CHEMICAL CONSTITUENTS FROM METHANOL SOAKING OF *Swietenia macrophylla* King.

By

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A project report submitted to the Department of Chemical Science

Faculty of Science

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Bachelor of Science (Hons) Chemistry

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ABSTRACT

CHEMICAL CONSTITUENTS FROM METHANOL SOAKING OF *SWIETENIA MACROPHYLLA*

CHEONG WEI LI

Leaves of *Swietenia macrophylla* were collected in Selangor, Malaysia. Three compounds were isolated from the methanol extract of the plant's leaves. The compounds were isolated by using various chromatographic techniques such as flash column chromatography and centrifugal thin layer chromatography. Structure elucidation and characterization of the isolated compounds were completed by spectroscopic methods. The instruments used included nuclear magnetic resonance spectrometer (NMR), infrared spectrometer (IR), and ultraviolet-visible light spectrophotometer (UV-Vis). Based on the spectral data obtained as well as by comparison with the literature data, two compounds had been identified. Compound A is a mixture of paraffin wax with squalene, compound B is cis-9-octadecenoic acid, methyl ester. Identification of compound C is incomplete due to time constraint.

ABSTRAK

KANDUNGAN KIMIA DARI METANOL RENDAMAN DALAM *SWIETENIA MACROPHYLLA*

CHEONG WEI LI

Daun *Swietenia macrophylla* yang dikaji telah dikumpul di Selangor, Malaysia. Tiga sebatian diperolehi daripada ebstrak metanol daun tersebut. Sebatian-sebatian tersebut diasing dengan menggunakan pelbagai teknik kromatografi seperti kromatografi turus kilat and kromatografi sentrifugal lapisan tipis. Pencirian struktur disempurnakan dengan pelbagai kaedah spektroskopi. Peralatan yang telah digunakan untuk analisis termasuk spectrometer resonans magnetik nuklear (NMR), spectrometer inframerah (IR), spektrofotometer ultraungu-cahaya terlihat (UV-Vis) dan polarimeter. Berdasarkan data-data spectra dan perbandingan dengan data-data kesusasteraan, dua sebatian telah dikenal pasti, sebatian A merupakan campuran sebatian skualen dan rantaian panjang alkan (lilin) manakala sebatian B ialah metil ester asid sis-oktadesenoik. Pencirian dan pengenalan sebatian C tidak dapat dilangsungkan disebab kesuntukan masa.

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Further appreciation goes to my seniors for their help, sharing of information and experience, as well as their support throughout the project. Lastly, I would like to extend my sincere appreciation to my family for helping me in collection of the studied plant.

DECLARATION

I hereby declare that the project report is based on my original work except for the quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

_____

(CHEONG WEI LI)

Date: _____

APPROVAL SHEET

This project report entitled "**CHEMICAL CONSTITUENTS FROM METHANOL SOAKING OF *SWIETENIA MACROPHYLLA* KING**" was prepared by CHEONG WEI LI and submitted as partial fulfillment of the requirements for the degree of Bachelor of Science (Hons) Chemistry at Universiti Tunku Abdul Rahman.

Approved by:

Supervisor,

_____ Date: _____

(Assoc.Prof.Dr.Lim Tuck Meng)

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: _____

PERMISSION SHEET

It is hereby certified that **CHEONG WEI LI** (ID No: **13ADB08245**) has completed this final year project entitled "**CHEMICAL CONSTITUENTS FROM METHANOL SOAKING OF *SWIETENIA MACROPHYLLA* KING**" under the supervision of **DR. LIM TUCK MENG** from the Department of Chemical Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

_____

(CHEONG WEI LI)

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LIST OF ABBREVIATIONS

AR	Analytical Reagent
BHT	Butylated hydroxyl toluene
$CDCl_3$	Deuterium chloroform
$CHCl_3$	Chloroform
COSY	Correlation Spectroscopy
CTLC	Centrifugal Thin Layer Chromatography
DCM	Dichloromethane
DEPT	Distortionless Enhancement by Polarization Transfer
DPPH	2,2-diphenyl-1-picrylhydrazyl
EBV_EA	Epstein-Barr virus early-antigen
FCC	Flash column chromatography
FRIM	Forest Research Institute Malaysia
FT	Fourier transform
GC	Gas chromatography
HMQC	Heteronuclear Multiple Quantum Correlation
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High Performance Liquid Chromatography
IR	Infrared
IC_{50}	50 % inhibitory concentration
J	Coupling constant

m	multiplet
MCC	Microbial Culture Collection
MTCC	Microbial Type Culture Collection
MeHg	Methyl mercury
MS	Mass spectrometer
MTT	[3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide]
NMR	Nuclear Magnetic Resonance
ppm	Parts per million
R_f	Retardation factor
s	singlet
t	triplet
TLC	Thin Layer Chromatography
UV-Vis	Ultraviolet-visible
1D	One dimensional
2D	Two dimensional
λ_{max}	Wavelength of maxima absorption
δ_C	^{13}C NMR chemical shift
δ_H	^{1}H NMR chemical shift

CHAPTER 1

INTRODUCTION

1.1 Natural products

Natural products are defined as the compounds isolated from nature which can be extracted from various natural sources such as plant, animal, marine, and microbes (Baker et al., 2007). The history of the extraction of natural products have long been known since Mesopotamian and Egyptian times, where people produce perfumes or pharmaceutically-active oils and waxes as their business (Bart and Pilz, 2011). Nowadays, natural products were widely utilized as dyes, polymers, fibers, glues, oils, waxes, flavoring agents, perfume and drugs. Besides that, biological properties of various natural products have been recognized thus further research in the possible of natural products to play roles in the search of new drugs, antibiotics, insecticides and herbicides are the focus of scientists currently (Croteau, Kutchan and Lewis, 2000).

Natural products are divided into three categories, which are primary metabolites, high molecular weight polymeric materials, and secondary metabolites (Hanson, 2003). Primary metabolites are the compounds that found in all plants which are essential for reproduction and metabolism of cells. High molecular weight polymeric materials are those compounds that take part in formation of cellular structure. Secondary metabolites refer to compounds that play a role in the interaction of the cell with its surroundings, which are not necessary for a cell to live. However, secondary metabolites usually protect living organisms against environment stresses, both biotic and abiotic conditions. These compounds often differ between individuals from the same population of species in respect of their amounts and types (Pagare et al., 2015).

1.2 Classification of Secondary Metabolites

The classification of secondary metabolites is based on the structure of the compounds. Secondary metabolites can be further classified into terpenes, alkaloids, flavonoids and steroids (Croteau, Kutchan and Lewis, 2000).

1.2.1 Terpenes

Terpenes comprise the biggest group of secondary metabolites. Terpenes are made up of five carbon (isoprene) units and joined in a “head-to-tail” fusion or “head-to-head” fusion. Some products are also formed by joining “head-to-middle fusion (Croteau, Kutchan and Lewis, 2000). The isoprene units and patterns of isoprene unit assembly are shown in Figure 1.1. In addition, terpenes may also undergo oxidation or rearrangement of carbon skeleton to form terpenoids. Terpenoids from plants are usually toxic and feeding deterrents to herbivores, or are attractants, and many possess pharmacological activity.

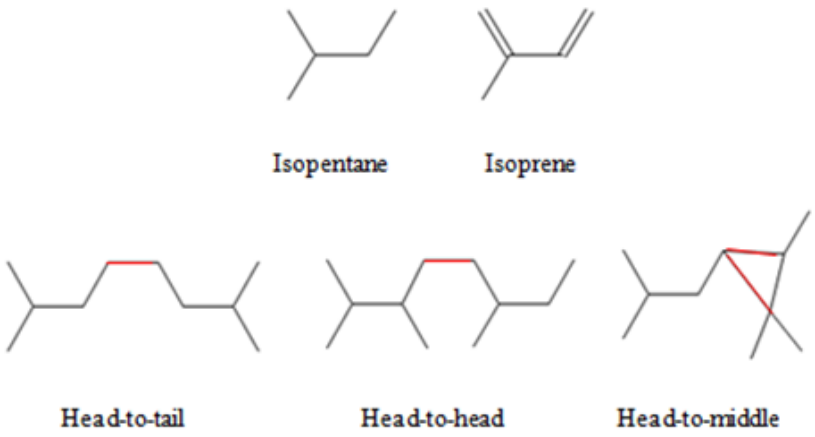
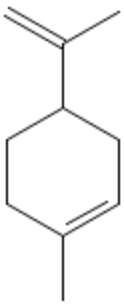


Figure 1.1: Structure of isoprene unit and ways of isoprene units joining up together.

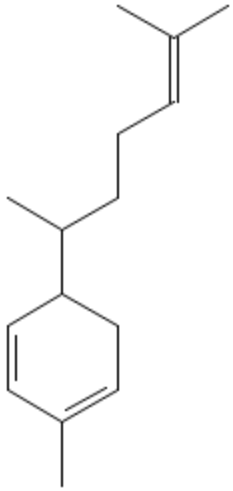
Terpenes can be further divided into groups based on the isoprene units present in the compound. There are hemiterpenes (made up of single isoprene unit), monoterpenes, diterpenes, sequiterpenes, triterpenes, tetraterpenes and polymeric terpenes (Tesso, 2005). Some examples of terpenes are shown in Figure 1.2 and the classification of terpenes according to isoprene unit is shown in Table 1.1.

Table 1.1: Classification of Terpenes (Tesso, 2005).

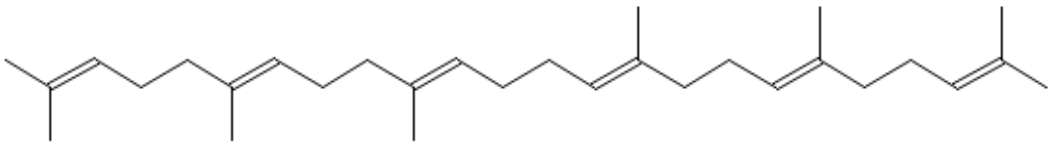
Types of Terpenes	**Number of Carbon**	**Number of Isoprene Unit**
Hemiterpenes	5	1
Monoterpenes	10	2
Sesquiterpenes	15	3
Diterpenes	20	4
Triterpenes	30	6
Tetraterpenes	40	8
Polymeric terpenes	>40	>8



[1] Limonene (monoterpene)



[2] Zingiberene (sesquiterpene)

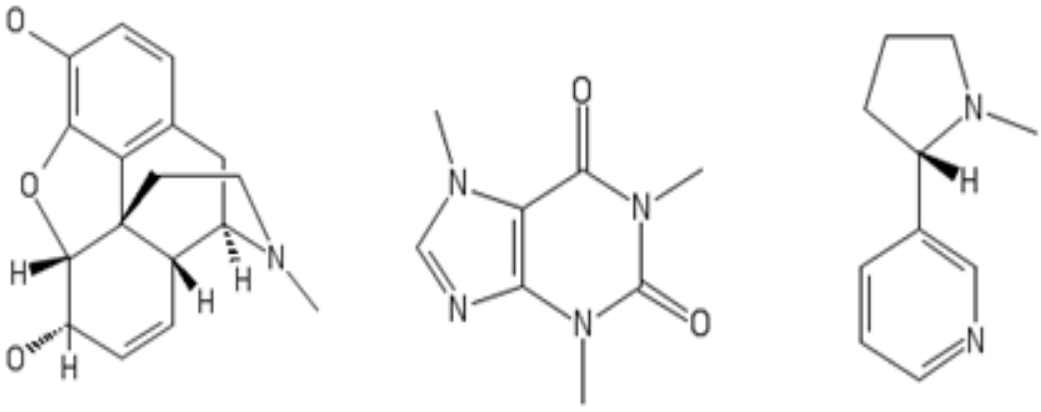


[3] Squalene (triterpene)

Figure 1.2: Some examples of terpenes.

1.2.2 Alkaloids

Alkaloids are defined as the low molecular weight compounds that contain at least one basic nitrogen atom and mostly derived from amino acids. Alkaloids are secondary metabolites that have basic properties due to the presence of nitrogen atom, which having a lone pair electrons (Zulak et al., 2006). Therefore, alkaloids usually have bitter taste, and some of them act as poisonous agents to protect plants from insects and herbivores. Some examples of alkaloids are shown in Figure 1.3.



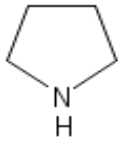
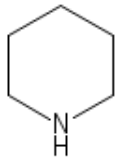
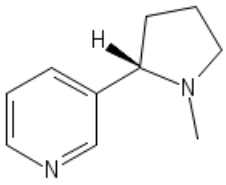
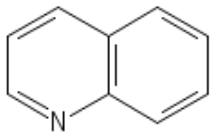
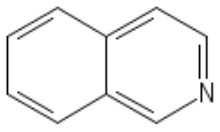
[4] Morphine **[5]** Caffeine **[6]** Nicotine

Figure 1.3: Some examples of alkaloids.

Classification of alkaloids is complex due to alkaloids the variety of molecular structure. There are several methods for classifying alkaloids. The first method is based on biosynthetic classification, where alkaloids are classified according to the same precursor where alkaloids are biosynthetically produced from. The second method is by taxonomic classification, which particularly classified the alkaloids according to their distribution in a variety of plant families, for instance: Cannabinaceous alkaloids, Rubiaceous alkaloids. The last method of classifying is according to the heterocyclic ring system of alkaloids, which commonly known as chemical classification. This method is the most widely accepted and common mode of the classification (Saxena et al., 2013).

To discuss further on chemical classification, alkaloids are classified into a few groups, which contain different heterocyclic ring system. Table 1.2 shows the classification of alkaloids.

Table 1.2: Classification of Alkaloids and Examples

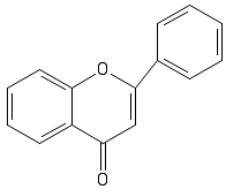
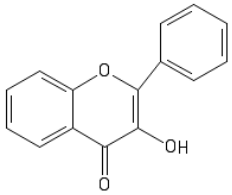
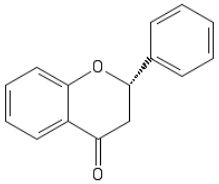
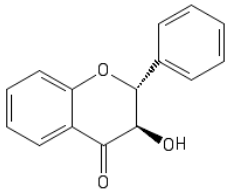
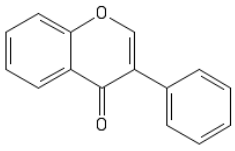
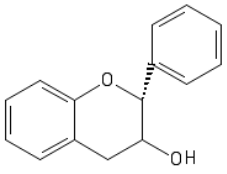
Types of Alkaloids	Ring Systems	Examples
Pyrrolidine Alkaloids	 Pyrrolidine (Tetrahydropyrrole) ring	Hydrine
Pyridine Alkaloids	 Piperidine (Hexahydropyridine) Ring	Coniine
Pyrrolidine-pyridine Alkaloids	 Pyrrolidine and piperidine rings	Nicotine
Quinoline Alkaloids	 Quinoline ring	Quinine
Isoquinoline Alkaloids	 Isoquinoline ring	Heroine

1.2.3 Flavonoids

Flavonoids are polyphenolic compounds that are widespread in nature. They are also the major coloring component of flowering plants. In plants, they act as antioxidants, antimicrobials, feeding repellants and visual attractors (Pietta, 2000). In human diet, more than 4,000 flavonoids have been recognized, and they are found mostly concentrated in fruits, vegetables and wines (Saxena et al., 2013).

Flavonoids are generally made up of basic structure with skeleton of diphenylpropane, which consists of two benzene rings, linked by three carbon chain that forms a closed pyran ring. Their structures are also referred as C6-C3-C6 (Kumar and Pandey, 2013). Flavonoids can be further divided into a variety of classes such as flavone, flavonols and flavonones and others. The differences in these classes depend on the level of oxidation and pattern of substitution of the carbon ring. General structures of each class are shown in Table 1.3.

Table 1.3: Classification of Flavonoids.

Class	Backbone Structure	Examples
Flavones		Luteolin, Apigenin
Flavonols		Quercetin, Kaempferol
Flavonones		Hesperetin, Naringenin
Flavanonol		Taxifolin
Isoflavones		Genistein, Daidzein
Flavan-3-ols		Catechin, Epicatechin

1.2.4 Steroids

Steroids are widely distributed in animals and plants, which the basic structure is made up of seventeen carbon atoms, based on the 1,2- cyclopentenophenanthrene skeleton as shown in Figure 1.4 (Schaller, 2003). Biosynthetically, steroids are derived from S-squalene-2,3-epoxide via acetate mevalonate pathway (Gunaherath and Gunatilaka, 2014).

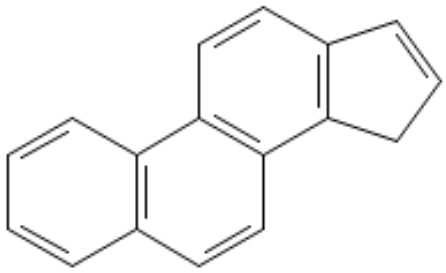
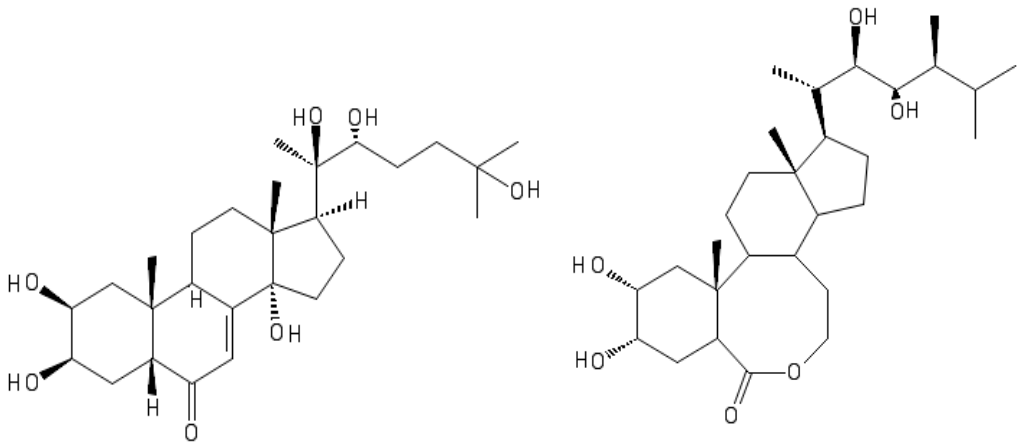


Figure 1.4: 1,2- cyclopentenophenanthrene

In human diet, plant steroids are found widely in most type of the food, such as spinach and suma root that consist of high concentration of β-ecdysterone. Steroid such as brassinosteroids found in small group of plants also have been reported to exhibit an essential role to promote the growth of plant (Gunaherath and Gunatilaka, 2014). Figure 1.5 shows the structures of β-ecdysterone and brassinosteroids.



[7] **[8]**

Figure 1.5: **[7]** β-ecdysterone and **[8]** brassinosteroids

1.3 Medicinal Plants

Medicinal plants are defined as the plants that contain therapeutic properties. Plants constitute one of the major raw materials of drugs, which have been used in treating various human diseases over thousands of years (Paritala et al., 2015). In ancient time, plants were used in foodstuffs with curative properties that help mankind to sustain its health, as well as applied as herbal remedies to treat certain diseases (Moghadamtousi et al., 2013).

In modern society, scientists are interested on the utilization of plants as drugs due to their harmonious nature with human biological system. For instance, plant-based antimicrobials are effective in treating infectious diseases while simultaneously reduce the side effects that are often associated with synthetic antimicrobials (Paritala et al., 2015). Therefore, scientific research had been conducted on medicinal plants, relies on identification of the bioactive components in plants, which leads to the development of new drugs or pharmaceutical products.

Despite of the development of drugs from medicinal plant, continuously research and investigation of biological activities of medicinal plants have also been conducted in order to find out the other possible applications of medicinal plants. In recent days, extensive research have been conducted to search for the potential plants which can be used in the management of agriculture and household pests (Paritala et al., 2015).

1.4 Plant of Interest

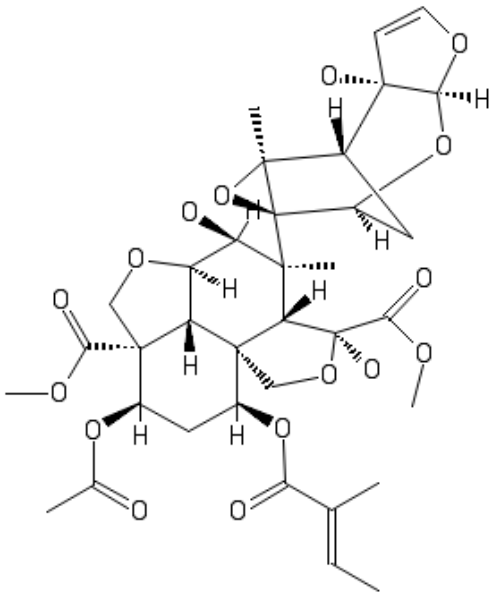
1.4.1 Family: Meliaceae

Meliaceae, or known as Mahogany family which shows Sapindales as order. It is a flowering plant family that consists of trees and shrubs, where the plants range in size from magnificent forest trees to small shrubs. The family Meliaceae consists of 575 species in 50 genera of trees and shrubs, which are mostly found in tropical to subtropical region (Yadav et al., 2015).

Economically, Meliaceae is crucial for its high quality timber. Trees of the genera *Swietenia, Entandrophragma, and Cedrela* in this family are regarded as "genuine mahogany", well-known as the source of mahogany wood. The mahogany wood is a glossy pink-red colour, with medium specific gravity and good wood machining; hence it is widely used in fine furniture, civil constructions, boats, ships, cabinet making and others purposes (Ribeiro da Silva et al., 2013).

Chemically, the family Meliaceae is well known due to the occurrence of limonoids. Limonoids are derived from the tetracyclic triterpenes, or known as tetranotriterpenoids. Limonoids are abundant in Meliaceae family and possess a

range of biological activities such as anti-feedant, toxic or growth-reducing properties to different species of insects. For instance, azadirachtin (as shown in Figure 1.6) is a well-known limonoid for its anti-feedant properties towards 200 species of insects (Roy and Saraf, 2006). Despite of limonoids, other compounds such as mono-, di-, sesqui-, and triterpenoids, coumarins, chromones, lignans, flavonoids and other phenolics compounds have also been isolated in Meliaceae. Limonoids and terpenoids are isolated as the major chemical constituents in Meliaceae plants. The major biological activities shown by most of the species are cytotoxic activity, antimicrobial activity, anti-feedant and anti-malarial activity (Yadav et al., 2015).



[9]

Figure 1.6: Structure of azadirachtin (limonoid)

1.4.2 Genus: *Swietenia*

Swietenia is only found in the neotropics region. It consists of three species, which are *Swietenia mahagoni* Jacq., *Swietnia humilis* Zucc. and *Swietenia macrophylla* King. Two natural hybrids are also found in some regions where the two species overlap, which are the cross between *Swietenia macrophylla* and *Swietenia humilis* and cross between *Swietenia macrophylla* and *Swietenia mahagoni* (Preciado et al., 2016).

In Malaysia, *Swietenia macrophylla* King is the most common found species. It is one of the earliest exotic timber trees brought in by Forest Research Institute Malaysia (FRIM) along with other trees such as *Tectona grandis* and *Gmelina arborea.* The trees can be easily found along the roadside, for shading purpose (Forest Research Institute Malaysia, 2014).

1.4.3 *Swietenia macrophylla* King

1.4.3.1 Botany Name and Common Names

Botany name of the plant is *Swietenia macrophylla* King., commonly known as cheria mahogany, “pokok tunjuk langit” or “sky fruit” in Malaysia due to the upward trend of its fruits towards the sky. The plant commonly known as big-leaved mahogany in England, Mahoni in Indonesia, Echtes mahagoni in Germany and “大叶桃花心木” in China (Moghadamtousi et al., 2013).

1.4.3.2 Distribution

Swietenia macrophylla King is found mainly in tropical region, which inhabits both wet and dry forests. It grows natively throughout the tropical regions of America, naturally distributed from southern Mexico, through Central America, to the northern South America. It is also found in west India, Malaysia and Southern China (Lowe et al., 2003).

1.4.3.3 Morphology

Tree of *Macrophylla* is in medium sized to large tree taller than 30 m (Figure 1.7). The bark of the tree is dark reddish brown, and the leaves are up to 35 to 50 cm, with 4 to 6 pairs of leaflets. The flowers are small yellow-cream colored panicles (Masoud Eid, Elmarzugi and El-Enshasy, 2013). The flowers are unisexual, with staminate and pistilate flowers which are insect-pollinated, and have a functional self-incompatibility system.

The fruit is woody, light brown color with usually 5-lobed capsule. Fruit is commonly known as sky fruit as it usually point upward towards the sky (Figure 1.7). The fruits split open from the apex or the base when they are ripe and dry. Seeds are hanging from the columella by their wing, usually with 35 to 45 seeds per fruit. The seeds are brown in colour, oblong, compressed, crested and extended into a wing at attachment end, which has 7.5 to 15 cm long including the wing, with extensive air spaces. The seeds are dispersed by wind. Flowering and fruiting is distinctly seasonal for this species (Lowe et al., 2003). Figures 1.8 illustrates the flowers, fruits, seeds and leaves of *Swietenia macrophylla*.

Figure 1.7: *Swietenia macrophylla* tree and fruit at Penang, Malaysia.

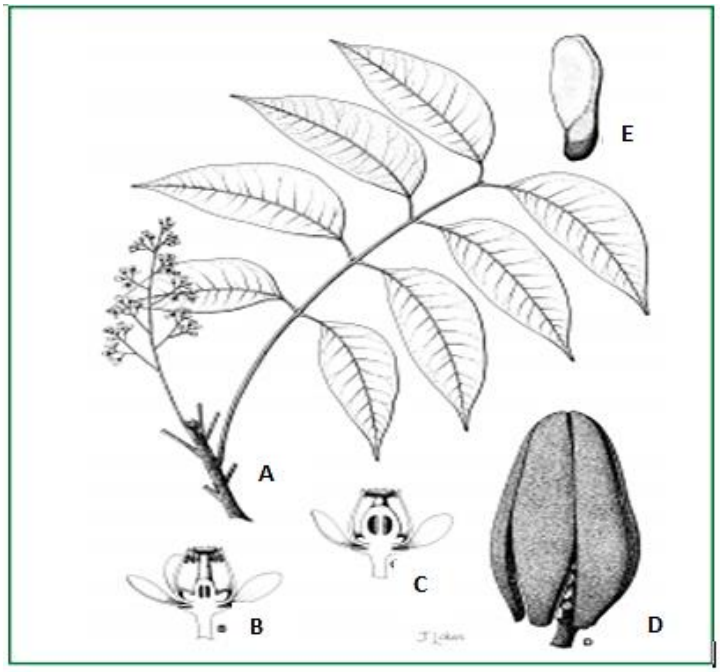


Figure 1.8: Flowers, fruits, seeds and leaves of Swietenia *macrophylla*.

A, the flowering branchlet; **B**, male flower and **C**, female flower; **D**, fruit; **E**, seed.

1.4.3.4 Taxonomic Classification

Table 1.4: Taxonomy classification of *Swietenia macrophylla* King. (IUCN Red List, 2015)

Kingdom	**Plantae**
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Sapindales
Family	Meliaceae
Genus	*Swietenia*
Scientific name	*Swietenia macrophylla*
Species authority	King

1.4.4 Traditional Uses of Plant

The ethnomedical uses of *Swietenia macrophylla* had been reported in various countries, mostly distributed in Asia region. Seed of this plant is the most common part used by people for treatment of various human ailments. In Malaysia, the seed is used traditionally by the natives and common folks to treat hypertension and diabetes. The seed is taken by direct chewing and swallowing (Moghadamtousi et al., 2013). In India, East Midnapore and West-Bengal, the seed is used traditionally by the natives to cure diarrhea (Maiti, Dewanjee and Mandal, 2007a). Furthermore, the natives also utilize the seed for curing of skin diseases and infections caused by wounds (Maiti, Dewanjee and Mandal, 2007b).

In Indonesia, the seed extract has also been used to treat malaria. The crushed seeds mixed with *Attalea phalerata* seed oil can be used to treat the skin problem such as allergy and to heal wound. It is also reported that the seeds of the plant also use as an abortion medicine in the ethnic group of Bolivian Amazonian (Moghadamtousi et al., 2013). Meanwhile, the bark of the plant has been used to treat diarrhea and fevers in Mexico. It is also used as an astringent for wound (Moghadamtousi et al., 2013).

1.5 Objectives

This research has the following objectives:

1. To extract, isolate and purify the compounds from *Swietenia macrophylla* King by chromatographic techniques.

2. To characterize the pure compounds by spectroscopic methods.

3. To elucidate the structure of isolated compounds based on the spectral data.

CHAPTER 2

LITERATURE REVIEW

2.1 Chemical Constituents of *Swietenia macrophylla*

Swietenia macrophylla has been well known for its wood quality in early years. It is economically important in the timber industry which its wood is widely used in furniture, musical instruments and civil constructions. Despite of its economic uses, people from different countries have made use of *Swietenia macrophylla* as traditional medicine to treat various diseases. Therefore, scientific inspection on the bioactive substances contain in the plant as well as evaluation on the biological activities shown in Swietenia *macrophylla* have been conducted by researchers. Even though a lot of pure compounds have been isolated from *Swietenia macrophylla* as well as their biological activities have been proven successfully, yet there is still a continuously research carried on *Swietenia macrophylla* in order to prospect for new compounds and potential biological activities of the plant.

In the earlier year, phytochemical investigations on *Swietenia macrophylla* were primarily focused on the seeds. In 1951, Chakraborty and Guha-Sircar had successfully isolated two compounds from the seeds, and in 1951, they identified them as mexicanolide-type limonoids and named as swietenolide **[10]** and swietenine **[11]**. In 1976, another limonoid also had been isolated from the seeds by Chan et al. and named as swietenolide diacetate **[12]**. In 1983, another mexicanolide-type limonoid, named 8,30-epoxyswietenine acetate **[13]** was isolated from the seeds by Taylor and Taylor using HPLC technique. The structure of compounds [10] – [13] are shown in Figure 2.1.

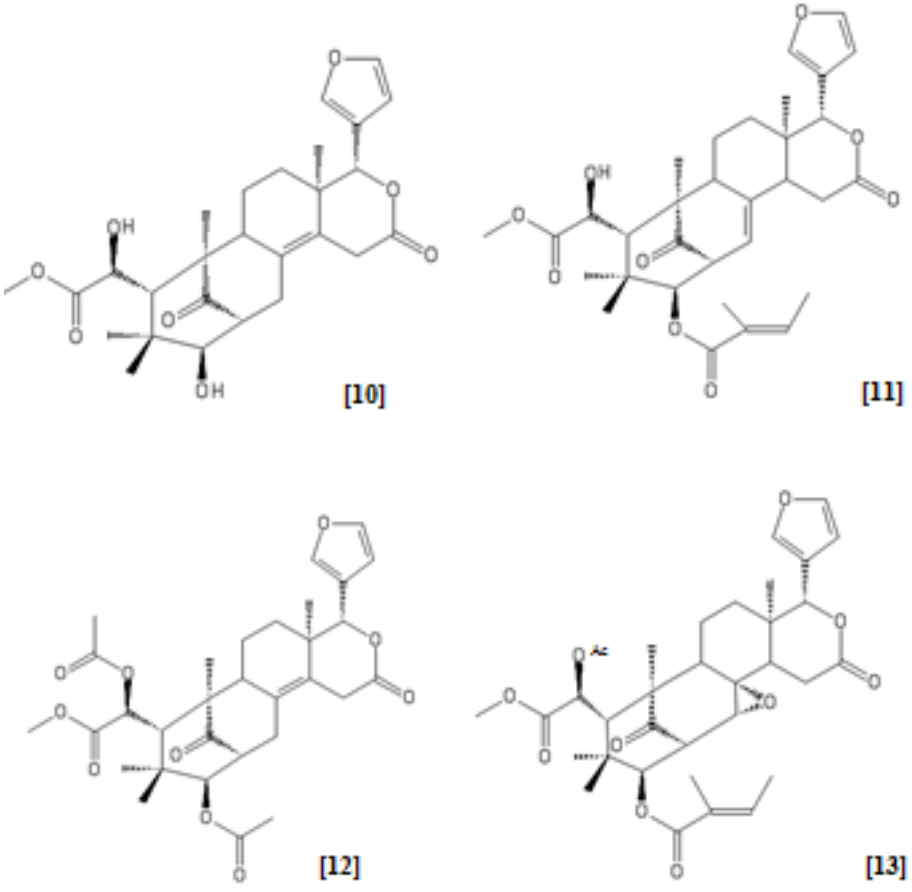
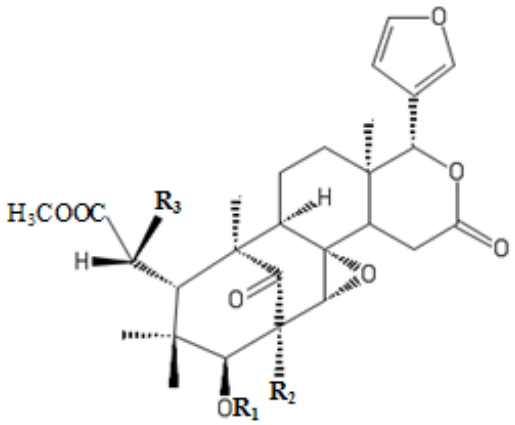


Figure 2.1: Structures **[10]** to **[13]** isolated from the seeds of *Swietenia macrophylla.*

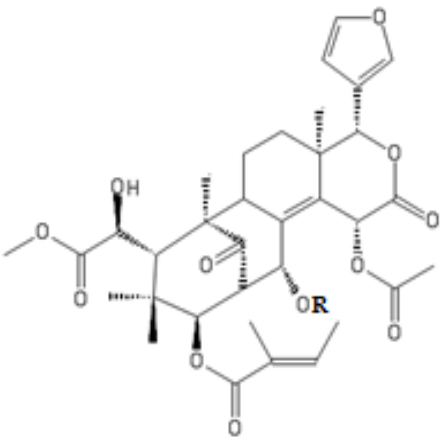
A subsequent study by Kojima, Isaka and Ogihara (1998) had successfully isolated out five new limonoids, named methyl 3β-tigloxy-2-hydroxy-1-oxo-meliac-8(30)-enate **[14]**, methyl 3β-tigloxy-2,6-dihydroxy-1-oxo-meliac-8(30)-enate **[15]**, methyl 3β-tigloxy-2-hydroxy-8α,30α-epoxy-1-oxo-meliacate **[16]**, methyl 3β-isobutyryloxy-2,6-dihydroxy-8α,30α-epoxy-1-oxo-meliacate **[17]** and methyl 3β-acetoxy-2,6-dihydroxy-8α,30α-epoxy-1-oxo-meliacate **[18]**. Figure 2.2 shows the limonoid structures mentioned above.



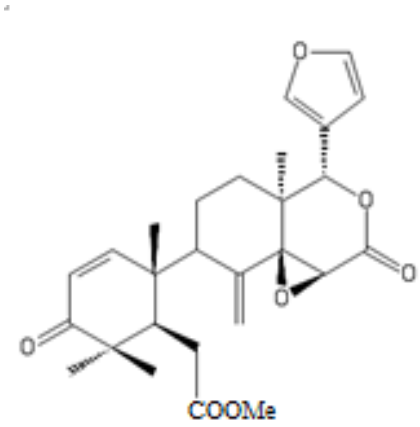
	R_1	R_2	R_3
[14]	tigloyl	OH	H
[15]	tigloyl	OH	OH
[16]	Ac	OH	OH
[17]	tigloyl	OH	H
[18]	Isobutyryl	OH	OH

Figure 2.2: Structures isolated from seeds of *Swietenia macrophylla* by Kojima et al. (1998).

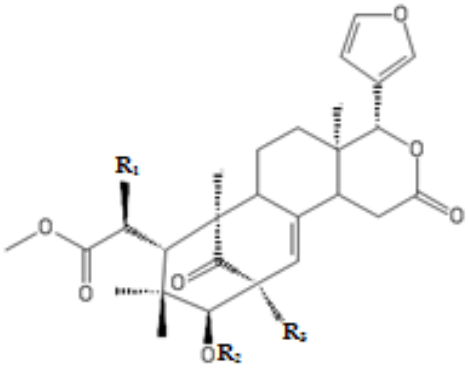
In 1999, Mootoo et al. had managed to isolate out several limonoids from the seeds, including augustineolide **[19]**, andirobin **[20]**, proceranolide **[21]**, 3β,6-dihydroxydihydrocarapin **[22]**, 7-deacetoxy-7-oxogedunin **[23]**, 6-*O*-acetyl-swietenolide **[24]**, 3β,14-dihydroxymexicanolide **[25]**, 3-*O*-tigloyl-swietenolide **[26]**, khayasin T **[27]**, swietemahonin E **[28]**, swietemahonin G **[29]** and 6-deoxyswietenine **[30]**. Structures of these isolated limonoids are shown in Figure 2.3.



[19] R = $COCH(CH_3)_2$



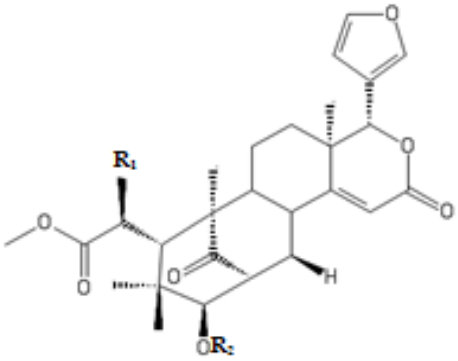
[20]



[21] R_1 = H , R_2 = $COCH(CH_3)CH_2CH_3$

[24] R_1 = OAc , R_2 = H

[30] R_1 = H , R_2 = $COC(CH_3)CH(CH_3)$



[22] R_1 = OH , R_2 = H

[27] R_1 = H , R_2 = $COC(CH_3)CH(CH_3)$

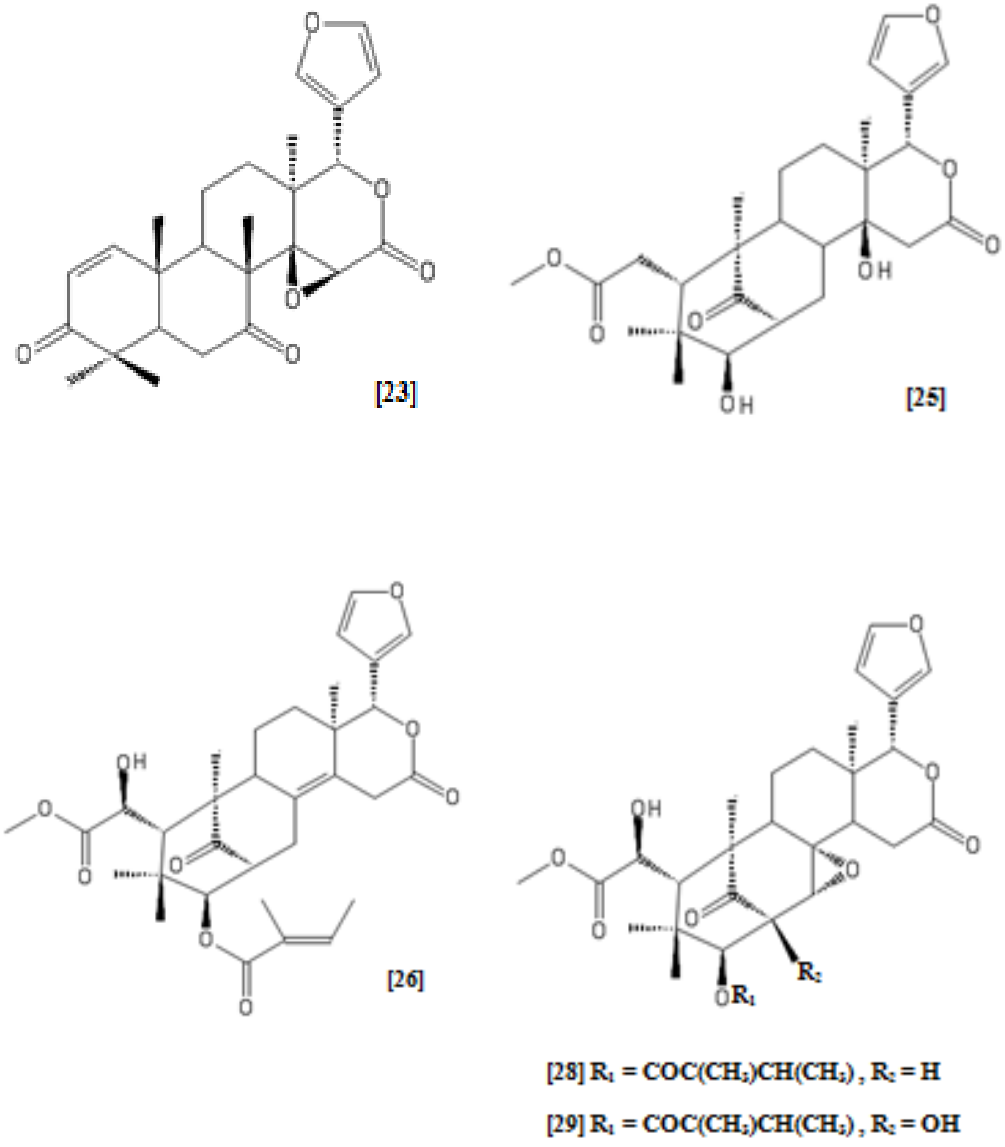


Figure 2.3: Structures isolated by Mootoo et al. (1999) from seeds of *Swietenia macrophylla.*

In the recent research, Chen et al. (2015) had successfully isolated another new compound from the seeds of *Swietenia macrophylla*, named Swietemacrophin **[31]**, together with five known compounds, humilinolide F **[32]**, swietemahonin E **[33]**, 3,6-*O*,*O*-diacetylswietenolide **[34]**, 3-*O*-tigloyl-swietenolide and swietenine. Figure 2.4 shows the structures of compounds mentioned above.

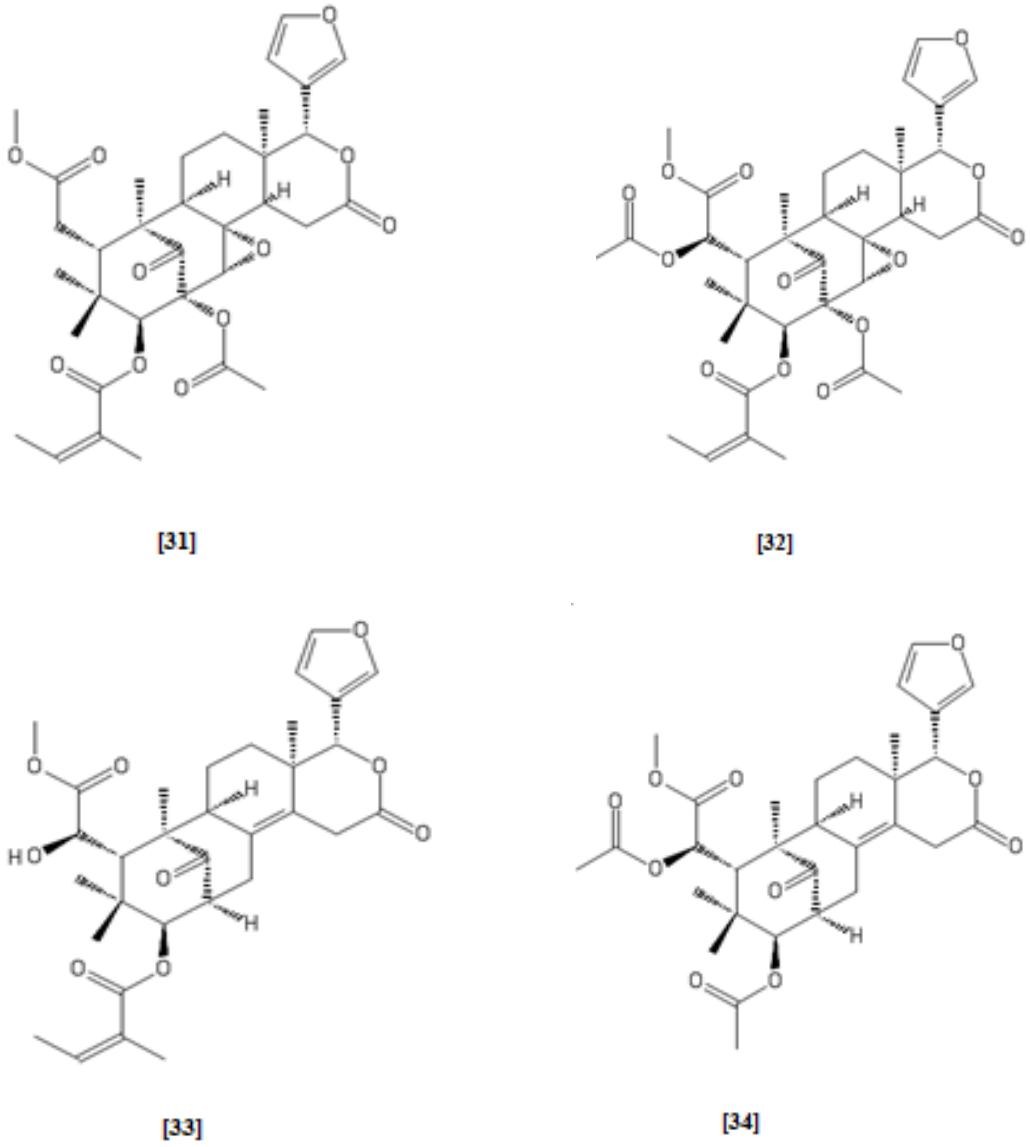


Figure 2.4: Structures isolated from seeds of *Swietenia macrophylla.*

Apart from the seeds, the other parts of *Swietenia macrophylla* have also been studied in recent year. In 2003, Marisi et al. had isolated essential oils from the matured leaves of *Swietenia macrophylla* named himachalene **[35]**, germacrene-D **[36]**, germacrene-A **[37]**, cadina-1,4-diene **[38]**, hexadecanoic acid **[39]** and ethyl hexadecanoate **[40]**. Figure 2.5 shows the structure of essential oils.

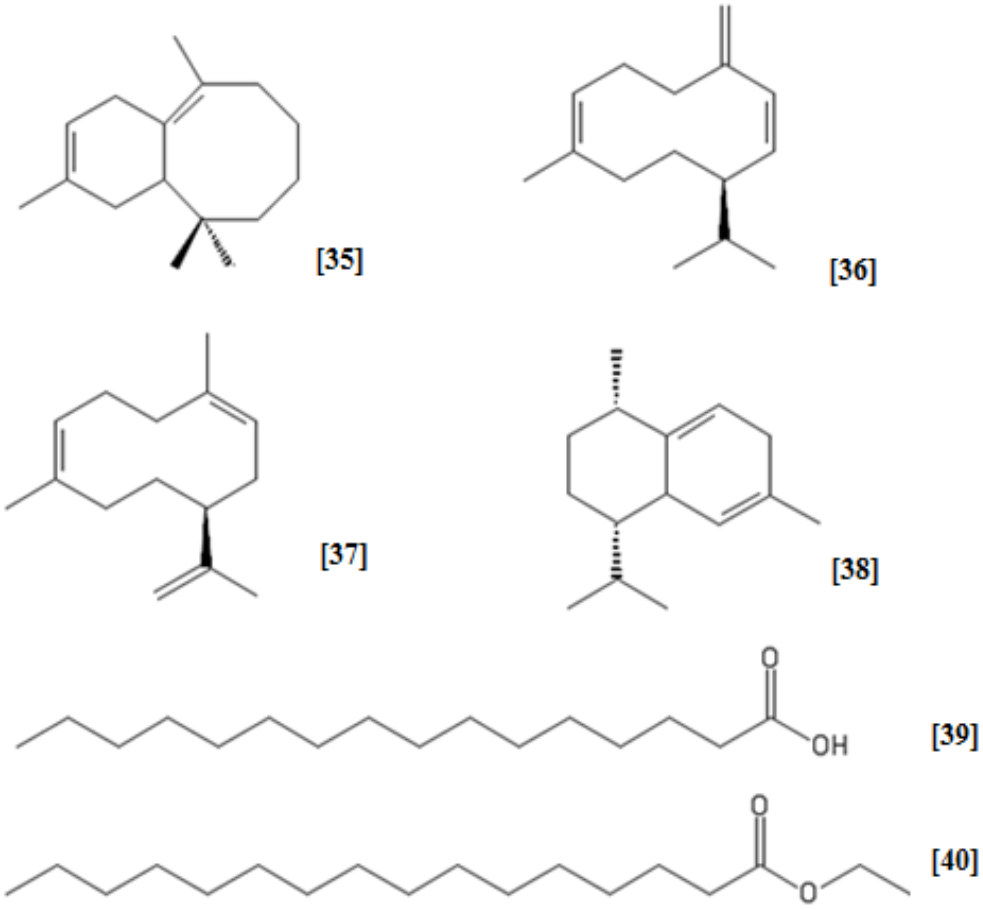
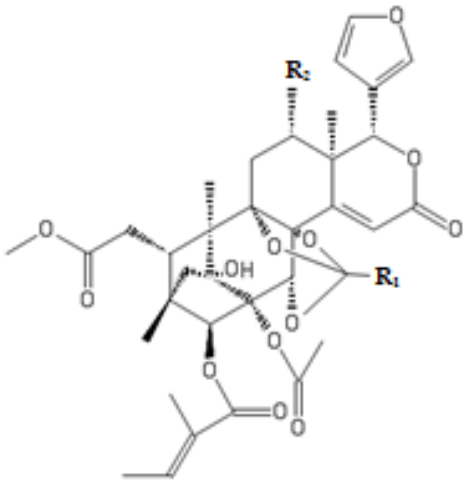


Figure 2.5: Structures of essential oils isolated from leaves of *Swietenia macrophylla.*

Aside from essential oils, Tan et al. (2009a) also reported that new phragmalin-type limonoids named swietephragmin H **[41]**, swietephragmin I **[42]** and swietephragmin J **[43]** as well as a new polyhydroxylated phragmalin, named swietemacrophine **[44]** had been isolated out from the dichloromethane extracts of the leaves of *Swietenia macrophylla*. In recent study, Liu et al. (2012) had reported a new phragmalin-type limonoids name swietenine J **[45]** from methanolic extract of the leaves of *Swietenia macrophylla* collected from Guangzhou, China. Structures of these compounds are shown in Figure 2.6.



[41] $R_1 = CH_2CH_3$, $R_2 = H$

[42] $R_1 = CH_3$, $R_2 = H$

[43] $R_1 = CH_2CH_3$, $R_2 = OH$

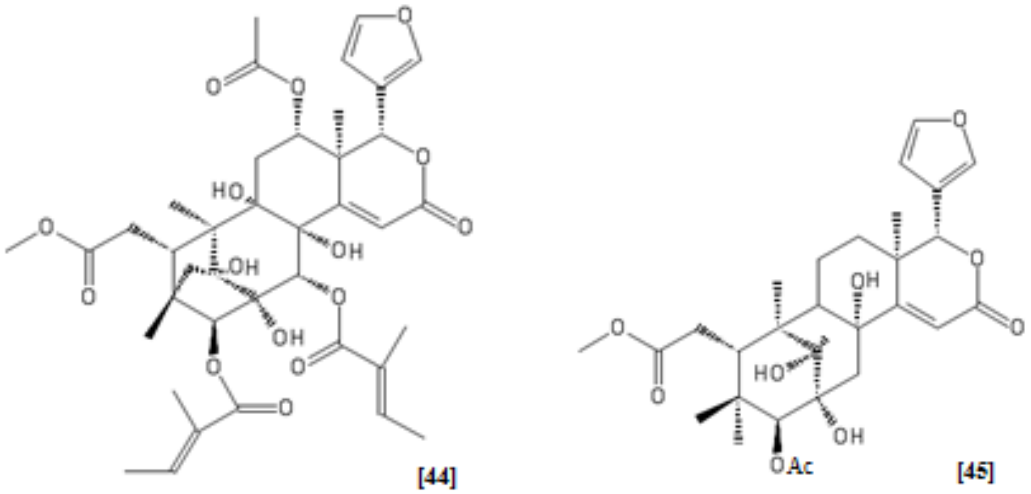


Figure 2.6: Limonoids isolated from leaves of *Swietenia macrophylla*.

In addition, a new phenylpropanoid-substituted catechin, named swietemacrophyllanin **[46]** had been isolated from the bark of *Swietenia macrophylla* by Falah, Suzuki and Katayama (2008). Figure 2.7 shows the structure of swietemacrophyllanin.

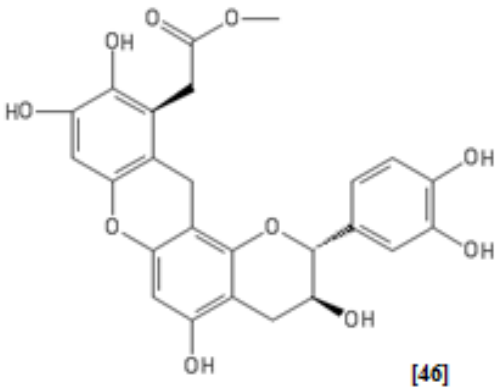


Figure 2.7: Swietemacrophyllanin **[46]** isolated from bark of *Swietenia macrophylla*.

2.2 Biological Activities

2.2.1 Antioxidant Activity

Falah, Suzuki and Katayama (2008) had reported the findings of a new compound, swietemacrophyllanin together with two known compounds catechin and epicatechin from the bark of *Swietenia macrophylla*. The antioxidant activities of these compounds were evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay, and they found that swietemacrophyllanin had the strongest antioxidant activitivitty with an IC_{50} value of 56 μg/mL compared with Trolox (standard reference).

Besides, the leaves of plant also show strong antioxidant property. In 2009a, Tan et al. studied the antioxidant activities of methanol, hexane and dichloromethane of leaf extracts by using DPPH free radical scavenging assay. Among these extracts, methanol extract had the strongest radical scavenger (IC_{50} = 7.67 μg/mL), which was higher than catechin (IC_{50} = 7.83 μg/mL) and was comparable to BHT.

2.2.2 Antimicrobial Activity

Maiti et al. (2007) had tested the antimicrobial efficacy of methanol and aqueous extract of *Swietenia macrophylla* seed against selected pathogenic bacterial and fungal strains by using disc diffusion and micro dilution assay methods. The selected bacterial strains were *Pseudomonas aeruginosa* MTCC 424, *Bacillus cereus* MTCC 430, *Klebsiella penumoniae* MTCC 109, *Staphylococcus aureus* MTCC 96, *Escherichia coli* MTCC 443, *Salmonella typhimurium* MTCC 98, *Micrococcus luteus* MCC 106, with streptomycin and gentamicin as standard antibacterial drugs. The fungal strains selected were *Aspergillus niger* MTCC 16404, *Candida albicans* MTCC 183, *Crytococcus albidus* MTCC 2661 and *Aspergillus flavus* MTCC 1973, with fluconazole as antifungal drug. The result reported that methanolic seed extract exhibited significant antimicrobial activity in term of antibacterial and antifungal activity.

The antimicrobial activity of the leaf extracts in methanol, dichloromethane and hexane were tested against four bacteria, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* and fungus, *Candida albicans* (Tan et al., 2009b). As a result, the methanol extract showed significant antifungal properties as well as found to be active against the Gram-positive bacteria tested.

2.2.3 Anticancer and Antitumor Activity

In the early of 1996, Guevara et al. had reported the antitumor activity of the *Swietenia macrophylla* seed. The ethanol extract of seed was investigated using Epstein-Barr virus early-antigen (EBV_EA) activation, with 12-0-tetradecanoylphorbol-13-acetate as tumor promoter, and it was found that there had considerable inhibitory activity on the EBV_EA activation.

Later, Goh and Kadir in 2011 revealed that the anticancer activity shown in ethyl acetate fraction of *Swietenia macrophylla* seed. The seed extract was assessed against human cancer cell lines named Ca Ski, HCT116, KB and MCF-7 by using MTT assay. Results showed that the seed extract induced the collapse of the mitochondrial membrane potential after 24 hours as well as caused depletion in total intracellular glutathione.

2.2.4 Antidiabetic Activity

Swietenine, a limonoid isolated from *Swietenia macrophylla* had reported to show antidiabetic activity (Dewanjee et al., 2009). The study showed that intake of swietenine at the dose of 25 and 50 mg/kg body weight had significantly lowered the fasting blood glucose level of type 2 diabetic rats. This findings was supported by Kalaivanan and Pugalendi (2011), which they supplied the seed extract at different dosage daily to the streptozotocin-induced diabetic rats, and they found the lowering of blood glucose level on the diabetic rats.

2.2.5 Other Activities

Maiti, Dewanjee and Mandal (2007) had reported the antidiarrhoeal activity in the petroleum ether extract of seed of *Swietenia macrophylla.* The antidiarrhoel effect was investigated in rats and significant result was observed by reduction in the rate of defecation.

In 2000, Munoz et al. reported that the bark extract of *Swietenia macrophylla* as well as the decoction of seeds showed the antimalarial activity. For the seed

extract, it was investigated against *Plasmodium falciparum* while the bark extract was tested against *Plasmodium vinckeipetteri* 279BY. The seed extract showed considerable antimalarial activity and the bark extract showed a strong antimalarial activity compared to seed extract (78 % inhibition on the rodent malaria *Plasmodium vinkeipetteri* 279BY at 100 µg/mL).

In the most recent study, Pamplona et al. (2015) reported that the leaves of *Swietenia macrophylla* showed potential cytoprotective effects on an *in vitro* model of neurodegeneration, by using primary cerebella cultures exposed to methyl mercury (MeHg). The evidence of cytoproctective observed when the acure (24 hours) and sub-chronic (72 hours) exposure of primary cerebellar cultures to MeHg resulted in decreased of cell viability time.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials and Instruments

Organic solvents used throughout the project are listed in Table 3.1. In Table 3.2, the types of silica gel used for chromatography are listed. Table 3.3 shows the types of instrument used for extraction, isolation and characterization in this project.

Table 3.1: List of organic solvents and its grade.

Solvents	Grade/ Purity	Brand
Methanol	AR	Fisher Scientific
Chloroform	AR	MERCK
Dichloromethane	AR	R&M
n-Hexane	AR	QReC
Deuderated chloroform	99.8 %	MERCK

Table 3.2: Types of silica gel

Types of Silica gel	Types of Chromatography	Brand
Silica gel 60 (230-400 mesh)	FCC	MERCK
Silica gel 60 F_{254}	TLC	MERCK
Silica gel 60 PF_{254} containing gypsum	CTLC	MERCK

Table 3.3: List of instrument

Instruments	Company	Model
Rotatory evaporator	Buchi	R-200
UV-Vis spectrophotometer	Thermo Scientific	GENESYS 10S
IR spectrometer	Perkin Elmer	RX1
Mass spectrometer	Agilent Technologies	ESI-MS
FT-NMR spectrometer	JEOL	JNM-ECX 400 MHz

3.2 Collection of Plant Material

Leaves of *Swietenia macrophylla* King were collected along the roadside of Kajang, Selangor. The identification of the species is based on the physical appearance of plant, which has a significance “sky fruit” on top of the trees. Approximate 8 kg of fresh leaves were collected, washed and dried under the sun for two weeks.

3.3 Extraction of Plant Materials

Dried leaves of *Swietenia macrophylla* King were grinded, and 4.5 kg of dried leaves powder were obtained. The dried powder was soaked separately in two 2 L conical flasks and three 5 L conical flasks by using methanol as the solvent. The soaking process was repeated four times, with each soaking time took about a week (4 x 7 days).

The methanol extracts being collected after each soaking process, followed by filtration through cotton wool. The filtrate was later being concentrated by rotatory evaporator (model R-200, Buchi) and the concentrated filtrates were

combined as crude product. From methanol extraction, 300 g of crude product was obtained.

3.4 Isolation of Compounds

The separation and isolation of compounds were carried out using chromatographic methods. Before the separation of compounds using various chromatographic methods, determination of solvent system is an important session, which a good solvent system will provide a good separation.

3.4.1 Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is a type of planar chromatography, divided into normal phase (with polar adsorbent) and reverse phase (with non-polar adsorbent). It is a technique that is frequently used to determine the solvent system before separation process.

TLC has its advantages, which is a quick, simple and inexpensive method for the researcher to identify the number of components in a mixture. Additionally, it also allows the researcher to monitor the effectiveness of separation by observing the separation of components in certain solvent system and hence allowing researcher to determine the best solvent system before conducting the separation process.

3.4.1.1 Preparation of Thin Layer Chromatography

Normal phase TLC was used in the project. The preparation of TLC plate is quick and simple. Generally, TLC plate was cut into a 10 cm height, with variable width length dependent on how many samples spotted onto the plate. A baseline was drawn 1 cm from the bottom of the plate as well as the solvent front is drawn 1 cm from the top of plate. The sample in solution form was spotted on the baseline using capillary tube and each sample is spotted 0.5 cm apart from each other in order to minimize the chance of overlapping.

Mobile phase was introduced into a sealed developing chamber and left for few minutes to allow for the chamber to be saturated with mobile phase. The TLC plate was later placed into the chamber to allow the development of plate until the

solvent movement reaches the solvent front. The developed TLC (Figure 3.1) was visualized and spotted under UV lamp (254 nm and 356 nm) as well as by iodine staining.

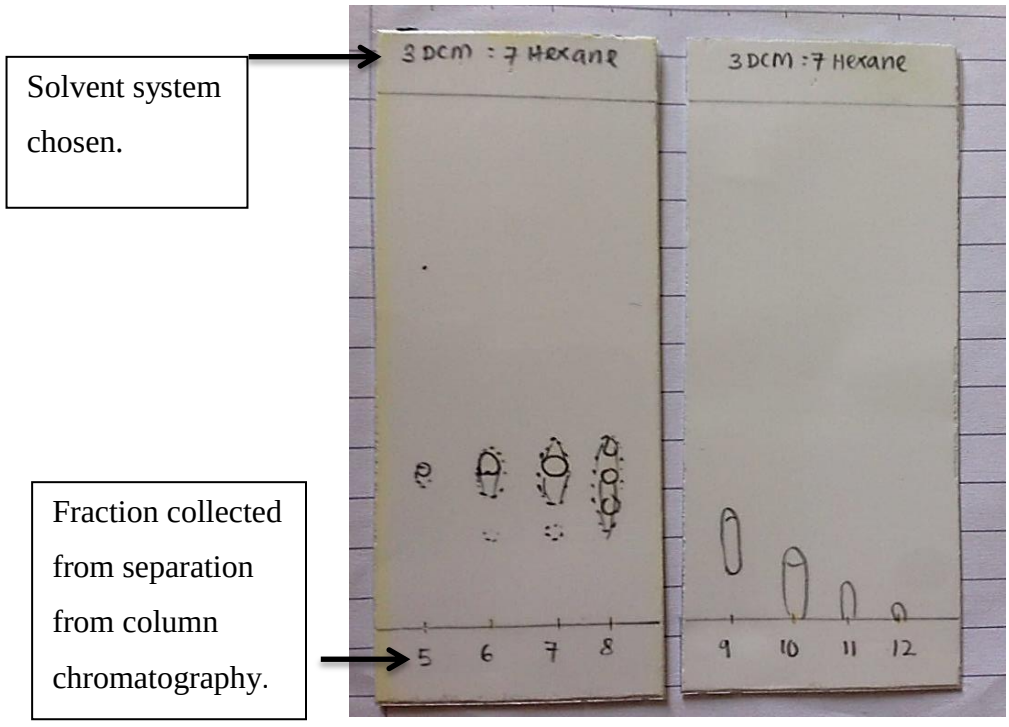


Figure 3.1: Developed TLC plates. Solid line refers to the UV visualized spot; Dotted line refers to spot visualized using iodine staining.

3.4.1.2 Identification of Compounds using TLC

The identification of compound using TLC technique is supported by comparing the retardation factor (R_f) of sample with R_f of known compound. The R_f value of the sample can be obtained by determining the migration rate of compound relative to the distance of solvent front. The retardation factor, R_f formula is shown as below:

$$R_f = \frac{Distance\ travelled\ by\ compounds\ (cm)}{Distance\ travelled\ by\ solvent\ (cm)}$$

By using the R_f value as reference, the spots with similar R_f can be regarded as same compound, which grouped together into a fraction. For instance, fractions 5 to 7 (Figure 3.1) have the similar R_f value, which can be recombined into a fraction.

To achieve a consistent and reproducible R_f value, the size of sample spot should be in the same size, small and concentrated. If the sample is too diluted, the spot on TLC plate is hard to be visualized. In contrast, a too concentrated spot will cause a tailing which affects the separation efficiency of compounds on plate. Meanwhile, if the size of the spot is too big, a broadening spot will be observed, which might overlap with other spot.

3.4.1.3 Determination of Solvent System using TLC

The choice of solvent system to be used in column chromatography can be determined through TLC by monitoring the R_f value. An ideal solvent system should separate the spot in between R_f value of 0.2 to 0.5 in order to achieve a good separation. R_f values that are more than 0.5 will cause the compounds to be eluted out fast, hence less separation will occur.

To monitor the R_f value within the ideal range, the polarity of solvent is important. By modifying the polarity of solvent system, the R_f value of sample spot can be increased (by increasing the polarity of solvent system) or decreased (by decreasing the polarity of solvent system).

3.4.2 Column Chromatography

Column chromatography is a common method used to separate compounds in mixture. Unlike the planar chromatography, column chromatography has its stationary phase or resin packed into a column, the mobile phase is passed through the column by gravity or pressure force. The working principle is based on the adsorption and interaction of compounds with stationary phase and mobile

phase. There are various types of column chromatography; yet only traditional gravity column chromatography and flash column chromatography were used in the project.

3.4.2.1 Gravity Column Chromatography

Gravity column chromatography is the traditional column chromatography, which work by applying the crude sample on top of a bed of silica gel loaded in a glass column. The mobile phase was eluted through the silica gel (stationary phase) in the column by gravity force. The separation in the mixture was based on the different affinity of the compounds towards mobile and stationary phases, which causes each compound to migrate through the column at different migration rate as well as eluted out at different times.

3.4.2.2 Flash Column Chromatography (FCC)

Flash column chromatography (FCC) is a method that was popularized by Clark Still of Columbia University, as an alternative of gravity-fed chromatography. It is also known as medium pressure chromatography (Naumiec et al., 2013). Two

differences of FCC compared to conventional gravity column chromatography: first, for FCC a smaller size of silica gel particles (230-400 mesh) are used, and second, instead of gravity force, the solvent is driven through the column with a pressurized gas. These modifications resulted in a rapid and high resolution separation process.

3.4.2.3 The Column Preparation

Before the separation and isolation performed, some criteria needed to consider. First of all, the quantity of silica gel required need to be calculated. The quantity of silica gel depended on the amount of sample, where the amount of silica gel was measuring in 1:50 ratio. For instance, 1 g of crude sample required 50 g of silica gel. Second, the selecting of column diameter and length is important, as different sample size required different size of column. The appropriate column size will give better separation. Third, the types of sample packing also need to be considered. The sample can be packed by using dry method and wet method. Dry method involved the deposition of sample on silica gel, and wet method involved the direct dissolving of sample in minimum amount of solvent. The choice of packing method depends on the solubility of sample in the solvent.

For the sample that can fully dissolve in the selected solvent system, wet packing is preferred. A minimum amount of solvent is used to dissolve the sample, and the sample is introduced directly into the column. For sample which is insoluble in the selected solvent system, dry packing is preferred. The sample is first being dissolved in any other solvent, followed by addition of silica gel into it. The solution is then being concentrated using rotatory evaporator and the concentrated silica-sample is left overnight for complete dryness. The dry silica-sample is then readily introduced into the column.

3.4.2.4 Separation Process

Normal phase gravity column chromatography was used in the separation of crude product (150 g) as shown in Figure 3.2. The solvent system selected was 100 % chloroform. An activated silica gel was prepared and made into slurry form by mixing with solvent. The slurry of silica gel was decanted into the glass column via a filter funnel, and settling of gel was done by tapping the side of glass column using a rubber hose. Tapping process was important in order to ensure the silica gel was compactly packed and prevent the trapping of air bubbles in the column. The sample was then introduced into the column when the solvent level was drained close above to the surface of silica layer.

Separation can be started when the sample layer has entered completely into the silica layer. The separation of sample was carried out by gradient elution, with increasing the polarity of solvent throughout the separation process. A total of 26 fractions were collected at the first separation.

The following separation and isolation process was carried out using FCC instead of gravity column chromatography. The flow of packing the column was the same as that of gravity column chromatography; the only difference is the elution process was carried out by introducing the compressed nitrogen gas from the top of the column. This increases the rate of solvent flow as well as reduces the time of separation.

Figure 3.2: Separation of crude product (150 g).

3.5 Purification

The purification in this project is accomplished by using centrifugal thin layer chromatography (CTLC). The stationary phase consists of gypsum silica gel that was coated on a glass plate. The preparation of stationary phase was done by mixing 50 g of silica 60 PF_{254} containing gypsum with 100 mL of cold distilled water. The slurry of silica gel was shake vigorously and poured on glass plate with cellophane tape attached on the edge of the plate to contain the gel. The glass plate was left to dry for 30 minutes in room temperature and further drying at 50 ^{o}C in oven for overnight. Later, the dried plate was smoothen by using two scrappers.

The CTCL was set up as shown in Figure 3.3. The plate was locked to a rotor and being covered. The solvent was introduced into the plate via the inlet tube, and hence the stationary phase was saturated with the solvent before the separation occurs. Later, the sample was dissolved in a minimum amount of solvent and introduced drop by drop into the stationary phase. Solvent was introduced again to separate the sample, and with the spinning motor the glass plate was spinned thus force the solvent and sample to move in a uniform circular motion outward from the centre. The separation can be visualized by using UV lamp (245 nm) and different bands were collected into different fractions.

Figure 3.3: The set-up of CTCL.

3.6 Structure Elucidation

The isolated compounds were characterized by using various spectroscopic methods. The structure elucidation of compounds were accomplished by using instruments such as nuclear magnetic resonance spectrometer (NMR), infrared spectrometer (IR), ultraviolet-visible light spectrophotometer (UV-Vis) and mass spectrometer (MS).

3.6.1 Nuclear Magnetic Resonance (NMR)

Nuclear magnetic resonance spectroscopy (NMR), is a research technique that performed analytical work based on magnetic properties of certain atomic nuclei. NMR is useful tool used to obtain physical, chemical, electronic and structural information about a molecule. There are two major categories of NMR: one-dimensional (1D) NMR and two-dimensional (2D) NMR. 1 D NMR experiments include proton NMR (^{1}H NMR), Carbon-13 NMR (^{13}C NMR), Distortionless Enhancement by Polarization Transfer (DEPT). 2D NMR experiments include Correlation Spectroscopy (COSY), Heteronuclear Multiple Quantum Correlation (HMQC) and Heteronuclear Multiple Bond Correlation (HMBC). Both 1D and 2D NMR experiments were used in this project.

For the preparation of NMR analysis, the sample must be dried and without non-deuterated solvent. Therefore, the sample was subjected to rotatory evaporator in order to remove any non-deuterated solvents. The dried sample was dissolved in a minimum amount of deuterated chloroform ($CDCl_3$) and transferred into a clean and dry NMR tube. Deuterated chloroform was topped up to the level of 4 cm height of the tube, and the tube was capped and labeled. The NMR tube containing sample was ready to send for NMR analysis.

3.6.2 Infrared Spectroscopy (IR)

Infrared Spectroscopy (IR) is another analytical tool that is widely used in industry for research and development. It deals with the infrared region and commonly used to identify functional groups present in a molecule. The sample preparation can be varied according to the state of sample. For liquid sample, a drop of liquid sample was sandwiched between two transparent salt plates, hence made the sample into a thin film. For solid sample, it can be prepared by Nujol mulls, KBr pellet as well as thin film method.

Thin film method was used in this project. For solid sample, a minimum amount of anhydrous chloroform was used to dissolve the sample, and a few drops of sample were added onto a salt plate. An even film can be obtained after the solvent fully evaporated. After that, the sample was sandwiched between two salt plates. For liquid sample, a drop a liquid sample was spread evenly on a salt plate, and a second plate was placed on top to sandwich the sample in between two plates, thus forming a nice even film. The salt discs with sample were held in the cell holder and ready to be analyzed using IR spectrometer.

3.6.3 Ultraviolet-Visible Light Spectrophotometry (UV-Vis)

Ultraviolet-visible spectrophotometry (UV-Vis) is an absorption spectroscopy that deals with the ultraviolet-visible spectral region. It does not completely work on identify the structure of a molecule, but it is useful for detecting the compounds with many double bonds.

The sample was prepared by dissolving in chloroform to give homogeneous solution. The sample solution was added into a quartz cuvette. Before analyzing the sample, chloroform was used as blank for baseline correction. After baseline correction, the quartz cuvette with sample was place into the cuvette holder inside the spectrophotometer for UV-Vis analysis. The scanning range was set from 800 nm to 200 nm and the maximum absorption, λ_{max} was recorded.

3.6.4 Mass Spectrometer (MS)

Mass spectrometry (MS) is a widely used analytical tool that helps to identify the molecular weight of a molecule. It ionizes the chemical species and sorts the ions according to their mass to charge ratio. Based on the masses of fragments, the possible structures of a molecule can be identified by comparing with the database. For the sample preparation, the weighted sample was dissolved in small volume of hexane, and transferred into a 2 mL sample vial for analysis.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Flowchart of Separation Process

The overall isolation process of the pure compounds has been illustrated using flowchart. The map of work done is shown in Figure 4.1, where the flowchart shows the isolation process starting from the crude by using 100 % chloroform as the solvent system, followed by the subsequent isolation process as well as purification process by using a mixture of dichloromethane (DCM) with hexane in different ratio.

Three pure compounds had been isolated and being labelled as Compound A (104.1 mg), Compound B (15.4 mg) and Compound C (32.9 mg).

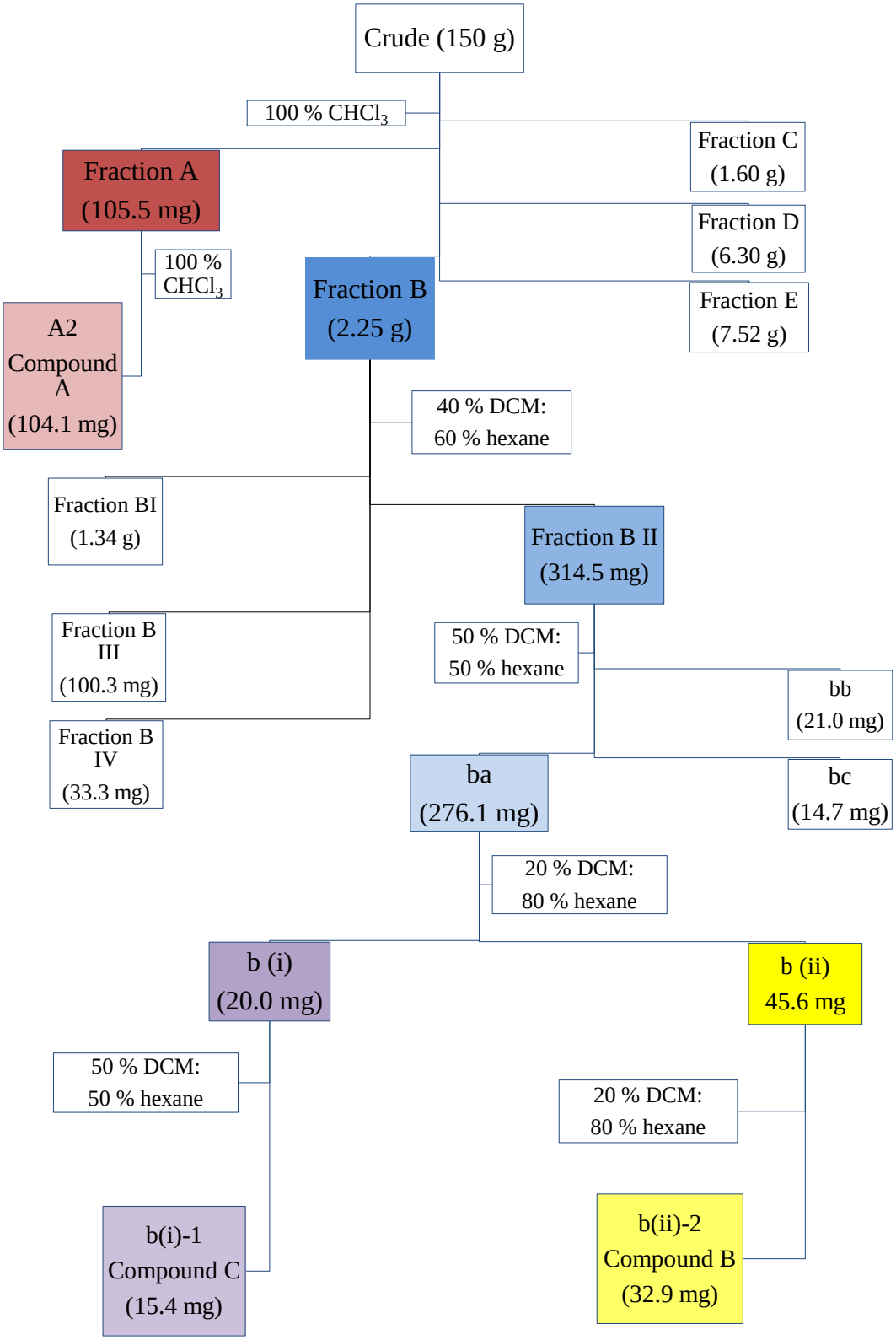


Figure 4.1: Flowchart of Separation Process.

4.2 Characterization of Compound A

Compound A was isolated as pale yellow solid with 104.1 mg. The R_f value of compound A was 0.81 in 100 % $CHCl_3$ (Figure 4.2). From the measurement of UV-Vis spectrophotometry, the UV (0.5 mg/mL, $CHCl_3$) λ_{max} is 240 nm with absorbance 1.330.

Figure 4.2: TLC plate of Compound A (100% $CHCl_3$).

The IR spectrum of compound A (Figure 4.3) shows strong and sharp peaks at 2913 cm^{-1} and 2847 cm^{-1} indicating the presence of aliphatic CH_2 and CH_3 chain. The sharp, medium band had been observed at 1462 cm^{-1} and weak band at methyl C-H bend 1375 cm^{-1}, plus a sharp band at 751 cm^{-1} (due to methylene rocking vibration) was indicative of long chain linear aliphatic structure. The information obtained from IR spectrum was tabulated in Table 4.1

Table 4.1: Interpretation of IR spectrum of Compound A.

Wavenumber, cm^{-1}	**Type of vibration**	**Assignment**
2913, 2847	sp^3 C-H stretch	Aliphatic CH_3 and CH_2
1462, 1375	sp^3 C-H bend	Aliphatic CH_3 and CH_2

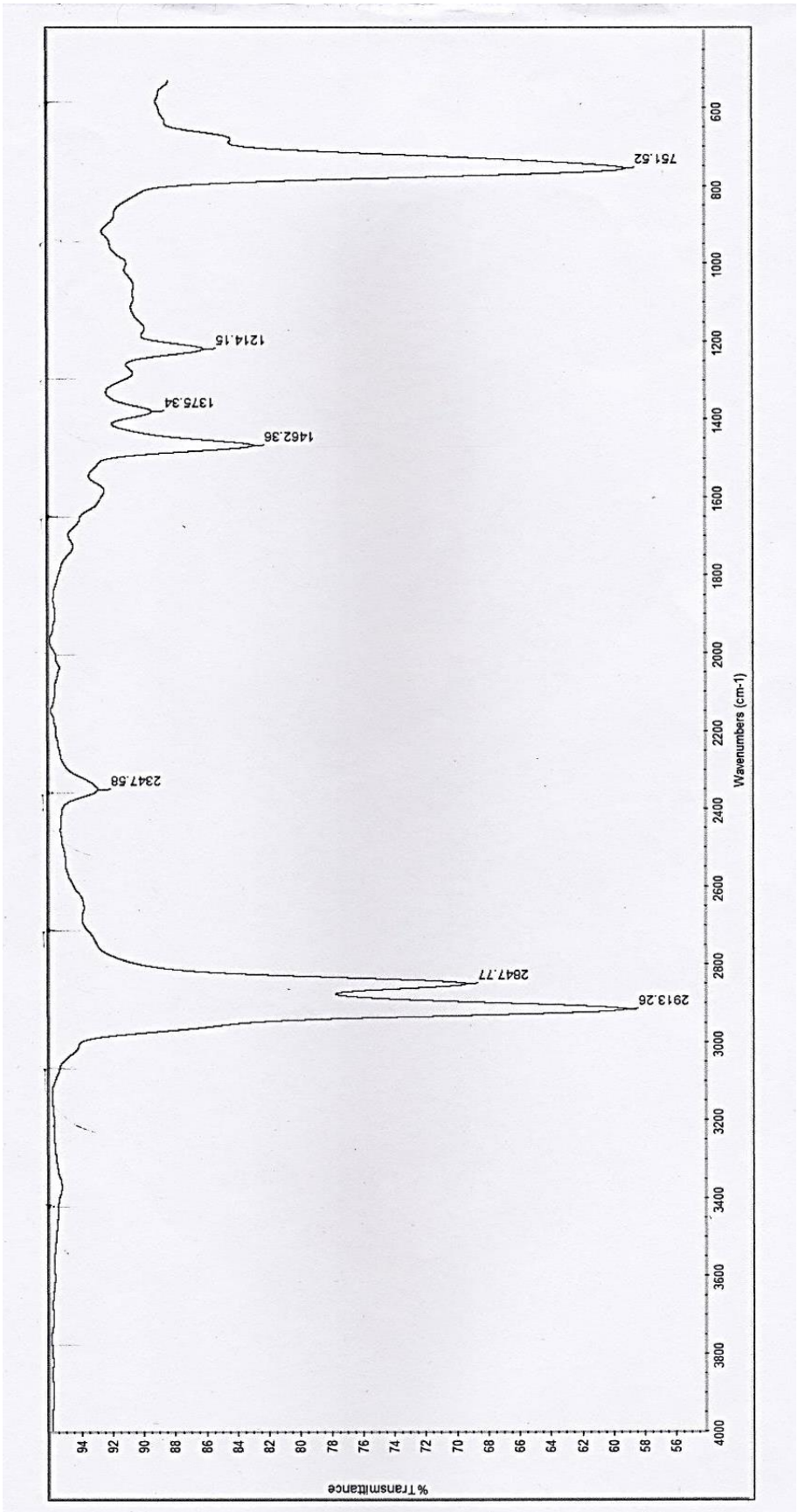


Figure 4.3: IR Spectrum of Compound A (thin film on NaCl, $CHCl_3$)

From ^{1}H NMR spectrum (Figure 4.4), a proton peak was observed at δ_H 0.87 ppm (t, J= 6Hz), and it was assigned as a methyl proton. The splitting of the proton peak gave information about the number of neighboring protons. Triplet splitting of the proton peak indicated that there were two adjacent protons. An intense peak was observed at δ_H 1.25 ppm (s), which was assigned to be an overlapping of methylene protons. Apart from the intense methylene proton peak mentioned earlier, other methylene proton peaks were observed at δ_H 1.60 ppm (s), 1.67 ppm (s), 1.96 ppm (m), 2.07 ppm (m). Besides, a peak was observed at δ_H 5.11 ppm indicating the present of vinylic protons in the compound.

From the ^{13}C NMR spectrum (Figure 4.5), 4 carbon peaks had been observed at range 10 ppm to 20 ppm and were assigned to be methyl carbons. Besides that, a number of peaks had been observed at range 20 ppm to 40 ppm and assigned to be methylene carbons. An intense carbon peak at δ_c 29.85 ppm indicated that there was overlapping of methylene carbons, therefore suggested that there was a long methylene carbon chain in compound A. In addition, 6 carbon peaks were observed at the olefinic carbons range, δ_c 124.42, 124.45, 131.40, 135.04 and 135.25 ppm, and it was suggested that compound A consists of 3 double bonds.

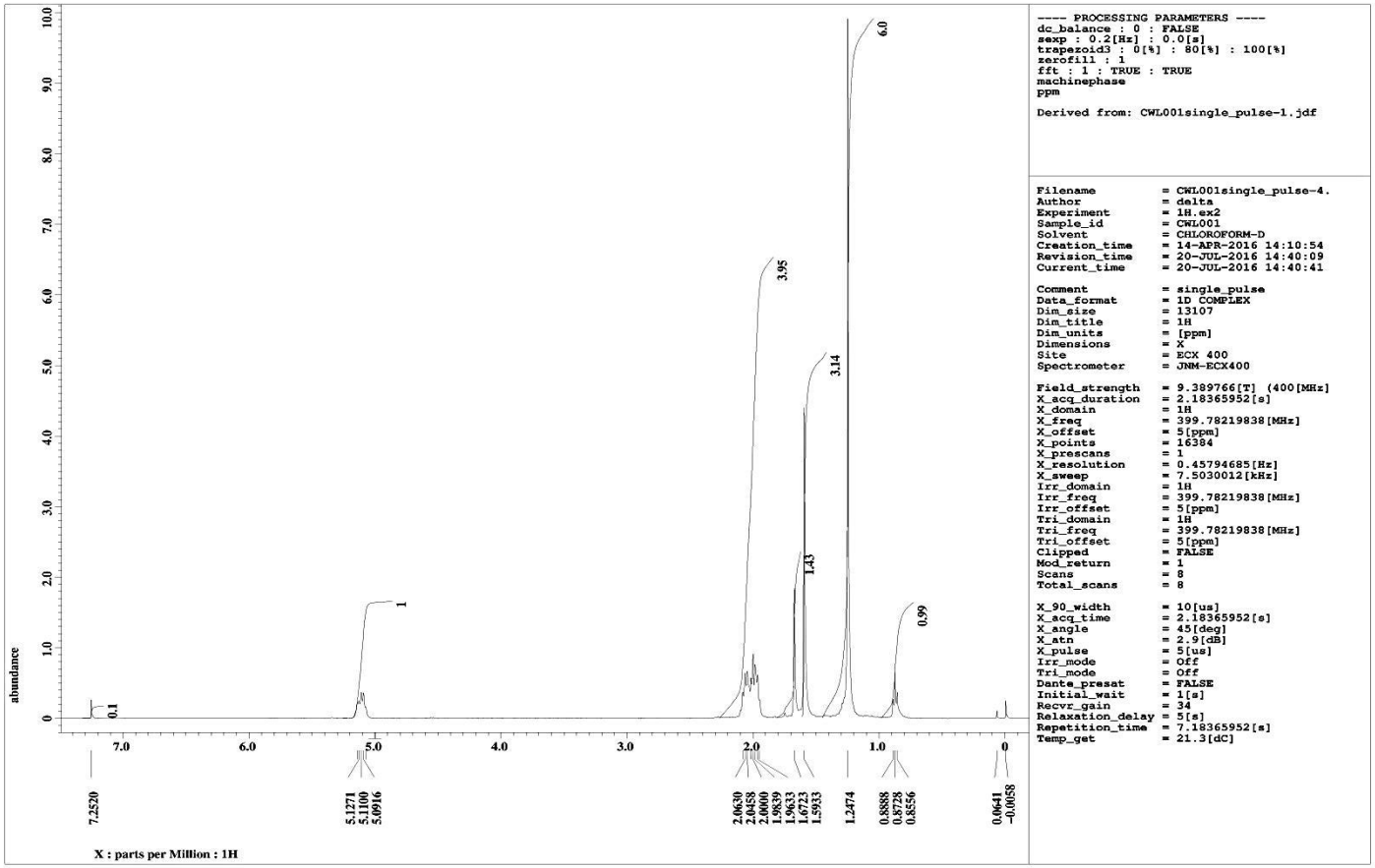


Figure 4.4: ^{1}H NMR Spectrum of Compound A ($CDCl_3$, 400 MHz)

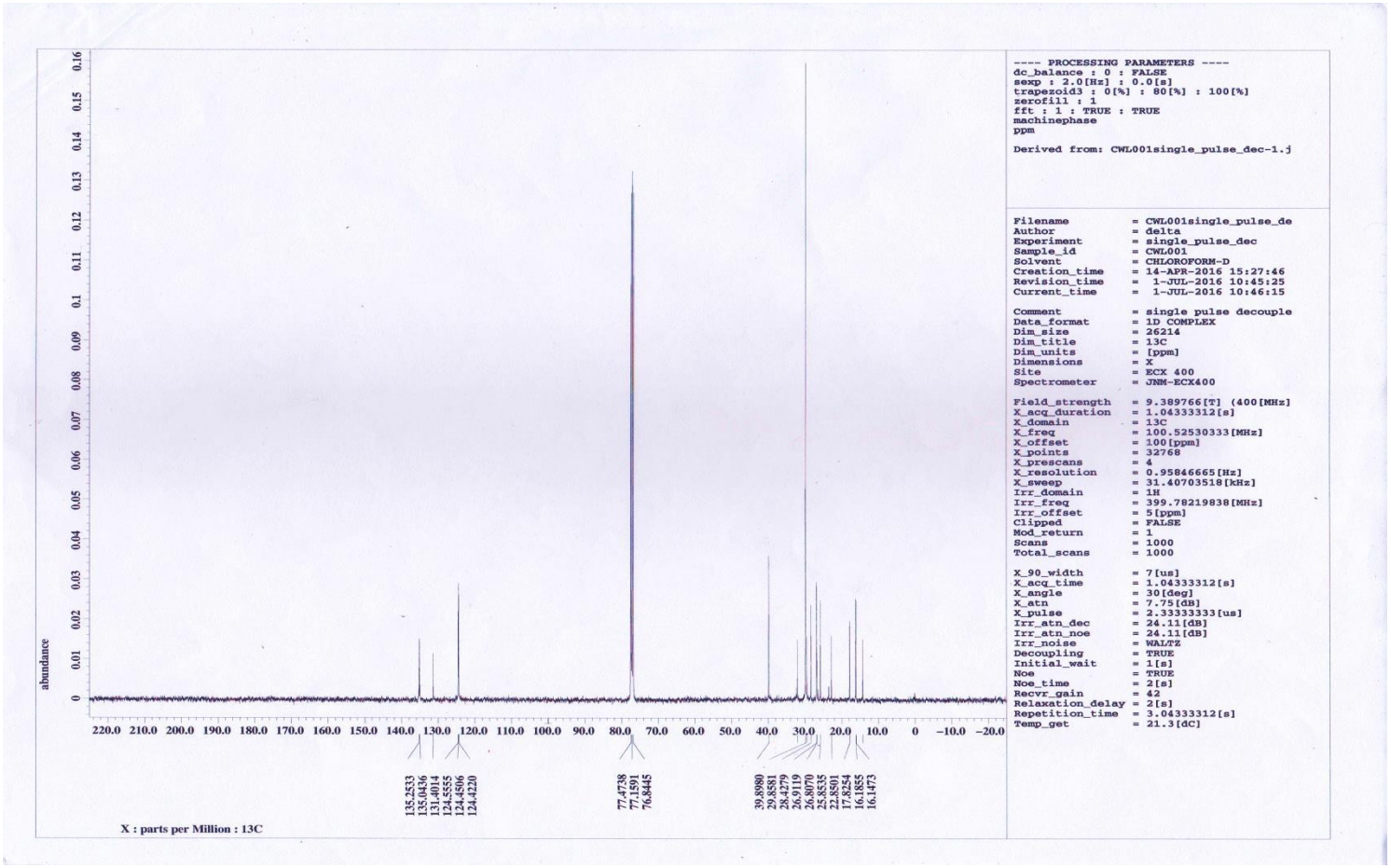


Figure 4.5: ^{13}C NMR Spectrum of Compound A ($CDCl_3$, 100 MHz)

Compound A was subjected to GC-MS analysis. Based on the GC analysis, three possible compounds had been detected in compound A, which were squalene with retention time at 22.91 minutes, tetratriacontane with retention time at 23.57 minutes and octacosane with retention time of 25.68 minutes. Compound A was suspected to be a mixture of squalene, tetratriacontane or octacosane.

From the COSY spectrum, correlations of H-1 (δ_H 0.87 ppm) and H-2 (δ_H 1.25 ppm) was observed. From HMBC spectrum, H-1 (δ_H 0.87 ppm) showed correlations with C-2, 3, 4, 5 (δ_C 22.85, 29.52, 29.68, 32.08 ppm), whereas from HMQC spectrum C-2, 3, 4, 5 showed the similar proton shift (δ_H 1.25 ppm). From COSY and HMBC spectra analysis, it can be concluded that there was only correlation found between H-1 and H-2, while H-1 and H-2 did not have any correlations with other signals. This indicated that compound A is not pure.

The first compound in the mixture compound A was named as compound A1. For the interpretation of structure, C-1 (δ_C 14.28 ppm) was bonded directly to H-1 (δ_H 0.87 ppm) and assigned to be a methyl carbon. The proton (H-1) showed triplet splitting (J = 6 Hz) indicated that the methyl carbon was bonded to carbon attached with two protons (-CH_2-). Besides that, C-2, 3, 4, 5 (δ_C = 22.85, 29.52, 29.68, 32.08 ppm) had the same proton shift at H-2 (δ_H 1.25 ppm) and were assigned to be methylene carbons.

Based on the spectral data, the proposed structure was illustrated in Figure 4.6. The compound was proposed to be a long aliphatic compound, which is a wax. Due to the overlapping of carbons, the exact number of carbons was difficult to be established. Based on the data shown, the compound is suspected to be octacosane and supported by the literature NMR value reported (HMDB, 2015). The comparison of NMR data of compound A with literature value is shown in Table 4.2.

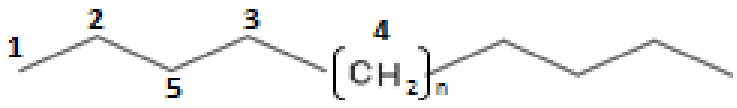


Figure 4.6: Proposed structure for Compound A1.

Table 4.2: Comparison of NMR data of Compound A1 with Literature value.

Carbon	**δ_C**	**δ_H**	***δ_C**	***δ_H**
1	14.28	0.87 (t, J= 6 Hz)	14.12	0.88
2	22.85	1.25 (s)	22.76	1.26
3	29.52	1.25 (s)	29.45	1.26
4	29.86	1.25 (s)	29.79	1.26
5	32.08	1.25 (s)	32.02	1.26

*δ = literature value

Interpretation of another compound in compound A (named as compound A2) had been done by excluding the chemical signals observed for compound A1. 6 carbons peaks were observed at olefinic carbon range, δ_C 124.42, 124.45, 124.55, 131.40, 135.04, 135.25 ppm, indicated that the compound has 3 double bonds. From HMBC spectrum, C-17 (δ_C 131.40 ppm) was a quaternary, olefinic carbon that correlated with protons on C-6, 9, 11. Protons attached to C-6, δ_H 1.59 ppm (s) and C-9, δ_H 1.67 ppm (s) showed singlet splitting, indicated that there were no neighboring protons, hence suggested that C-6 and C-9 were bonded directly to the quaternary, olefinic carbon. Besides, C-6 also showed correlation with proton at δ_H 5.11 ppm, indicated that there was an olefinic carbon attached with proton (H-C=C) at 2 or 3 bonds apart from C-6. The proposed fragment structure was showed in Figure 4.7.

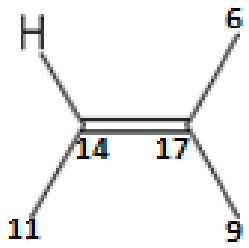


Figure 4.7: Fragment structure of compound A2.

In addition, from COSY, proton at δ_H 5.11 ppm was found to correlate to proton at δ_H 2.05 ppm, as well as to proton δ_H 1.98 ppm. With the help of HMBC spectrum, a complete structure had been illustrated in Figure 4.8.

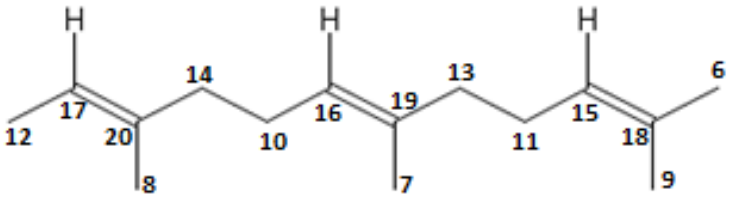


Figure 4.8: Possible structure of compound A2.

The proposed structure was suggested to be squalene (Figure 4.9), with symmetrical structure. This can be confirmed by comparing with the reported literature value in Table 4.3 (Pogliani et al., 1994).

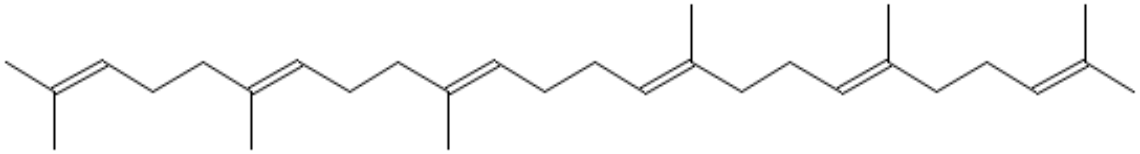


Figure 4.9: Structure of Squalene.

Table 4.3: Comparison of NMR data of Compound A2 with Literature value.

Carbon	**δ_C**	**δ_H**	***δ_C**	***δ_H**
6	16.14	1.59 (s)	15.93	1.62
7	16.18	1.59 (s)	15.98	1.61
8	17.82	1.59 (s)	17.60	1.61
9	25.85	1.67 (s)	25.63	1.68
10	26.80	2.05 (m)	26.66	2.01
11	26.91	2.05 (m)	26.79	2.08
12	28.43	1.98 (m)	28.28	2.03
13	39.90	1.98 (m)	39.74	1.99
14	39.91	1.98 (m)	39.76	2.01
15	124.42	5.11 (m)	124.30	5.13
16	124.45	5.11 (m)	124.34	5.17
17	124.56	5.11 (m)	125.45	5.11
18	131.40	-	131.01	-
19	135.04	-	134.74	-
20	135.25	-	134.94	-

4.3 Characterisation of Compound B

Compound B was obtained as a pale yellow oil (32.9 mg) with R_f value: 0.31 (20% DCM: 80% hexane). The TLC plate of compound C is showed in Figure 4.10. The solid line indicated a spot under UV lamp and dotted line spotted under iodine staining. From the measurement of UV-Vis spectrophotometry, the UV (1.0 mg/mL, $CHCl_3$) λ_{max} is 245 nm with absorbance 1.683.

Figure 4.10: TLC plate of compound B (20% DCM: 80% hexane)

The infrared (IR) spectrum of compound B is shown in Figure 4.11. From the IR spectrum, some important peaks had been detected. Strong and sharp peaks with wavenumber of 2918 cm^{-1} and 2893 cm^{-1} indicated that there are aliphatic CH_3

and CH_2 group (sp^3 C-H stretch) in compound B. The sp^3 C-H bend also have been observed at 1366 and 1452 cm^{-1}.

A significant sharp peak observed at about 1700 cm^{-1} indicates there is a carbonyl C=O stretch. The carbonyl group stretch can be due to the presence of an aldehyde, ketone, carboxylic acid or ester. However, no strong, broad band of O-H stretch had been observed around 3200 to 3600 cm^{-1}, thus indicated that the compound does not have alcohol or carboxylic acid functionality groups. By referring to the IR spectrum, the wavenumber at 1740 cm^{-1} showed that it is an ester as ester carbonyl stretch has been reported at range of 1725 – 1750 cm^{-1} (Coates, 2006). The present of alkoxy (C-O) stretch at range around 1000 to 1300 $cm^{=1}$ further confirmed the presence of ester as one of the functional groups in compound B, not aldehyde or ketone group.

Weak to medium peaks were observed at wavenumbers 1593 cm^{-1} and 1650 cm^{-1}, and these peaks were assigned to be an alkenyl C=C stretch, as well as the out-of-plane bending of C-H also observed at 718 cm^{-1}, a medium , broad peak. According to Coates (2006), trans-disubstituted alkene has sp^2 C-H out-of-plane bend at wavenumber around 960 to 970 cm^{-1} while cis-disubstituted alkene will show wavenumber at 700 cm^{-1}. By referring to the IR spectrum of compound B, it

could be suggested that compound B is probably an alkene group with cis configuration.

The information obtained from IR spectrum of compound B suggested that compound B is an unsaturated compound with cis-alkene disubstituted configuration, and having an ester functional group. The information had been summarized in Table 4.4.

Table 4.4 Interpretation of IR spectrum of compound B

Wavenumber, cm^{-1}	**Type of vibration**	**Assignment**
2918, 2893	sp^3 C-H stretch	Aliphatic CH_3 and CH_2
1366, 1452	sp^3 C-H bend	Aliphatic CH_3 and CH_2
1740	C=O stretch	Ester functional group
1168, 1014	C-O stretch	Alkoxy group
1593, 1650	C=C stretch	Alkene
718	sp^2 C-H bend	Cis- disubstituted alkene

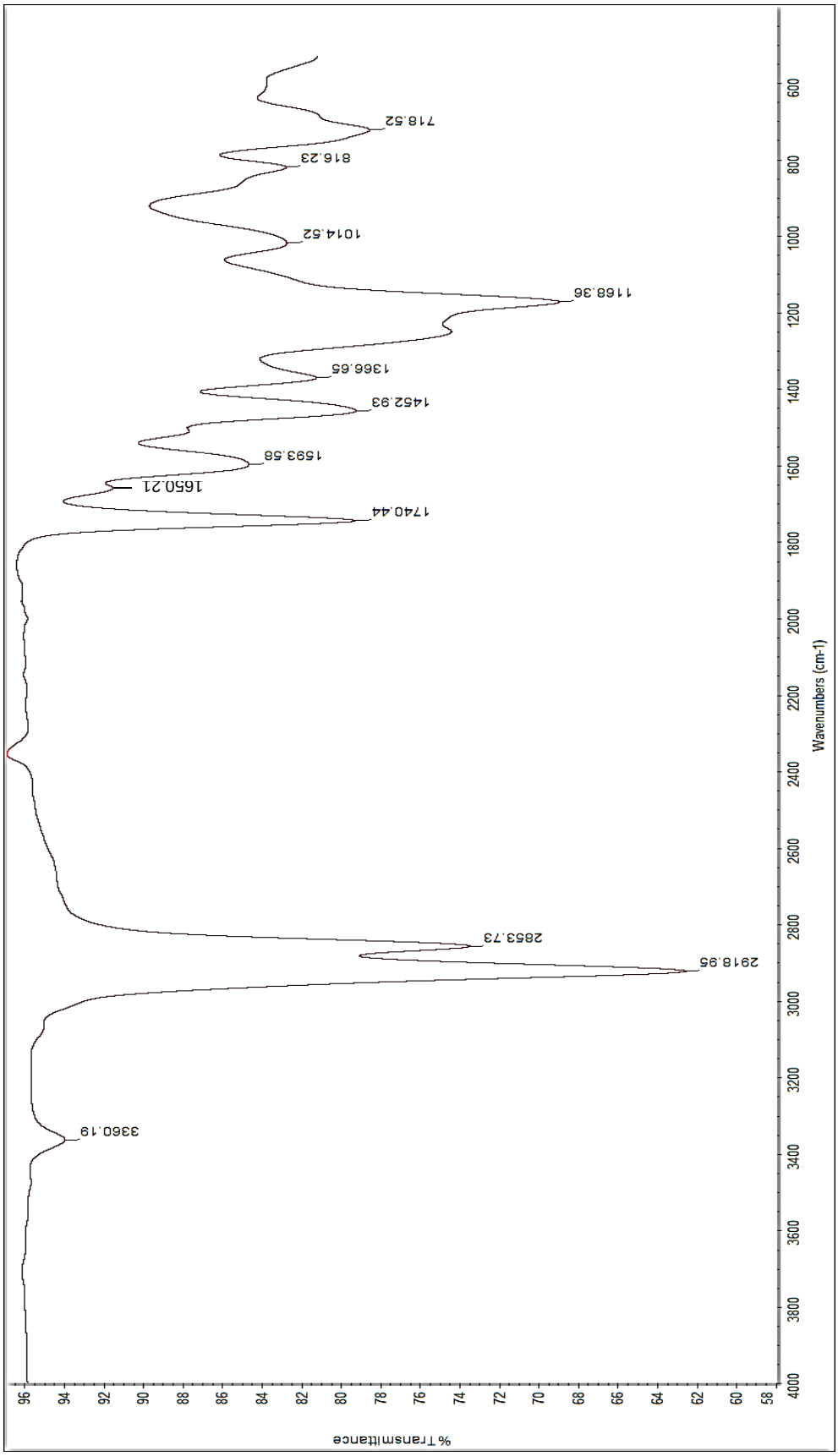


Figure 4.11: IR Spectrum of Compound B (thin film on NaCl, $CHCl_3$)

From ^{1}H NMR spectrum (Figure 4.12), compound B showed a signal at δ_H 5.32 ppm (m) and was assigned to be a vinylic proton (**H**-C=C). The peak at δ_H 3.63 ppm (s), the proton was assigned to a proton in an α-position to an oxygen (RO-C**H**). Peaks observed at range δ_H 1.23 to 2.30 ppm were suggested to be methylene protons. For proton peak less than 1.0 ppm, it was suggested to be methyl proton.

From ^{13}C NMR spectrum (Figure 4.13), a total of 19 carbons had been observed. A carbon peak with chemical shift, δ_c 14.21 was suggested to be a methyl carbon. Methylene carbon peaks resonated between δ_c 20 ppm to δ_c 30 ppm, and from the spectrum, a total of 14 carbon peaks were observed (δ_c 22.79, δ_c 25.06, δ_c 27.26, δ_c 27.31, δ_c 32.03, δ_c 34.21 and 8 other carbons at range : δ_c 29.19 - δ_c 29.86).

A peak had been observed at δ_c 51.53 and was suggested to be an alkoxy carbon. Another peak at δ_c 174.46 also been observed indicated the present of carbonyl group (C=O) probably due to an ester group. Lastly, two carbon peaks fall at the unsaturated carbon range with chemical shift δ_c 129.83 and δ_c 130.09, indicated that compound B has an alkene group (C=C).

From DEPT spectrum, the carbon types were confirmed, whereas there were 2 methyl carbons (δ_c 14.21 ppm and δ_c 51.53 ppm), 14 methylene carbons (δ_c 22.79 ppm to δ_c 34.21 ppm), 2 methine carbons (δ_c 129.83 ppm and δ_c 130.09 ppm) and 1 quaternary carbon (δ_c 174.46 ppm).

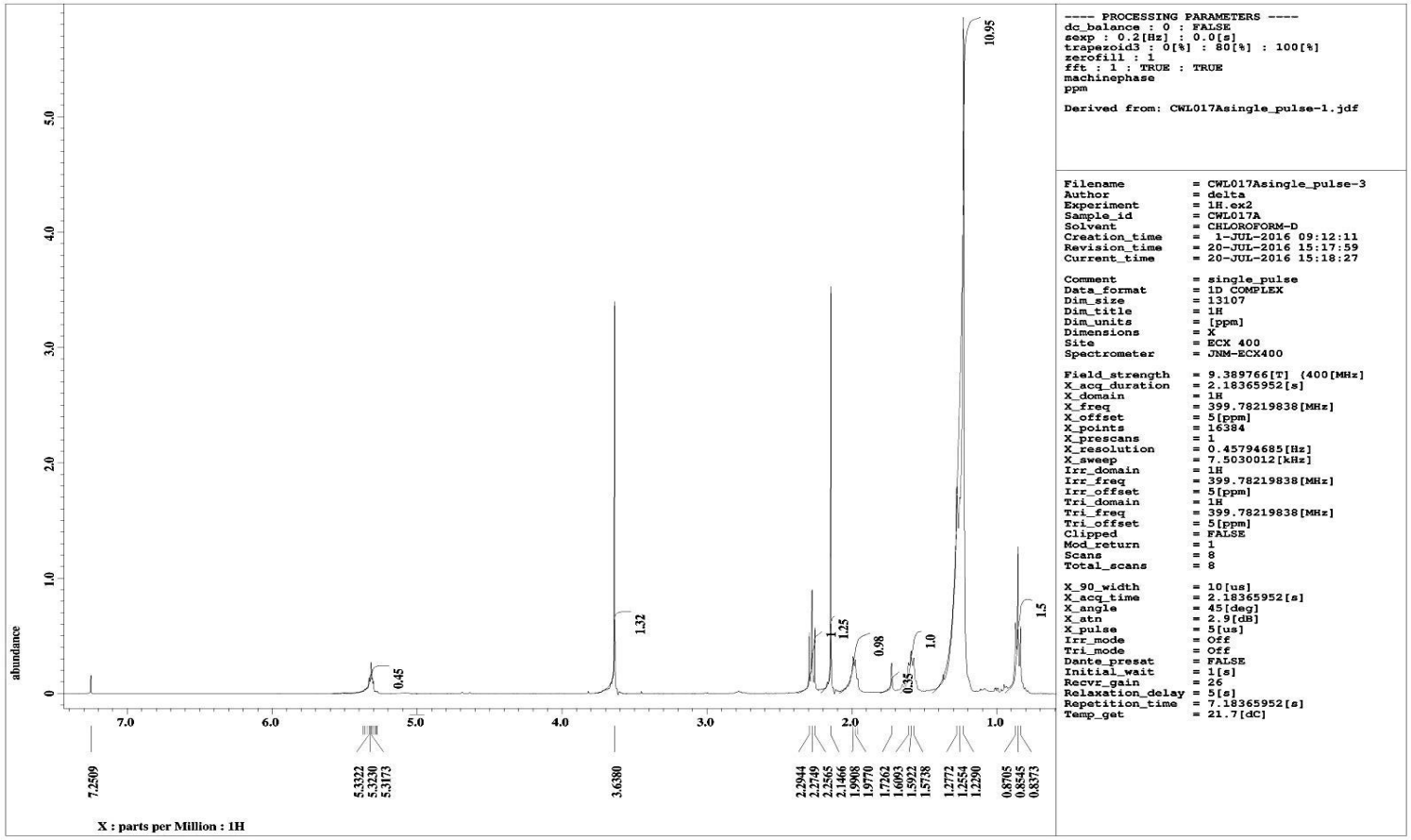


Figure 4.12: ^{1}H NMR Spectrum of Compound B ($CDCl_3$, 400 MHz)

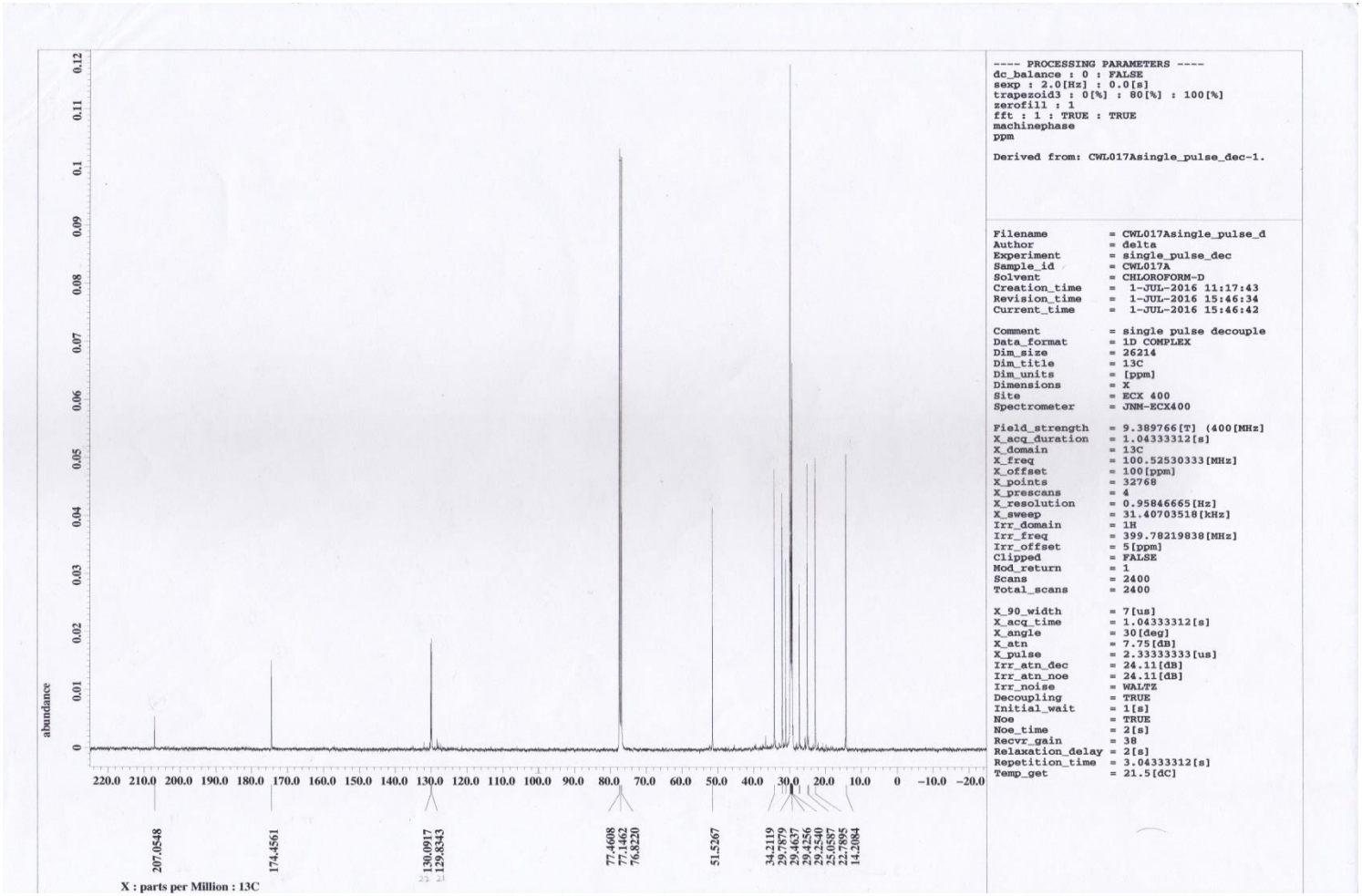


Figure 4.13: ^{13}C NMR Spectrum of Compound B ($CDCl_3$, 100 MHz)

The assignment of chemical shifts of carbon, proton and their correlations were tabulated in Table 4.5.

Table 4.5: ^{13}C and ^{1}H NMR data and their correlations in Compound B.

Carbon	δ_c	δ_H	HMBC
1	174.46	-	C-3, 15, 16
2	34.21	2.27(t, J = 7.6 Hz)	C-3
3	25.06	1.60 (m)	C-6
4	29.86	1.25 (s)	C-3, 5, 15
5	29.78	1.25 (s)	C-3, 5, 15
6	29.63	1.25 (s)	C-3, 5, 15
7	29.46	1.25 (s)	C-3, 5, 15
8	27.31	1.99 (m)	C-18
9	130.09	5.32 (m)	C-5
10	129.83	5.32 (m)	C-4
11	27.26	1.99 (m)	C-17
12	29.23	1.25 (s)	C-2, 4, 14
13	29.25	1.25 (s)	C-2, 4, 14
14	29.36	1.25 (s)	C-2, 4, 14
15	29.42	1.25 (s)	C-2, 4, 14
16	32.03	1.25 (s)	C-1, 2

17	22.79	1.25 (s)	C-1
18	14.21	0.85 (t, J = 6.6 Hz)	C-2
19	51.53	3.64 (s)	-

From the HMBC spectrum, carbon-1 which was a quaternary, carbonyl group carbon showed correlation with C-19. Carbon-19 at δ_c 51.53 ppm is a methoxy carbon, and its proton at δ_H 3.64 showed a singlet splitting, indicated that there are no neighboring protons around C-19. Therefore, it was suggested that the C-19 was only bonded directly to C-1, no other correlations were shown. As for carbon-19, it also shows further correlations with C-3 and C-2. The structure fragment of compound B is illustrated at Figure 4.14.

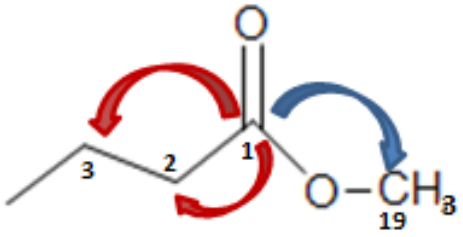


Figure 4.14: Fragment structure (ester functional group) of Compound B.

Besides, olefinic carbons (C-10, C-9) showed correlations with carbon at δ_c 27.26 and 27.31 ppm respectively, indicated that the olefinic carbons bonded directly to methylene carbons (C-11 and C-8). A chain of methylene carbons (C-7, 6, 5, 4) showed correlations with carbon-8, while another chain of methylene carbons (C-12, 13, 14, 15) were correlated with carbon-11, indicated that there were two methylene carbons chain attached to C-11 and C-8 respectively. The proposed fragment structure was shown in Figure 4.15.

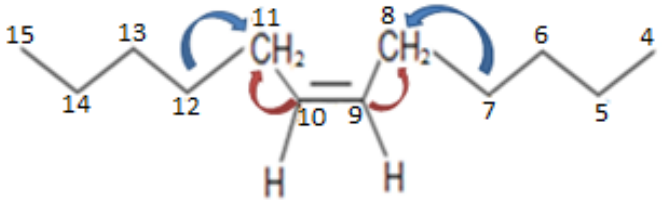


Figure 4.15: Fragment structure (alkene group) of Compound B.

According to the analysis of NMR data and IR spectrum, compound B was proposed to be a cis-9-octadecenoic acid, methyl ester. This can be further confirmed by the result obtained from ESI-MS, where the molecular mass showed 296 g/mol in mass spectrum, and this matched with the molecular mass of the proposed structure. Figure 4.16 shows the structure of cis-9-octadecenoic acid, methyl ester.

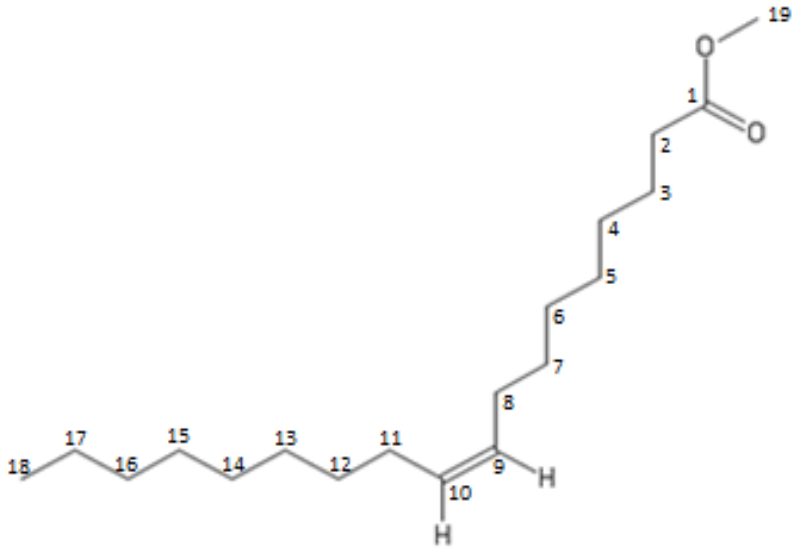


Figure 4.16: Structure of cis-9-octadecenoic acid, methyl ester.

4.4 Characterization of Compound C

Compound C was obtained as a yellowish oil (15.4 mg), with R_f value of 0.55 in 40% DCM: 60 % hexane. From the ^{13}C NMR spectrum, a total of 18 carbon peaks had been observed, and the type of carbons had been confirmed via DEPT spectrum. There were 2 methyl carbons, 9 methylene carbons, 6 methine carbons and 1 quaternary carbon revealed by DEPT. By referring to the chemical signals, 6 methine carbons in the range of δ_C 120 to 140 ppm has been assigned to be olefinic carbons.

Besides, a peak had been observed at δ_C 51 ppm and δ_C 174 ppm respectively, indicated that there was an alkoxy group as well as ester group present in compound C. According to DEPT spectrum, the carbon type at δ_C 51 ppm is methyl carbon, hence inferring that the alkoxy group is a methoxy carbon. The ^1H NMR and ^{13}C NMR are shown in Figures 4.17 and 4.18.

Characterization of compound C is incomplete due to time constraint, yet the fragment structure of compound B had been proposed in Figure 4.19. From ^1H NMR spectrum, the proton attached to methoxy carbon δ_H 3.65 ppm was observed without splitting (singlet). Based on the HMBC spectrum, the methoxy carbon

was correlated to the ester carbon δ_C 174 ppm, and it is suggested that the methoxy carbon is the end chain of the structure, therefore the methoxy proton shows only singlet splitting.

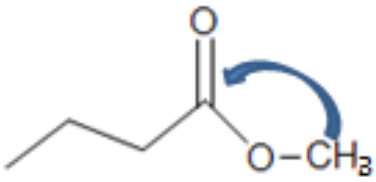


Figure 4.19: Fragment structure of compound C.

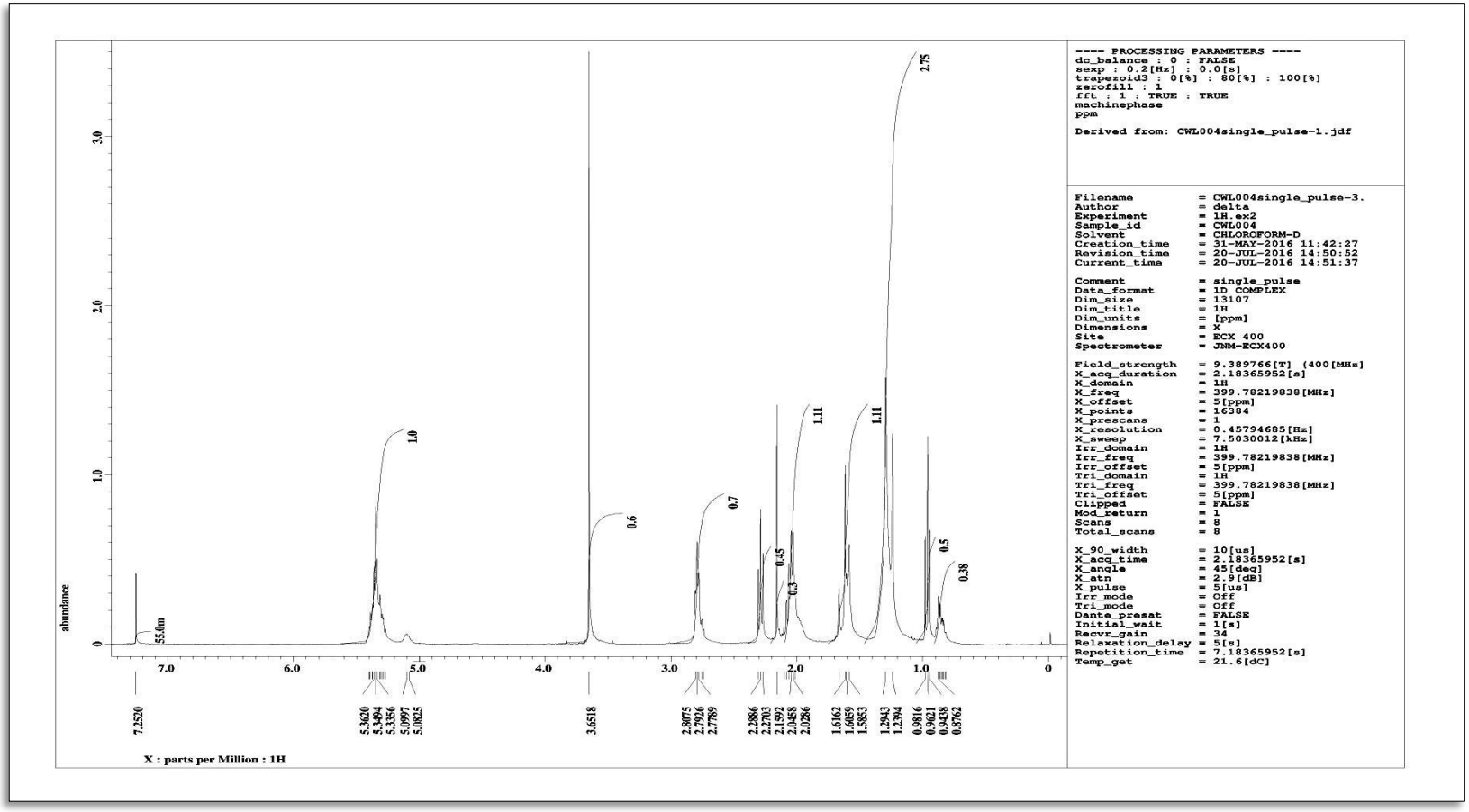


Figure 4.17: ^{1}H NMR Spectrum of Compound C ($CDCl_3$, 400 MHz)

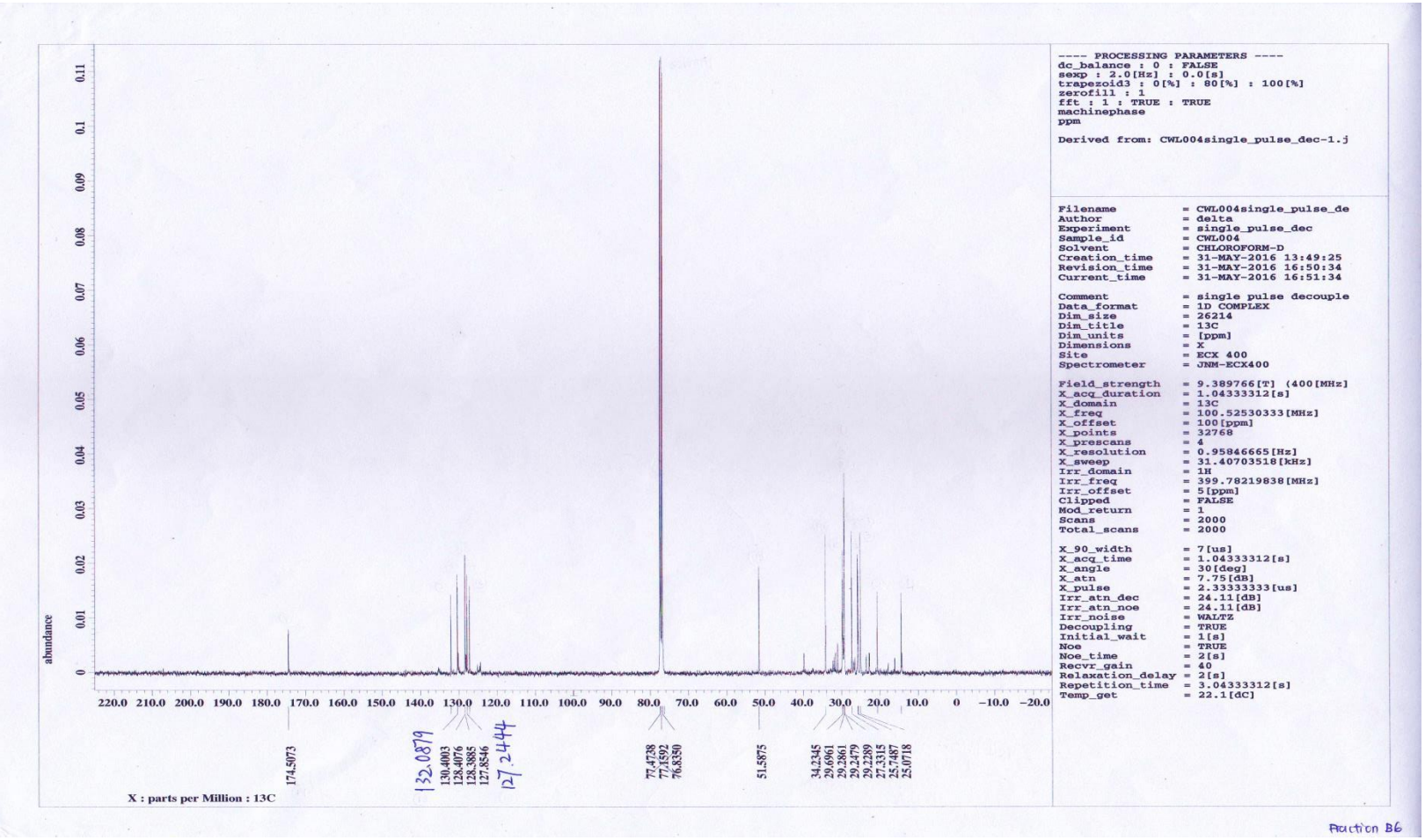


Figure 4.18: ^{13}C NMR Spectrum of Compound C ($CDCl_3$, 100 MHz)

CHAPTER 5

CONCLUSION

5.1 Conclusion

Two compounds and a mixture were isolated from the methanol leaves extract of *Swietenia macrophylla.* Chromatography technique such as thin layer chromatography, flash column chromatography and centrifugal thin layer chromatography were used to isolate and purify the compounds. Characterization of compounds were accomplished using various spectroscopic methods.

Based on the analysis, compound A was identified as a mixture of paraffin wax and squalene. The paraffin wax was suspected to be octacosane. Compound B was identified as cis-9-octadecenoic acid, methyl ester while the structure of compound C is unable to be completed due to time constraint. Partial structure was proposed for compound C suggested that compound C was a compound contain methyl ester functional group.

5.2 Future Study

More chemical constituents in the leaves of Swietenia *macrophylla* are expected to be isolated in future. The isolation of known and novel compounds is one of the major goals in future studies. Different types of isolation processes as well as advance chromatographic methods can be utilized in order to improve the isolation and separation process of the compounds.

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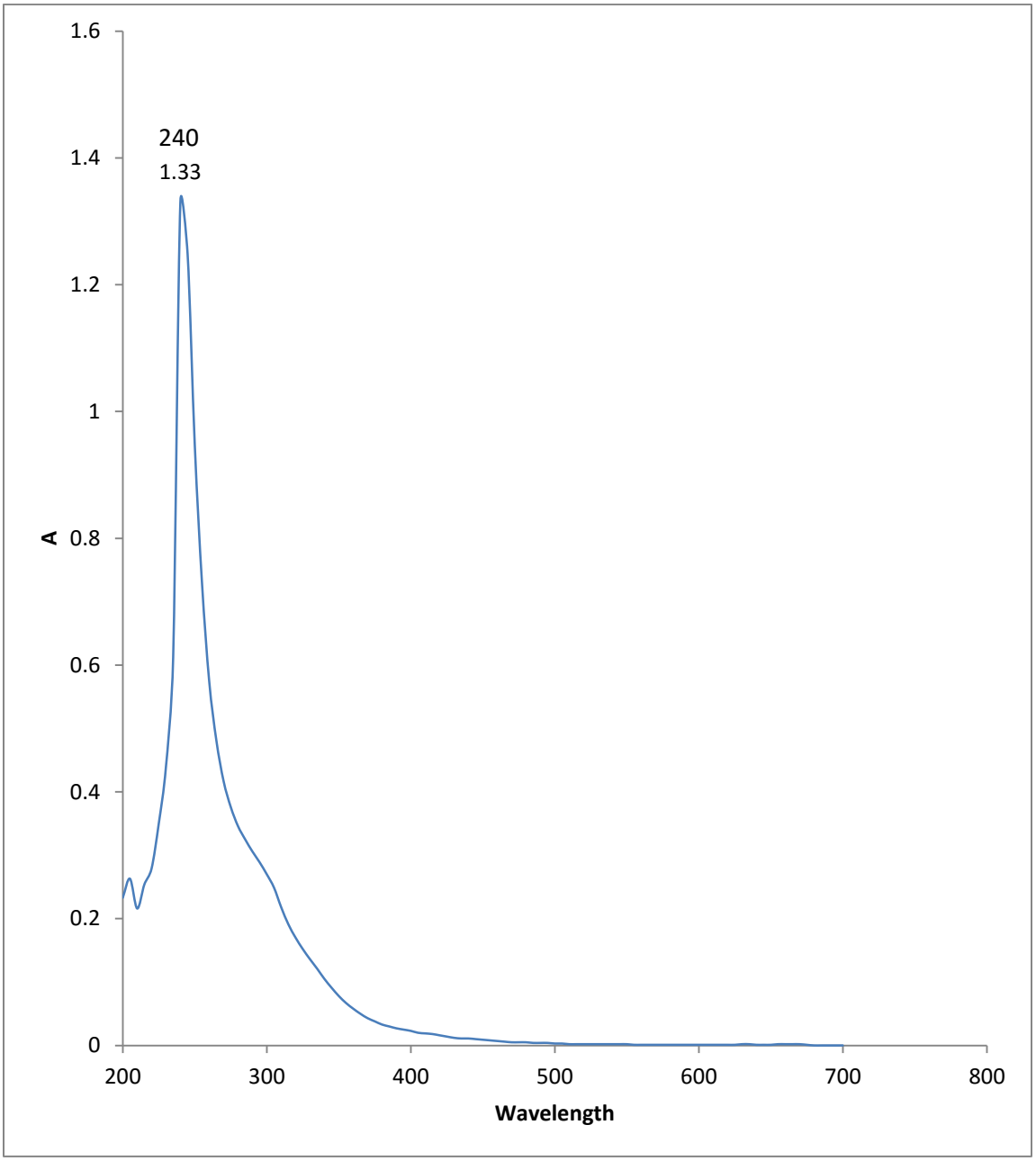
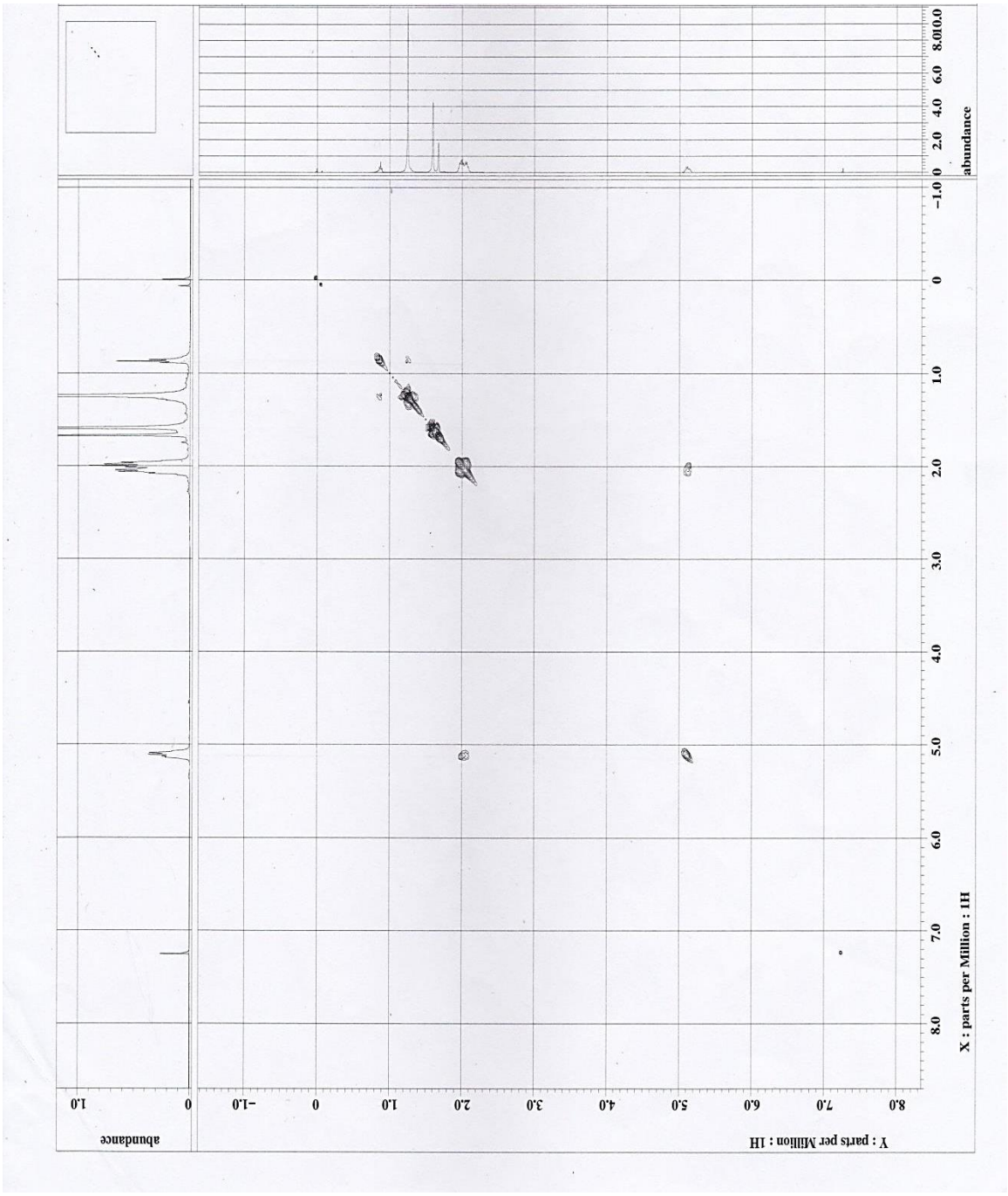
APPENDIX A

UV-Vis Spectrum of Compound A



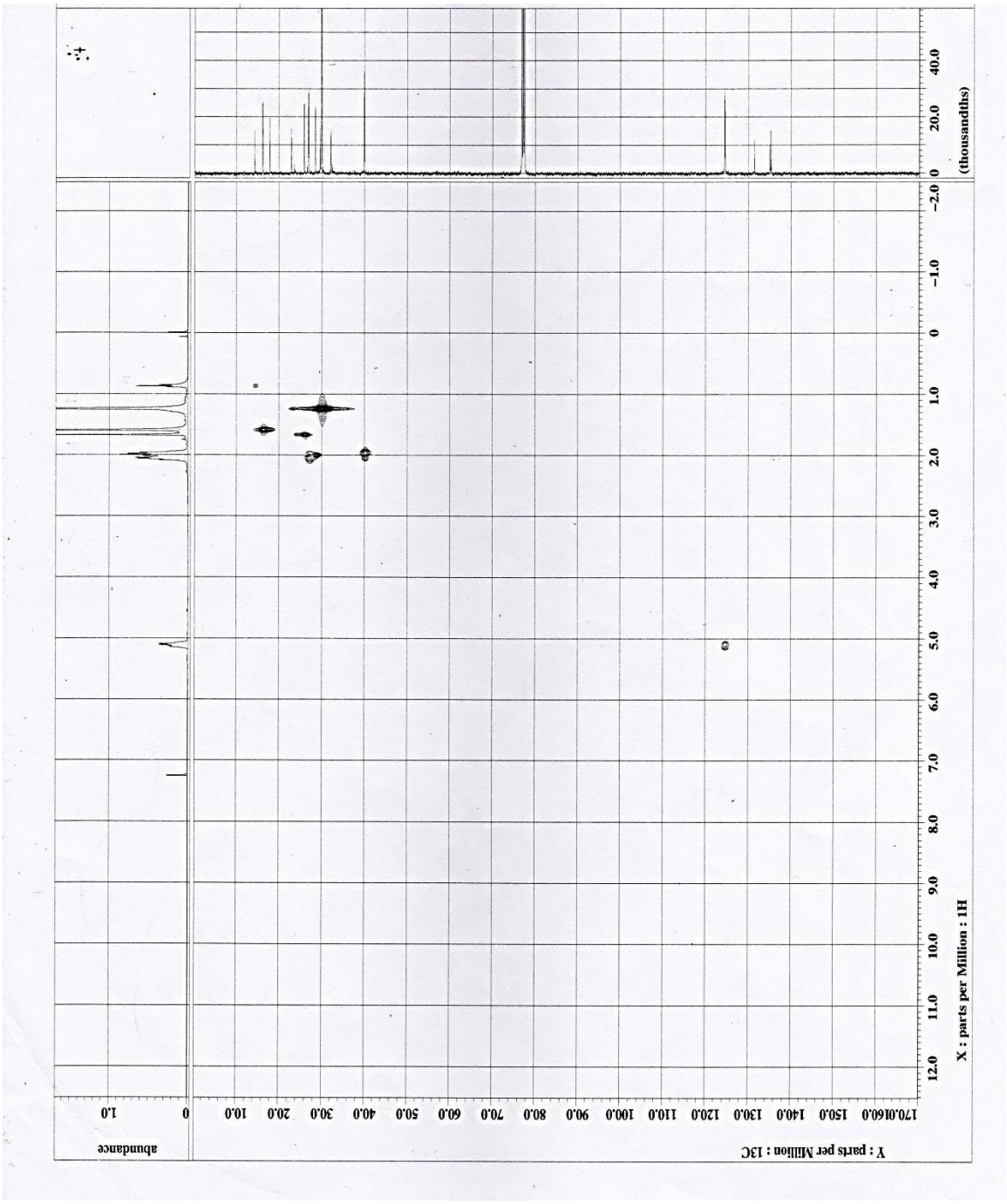
APPENDIX B

COSY NMR Spectrum of Compound A



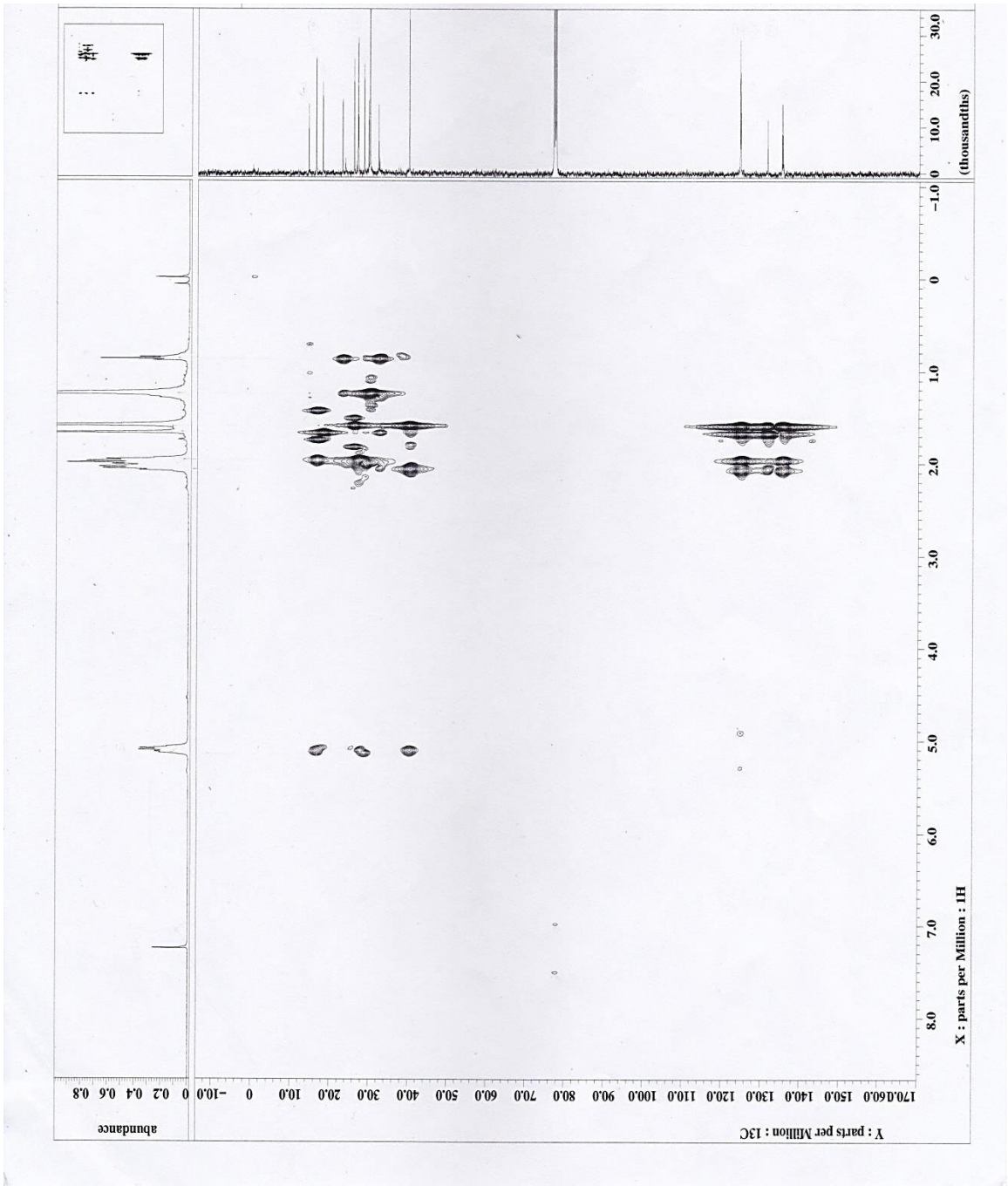
APPENDIX C

HMQC NMR Spectrum of Compound A



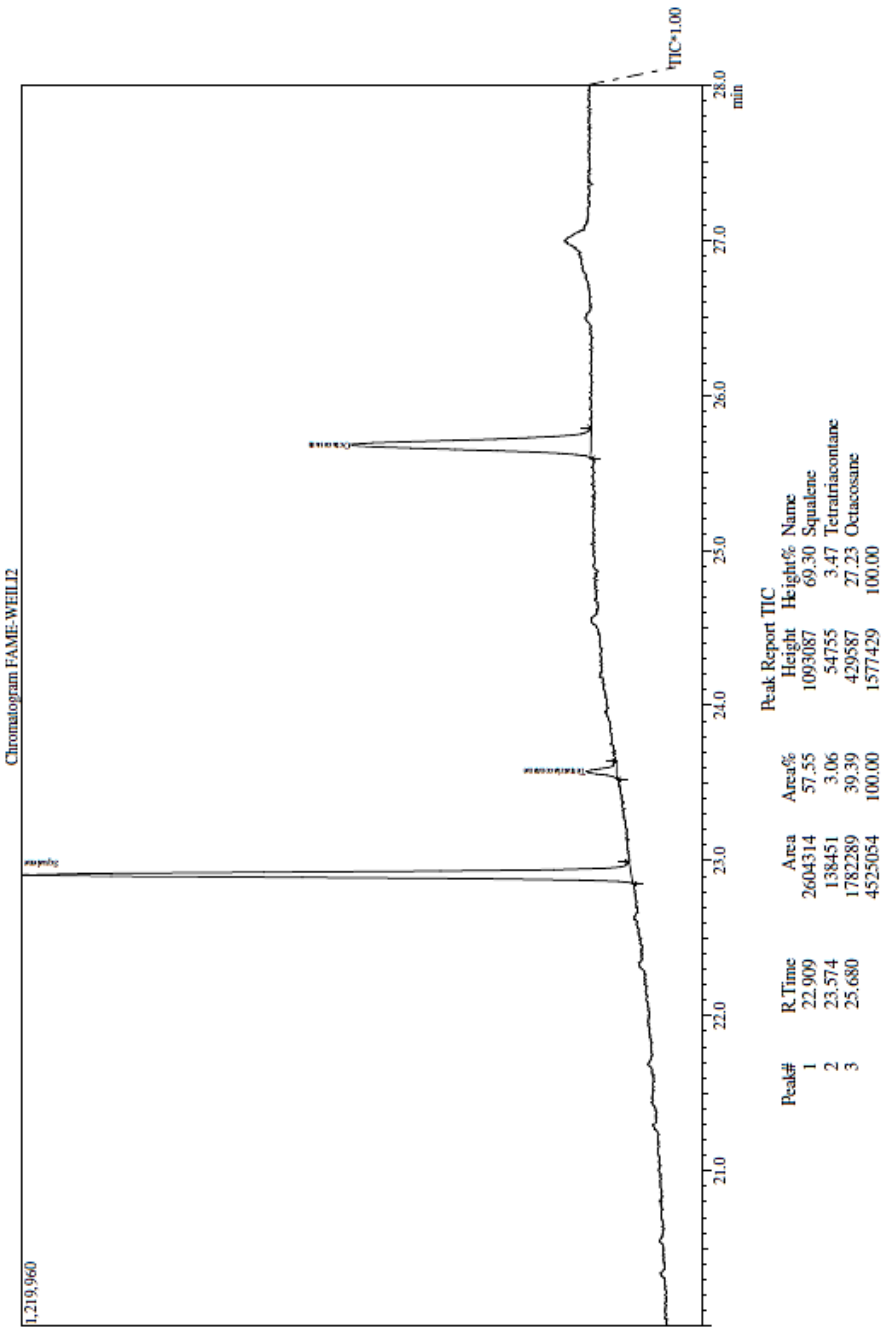
APPENDIX D

HMBC NMR Spectrum of Compound A



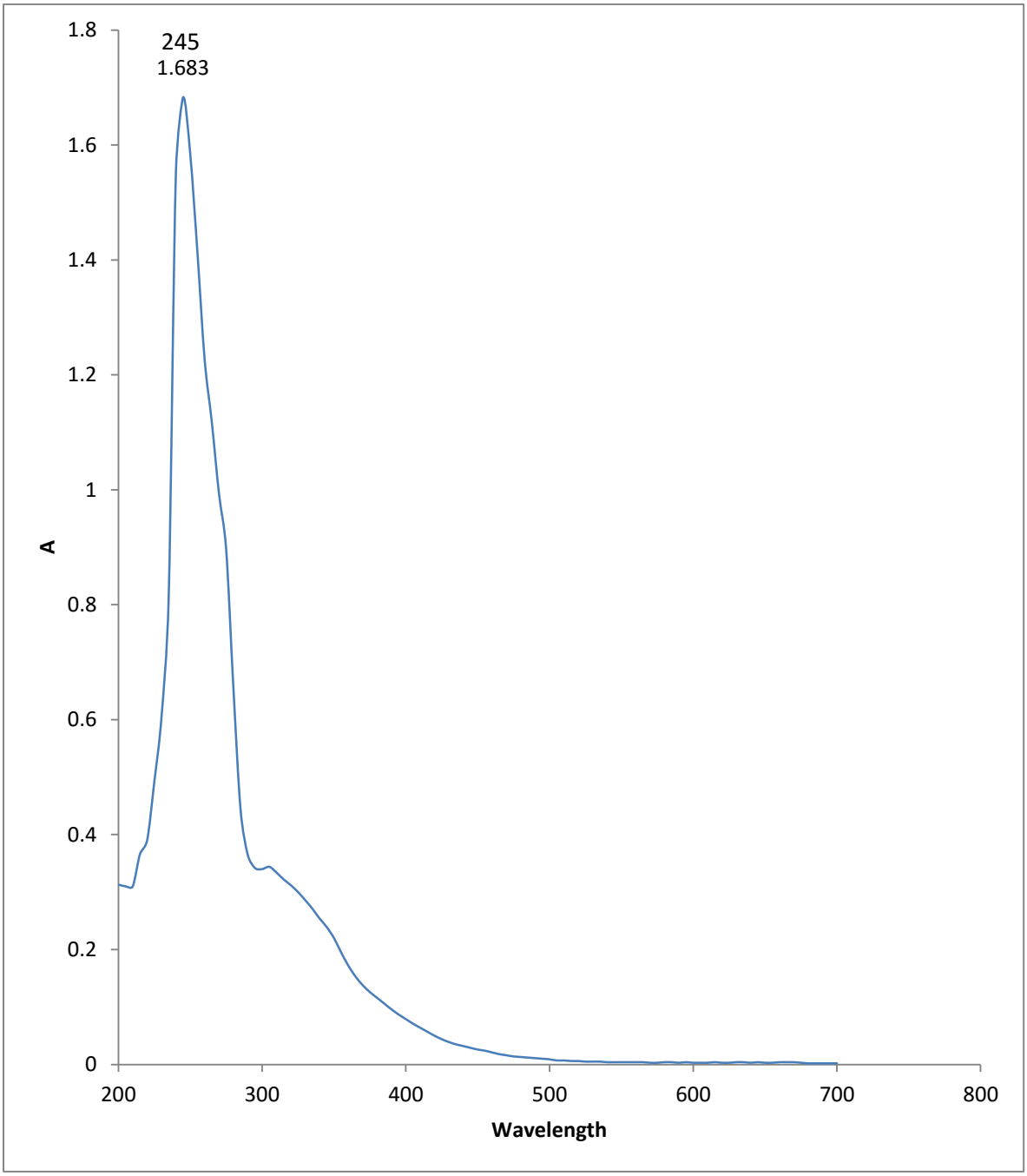
APPENDIX E

GC Spectrum of Compound A



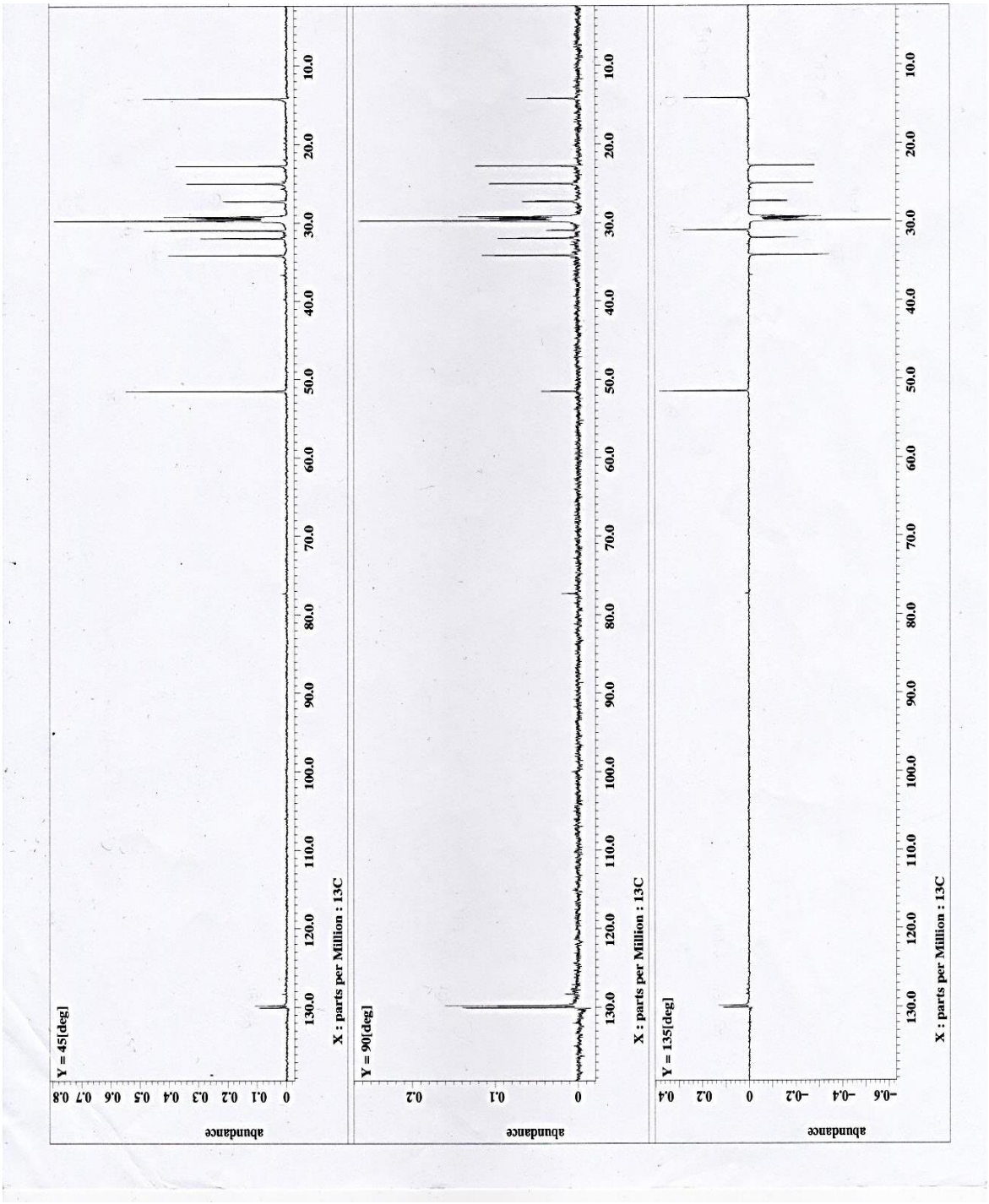
APPENDIX F

UV-Vis Spectrum of Compound B



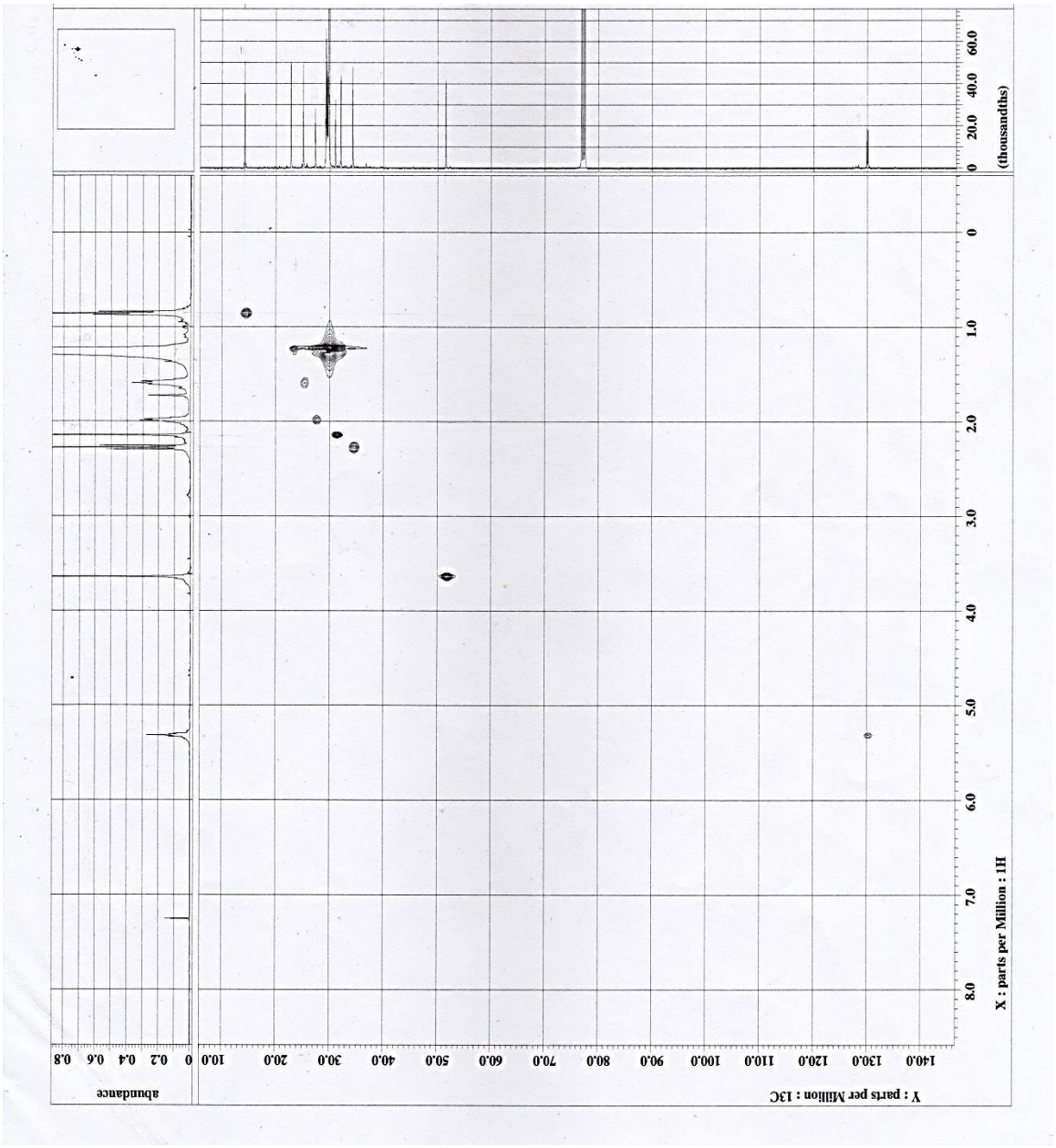
APPENDIX G

DEPT NMR Spectrum of Compound B



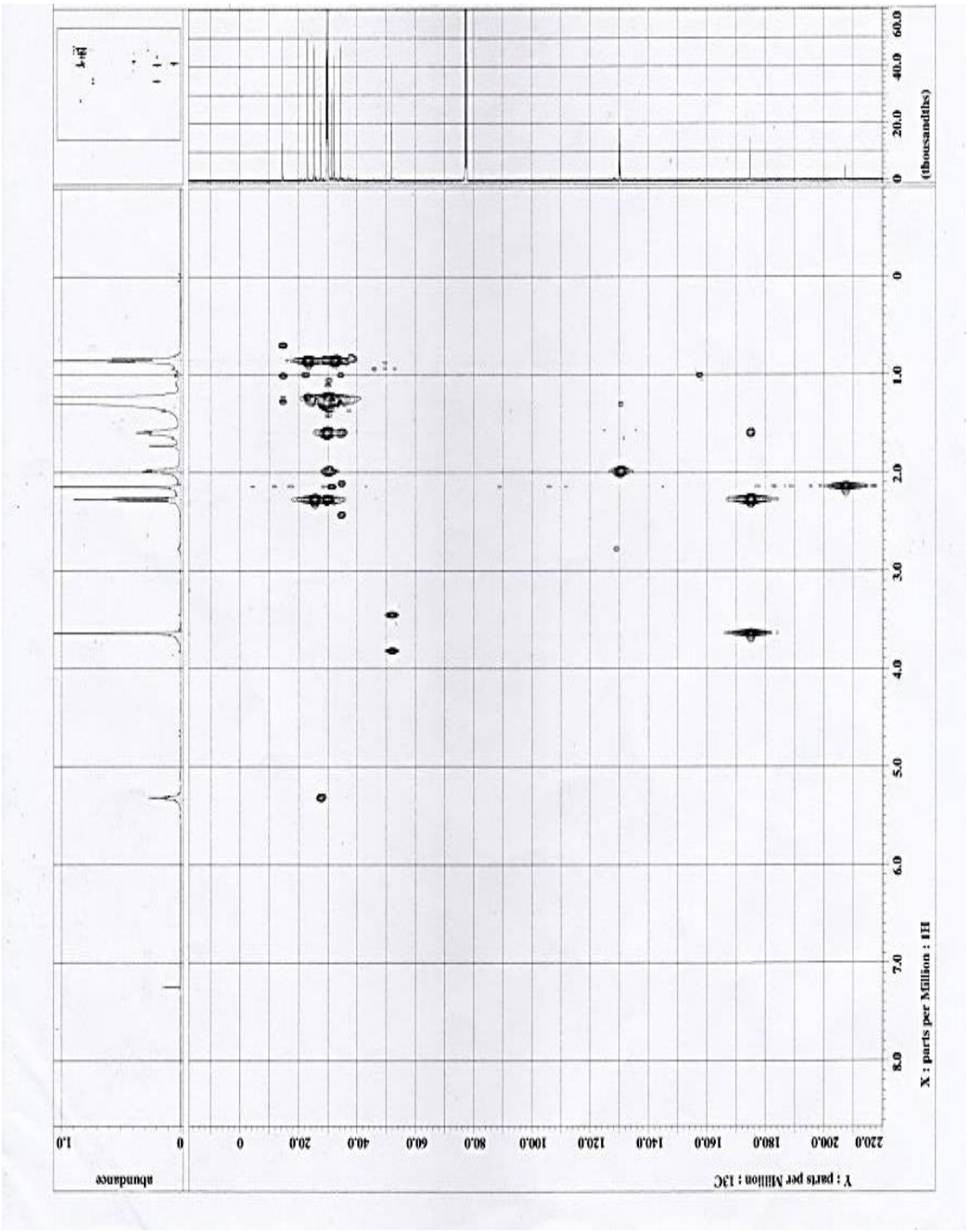
APPENDIX H

HMQC NMR Spectrum of Compound B



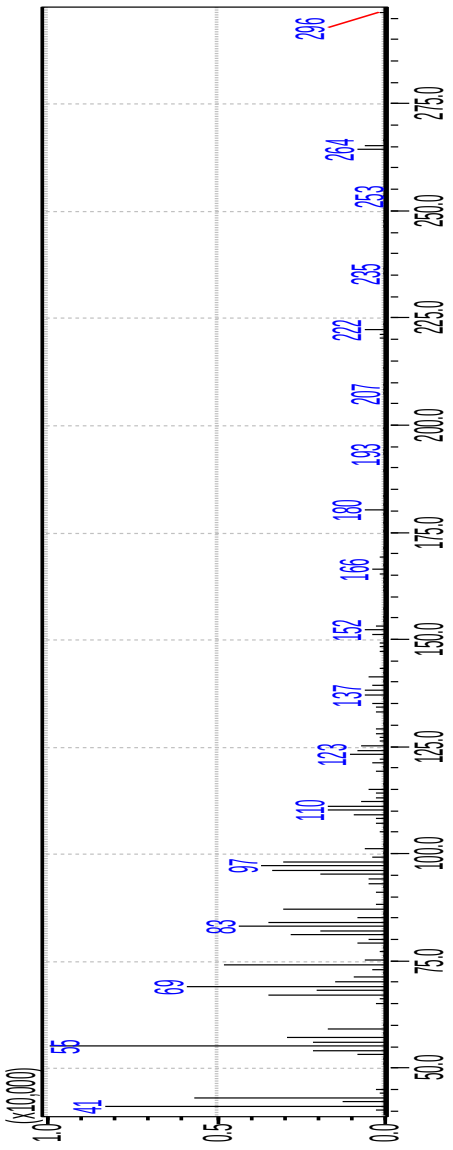
APPENDIX I

HMBC NMR Spectrum of Compound B



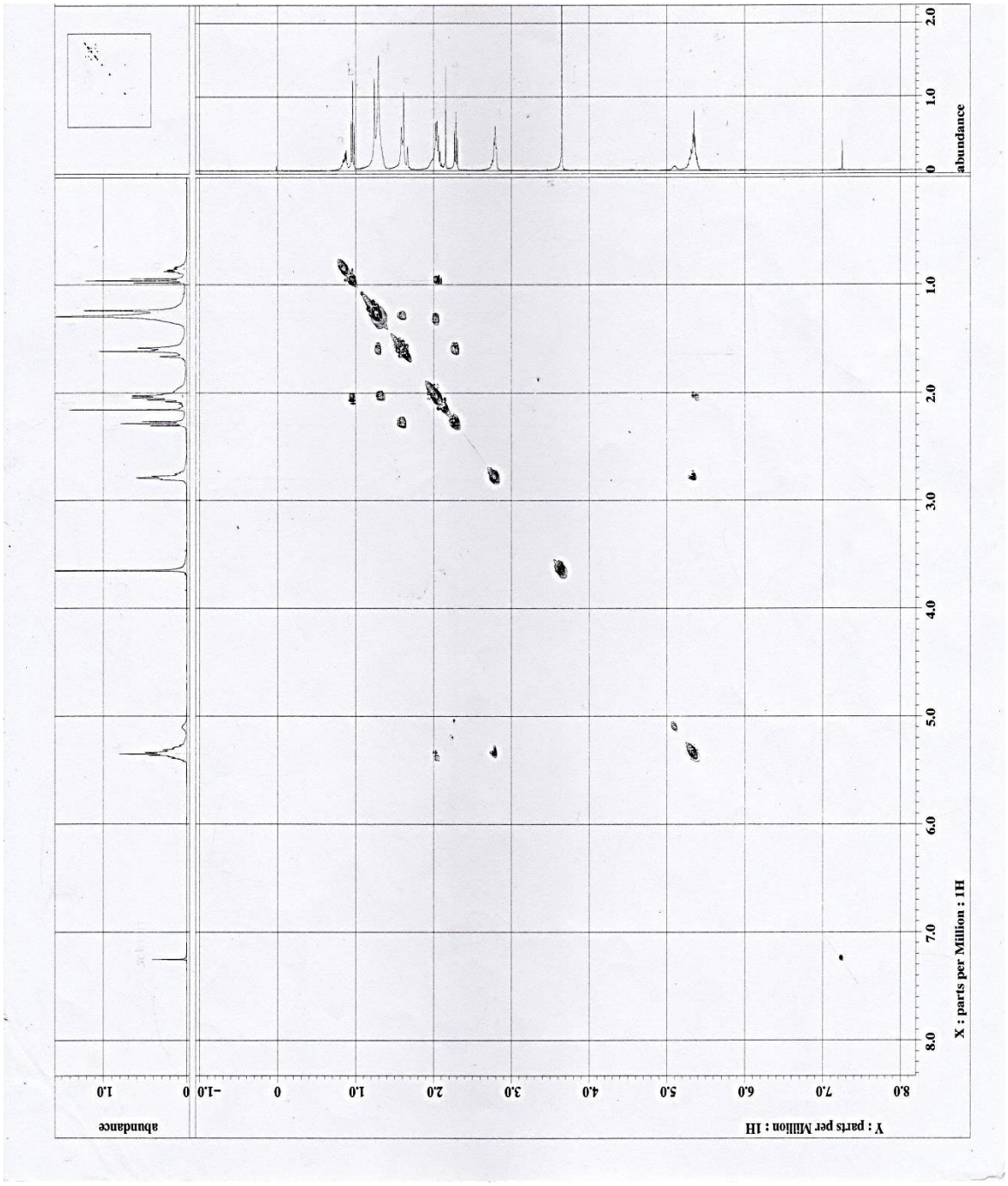
APPENDIX J

Mass Spectrum of Compound B



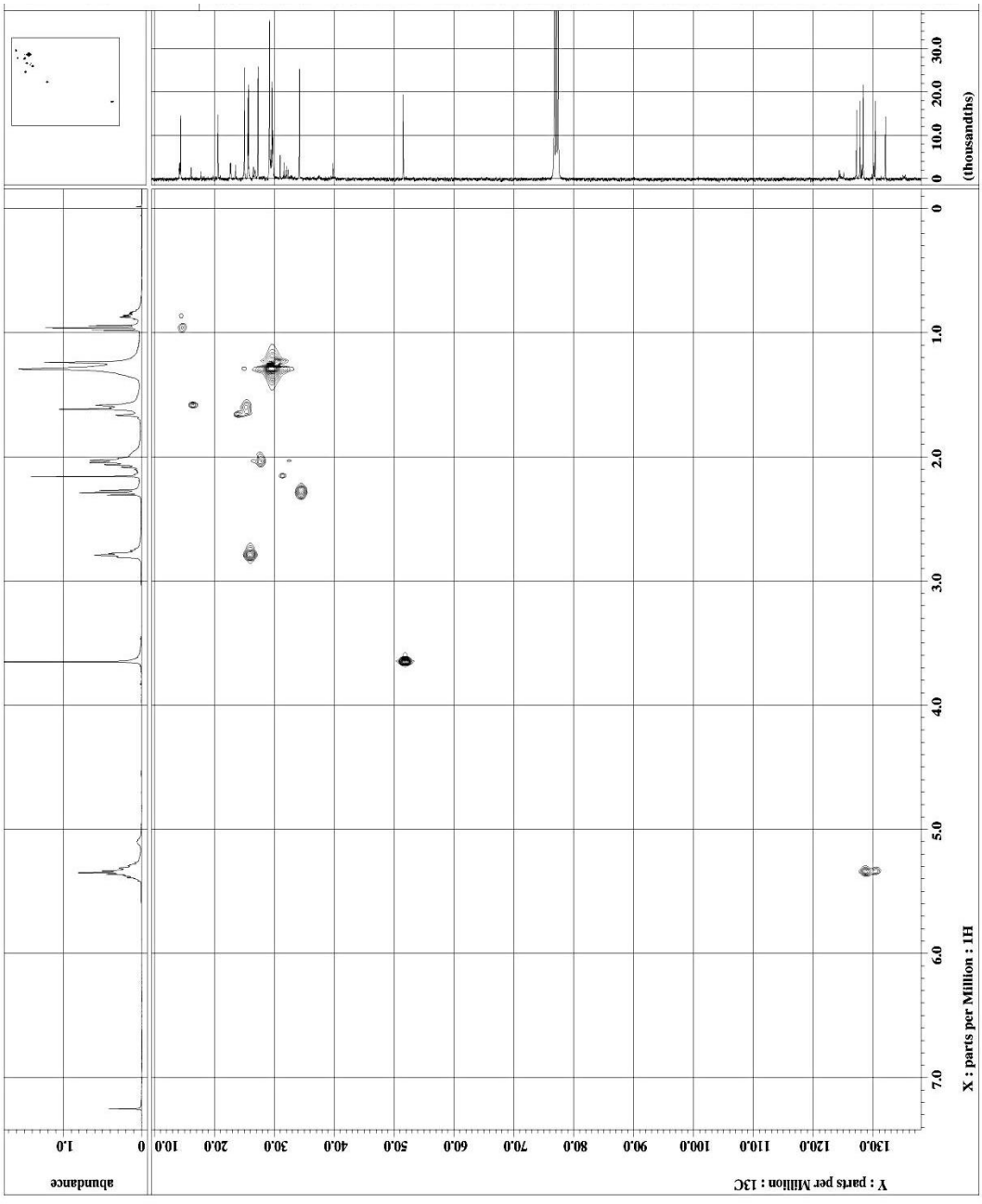
APPENDIX K

COSY NMR Spectrum of Compound C



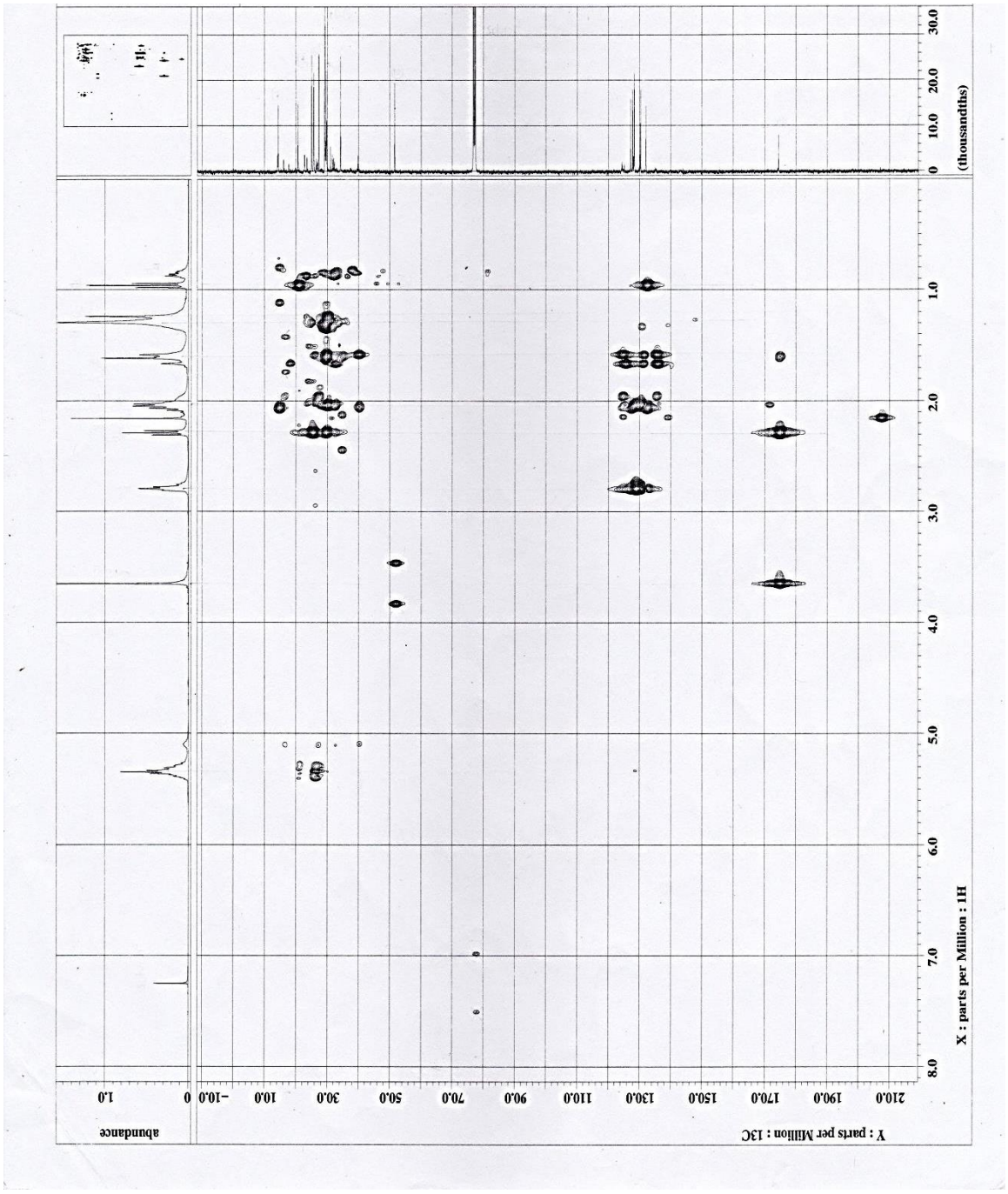
APPENDIX L

HMQC NMR Spectrum of Compound C



APPENDIX M

HMBC NMR Spectrum of Compound C



APPENDIX N

DEPT NMR Spectrum of Compound C