ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

PHYTOCHEMICAL INVESTIGATION ON THE LEAVES OF *LAGGERA TOMENTOSA* (ETHANOL EXTRACT)

By: Abinet Haile

ADVISOR: Nigist Asfaw (Ph. D)

JULY 2007

PHYTOCHEMICAL INVESTIGATION ON THE LEAVES OF LAGGERA TOMENTOSA (ETHANOL EXTRACT)

A GRADUATE PROJECT SUBMITTED TO THE OFFICE OF RESEARCH AND GRADUATE PROGRAMME OF ADDIS ABABA UNIVERSITY

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

BY

ABINET HAILE

JULY 2007

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

PHYTOCHEMICAL INVESTIGATION ON THE LEAVES OF LAGGERA TOMENTOSA (ETHANOL EXTRACT)

BY

ABINET HAILE

DEPARTMENT OF CHEMISTRY

SCIENCE FACULTY

JULY 2007

APPROVED BY THE EXAMINING BOARD:

Dr. Nigist Asfaw _____
Advisor

Dr. Ashebir Fisiha _____
Chairman

Prof. Ermias Dagne _____
Examiner

Dr. Gizachew Alemayehu _____
Examiner

Dr. Vitaly Dubina _____
Examiner

DECLARATION

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

Name: Abinet Haile
Signature: _____

Place and date of submission
Chemistry Department, Addis Ababa University
July 2007

This project has been submitted for examination with approval as University advisor

Advisor	Signature
Dr. Nigist Asfaw	_____

Date: July 30, 2007

To: Dr. Gizachew Alemayehu
Head, Department of Chemistry, AAU

This is to confirm that Abinet Haile has incorporated the comments of the examining board in the final version of his M.Sc. project.

Sincerely yours,

_____

Dr. Nigist Asfaw

Acknowledgement

I have the gratification of expressing my great indeptness to my advisor Dr. Nigist Asfaw for her unreserved and invaluable guidance, critical advice, and many useful suggestions throughout this work and for her motherly advice in any aspect.

I am particularly grateful to Dr. Wondimagegn Mammo for his immediate response when support is needed and also for his consistent help and encouragement throughout the project work.

My acknowledgement also goes to Prof. Ermias Dagne and ALNAP for their help in allowing me to use the NMR laboratory.

I greatly acknowledge W/t Senait Dagne for her continous follwup and helping in column chromatography.

I must express my thanks to Ato Yoseph Atlaw and Ato Masreshaw Asnakew for their help in running the NMR spectrum within a short period of time and for their patience.

My gratefulness goes to W/t Medhanit Mammo for running the IR spectrum of the isolated compounds.

I have a great pleasure in thanking my family and all friends for their being with me and for their continuous encouragement.

Table of Contents

List of Tables

List of Figures

List of Schemes

List of Appendices

ABSTRACT

Phytochemical Investigation on the Leaves of *Laggera tomentosa* (Ethanol extract)

By

Abinet Haile

Advisor: Dr. Nigist Asfaw

Laggera tomentosa is a perennial fragrant bushy herb endemic to Ethiopia. It is a well known medicinal plant. The juice of the crushed plant is ingested as a treatment for stomach-ache and is also used against migraine. Phytochemical investigation on the ethanol extract of the leaves of this plant resulted in the isolation of one sesquiterpene and one flavone, namely 3-(3'-acetoxy-2'-hydroxy-2'-methyl butyryl)- cuauhtemone (**LTE-7**) and 3',5,6-trihydroxy-3,6-dimethoxyflavone (**LTE-8**), respectively.

1. Introduction

1.1. Natural Products

Plants and animals produce an amazingly diverse range of chemicals. These chemical products of plants and animals can be classified into primary and secondary metabolites. Primary metabolites are those which are common to all species and can be subdivided into proteins, carbohydrates, lipids and nucleic acids. These four groups of materials are defined according to the chemical structures of their members. The secondary metabolites are often referred to as "natural products". These can be subdivided into terpenoids, alkaloids, shikimates and polyketides. The classification is based on the means by which the materials were made. The reaction path leading to a particular natural product is called the *biosynthetic pathway*, and the corresponding event is known as the *biogenesis* **[1]**. Different plant and animal species can employ dramatically different biosynthetic pathways to produce the same metabolite **[2]**.

Individual secondary metabolites may be common to a number of species or may be produced by only one organism. Relative species often have related patterns of secondary metabolite production and so a species can be classified according to the secondary metabolite they produce. Such a classification is known as *chemical taxonomy*. Occasionally, two plants are found to have identical physical aspects which botanists use for classification, but differ in the secondary metabolites they produce. For example, two flowers may look identical but one is odorless while the other possesses a strong scent due to the production of a fragrant terpenoid chemical. Such different strains are known as *chemotypes* **[1]**.

Natural products, as the term implies, are those chemical compounds derived from living organisms, plants, animals, insects, and the study of natural products is the investigation of their structure, formation, use, and purpose in the organism **[3]**. Natural products isolated from plants arid microorganisms have been providing noble, clinically active drugs. The key to the success of discovering naturally occurring therapeutic agents rests on bioassay-guided fractionation and purification procedures **[4]**.

Natural product chemistry covers the chemistry of naturally occurring organic compounds, their biosynthesis, function in their own environment, metabolism, and more conventional branches of chemistry such as structure elucidation and synthesis **[2]**.

Until the late 1800's, organic chemistry was almost exclusively the study and use of natural products. The purpose of these compounds in the organisms and their formation was little understood or investigated, primarily due to the lack of appropriate techniques and structural theory. The natural products that were studied and used tended to be the compounds that occurred in the largest amounts, mostly in plants, and were most easily isolated in a pure, or sometimes not very pure, form by techniques such as simple distillation, steam distillation, or extraction with acid or base.

Originally teas or decoctions (aqueous extracts) or tinctures or elixirs (alcoholic extracts) were used to prepare and administer herbal remedies - these were usually the starting points for isolation work. We now employ different solvents, e.g., ethanol to extract, hexane to concentrate non-polar constituents, methanol to concentrate polar constituents, and modern isolation techniques include all types of chromatography, often guided by bioassays, to isolate the active compounds. Up until the 1950's, the structures of natural products were determined by degradative techniques, and a structure was not proven until the compound had been synthesized in an unambiguous manner. Stereochemistry was not often determined. Now, structures are elucidated primarily by spectroscopic techniques, and the stereochemistry is an important feature of the structure **[3]**.

1.2. Genus *Laggera*

Laggera is a genus with *ca.* 20 species mainly found in tropical Africa and south east Asia. *Laggera alata* (D. Don) SCH.-BIP. ex. OLIVER and *Laggera pterodonta* (DC) BENTH (Compositae) are employed as traditional herbal medicines because of their anti-inflammatory and antibacterial activities. The green herbal medicinal plant *Laggera pterodonta* shows antileukemia, antiphlegm, and antibronchitis activities. Recently, much attention has been paid to *Laggera* species and their chemical contents because of their multifaceted activities. Extensive studies of *Laggera* have led to the identification of many compounds, such as monoterpenes, sesquiterpenes, cyclitols, and flavonoids. The

phytochemical progress and list of the compounds isolated from the genus *Laggera* in the past decades are summarized below **[5]**.

Chemical constituents

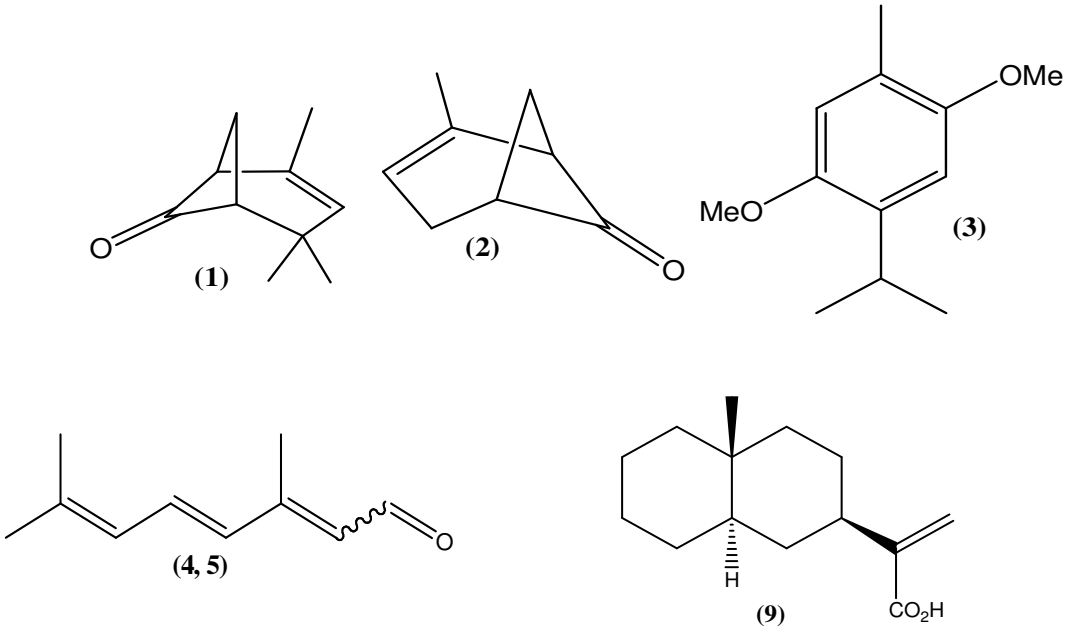
Since 1960s, more than 30 chemical constituents have been isolated from the genus *Laggera*, including monoterpenes, sesquiterpenes, flavonoids, and cyclitols **(Table 1) [5]**.

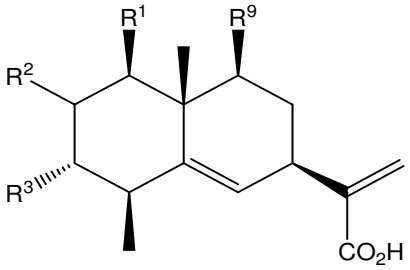
Table 1: Chemical constituents of the genus *Laggera*

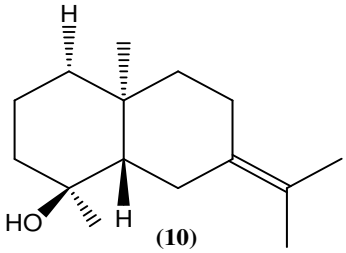
No.	Compound name	Source	Ref.
1	(1S,5R)-(-)-2,4,4-Trimethylbicyclo [3.1.1]hept-2-en-6-one	*Laggera tomentosa*	**[6]**
2	2,7,7-Trimethylbicyclo[3.1.1] hept-2-en-6-one	*Laggera tomentosa*	**[6]**
3	1,4-Dimethoxy-5-methyl-2-(1-methylethyl)benzene	*Laggera tomentosa*	**[6]**
4	(2*E*,4*E*)-3,7-Dimethylocta-2,4,6-trienal	*Laggera tomentosa*	**[6]**
5	(2*Z*,4*E*)-3,7-Dimethylocta-2,4,6-trienal	*Laggera tomentosa*	**[6]**
6	1β-Hydroxypterodontic acid	*Laggera pterodonta*	**[7]**
7	2α,3β-Dihydroxypterodontic acid	*Laggera pterodonta*	**[7]**
8	2β-Acetoxypterodontic acid	*Laggera pterodonta*	**[7]**
9	2α-Acetoxycostic acid	*Laggera pterodonta*	**[7]**
10	*ent*-7(11)-Selinen-4-ol	*Laggera pterodonta*	**[7]**
11	Pterodontriol D	*Laggera pterodonta*	**[7]**
12	Pterodontriol B	*Laggera pterodonta*	**[7]**
13	Pterodondiol C	*Laggera pterodonta*	**[7]**
14	5α,11-Dihydroxyeudesm-3-en-2-one	*Laggera pterodonta*	**[7]**
15	1β,3α,9β -Trihydroxyeudesma-5,11(13)-dien-12-oic acid	*Laggera pterodonta*	**[8]**
16	1β,3α-Dihydroxyeudesma-5,11(13)-dien-12-oic acid	*Laggera pterodonta*	**[8]**

17	Pterodontoside A	*Laggera pterodonta*	**[8]**
18	Pterodontoside B	*Laggera pterodonta*	**[8]**
19	Pterodontoside C	*Laggera pterodonta*	**[8]**
20	Pterodontoside D	*Laggera pterodonta*	**[8]**
21	Pterodontoside E	*Laggera pterodonta*	**[8]**
22	Pterodontoside F	*Laggera pterodonta*	**[8]**
23	*ent*-Eudesmane-2α,4β,11-triol 11-O-β-d-glucopyranoside	*Laggera pterodonta*	**[8, 9]**
24	Isointermedeol	*Laggera alata*	**[10]**
25	7-Epi-α-eudesmol	*Laggera alata*	**[10]**
26	7-Epi-β-eudesmol	*Laggera alata*	**[10]**
27	7-Epi-γ-eudesmol	*Laggera alata*	**[10]**
28	β-Dihydroagarofuran	*Laggera alata*	**[10]**
29	7-Epieudesm-11(13)-ene-3β,4α-diol	*Laggera crispata*	**[11]**
30	3α-(Angeloyloxy)-4α,11-dihydroxyeudesm-6-en-8-one	*Laggera crispata*	**[11]**

Figure 1: List of structures of compounds from the genus *Laggera*

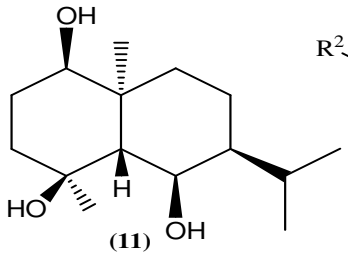




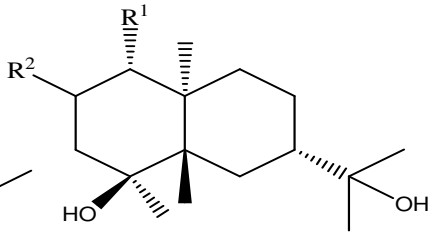


(10)

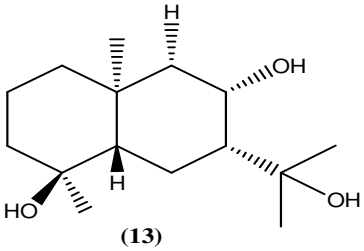
	R^1	R^2	R^3	R^4
6	OH	H	H	H
7	H	α-OH	β-OH	H
8	H	β-OAc	H	H
15	OH	H	α-OH	OH
16	OH	H	α-OH	H
17	OGlc	H	H	H
18	H	H	H	Oglc



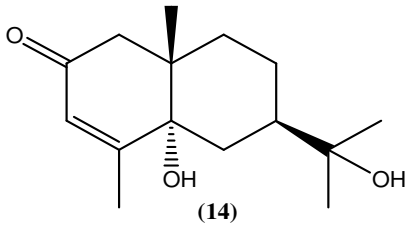
(11)



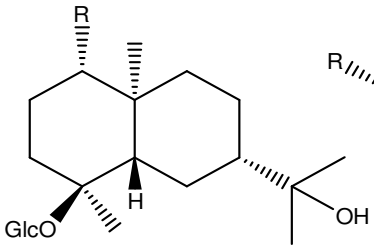
	R^1	R^2
12	OH	H
22	OGlc	H



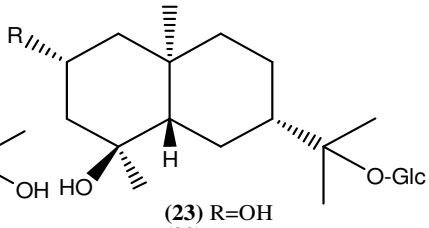
(13)



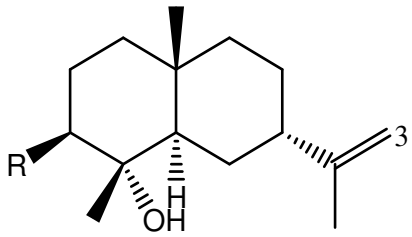
(14)



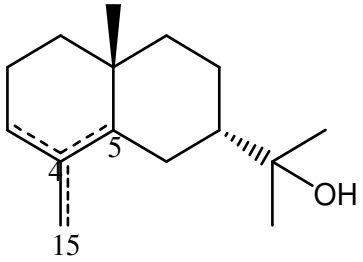
(19) R=H
(21) R=OH



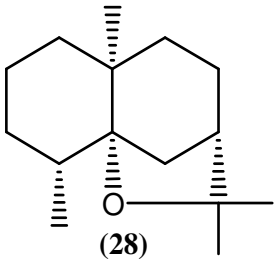
(23) R=OH
(20) R=H



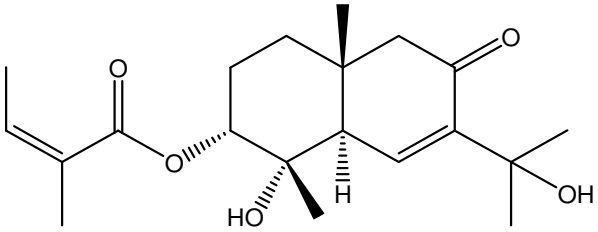
(24) R=H
(29) R=OH



(25) $\Delta^{3,4}$
(26) $\Delta^{4,15}$
(27) $\Delta^{4,5}$



(28)



(30)

1.3. *Laggera tomentosa*

Laggera tomentosa SCH. Bip. ex Oliv. and Hiern (Astraceae) known locally as "*keskese*", is a perennial fragrant bushy herb (0.5 – 1.2m high) and occurs in Ethiopia only. It is found in Tigray, Gondor, Gojjam, Wollo, Shewa and Arsi on dry hill and mountain slopes at an altitude of 2345 – 2950m. Locally, it is a well known medicinal plant. The juice of the crushed plant is ingested as a treatment for stomach-ache, and is also used against migraine. It can also be used as a fumigant and for cleansing milk containers **[12]**. Phytochemical study on the essential oil of *Laggera tomentosa* has been reported before **[6, 13]**. A literature survey indicated that there are no published reports on the solvent extraction of the plant. However, two M. Sc. projects on the solvent extract of *Laggera tomentosa*, indicated the isolation and characterization of five compounds, namely 4-O-acetylcuauthemone-3-O-angelate, 4-O-acetylcuauthemone-3-O- (2'-methyl-2'-hydroxy-3-acetoxy butyrate), 3-(3'-acetoxy-2'-hydroxy-2'-methylbutyl)-cuauthemone, 3',5,6-trihydroxy-3',4',7-trimethoxyflavone, 3',4',5,7-tetrahydroxy-3,6-dimethoxyflavone. **[14, 15]**.

1.4. Terpenes

Using a simple five carbon building block, nature creates an array of terpenoid chemicals with an infinite variety of structural variation and vast range of biological functions, which is known as *terpenoids* **[1]**. Terpenoids are structurally highly diverse and abundant family of natural compounds, of which only a limited number function in primary life processes, such as steroidal compounds as structural components of membranes, phytol and carotenoid pigments in photosynthesis, and the prenylated moieties ubiquinone and plastoquinone. Most terpenoids have been found to perform secondary functions, not only in defense against insect herbivores, but also as components of the scent of flowers and as key agents in metabolic processes **[16]**. Terpenoids have been used in perfumery, cosmetics and medicine for thousands of years and are still extracted from natural sources for these uses.

Terpenoids are defined as materials with molecular structures containing carbon back bones made up of isoprene (2-methylbuta-1,3-diene) units. Isoprene contains five carbon atoms and therefore, the number of carbon atoms in any terpenoid is a multiple of five. Degradation products of terpenoids in which carbon atoms have been lost through chemical and biochemical processes may contain different number of carbon atoms, but their overall structure will indicate their terpenoid origin and they will still be considered as terpenoids.

The generic name “terpene” was originally applied to the hydrocarbons found in turpentine, the suffix “-ene” indicating the presence of olefinic bonds. Each of these materials contains two isoprene units, hence ten carbon atoms. Related materials containing 20-carbon atoms are named as *diterpenes*. The relationship to the isoprene was discovered latter, by which the terms monoterpene and diterpene were well established. Hence the most basic member of the family, i.e. those containing only one isoprene unit, came to be known as *hemiterpenoids* **(Table 1.1) [1]**.

Table 2: Classification of Terpenoids

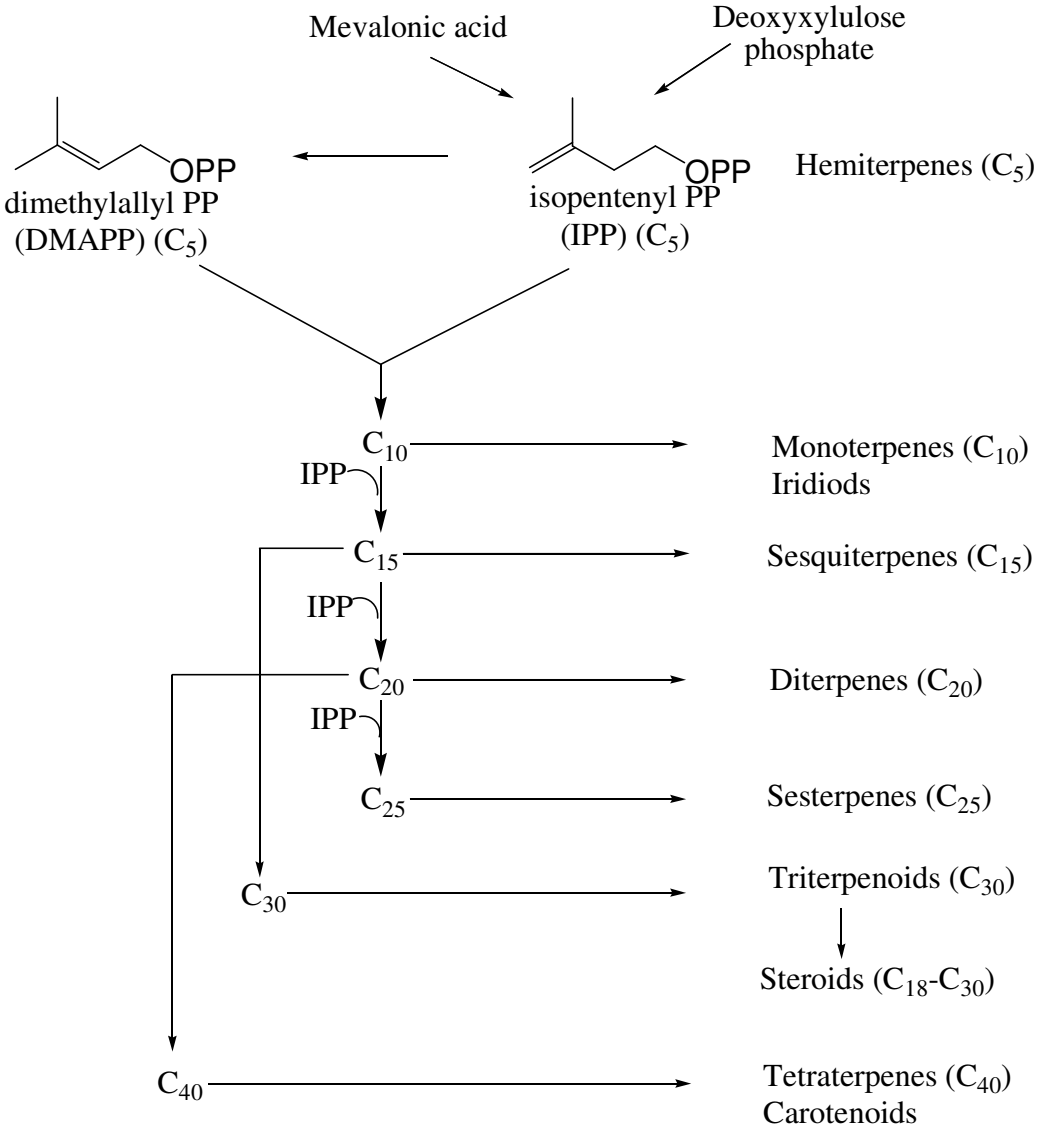
Name	Number of isoprene units	Number of carbon atoms
Hemiterpenoid	1	5
Monoterpenoids	2	10
Sesquiterpenoids	3	15
Diterpenoids	4	20
Sesterterpenoids	5	25
Triterpenoids	6	30
Tetraterpenoids	8	40
Polyisoprenoids	>8	>40

Terpenoid Biosynthesis

Terpenoid biosynthesis begins with the commitment of three molecules of acetate to the formation of isopentenyl diphosphate (which contains five carbon atoms: C_5). The subsequent key reaction in terpenoid biosynthesis is the isomerization of isopentenyl diphosphate (IPP) to dimethylallyl diphosphate (DMAPP). Condensation of these C_5 isomers by a prenyltransferase forms geranyl diphosphate (GPP) (C_{10}) as a precursor for monoterpenes. Subsequent addition of isopentenyl diphosphate results in the formation of farnesyl diphosphate (FPP) (C_{15}) as a precursor for sesquiterpenes, and geranyl geranyl diphosphate (GGPP) (C_{20}) as a precursor for diterpenes. Terpene syntheses transform these common linear prenyl diphosphate intermediates to a wide variety of mostly cyclic products. These compounds are then often modified further by oxidations, reductions, and isomerization to form an even greater structural variety **[16]**.

The study of plant terpenoid biosynthesis has made rapid progress in recent years. Isoprenoids are synthesized by consecutive condensations of their five-carbon precursor, isopentenyl diphosphate, to its isomer, dimethylallyl diphosphate. Two pathways for these precursors are known. One is the mevalonate pathway, which operates in eukaryotes, archaebacteria, and cytosols of higher plants. Mevalonate is especially important for sterol biosynthesis. The other is a recently discovered pathway, the non-mevalonate or the 2-C-methylerythritol-4-phosphate pathway, which proceeds via 1-deoxyxylulose-5-phosphate pathway, which is used by many eubacteria, green algae, and chloroplasts of higher plants and important for carotenoid biosynthesis **[17]**.

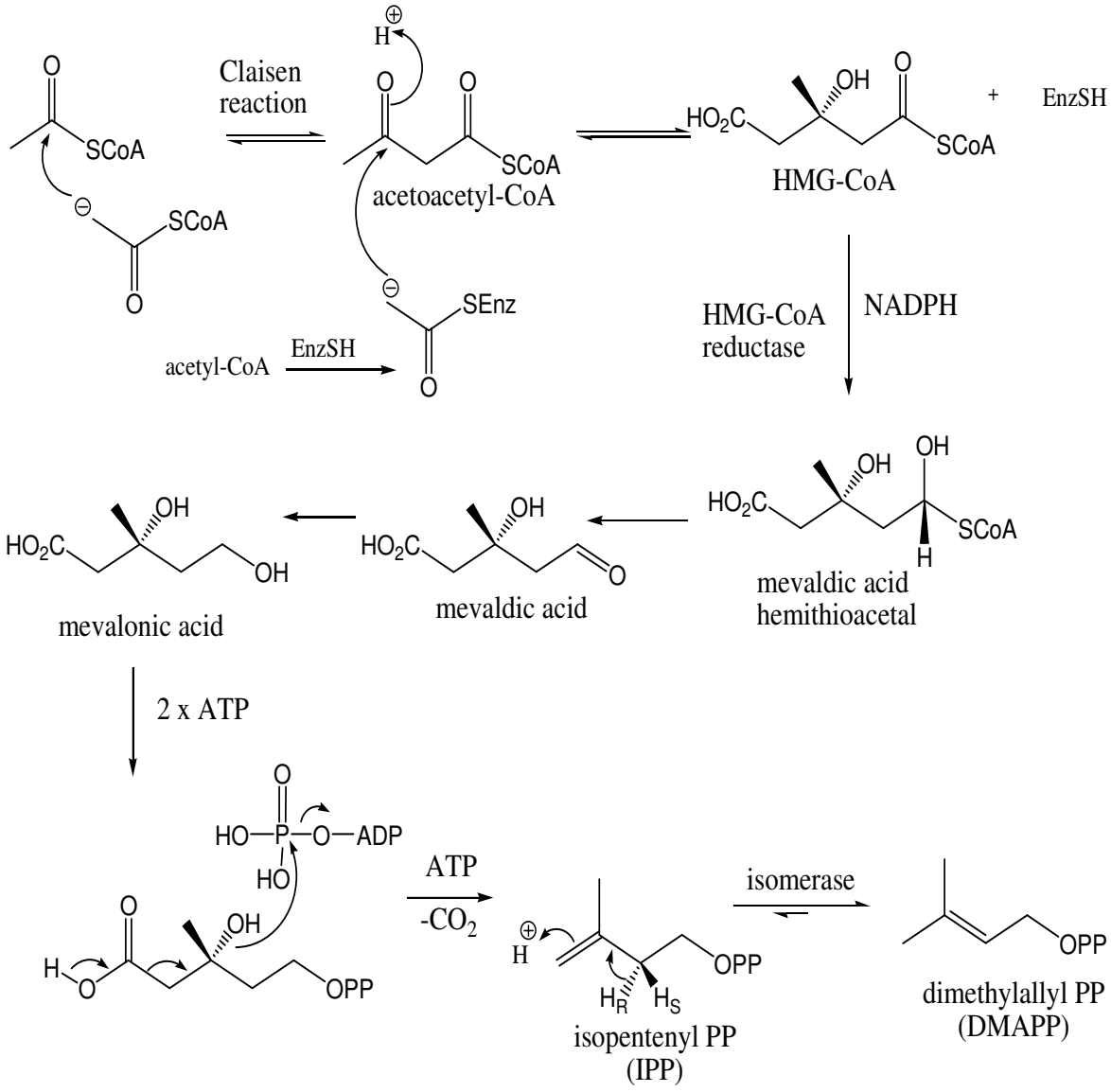
Condensation of IPP and its isomer, DMAPP, by class specific prenyltransferase (PTs) supplies the three central intermediates of the isoprenoid pathway, GPP, FPP, and GGPP. The basic terpene skeletons are then formed from the GPP, FPP, or GGPP by catalysis of terpene synthases (TPS) resulting in monoterpenes (10 carbon atoms), sesquiterpenes (15 carbon atoms), and diterpenes (20 carbon atoms), respectively. These enzymes function through the divalent metal ion-assisted generation of carbocation intermediates from the prenyl diphosphate precursor and give rise to the hundreds of cyclic and acyclic parent skeletons typical of plant terpenoids **[18]**.



Scheme: 1: Terpenoid Biosynthesis

1.4.1. Mevalonate pathway for isoprenoid biosynthesis

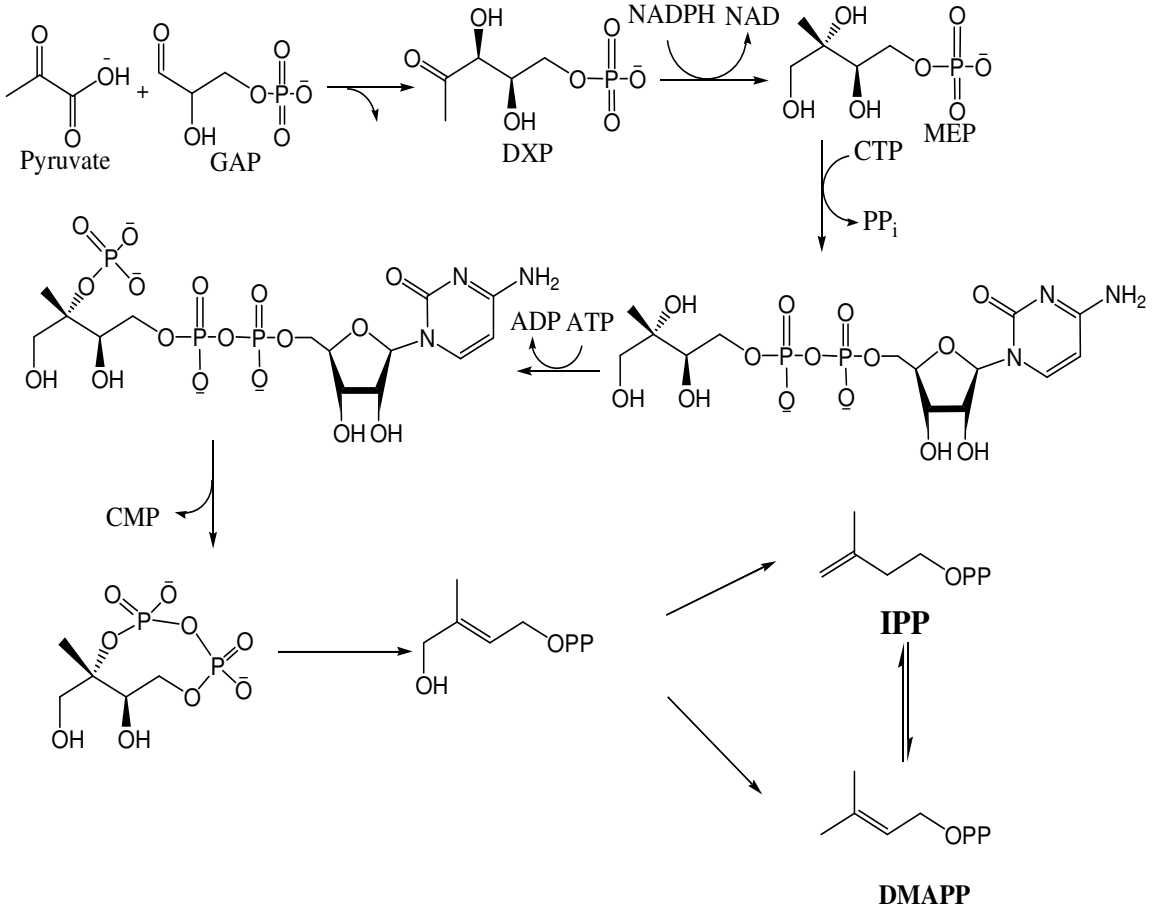
After cellular uptake of the acetate, it is converted to acetyl-CoA and condensed with another unit of acetyl-CoA to generate acetoacetyl-CoA. Addition of a third acetyl-CoA yields (S)-3-Hydroxy-3-Methylglutaryl-CoA (HMG-CoA), which is subsequently reduced by the enzyme HMG-CoA reductase to (R)-mevalonic acid (MVA). MVA then undergoes a series of phosphorylations, lose of CO_2 and inorganic phosphate to give IPP, which can be isomerized by IPP-DMAPP isomerase (idi) to DMAPP **(Scheme 2)**.



Scheme: 2 Mevalonate pathway of isoprenoid biosynthesis **[19]**

1.4.2. Deoxyxylulose Phosphate Pathway for Isoprenoid Biosynthesis

This pathway begins with conversion of glucose into glyceraldehyde-3-phosphate (GAP) and pyruvate, followed by thiamine-mediated decarboxylation of pyruvate. Condensation with GAP generates 1-deoxy-D-xylulose-5-phosphate (DXP). DXP then undergoes a rearrangement and reduction to give 2-*C*-methyl-D-erythritol-4-phosphate (MEP). This step represents the first committed step of the non-MVA pathway, since DXP is used in other biosynthetic pathways. After several transformations, the cyclic diphosphate is made. At this point, the $[4Fe\text{-}4S]^{2+}$ metal cluster sequentially transfers two electrons to open the diphosphate and eliminate the inactivated secondary hydroxyl group. A similar iron-sulfur cluster performs a second two-electron transfer to yield an allylic anion that can give either IPP or DMAPP upon protonation **(Scheme 3) [20]**.



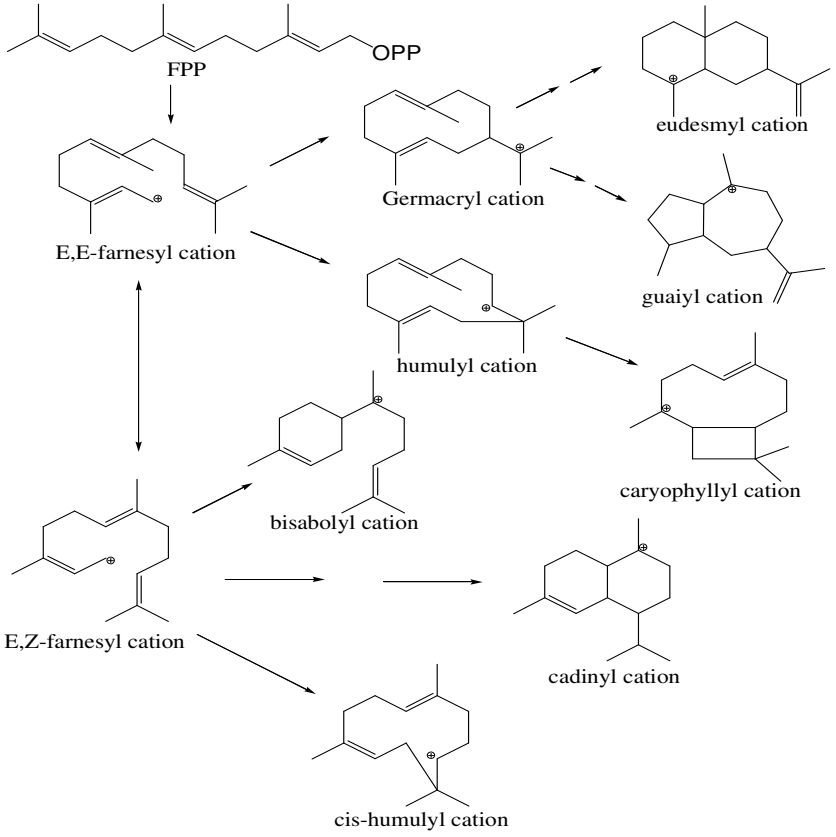
Scheme: 3 The deoxyxylulose phosphate (methylerythritol) pathway for isoprenoid biosynthesis **[19]**

1.5. Sesquiterpenes

Sesquiterpenoids are defined as the group of 15 carbon compounds derived by the assembly of 3 isoprenoid units and they are found mainly in higher plants but also in invertebrates. Sesquiterpenes, with monoterpenes, are an important constituent of essential oils in plants. They are the most diverse group of isoprenoids. In plants, they function as pheromones and juvenile hormones. Sesquiterpene structures present several acyclic, mono-, bi-, tri-, and tetracyclic systems.

Sesquiterpenoid biosynthesis

Addition of further C_5 isoprene diphosphate (IPP) unit to geranyldiphosphate in an extension of the prenyl transferase reaction leads to the fundamental sesquiterpene precursor, farnesyl diphosphate (FPP). FPP can then give rise to linear and cyclic sesquiterpenes (**Scheme 4**) **[19]**.



Scheme: 4 Biosynthesis of sesquiterpenes

1.6. Flavonoids

Flavonoids are a class of low-molecular-weight phenolic compounds that are widely distributed in the plant kingdom. Over 6000 naturally occurring flavonoids have been described, and many of them are common in higher plants. These compounds frequently serve as pigments in plants, but are also involved in many biological interactions. Flavonoids are built upon a C6-C3-C6 flavone skeleton in which the three-carbon bridge between the phenyl groups is commonly cyclized with oxygen. Based on the degree of unsaturation and oxidation of the three-carbon segment, flavonoids are divided in several classes (**Fig.2**). Most flavonoids reported in the literature are glycosides of a relatively small number of flavonoid aglycons, which are generally water-soluble and accumulate in the vacuoles of plant cells.

A significant role of flavonoids that has been under very active research recently, is their possible beneficial influence on human health. Flavonoids have been found to own potent antioxidant and free radical scavenging activities *in vitro*. There is growing evidence from human consumption studies supporting a protective role of flavonoids in cardiovascular diseases and cancer. Many flavonoids have been found to possess antiviral, antibacterial, antifungal or anti-allergenic properties. However, because of the wide variety of different flavonoids, their possible interactions with other substances, and the complexity of their metabolism in the human system, more research in this area is still needed **[21]**.

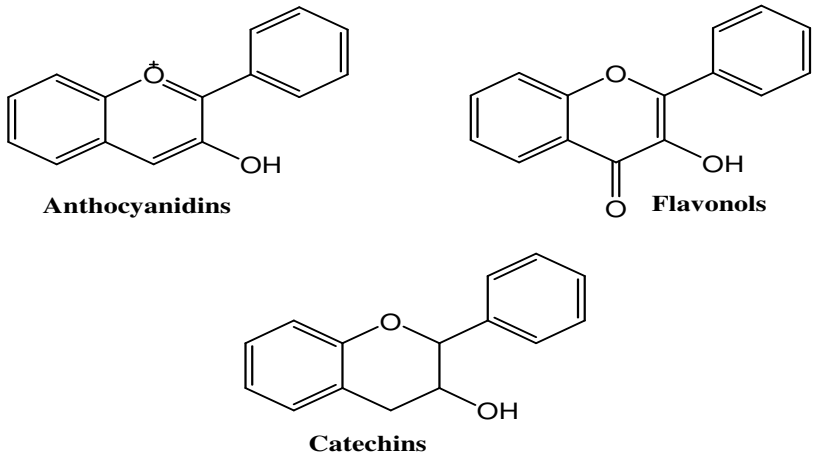
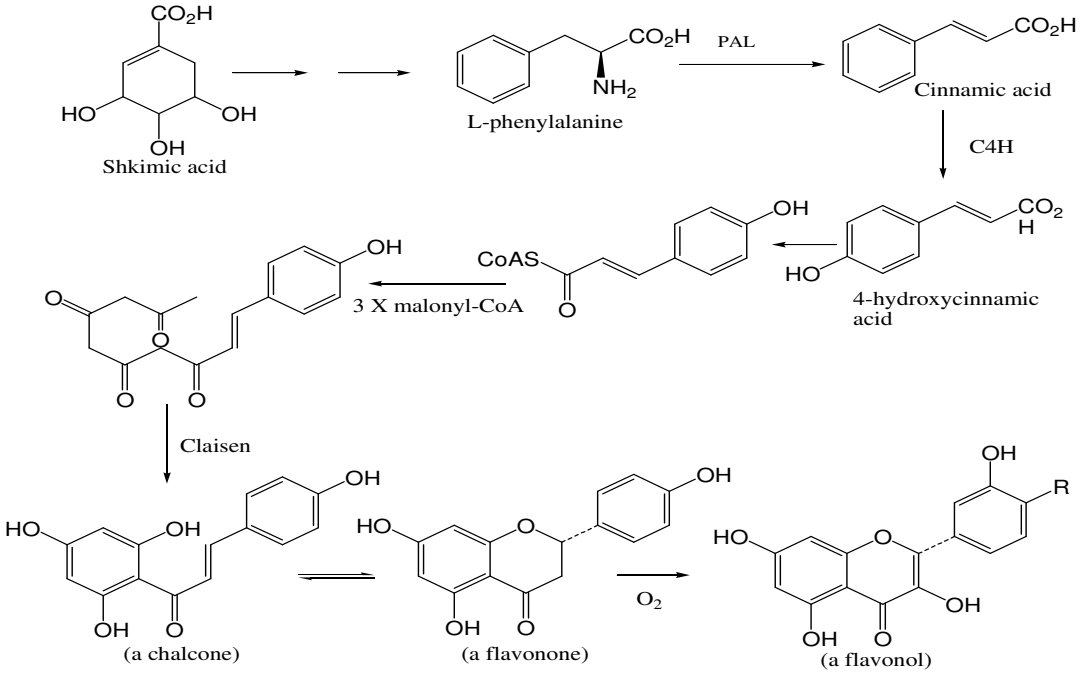


Figure 2: Classes of Flavonoids

Flavonoid biosynthetic pathway

Flavonoids are synthesized via the phenylpropanoid pathway. Phenylalanine ammonia lyase (PAL) catalyzes the conversion of phenylalanine to cinnamate. PAL also shows activity with converting tyrosine to *p*-coumarate, albeit to a lower efficiency. The cinnamate-4-hydroxylase (C4H) catalyzes the synthesis of *p*-hydroxycinnamate from cinnamate and 4-coumarate:CoA ligase (4CL) converts *p*-coumarate to its coenzyme-A ester, activating it for reaction with malonyl CoA. The flavonoid biosynthetic pathway starts with the condensation of one molecule of 4-coumaroyl-CoA and three molecules of malonyl-CoA, yielding naringenin chalcone. This reaction is carried out by the enzyme chalcone synthase (CHS). Chalcone is isomerised to a flavanone by the enzyme chalcone flavanone isomerase (CHI). From these central intermediates, the pathway diverges into several side branches, each resulting in a different class of flavonoids. Flavanone-3-hydroxylase (F3H) catalyzes the stereospecific 3ß-hydroxylation of (2S)-flavanones to dihydroflavonols. The overview of the flavonoid pathway is presented in **Scheme 5**. There is evidence that the enzymes involved in flavonoid metabolism might be acting as membrane-associated multienzyme complexes, which has implications on the overall efficiency, specificity, and regulation of the pathway **[21]**.



Scheme: 5 Biosynthesis of Flavonoids **[19]**

1.7. Objectives of the project

The main objective of this work is to undertake phytochemical study on the ethanol extract of the leaves of *Laggera tomentosa*. And to elucidate structures of the components of the ethanol extract of the plant. Except for reports on the essential oil constituents of this species, there are no published reports on the chemistry of the plant. The plant was selected for this study because it is endemic to Ethiopia and important in traditional medicine.

2. Result and discussion

Two compounds were isolated and characterized from the ethanol extract of leaves of *Laggera tomentosa*, **LTE-7** and **LTE-8**. Structure elucidation of the compounds was based on the spectroscopic data obtained and in comparison with data in the literature for similar compounds. The characterizations of the two compounds are described below.

2.1. Characterization of LTE-7

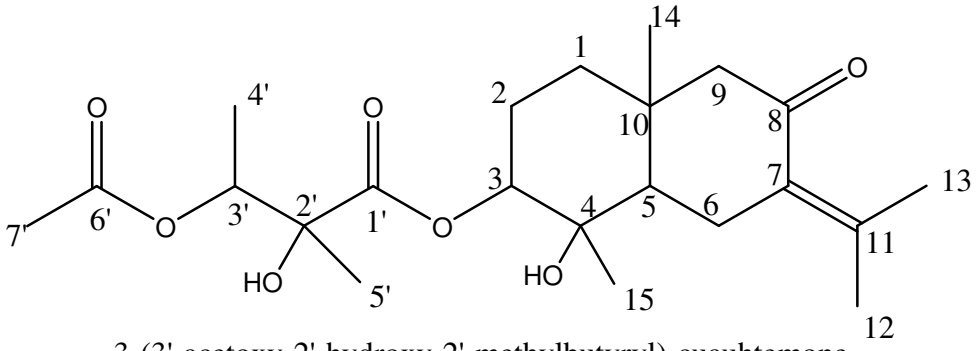
The compound **LTE-7** was isolated as a white solid with melting point of 138^0C and R_f value of 0.4 in $CHCl_3$: EtOAc (1:1). In the IR spectrum **(Appendix-7)** a sharp **absorption** band at 3510 cm^{-1} indicated the presence of OH group. Two intense absorption bands at 1733 cm^{-1} and 1720 cm^{-1} indicated the presence of two ester carbonyl groups. An absorption band at 1667 cm^{-1} indicated the C=O stretch of an α,β-unsaturated carbonyl group. The UV spectrum **(Appendix-8)** displayed an absorption band at λ_{max} (in $CHCl_3$) 285 nm indicating the presence of α,β-unsaturated carbonyl chromophore.

Inspection of the ^{1}H-NMR spectrum **(Appendix-1)** of the compound showed a 1H quartet at δ 5.15 indicating a methine attached to a methyl group and oxygen on the other side. And a 1H singlet at 4.90 indicated the presence of methine attached to oxygen. A broad singlet at δ3.72 indicated the presence of OH group. The spectrum also indicated that there are six 3H singlets at δ 2.06, 2.01, 1.84, 1.42, 1.29 and 0.96 indicating the presence of six methyl groups attached to quaternary carbons. A 3H doublet at δ 1.31 showed the presence of a methyl group attached to a methine.

By comparing the ^{13}C and DEPT-135 NMR spectra **(Appendix-2 and Appendix-3)**, there existed a quaternary carbon atom at δ 202.38, which indicated the presence of conjugated carbonyl group. The quaternary carbon peaks at δ 174.75 and 169.96 indicated two ester carbonyl groups. The peaks at δ 146.30 and 130.46 indicated the existence of two olefinic carbon atoms. In addition there existed three quaternary carbon atoms at δ 76.44, 72.14 and 35.73. The DEPT-135 NMR displayed four downward peaks at δ 59.66, 33.33, 25.48 and 23.87, which showed the presence of four methylene groups. There were ten peaks left which were assigned as three methine (δ 78.87, 74.45 and

46.47) and seven methyl groups (δ 23.72, 23.01, 22.40, 21.42, 21.08, 18.71 and 13.32) by comparing it with HSQC **(Appendix-5)**.

From the spectroscopic data obtained for the compound and with comparison of M. Sc. project (Kibrom) **[19]**, the following structure was proposed for the compound **LTE-7(Figure 3)**.



3-(3'-acetoxy-2'-hydroxy-2'-methylbutyryl)-cuauhtemone

Figure 3: LTE-7

The 2D NMR spectra of the compound **LTE-7** further supported the proposed structure. From HSQC spectrum **(Appendix-5)**, the protons at δ 4.90 (1H, t) and 5.15 (1H, q, J=6.4) correlated with the carbon peaks at δ 78.87 and 74.45, respectively. And the proton peak at δ 1.31 (3H, d, J=6.4) correlated with the carbon peak at δ 13.32. The HSQC result is summarized in **Table 3**.

Table 3: HSQC correlation of **LTE-7**

Carbon No	**Hydrogen number**	**Remark**
C-3 (δ 78.87)	δ 4.90 (1H, *s*)	CH
C-3' (δ 74.45)	δ 5.15 (1H, *q*)	CH
C-9 (δ 59.66)	δ 2.25 (2H, *s*)	CH_2
C-5 (δ 46.47)	δ 1.93 (1H, *dd*)	CH
C-1 (δ 33.33)	δ 1.48 (1H, *m*) & δ 1.26 (1H, *m*)	CH_2
C-6 (δ 25.48)	δ 2.90 (1H, *dd*) & δ 2.18 (1H, *dd*)	CH_2
C-2 (δ 23.87)	δ 1.81 (2H, *m*)	CH_2
C-12 (δ 23.72)	δ 2.06 (3H, *s*)	CH_3
C-13 (δ 23.01)	δ 1.84 (3H, *s*)	CH_3
C-5' (δ 22.40)	δ 1.42 (3H, *s*)	CH_3
C-15 (δ 21.42)	δ 1.29 (3H, *s*)	CH_3
C-7' (δ 21.08)	δ 2.01 (3H, *s*)	CH_3
C-14 (δ 18.71)	δ 0.96 (3H, *s*)	CH_3
C-4' (δ 13.32)	δ 1.31 (3H, *d*)	CH_3

The HSQC spectrum showed that there is a pair of diastereotopic proton, δ 2.90 and δ 2.18 (C-6). The protons at carbon number 6 are coupled with the protons at carbon number 5. The correlation between the two hydrogens is shown in **Figure 4**.

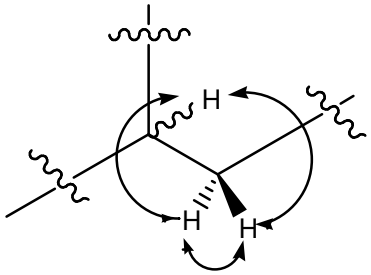


Figure 4: The correlation between H-6 and H-5 of **LTE-7**

The COSY spectrum **(Appendix-4)** also supported the predicted structure of compound **LTE-7**. **Table 4** shows the COSY correlation of **LTE-7**, which shows the near by hydrogen.

Table 4: $^{1}H \leftrightarrow {}^{1}H$ COSY correlation of **LTE-7**

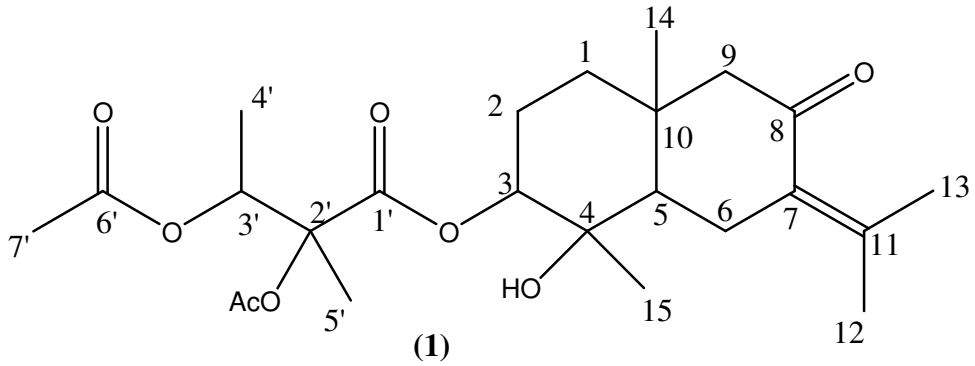
Carbon No	**$^{1}H \leftrightarrow {}^{1}H$ COSY**
C-3' (δ 74.45)	H-3'↔H-4'
C-3 (δ 78.87)	H-3↔H-2
C-6 (δ 25.48)	H-6A↔H-5, H-6B
C-9 (δ 59.66)	H-9↔H-14, H-5
C-6 (δ 25.48)	H-6B↔H-5, H-12, H-6A, H-13
C-12 (δ 23.72)	H-12↔H-13, H-6B, H-9, H-6A
C-7' (δ 21.08)	H-7'↔
C-5 (δ 46.47)	H-5↔H-6A, H-9, H-6B
C-13 (δ 23.01)	H-13↔H-6B, H-12
C-2 (δ 23.87)	H-2↔H-1A, H-1B
C-1 (δ 33.33)	H-1A↔H-2, H-1B
C-5' (δ 22.40)	H-5'↔H-1B
C-4' (δ 13.32)	H-4'↔H-3'
C-15 (δ 21.42)	H-15↔
C-1 (δ 33.33)	H-1B↔H-2, H-1A
C-14 (δ 18.71)	H-14↔H-9, H-1A

The COSY spectrum also showed that the proton peak at δ 1.31 (H-4') correlated with the proton peak at δ 5.15 (H-3').

Table 5: HMBC correlation of **LTE-7**

Carbon no	Protons correlated
C-8	C-8↔H-6A, H-9, H-6B
C-1’	C-1’↔H-3’, H-3, H-5’
C-6’	C-6’↔H-3’, H-7’
C-11	C-11↔H-6A, H-12, H-13
C-7	C-7↔H-6A, H-6B, H-12, H-5, H-13
C-3	C-3↔H-15, H-1B
C-2’	C-2’↔H-5’, H-4’, H-15
C-3’	C-3’↔H-5’, H-4’
C-4	C-4↔H-5, H-15, H-1B
C-9	C-9↔H-5, H-14
C-5	C-5↔H-3, H-6A, H-9, H-6B
C-10	C-10↔H-6A, H-9, H-6B, H-5, H-1A, H-1B, H-14
C-1	C-1↔H-3, H-9, H-14
C-6	C-6↔H-5
C-2	C-2↔H-3, H-1A, H-1B
C-1	C-1↔H-6B, H-13
C-13	C-13↔H-12
C-5’	C-5’↔H-3’, H-7’
C-15	C-15↔H-3, H-6B, H-5, H-1A
C-7’	C-7’↔
C-14	C-14↔H-9, H-5
C-4’	C-4’↔H-3’, H-5’

The ^{1}H-NMR data obtained for **LTE-7** is comparable with the data for the compound isolated from *Pluchea indica,* compound 1 **(Figure 5) (Table 6) [22]**.



(1)

Figure 5: Compound **(1)**

Table 6: Comparison of ^{1}H-NMR of **LTE-7** with compound **(1)**.

Carbon No	^{1}H-NMR	
	LTE-7	Compound 1
1	1.26 (*m*), 1.48 (*m*)	1.33 (*dt*), 1.48 (*ddd*)
2	1.81 (*m*)	1.81 (*m*), 1.84 (*m*)
3	4.90 (*t*)	5.02 (*t*)
5	1.93 (*dd*)	1.92 (*dd*)
6	2.90 (*dd*), 2.18 (*m*)	3.01 (*dd*), 2.17 (*dd*)
9	2.25 (*s*)	2.17 (*d*), 2.25 (*d*)
12	2.06 (*s*)	2.10 (*s*)
13	1.84 (*s*)	1.86 (*s*)
14	0.96 (*s*)	0.98 (*s*)
15	1.29 (*s*)	1.28 (*s*)
3’	5.15 (*q*)	5.24 (*q*)
4’	1.31 (*d*)	1.26 (*d*)
5’	1.42 (*s*)	1.66 (*s*)
7’	2.01 (*s*)	2.09 (*s*)

Based on spectroscopic data and literature survey, this compound (**LTE-7**) was characterized to be 3-(3’-acetoxy-2’-hydroxy-2’-methylbutyryl)-cuauhtemone.

2.2. Characterization of LTE-8

LTE-8 is a yellowish solid with melting point 208 ^{o}C and R_f value of 0.4 in the solvent system $CHCl_3$:EtOAc=1:1. A strong IR absorption band **(Appendix-12)** at 3491 cm^{-1} showed the presence of a free OH group and broad absorption band around 3485 cm^{-1} indicate the presence of an aromatic OH group. In the UV spectrum **(Appendix-13)** λ_{max}=343 nm (in $CHCl_3$) was observed due to bathochromic shift on cyclic enone. The absorption at λ_{max}=280 nm and 240 nm indicate the presence of aromatic system.

The ^{1}H NMR spectrum **(Appendix-9)** displayed four aromatic protons at δ 7.73 (1H, *s*), 7.69(1H, *d, J=4Hz*), 7.06 (1H, *d*, *J=4Hz*), and 6.58 (1H, *s*). This result confirmed that the protons at δ 7.69 and δ 7.06 are coupling partners. And the proton singlet at δ 12.44 showed the presence of aromatic OH group. In addition the peaks at δ 4.02 (3H, *s*), 4.01 (3H, *s*), and 3.88 (3H, *s*) indicated that there are three methoxy groups on the compound.

The ^{13}C NMR spectrum **(Appendix-10)** revealed 18 carbons, which were identified as three methoxy (δ 60.20, 56.51, 56.15), four aromatic methine carbons (δ 122.64, 114.59, 110.95 and 90.19) the DEPT-135 spectrum **(Appendix-11)** only shows these carbon peaks, eleven quaternary carbon atoms (δ 178.74, 156.22, 152.88, 149.75, 148.32, 146.35, 145.27, 138.69, 129.21, 122.60, 106.38) which were deduced from the DEPT-135 NMR spectrum. The carbon peak at around δ 178.74 indicated the presence of α,β-unsaturated carbonyl carbon. This compound was reported before by Kibrom **[19]**. Even if the 2D NMR spectrum is not run for this case, the structure of the compound is deduced by comparing it with his work. The position of the methoxy group in the structure was determined from the HMBC spectra **[19]**.

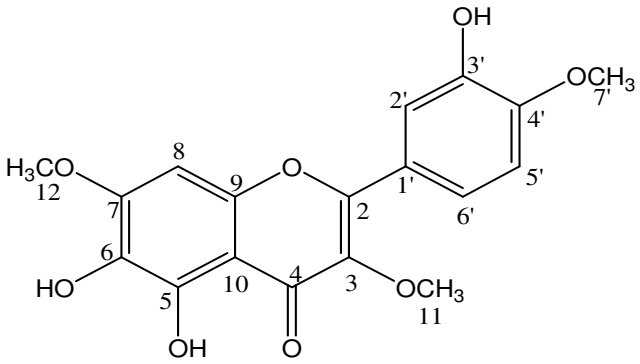
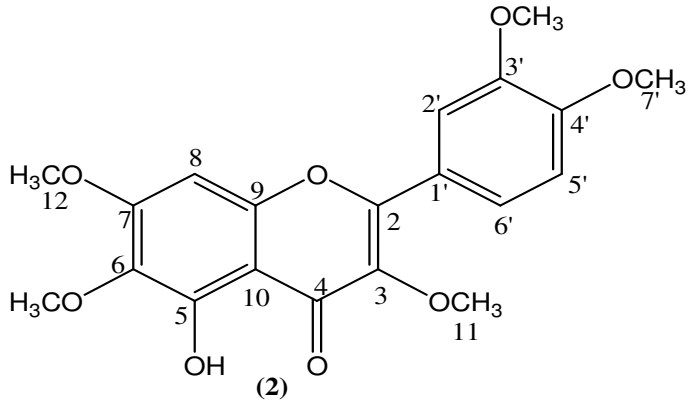


Figure 6: LTE-8

The ^{13}C-NMR data obtained for **LTE-8** is comparable with the data for compound 2 **(Figure 7) (Table 7) [23]**.



(2)

Figure 7: Compound **(2)**

Table 7: Comparison of the ^{13}C-NMR of **LTE-8** and compound **(2)**.

Carbon No	^{13}C (ppm)	
	LTE-8	Compound 2
2	156.22	151.4
3	138.69	138.0
4	178.74	180.0
5	145.27	151.9
6	129.21	131.5
7	152.88	158.0
8	90.19	89.8
9	149.75	155.0
10	106.38	105.7
1’	122.60	122.1
2’	110.95	110.7
3’	148.32	148.0
4’	146.35	150.8
5’	114.59	110.3
6’	122.64	121.5

Based on spectroscopic data and literature survey, the compound **LTE-8** is 3’,5,6-trihydroxy-3,4’,7-trimethoxyflavone.

3. Conclusion

The genus *Laggera* includes ca. 20 species, and some of them have been used as traditional herbal medicines. The chemical studies on these *Laggera* species have revealed that some components exhibit strong bioactivity. Most species of the genus *Laggera* have received little attention; much more phytochemical and biological studies should be carried out on these plants in order to disclose their active principles **[5]**.

Two compounds, one sesquiterpene (**LTE-7**) and one flavone (**LTE-8**), were isolated in this project, namely 3-(3'-acetoxy-2'-hydroxy-2'-methyl butyryl)-cuauhtemone and 3',5,6-trihydroxy-3,4',7-trimethoxyflavone respectively. The two compounds (**LTE-7** and **LTE-8**) were reported by Kibrom **[19]**, the first compound **(LTE-7)** being reported for the first time.

4. Experimental

4.1. General

TLC was performed on a 0.25 mm thick silica gel GF_{254} (Merck). Ultraviolet-Visible (UV-Vis) spectra were determined using a GENESY'S 2PC spectrometer (200 – 400 nm) at room temperature. Infrared (IR) spectra were measured on a Perkin-Elmer BX spectrometer (400 – 4000 cm^{-1}) in KBr pelletes, since both samples are solid. Melting points were recorded using Thomas HOOVER capillary melting point apparatus. All NMR experiments were recorded using a Bruker 400 MHz Advance NMR spectrometer. Components of the *Laggera tomentosa* on TLC were detected by their UV fluorescence and by spraying with 1% vanillin in H_2SO_4.

4.2. Plant Material

Laggera tomentosa was collected from Daletti, western Shewa of Ethiopia (26 Km far from Addis Ababa near Alemgena) in November 2005. A voucher specimen (SD 6487) is deposited at the National Herbarium (ETH), Department of Biology, Addis Ababa University, Addis Ababa.

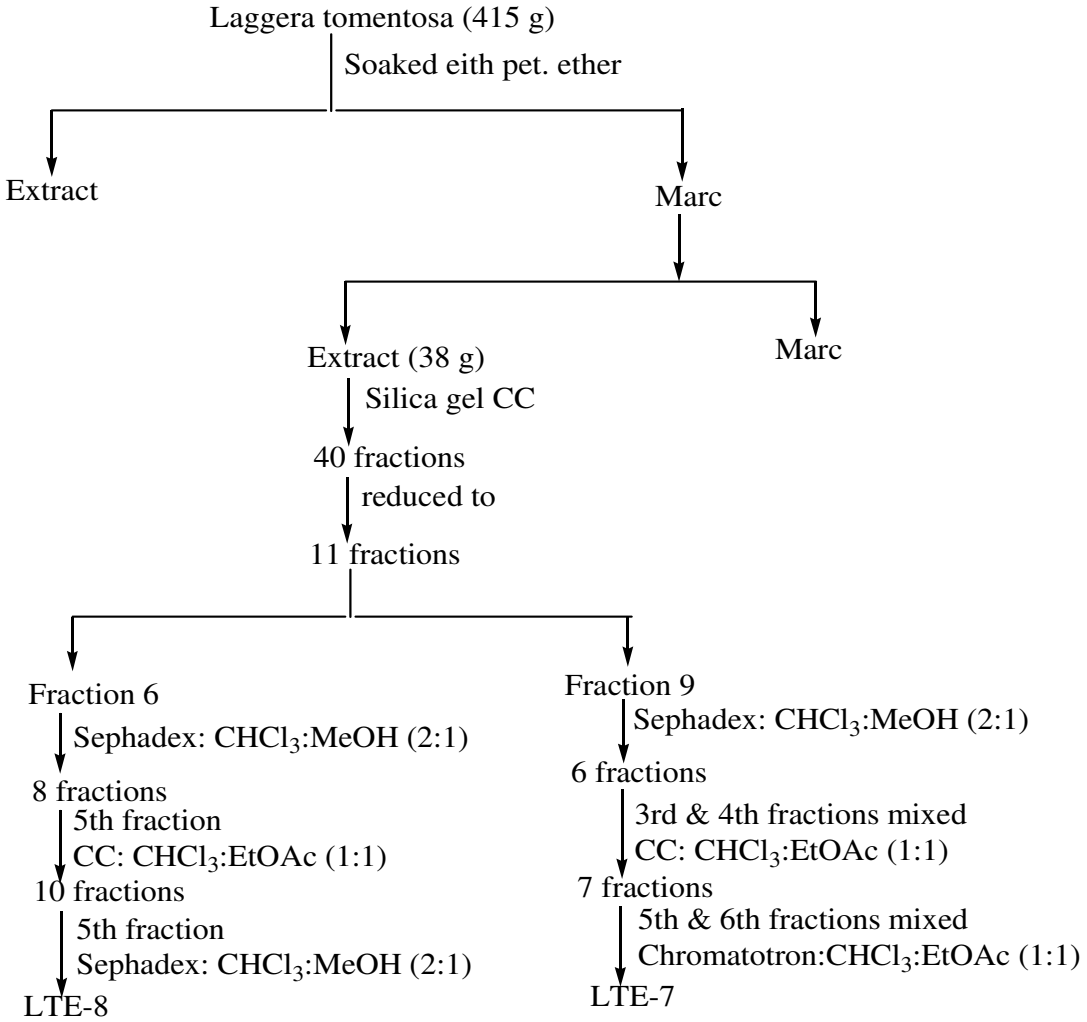
4.3. Coding System

In LTE, L-stands for the genus name *Laggera*, and T-stands for the species name *tomentosa* and E-stands for the ethanol extract. The number behind LTE indicates the location of the compound starting from the highest R_f value to the lowest. Thus, **LTE-7** stands for the seventh compound in the *Laggera tomentosa* ethanol extract, which is less polar, and **LTE-8** stands for the eighth compound in the *Laggera tomentosa* ethanol extract, which is more polar.

4.4. Isolation and Analysis

415 g of powdered leaf of *Laggera tomentosa* was first extracted with petroleum ether. The mark from the extract was then soaked with ethanol for 24 hrs. The filtrate then is concentrated under a reduced pressure Rotavapor. The yield then be 38g jelly solid.

15g of the crude extract from ethanol was applied to a silica gel (160 g) column chromatography and eluted with neat $CHCl_3$. The solvent system is changed continuously from $CHCl_3$: EtOAc (9:1) to $CHCl_3$: EtOAc (1:1). 40 fractions were collected and TLC analysis was made. According to their TLC result, the 40 fractions were reduced to 11 fractions. This is made by comparison of their R_f value, fractions having similar R_f value are mixed. **Scheme 6** illustrates the isolation of **LTE-7** and **LTE-8**.



Scheme: 6 Isolation of the components of **LTE**

4.4.1. Isolation of LTE-7

Fraction 9 from column chromatography was passed through sephadex LH-20 using $CHCl_3$: MeOH (2:1) as eluent and six fractions were collected. The third and fourth fractions have the same R_f value when TLC is run. So they are mixed and passed through column chromatography (20g silica gel) using $CHCl_3$: EtOAc (1:1) as eluent. Seven fractions were collected. The fourth and fifth fractions were mixed and passed through chromatotron in the solvent system $CHCl_3$: EtOAc (1:1). The purification using chromatotron yielded 128 mg of **LTE-7**.

Its TLC run with $CHCl_3$: EtOAc (1:1) showed brown color after spraying 1% vanillin in H_2SO_4 and heated on an oven (R_f=0.4). The compound obtained (**LTE-7**) is a colorless solid with melting point of 140^oC.

IR: (KBr) ν_{max}: 3509, 2941, 1732, 1720, 1667, 1583, 1438, 1379, 1267, 1205, 1149, 1106, 1080, 1066, 1019, 966 cm^{-1}; UV-Vis spectrum: λ_{max} ($CHCl_3$) = 285, 324 nm; ^1H NMR (400 MHz, $CDCl_3$,): δ 5.15 (1H, *q, J=6.4,* H-3'), 4.90 (1H*, t*, H-3), 3.72 (1H*, s*, OH), 2.90 (1H, *dd*, H-6A), 2.25 (2H, *s*, H-9), 2.18 (1H, *m*, H-6B), 2.06 (3H, *s*, H-12), 2.01 (3H, *s*, H-7'), 1.93 (1H, *dd*, H-5), 1.84 (3H, *s*, H-13), 1.81 (2H, *m*, H-2), 1.48 (1H, *m*, H-1A), 1.42(3H, *s*, H-5'), 1.31 (3H, *d, J=6.4*, H-4'), 1.29 (2H, *s*, H-15), 1.26 (1H, *m*, H-1B), 0.96 (3H, *s*, H-14); ^{13}C NMR (100 MHz, $CDCl_3$): δ 202.38 (C-8), 174.75 (C1'), 196.96 (C-6'), 146.30 (C-11), 130.46 (C-7), 78.87 (C-3), 76.44 (C-2'), 74.45 (C-3'), 72.14 (C-4), 59.66 (C-9), 46.47 (C-5), 35.73 (C-10), 33.33 (C-1), 25.48 (C-6), 23.87 (C-2), 23.72 (C-12), 23.01 (C-13), 22.40 (C-5'), 21.42 (C-15), 21.08 (C-7'), 18.71 (C-14), 13.32 (C4').

4.4.2. Isolation of LTE-8

Fraction 6 from column chromatography was passed through sephadex LH-20 using $CHCl_3$: MeOH (2:1) as eluent and 8 fractions were collected. The fifth fraction is then subjected to Column chromatography (25g silica gel) in the solvent system $CHCl_3$:EtOAc (1:1). 10 fractions were collected and the fifth fraction is passed through sephadex. After purification with sephadex 3 mg of **LTE-8** is obtained.

LTE-8 is a yellowish solid with melting point of 208°C. Its TLC run with $CHCl_3$: EtOAc (1:1) showed yellow color after spraying with 1% vanillin in H_2SO_4.

IR: (KBr) ν_{max}: 3491, 2928, 1676, 1577, 1515, 1485, 1458, 1427, 1355, 1275, 1246, 1213, 1165, 1135, 1031, 988, 819, 794; UV-Vis spectrum: λ_{max} ($CHCl_3$)= 240, 280, 343 nm; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.44 (1H, *s*, OH), 7.73 (1H, *s*, H-2'), 7.69 (1H, *d*, *J=8.8 Hz*, H-6'), 7.07 (1H, *d*, *J=8.8 Hz*, H-5'), 6.58 (1H, *s*, H-8), 4.02 (3H, *s*, H-12), 4.01 (3H, *s*, H-7'), 3.88 (3H, *s*, H-11); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.74 (C-4), 156.22 (C-2), 152.88 (C-7), 149.75 (C-9), 148.32 (C-3'), 146,35 (C-4'), 145.27 (C-5), 138.69 (C-3), 129.21 (C-6), 122.64 (C-6'), 122.60 (C-1'), 114.59 (C-5'), 110.95 (C-2'), 106.38 (C-10), 90.19 (C-8), 60.20 (C-11), 56.51 (C-12), 56.15 (C-7').

5. Reference

[1] http://www.rsc.org/ebooks/archive/free/BK9780854046812/BK9780854046812-00001.pdf

[2] Koskinen A., *Asymmetric synthesis of natural products*, **1993**, John Wiley & Sons Ltd, University of Oulu, Oulu, Finland.

[3] http://www.people.vcu.edu

[4] http://www.arbec.com.my/top

[5] Kiyota H., Shi Q., Huo C., Li X., *Chemistry and Biodiversity*, **2007**, 4, 105

[6] Asfaw, N.; Stroresund, H. J.; Skatterbol, L.; Aasen, A., *Phytochemistry*, **1999**, 52, 1491.

[7] Zhao, Y.; Yue, J. M.; Lin, Z.W.; Ding, J. K.; Sun, H. D., *Phytochemistry*, **1997**, 44, 459.

[8] Zhao, Y.; Yue, J. M.; Lin, Z.W.; Ding, J. K.; Sun, H. D.,*J. Nat. Prod*. **1997**, 60, 545.

[9] Li, S. L.; Ding, J. K.; Jiang, B.; Na, B., *Phytochemistry*, **1998**, 49, 2035.

[10] Raharivelomanana, P.; Bianchini, J. P.; Ramanoelina, A. R. P.; Rasoarahona, J. R. E.; Faure, R.; Cambon, A., *Phytochemistry*, **1998**, 47, 1085.

[11] Ahmed, A. A.; El-Seed, H. R.; Mahmoud, A. A.; El-Douski, A. E. A.; Zeid, I. F.; Bohlin, L., *Phytochemistry*, **1998**, 49, 2421.

[12] Mesfin, T., *The Flora of Ethiopia and Eritrea*, **2004**, Vol. 4, Part 2, The National Herbarium, Addis Ababa University, Addis Ababa and Uppsala University, Uppsala, p. 140 – 144.

[13] Asfaw, N.; Stroresund, H. J.; Skatterbol, L.; Aasen, *J. Essent. Oil Res*., **2003**, 15, 102

[14] Kibrom G., M. Sc. Project, A. A. U., Department of Chemistry, Addis Ababa, 2006.

[15] Dibaba A., M. Sc. Project, A. A. U., Department of Chemistry, Addis Ababa, 2006.

[16] Hoeven, R. S. V.; Monforte, A. J.; Breeden, D.; Tanksley, S. D.; Steffens, J. C. *Plant Cell*. **2000**, 12, 2283

[17] Tomohisa K.; *Bioscience, Biotechnology, and Biochemistry***, 2002**, 66, 1619

[18] Martin, D.; Tholl,D.; Gershenzon, J.; Bohlmann, J., *Plant Physiol*, **2002**, 129, 1003

[19] Dewick,P. M., *Medicinal Natural Products*, 2nd ed., **2004**, John Wiley and Sons Ltd, University of Nottingham, Nottingham, UK.

[20] Felix R.; Wolfgang E.; Juraithip W.; Stefan H.; Christoph A. S.; Adelbert B., *Eur. J. Biochem.* **2001**, 268, 3190-3197.

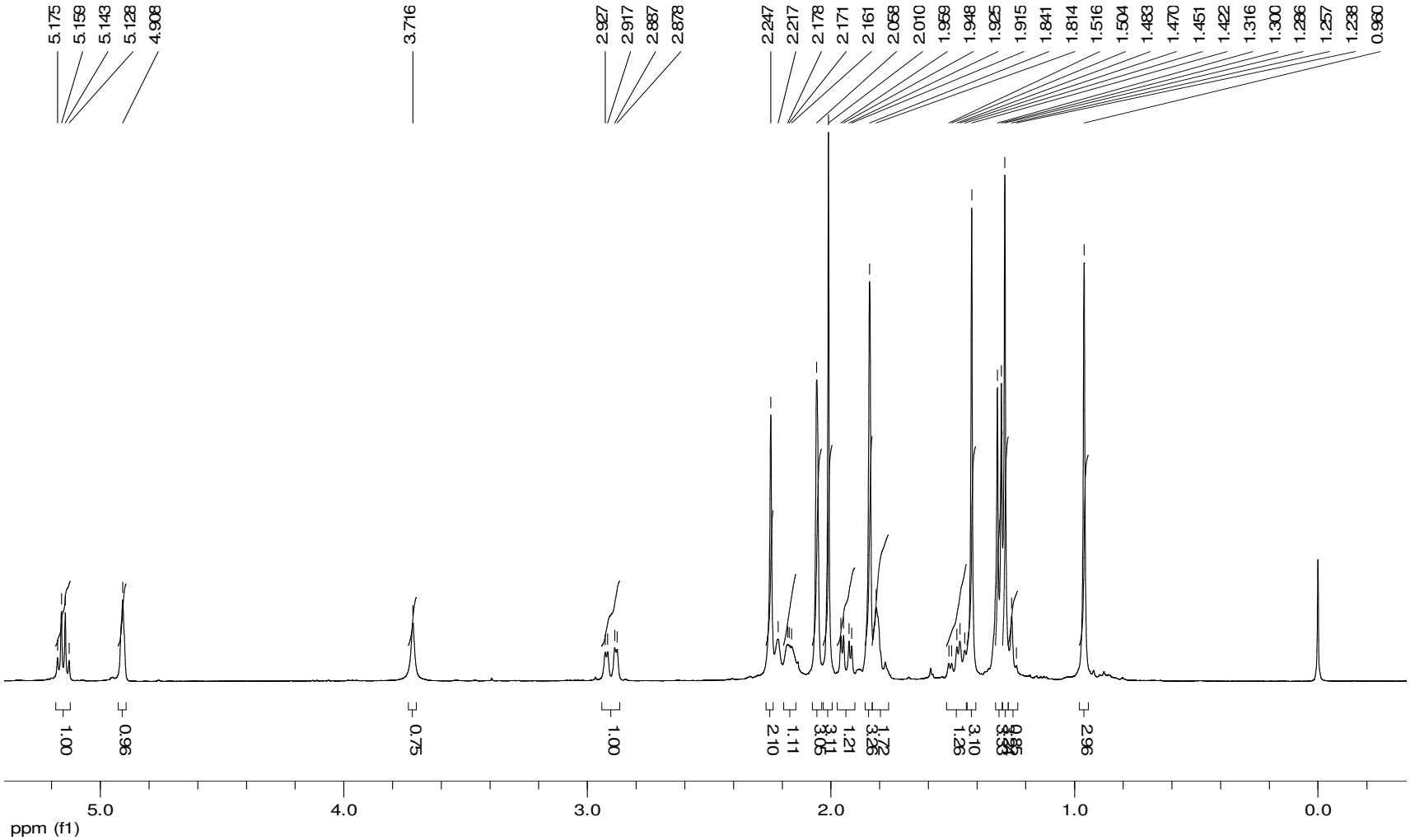
[21] Laura J., *Flavonoid Biosynthesis in Bilbery*, **2003**, Department of Biology, University of Oulu, Oulu.

[22] Ruangrungsi, N.; Rodkird, S.; Tantivatana, P., *Journal of Natural Products*, **1983**, 46, 671

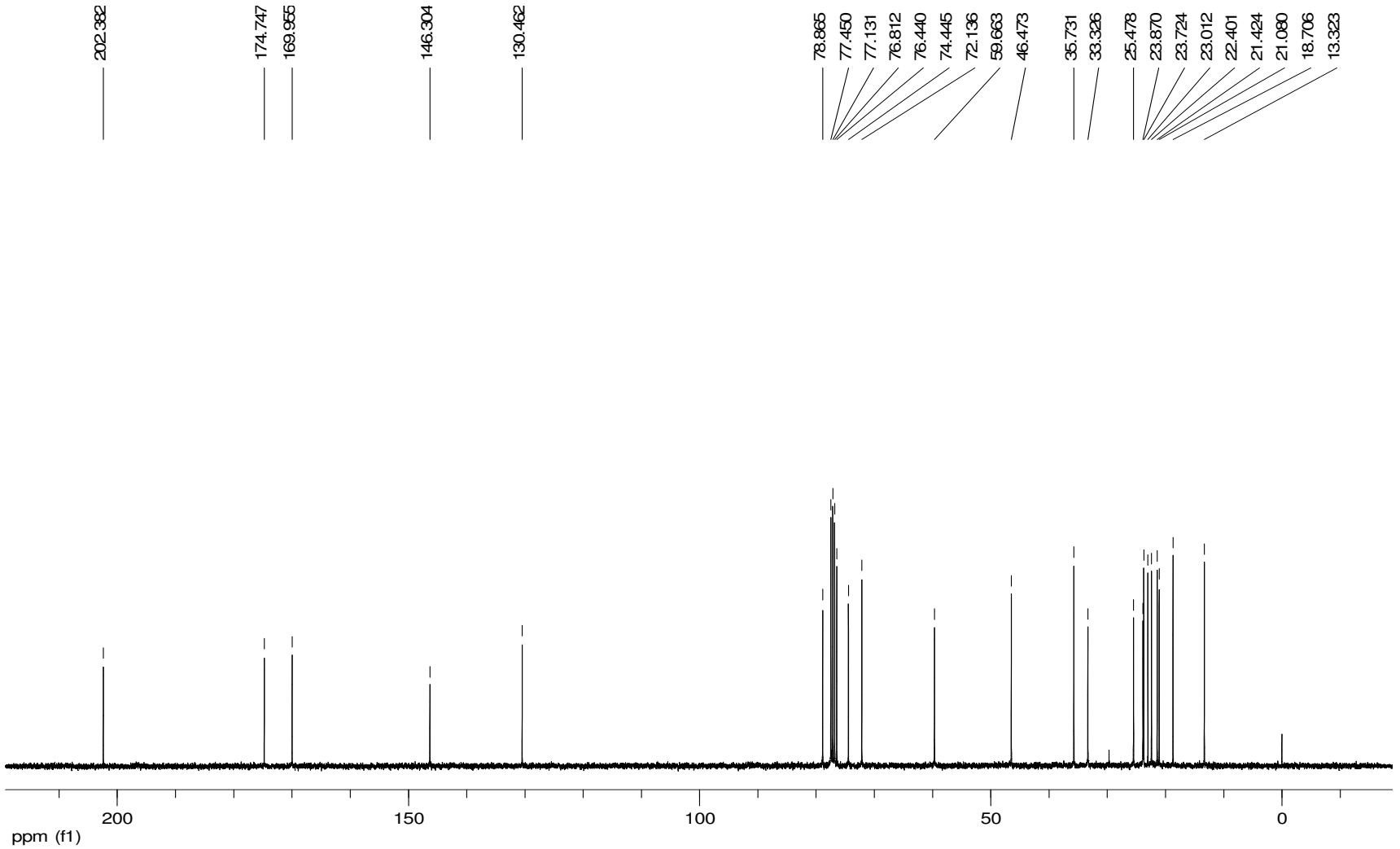
[23] Wenkert, E.; Gottliebh, *Phytochemistry*, **1977**, 16, 1811

APPENDICES

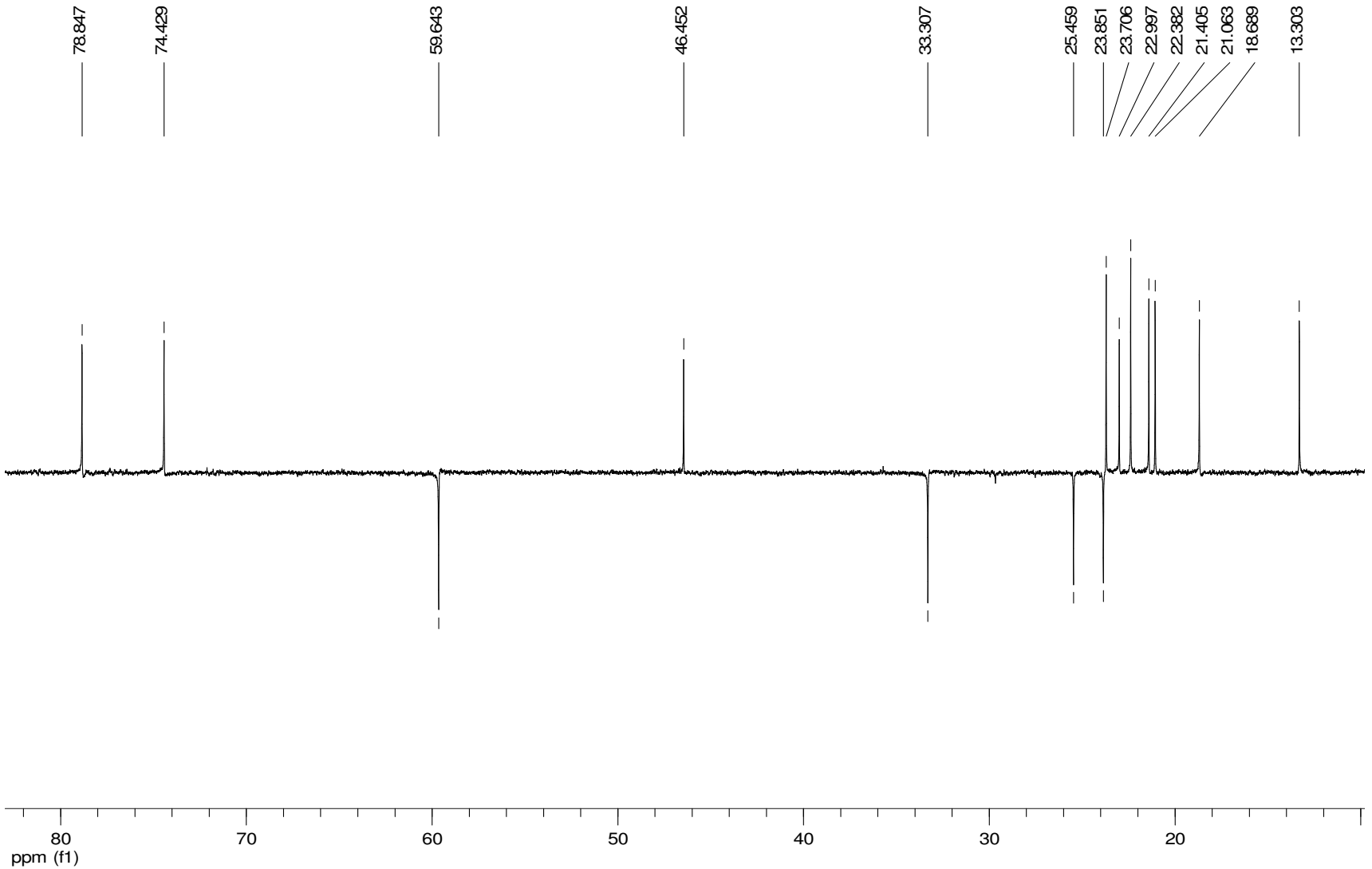
APPENDIX-1: ^{1}H-NMR of LTE-7



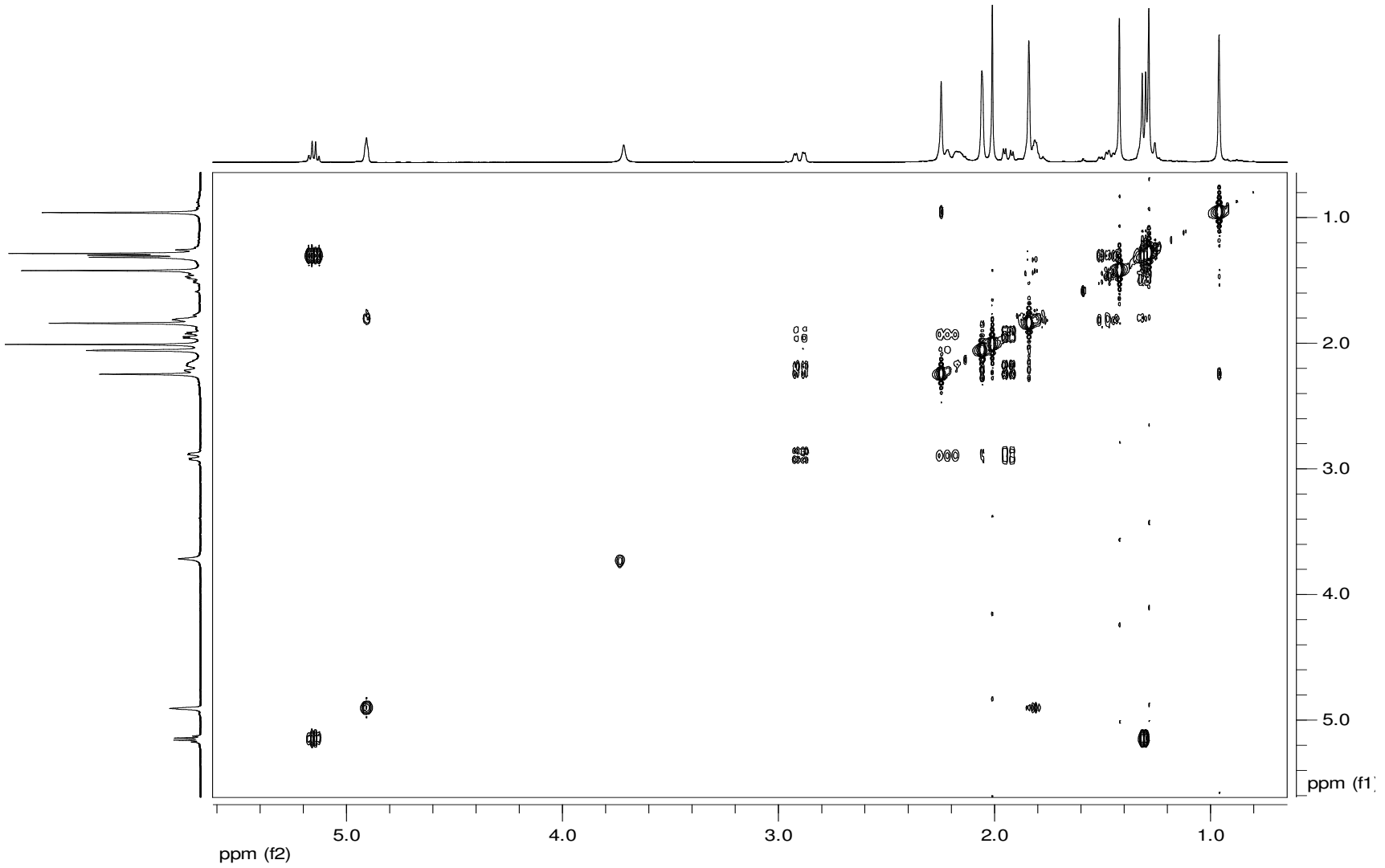
APPENDIX-2: ^{13}C-NMR of LTE-7



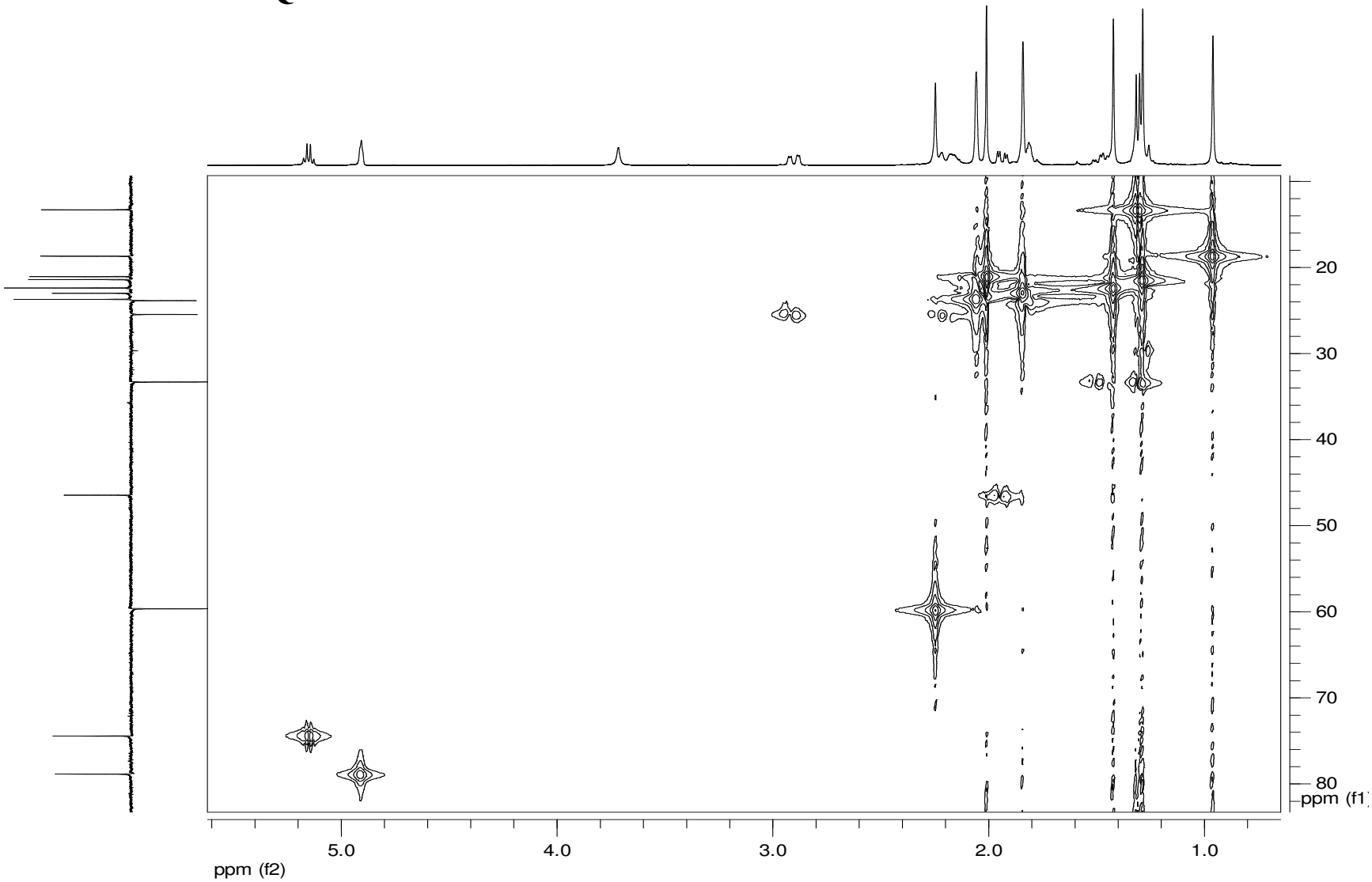
APPENDIX-3: DEPT-135 NMR of LTE-7



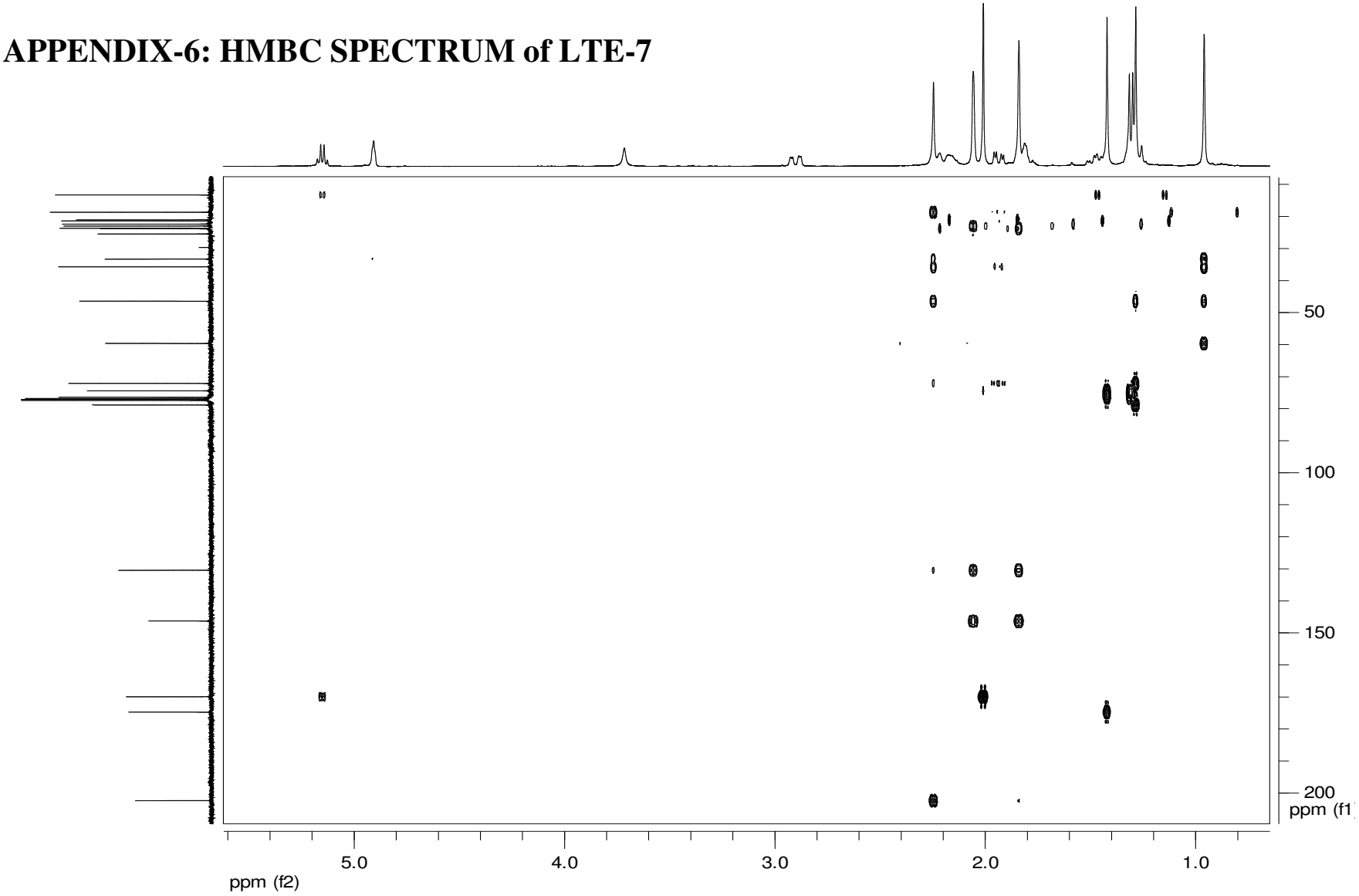
APPENDIX-4: COSY SPECTRUM OF LTE-7



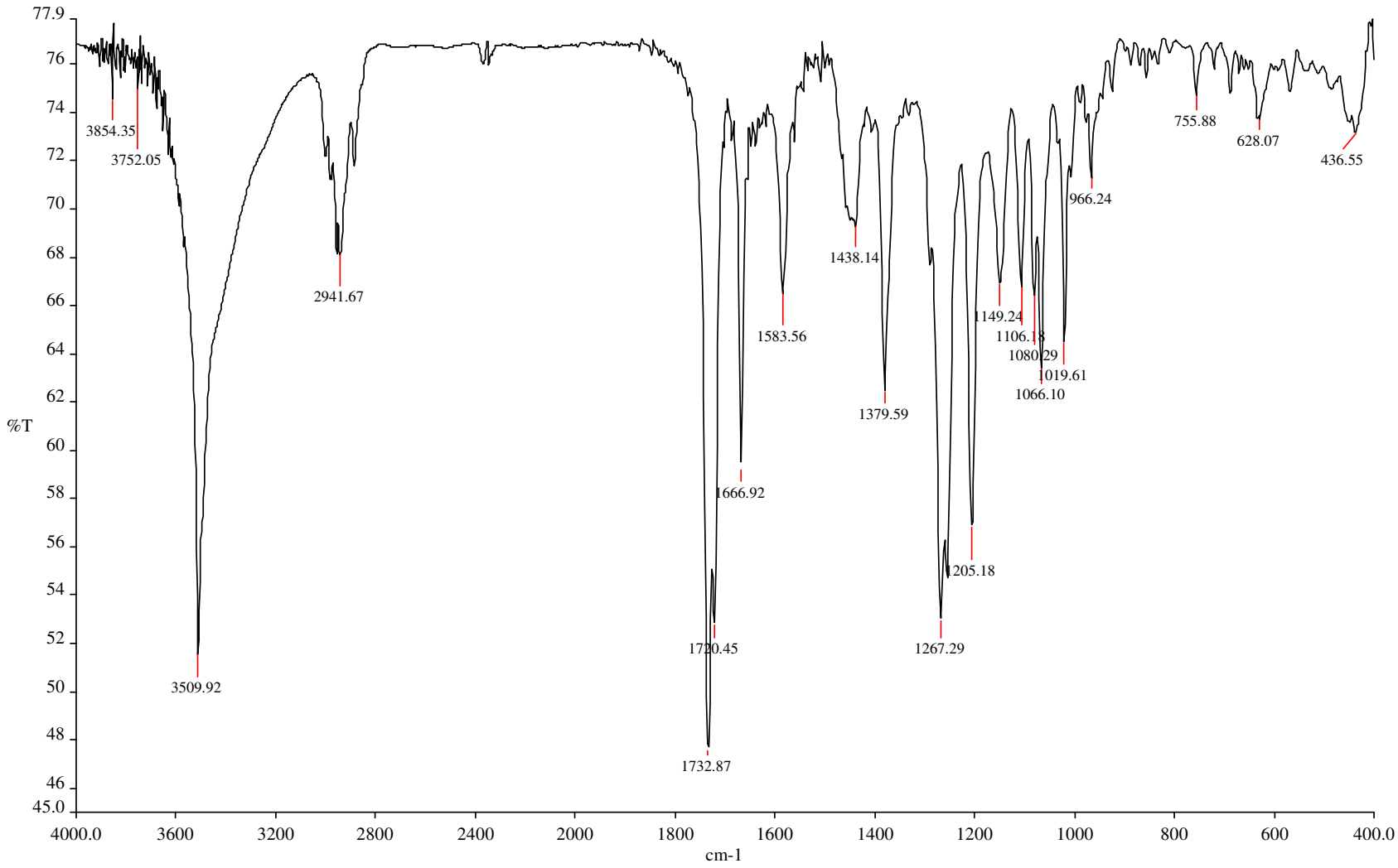
APPENDIX-5: HSQC SPECTRUM of LTE-7



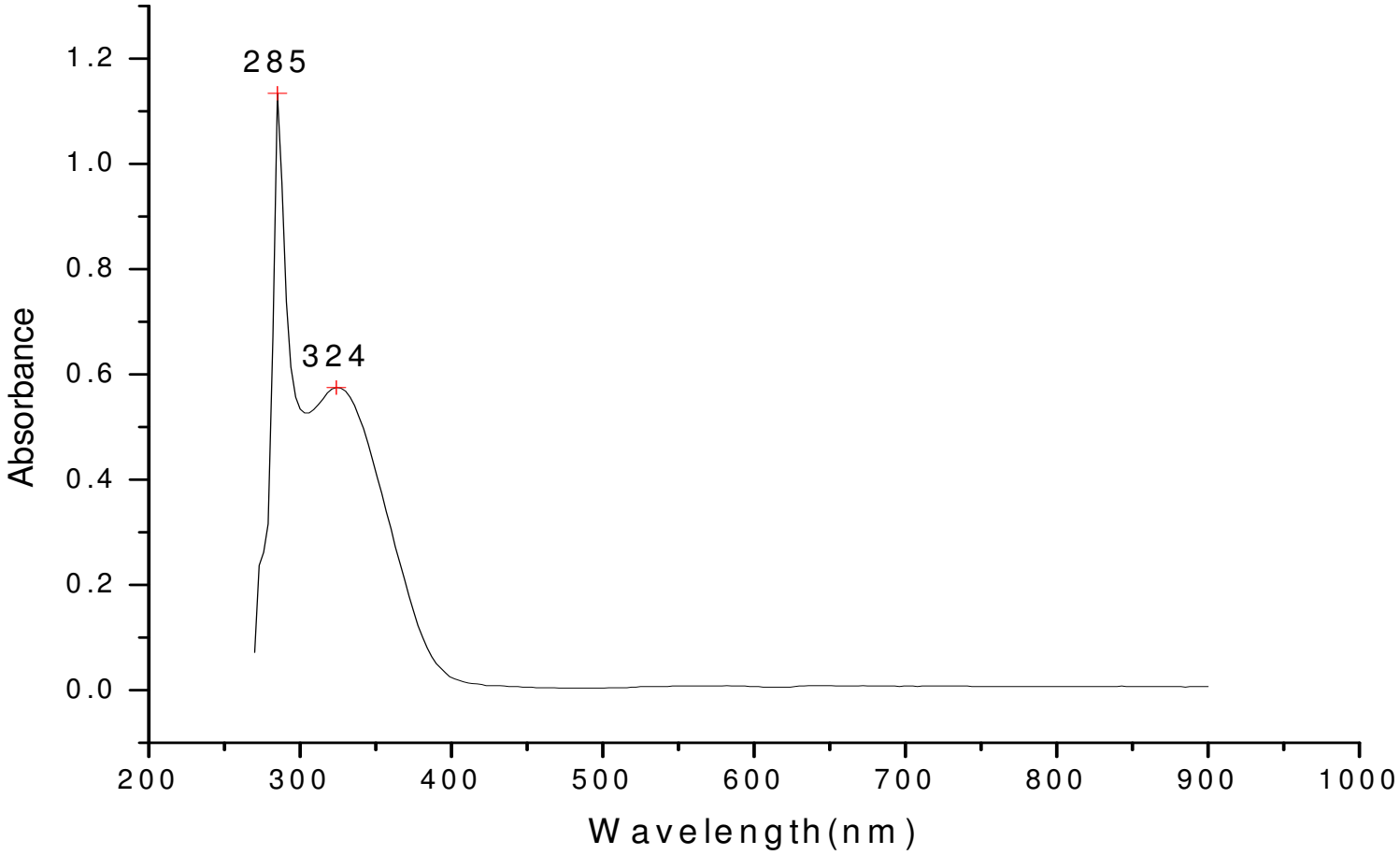
APPENDIX-6: HMBC SPECTRUM of LTE-7



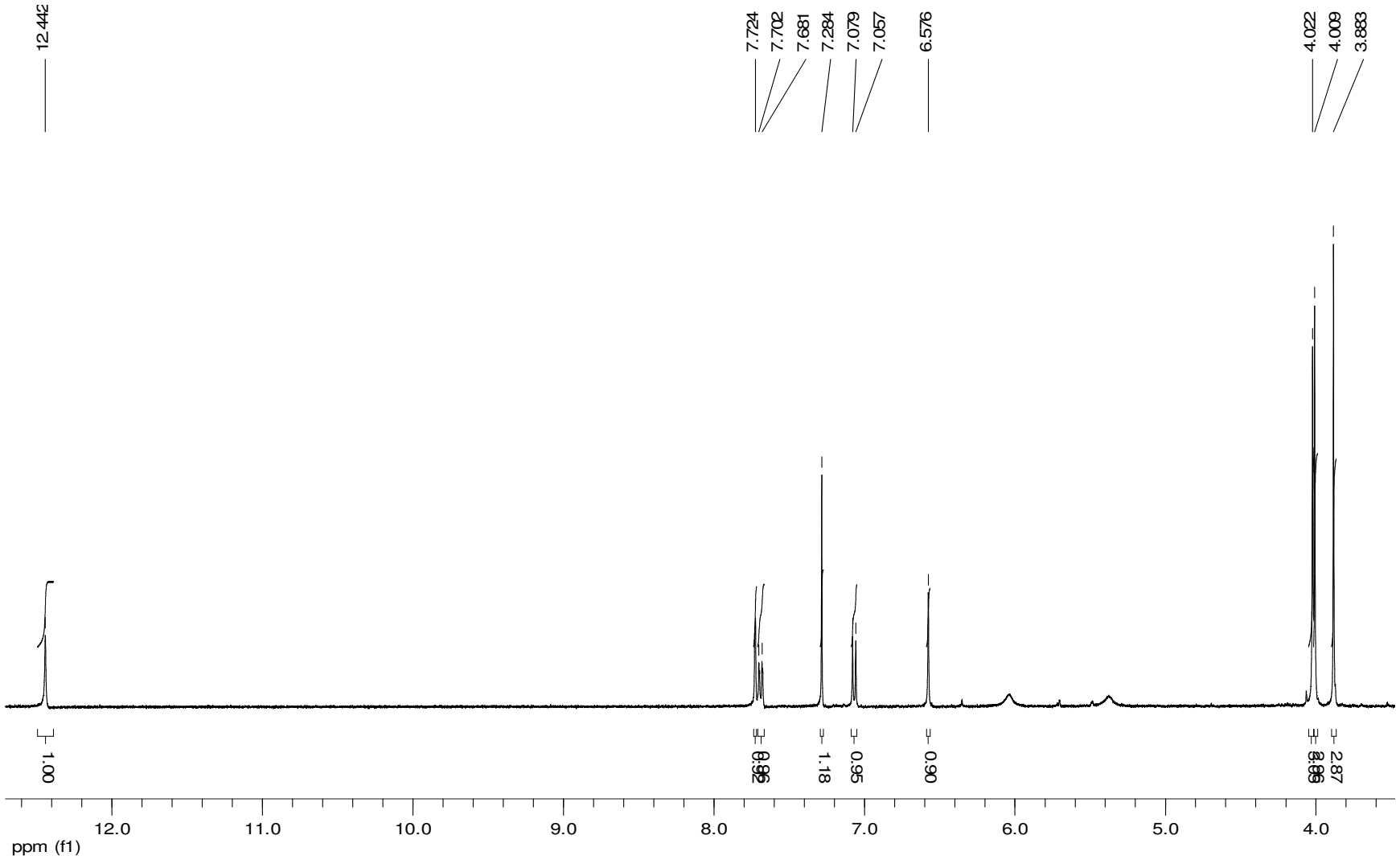
APPENDIX-7: IR SPECTRUM of LTE-7



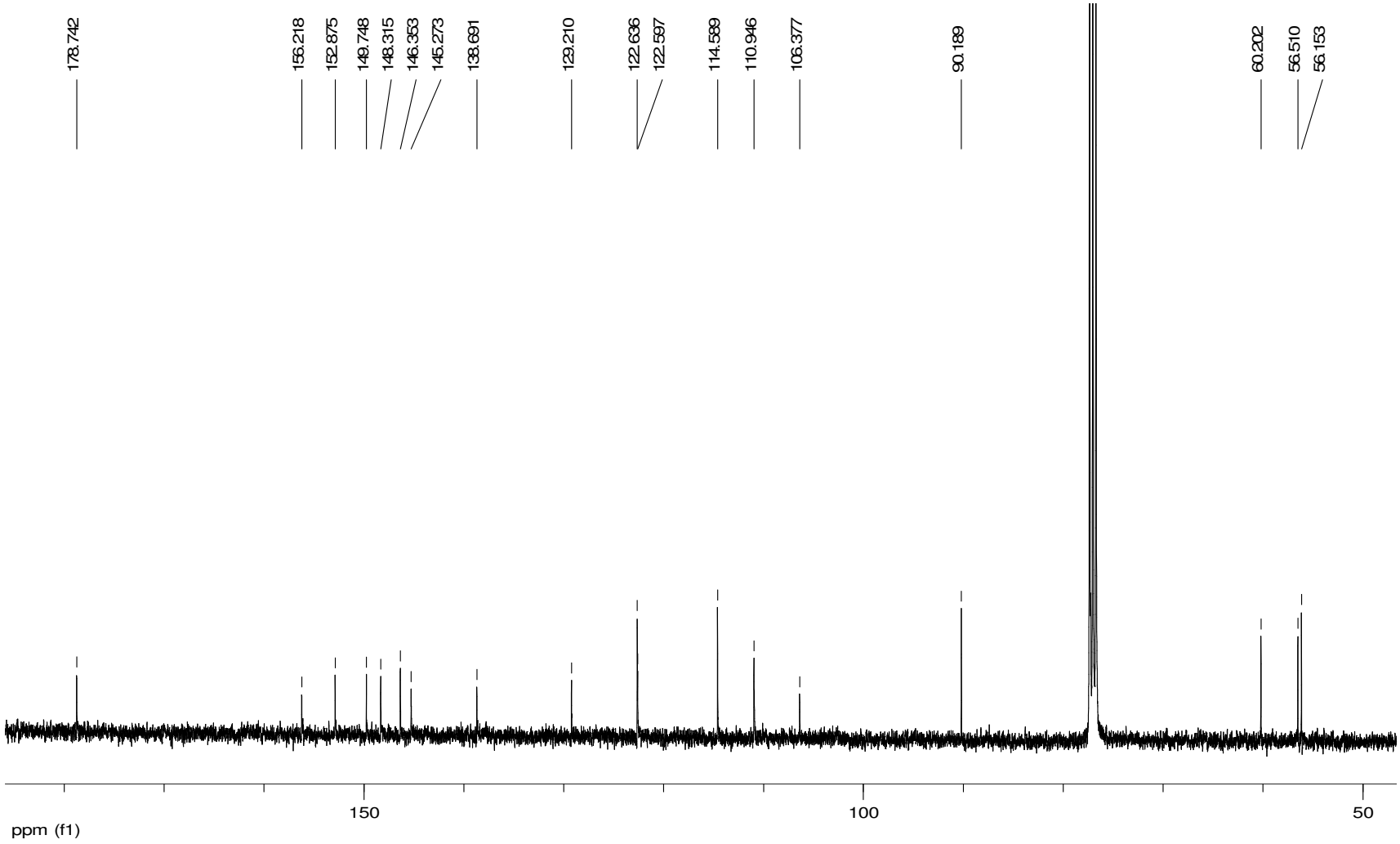
APPENDIX-8: UV SPECTRUM of LTE-7



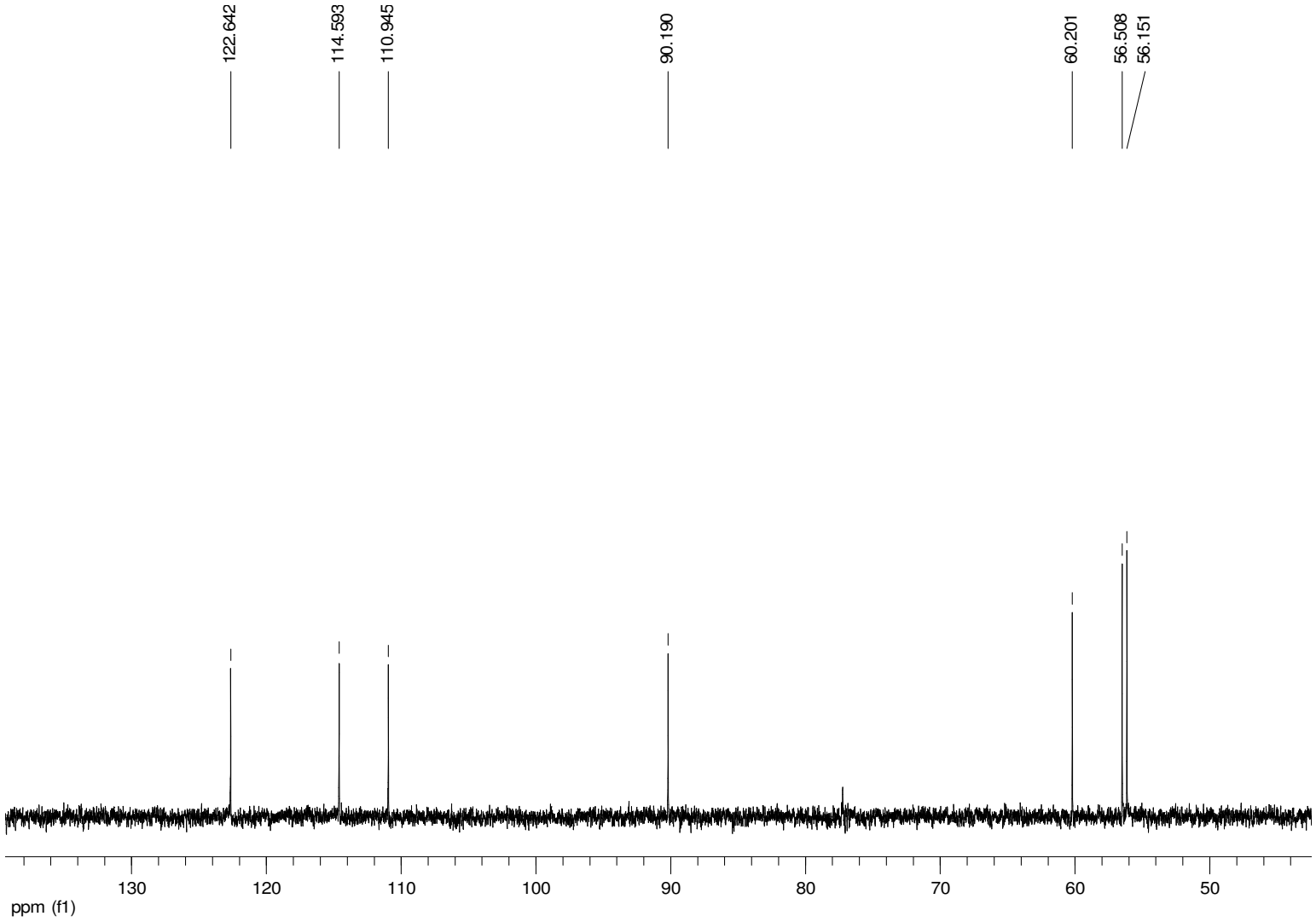
APPENDIX-9: ^{1}H-NMR of LTE-8



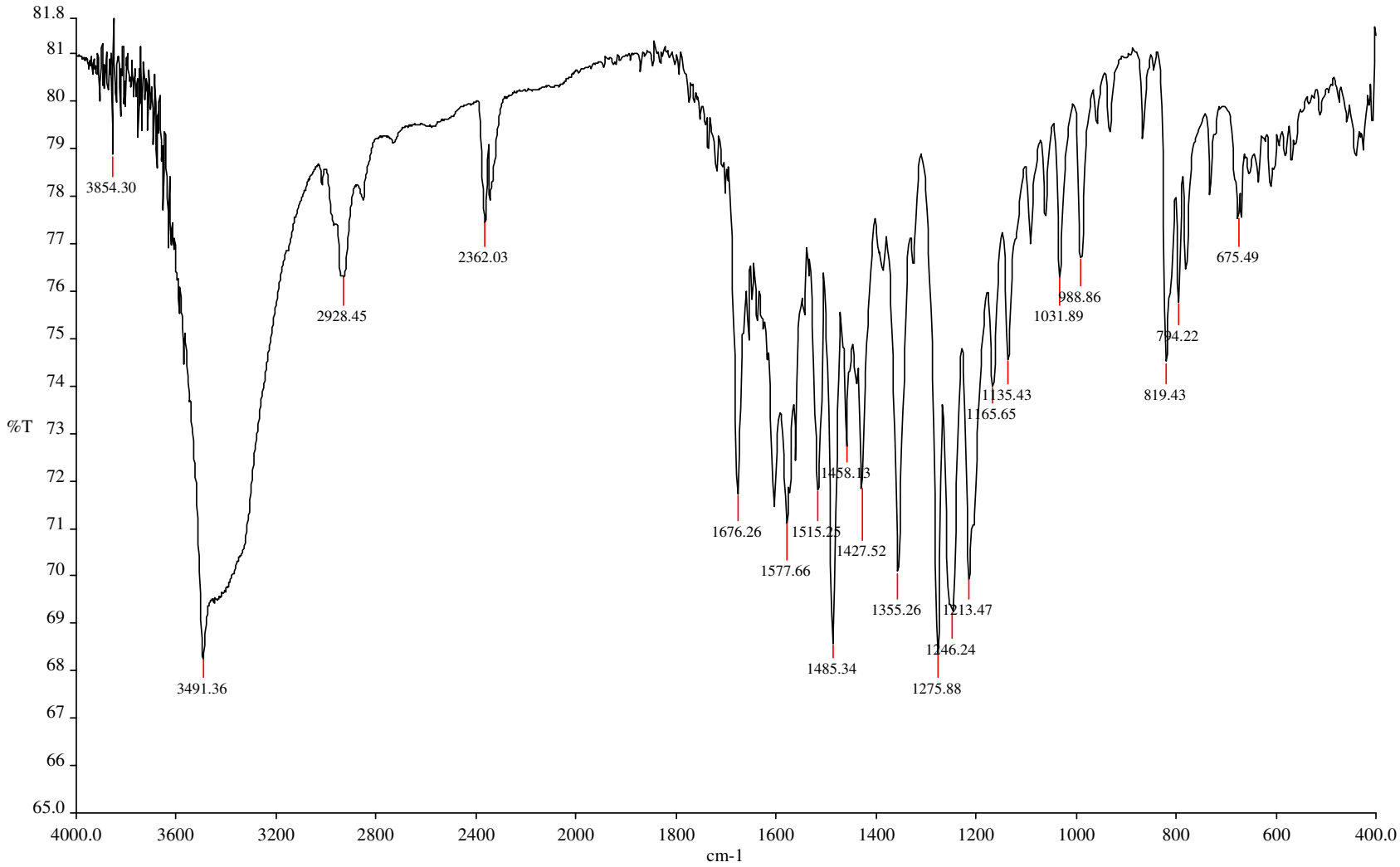
APPENDIX-10: ^{13}C-NMR of LTE-8



APPENDIX-11: DEPT-135 NMR of LTE-8



APPENDIX-12: IR SPECTRUM of LTE-8



APPENDIX-13: UV SPECTRUM of LTE-8