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DEPARMENT OF CHEMISTRY

GRADUATE PROJECT (Chem.774)

PHYTOCHEMICAL INVESTIGATION ON THE LEAVES OF *DODONAEA VISCOSA* var. *ANGUSTIFOLIA*

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A Graduate Project Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry

July, 2009

Declaration

I, the undersigned, declare that this MSc project is my original work and has not been presented for any degree in any other university and that all sources of materials used for this project have been duly acknowledged.

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Signature: _____

This MSc. project has been submitted for examination with my approval as a university advisor.

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Date and place of submission: Department of Chemistry

Addis Ababa University

July 2009

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PHYTOCHEMICAL INVESTIGATION ON THE LEAVES OF *DODONAEA VISCOSA* var. *ANGUSTIFOLIA*

By: Dessalegn Bekele

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ABSTRACT

The petroleum ether extract of the leaves of Dodonaea viscosa var. angustifolia afforded a diterpene 5-(2-(furan-3-yl)ethyl)-3,4,4a,5,6,7,8,8a-octahydro-8 hydroxy-5,6,8a-trimethylnaphthalene-1-carboxylic acid (compound Dc-8B) whereas the chloroform extract gave two compounds; a diterpene hautriwaic acid (compound D-16) and a flavonoid Santin (5,7-dihydroxy-3,6,4'-trimethoxy flavone) (compound D_{26}-3B). Structural elucidation of these compounds were conducted using ^{1}H NMR, ^{13}C NMR, DEPT, 2D NMR (COSY, HSQC, & HMBC), and UV-Vis spectra.

1. INTRODUCTION

1.1 General

Organic chemistry as it stands today has developed largely from the chemistry of natural products. With the advent of modern spectroscopic techniques such as multidirectional NMR spectroscopy, mass spectroscopy, and improved chromatographic techniques, a host of new organic substances from terrestrial and marine organisms are being discovered, many of which have interesting bioactivities [1].

Natural products include exudates and pure compounds (e.g., alkaloids, coumarins, flavonoids, glycosides, lignans, steroids, sugars, terpenoids, etc.) isolated from plants, animals or microorganisms. In most cases the term natural product refers to secondary metabolites, which are small molecules (mol wt < 200 amu) produced by an organism, that are not strictly necessary for the survival of the organism [2].

The use of natural products, especially plants, for healing is an ancient and universal as medicine itself. The therapeutic use of plants certainly goes back to centuries ago. It has been recorded that Hippocrates used approximately 400 different plant species for medicinal purposes. Natural products played a prominent role in ancient traditional medicine systems, such as Chinese, Ayurveda, and Egyptian, which are still in common use today. According to the World Health Organization (WHO), 75% of people still rely on plant-based traditional medicines for primary health care globally [3].

Nature has been a source of therapeutic agents for thousands of years, and an impressive number of modern drugs have been derived from natural sources, many based on their use in traditional medicine. Over the last century, a number of top-selling drugs have been developed from natural products (vincristine from *Vinca rosea*, morphine from *Papaver somniferum*, Taxol® from *Taxus brevifolia*, etc.). In recent years, a significant revival of interest in natural products as a potential source for new medicines has been observed among the academia as well as pharmaceutical companies [3].

Apart from natural product-derived modern medicines, natural products are also used directly in the "natural" pharmaceutical industry, which is growing rapidly in Europe and North America, as well as in traditional medicine programs being incorporated in to the primary health care systems of Mexico, the People's Republic of China, Nigeria, and other developing counties [3].

Advent, introduction, and development of several new and highly specific *in vitro* bioassay techniques, chromatographic methods, and spectroscopic techniques, especially nuclear magnetic resonance (NMR) spectroscopy, have made it much easier to screen, isolate, and identify potential drug lead compounds quickly and precisely. Automation of these methods now makes natural products viable for high-throughput screening (HTS) [4].

2. LITERATURE REVIEW

2.1 Botanical background

Dodonaea viscosa is grouped under division Angiosperm, subclass Dicotyledonae and family Sapindaceae. It was named *Dodonaea viscosa* by Phillip Miller in 1754, and is also known by common names: Sticky Hop-bush, Giant Hop-bush, Broad leaf Hop-bush, Candlewood, Narrow leaf Hop-bush, Native Hop, Native Hop Bush, Soap wood, etc. [5].

Dodonaea viscosa is spreading or erect shrub or tree up to about 5 m long and branchlets are angled to flattened, usually slightly ribbed, vary from smooth to covered with minute soft hairs. Leaves are simple, stalkless or petiolate, linear to spoon-shaped, rarely wedged shaped, 1-15.5 cm long,1-25 mm wide, evergreen, if crushed the foliage is sticky, afforded by an evenly distributed coat of resin. Flowers are in terminal panicles, 3-4 sepals, lanceolate to ovate [5]. *Dodonaea viscosa* var. *angustifolia* is found evenly distributed in Woina-Dega climatic area of East Wollega zone of Oromiya administrative region at about 1554 - 2149 m.a.s.l.

Figure 1 *Dodonaea viscosa* leaves

2.2 Flavonoids

More than 1300 different flavonoid compounds have been isolated from plants. The Flavonoids have two benzene rings separated by propane unit (**1**) and are derived from flavones. The more conjugated compounds often are brightly colored. They are generally found in plants as their glycosides, with hexoses such as glucose, galactose, and rhamnose, and pentoses such as arabinose and xylose as the most commonly found sugars. The sugars can be attached singly or in combination with each other. The different classes within the group are distinguished by the number and position of the hydroxy, methoxy, and sugar substituents. These include the catechins, leucoanthocyanidins, flavanones, flavanonols, flavones, flavonols, chalcones, aurones, and isoflavones whose general structures are shown in Figure 2 [4, 6].

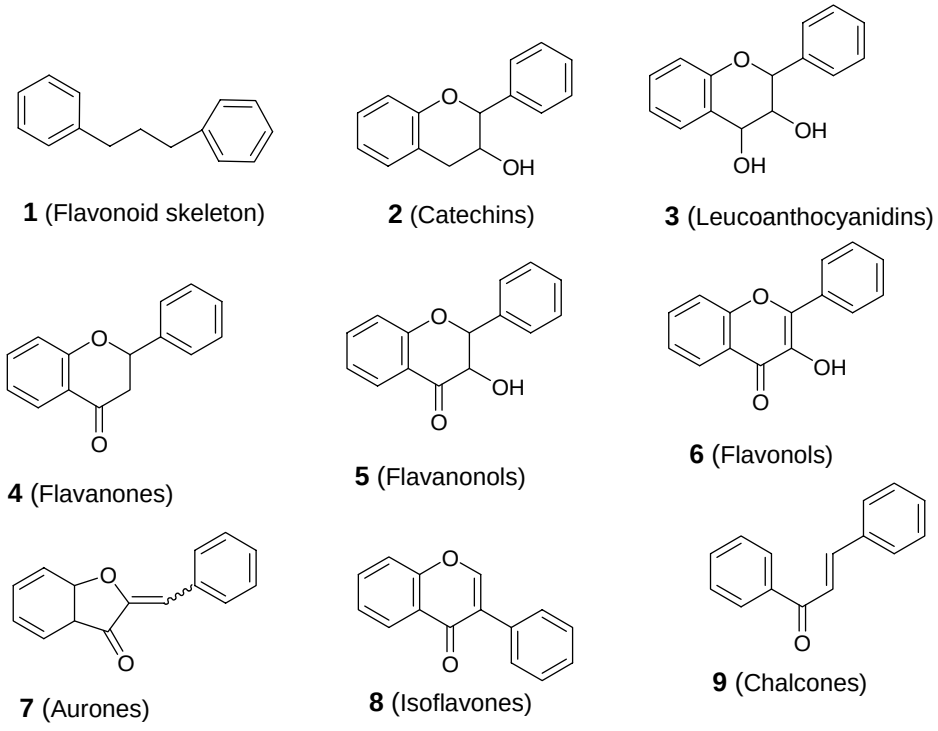
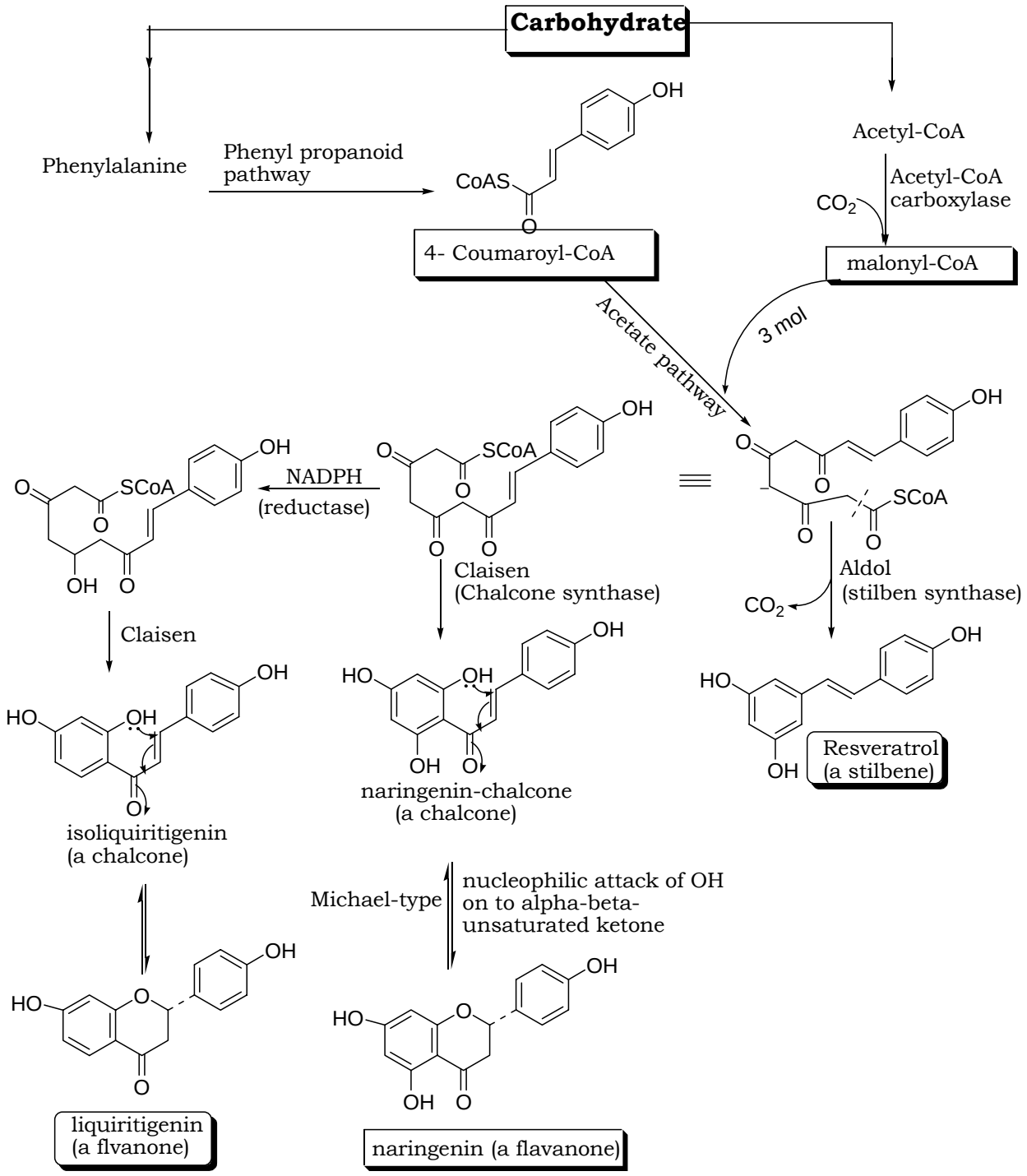


Figure 2: Common classes of flavonoids

2.2.1 Biosynthesis of Flavonoids

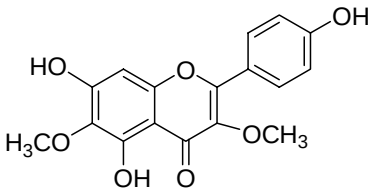
Flavonoids are produced from 4-coumaroyl-CoA starter units (from which ring-B and part of the heterocyclic ring of the flavonoid skeleton are provided) where as ring-A originates from three acetate units via three molecules of malonyl-CoA (formed from acetyl-CoA and CO_2 catalyzed by acetyl CoA carboxylase). Both precursors are derived from carbohydrates. The key enzyme for the formation of the flavonoid skeleton is chalcone synthase (CHS), which catalyzes the stepwise condensation of three acetate units from malonyl-CoA with 4-coumaroyl-CoA by Aldol or Claisen-like reaction to form C_{15} intermediate 2',4',6',4-tetrahydroxychalcone. Enzymes stilbene synthase and chalcone synthase couple a 4-coumaroyl-CoA unit with three malonyl-CoA units giving stilbenes, e.g., resveratrol, or chalcones, e.g., naringenin-chalcone, respectively. Chalcones act as precursors for a vast range of flavonoid derivatives and most contain six-membered heterocyclic rings, formed by Michael type nucleophilic attack of phenol group on to α,β-unsaturated ketone giving a flavanone [7, 8].



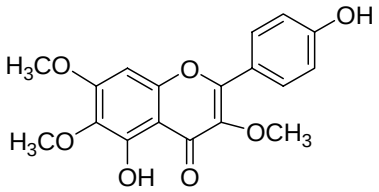
Scheme 1. Biosynthesis of Flavonoids

2.2.2 The major Flavonoids of *Dodonaea viscosa* var. *angustifolia*

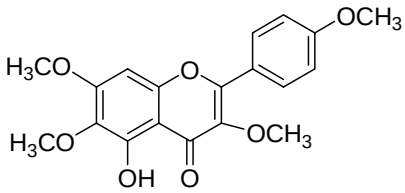
HPLC finger printing of the crude extract of *Dodonaea viscosa* var. *angustifolia* has revealed the presence of diterpenoid acids: such as hautriwaic acid (**25**), dodonic acid (**26**), and structurally related diterpenoids, reducing sugars, biologically active saponins, tannins, quinones, triterpene steroids as well as several flavonoids such as flavones, e.g., santin (5,7-dihydroxy-3,6,4'-trimeth-oxyflavone) (**13**), penduletin (**11**), aliarin (**14**) and a flavonoid with an isoprenoid sidechain:5,7-dihydroxy-3'-(3-hydroxymethylbutyl)-3,6,4'-trimethox-yflavone (**15**), flavanones, e.g., pinocembrin (**16**) [9,10,11]. The flavonoids are known to have medicinal properties including antibacterial and antiviral activity. Studies have shown they are synthesized by plants in response to microbial infection and have effective *in vitro* antimicrobial activity against a wide variety of microorganisms. This activity is probably due to their ability to complex with extracellular and soluble proteins and bacterial cell walls [12, 13].



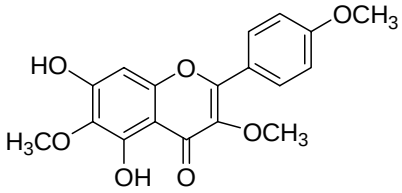
10 (5,7,4'-trihydroxy-3,6-dimethoxyflavone)



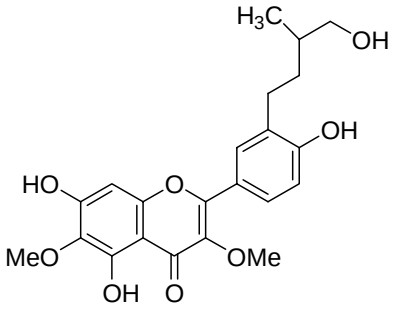
11 [5,4'dihydroxy-3,6,7-trimethoxyflavone (**Penduletin**)]



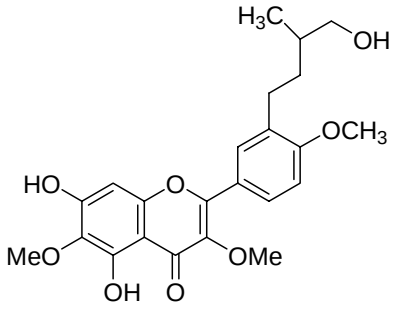
12 (5-hydroxy-3,6,7,4'-tetramethoxyflavone)



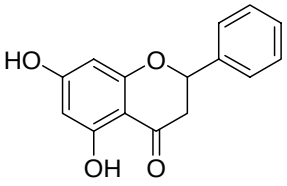
13 [5,7-dihydroxy-3,6.4'-trimethoxyflavone (**Santin**)]



14 [5,7,4'-trihydroxy-3'(3-hydroxymethylbutyl)-3,6-dimethoxyflavone (**aliarin**)]



15 (5,7-dihydroxy-3'-(3-hydroxymethylbutyl)-3,6,4'-trimethoxyflavone)



16 [5,7-dihydroxyflavanone (**pinocembrin**)]

Figure 3: The major flavonoids of *Dodonaea viscosa.*

2.3 Tannins

Tannins consist of mainly gallic acid residues that are linked to glucose *via* glycosidic bonds (**17**) and are common to vascular plants existing primarily within woody tissues. Tannins consist of various phenolic compounds that react with proteins to form water-insoluble copolymers. This reaction with proteins has been used industrially for the conversion of animal skin into leather. Plant tissues that are high in tannin content have a highly bitter taste and are avoided by most feeders. Tannins may be either condensed or hydrolyzable. Condensed tannins are formed biosynthetically by the condensation of catechins to form polymeric networks. Hydrolyzable tannins are derived from gallic acid [4].

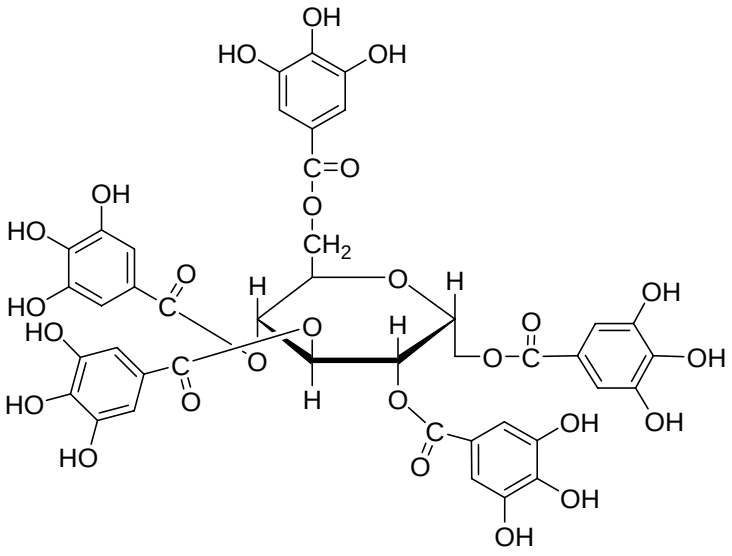


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Figure 4: Basic structure of tannins

2.4 Saponins

Saponins are high-molecular-weight triterpene glycosides containing a sugar group attached to either a sterol or other triterpene as shown in Figure 5. They are widely distributed in the plant kingdom and are composed of two parts: glycone (sugar) and aglycone or genin (triterpene). Typically, they have detergent properties, readily form foams in water, have a bitter taste, and are piscicidal (toxic to fish). Many of the plants that contain saponins have been used historically as soaps. These include soaproot (*Chlorogalum pomeridianum*), soapbark (*Quillaja saponaria*), soapberry (*Sapindus saponaria*) and soapnut (*Sapindus mukurossi*). Saponins may be mono-or polydesmodic, depending on the number of attached sugar moieties. Biosynthetically, the saponins are comprised of six isoprene units and are derived from squalene. Saponins are constituents of many plant drugs and folk medicine, especially among Asian peoples. This has lead to great interest in the investigation of their pharmacological properties. The ammonium and calcium salts of glycyrrhizinc acid (a naturally occurring saponin) are referred to as the glycyrrhizins. At 50 to 100 times sweeter than sucrose, these are the active ingredients in licorice root (*Glycyrrhiza glabra*), with expectorant, bacteriostatic, and antiviral activity. The ginsenosides are one of many triterpene saponins from ginseng (*Panax ginseng*) believed to be responsible for its immunostimulant activity [4, 8].

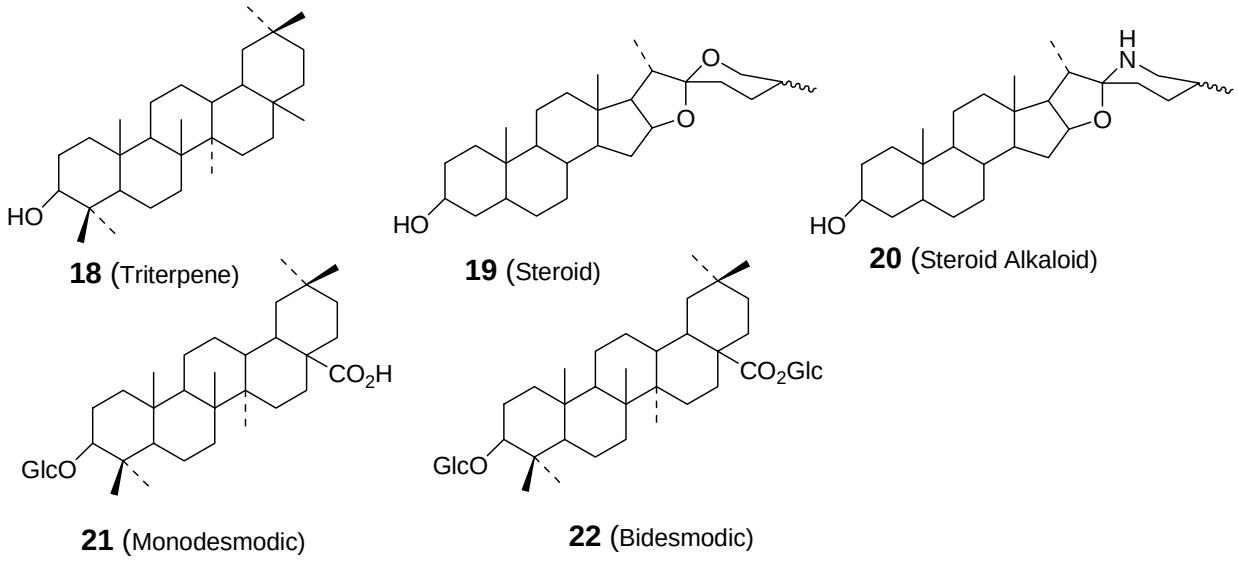


Figure 5: Classification of saponins

2.4.1 Biologically active saponins

The *Dodonaea* saponin esters are isolated from the methanol extract of the seed of D. *viscosa* and have structural similarity with antioxidant saponin esters from *Aesculus hippocastanum*. *Dodonaea* saponins also showed remarkable activity with 100% lethality at a concentration of 25 ppm, when subjected to the Viscarin-Carrageenin oedema test on a rat paw [14].

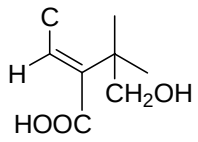
2.5 Diterpenes from *Dodonaea viscosa*

The carbon skeleton of terpenes is built from the combination of two or more isoprene units, which are linked in a head–to-tail manner. The number of carbon units they contain classifies terpenes as hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterpenes (C_{25}), triterpenes (C_{30}) and tetraterpene (C_{40}). Terpenoids are oxygen containing

analogous of terpenes. They are distributed in the plant kingdom, especially in those plants that have abundant chlorophyll [15].

The diterpenes are a widely varied group of compounds based on four isoprene groups, most of which are of limited distribution in the plant kingdom. Because of their higher boiling points they are not considered to be essential oils, instead they are classically considered to be resins, the material that remains after steam distillation of a plant extract. The diterpenes arise from geranyl geranyl pyrophosphate (GGPP), which is formed by addition of a further isopentenyl pyrophosphate (IPP) molecule, to farnesyl pyrophosphate [15, 16].

A diterpenoid acid: hautriwaic acid (**25**) was first isolated and characterized as monohydroxy carboxylic acid from the leaves of *Dodonaea viscosa* in 1936 by Kotake and Kuwatal and the full structure was determined in 1970 by Hong-Yen Hsti, and Yuh Pan Chen [17]. Hautriwaic acid, $C_{20}H_{28}O_4$, has an α,β-disubstituted acrylic acid group (**23**). According to the literature [17], upon heating above the melting point, hautriwaic acid (**25**) was converted into a γ-lactone (**24**): $C_{20}H_{26}O_3$. Other diterpenes like dodonic acid (**26**) and methyl-dodonate (**27**) were also identified from this plant [18].



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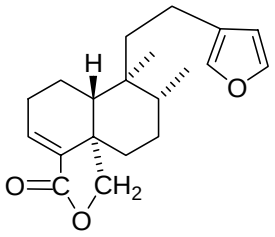

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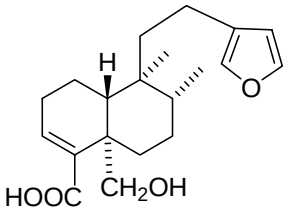
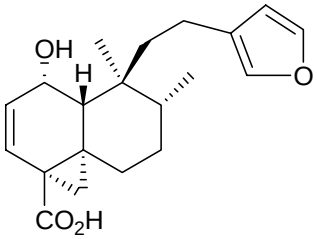
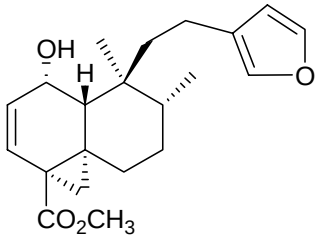


25 (Hautriwaic acid)



26 (Dodonic acid)

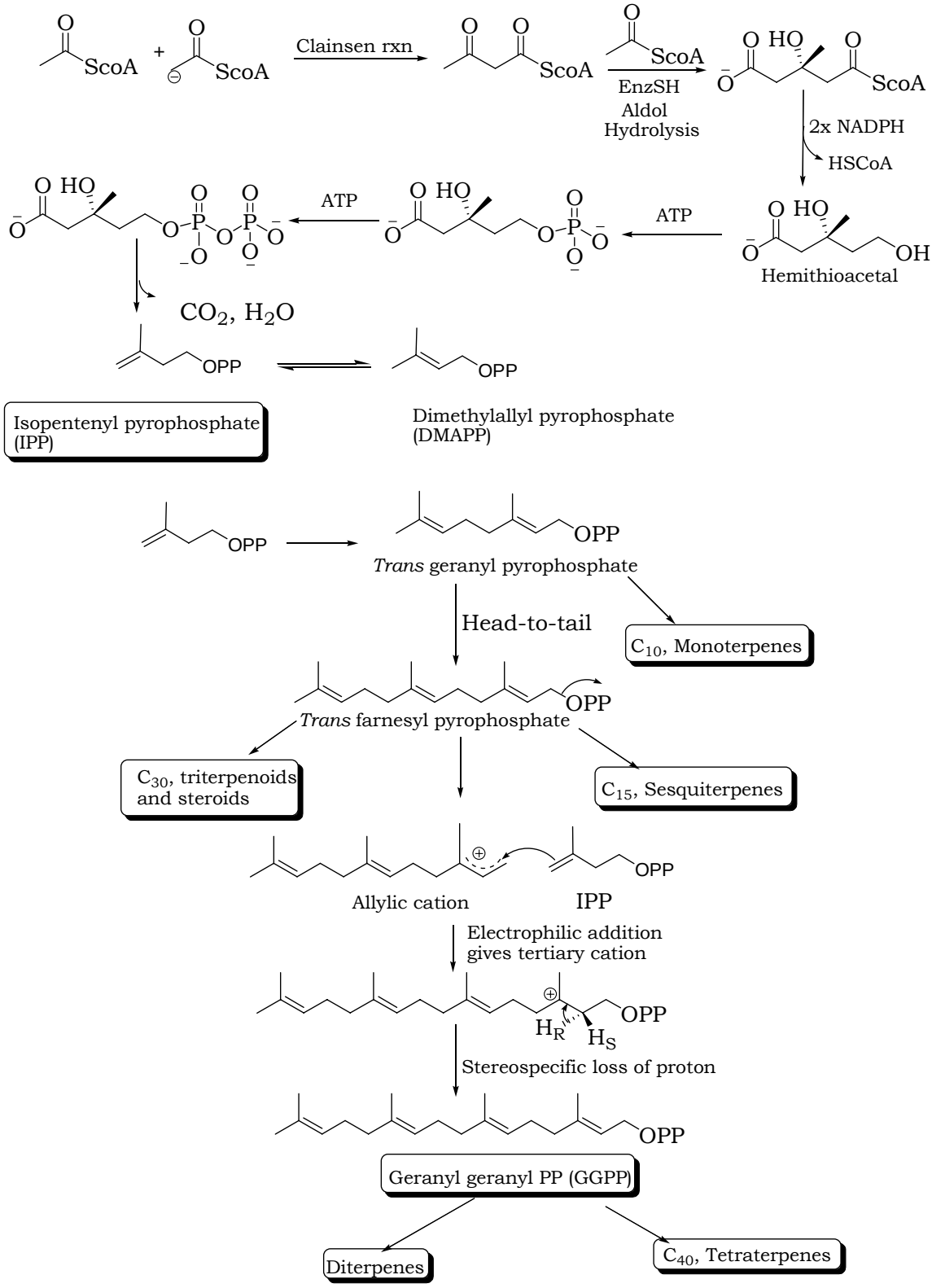


27 (Methyldodonate)

Figure 6: Structures of some diterpenoids from *Dodonaea viscosa.*

2.5.1. Biogenesis of Terpenes

Terpenes have a common biogenetic origin based on isopentyl pyrophosphate, which is formed from mevalonic acid via hydroxymethyl glutarate in the acetate/mevalonate pathway or glyceraldehydes-3-phosphate in the non-mevalonate pathway (Scheme 2). The starting point in the mevalonate pathway is the condensation of two molecules of acetylcoenzyme A to form acetoacetyl coenzyme A [19].



Scheme 2. Biogenesis of terpenoids

2.6 Pharmacognostic study

Dodonaea viscosa Jacq., was reported for medicinal use largely as analgesic, anti-inflammatory, antiviral, spasmolytic, laxative, antimicrobial and hypotensive agent [20]. In India, the infusion of leaves was used to treat rheumatism, gout, hemorrhoids, fractures and snake bites. The leaves were reported also to possess local anesthetic, smooth muscle relaxant, antifungal, and anti-ulcerogenic activity. The ethanol extract of the leaves has shown anti-ascariasis, anthelmintic, cardiac depressant, hypotensive, uterine relaxation and vasoconstrictor activity in different experimental models [21].

2.6.1 Antifungal, anti-inflammatory and anti-bacterial activity of *Dodonaea viscosa*.

Dodonaea viscosa var. *angustifolia* has antifungal properties and at a concentration of 50 mg/ml, it can eliminate *Candida albicans* within 30 s from HIV-infected patients. The report that this plant extract has an analgesic effect [22, 23] is a further advantage because patients with oral candidiasis have painful mouths and experience difficulty with eating and swallowing. These properties suggest that the extract has the potential to be used as an effective mouth rinse for the prevention of recurrent oral candidiasis by reducing the number of *Candida* in the mouth to an acceptable level [23, 24].

The *D. viscosa* leaves aq. ethanolic extract possess anti-inflammatory activity. An oral dose of 300 mg/kg inhibited the edema induced in rats by carrageenin injection and no toxic symptom or mortality was observed in 14 days of study in mice. This result seems to support the use of *D. viscosa* var. *angustifolia* leaves ethanolic extract in relieving inflammation [25, 26, and 27].

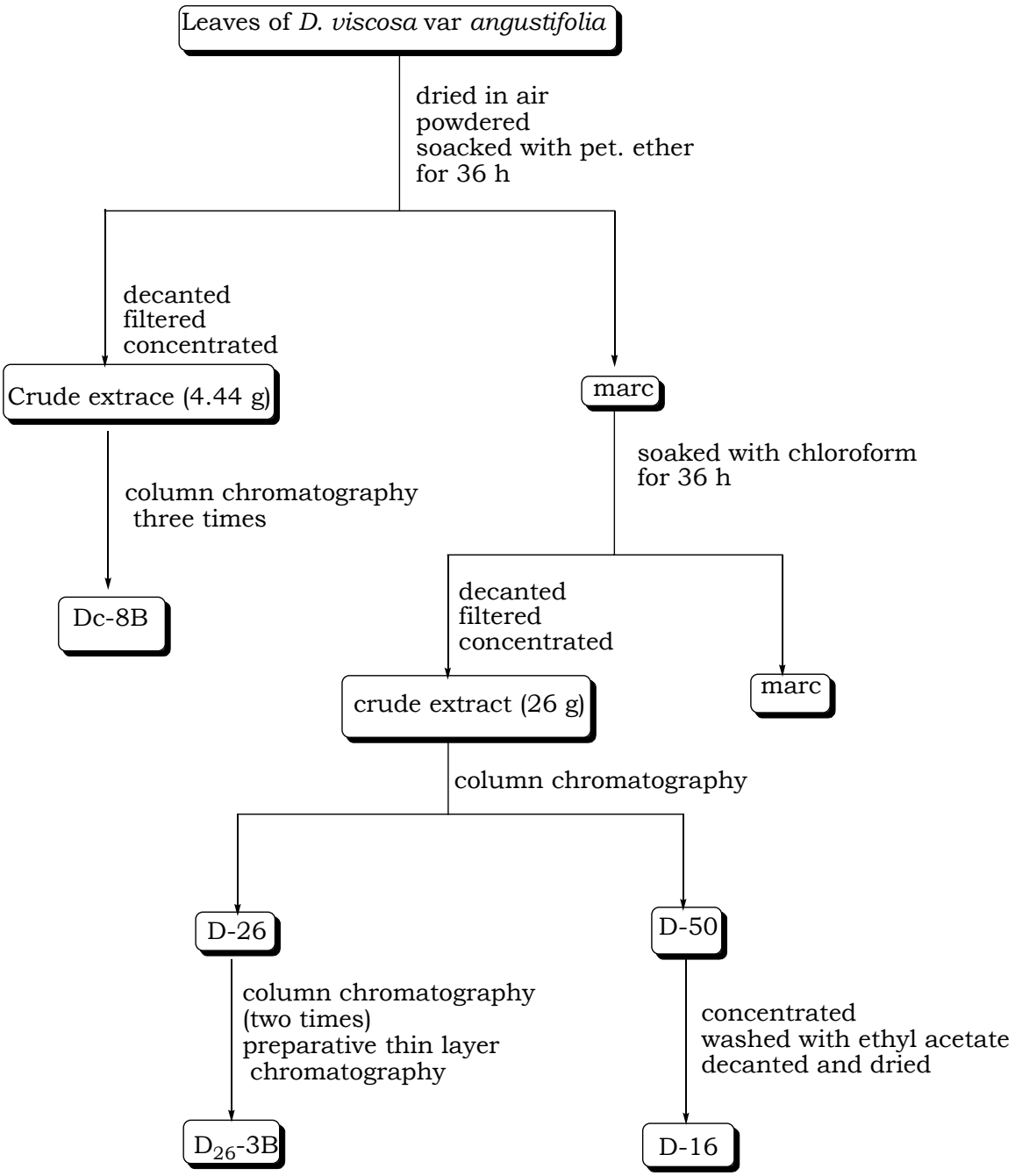
The crude extract of *Dodonaea viscosa* Jaeq. leaves has an inhibitory effect against *Staphylococcus aureus*, *Streptococcus pyogenes,* and *Corynebacterium dephtherieae*, but no activity against *Escherichia coli* and *Pseudomonas aeruginosa,* thereby suggesting potential against notable gram-positive organisms [28, 29].

3. OBJECTIVES OF THE STUDY

Dodonaea viscosa var. angustifolia is one of the cultural medicinal plants found in the western part of Ethiopia around Gidda Ayana town, 110 km north of Nekemte town. The main objectives of this project work are, to isolate pure compounds from the leaves of *Dodonaea viscosa var. angustifolia* and elucidate their structures by the use of spectroscopic methods (NMR, and UV-VIS).

4. RESULTS AND DISCUSSION

Air-dried leaves (300 g) of *Dodonaea viscosa var. angustifolia* were subjected to exhaustive extraction successively with petroleum ether and chloroform. The solvent from each extract was removed under reduced pressure using rotavapor to obtain a petroleum ether extract (4.44 g) and a chloroform extract (26 g), respectively. Chromatographic purification of the petroleum extract gave a compound coded Dc- 8B whereas the chloroform extract yielded compounds coded D26-3B and D-16. The structures of these compounds have been elucidated based on spectroscopic evidences and the method used to extract the plant material is shown in Scheme 3.



Scheme 3. Flow chart used to extract plant material and isolate compounds.

4.1. Characterization of compound Dc-8B

Compound Dc-8B is a white crystalline solid obtained from fraction 8 of petroleum ether extract and is soluble in chloroform. It is optically active with specific rotation of $[\alpha]_D$ = -2.4^0 and melting point was measured to be within a range of 92.8-94.6^{0}C. The UV spectrum of compound Dc-8B (Appendix 1.1) also displays absorbance peaks at λ_{max} = 249, 273, 314 and 333 nm which indicates the presence of $\Pi \rightarrow \Pi^*$ transition of C=C double bond, $\Pi \rightarrow \Pi^*$ transition of C=O (carbonyl functionality), and $n \rightarrow \Pi^*$ transitions of C=O bond.

The ^{1}H NMR spectrum (Appendix 1.2) of Dc-8B in $CDCl_3$ (Table 1) suggested the presence of three methyl signals at δ 0.88 (3H, d), 1.25 (3H, s) and 0.78 (3H, s). The NMR spectrum also shows two methine protons at δ 1.53 (1H, m) and 1.40 (1H, dd), attached to stereogenic centers. Three methylene groups at δ1.59, 1.63 (2H, m), δ 1.64, 1.67 (2H, m) and δ1.71, 1.74 (2H, m) most probably represent the three methylene protons adjacent to stereogenic center. Two methylene protons at δ 2.18 (2H, dd) and 2.32 (2H, ddd) indicated complex spectra which may arise from the coupling taking place with each of the geminal diastereotopic methylene protons adjacent to stereogenic center. Methine proton at δ 3.78 (1H, dd) was attached to carbon containing electronegative atom oxygen and the doublet of doublet peak observed was most probably due to coupling with diastereotopic methylene protons adjacent to the stereogenic center. Methine protons at δ 7.20 (1H, t), 6.27 (1H, dd), 7.22 (1H, s) and 7.37 (1H, dd) were attached to sp^2-carbon. Broad peak at about δ 8.85 (1H) indicates acidic hydrogen most probably of the carboxylic acid functional group in the compound.

Table 1. ^{1}H NMR spectral data for compound Dc-8B

Carbon No.	Proton chemical shift (δ in ppm)	No. of hydrogen, multiplicity	Remark
C-1	0.88	3H, d	CH_3
C-2	1.25	3H, s	CH_3
C-3	1.59,1.63	2H, m	CH_2
C-4	0.77	3H, s	CH_3
C-5	1.64,1.67	2H, m	CH_2
C-6	1.71,1.74	2H, m	CH_2
C-7	1.53	1H, m	CH
C-8	2.18	2H, dd	CH_2
C-9	2.32	2H, ddd	CH_2
C-10	-	-	Quaternary carbon
C-11	-	-	Quaternary carbon
C-12	1.40	1H, dd	CH
C-13	3.78	1H, dd	CH
C-14	6.27	1H, dd	CH
C-15	-	-	Quaternary carbon
C-16	7.22	1H, s	CH
C-17	-	-	Quaternary carbon
C-18	7.37	1H, dd	CH
C-19	7.20	1H, t	CH
C-20	-	-	Quaternary carbon

The ^{13}C NMR spectrum (Appendix 1.3, Table 2) of Dc-8B analyzed with the aid of DEPT-135 (Appendix 1.4) showed two quaternary carbons in the aliphatic region at δ38.6 and δ 44.7, two quaternary carbons in the aromatic region at δ140.3 and δ125.3, one quaternary oxygenated carbon at δ173.4. In addition, three methine carbon signals were observed in the aliphatic region at δ33.8, δ45.6 and δ74.4. More importantly, the methine carbon at δ74.4 is most probably attached to an electronegative oxygen atom. The ^{13}C NMR spectrum also shows the presence of four methine carbons in the aromatic region at δ110.9, δ138.4, δ142.8 and δ144.1. As confirmed by the DEPT-135 spectrum, there are five methylene carbons (δ17.2, δ17.9, δ35.6, δ38.6) and three methyl carbons (δ15.6, δ16.7 and δ17.6) in the aliphatic region.

Table 2. Proton-decoupled ^{13}C NMR data for compound Dc-8B

Carbon		Remark	Carbon		Remark
No	δ (ppm)		No	δ (ppm)	
C-1	14.6	CH_3	C-11	44.7	Quaternary carbon
C-2	16.7	CH_3	C-12	45.6	CH
C-3	17.2	CH_2	C-13	74.4	CH
C-4	17.6	CH_3	C-14	110.9	CH
C-5	17.9	CH_2	C-15	125.3	Quaternary carbon
C-6	27.5	CH_2	C-16	138.4	CH
C-7	33.8	CH	C-17	140.3	Quaternary carbon
C-8	35.6	CH_2	C-18	142.8	CH
C-9	38.6	CH_2	C-19	144.1	CH
C-10	38.6	Quaternary carbon	C-20	173.4	Quaternary carbon

Heteronuclear multiple-quantum correlation spectroscopy (HMQC) experiment (Appendix 1.5) also correlates the chemical shifts of protons with directly bonded carbon atoms. The HMQC NMR spectrum showed that, the three-methyl protons at δ0.88, δ1.25, and δ0.78 correlate with carbon signals at δ15.7, δ16.7, and δ17.6, respectively. Moreover, the HMQC clearly shows the methine proton at δ3.78 (1H, dd) correlates with the oxygenated carbon at δ74.4. Table 3 shows the correlations of protons to carbons as evidenced by the HMQC spectrum.

Table 3. HMQC data for compound Dc-8B

DEPT		Proton No.	^{1}H NMR δ(ppm)	Multiplicity	Remark
Carbon No.	δ(ppm)				
C-1	14.6	1	0.88	d	CH_3
C-2	16.7	2	1.25	s	CH_3
C-3	17.2	3	1.59,1.63	m	CH_2
C-4	17.6	4	0.77	s	CH_3
C-5	17.9	5	1.64,1.67	m	CH_2
C-6	27.5	6	1.71,1.74	m	CH_2
C-7	33.8	7	1.53	m	CH
C-8	35.6	8	2.18	dd	CH_2
C-9	38.6	9	2.32	ddd	CH_2
C-12	45.6	12	1.40	dd	CH
C-13	74.4	13	3.78	dd	CH
C-14	110.9	14	6.27	dd	CH
C-16	138.4	16	7.22	s	CH
C-18	142.8	18	7.37	dd	CH
C-19	144.1	19	7.20	t	CH

The ^{1}H-^{1}H Correlation Spectroscopy (Homonuclear COSY) experiment (Appendix 1.6) showed the correlation between H-3 at δ1.59, δ1.63 and H-8 at δ2.18, H-5 at δ1.64, δ1.67 and H-7 at δ1.53, H-5 and H-13 at δ 3.78. The COSY experiment also showed correlations between H-6 at δ1.71, δ1.74 and H-9 at δ2.32, H-6 and H-12 at δ1.40 indicating the three-diastereotopic methylene protons. Similarly, other strong correlations were observed from COSY experiment as shown in Table 4.

Table 4. ^{1}H-^{1}H COSY data for compound Dc-8B

Carbon No.	^{1}H NMR δ (in ppm)	^{1}H-^{1}H COSY
C-1	0.88	H-1↔H-5, H-7
C-2	1.25	-
C-3	1.59,1.63	H-3↔ H-8
C-4	0.77	-
C-5	1.64,1.67	H-5↔ H-1, H-7, H-13
C-6	1.71,1.74	H-6↔ H-9, H-12
C-7	1.53	H-7↔ H-1, H-5
C-8	2.18	H-8↔ H-3,
C-9	2.32	H-9↔ H-19, H-6
C-12	1.40	H-12↔ H-6
C-13	3.78	H-13↔ H-5
C-14	6.27	H-14↔H-18, H-16
C-16	7.22	H-16↔ H-14, H-18
C-18	7.37	H-18↔ H-14, H-16
C-19	7.20	H-19↔ H-9

From ^{1}H-^{1}H COSY, ^{13}C NMR, DEPT and ^{1}H NMR spectral data the partial structures shown in Figure 7 can be suggested.

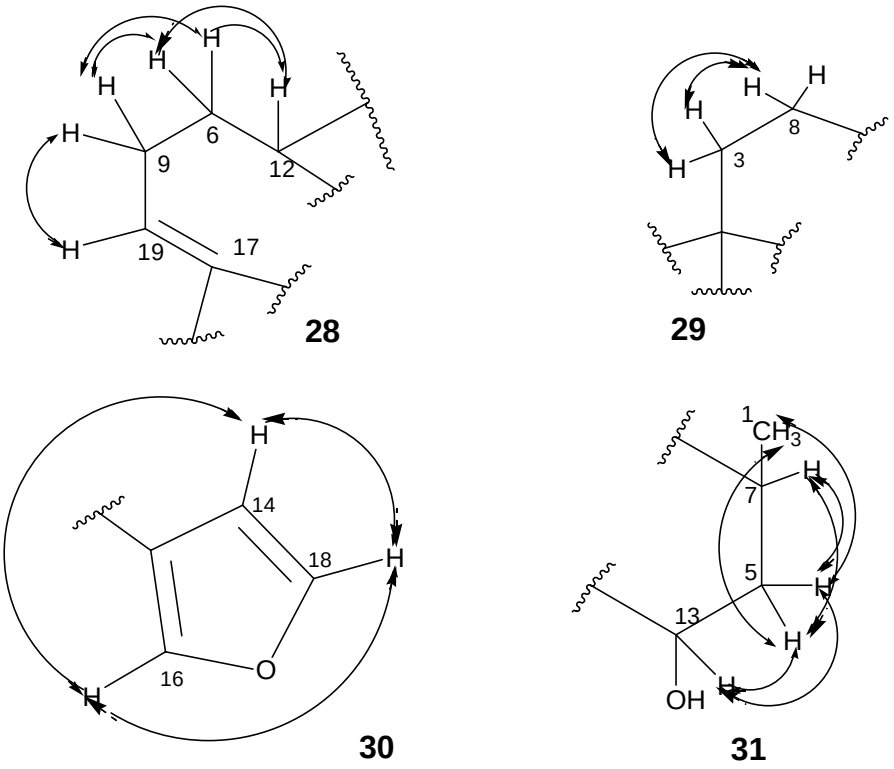
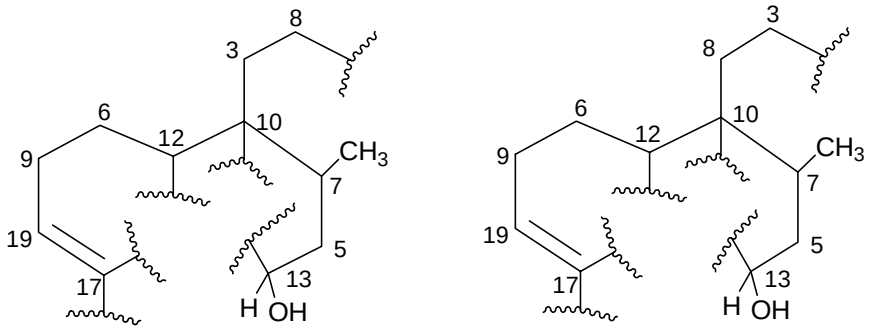


Figure 7: Partial structures based on ^{1}H-^{1}H COSY spectral data of Dc-8B

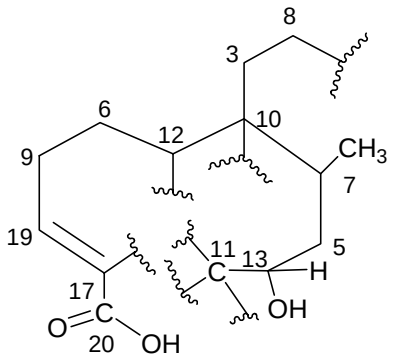
In fact, differentiating between the suggested partial structures based on proton multiplicity is difficult, since the protons are not well resolved in the aliphatic region. To identify the correct structure, finding the stereogenic centers which are attached to any of these is necessary. From the HMBC spectrum (Appendix 1.7) and Table 5, methine proton H-12 correlates with the quaternary carbon C-10 at δ38.7, and methylene proton H-8 correlates with C-10. Similarly the methyl proton H-1 correlates with C-10, based on this information the partial structures **32** and **33** maybe assigned.



32 **33**

Figure 8: Comparative partial structures based on HMBC spectral data of Dc-8B

To differentiate between these two suggested structures, there is a need to see other correlations from HMBC; hence, C-3 (δ17.3) shows correlation with H-12 (δ1.40) indicating structure **32** to be more preferable than **33**. In addition, the methine proton H-13 show correlation with a quaternary carbon C-11 (δ44.7), H-19 (δ7.20) shows correlation with a carbonyl carbon C-20 (δ173.4). Based on the information proposed structure **32** can be improved to give structure **34**.



34

Figure 9: Suggested partial structure based on HMBC spectral data of Dc-8B

From HMBC, again there is correlation between H-19 and C-11 indicating a quaternary carbon C-11 has to be connected to C-17 to form a cyclic system. Moreover, there is strong correlation between H-12 and C-11 indicating the quaternary carbon C-11 must be attached again to C-12. Strong correlation of methyl hydrogen H-2 (δ0.88) with C-11 was observed indicating the quaternary carbon C-11 has to incorporate methyl group. From the above information, two possible structures can be deduced for compound Dc-8B. To identify the correct structure the HMBC data given in Table5 was found to be most useful.

Table 5. HMBC data for compound Dc-8B

Carbon No.	^{13}C δ(ppm)	HMBC (1H→13C)
C-1	14.6	H-1↔C-5, H-1↔C-7, H-1↔C-10
C-2	16.7	H-2↔C-11, H-2↔C-12, H-2↔C-13, H-2↔C-17
C-4	17.6	H-4↔C-7, H-4↔C-10, H-4↔C-12
C-5	17.9	H-5↔C-7
C-8	35.6	H-8↔ C-10
C-9	38.6	H-9↔ C-6, H-9↔ C-17
C-12	45.6	H-12↔C-3, H-12↔C-6, H-12↔C-10, H-12↔C-11
C-13	74.4	H-13↔C-2, H-13↔C-17
C-14	110.9	H-14↔C-15, H-14↔C-16, H-14↔C-18
C-16	138.4	H-16↔C-14, H-16↔C-15, H-16↔C-18,
C-18	142.8	H-18↔C-14, H-18↔C-15, H-18↔C-16
C-19	144.1	H-19↔C-11, H-19↔C-20

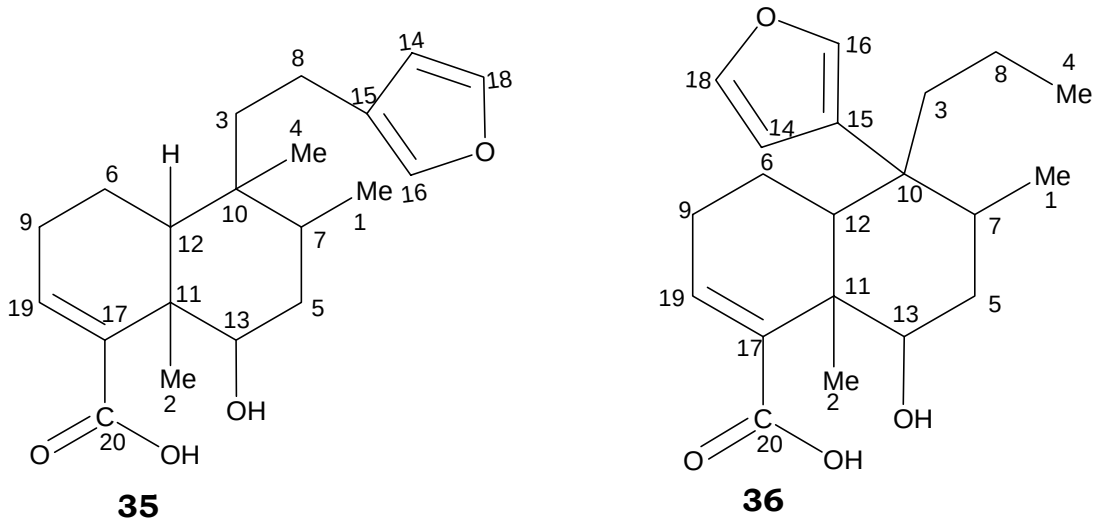


Figure 10: The possible suggested structure for compound Dc-8B

Comparing structures **35** and **36** with the help of full HMBC data, it was found out that there is no correlation between methylene hydrogen H-8 and methyl carbon C-4 or there is no correlation between methyl hydrogen H-4 and methylene carbon C-8. In addition there is no correlation between methine proton H-12 and C-15. In fact, HMBC also suggested strong correlation between H-4 and C-10. From this information and the whole data of HMBC, HMQC, ^{1}H-^{1}H COSY, DEPT, ^{13}C NMR, ^{1}H NMR and UV, the suggested structure **35** is most probably the correct structure of compound Dc-8B and to our knowledge compound **35** (Dc-8B) has not been reported from *Dodonaea viscosa* var. *angustifolia*.

4.2. Characterization of compound D-16

The compound D-16 was obtained as white needle-like crystals soluble in methanol but insoluble in ethyl acetate. Its melting point was measured to be within a range of 163.7-165.3^{0}C (179-179.5^{0}C literature value [17]). It displays UV absorption peaks in ethanol (Appendix 2.1) at λ_{max} = 280 nm indicating the presence of $\Pi \rightarrow \Pi^*$ electronic transitions for C=C double bond and the absence of conjugation.

The ^{1}H NMR spectrum (Appendix 2.2) of D-16 in DMSO indicates the presence of two methyl signals at δ 0.76 (3H, s) and δ 0.80 (3H, d), five diastereotopic methylene protons at δ 1.81, 1.53 (2H, m), δ 2.08, 2.27 (2H, m), δ 1.02, 2.30 (2H, dt), δ 1.40, 1.59 (2H, m) and δ 3.68, 3.92 (2H, dd). More specifically, the ^{1}H NMR of compound D-16 shows the presence of primary alcoholic group attached to a quaternary carbon atom at δ 3.68 and 3.92 (AB pattern). The NMR spectrum also showed two methylene protons in the aliphatic region at δ 2.24 (2H, ddd) and δ 1.36 (2H, dd) and two methine protons in the aliphatic region at δ 1.58 (1H, m) and δ 1.39 (1H, dd). Moreover, one methine proton in the olefinic region at δ 6.59 (1H, t) and three methine protons at δ 6.35 (1H, d), δ 7.43 (1H, s) and δ 7.53 (1H, d) in aromatic region were shown in Table 6.

Table 6. ^{1}H NMR data of compound D-16.

Carbon No.	Proton chemical shift (δ in ppm)	No. of hydrogen, multiplicity	Remark
C-1	0.80	3H, d	CH_3
C-2	1.81, 1.53	2H, m	CH_2
C-3	2.08, 2.27	2H, m	CH_2
C-4	0.76	3H, s	CH_3
C-5	2.24	2H, ddd	CH_2
C-6	1.36	2H, dd	CH_2
C-7	1.02, 2.30	2H, td	CH_2
C-8	1.58	1H, m	CH
C-9	1.40, 1.59	2H, m	CH_2
C-10	---	---	Quaternary carbon
C-11	---	---	Quaternary carbon
C-12	1.39	1H, dd	CH
C_{13}	3.68, 3.92	2H, dd	CH_2
C-14	6.35	1H, d	CH
C-15	---	---	Quaternary carbon
C-16	6.59	1H, t	CH
C-17	7.43	1H, s	CH
C-18		---	Quaternary carbon
C-19	7.53	1H, d	CH
C-20	---	---	Quaternary carbon

The ^{13}C NMR spectrum (Appendix 2.3) of D-16 in DMSO analyzed with DEPT-135 (Appendix 2.4, Table 7), shows the presence of two quaternary carbons in the aliphatic region at δ39.8 and δ 42.9, two quaternary carbons in the olefinic region at δ 141.3 and δ 126.5 and one quaternary oxygenated carbon at δ170.6. In addition, two methine carbons in the aliphatic region at δ 36.9 and δ 47.4 and four methine carbons in the olefinic region at δ 139.3, δ 112.5, δ 139.9 and δ 144.2. Comparing the chemical shift of C-14 (δ 111.7), C-17 (δ 139.1) and C-19 (δ 143.4); it can be seen that C-19 and C-17 are deshielded by the electronegative oxygen atom directly connected to them while C-14 is shielded by the resonance between the Π-bond and the unshared pair of electron on oxygen. Hence, the signal due to C-14 is found up field shifted than the other two. Moreover, C-17 and C-19 are both influenced by the resonance effect to the same extent but the presence of alkyl at adjacent position shielded C-17 inductively largely than the C-19 found three bonds away from the alkyl substituent. Hence, the signal due to C-17 is also found up field from the C-19 signal in its ^{13}C NMR spectrum. The DEPT-135 spectrum also confirms the presence of seven methylene carbon signals at δ 17.1, 18.1, 26.8, 27.3, 32.5, 38.9 and 64.6. Out of them, the methylene at δ 64.6 is most probably an oxygenated carbon as it is relatively found down field of the others. The ^{13}C NMR spectrum of D-16 (DMSO-d_6) also shows the presence of two methyl carbons at δ 17.1 and 19.5.

Table 7. Proton decoupled ^{13}C NMR data for compound D-16

Carbon		Remark	Carbon		Remark
No.	δ (ppm)		No.	δ (ppm)	
C-1	16.3	CH_3	C-11	42.9	Quaternary carbon
C-2	17.1	CH_2	C-12	46.6	CH
C-3	18.1	CH_2	C-13	64.6	CH_2
C-4	18.7	CH_3	C-14	111.6	CH
C-5	26.8	CH_2	C-15	126.5	Quaternary carbon
C-6	27.3	CH_2	C-16	138.4	CH
C-7	32.5	CH_2	C-17	139.1	CH
C-8	36.2	CH	C-18	141.3	Quaternary carbon
C-9	38.9	CH_2	C-19	143.4	CH
C-10	39.8	Quaternary carbon	C-20	170.6	Quaternary carbon

The HMQC spectrum (Appendix 2.5) shows correlations such that the two methyl protons at δ 0.80 and δ 0.76 correlate with the carbon signal at δ 17.1 and 19.5, respectively. The two aliphatic methylene protons at δ 1.58 and 1.39 correlate with the carbon signals at δ 36.2 and δ 46.6, respectively. HSQC also shows the correlation of oxygenated methylene group at δ 3.68 and δ 3.92 with carbon at δ 64.6. The HMQC data of compound D-16 is presented in Table 8.

Table 8. Observed correlations in HMQC data of compound D-16

DEPT		Proton No.	^{1}H NMR δ(ppm)	Multiplicity	Remark
Carbon No.	δ(pp)				
C-1	16.3	1	0.80	d	CH_3
C-2	17.1	2	1.81, 1.53	m	CH_2
C-3	18.1	3	2.08, 2.27	m	CH_2
C-4	18.7	4	0.76	s	CH_3
C-5	26.8	5	2.24	ddd	CH_2
C-6	27.3	6	1.36	dd	CH_2
C-7	32.5	7	1.02, 2.30	td	CH_2
C-8	36.2	8	1.58	m	CH
C-9	38.9	9	1.40, 1.59	m	CH_2
C-12	46.6	12	1.39	dd	CH
C-13	64.6	13	3.68, 3.92	dd	CH_2
C-14	111.7	14	6.35	d	CH
C_{-16}	138.4	16	6.59	t	CH
C-17	139.1	17	7.43	s	CH
C-19	143.4	19	7.53	d	CH

The ^{1}H-^{1}H COSY experiment (Appendix 2.6) shows the correlation between H-2 at δ 1.81, 1.53 with H-5 at δ 2.24 and H-2 with H-2. Similarly, H-3 at δ 2.08, 2.27 correlates with H-8 at δ 1.56 and H-9 at δ 1.40, 1.59. H-7 at δ 1.02, 2.30 correlate with H-6 at 1.36, H-7 and H-12 at δ 1.39. H-9 at δ 1.40, 1.59 correlates with H-3, moreover H-13 at δ 3.68, 3.92 correlates with H-13,

indicating the five-methylene protons to be diastereotopic. Similarly, there are other strong correlations observed from COSY experiment as shown in Table 9.

Table 9. Observed correlations in ^{1}H-^{1}H COSY spectral data of compound D-16

Carbon No.	^{1}H NMR δ (in ppm)	^{1}H-^{1}H COSY
C-1	0.80	H-1↔H-8
C-2	1.81, 1.53	H-2↔H-2, H-2↔H-5
C-3	2.08, 2.27	H-3↔ H-8, H-3↔ H-9
C-5	2.24	H-5↔H-2, H-5↔H-16
C-6	1.36	H-6↔H-7
C-7	1.02, 2.30	H-7↔H-6, H-7↔H-7, H-7↔H-12
C-8	1.58	H-8↔H-1, H-8↔H-^{1}
C-9	1.40, 1.59	H-9↔H-3
C-12	1.39	H-12↔H-7
C-13	3.68, 3.92	H-13↔H-13
C-14	6.35	H-14↔H-19
C-16	6.59	H-16 ↔H-5
C-17	7.43	H-17↔H-19
C-19	7.53	H-19↔H-14, H-19↔H-17

The information obtained from ^{1}H NMR, ^{13}C NMR, DEPT and ^{1}H-^{1}H COSY can suggest partial structures **37**, **38**, **39**, **40** and **41**.

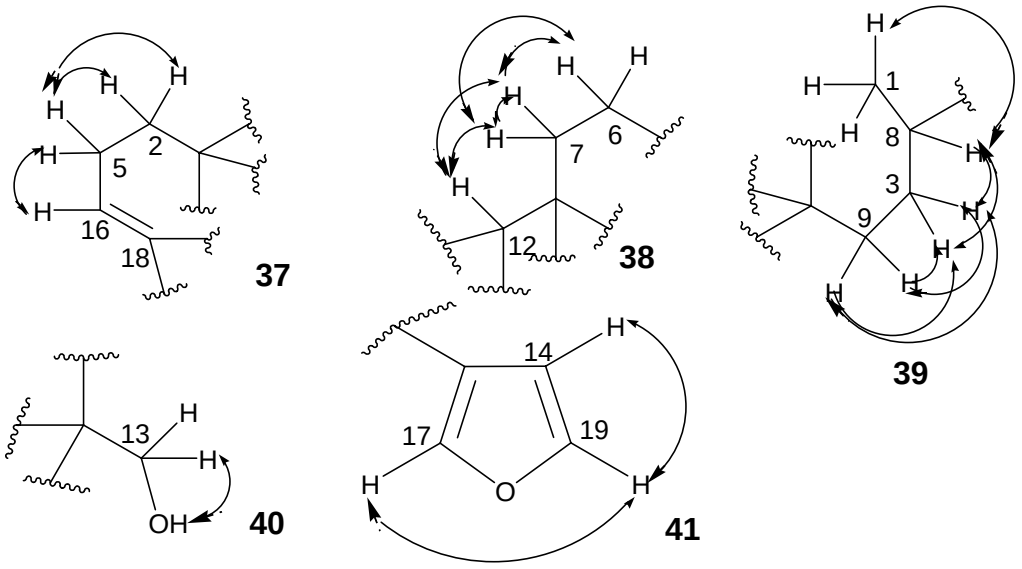


Figure 11: Suggested partial structural fragments based on ^{1}H-^{1}H COSY spectral data of D-16.

The next task to be done is identifying the stereogenic center to which each of the diastereotopic methylene carbons are attached. This can be done by analyzing the HMBC spectrum (Table 10, Appendix 2.7) of compound D-16. Thus, H-1 (δ 0.80) correlates with C-10 (δ 39.8), H-4 (δ 0.76) correlates with C-10, H-4 correlates with C-12 (δ 46.6), and H-12 (δ 1.39) correlates with C-2 (δ 17.1). From these correlations we can find out that, since C-1 is directly attached to C-8, the observed correlation between H-1 and C-10 may be a three bond correlation in which C-10 is attached to C-8. Moreover, C-12 is a methine carbon containing only H-12 and H-4 correlates with both C-12 and C-10 indicating the methyl carbon C-4 is most probably attached to C-10. C-12 should be connected directly to C-10 to have the expected three bond correlation between H-4 and C-12; consequently C-10 also fulfills the expected stereogenicity. In similar way H-12 correlates with C-11 (δ 42.9), H-13 (δ 3.68, 3.92) correlates with C-18 (δ 141.3), H-16 (δ 6.59) correlates with C-11, and H-

16 correlates with C-20 (δ 170.6). From these correlations, the methine carbon C-16 in the olefinic region is found connected to C-5 (δ 26.8) and C-18 at both ends. Hence, the observed correlation between H-16 and C-11 should be a three bond correlation in which C-11 is most probably found attached to C-18. In addition, H-16 also shows correlation with C-20 (a carbonyl carbon), indicating the expected carboxylic functional group is most probably attached to C-18 and C-18 is a quaternary carbon as expected from ^{13}C NMR and DEPT experiment. On the other hand, the observed correlation between H-12 and C-11 should be a two-bond correlation in which C-11 has to be directly connected to C-12; hence, C-12 also fulfills the expected stereogenicity. The correlation observed between H-13 and C-18 indicated H-13 to be three or more bonds away from C-18 since C-18 is a quaternary carbon bearing the carboxylic functional group on one hand and the expected quaternary C-11 on another hand. Therefore, C-13 is most probably attached to C-11.

Table 10. Observed correlations in HMBC spectral data of compound D-16

Carbon NO.	^{13}C NMR δ(ppm)	HMBC
C-1	16.3	H-1↔C-8, H-1↔ C-10
C-4	18.7	H-4↔ C-8, H-4↔ C-10, H-4↔ C-12
C-12	46.6	H-12↔C-2, H-12 ↔ C-10, H-12↔ C-11
C-13	64.6	H-13↔C-18
C-14	111.7	H-14 ↔ C-15, H-14 ↔ C-17, H-14 ↔ C-19
C-16	138.4	H-16 ↔ C-11, H-16 ↔ C-20
C-17	139.1	H-17↔C-14, H-17↔C-15, H-17↔C-19
C-19	143.4	H-19↔C-14, H-19↔C-15, H-19↔C-17

In the aromatic region, proton-carbon correlations were observed such that H-14 (δ 6.35) correlates with C-15 (δ 126.5), C-17 (δ 139.1) and C-19 (δ 143.9). H-17 correlates with C-14 (δ 111.7), C-15 and C-19. Similarly, H-19 (δ7.53) correlates with C-14, C-15 and C-17. This shows the aromatic moiety consisting of four carbons and oxygen atom is most probably a furan. The fact that the furan ring is substituted at the 3-position is evident from the ^{1}H and ^{13}C NMR spectra. The above spectroscopic evidence allows the structural fragments to be assembled together to give fragments **42**, **43** and **44** shown in Figure 12.

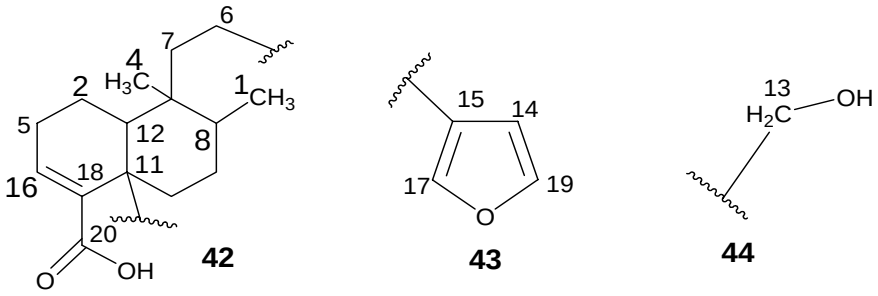


Figure 12: Suggested structural fragments for compound D-16 based on HMBC.

The fragments in Figure 12 can be brought together to give two possible structures **45** and **46** for D-16.

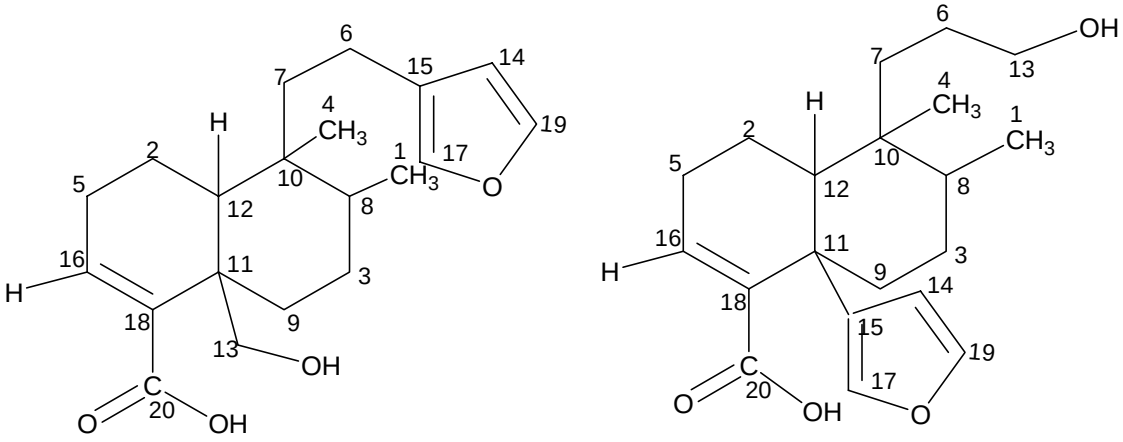


Figure 13: Suggested structures for compound D-16

The ^{1}H NMR spectrum displayed signals attributable to H-13 as AB doublets at δ 3.68 and 3.92 indicating the presence of diastereotopic methylene protons on C-13. Thus, C-13 should be adjacent to a chiral center; hence, structure **46** can be rejected since a triplet signal is expected for H-13 according to the structure. Based on the above information and full spectral data obtained from HMBC, ^{1}H-^{1}H COSY, HSQC, DEPT, ^{13}C NMR and ^{1}NMR experiments structure **45** is assigned to D-16. An attempt to compare the ^{1}H and ^{13}C NMR data presented above for compound **45** with the previously identified hautriwaic acid [17] was not satisfactory since it was reported on short communication paper, the full ^{1}H and ^{13}C NMR data were not reported and only selected proton correlations which closely agree with those described above for compound **45** were given.

4.3. Characterization of compound D_{26}-3B

Compound D_{26}-3B was obtained as yellow solid from the $CHCl_3$ extract. It is optically inactive and has melting point within a range of 163.5 -164.6^{0}C. The UV-VIS spectrum (in $CHCl_3$) (Appendix 3.1) showed maximum absorption bands at λ_{max}= 273 nm indicating the presence of $\Pi \rightarrow \Pi^*$ transition of C=C double bond and 334 nm indicating the presence of $\Pi \rightarrow \Pi^*$ transition of C=O (carbonyl functionality) and $n \rightarrow \Pi^*$ transition of C=O bond which showed the molecule has conjugation.

The ^{1}H NMR spectrum (Appendix 3.2) of D_{26}-3B suggested the presence of three methyl signals at δ 3.90 (3H, s), δ 3.85 (3H, s), and δ 4.05 (3H, s), three methine protons in the aromatic region at δ 6.57 (1H, s), δ 7.01 (1H, d) and δ 8.15 (1H, d). The spectrum also showed a broad signal at δ 6.35 indicating an OH signal in the compound, moreover, a signal at δ 12.09 (1H, s) indicates the presence of an acidic proton.

Table 11. ^{1}H NMR data of compound D_{26}-3B

Carbon No.	Proton chemical shift (δ in ppm)	No. of hydrogen, multiplicity	Remark
C-1	3.90	3H, s	CH_3
C-2	3.85	3H, s	CH_3
C-3	4.05	3H, s	CH_3
C-4	6.57	1H, s	CH
C-5	---	---	Quaternary carbon
C-6	7.02	2H, d	CH
C-7	---	---	Quaternary carbon
C-8	---	---	Quaternary carbon
C-9	8.07	2H, d	CH
C-10	---	---	Quaternary carbon
C-11	---	---	Quaternary carbon
C-12	---	---	Quaternary carbon
C-13	---	---	Quaternary carbon
C-14	---	---	Quaternary carbon
C-15	---	---	Quaternary carbon
C-16	---	---	Quaternary carbon

The ^{13}C NMR spectrum (Appendix 3.3, Table 12) of D_{26}-3B analyzed with the aid of the DEPT-135 spectrum (Appendix 3.4) showed ten quaternary carbons, which were attributed to five oxygenated aromatic carbons at δ 130.1, 151.8, 152.2, 155.1 and 161.7, two oxygenated olefinic carbons at δ 138.4 and 156.2,

two aromatic quaternary carbons at δ 106.1 and 122.7, and a carbonyl carbon at δ 179.2. Three methine carbon signals in the aromatic region at δ 93.2, 114.1 and 130.2 of which two are due to symmetrically placed aromatic carbons on a *para* substituted aromatic ring. Three methyl carbons at δ 55.4, 60.2 and 60.9 indicate the presence of three methoxy groups.

Table 12. Proton decoupled ^{13}C NMR data for compound D_{26}-3B.

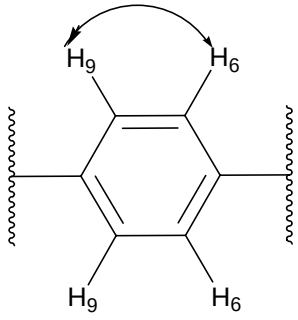
Carbon		Remark	Carbon		Remark
No.	δ (ppm)		No.	δ (ppm)	
C-1	55.5	CH_3	C-9	130.2	CH
C-2	60.2	CH_3	C-10	138.4	Quaternary carbon
C-3	60.9	CH_3	C-11	151.8	Quaternary carbon
C-4	99.2	CH	C-12	152.2	Quaternary carbon
C-5	106.1	Quaternary carbon	C-13	155.1	Quaternary carbon
C-6	114.1	CH	C-14	156.2	Quaternary carbon
C-7	122.7	Quaternary carbon	C-15	161.7	Quaternary carbon
C-8	130.1	Quaternary carbon	C-16	179.2	Quaternary carbon

The HMQC NMR spectrum showed correlations such that the methyl protons at δ 3.90, δ 3.85 and δ 4.05 correlate with carbons at δ 55.4, δ 60.2 and δ 60.9, respectively. In addition, aromatic protons at δ 6.57, 7.01 and 8.15 also correlate with aromatic carbons at δ 93.2, 114.1 and 130. 2, respectively, as shown in Table 13.

Table 13. HMQC data for compound D_{26}-3B

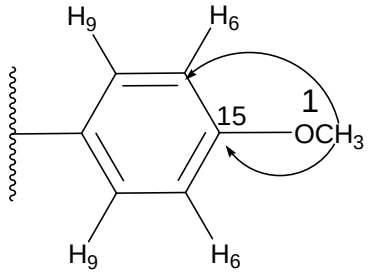
DEPT		Proton No.	^{1}H NMR δ(ppm)	Multiplicity	Remark
Carbon No.	δ(ppm)				
C-1	55.5	1	3.90	s	CH_3
C-2	60.2	2	3.85	s	CH_3
C-3	60.9	3	4.05	s	CH_3
C-4	93.2	4	6.57	s	CH
C-6	114.1	6	7.02	d	CH
C-9	130.2	9	8.07	d	CH

The ^{1}H-^{1}H COSY spectrum (Appendix 3.6) showed the correlation between two aromatic protons each symmetrically placed on *para* substituted benzene ring H-6 at δ 7.01 and H-9 at δ8.15. The two protons are found coupled to each other but no other ^{1}H-^{1}H correlations are observed from its COSY spectrum.



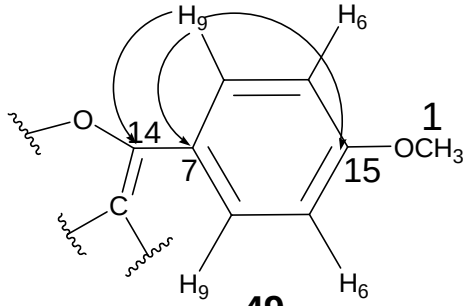
47

The HMBC spectral data (Appendix 3.7, Table 14) reveals that H-1 (δ 3.9) correlates with C-6 (δ 114.1) and C-15 (δ 161.7) indicating one of the quaternary carbons on the *para* substituted benzene ring is C-15 and the methoxy group bearing C-1 (δ 55.4) is found attached to it. Moreover, the correlation observed between H-1 and C-6 showed the position of C-6 to be *ortho* to C-15. From this information a partial structure can be deduced as



48

Additional correlations observed between H-9 (δ 8.15) with C-7 (δ 122.7 strong), C-14 (δ 156.2) and C-15 (δ 161.7) showed the partial structure can further be extended to substructure **49**. As confirmed from ^{13}C NMR and DEPT experiments, C-14 is a quaternary oxygenated carbon at olefinic position.

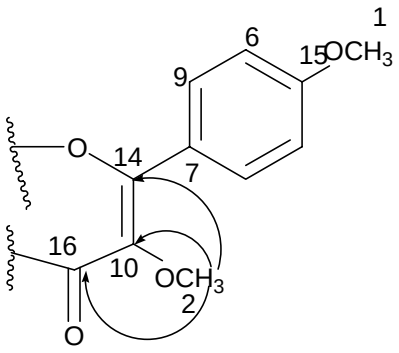


49

Table 14.Observed correlations in HMBC data of compound D_{26}-3B

Carbon No.	^{13}C δ(ppm)	HMBC ($^{1}H \rightarrow ^{13}C$)
C-1	55.5	H-1↔C-6, H-1↔C-15
C-2	60.2	H-2↔C-10, H-2↔C-14, H-2↔C-16,
C-3	60.9	H-3↔C-5, H-3↔C-8, H-3↔C-11
C-4	93.2	H-4↔C-5, H-4↔C-8, H-4↔C-11, H-4↔C-12, H-4↔C-13, H-4↔C-14
C-6	114.1	H-6↔C-7, H-6↔C-9, H-6↔C-14, H-6↔C-15
C-9	130.2	H-9↔C-7, H-9↔C-14, H-9↔C-15
OH on C-13		H_{OH}↔C-5, H_{OH}↔C-8, H_{OH}↔C-8, H_{OH}↔C-11, H_{OH}↔C-12, H_{OH}↔C-13, H_{OH}↔C-16

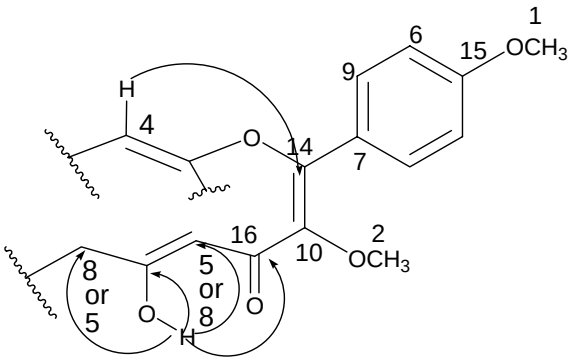
The HMBC spectrum also showed a correlation between H-2 (δ 3.85) with C-10 (δ 138.4), C-14 and C-16 (δ 179.2). In connection to this, ^{13}C NMR and DEPT spectra showed C-14 is a quaternary carbon, C-16 is a carbonyl carbon, C-10 is again a quaternary oxygenated carbon at olefinic position, and hence a methoxy group containing C-2 is most probably attached to C-10. The attachment of C-10 to both C-14 and the carbonyl carbon C-16 could also be confirmed. From this information, the above partial structure can be further extended as



50

Figure 14: Partial structure of compound D_{26}-3B based on HMBC data.

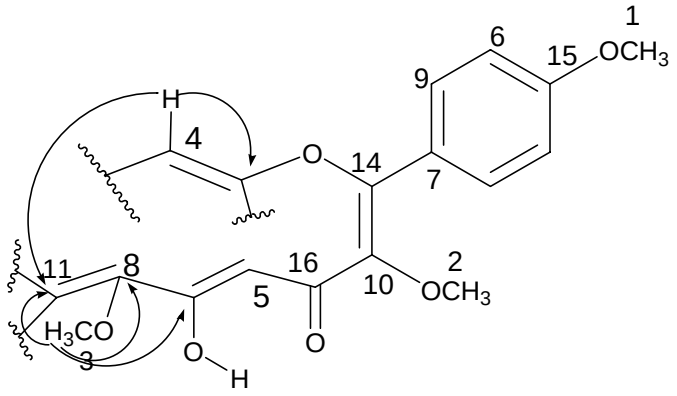
The HMBC spectrum further displayed correlations between H-4 and C-14, C-12 (δ 152.2), C-11 (δ 151.8), C-13 (δ 155.1), C-15 and C-8 (δ130.1). Since the signal due to H-4 is a singlet in aromatic region and the corresponding ^{13}C signal of C-4 appeared at δ 93.2, C-4 can not be at oxygenated position. Therefore, the observed correlation between H-4 and C-14 should be a four bond correlation across "O" linkage. The acidic proton of hydroxyl group at δ 12.90 can be attributed to the OH proton at C-13 which is chelated with the carbonyl oxygen. The strong correlations observed with quaternary carbons C-5 and C-8 could be used to place them at *ortho* position to the chelated hydroxyl group and the following partial structure can be suggested.



51

Figure 15: Partial structure of compound D_{26}-3B based on HMBC data.

To ascertain the exact positions of C-5 and C-8, observing further correlations is necessary. Hence strong correlation is observed in the HMBC spectrum between the methoxy protons signal at δ 4.05 and C-8 indicating C-8 to be oxygenated and connected to methoxy group. Similarly, the quaternary carbon C-5 is not at oxygenated position as confirmed by its ^{13}C NMR spectrum. Moreover, weak correlations observed between the methoxy protons signal at δ 4.05 and C-11, and C-15 indicates a four-bond correlation connecting C-11 to C-8. Therefore, a quaternary carbon C-5 is most probably located between C-16 and the carbon bearing the chelated OH group.

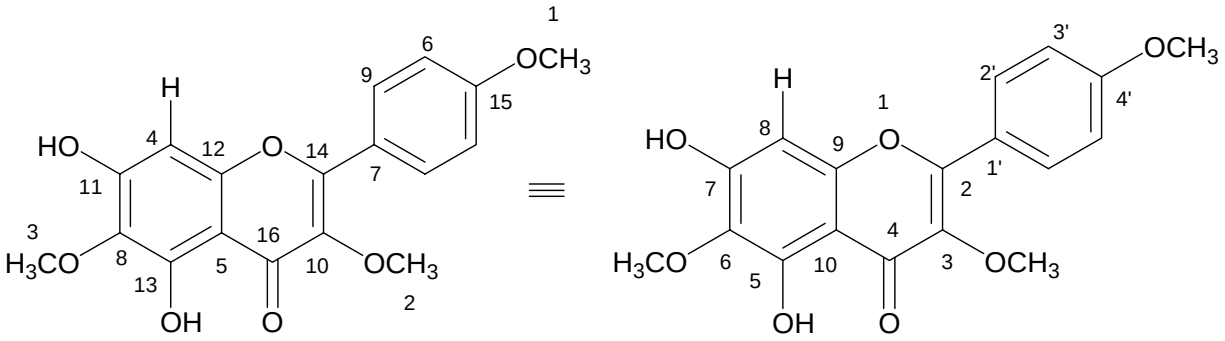


52

Figure 16: Partial structure of compound D_{26}-3B based on HMBC data.

The strong correlation observed between H-4 and C-11 or C-12 placed both C-11 and C-12 (oxygenated carbon) at *ortho* position to C-4, moreover, the observed correlation between H-4 and C-5, results in a ring closure between C-12 and C-5 to form a six-membered aromatic ring. The correlation observed between the chelated hydroxyl proton and C-13 confirms the position of hydroxyl group to be on C-13. In a similar way a quaternary carbon C-11 (δ 151.8) at oxygenated position most probably contains an OH proton whose chemical shift was indicated at δ 6.80 in its ^{1}H NMR spectrum. Based on the above information and full spectral data of UV-VIS, ^{1}H NMR, ^{13}C NMR, DEPT,

^{1}H-^{1}H COSY, HMQC, and HMBC, the correct structure of compound D_{26}-3B most probably is the same as the known flavonoid santin (5,7-dihydroxy-3,6,4'-trimethoxy flavones) (compound **13**) [11].



53 (D_{26}-3B) Santin (5,7-dihydroxy-3,6,4'-trimethoxy flavone)

5. EXPERIMENTAL SECTION

5.1. General

^{1}H, ^{13}C, DEPT-135 and 2D NMR spectra were recorded on a Bruker Avance 400 spectrometer in $CDCl_3$ and deutrated DMSO. The ultra-violet and visible (UV-Vis) spectra were taken on GENESY'S 2PC UV-Vis scanning spectrometer (200-800 nm). Melting points were recorded on Mettler FP-85 cell using FP-90 central processing unit. Analytical thin layer chromatography was run with precoated 0.2 mm silica gel 60 F_{254} on aluminum foil and compounds on TLC were detected under UV lamp at 254 and 346 nm. Column chromatography was done by using silica gel 60, particle size 0.063-0.200 nm (70-230 mesh ASTM). Preparative thin layer chromatography was done on 1 mm silica gel plates prepared by coating silica gel GF_{254} with 13% calcium sulfate and fluorescent indicator on glass plates.

5.2. Sample Collection

The leaves of *Dodonaea viscosa* var. *angustifolia* were collected in Feb 2009, from the western part of Ethiopia around Gidda Ayana town. The collection was carried out from two localities called Lagadhera and Yanadoro 1 Km North and 5 Km South-East of Gidda Ayana, respectively. The plant specimen collected was identified by Prof. Ensermu Kelbesa of the Biology Department (AAU) and a voucher specimen (DES-001) was deposited at the National Herbarium of Ethiopia, Addis Ababa University, Faculty of Science.

5.3. Extraction and Isolation

5.3.1. Petroleum ether extract

The air-dried and ground leaves of *Dodonaea viscosa* var. *angustifolia* (300 g) were soaked with 550 ml petroleum ether for 36 hours. The extract was decanted, filtered on fluted filter paper and evaporated under reduced pressure by using rotary evaporator to afford 4.44 g of crude extract which was adsorbed on 12 g silica gel to carry out column chromatography. The crude extract was further fractionated by a solvent system of petroleum ether: ethyl acetate (7:3) with an RF value of 0.4 for compound Dc-8B. 144.5 g silica gel was subjected to column packing and eluted with a solvent system of petroleum ether: ethyl acetate (7:3) to afford 20 fractions. Out of the 20 fractions collected, fractions 17 to 20 were mixed together, concentrated under reduced pressure and packed twice with a solvent system of petroleum ether: ethyl acetate (8:2). After 24 hour a white crystal was obtained at the bottom of the 8th fraction and washed repeatedly with hexane to remove impurities.

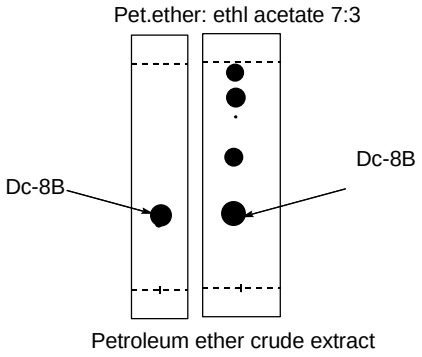


Figure 18: TLC of petroleum ether crude extract

5.3.2. Chloroform extract

The solvent free marc of petroleum extract was then soaked with chloroform (450 ml) for 36 hours. The chloroform extract was then decanted, filtered on fluted filter paper and concentrated under reduced pressure to afford 26 g of crude extract. The crude extract was adsorbed on 15 g silica gel and then subjected to column chromatography and eluted with gradients of solvent system to afford 60 fractions as shown below.

1. Petroleum ether (100%) 1-5 fractions
2. Petroleum ether: ethyl acetate (9:1) 7-12 fractions
3. Petroleum ether: ethyl acetate (8:2) 13-17 fractions
4. Petroleum ether: ethyl acetate (7:3) 18-24 fractions
5. Petroleum ether: ethyl acetate (6:4) 25-31 fractions
6. Petroleum ether: ethyl acetate (5:5) 32-35 fractions
7. Petroleum ether: ethyl acetate (4:6) 36-39 fractions
8. Petroleum ether: ethyl acetate (3:7) 40-42 fractions
9. Petroleum ether: ethyl acetate (2:8) 43-46 fractions
10. Petroleum ether: ethyl acetate (1:9) 47-50 fractions
11. Ethyl acetate (100%) 51-52 fractions
12. Ethyl acetate: methanol (9:1) 53-56 fractions
13. Ethyl acetate: methanol (8:2) 57-60 fractions

Based upon their TLC profile fractions from 25-35 were mixed together and labeled as D-26. Similarly fractions from 47-60 have similar composition and labeled as D-50 after mixing together. D-26 was concentrated and subjected to column chromatography after adsorbing on 5 g silica gel to afford three fractions each about 80 ml and labeled D_{26}-1, D_{26}-2 and D_{26}-3. From the three fractions, D_{26}-3 was concentrated and subjected to preparative thin layer chromatography with a solvent system of petroleum ether: ethyl acetate (3:2) and the band corresponding to the major compound was carefully taken from the plate, washed with a mixture of ethyl acetate: methanol (9:1), concentrated under reduced pressure, allowed to dry in vacuum oven for 8 hours and labeled as D_{26}-3B for NMR analysis.

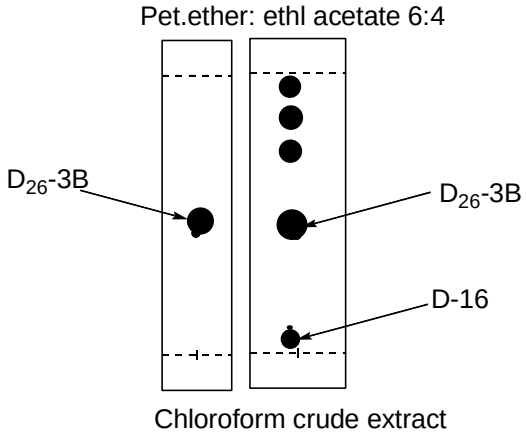


Figure 19: TLC of chloroform extract

Fraction D-50 was concentrated under reduced pressure and in to it was added ethyl acetate and allowed to stand for one hour. After one hour the impurities were found dissolved in ethyl acetate while white needle-like crystals were found deposited at the bottom of the flask which were found to be soluble in methanol. These needle-like crystals were labeled as D-16 for NMR analysis.

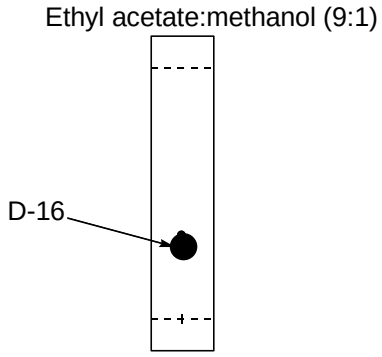


Figure 20: TLC of compound D-16

6. SPECTRAL DATA

Dc-8B (**35**): White crystalline solid, m.p. 92.8-94.6^{0}C, RF 0.4 in pet. Ether: EtOAC (7:3), $[\alpha]_D$=-2.4^0, UV-Vis λ_{max} (ethanol) nm: 249, 273, 314 and 333. ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, d, CH_3), 1.25 (3H, s, CH_3), 1.59, 1.63 (2H, m, CH_2), 0.77(3H, s, CH_3), 1.64, 1.67 (2H, m, CH_2), 1.71, 1.74 (2H, m, CH_2), 1.53 (1H, m, CH), 2.18 (2H, dd, CH_2), 2.32 (2H, ddd, CH_2), 1.40 (1H, dd, CH), 3.78 (1H, dd, CH), 6.27 (1H, dd, CH), 7.22 (1H, s, CH), 7.37 (1H, dd, CH) and 7.20 (1H, t, CH). ^{13}C NMR ($CDCl_3$) δ 14.6 (C-1), 16.7 (C-2), 17.2 (C-3), 17.6 (C-4), 17.9 (C-5), 27.5 (C-6), 33.8 (C-7), 35.6 (C-8), 38.6 (C-9, C-10), 44.7 (C-11), 45.6 (C-12), 74.4 (C-13), 110.9 (C-14), 125.3 (C-15), 138.4 (C-16), 140.3 (C-17), 142.8 (C-18), 144.1 (C-19) and 173.4 (C-20).

D-16 (**45**): White needle-like crystals, m.p. 163.7-165.3^{0}C (lit. [17] 179-179.5^{0}C), UV-Vis λ_{max} (ethanol) nm: 280. ^{1}H NMR (DMSO) δ 0.80 (3H, d, CH_3), 1.81, 1.53 (2H, m, CH_2), 2.08, 2.27 (2H, m, CH_2), 0.76 (3H, s, CH_3), 2.24 (2H, ddd, CH_2), 1.36 (2H, dd, CH_2), 1.02, 2.30 (2H, td, CH_2), 1.58 (1H, m, CH), 1.40, 1.59 (2H, m, CH_2), 1.39 (1H, dd, CH), 3.68, 3.92 (2H, dd, CH_2), 6.35 (1H, d, CH), 6.59 (1H, t, CH), 7.43 (1H, s, CH) and 7.53 (1H, d, CH). ^{13}C NMR (DMSO) δ 16.3 (C-1), 17.1 (C-2), 18.1 (C-3), 18.7 (C-4), 26.8 (C-5), 27.3 (C-6), 32.5 (C-7), 36.2 (C-8), 38.9 (C-9), 39.8 (C-10), 42.9 (C-11), 46.6 (C-12), 64.6 (C-13), 111.6 (C-14), 126.5 (C-15), 138.4 (C-16), 139.1 (C-17), 141.3 (C-18), 143.4 (C-19) and 170.6 (C-20).

D_{26}-3B (**53**): Yellow solid, m.p. 163.5-164.4^{0}C, RF 0.45 in pet. Ether: EtOAC (6:4), UV-Vis λ_{max} ($CHCl_3$) nm: 273 and 334. ^{1}H NMR ($CDCl_3$) δ 3.90 (3H, s, CH_3), 3.85 (3H, s, CH_3), 4.05 (3H, s, CH_3), 6.57 (1H, s, CH), 7.02 (2H, d, CH) and 8.07 (2H, d, CH). ^{13}C NMR ($CHCl_3$) δ 55.5 (C-1), 60.2 (C-2), 60.9 (C-3), 99.2 (C-4), 106.1 (C-5), 114.1 (C-6), 122.7 (C-7), 130.1 (C-8), 130.2 (C-9), 138.4 (C-10), 151.8 (C-11), 152.2 (C-12), 155.1 (C-13), 156.2 (C-14), 161.7 (C-15) and 179.2 (C-16).

7. CONCLUSION

Dodonaea viscosa is a medicinal plant widely used around the world as analgesic, anti-inflammatory, antiviral, antifungal, anti-ulcerogenic, pasmolytic, laxative, antimicrobial, hypotensive, rheumatism, gout, hemorrhoids, fractures and snake bites. In the course of this project work, three compounds (**35**, **45** and **53**) were identified from the leaves of *Dodonaea viscosa* var. *angustifolia* Out of these compound **35** was isolated from petroleum ether extract and the remaining two (**45** and **53**) were isolated from chloroform extract. Moreover the structural elucidations of these compounds were done by using spectroscopic methods NMR and UV-Vis.

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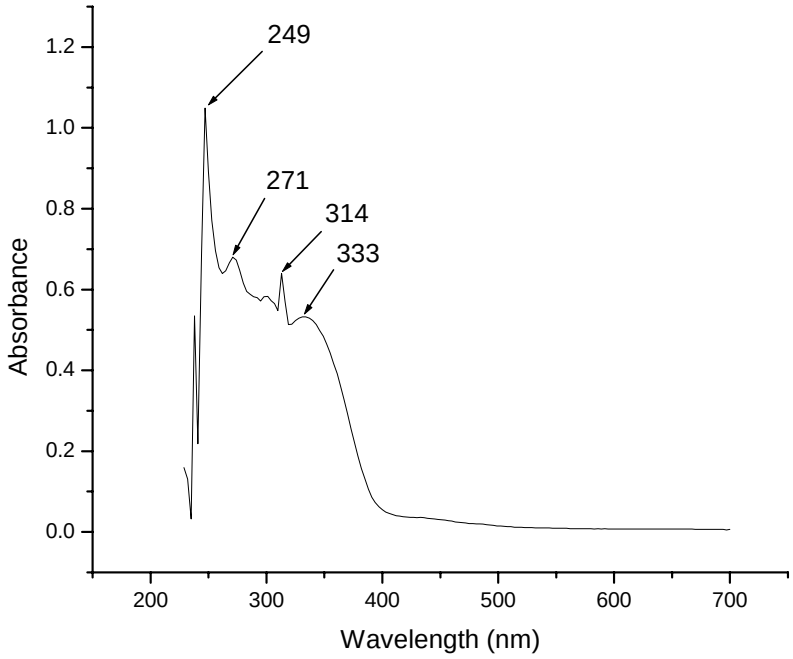
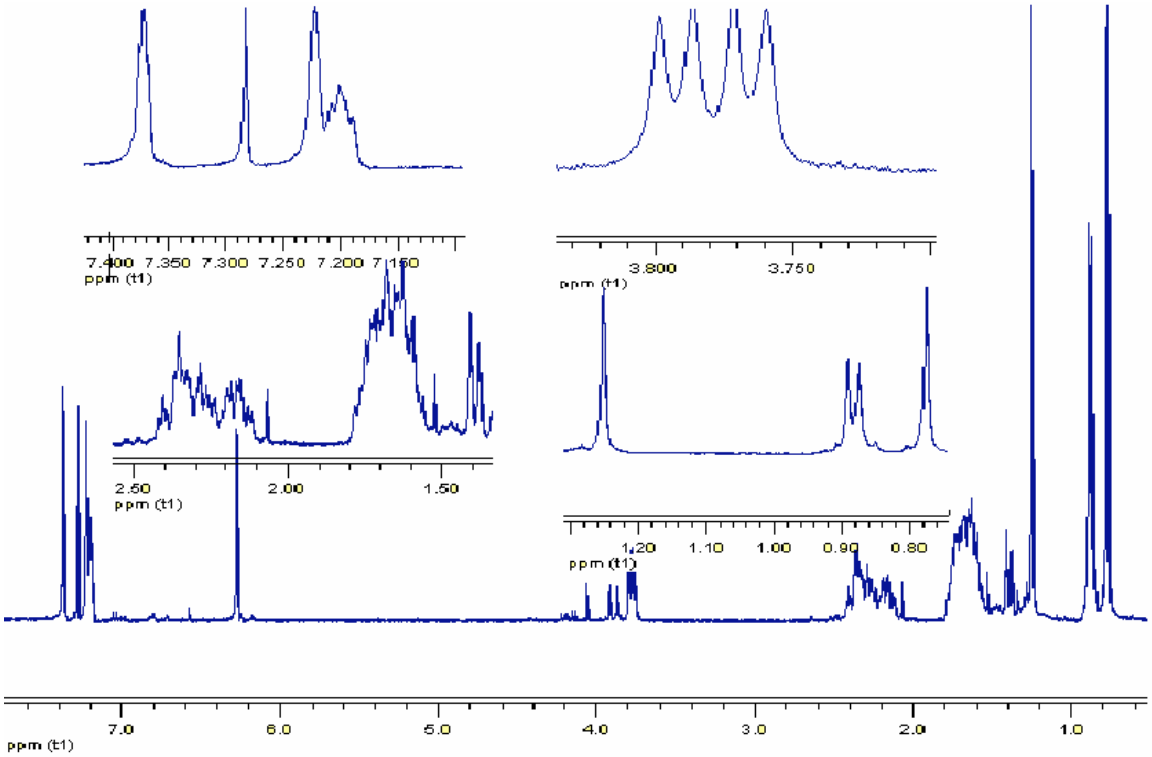
9. APPENDICES

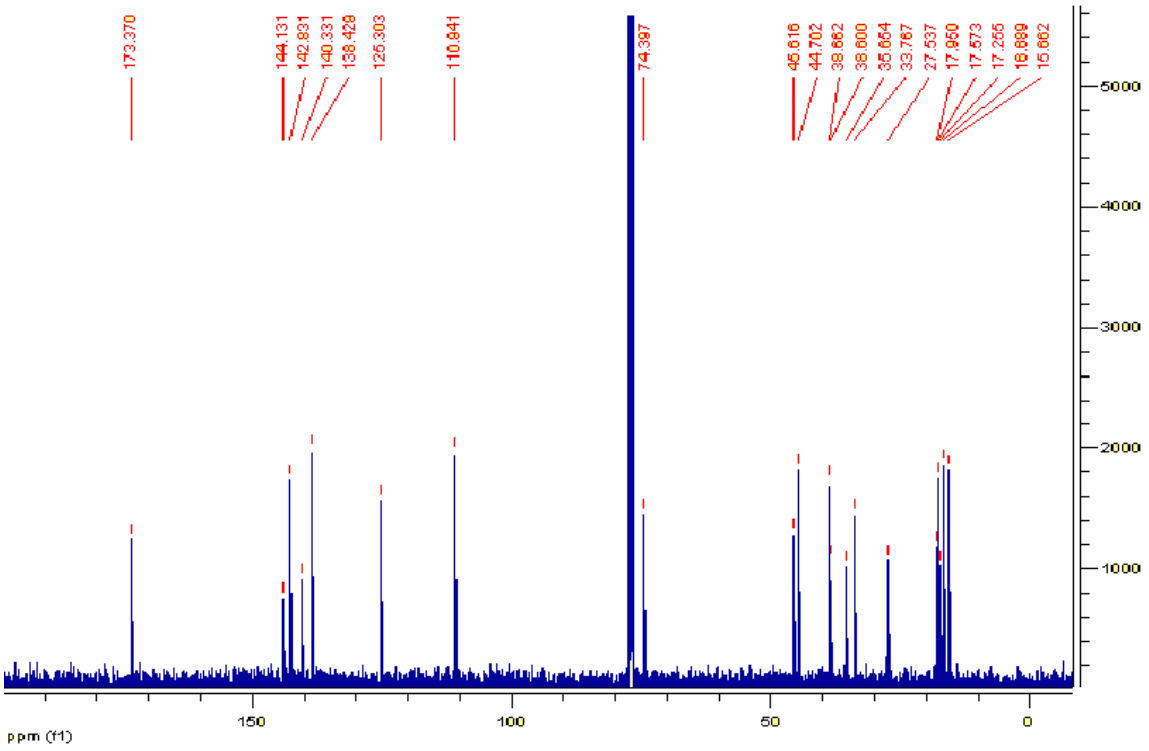
Appendix: 1.1. UV-Visible spectrum of compound Dc-8B in ethanol.



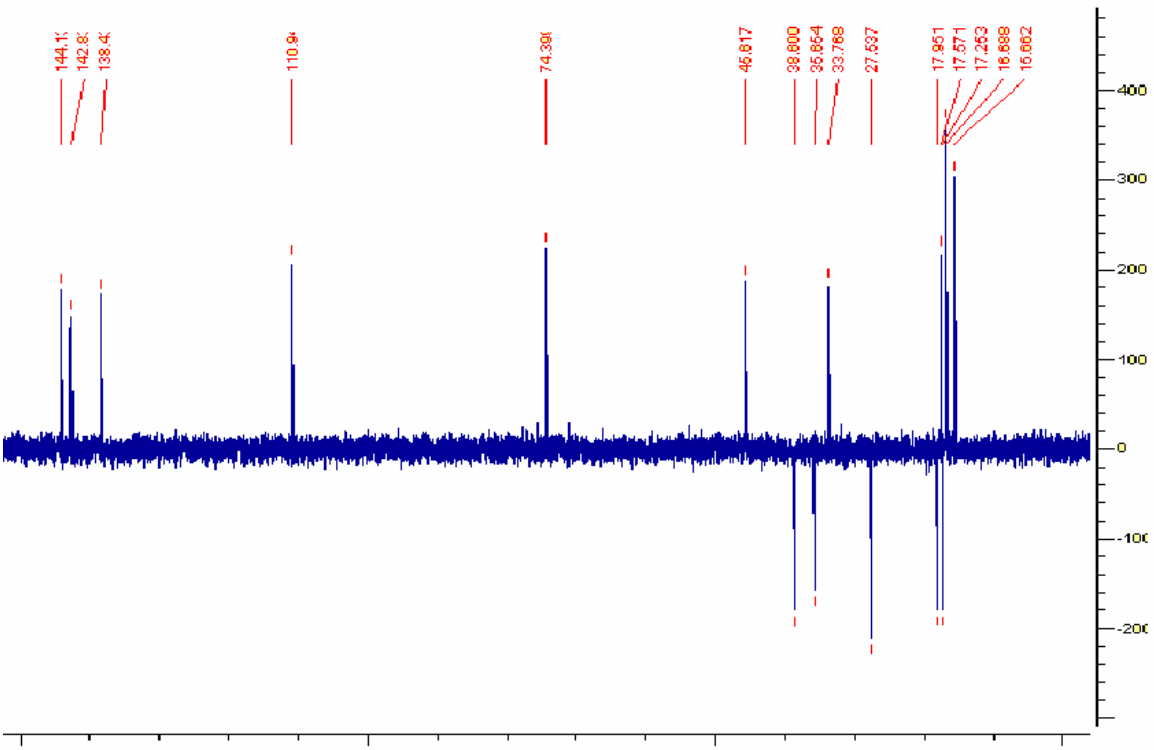
Appendix 1.2. ^{1}H NMR spectrum of compound Dc-8B in $CDCl_3$.



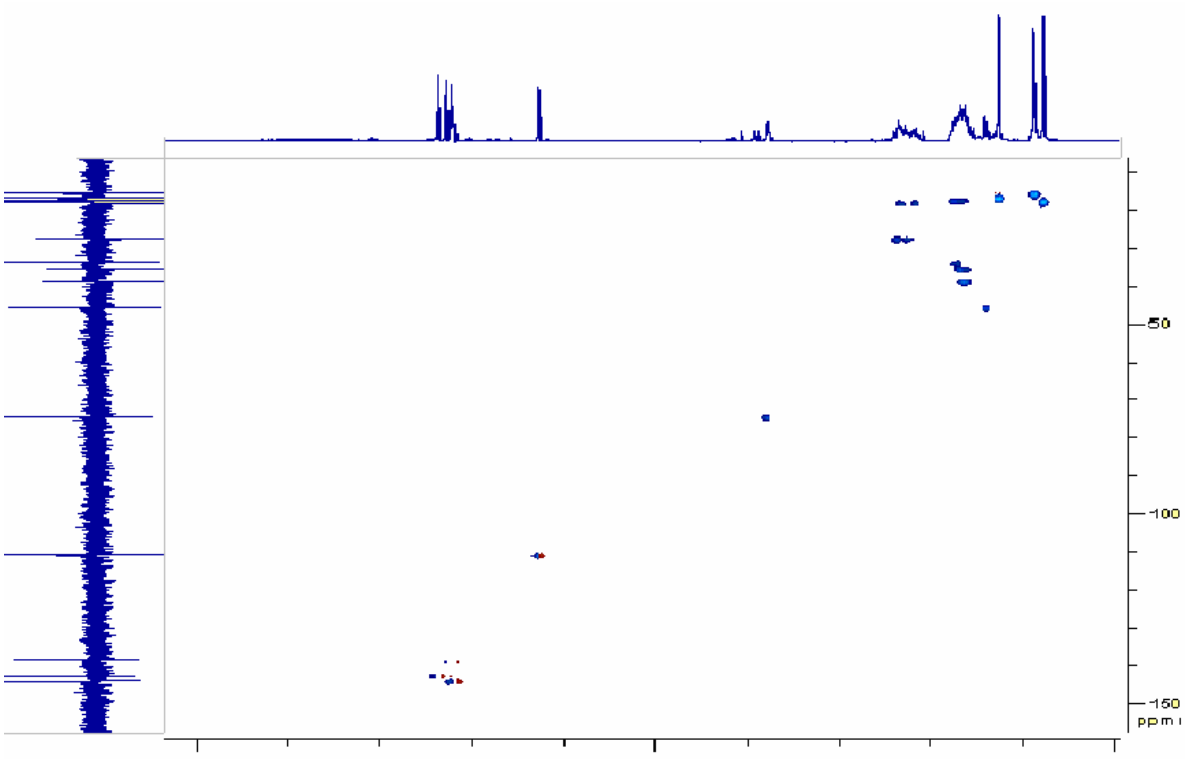
Appendix 1.3. ^{13}C NMR spectrum of compound Dc-8B in $CDCl_3$.



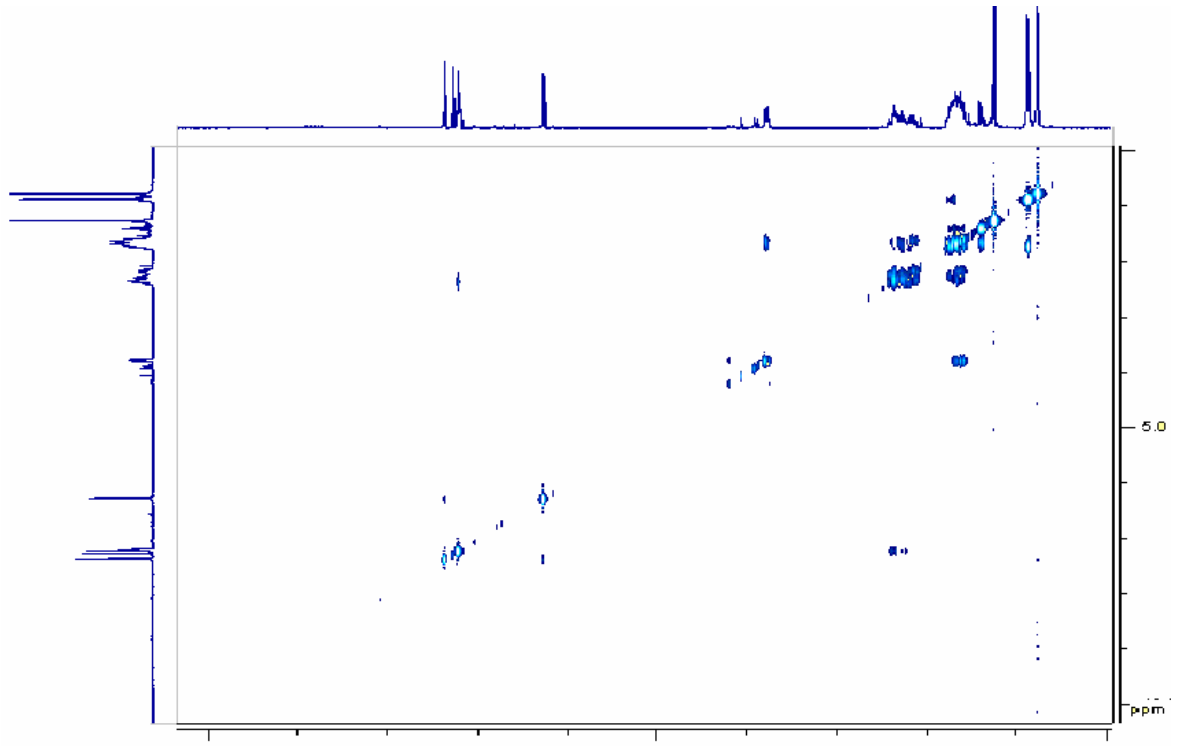
Appendix 1.4. DEPT-135 spectrum of compound Dc-8B.



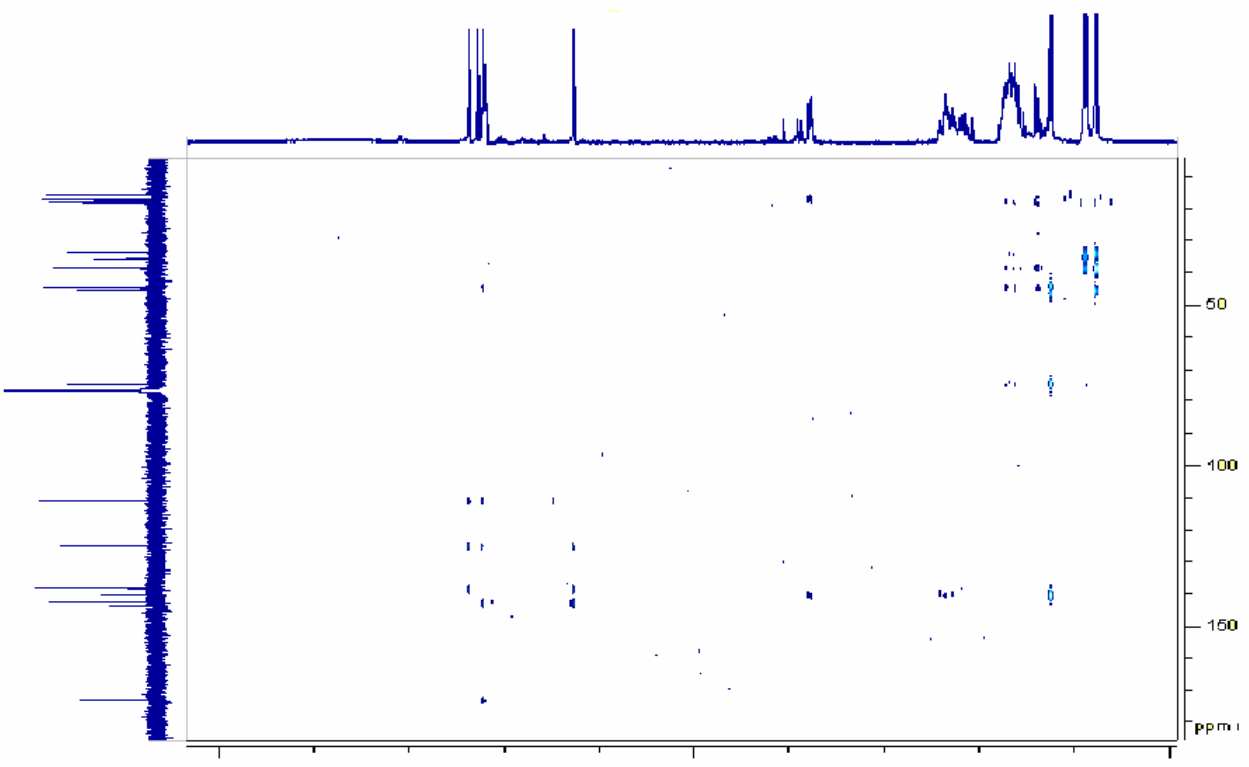
Appendix 1.5. HMQC spectrum of compound Dc-8B in $CDCl_3$.



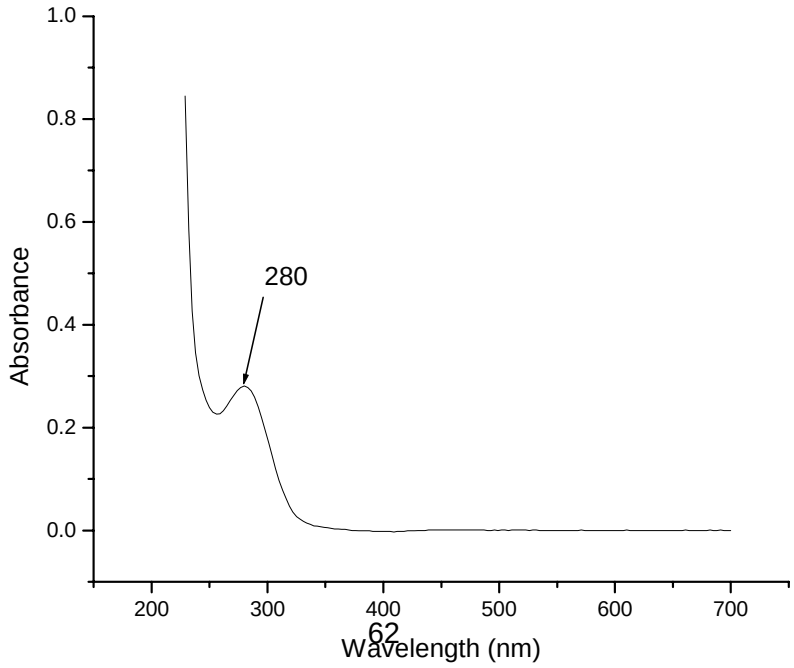
Appendix 1.6. ^{1}H-^{1}H COSY spectrum of compound Dc-8B in $CDCl_3$.



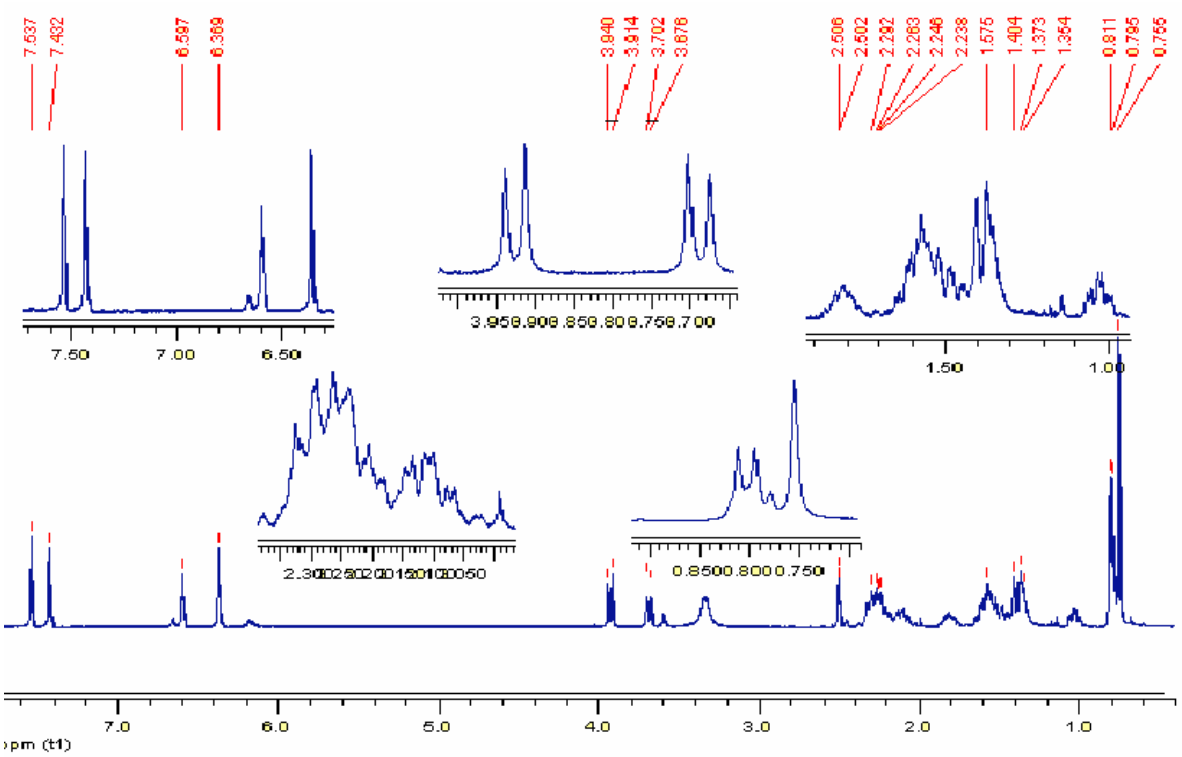
Appendix 1.7. HMBC spectrum of compound Dc-8B in $CDCl_3$.



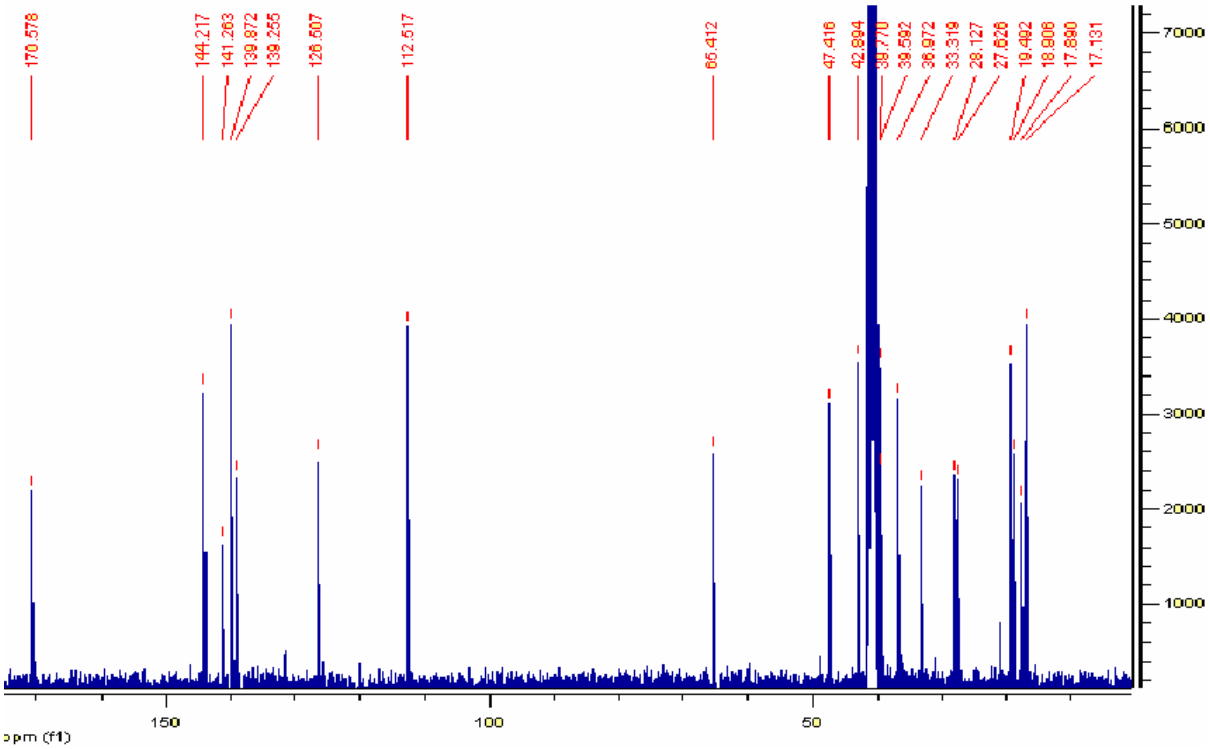
Appendix: 2.1. UV-Visible spectrum of compound D-16 in ethanol.



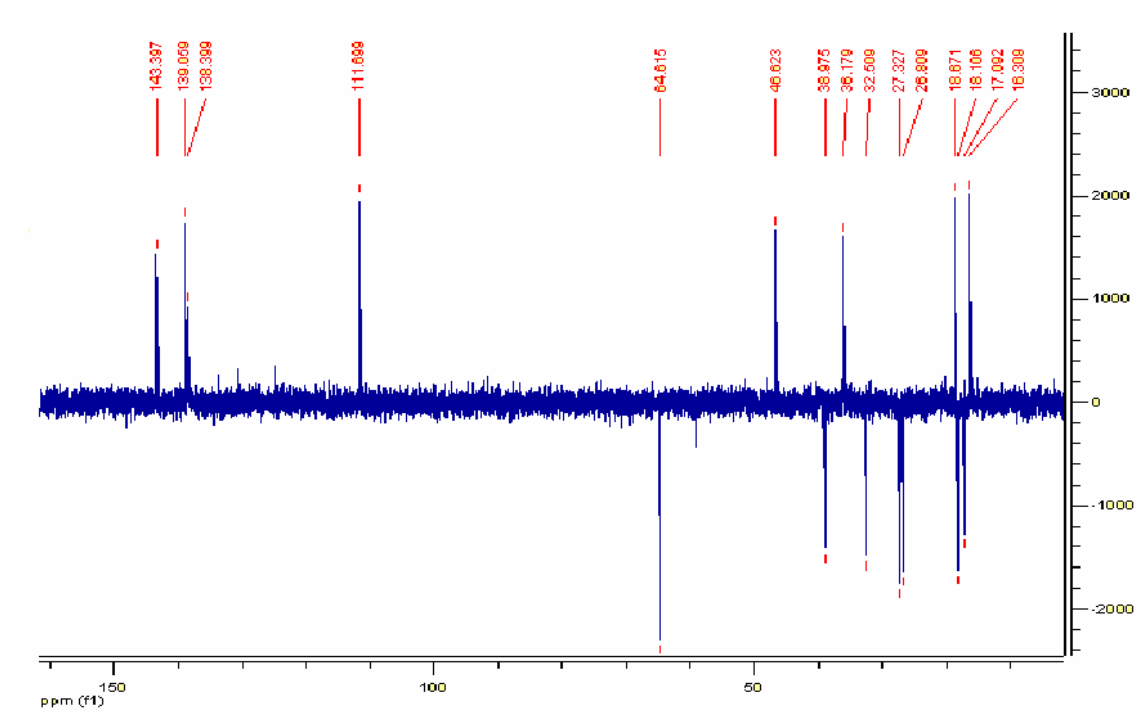
Appendix 2.2. ^{1}H NMR spectrum of compound D-16 in DMSO-d_6.



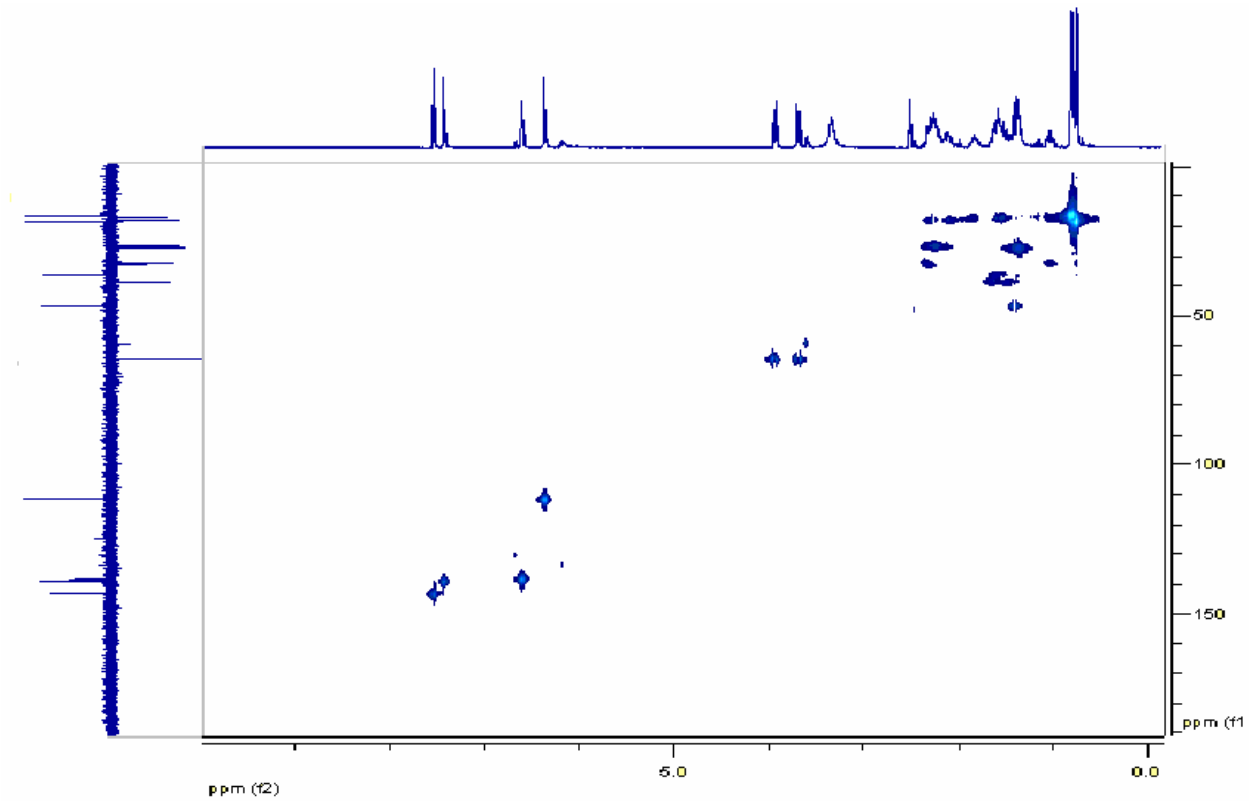
Appendix 2.3. ^{13}C NMR spectrum of compound D-16.



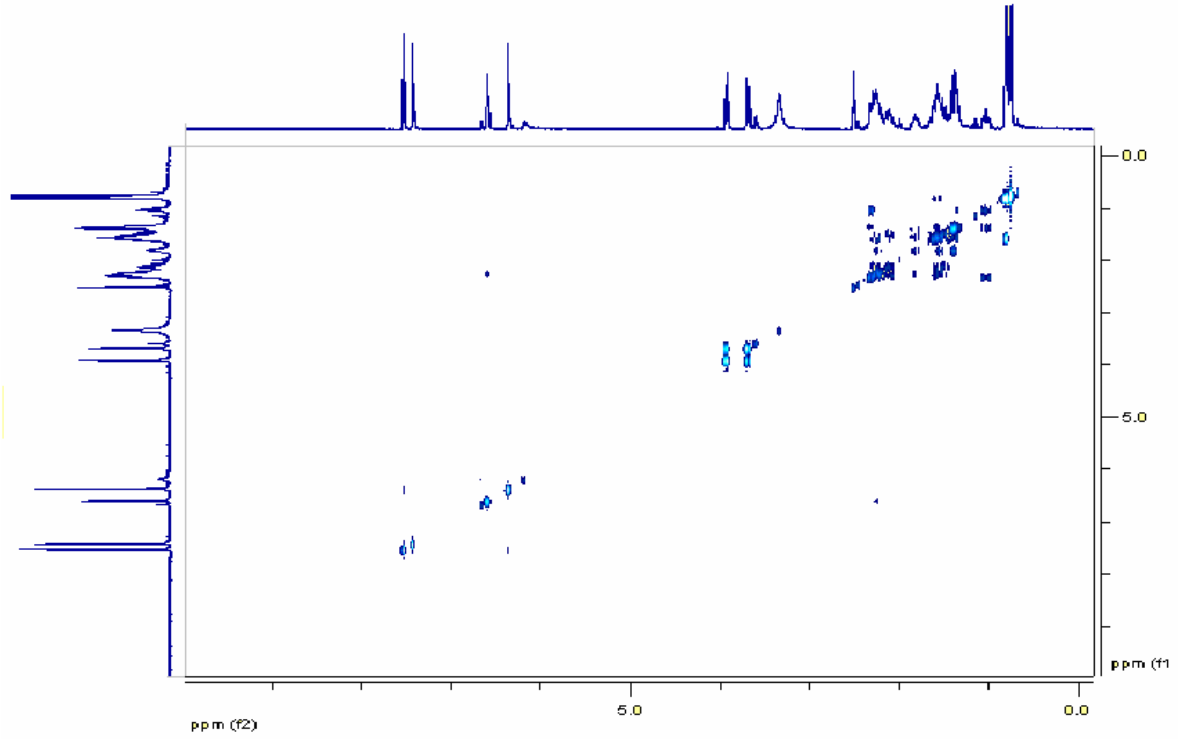
Appendix 2.4. DEPT-135 spectrum of compound D-16 in DMSO-d_6.



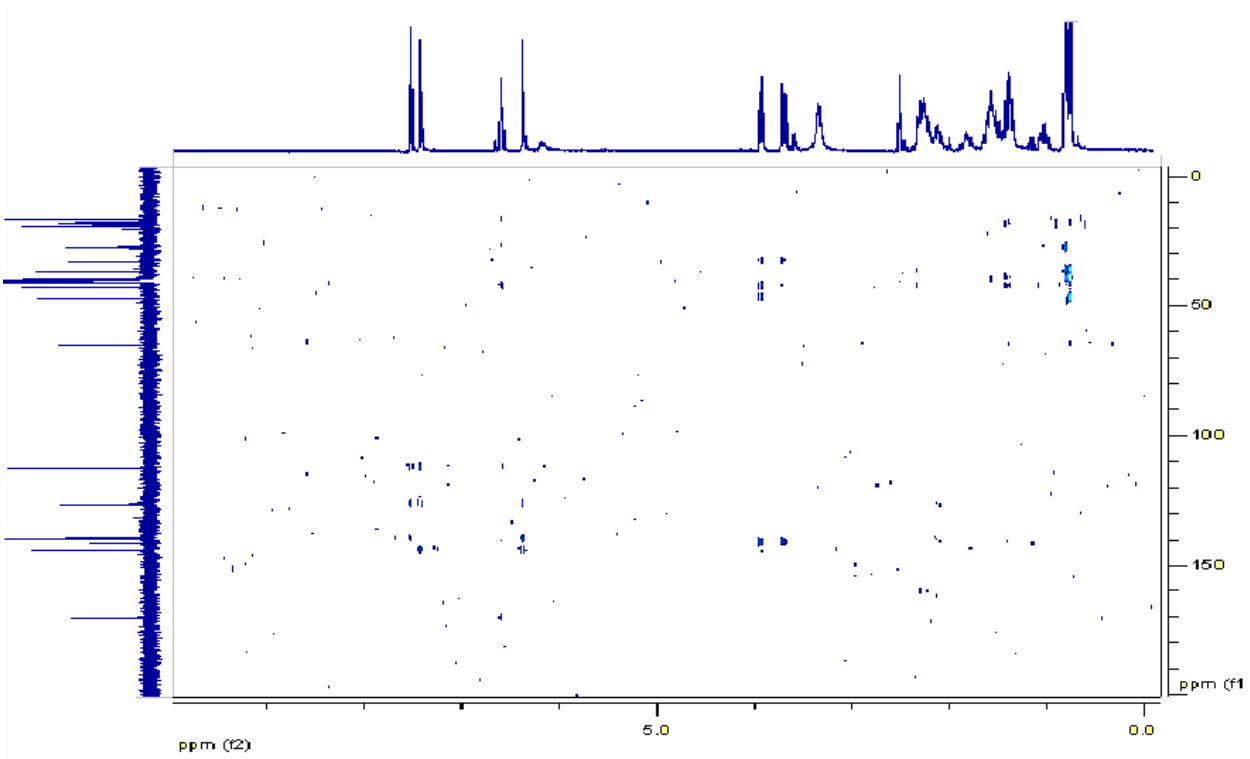
Appendix 2.5. HMQC spectrum of compound D-16 in DMSO-d_6.



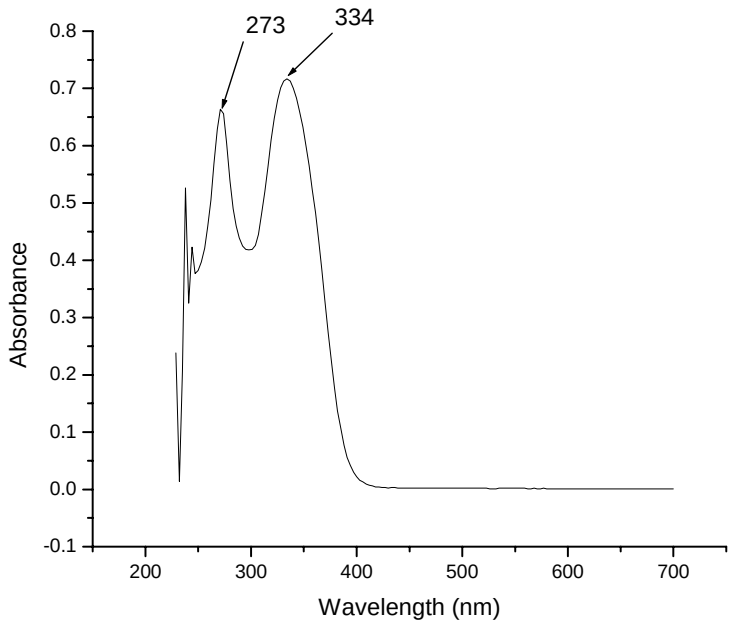
Appendix 2.6. ^{1}H-^{1}H COSY spectrum of compound D-16 in DMSO-d_6.



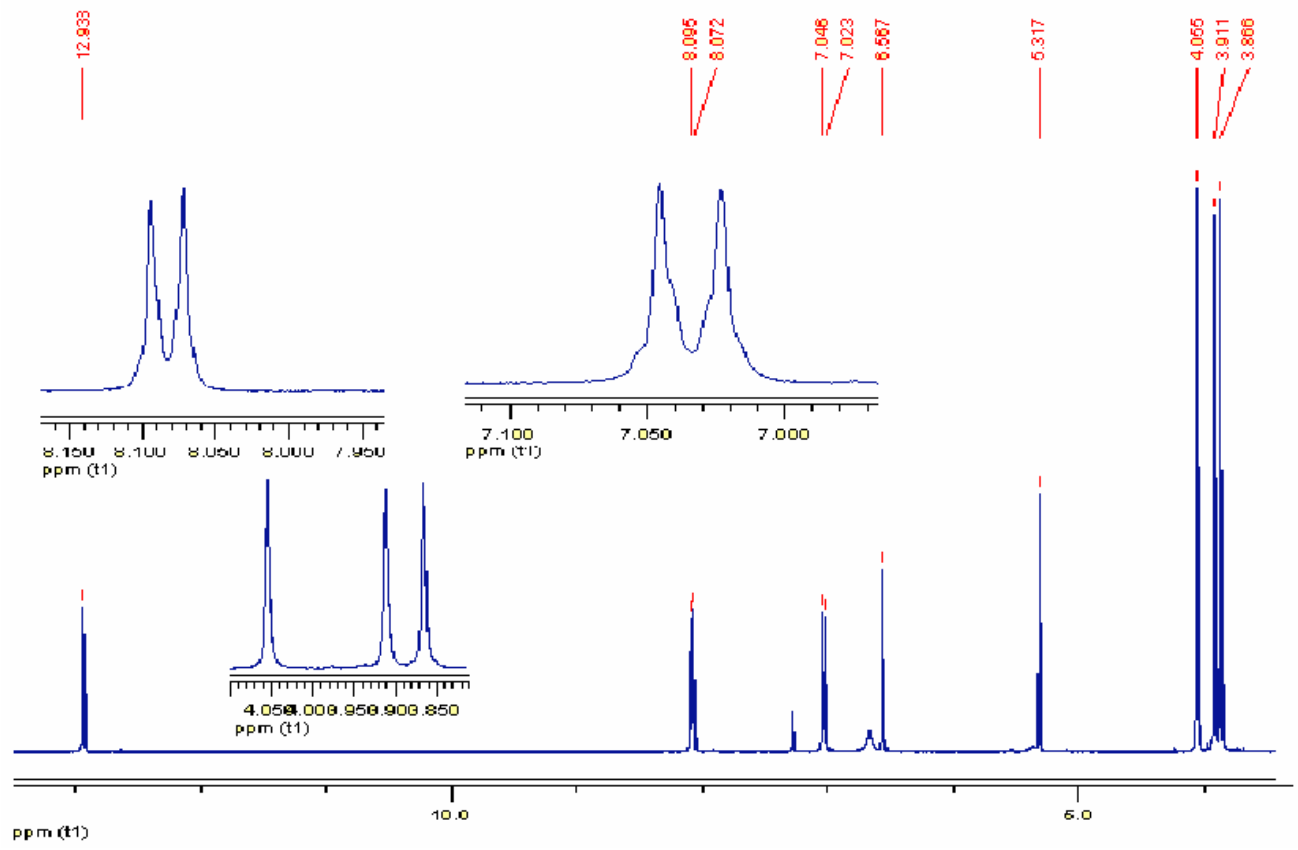
Appendix 2.7. HMBC spectrum of compound D-16 in DMSO-d_6.



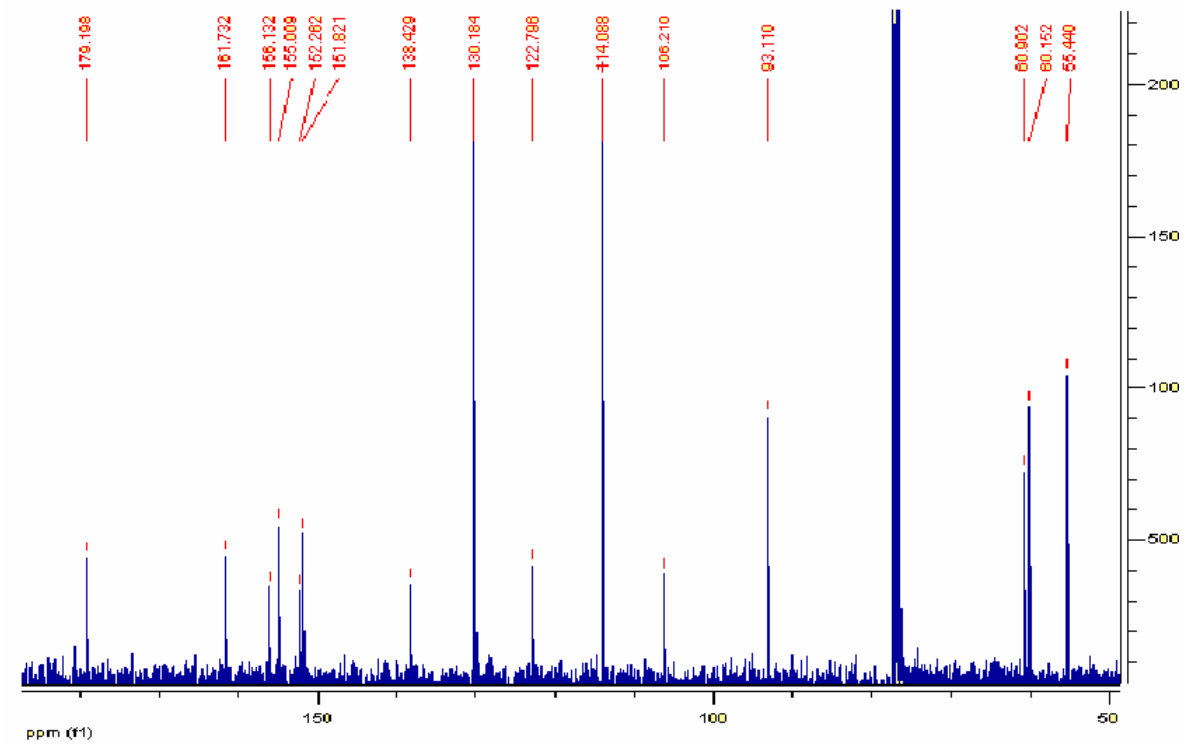
Appendix: 3.1. UV-Visible spectrum of compound D_{26}-3B in $CHCl_3$



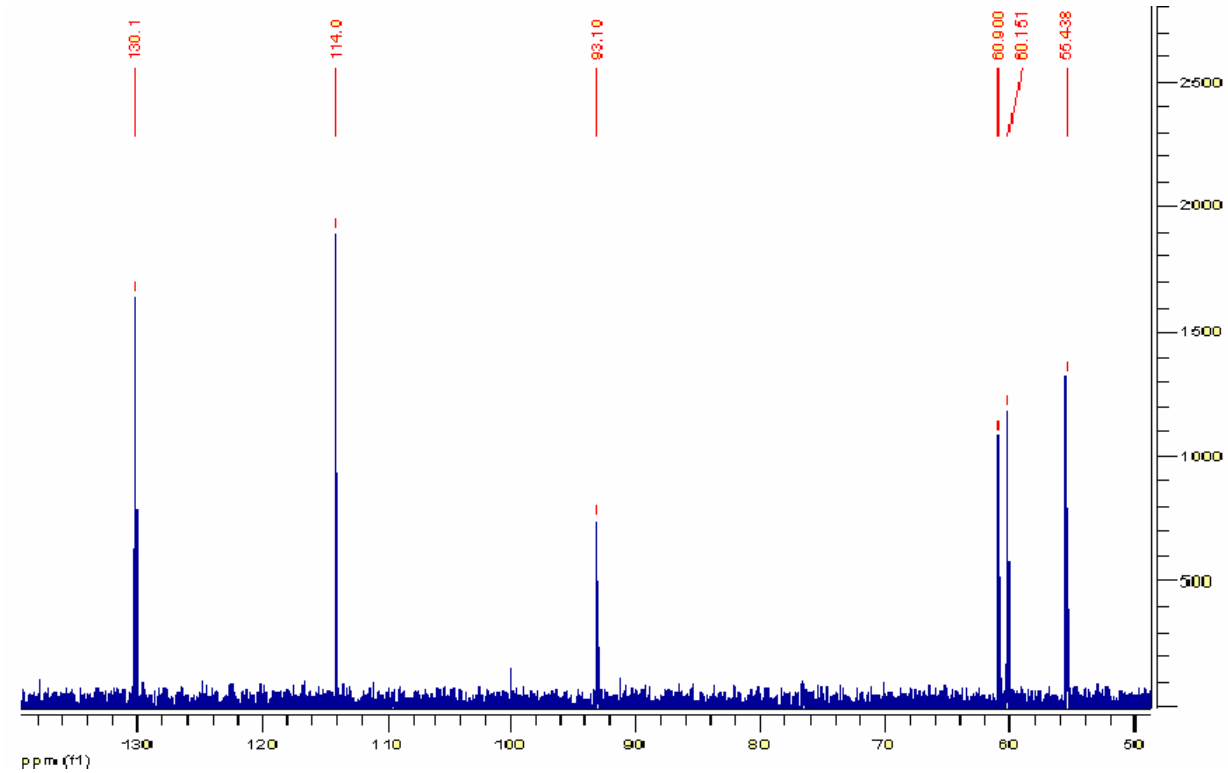
Appendix 3.2. ^1H NMR spectrum of compound D_{26}-3B in $CDCl_3$



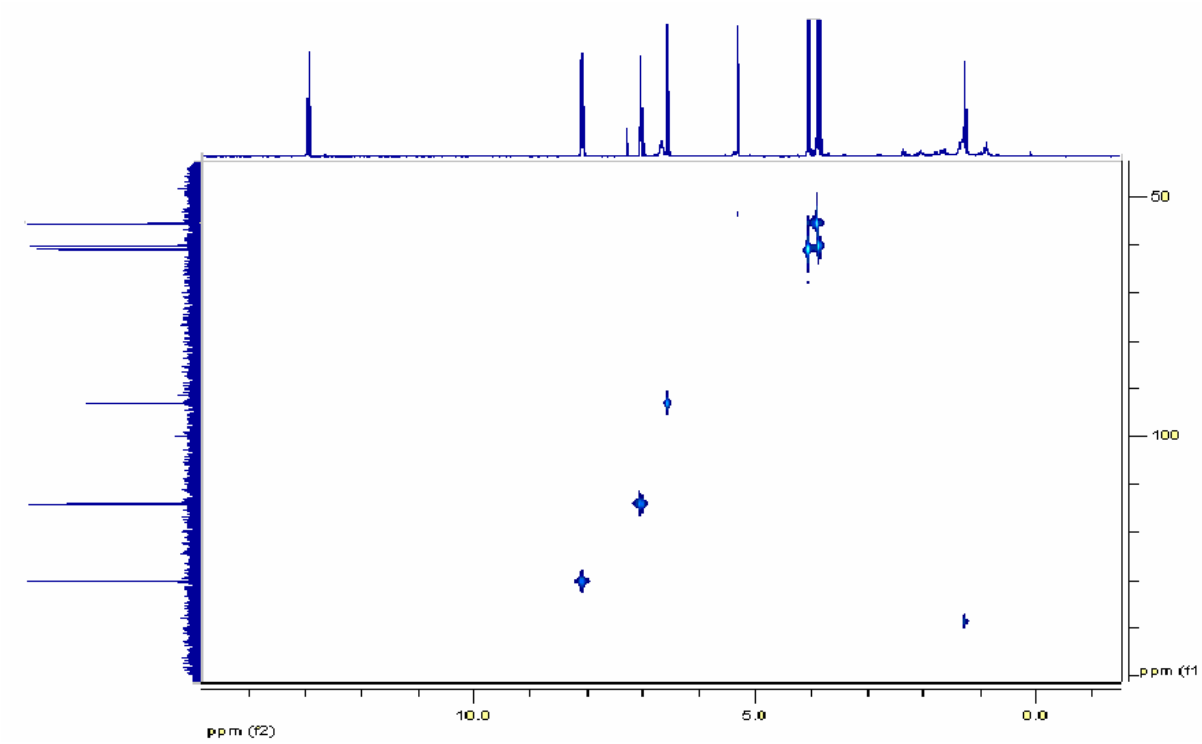
Appendix 3.3. ^{13}C NMR spectrum of compound D_{26}-3B in $CDCl_3$



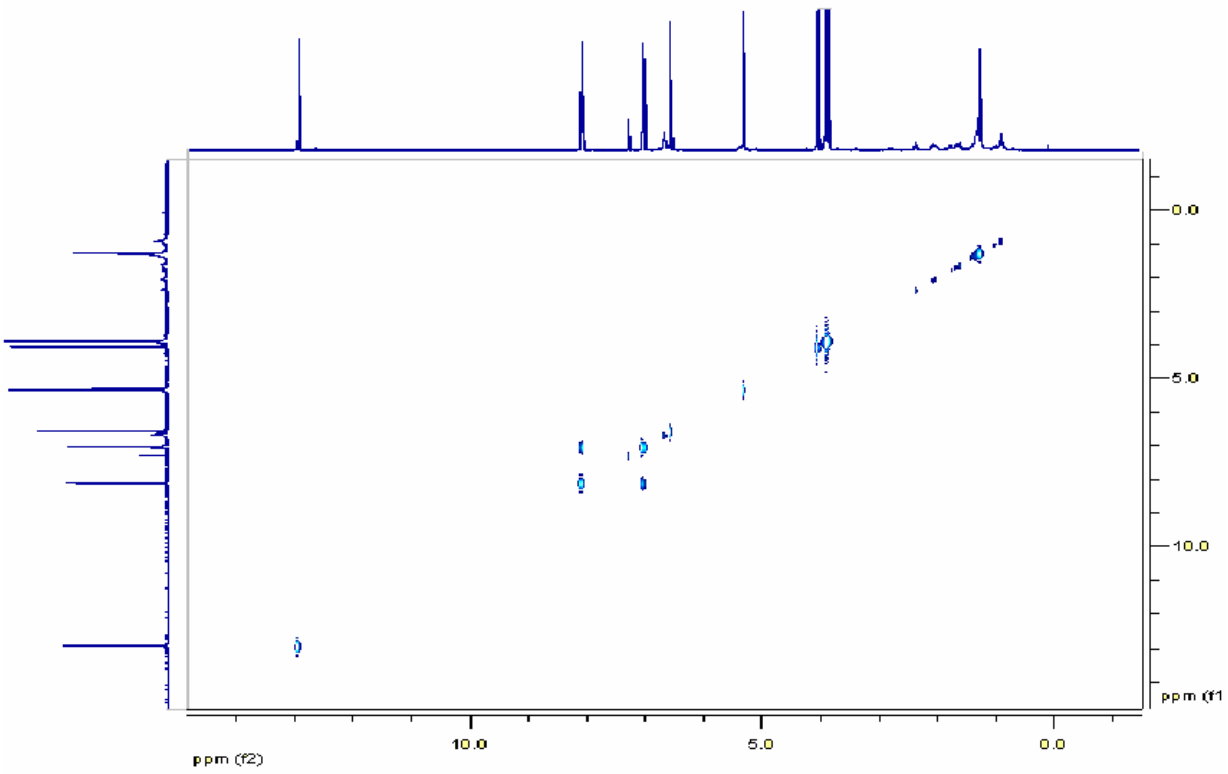
Appendix 3.4. DEPT-135 spectrum of compound D_{26}-3B in $CDCl_3$



Appendix 3.5. HMQC spectrum of compound D_{26}-3B in $CDCl_3$.



Appendix 3.6. ^{1}H-^{1}H COSY spectrum of compound D_{26}-3B in $CDCl_3$.



Appendix 3.7 HMBC spectrum of compound D_{26}-3B in $CDCl_3$.