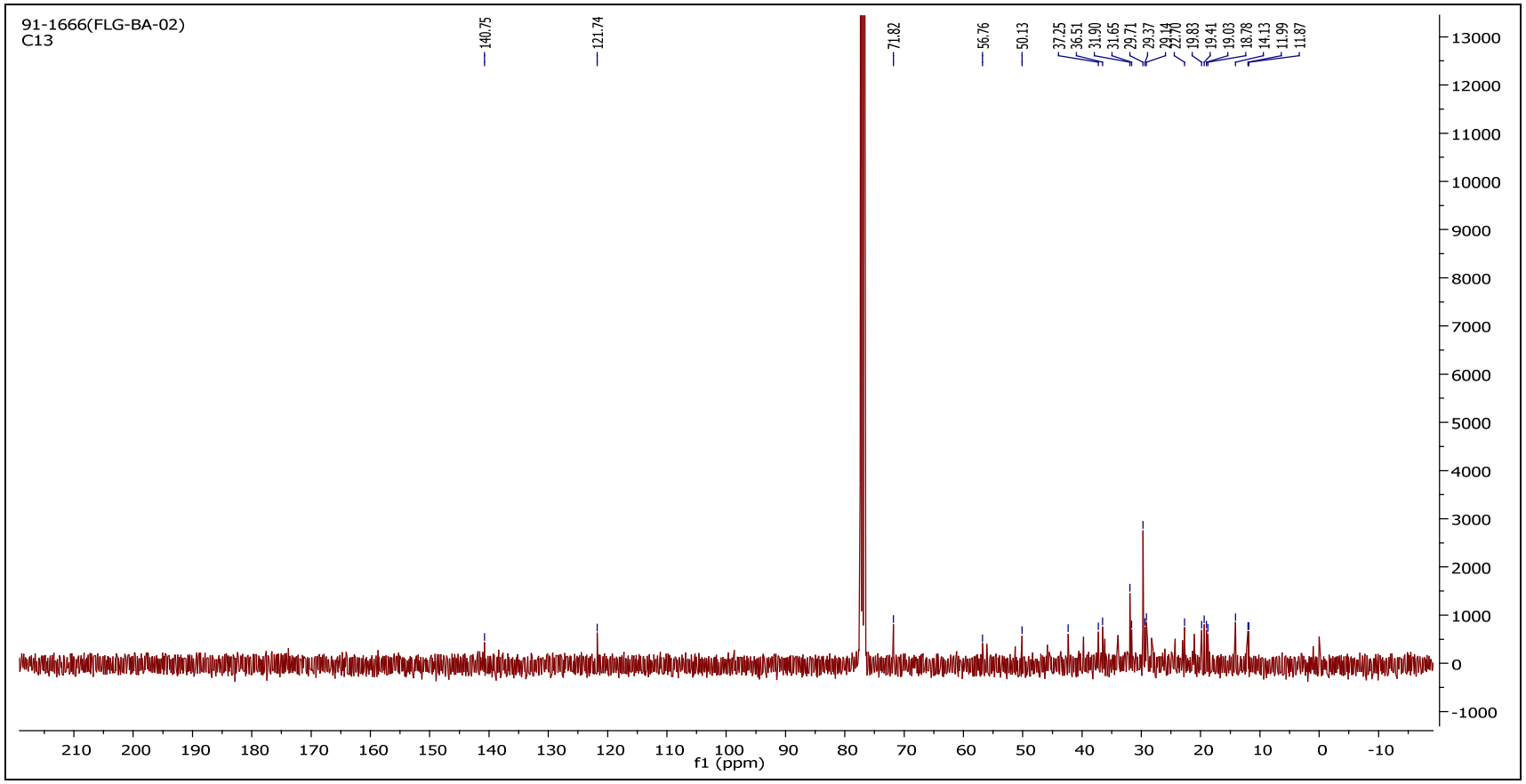
Appendix 31: HMBC spectrum of 1,11-dimethoxycanthin-6-one **(5)**



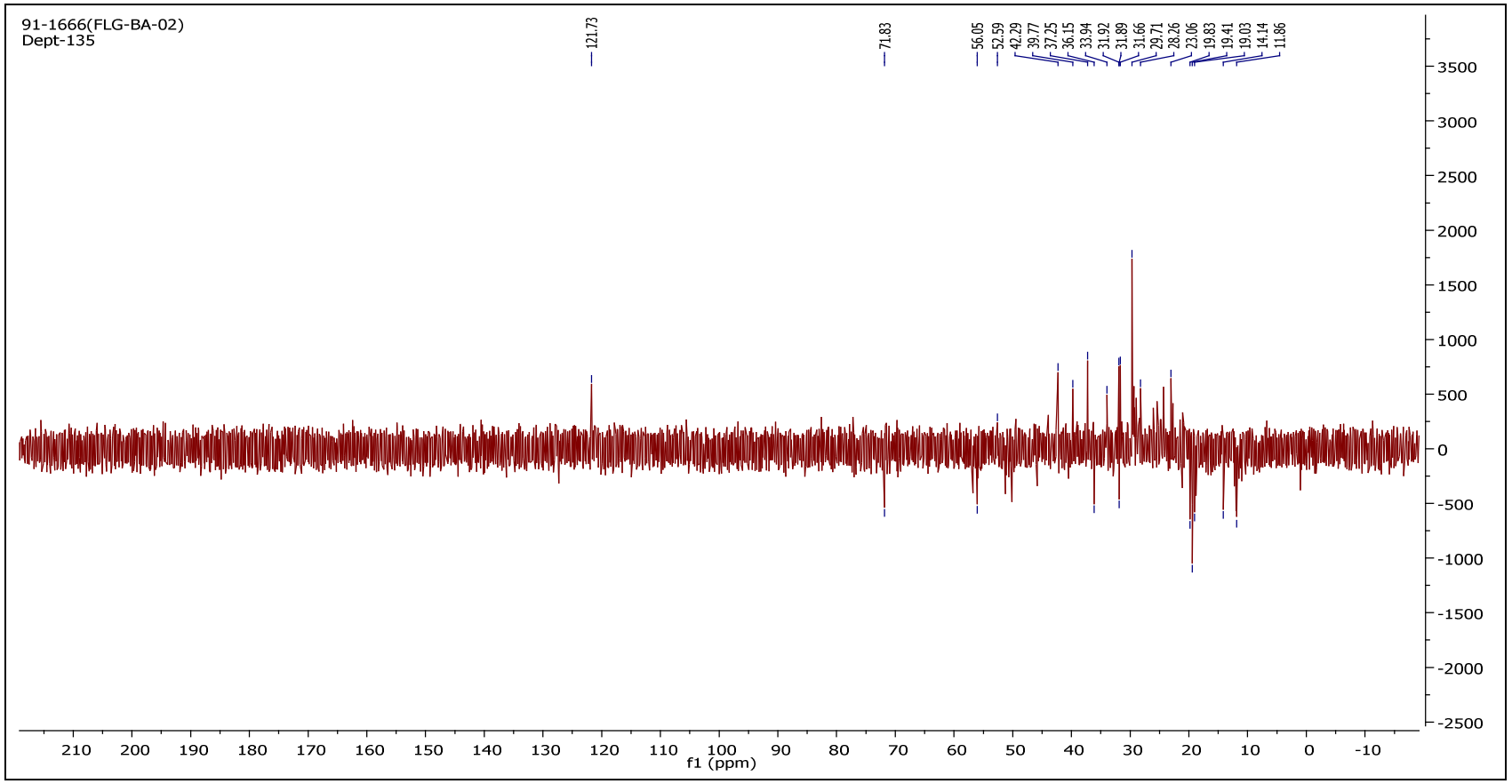
Appendix 32: ^{1}H NMR spectrum of compound **6**



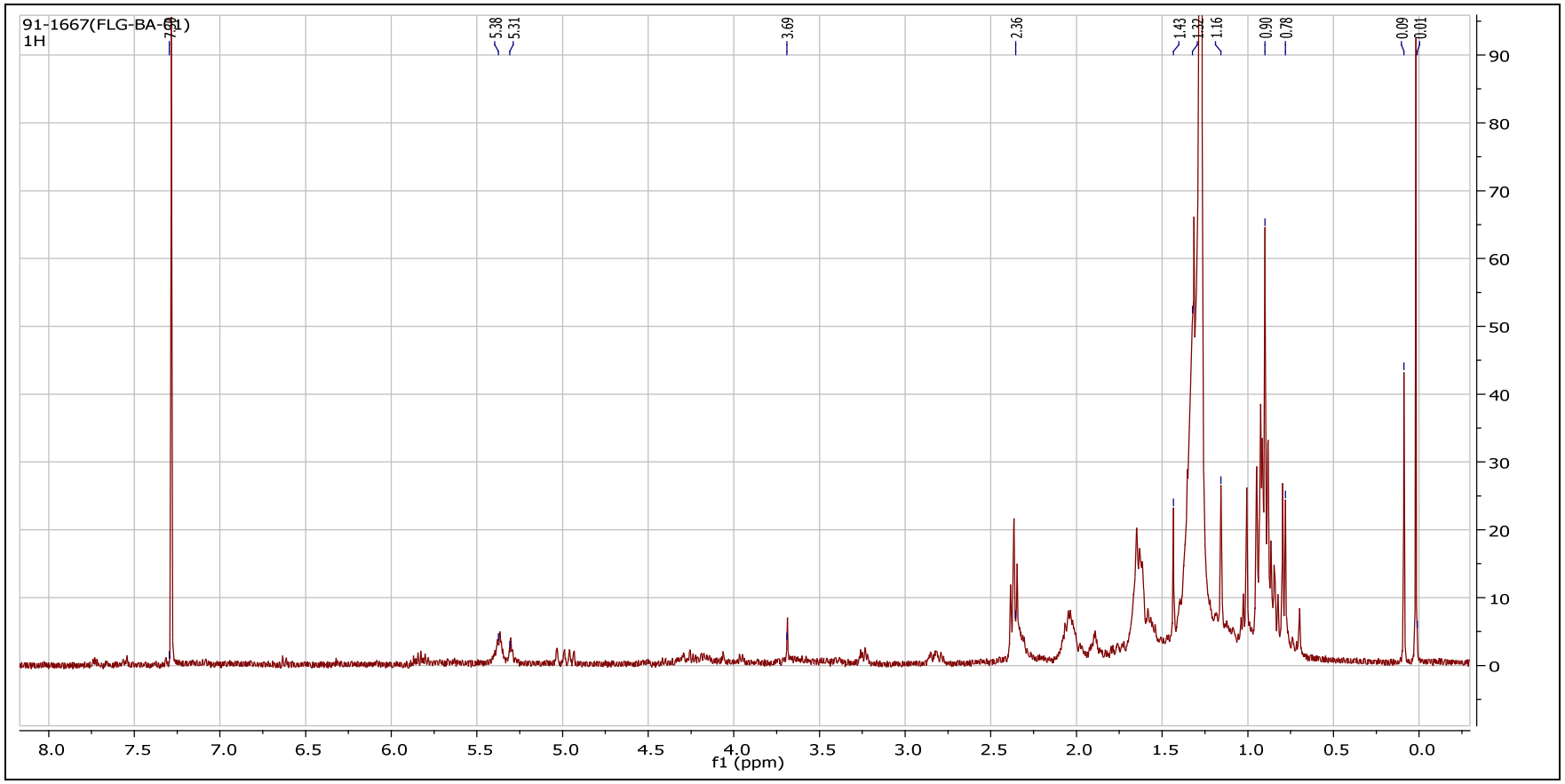
Appendix 33: ^{13}C spectrum of compound **6**



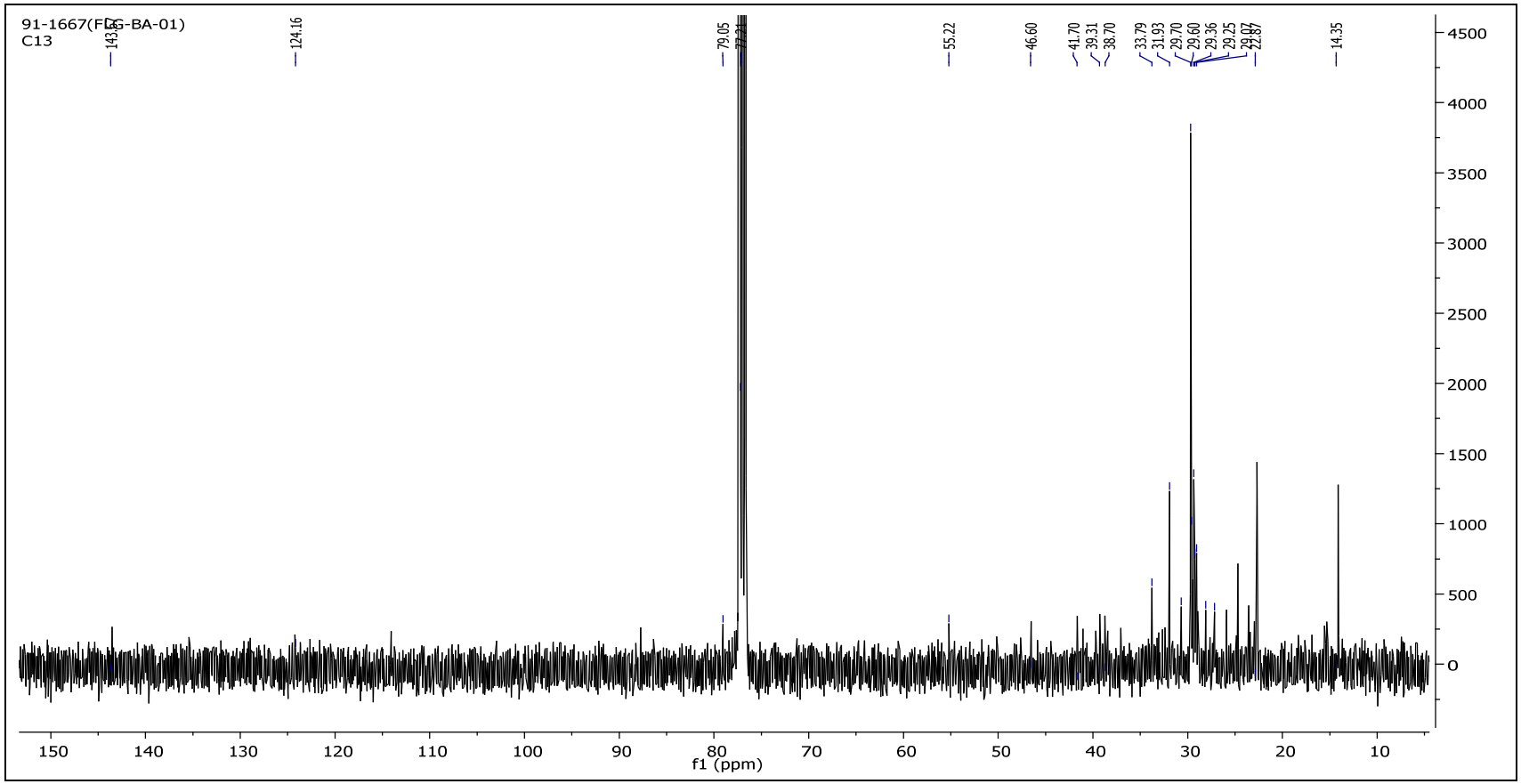
Appendix 34: DEPT-135 of compound **6**



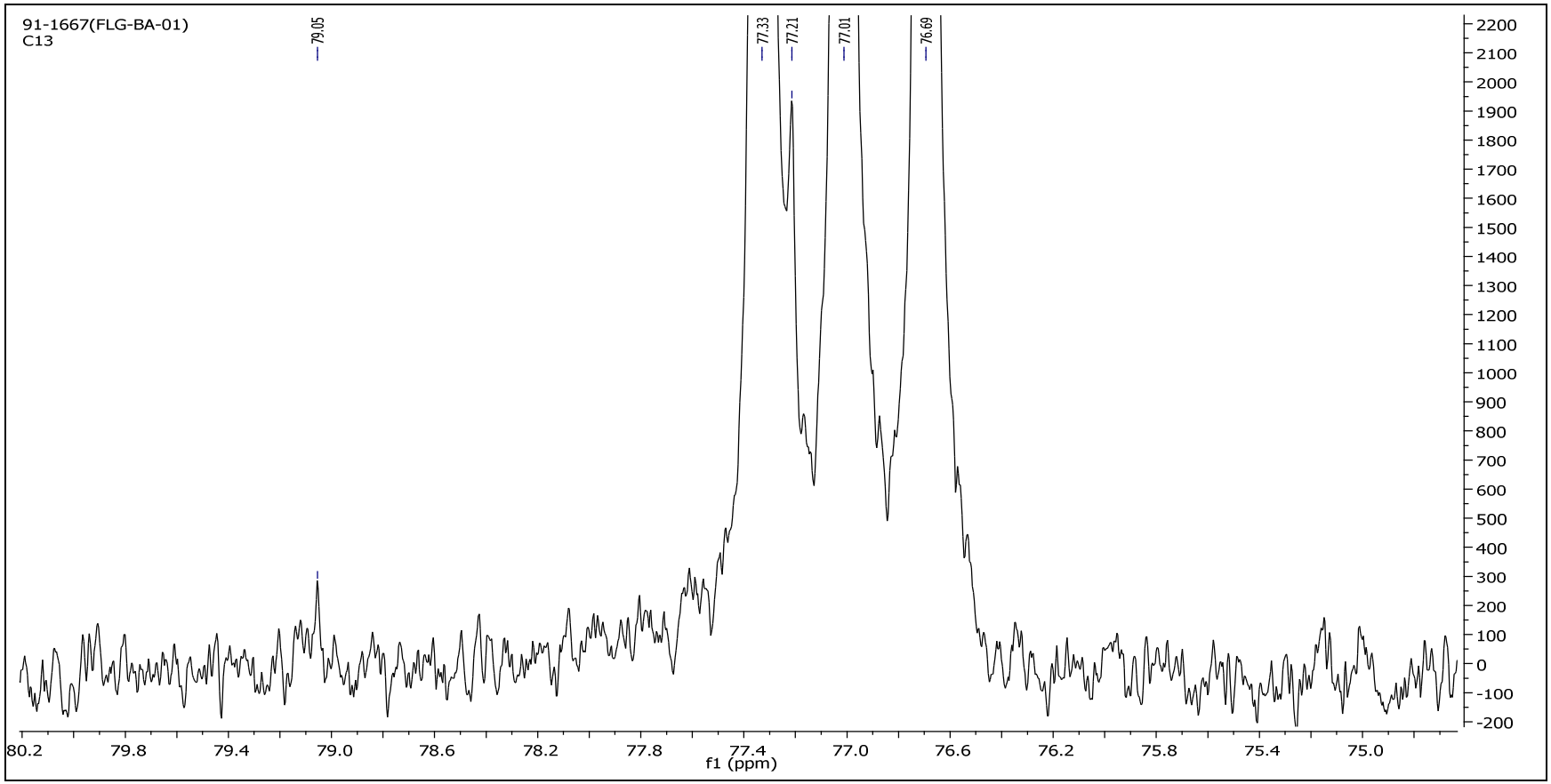
Appendix 35: ^{1}H NMR spectrum of compound **7**



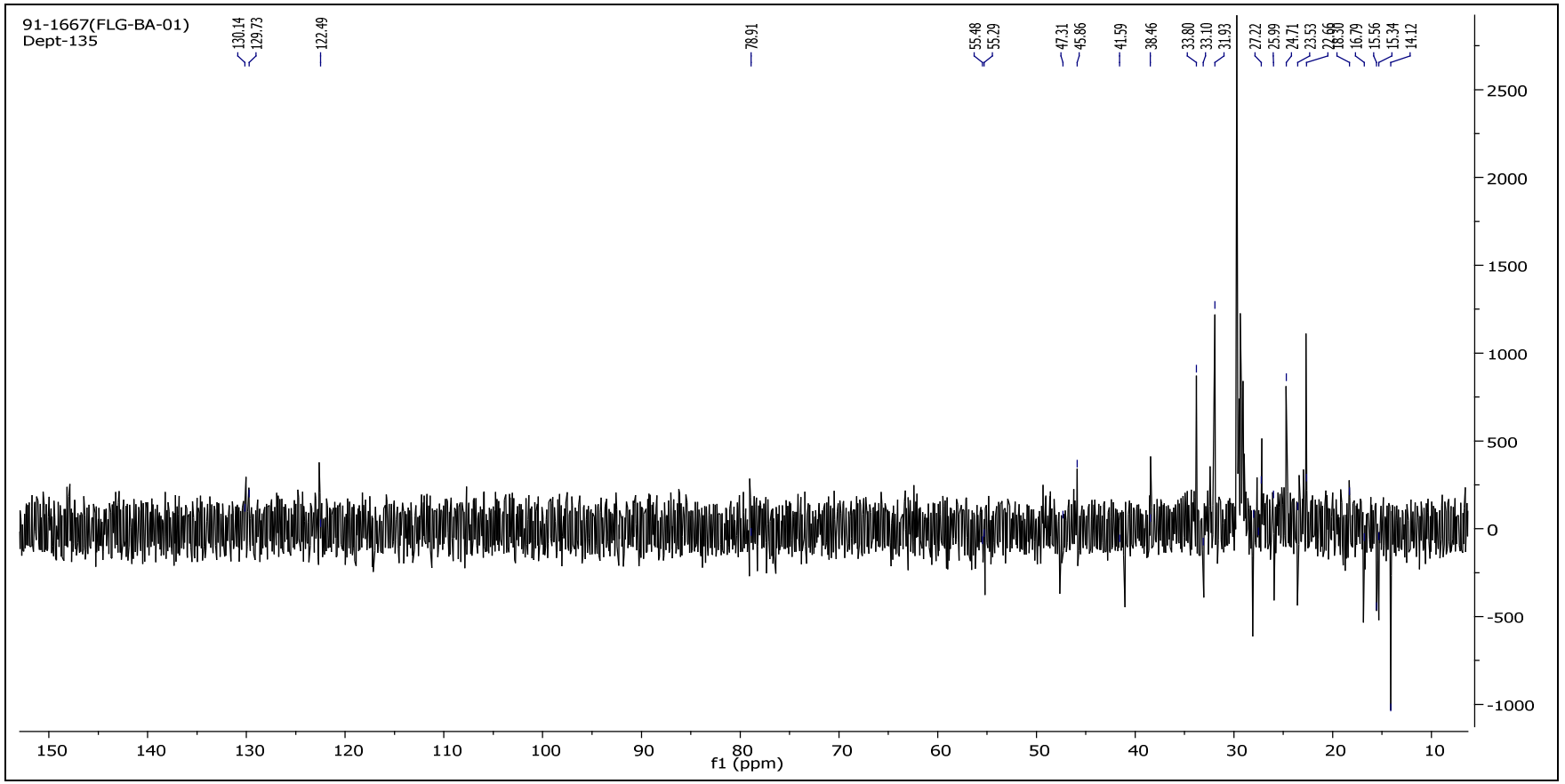
Appendix 36: ^{13}C NMR spectrum of compound **7**



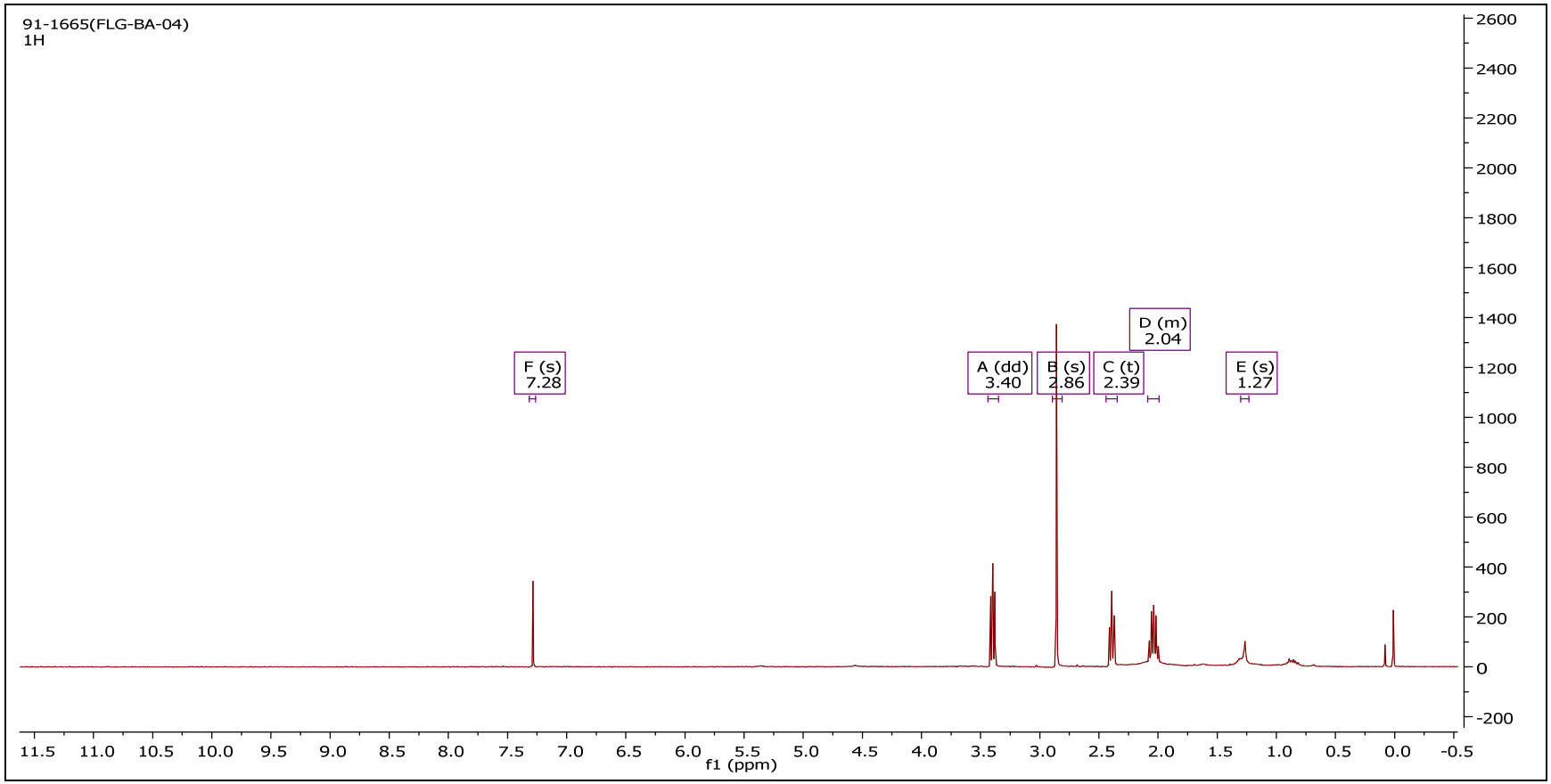
Appendix 37: ^{13}C NMR spectrum of compound 7 (peaks from 70-80ppm to show d/c with compound **6**



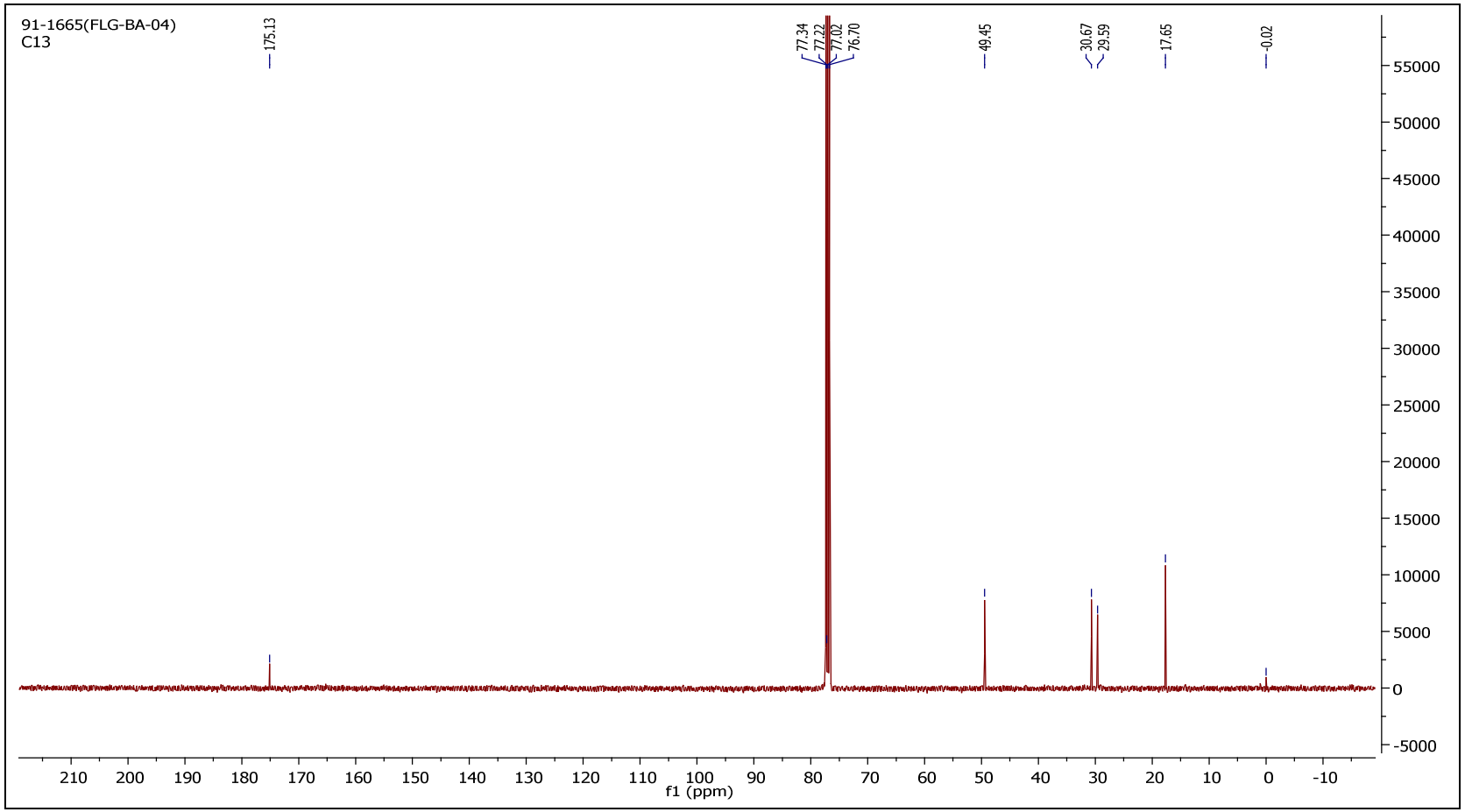
Appendix 38: DEPT-135 spectrum of compound **7**



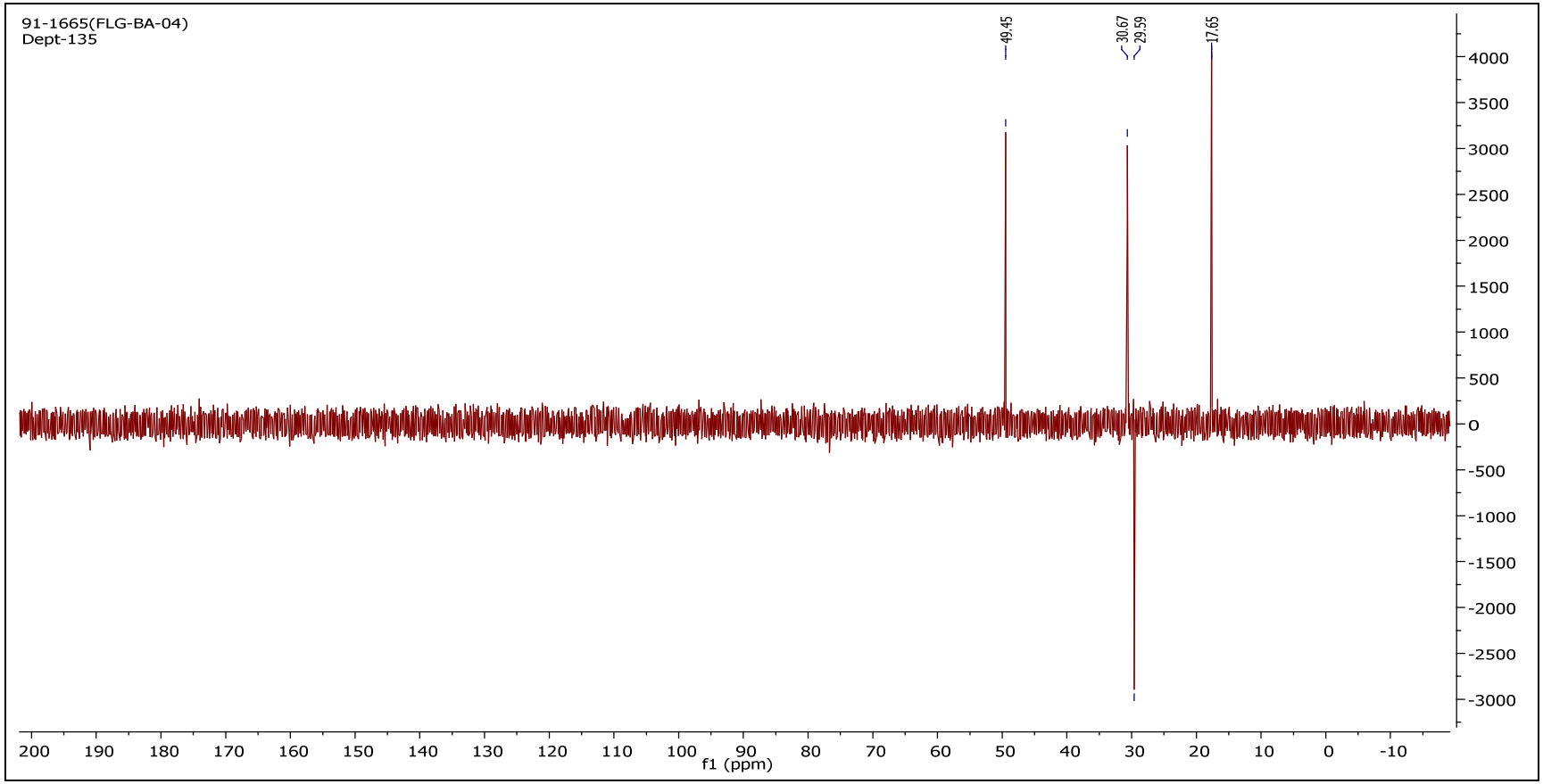
Appendix 39: ^{1}H NMR spectrum of compound **8**



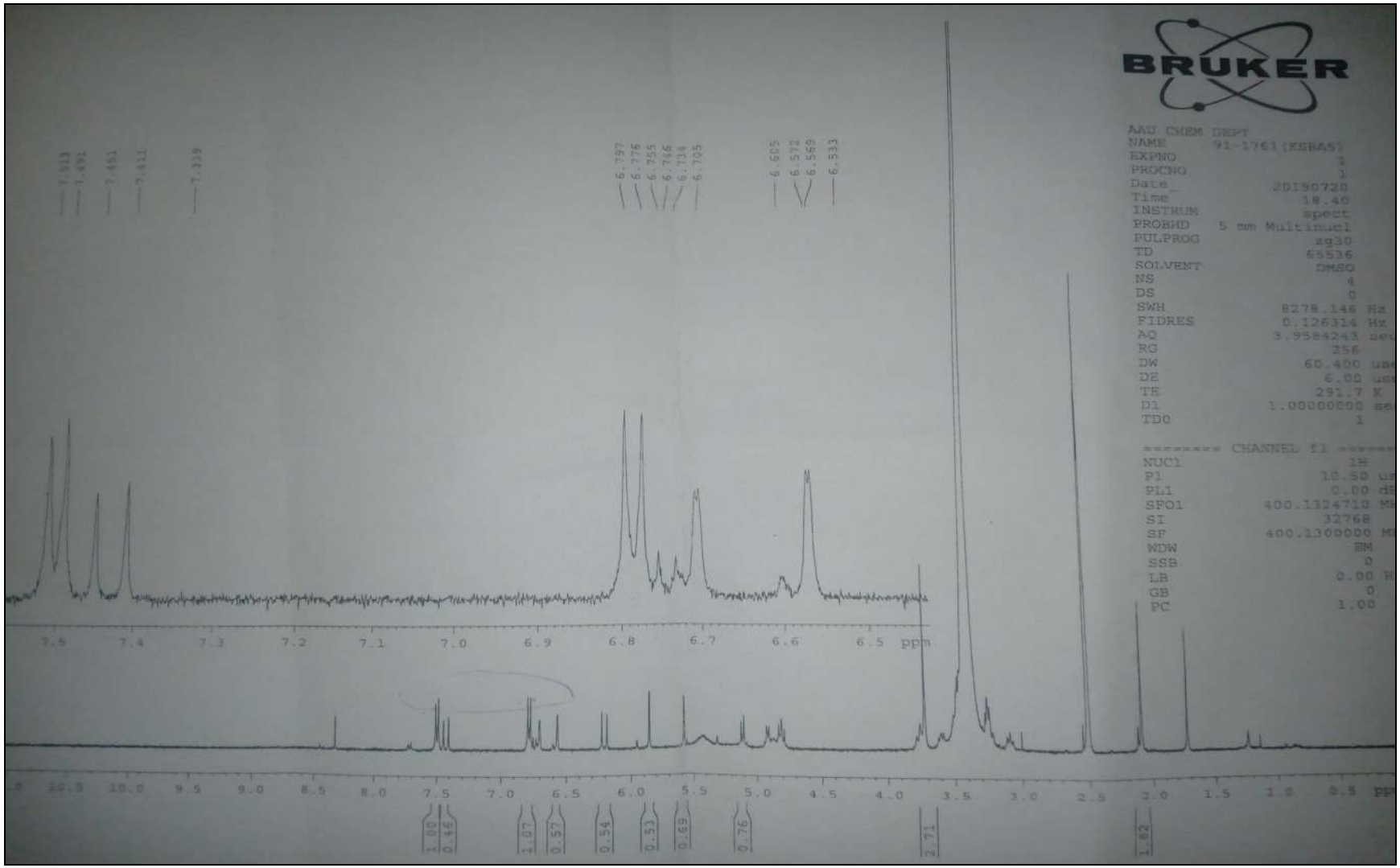
Appendix 40: ^{13}C NMR spectrum of compound **8**



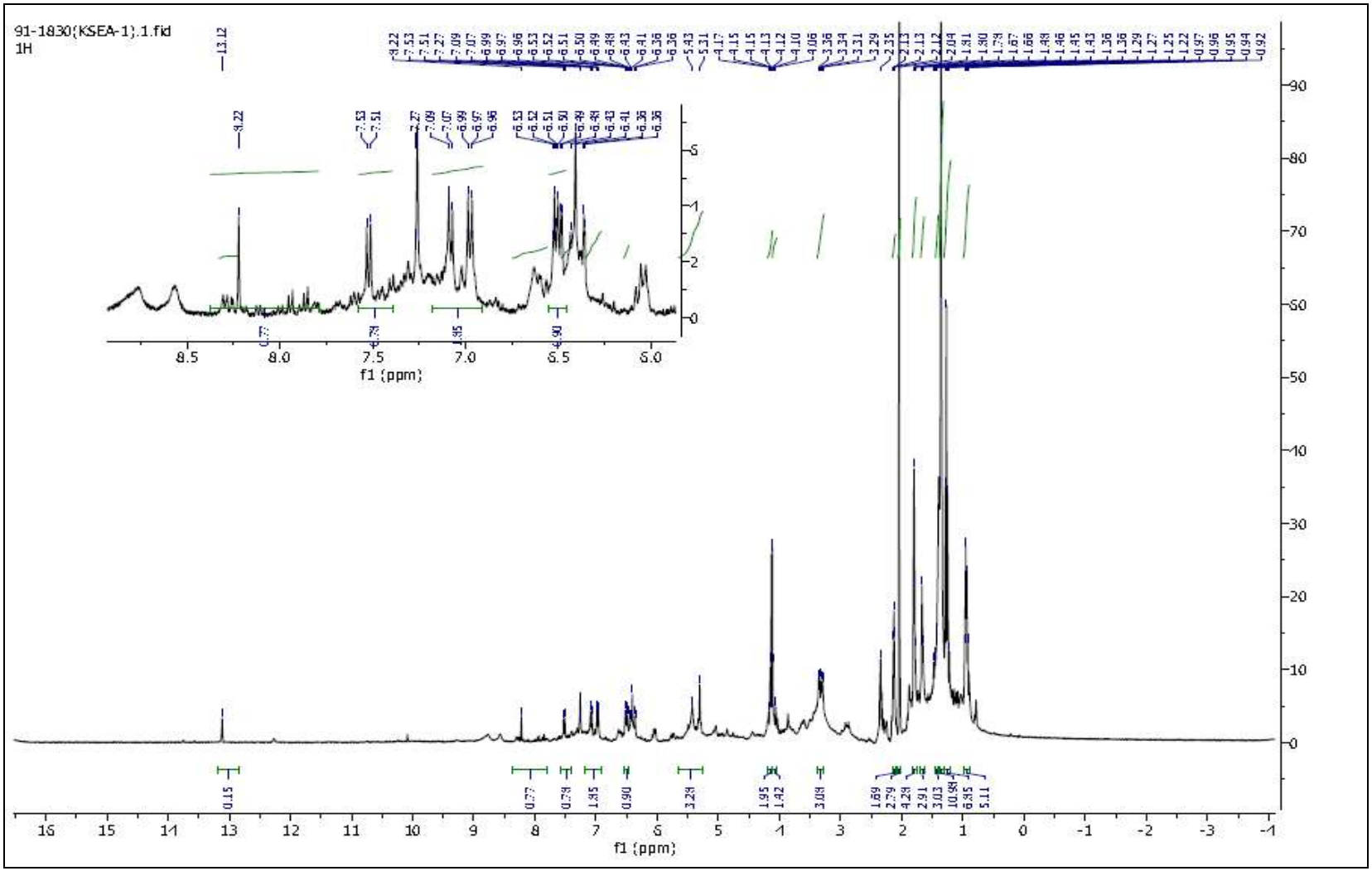
Appendix 41: DEPT-1315 NMR spectrum of compound **8**



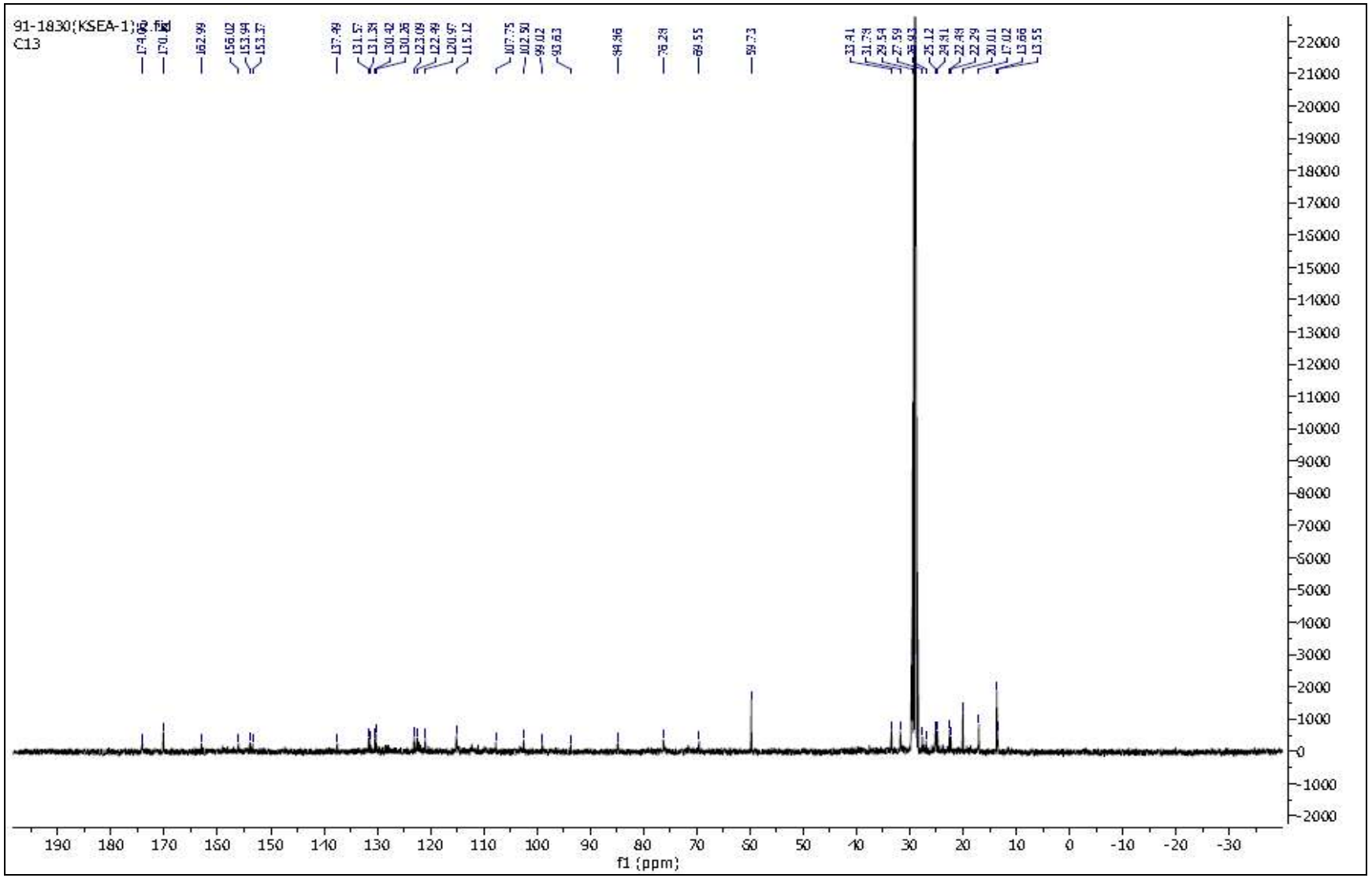
Appendix 42: ^{1}H NMR spectrum of compound **10**



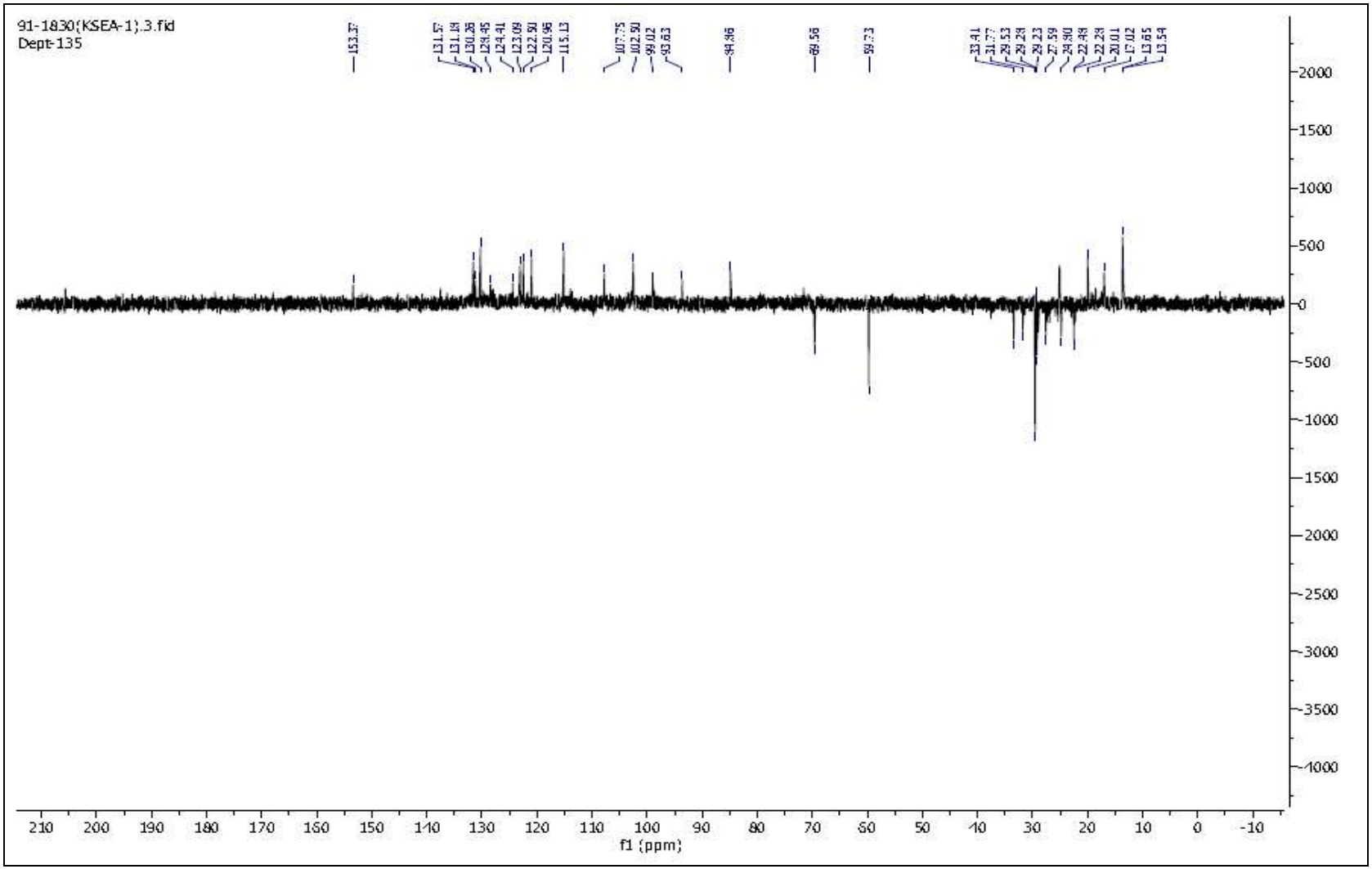
Appendix 43: ^{1}H NMR spectrum of compound **11**



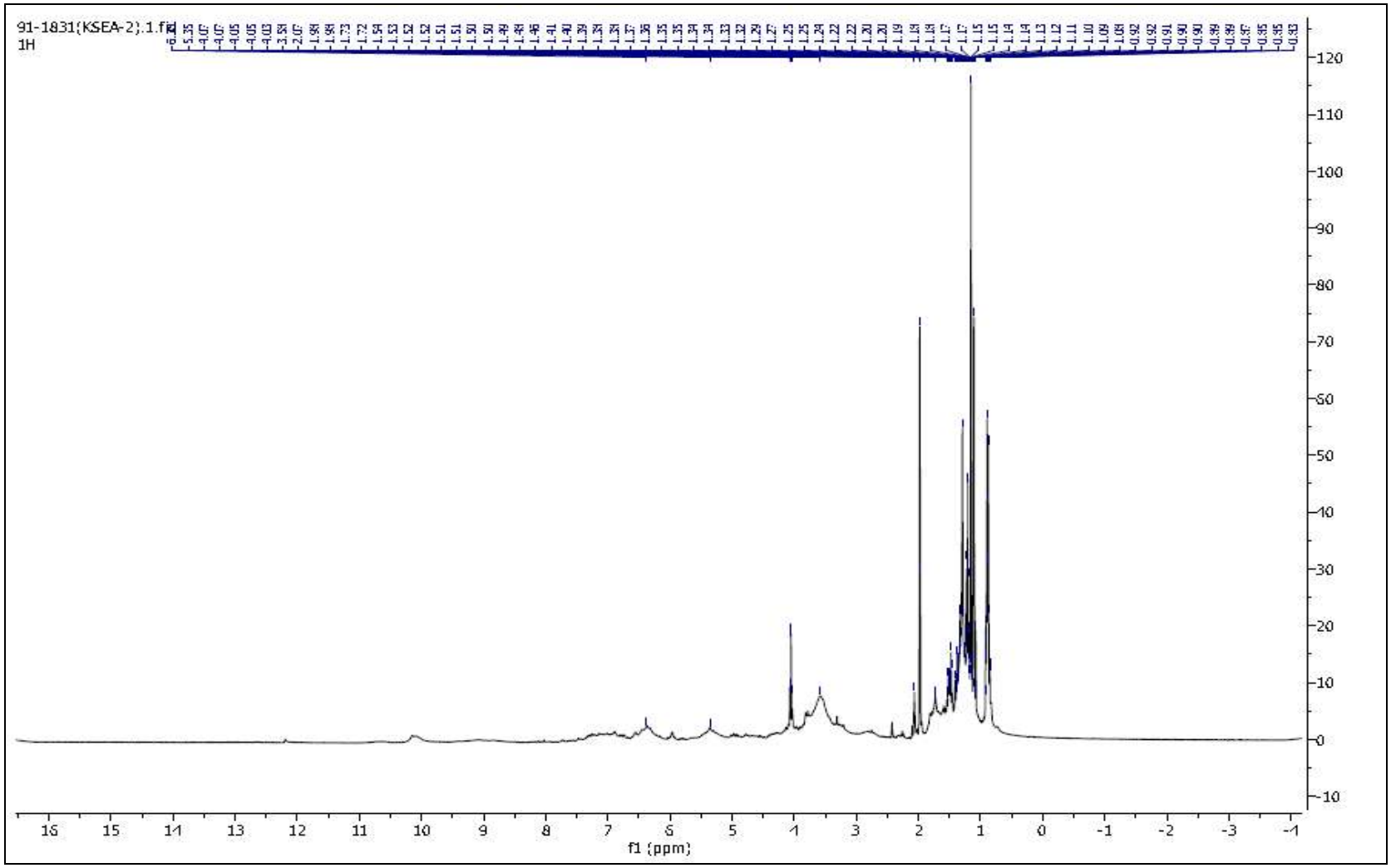
Appendix 44: ^{13}C NMR spectrum of compound **11**



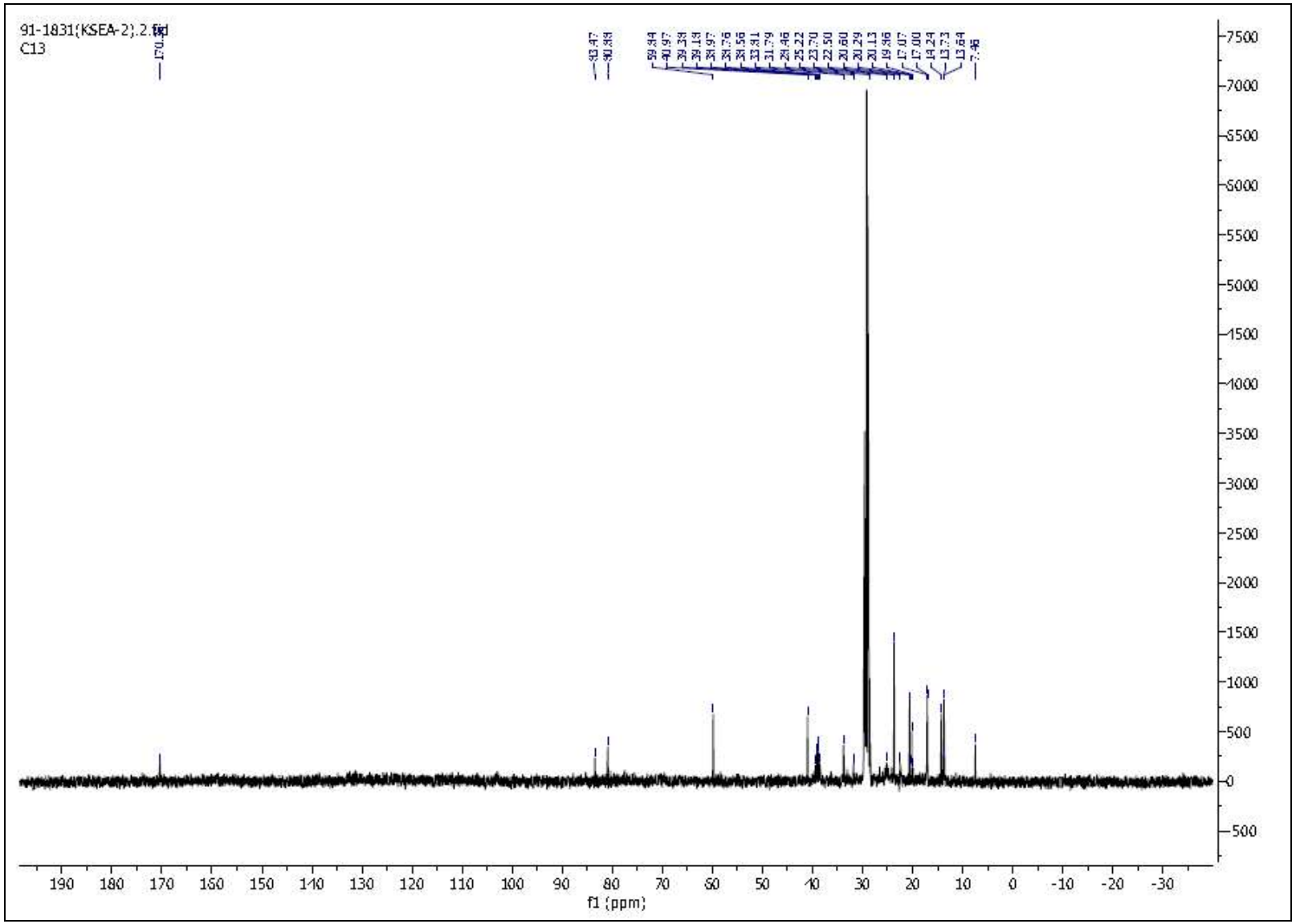
Appendix 45: DEPT-135 spectrum of compound **11**



Appendix 46: ^{1}H NMR spectrum of compound **12**



Appendix 47: ^{13}C NMR spectrum of compound **12**



Appendix 48: DEPT-135 spectrum of compound **12**