INVESTIGATION OF CHEMICAL CONSTITUENTS FROM *GARCINIA PARVIFOLIA* AND THEIR ANTIOXIDANT ACTIVITIES

By

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ABSTRACT

INVESTIGATION OF CHEMICAL CONSTITUENTS FROM *GARCINIA PARVIFOLIA* AND THEIR ANTIOXIDANT ACTIVITIES

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Garcinia species are rich in phenolic compounds which are potential antioxidants. In line with the search for natural antioxidant, the stem bark of *Garcinia parvifolia* was investigated for its phytochemicals and antioxidant activity. The plant material was subjected to sequential solvent extraction by using dichloromethane, ethyl acetate and methanol. The crude extracts obtained were subsequently fractionated and purified via column chromatography to give pure compounds. From the crude ethyl acetate extract, three xanthone derivatives and a terpenoid were successful isolated, namely brasixanthone B **[67]**, 1,3,6-trihydroxy-2,4-bis(3-methylbut-2-enyl)xanthone [**68]**, rubraxanthone **[69]** and tetraprenyltoluquinone (TPTQ) **[70]**. The structures of isolated compounds were characterized and elucidated via various spectroscopic techniques including 1D- and 2D-NMR, LC-MS, UV-Vis and IR analyses. In the DPPH assay, the crude dichloromethane and ethyl acetate extracts showed significant antioxidant activity with IC_{50} values of 40.0 and 44.5 µg/ml, respectively. Meanwhile, isolated compounds **68** and **69** were tested to give weak activity with IC_{50} values of 185 and 195 µg/ml, respectively, whereas compounds **67** and **70** were found to be inactive in the assay showing IC_{50} values of more than 240 µg/ml.

ABSTRAK

Spesies *Garcinia* adalah kaya dengan kompaun fenolik yang berpotensi antioksidan. Selaras dengen mencari antiokdisen semula jadi, kulit kayu batang *Garcinia parvifolia* telah disiasat bagi fitokimia dan aktiviti antioksidan. Bahan tumbuhan tetah diestrak berturutan dengan menggunakan diklorometana, etil asetat and metanol. Ekstrak mentah yang diperolehi kemudiannya diasingkan dan melalui kolum kromatografi untuk menghasilkan kompaun tulen. Daripada ekstrak etil asetat, tiga xanthona derivatif dan satu terpenoid telah diasingkan, iaitu brasixanthone B **[67]**, 1,3,6-trihydroxy-2,4-bis(3-methylbut-2-enyl)xanthone **[68]**, rubraxanthone **[69]** and tetraprenyltoluquinone (TPTQ) **[70]**. Struktur-struktur kompaun diperolehi telah dikenal pastikan melalui pelbagai spektroskopi analisis, termasuklah 1D- and 2D-NMR, LC-MS, UV-Vis dan IR. Dalam kaedah DPPH, ekstrak mentah diklorometana dan etil asetat menunjukkan ciri-ciri antioksidan yang nyata dengan nilai IC_{50} iaitu 40.0 and 44.5 μg/ml. Sementara itu, kompaun 68 dan 69 menunjukkan activiti yang lemah dengan nilai IC_{50} iaitu 185 and 195 μg/ml manakala kompaun 66 dan 70 didapati tidak aktif dalm ujian tersebut, menunjukkan nilai IC_{50} yang melebihi 240 μg/ml.

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DECLARATION

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

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(CHIA LUN CHANG)

APPROVAL SHEET

The project report entitled "INVESTIGATION OF CHEMICAL CONSTITUENTS FROM *GARCINIA PARVIFOLIA* AND THEIR ANTIOXIDANT ACTIVITIES" was prepared by CHIA LUN CHANG and submitted as partial fulfillment of the requirements for the degree of Bachelor of Science (Hons) Chemistry at Universiti Tunku Abdul Rahman.

Approved by:

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(Dr. Lim Chan Kiang)

Supervisor

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UNIVERSITI TUNKU ABDUL RAHMAN

Date: _____

PERMISSION SHEET

It is hereby certified that **Chia Lun Chang** (ID No: **12ADB02637**) has completed this final year project entitled "**INVESTIGATION OF CHEMICAL CONSTITUENTS FROM *GARCINIA PARVIFOLIA* AND THEIR ANTIOXIDANT ACTIVITIES**" under the supervision of Dr. Lim Chan Kiang 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,

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(CHIA LUN CHANG)

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LIST OF ABBREVIATIONS

DPPH	1,1-diphenyl-2-picrylhydrazyl
BPX5	5% phenyl 95% methyl polysilphenylene / siloxane phase
A_0	Absorbance of the blank (negative control)
A_1	Absorbance of the test sample
α	Alpha
β	Beta
C=C	Carbon=Carbon
^{13}C	Carbon-13
C-H	Carbon-Hydrogen
C-O	Carbon-Oxygen (or Carbinol)
C=O	Carbon-Oxygen (or Carbonyl)
cm	Centimeter
δ	Chemical shift
δ_C	Chemical shift of carbon
δ_H	Chemical shift of proton
c	Concentration of sample in g/mL
J	Coupling constant in Hertz
^oC	Degree in Celsius
Acetone-d_6	Deuterated acetone
$CDCl_3$	Deuterated chloroform
DCM	Dichloromethane
d	Doublet
dd	Doublet of doublets
EtOAc	Ethyl acetate
FTIR	Fourier-Transform Infrared Spectroscopy
GC-MS	Gas Chromatography-Mass Spectrometry
IC_{50}	Half maximal inhibitory concentration

Hz	Hertz
J_{CH}	Heteronuclear coupling between carbon and proton
HMBC	Heteronuclear Multiple Bond Coherence
HMQC	Heteronuclear Multiple Quantum Coherence
HPLC	High Performance Liquid Chromatography
HRESIMS	High Resolution Electrospray Ionization Mass Spectrometry
IR	Infrared
kg	Kilogram
LC-MS	Liquid Chromatography-Mass Spectrometry
m/z	Mass-to-charge ratio
λ_{max}	Maximum wavelength
µg	Microgram
µL	Microliter
µmol	Micromole
mg	Miligram
mL	Mililiter
mm	Milimeter
mM	Milimole
mol	Mole
m	Multiplet
nm	Nanometer
NMR	Nuclear Magnetic Resonance
1D-NMR	One Dimension Nuclear Magnetic Resonance
O-H	Oxygen-Hydrogen (or Hydroxyl)
ppm	Part per million
KBr	Potassium bromide
^{1}H	Proton
R_f	Retention factor
s	Singlet

TMS	Tetramethylsilane
TLC	Thin Layer Chromatography
TPA	Tissue Plasminogen Activator
t	Triplet
2D-NMR	Two Dimension Nuclear Magnetic Resonance
UV-Vis	Ultraviolet-Visible

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Nature has been the major source of medicinal agents which provides ethnomedicinal properties for thousands of years. Historically, natural products play a prominent role as natural medicine in therapeutic development. Natural products are the organic compounds produced by living organisms, mostly derived from plant species that usually possess pharmacological or biological effects (James, 2012). Phytochemistry is a sub-division of natural product chemistry which focuses on the study of plant derived metabolites.

Metabolites are known as the intermediates in the metabolic process in nature which can be classified into primary and secondary metabolites (Ana, 2012). The examples of primary metabolites are protein, carbohydrate, lipids, nucleic acids and enzyme. Primary metabolites are involved in the normal growth, development, respiration and reproduction which are typically crucial for the survival and metabolic processes of a living organism (James, 2003). Meanwhile, secondary metabolites are chemical compounds derived from living organisms, which are not directly involved in metabolic processes, and apparently not necessary for the survival, growth, development or reproduction process.

The common classes of secondary metabolites are xanthones, terpenoids, flavonoid, depsidone and phloroglucinol. Secondary metabolites perform several important functions, for instance, communication purpose, mediation of spatial competition, prevention of fouling, protection against UV radiation and chemical signaling to enhance adaptability to surrounding environment (Sonia, 2006). They are normally produced from complex combinations of biosynthetic pathways in order to defense against predators and pathogens, in providing reproductive advantage as intraspecific and interspecific attractants, and to create competitive advantage as poisons of rival species (Hailemichael, 2005). Simple principle building blocks such as amino acids, acetate, shikimate and mevalonate are often constructed in the form of secondary metabolites (Ma, 2011).

Plant kingdom has been well represented for their medicinal purposes over millions of years due to their ethnopharmacological properties which play an important role in the major sources of drug development (Phillipson, 2001). According to the World Health Organization (WHO News, 2002), the majority of people in the world are heavily relying on herbal medicine which serves as the sources of health products for their primary health care.

Throughout history, the first compendium of medical herbal in China, *Pen-Tsao*, which covered around 365 types of drugs, was compiled by Shen Nung in 2800 BC (Ari, 1993). Traditional Chinese Medicine such as Ginseng and Ling Zhi were used for the purpose to alleviate diabetes and stabilize blood pressure. Other important discovery was the introduction of medicinal mushrooms as anti-cancer

drug by the Japanese (Raymond and George, 2015). In addition, ancient civilizations of both China and India have provided written evidence for the employment of natural product in the medicine system. The earliest known written describing the remedies used for treatment of different kinds of diseases was recorded on a 4000 year old Sumerian clay tablet (Kong, et al., 2003). Besides, one of the best-known pharmaceutical records that included around 700 drugs was found in Egypt dated from 1500 BC (Wong, et al., 2009).

The early traditional medicines were mostly derived from natural plants through chemicals and pharmacological researches (Sarker and Nahar, 2012). Some examples of traditional medicines that were used in ancient time such as the mandrake as pain relief agent, turmeric as blood clotting drugs, and circulatory disorders can be treated by raw garlic. About nine pure natural products were isolated as anticancer agents between the years 1981 to 2006, such as vincristine and vinblastine (Newman and Cragg, 2007). In the early history, researchers utilized the advantages of natural compounds in order to investigate and study the natural medicine for drug application in the medicinal development.

Morphine, quinine and penicillin are among the famous and the earliest chemical compounds that were isolated as natural products. Morphine is an alkaloid which was introduced in 1826 as the first commercialized therapeutic agent by Merck. Morphine was derived from opium poppy *Papaver somniferum* for the purpose of pain relief during ancient Mesopotamia (James, 2003). From the bark of *Cinchona*, quinine was isolated as antimalarial drug in 1820 (Phillipson, 2001). Through

historical experiences, natural products also play an important role in development of new drug in the area of cancer and infectious diseases. For instance, vincristine and vinblastine were separated from *Catharanthus roseus,* which have been introduced as effective antineoplastic agents in 1994 (Sahu, et al., 2004).

Malaysia is a country in the tropical rain forest zone, with huge diversity of flora which potentially to be rich sources of medicinal herbs. In terms of commercialization, approximately 3700 pure compounds have been isolated from 15000 known plant species for applications as food additives and traditional medicine. The earliest report on phytochemicals screening in Malaysia was presented by Arthur in 1954, which included a total of 205 plants in Sabah (Bala and Ahmed, 2008). Moreover, the isolation of a series of pure compounds from *Guttiferae* species was reported by Goh et al., such as prenylated xanthones, neoflavonoids and biflavonoids in 1990s. The availability of large diversity of Malaysia flora catalyzed a rapid development in phytochemical research, subsequently enhanced the pharmaceutical drug discovery and medicinal system successfully (Phillipson, 2001).

Therapeutic drugs discovered from natural products were found to possess various biological activities such as anti-inflammatory, antimalarial, antineoplastic, antioxidant, antiplasmodial, antifungal, as well as anti-microbial (Goutam, 2009). The common techniques used in the natural products research nowadays are extraction, separation and isolation, and structural elucidation by using spectroscopic and spectrometric methods (Dias, Urban and Roessner, 2012). The

development of technologies such as chromatographic and spectroscopic techniques (UV-Vis, IR, MS and NMR) has enhanced the skills of structural elucidation and characterization of pure compounds (Tsuda, 2004).

The discovery of drugs derived from natural products has become more significant in the medicine system to serve as therapeutic agent in the years to come. Minimal side effects become the main reason that natural products are widely used in the pharmacological and medicinal field. In 21st century, the demand of natural products as health-care products is at all-time high as it possesses different significant values in different fields of study (Goutam, 2012). Thus, in future, natural products will remain as main character in meeting these demands through the study of world's biodiversity which has yet to be explored. Further investigation and development have become more significant in the aspects of natural products. However, there are several challenges in the research of natural products nowadays. The loss of biodiversity and development of the western drugs have become the main challenge for the natural products research (Rates, 2001).

1.2 Botany of Plant Species Studied

1.2.1 Taxonomy

Clusiaceae is a family of plants belongs to the order Malpighiales comprising of 37 genera and 1610 species of trees and shrubs, usually with milky sap and fruits or capsules for seeds. *Garcinia* is one of the largest genus in the Guttiferae family

that comprises of about 250 species which are found to confine to the warm humid tropics of the world (Bonnie, 1996). The plant studied, *Garcinia parvifolia* is classified as a member of Clusiaceae (or Guttiferae) family. Table 1.1 shows the taxonomy of *Garcinia parvifolia*.

Table 1.1: Taxonomy of *Garcinia parvifolia*

Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Malpighiales
Family	Clusiaceae / Guttiferae
Genus	*Garcinia*
Species	*Garcinia parvifolia*

1.2.2 Morphology

Garcinia parvifolia is an evergreen tree, usually small to medium-sized which grows up to 5 - 33 m high with a bole of about 23 cm, and it has a rather rough bark. The branches of *Garcinia parvifolia* are normally black and they contain yellow latex (Bonnie, 1996). Their leaves are often simple, opposite, glabrous with dark green above and paler green below. It is normally in 3.5 – 17 cm long, 2-7 cm wide, and the petiole is 0.5 -1.5 cm long with a shallow furrow on the anterior side. The flowers of the plants are 0.5 – 1 cm across which can be unisexual, monoecious or polygamous and they are pale yellow in colour. The sepals are pale

orange colour with 2.5 – 3 mm long. The petals are with 0.5 cm long usually pale yellow or light pink in colour (Lim, 2012). Generally, the fruits produced from *Garcinia parvifolia* are cherry-like which change from yellow to red when they are ripe. The flesh contributes about 63% of the total weight (11 – 19 g) of fruits. The flesh of fruits is similar to mangosteen-taste which is juicy, sweet and sours. Figure 1.1 illustrates the tree and fruits of *Garcinia parvifolia*.

Figure 1.1 *Garcinia parvifolia* tree and its fruits

1.2.3 Geographical Distribution and Habitat

Garcinia belongs to family of Clusiaceae, is native in tropical and subtropical countries of South East Asia such as Thailand, Brunei, Malaysia and Indonesia (Ittipon, et al., 2014). The plant studied, *Garcinia parvifolia* is widely distributed in tropical Africa, Asia, New Caledonia, and Polynesia (Vatcharin, et al., 2006). *Garcinia parvifolia* grows well in humid tropical environment and it is commonly found in primary and secondary forest and pear swamp forest (Lim, 2012). The cherry-like fruit produced by *Garcinia parvifolia* is locally known as ‘asam kandis’ or ‘asam kundong’ in Sabah and Sarawak (Siti, Jeffrey and Mohd, 2013).

1.2.4 Ethnomedicinal Uses and Pharmacological Studies

The wood of the trees are a source of resin. A wide variety of natural products including xanthones, triterpenes, flavonoids, coumarins, chalcones, depsidones and phloroglucinol were reported for their isolation from the genus of *Garcinia*. Phytochemical content of the plant *Garcinia parvifolia* was investigated due to their ethnomedicinal and pharmaceutical potentials (Leonardus, et al., 2006). The phytochemicals isolated from various parts of *Garcinia parvifolia* were found to possess biological and pharmacological properties such as antioxidant, antimicrobial, anticancer, antiplatelet, antiplasmodial and anti-inflammatory activities and were used for treatment of allergic reaction (Ee and Cheow, 2007).

One of the recent studies reported that the extracts obtained from *Garcinia parvifolia* are potential antifungal and antimalarial sources (Lathifah, et al., 2010). Some chemical researches also reported that depsidones isolated from the leaves of *Garcinia parvifolia* provides cytotoxic effect. The reported depsidone is garcidepsidone which was isolated from the chloroform soluble fraction of the plant (Xu, et al, 2000). Later, in 2006, a phloroglucinol isolated from the twigs of *Garcinia parvifolia*, namely parvifoliol E was reported to show good antioxidant activity, showing an IC_{50} of 0.02 μM. (Vatcharin, et al., 2006)

1.3 Problem Statement

Antioxidant is a molecule that inhibits oxidation that caused by free radicals. These free radicals normally induce oxidative stress and cause cellular damage and increase the risk of cancer. Therefore, some natural compounds which show antioxidant property provide protection against free radicals by stabilizing them to minimize the cell damage. Some secondary metabolites such as phenolic compounds are found to be good antioxidant in nature. Natural antioxidants are highly demanded in the worldwide market (Stoia and Oancea, 2011).

Nowadays, synthetic antioxidants are produced as alternatives to natural. However they exert toxic side effects especially at high concentration. It might cause pathological and carcinogenic effects (Rashmi, 2011). In addition, development of synthetic antioxidants is a costly and lengthy process. Thus, most of the synthetic antioxidants were not employed as food additive as they are not as health friendly as the natural antioxidants.

In line with the search for new natural antioxidants, *Garcinia parvifolia* was phytochemically investigated and evaluated for its antioxidant activity via DPPH assay. Previously study on this plant species has revealed it to be rich in phenolic compounds which are potential source of natural antioxidant (Ruma, Sunil and Prakash, 2013).

1.4 Objectives of Study

The objectives of performing this study are:

- To extract and isolate chemical constituents from the stem bark of *Garcinia parvifolia.*

- To identify and characterize the structures of isolated compounds via modern spectroscopic and spectrometric analyses.

- To investigate the antioxidant activity of the isolated compounds and crude extracts of *Garcinia parvifolia* via DPPH assay.

CHAPTER 2

LITERATURE REVIEW

2.1 Phytochemical Studies

Phytochemistry is the study of phytochemicals, also known as specialized compounds which are chemically derived from plants. Plants have built up its own chemical defenses against the environmental threats over millions of years, such as UV radiation, microbial attacks and reactive oxygen species. The phytochemical produced from plants play a defensive role against biotic and abiotic threats such as wounding, pathogen attack, and play an ecological role in regulating the interactions between plants, microorganisms, insect and animals. Phytochemicals that are derived from plants form the basis of traditional drug pharmacopeia and to serve as a proven source of therapeutic medicines (William and Douglas, 2012).

Plant-derived bioactive compounds which have been extensively studied were found to exhibit diverse and interesting biological activities such as antimalarial, antineoplastic, antioxidant, anti-ulcer, anti-inflammation and antimicrobial properties. There are several important classes of secondary metabolites which are commonly reported from the plant kingdom such as xanthones, coumarins, flavonoids, phloroglucinol, and terpenoids (Su, et al., 2008).

2.1.1 Xanthones

Xanthones are polyphenolic compounds that show unique chemical structures with a basic molecular formula of $C_{13}H_8O_2$, which is also named as 9*H*-xanthen-9-ones (Luo, et al., 2013). The basic chemical structure of xanthones composes of three aromatic rings in which the middle pyran ring is fused with two benzene rings through a carbonyl group and an ethereal oxygen. In fact, natural occurring xanthones and its derivatives are commonly found in higher plant families of Clusiaceae, Gentianaceae, Moraceae, Polygalaceae, also in fungi and lichens (Negi, et al., 2013). Figure 2.1 shows the basic skeleton of xanthone.

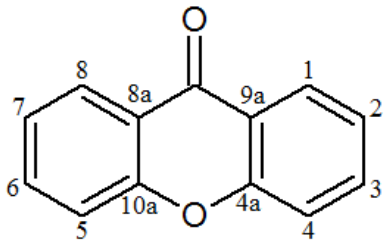


Figure 2.1: Molecular structure of xanthone

Various biological activities have been reported from xanthones apart from antioxidant activity, such as antimalarial, antifungal, anti-HIV activity, anticarcinogenic, antimicrobial, anti-allergic, antiviral, and antiasthmatic activity **(**Surat, 2006**)**. Some literatures also reported the use of xanthones as traditional medicine to treat fever and loss of appetite **(**Sarawut, 2014**)**.

2.1.2 Terpenoids

Terpenes are naturally-occurring organic compounds that are built up from isoprene (C_5H_8) units which are linked in a head-to-tail manner. Terpenes are basically made up of hydrocarbons whereas terpenoids consist of extra functional groups such as carbonyl and hydroxyl groups (Nita, Rajesh and Anju, 2014). In general, terpenoids are present in higher plants, and more than 23,000 individual structures have been identified mainly from vegetative tissues. Terpenoids can be grouped into monoterpenoids (C_{10}), sesquiterpenoids (C_{15}), diterpernoids (C_{20}), sesterterpenoids (C_{25}), triterpenoids (C_{30}) and carotenoids (C_{40}). Terpenoids are a large group of natural molecules majorly occur in plants, and other sources such as fungi and marine animals. Terpenoids are volatile natural compounds which provide fragrance in plants and flowers, and exhibit diverse pharmaceutical properties such as antitumor, antineoplastic and antibacterial (Parayil, et al., 2008). Figure 2.2 shows the molecular structure of isoprene unit.

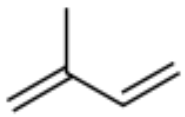


Figure 2.2: Molecular structure of isoprene (C_5H_8)

Monoterpernoids are the major aroma constituents of plants. Mostly, terpenoids are found to be colourless, volatile with steam, fragrant liquids which are lighter than water (Sam, 2008). For instance, camphor is belongs to monoterpenoids which can be obtained from *Cinnamomum camphora,* and to serve as protecting agent in clothes against the moths, stimulant for heart muscles and antiseptic agent.

2.1.3 Flavonoids

Flavonoids are a group of polyphenolic compounds which are widely distributed throughout the plant kingdom such as in the families of Malvaceae, Asteraceae, Lamiaceae, Clusiaceae and Gentianaceae. However, the family of Leguminosae is the most endowed with flavonoid constituents among all the plant families. The basic structure of flavonoids comprises of a diphenylpropane skeleton (C_6-C_3-C_6) in which the three-carbon bridge between the two phenyl rings is normally cyclized with oxygen to give the middle ring of flavonoids (Miguel, 2009).

There were about 3000 varieties of flavonoids which have been reported to be widely used in healthcare products due to their low level of toxicity (Agrawal, 2011). Flavonoids are usually present as aglycones, glycosides and methylated derivatives. In general, flavonoids occur in plants as glycosylated derivatives which serve as plant pigments that contribute yellow or orange colour in leaves, fruits and flowers. Naturally occurring flavonoids are categorized into 6 main subclasses depending on the oxidation state of the central pyran ring such as flavones, flavanones, flavonols, isoflavones, flavanols and anthocyanidins (Hamdoon, 2009). Figure 2.3 shows the basic molecular structure of flavonoids.

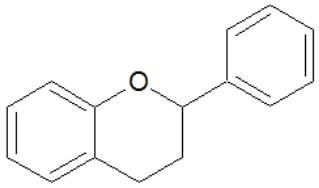


Figure 2.3: Basic molecular structure of flavonoid

The chemical nature of flavonoids is dependent on their structural class, degree of hydroxylation, degree of conjugation and level of polymerization (Agrawal, 2011). Various biological activities were reported from flavonoids, including anti-inflammatory, antibacterial, anti-allergic, and antiviral activities (Pier, 2000).

2.2 Chemistry of *Garcinia* Species

The genus *Garcinia* (family of Guttiferae) comprises of about 300 different species which are commonly found in tropical Asia, Africa and Polynesia. In Malaysia, *Garcinia* is best known as a genus of fruit trees. Throughout the decades of study, a variety of plant parts of *Garcinia* have been widely investigated such as fruits, stem barks, seeds, flowers, leaves and roots, mainly due to the presence of bioactive natural products with their valuable pharmacological properties. Phytochemical studies have revealed the genus *Garcinia* to be rich in a variety of secondary metabolites including xanthones, triterpenoids, phloroglucinols, depsidones, biflavonoids and benzophenones (Kuete, et al., 2007). Among the *Garcinia* species, *G. mangostana, G. cowa, G. indica, G. lanceaefolia, G. diversifolia, G. parvifolia and G. cambogia* have been extensively investigated which led to many important publications. Some of the phytochemicals were found to possess a wide variety of biological and ethnomedicinal properties such as antihypertensive, antimalarial, antioxidant, and antifungal (Sunit, et al., 2003).

2.2.1 Chemistry and Biological Activities of *Garcinia mangostana*

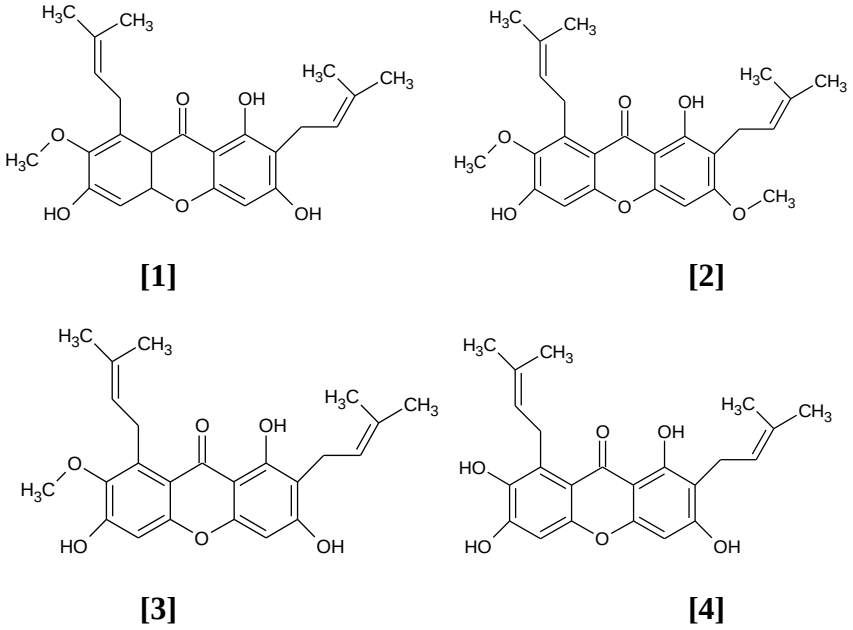
In the period from 2008 to 2013, there have been about 454 scientific papers reported on *Garcinia mangostana* which evidenced a great interest in the study of mangosteen plant especially its fruit hulls. The fruit hulls are found to be rich in various xanthones of pharmacological and ethnomedicinal importances (Dmitrily, 2009). Among the *Garcinia* species, *G. mangostana* is the earliest species to be studied for its phytochemical content and biological activities.

In year 1958, isolation of mangostin **[1]** and β-mangostin **[2]** from the fruits, bark and dried latex of *G. mangostana* were carried out by Yates and Stout (Bonnie, 1996). Through various chemical elucidation, mangostin **[1]** was identified as 1,3,6-trihydroxy-7-methoxy-2,8-di-(3-methyl-2-butenyl)-xanthone. Throughout the years of study, there are about 68 xanthones reported for their isolation from different parts of *G. mangostana*, in which 50 of them are commonly occurred in the fruit's pericarp. α-Mangostin **[3]** and γ-mangostin **[4]** are the two most abundant xanthones derived from the pericarp of mangosteen fruits. Apart from chemistry study, α-mangostin **[3]** and γ-mangostin **[4]** were found to exhibit anti-inflammatory effect in macrophage and adiposite cell (Sarawut, 2014).

Chemical investigation on the pericarp of *G. mangostana* led to the isolation of two new highly oxygenated prenylated xanthones from methanol extract, namely 8-hydroxycudraxanthone G **[5]** and mangostingone **[6]**. These two xanthone were appeared as yellow solid (Hyun, et al., 2006). Besides, a total of twelve known

xanthones were isolated from the dichloromethane extract which were cudraxanthone G **[7]**, 8-deoxygratanin **[8]**, garcinmangosone **[9]**, garcinone D **[10]**, garcinone E **[11]**, gartanin **[12]**, 1-isomangostin **[13]**, α-mangostin **[3]**, γ-mangostin**[4]**, mangostinone **[14]**, smeathxanthone A **[15]**, and tovophyllin A **[16]**.

From the biological point of view, the isolated xanthones were subjected to peroxynitrite-scavenging assay for evaluation of their antioxidant activity. The isolated xanthones that showed potent antioxidant activities were 8-hydroxycudraxanthone G **[5],** gartanin **[12]**, α-mangostin **[3]**, γ-mangostin **[4]**, and smeathxanthone A **[15]**, with reported IC_{50} values in the range of 2.2 to 12.2 μM. Besides, α-mangostin **[3]** has also been reported as an important anti-mycobacterial agent against *Mycobacterium tuberculosis* with a minimum inhibitory concentration of 6.25 μg/mL (Hyun, et al., 2006).



[1] **[2]**

[3] **[4]**

Figure 2.4: Structures of chemical constituents isolated from *Garcinia mangostana*

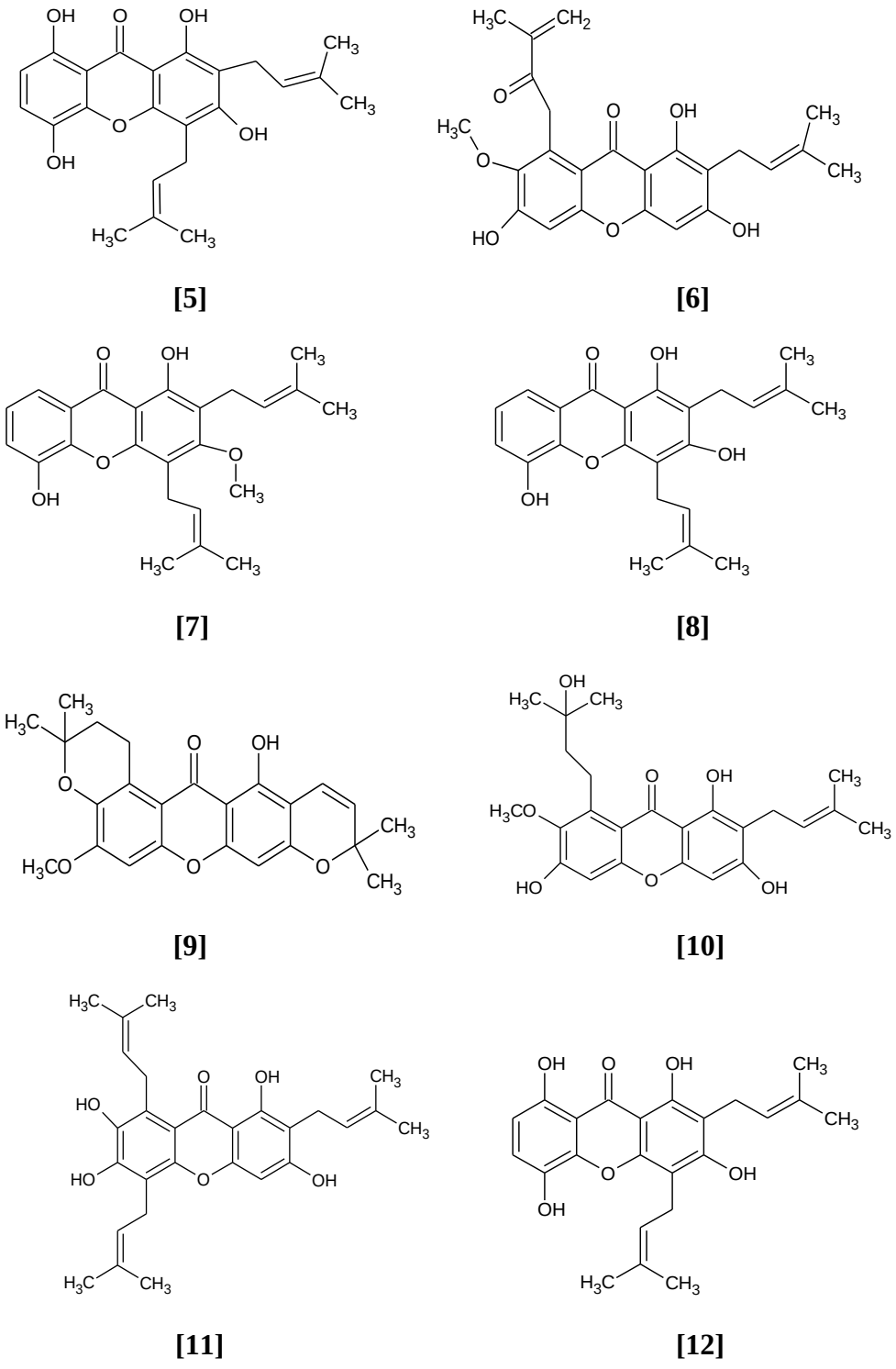
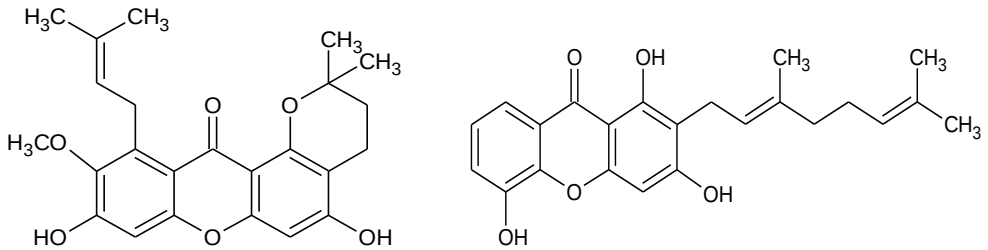
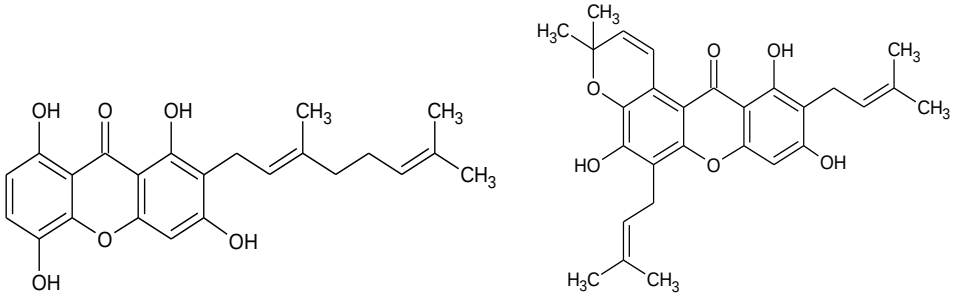


Figure 2.5: Structures of chemical constituents isolated from *Garcinia mangostana* (continued)



[13] **[14]**



[15] **[16]**

Figure 2.6: Structures of chemical constituents isolated from *Garcinia mangostana* (continued)

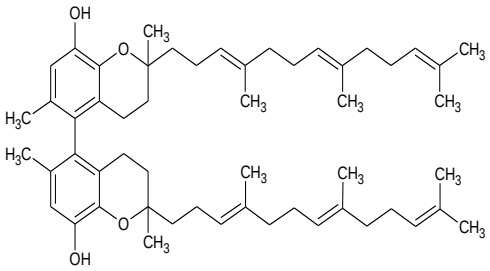
2.2.2 Chemistry and Biological Activities of *Garcinia cowa*

Garcinia cowa, namely Cha-muang in Thai language, is widely distributed throughout Myanmar, Thailand and Malaysia (Mahabusarakam, Chairerk and Taylor, 2005). This plant species has been extensively studied in terms of its phytochemicals and biological activities because of its valuable therapeutic uses in folk medicine as effective pharmacological agent. For instance, the fruit and leaves of *Garcinia cowa* are used for improvement of blood circulation, whereas the bark, root and latex of the plant are commonly used for treatment of vomiting, cough, ulcer and chronic diarrhea (Xia, et al., 2015).

In year 2014, two new compounds were reported by Ittipon and coworkers, namely garciniacowol **[17]** and garciniacowone **[18]** which were isolated from the acetone extract of the stem barks of *G. cowa*. Compound **[17]** was isolated as colourless viscous oil with a molecular formula of $C_{54}H_{78}O_4$ whereas compound **[18]** was separated in the form of yellow viscous oil with a molecular formula of $C_{28}H_{34}O_6$. Along with these, 13 known compounds were isolated from the same extract including parvifoliol F **[19]**, cowaxanthone **[20]**, norcowanin **[21]**, cowanin **[22]**, cowanol **[23]**, cowagarcinone B **[24]**, cowagarcinone D **[25]**, cowagarcinone E **[26]**, fuscaxanthone A **[27]**, fuscaxanthone C **[28]**, 6-O-methylmangostanin **[29]**, cowaxanthone D **[30]**, 1,7-dihydroxyxanthone **[31]**.

All these isolated compounds were further evaluated for their biological properties. Among these, garciniacowone **[18],** a xanthonoid compound showed the strongest antibacterial activities against MRSA SK1 and *S. aureus* with a minimum inhibitory concentration (MIC) of 2 µg/mL (Siridechakorn, et al., 2012).

In year 2015, a new compound namely 6-hydroxycalabaxanthone **[32]** was isolated as yellow amorphous powder. This compound showed potent cytotoxic effect against DU-145 prostate cancer cell line with an IC_{50} value of 6.4 µM (Fatma, 2015b).



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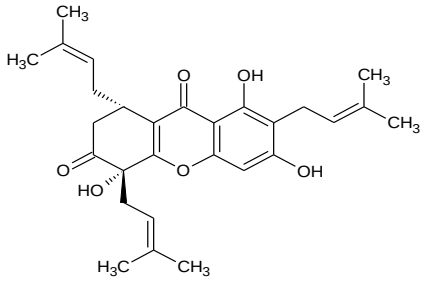

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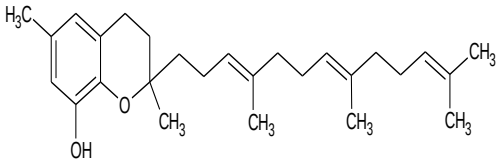

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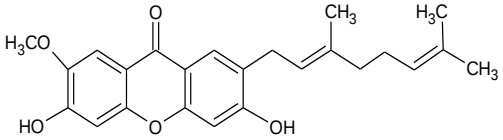

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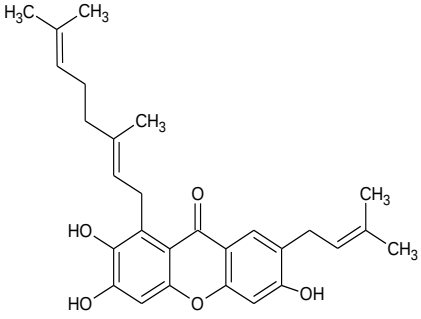

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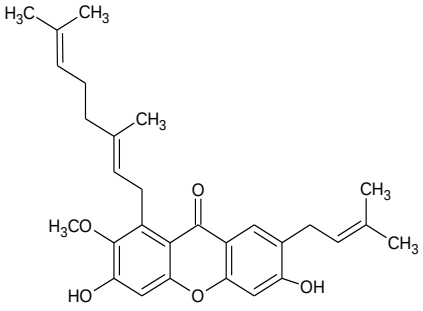

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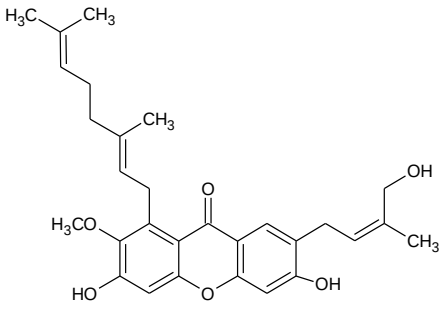

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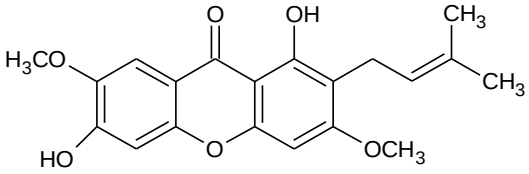
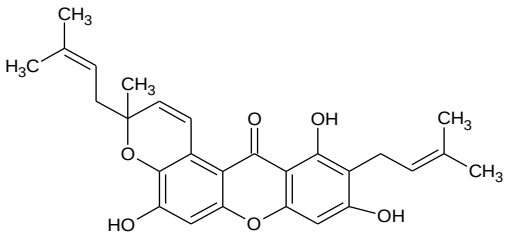


[24]

Figure 2.7: Structures of chemical constituents isolated from *Garcinia cowa*



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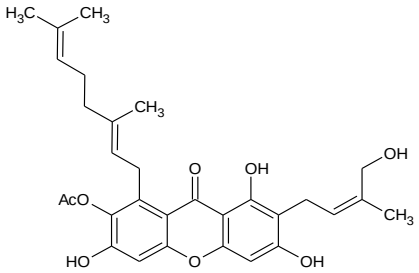

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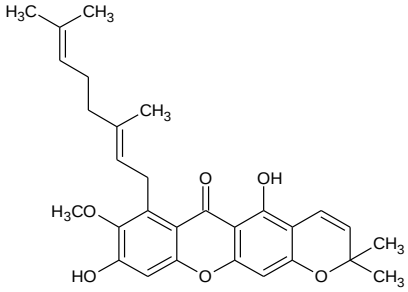

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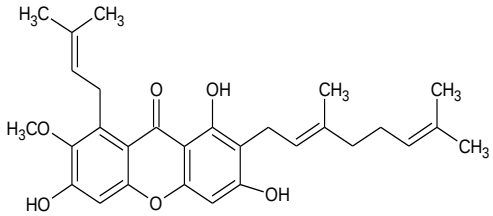

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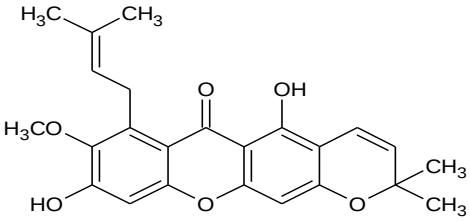

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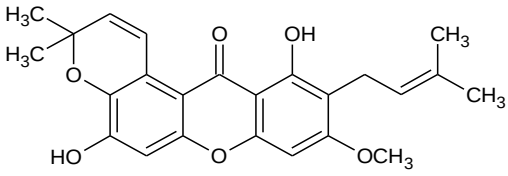

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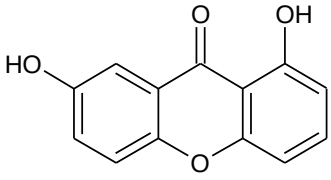
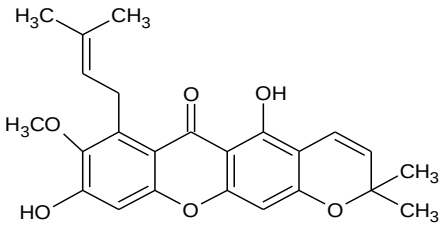


[31]



[32]

Figure 2.8: Structures of chemical constituents isolated from *Garcinia cowa*

(continued)

2.2.3 Chemistry and Biological Activities of *Garcinia parvifolia*

In this project, *Garcinia parvifolia* was selected as the plant material for phytochemical investigation in the search for chemically interesting substances and potential antioxidant agents. Previously, there were several chemical investigations reported on different parts of this plant including leaves, twigs, latex, fruits and barks, which led to the isolation of different classes of chemical constituents from this plant species such as depsidones, xanthones, phloroglucinol, flavonoid, alkaloids and terpenoids. In year 2000, Xu and coworkers reported the isolation of four novel prenylated depsidones from the leaves of *G. parvifolia*. These four isolated depsidones were obtained from the chloroform soluble fraction, namely garcidepsidone A **[33]**, garcidepsidone B **[34]**, garcidepsidone C **[35]** and garcidepsidone D **[36]**, respectively (Xu, et al., 2000).

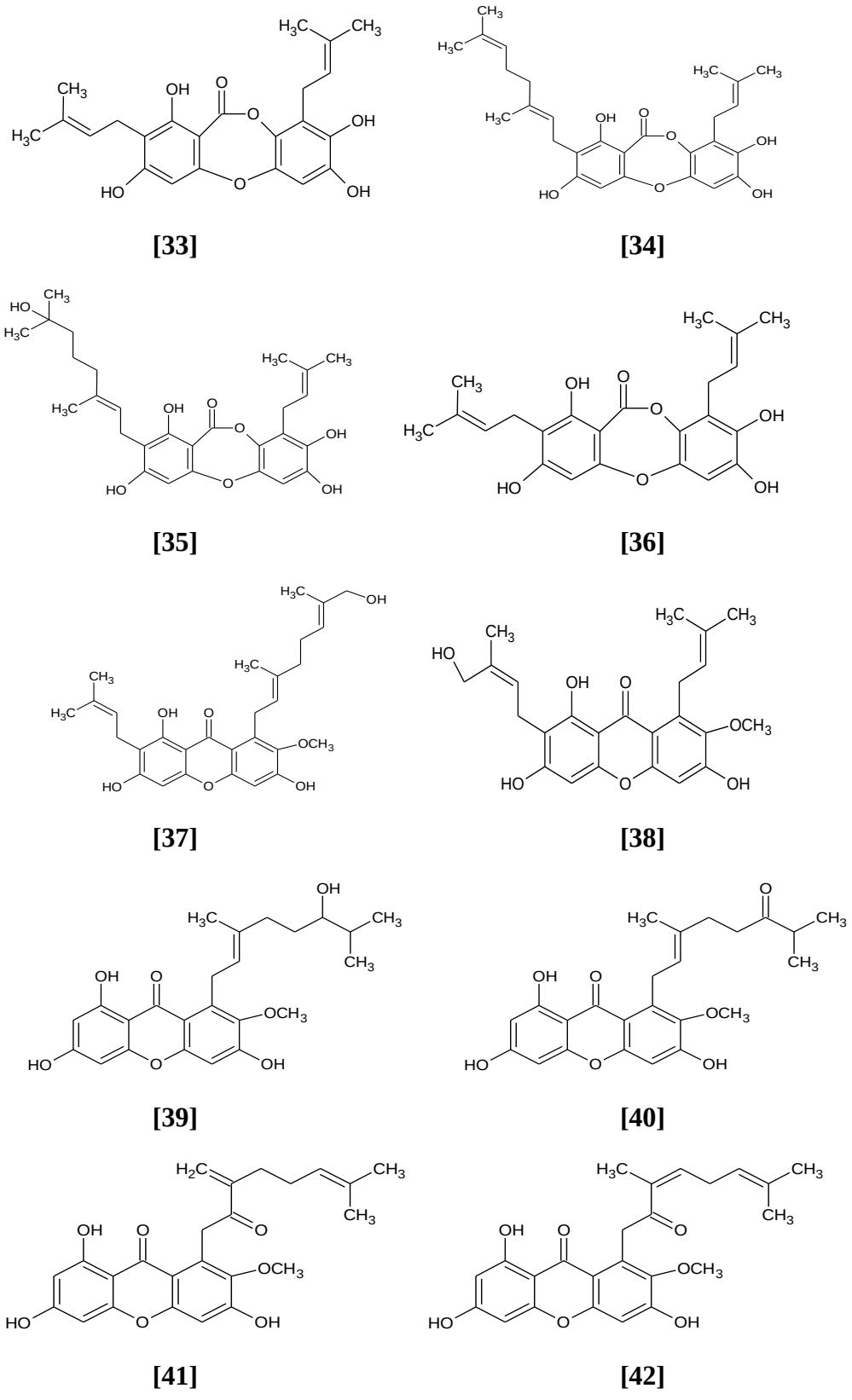
A year later, the same research group conducted another phytochemical investigation on the dried bark of *G. parvifolia* which led to isolation of nine new xanthones, namely parvixanthones A-I **[37-45]**. These xanthones were found to have a common 1,3,6,7-oxygenated pattern which structurally varied by different position of attachment of oxygenated isoprenyl or geranyl substituents in the xanthone skeleton.

In year 2006, from the leaves extracts of *G. parvifolia*, a new benzoquinone derivative, namely parvifoliquinone **[46]** and six known compounds were reported by Vatcharin and coworkers. The known compounds isolated were parvifoliol B

[47], parvifoliol C **[48]**, parvifoliol E **[49]**, garcidepsidone B **[34]**, nigrolineaisoflavone A **[50]** and mangostinone **[14]**.

From biological perspective, Vatcharin and coworkers revealed that the crude methanol extract from the leaves of *G. parvifolia* exhibited remarkable antibacterial activities against methicillin-resistance *Staphylococcus aureus* (MRSA). All the compounds isolated from this plant were subjected to antibacterial screening. Among these compounds, parvifoliol B **[47]** showed the best antibacterial activity against MRSA with a minimum inhibitory concentration of 32 µg/mL (Vatcharin, et al., 2006).

In the next year, chemical and biological investigations on *Garcinia parvifolia* by Ee and Cheow, reported the isolation of a triterpenoid, named α-amyrin **[51]** and two xanthones known as cowanin **[22]** and rubraxanthone **[52]**. These three pure compounds which were obtained from the hexane and chloroform stem bark extracts were further evaluated for their biological effects towards the larvae of *Aedes aegypti* and HL-60 cell line. However, only rubraxanthone **[52]** showed highly active against the larvae of *Aedes aegypti* (LC_{50} = 15.49 µg/mL), and HL-60 cell line (IC_{50} = 7.5 µg/mL) (Ee and Cheow, 2007).



[33] **[34]**

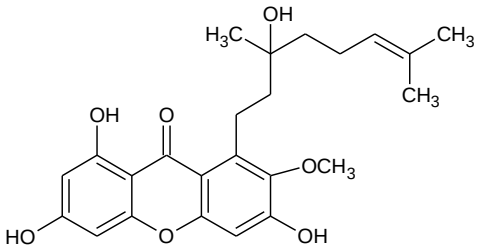
[35] **[36]**

[37] **[38]**

[39] **[40]**

[41] **[42]**

Figure 2.9: Structures of chemical constituents isolated from *Garcinia parvifolia*



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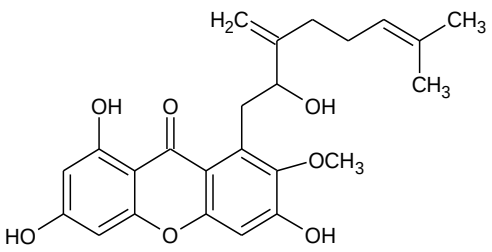

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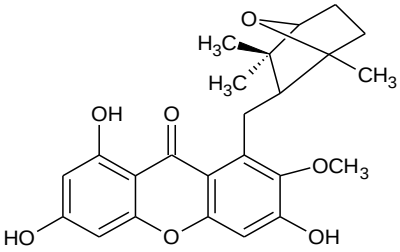

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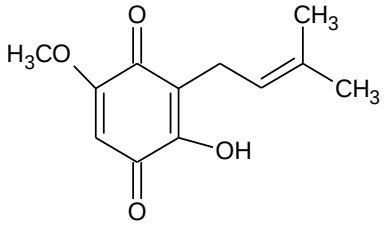

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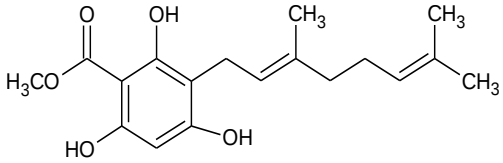

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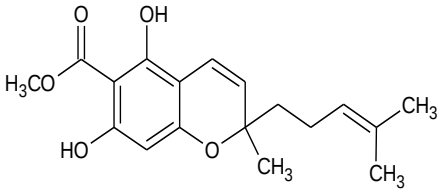

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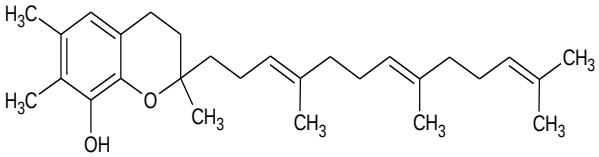
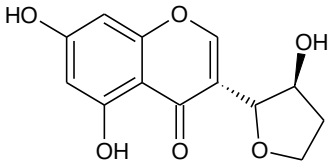
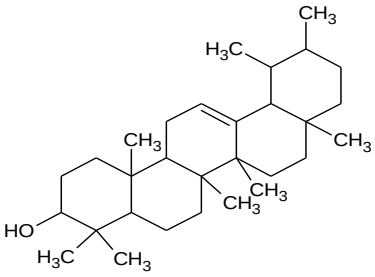


[49]



[50]



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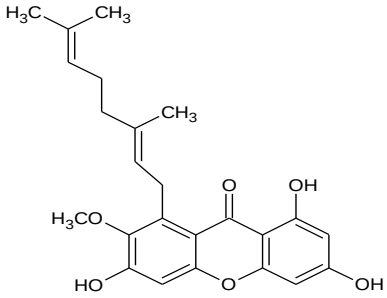


[52]

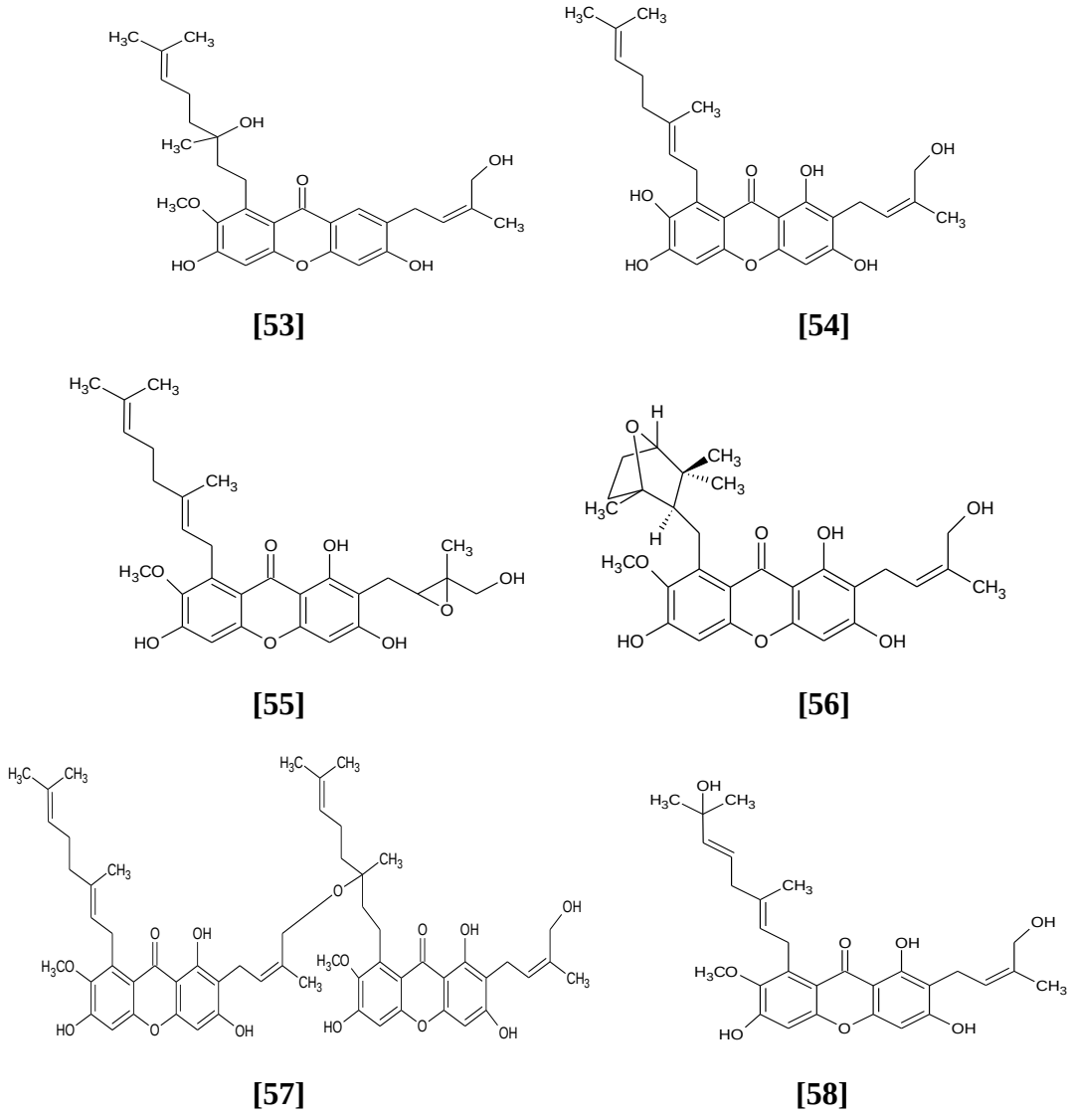
Figure 2.10: Structures of chemical constituents isolated from *Garcinia parvifolia* (continued)

2.2.4 Chemistry and Biological Activities of *Garcinia fusca* Pierre

Garcinia fusca Pierre is widely distributed in tropical Asian countries such as Thailand, Vietnam, Malaysia, India and Indonesia. In Thailand, *G. fusca* is known as 'Madan-paa' or 'Mak-Mong', meanwhile it is called as 'Bứa lứa' by the Vietnamese (Nguyen, 2015). The young leaves and fruits of this plant are usually used in food preparation and served as refreshing drink. Traditionally, *G. fusca* was introduced by botanist due to its interesting pharmaceutical characteristics, and used in folk medicine collections. For instance, the roots, barks and leaves of the plant are commonly used in the treatment of cough, fever and usually skin diseases. Several previous articles reported that *G. fusca* are rich in tetraoxygenated xanthones and biflavonoids (Jannarin, et al., 2013).

In year 2015, researcher Nguyen from University of Science in Vietnam carried out phytochemical and biological investigations on the bark of *G. fusca*. The air-dried and powdered bark of *G. fusca* was extracted with hexane and ethyl acetate. There were a total of eight new xanthones isolated from the two bark extracts. Pure compounds obtained from the hexane extract including fuscaxanthone I **[53]**, fuscaxanthone J **[54]**, fuscaxanthone K **[55]**, fuscaxanthone L **[56]**, fuscaxanthone M **[57]**. On the other hand, fuscaxanthone N **[58]**, fuscaxanthone O **[59]** and fuscaxanthone P **[60]** were derived from the ethyl acetate extract, simultaneously with other known compounds such as benzaldehyde derivative, namely 11-hydroxy-1-isomangostin **[61]**, griffipavixanthone **[62]**, morelloflavone **[63]**, volkinflavone **[64]**, GB-1a **[65]** and GB-2 **[66]**.

All the new xanthone isolated, except for fuscaxanthone K **[55]** and fuscaxanthone M **[57]** which were biologically inactive, exhibited significant cytotoxic effect against HeLa cells showing IC_{50} values in the range of 19.1-45.9 µM. Griffipavixanthone **[62]**, a bixanthone was reported to possess potent anti-angiogenic property, and exhibit high cytotoxic effect against HeLa cells with IC_{50} value of 7.9 µM (Jannarin, 2011).

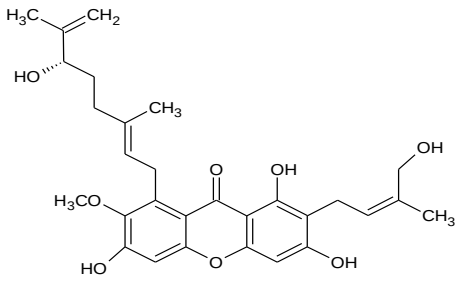


[53] **[54]**

[55] **[56]**

[57] **[58]**

Figure 2.11: Structures of chemical constituents isolated from *Garcinia fusca*



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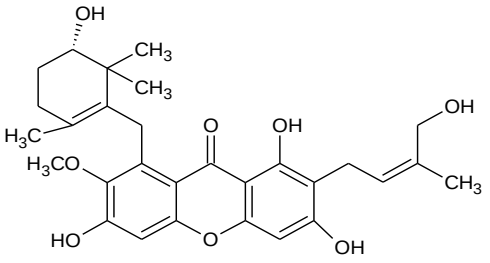

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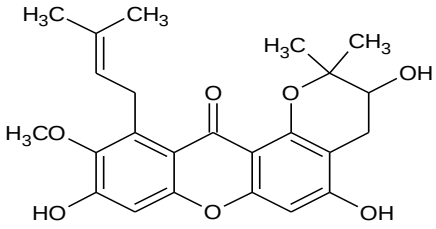

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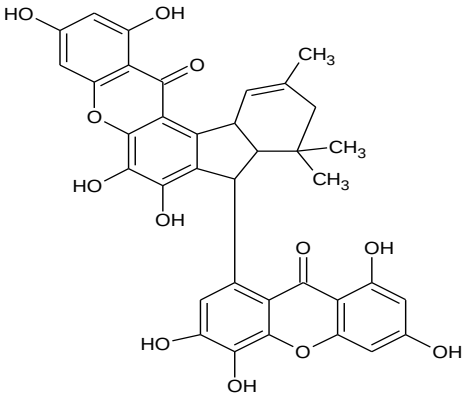
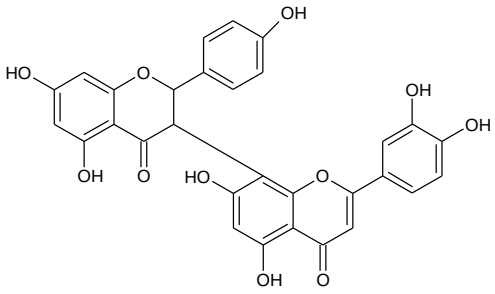


[62]



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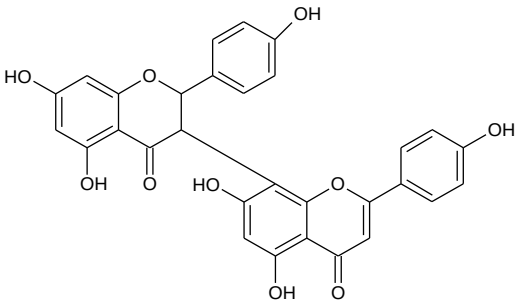

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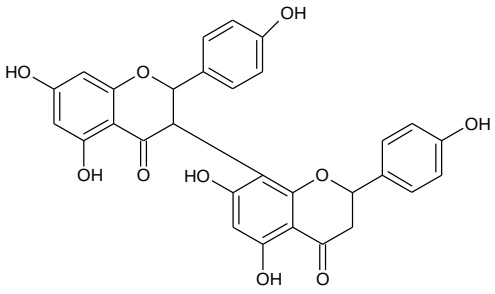

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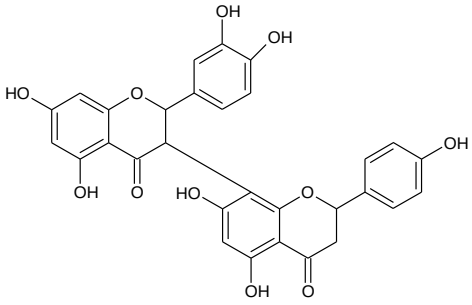


[66]

Figure 2.12: Structures of chemical constituents isolated from *Garcinia fusca* (continued)

2.3 Summary of Literature Review on the Genus *Garcinia*

The classes of natural products isolated from *Garcinia* species and their pharmacological importance are summarized in Table 2.1.

Table 2.1: Summary of literature data on the genus *Garcinia*

Plant Species	Classes of Compound	Biological Activities	References
G. atroviridis	Xanthones Benzophenones Flavonoids	Antioxidant Antimicrobial Cytotoxicity	Permana, et al., 2002 Nursakina, et al., 2012
G. bracteata	Xanthones	Antiproliferative Anticancer Antitumor	Tao, et al., 2013 Odile, et al., 2000
G. brasiliensis	Sesquiterpene Benzophenones	Anti-inflammatory Cytotoxicity Antioxidant Anti-allergic	Arwa, et al., 2015 Vanessa, et al., 2011
G. cambogia	Xanthones Benzophenones Organic acids	Antiobesity Antioxidant	Scott, et l., 2014 Kohsuke, et al., 2008
G. cantleyana	Benzophenones Xanthones Terpenoids Steroids	Antioxidant Cytotoxicity	Ibrahim and Fadlina, 2012 Khalid, et al., 2007
G. cochinchinensis	Benzophenones Xanthones	Anticancer Cytotoxicity	Binh, et al., 2013 Nguyen, et al., 2011

G. conrauana	Lactones Biflavonoids Benzophenones	Anti-asthma Antioxidant	Peter and Elizabeth, 1980 Raouf and Peter, 1982
G. cowa	Xanthones Phloroglucinols Organic acids Depsidones Flavonoids Terpenes Steroids	Antimalarial Antibacterial Anticancer Cytotoxicity	Negi, et al., 2013 Xu, et al., 2000 Fatma, et al., 2015a
G. dioica	Flavonoids Xanthones Alkaloids	Antimicrobial	Munekazu, et al., 1995
G. dulcis	Xanthones Flavonoids Benzophenones	Antibacterial Antioxidant Antimalarial	Mohd, et al., 2015
G. fusca	Xanthones Terpenoids Biflavonoids	Antivirus Anti-bacterial Antioxidant Anti-inflammatory Cytotoxic Antifungal	Nguyen, 2015 Jannarin, 2011 Jannarin, et al., 2013
G. griffithii	Xanthones Benzophenones	Cytotoxicity Antiplatelet Aggregation	Bonnie, 1996
G. hombroniana	Triterpenes Lanostanes Flavonoids Xanthones Organic acids	Antibacterial Antioxidant Antiplatelet aggregation	Dyary, et al., 2015 Jamila, et al., 2015

G. indica	Benzophenones Organic acids Flavonoids	Antioxidant Anti-glycation Antiulcer Antifungal Anticancer	Tang, et al., 2013. Prajakta, et al., 2015 Subhash, et al., 2009
G. kola	Biflavonoids Alkaloids	Antidiabetic Antibacterial Antifungal Antihepatotoxic	Benetode, 2015
G. lucida	Alkaloids Steroids Triterpenes	Antiprotozoal Antimicrobial Cytotoxicity	Anne, et al., 1990 Nicole, et al., 2003
G. macrophylla	Benzophenones	Cytotoxicity	Satyanshu, Shelly and Sunil, 2013
G. mangostana	Xanthones Phloroglucinols Depsidones Flavonoids Triterpenes	Antioxidant Anticancer Antitumor Anti-inflammatory Anti-allergic Anti-HIV Antituberculosis	Hyun, et al., 2006 Sunit, et al., 2003 Sarawut, 2014 Bonnie, 1996
G. merguensis	Xanthone Biflavonoid	Cytotoxicity Antibacterial Antioxidant	Nguyen, et al., 2003 Kongkiat, et al., 2013
G. multiflora	Xanthones Benzophenones Phloroglucinols Biflavonoids	Anti-HIV Antioxidant Anti-inflammatory Antiviral	Lee, et al., 2013 Chien, et al., 2008

G. oblongifolia	Xanthones Phloroglucinols Benzophenones Organic acids	Antibacterial Antienteroviral Cytotoxicity	Ittipon, et al., 2014 Zhang, et al., 2016
G. parvifolia	Xanthones Phloroglucinols Depsidones Flavonoids Terpenoids Benzophenones	Antioxidant Anti-inflammatory Antibacterial Cytotoxicity Antimicrobial	Vatcharin, et al., 2006 Xu, et al., 2000
G. polyantha	Xanthones Triterpenoids Organic acids Flavonoids	Antimalarial Antibacterial Antifungal Antioxidant	Gabin, et al., 2008 Lannang, et al., 2005 Stephen and Peter, 1986
G. shomburgkiana	Xanthones Benzophenones Biflavonoids Steroids	Cytotoxicity	Ito, et al., 2013
G. smeathmannii	Xanthones Benzophenones Triterpene	Antimicrobial Anticandidal Antifungal	Kuete, et al., 2007 Justin, et al., 2005
G.subelliptica	Xanthones Benzophenones Phloroglucinols	Antioxidant Cytotoxicity	Lin, et al., 2012 Hiroyuki, et al., 1998
G. xamthochymus	Xanthoness Phloroglucinols	Antioxidant Anticancer Antibacterial	Scott, et al., 2004 Manohar, et al., 2014 Nethracathi, at al., 2015

CHAPTER 3

EXPERIMENTAL

3.1 Plant Material

The plant studied in this project was *Garcinia parvifolia*. The stem bark of *Garcinia parvifolia* was collected from the jungle in Landeh, Sarawak. It was authenticated by Mr. Tinjan Anak Kuda, the botanist from Forest Department of Sarawak. A voucher specimen (UITM 3008) detailing the collection of plant was deposited at the herbarium of Universiti Teknologi MARA, Sarawak.

3.2 Chemical Reagent

The industrial grade solvents and materials used in the extraction, isolation and purification of chemical constituents from *Garcinia parvifolia* are summarized in Table 3.1. The analytical grade solvents and materials used in TLC analysis are listed in Table 3.2. The deuterated solvents used in NMR analysis are listed in Table 3.3. The materials and HPLC grade solvents used in LC- and GC-MS analysis are shown in Table 3.4. The analytical grade solvents and materials used in UV-Vis analysis are listed in Table 3.5. Chemical reagents and materials used in antioxidant assay are summarized in Table 3.6.

Table 3.1: Industrial grade solvents and materials used in the extraction, isolation and purification of chemical constituents

Materials / Solvents	Molecular formula	Density, ρ (g/cm^3)	Source, Country
Sea sand	-	-	Merck. Germany
Silica gel (60 Å)	SiO_2	-	Merck, Germany
Sephadex® LH-20	-	-	New Jersey, USA
Sodium sulphate anhydrous	Na_2SO_4	2.66	John Kollin Corporation, USA
n-Hexane	$CH_3(CH_2)_4CH_3$	0.659	Merck, Germany
Dichloromethane	CH_2Cl_2	1.325	Fisher Scientific, UK
Ethyl acetate	$CH_3COOC_2H_5$	0.902	Lab Scan, Ireland
Acetone	CH_3COCH_3	0.791	QREC, Malaysia
Methanol	CH_3OH	0.791	Mallinckrodit Chemicals, Philipsburg

Table 3.2: Analytical grade solvents and materials used in TLC analysis

Materials / Solvents	Molecular formula	Source, Country
TLC silica gel 60 F_{254}	-	Merck, Germany
Iodine	I_2	Fisher Scientific, UK
n-Hexane	$CH_3(CH_2)_4CH_3$	R & M Chemicals, UK
Dichloromethane	CH_2Cl_2	OREC, Malaysia
Ethyl acetate	$CH_3COOC_2H_5$	Fisher Scientific, UK

Table 3.3: Deuterated solvents used in NMR analysis

Materials / Solvents	Molecular formula	Source, Country
Deuterated chloroform	$CDCl_3$	Acros Organics, Belgium
Acetone-d_6	CD_3COCD_3	Acros Organics, Belgium
Methanol-d_4	CD_3OD	Acros Organics, Belgium

Table 3.4: HPLC grade materials used in LC- and GC-MS analysis

Materials / Solvents	Molecular formula	Density, ρ (g/cm^3)	Source, Country
Acetonitrile	CH_3CN	41.05	Fisher Scientific, UK
Methanol	CH_3OH	32.04	Fisher Scientific, UK
Nylon Syringe filter (0.5 µm)	-	-	Membrane solution, USA

Table 3.5: Analytical grade solvents and materials used in UV-Vis analysis

Materials / Solvents	Molecular formula	Source, Country
Chloroform	$CHCl_3$	Fisher Scientific, UK
Cuvette (quartz)	-	Membrane Solution, USA

Table 3.6: Chemical reagents and materials used in antioxidant assay

Chemical reagents / Materials	Source, Country
Kaempferol	Sigma-Aldrich, USA
Ascorbic acid (Vitamin C)	Sigma-Aldrich, USA
1,1-Diphenyl-2-picryhydrazyl (DPPH)	Sigma-Aldrich, USA
96-well plate	Techno Plastic, Switzerland

3.3 Instrument

3.3.1 Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) is a spectroscopic method that studies the physical phenomenon based upon the magnetic property of an atom's nucleus. It involves the absorption of radio frequency radiation by atomic nuclei in an applied magnetic field. NMR is one of the non-destructive techniques used to obtain the physical, chemical, electronic and structural information about a molecule. Thus, by analyzing the peaks or signals of NMR spectra, the structural composition of organic compounds can be determined. In this project, JEOL JNM-ECX 400 MHz spectrometer was used to obtain ^{1}H NMR, ^{13}C NMR, DEPT, HMQC and HMBC spectra of samples.

The common solvents used to dissolve the samples are deuterated-chloroform and deuterated acetone. Tetramethylsilane (TMS) was used as an internal standard and reference during analysis. The dissolved samples were separately transferred to NMR tubes up to 4 cm in height and the tubes were then capped and sealed with parafilm to avoid solvent evaporation. The selection of solvents is depending on the degree of solubility of compound in the solvent.

3.3.2 Infrared Spectrophotometer (IR)

Infrared (IR) spectrophotometer is a spectroscopic instrument that is used to study types of functional groups present in a compound. IR spectrum also provides specific fingerprint region that can be used for structural identification of compounds. The bands observed in IR spectra are due to the interaction of the electrical vector of electromagnetic radiation with the electric dipole of non-symmetrical bonds in the vibrational infrared region. In this project, Perkin Elmer 2000-FTIR spectrophotometer was used to analyse samples in the wavenumber range of 4000 to 400 cm^{-1}. Sample preparation was done by mixing the finely ground solid sample with powdered potassium bromide (KBr) evenly in a ratio of 1:10. Then, the mixture was pressed under high pressure which results in the KBr melts and seals the compound into a matrix. The resulted KBr pellet was inserted into a sample holder for IR analysis.

3.3.3 Ultraviolet – Visible Spectrophotometer (UV-Vis)

Ultraviolet-Visible (UV-Vis) spectrophotometer is a technique used to analyse the chromophores present in a molecule by studying its conjugated system. As atoms or molecules absorb energy, an electron is promoted from a ground state to an excited state, known as electronic transition. By measuring the energy difference between the excited and ground states, the characteristic absorption provides information about classes, structure and properties of compounds. In this project, the spectra of samples were obtained using Perkin Elmer Lambda 35, double-beam

spectrophotometer. Chloroform was used as blank, and to dissolve and dilute the samples for analysis. UV-Vis spectra were obtained in the wavelength range of 200 – 400 nm.

3.3.4 Liquid Chromatography – Mass Spectrometry (LC-MS)

Liquid Chromatography–Mass Spectrometry (LC-MS) is a coupled technique of liquid chromatography with mass spectrometry which is used to provide useful structural information for non-volatile compounds. In the sample preparation, 1 mg of sample was dissolved in 1 mL of HPLC grade solvent. The HPLC grade solvent was used in LC-MS to give a better quality of mass spectra. Sample solution was filtered through nylon syringe filter in order to remove impurities or undissolved solid. Agilent Technologies 6520 LC/MS equipped with an electrospray ionization source was used for compound analysis.

3.3.5 Melting Point Apparatus

Melting point apparatus is an instrument used to determine the melting point, as well as to assess the purity of a solid sample. A pure crystalline compound will give a narrow and sharp range of melting point as compared to impure substances. A little amount of solid sample was introduced into a hematocrit capillary tube and the tube was then inserted into a heating block. The temperature range was recorded when the compound started and completely melted. The melting point of compound was measured by Stuart SMP 10 melting point apparatus.

3.3.6 Polarimeter

Polarimetry is a non-destructive technique used to measure the optical activity exhibited by chiral active compound. For chiral compound, optical activity (specific rotation) was measured by observing the rotation of the plane of polarized light either clockwise or anti-clockwise when passing through it. Chloroform was used to dissolve and dilute the sample before it was placed in the polarimeter. In this study, JASCO, P-2000 polarimeter was used to measure the optical rotation of samples.

3.4 Chromatographic Methods

3.4.1 Column Chromatography

Column chromatography is widely used for isolation and purification of chemical constituents from the crude extracts. The purification of crude extracts involves partitioning of chemical constituents between stationary phase and mobile phase. In this study, gravity column chromatography packed with silica gel was selected as separating medium in which silica gel served as the stationary phase which was held in a glass column, meanwhile the mobile phase, a solvent mixture was flown through the column by gravitational force.

The sizes of the glass columns used were 25 mm, 30 mm and 80 mm in internal diameter, depending on the amount of sample available for purification. Silica

slurry was prepared by homogeneous mixing of silica gel with non-polar solvent, hexane. Then, the slurry of silica gel was introduced into the column and was left overnight for settlement of silica gel so that the column was densely packed. While waiting for the silica gel to settle down, the side of the column was tapped gently using a rubber pipe to eliminate air bubbles trapped in the packed column which might lead to cracked column.

The sample was prepared using dry packing method, in which the samples were blended with a minimal amount of silica gel, and was allowed to dry in the fume hood before it was introduced into the packed column. After the sample mixture was subjected to the packed column, mobile phase was introduced into the column in order to elute out the chemical compounds. Figure 3.1 shows the apparatus set up for column chromatography.

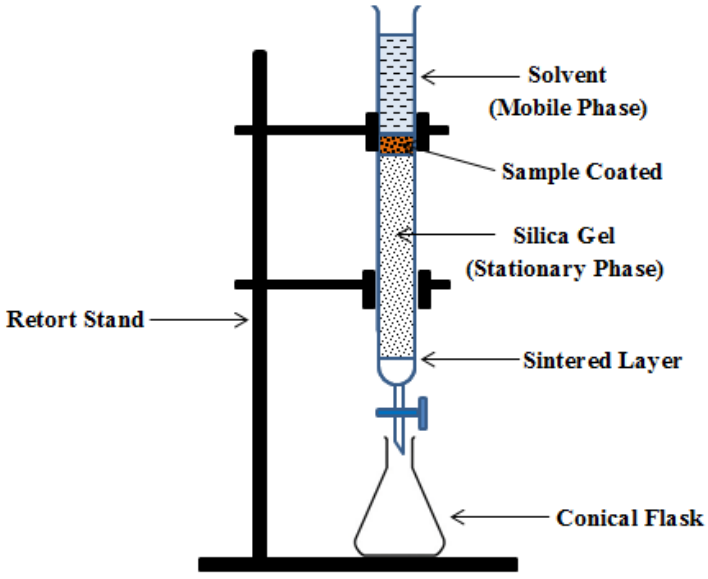


Figure 3.1: Apparatus set up for column chromatography

The solvent system applied was gradient elution in which the mobile phase was introduced in increasing solvent polarity. Solvent mixtures of hexane-dichloromethane, dichloromethane-ethyl acetate, ethyl acetate-acetone and acetone-methanol were used in order to separate compounds with various polarities out from the column. Separation was achieved through different affinities exhibited by chemical components toward both stationary and mobile phase, resulting in differences in migration rates. The eluent was collected according to volume or separated bands. The fractions obtained were concentrated using rotary evaporator and analysed using TLC. With the aids of developed TLC, the number of components present in the fractions can be determined quantitatively. The fractions which showed similar TLC pattern were combined, and fractions of interest were subsequently subjected for further purification.

3.4.2 Thin Layer Chromatography (TLC)

Thin layer chromatography is a type of planar chromatography, which a thin layer of adsorbent (silica gel) is coated on a flat plate (aluminium carrier sheet). In this study, Merck silica gel 60 F_{254} TLC plate was used to find out the appropriate solvent system to be introduced prior to column chromatographic separation. Besides, TLC also provides information about the chemical identity and composition of substances, and to check on the effectiveness of a purification step. TLC plate was cut into smaller size of 4 cm x 8 cm. Dissolved sample was spotted on the baseline which was drawn 0.5 cm from the bottom of TLC plate, using a microcapillary tube. Developing chamber was prepared containing a mixture of

solvents and a half piece of filter paper lying against the internal wall of chamber. Filter paper was used to saturate the solvent vapour atmosphere in the developing chamber. TLC plate with the applied sample was placed into the developing chamber and mobile phase (solvent mixture) moved up the stationary phase by capillary action. As the solvent moved up the plate, the components of sample were carried along up the plate until it reached the solvent front line, which is about 1.0 cm from the top of the TLC plate. The TLC plate was then removed from the developing chamber. After solvent evaporated from the plate, the TLC plate was visualized using UV light (254 nm and 365 nm), stained with iodine vapour, and tested with ferric chloride solution. The example of developed TLC plate is illustrated in Figure 3.2.

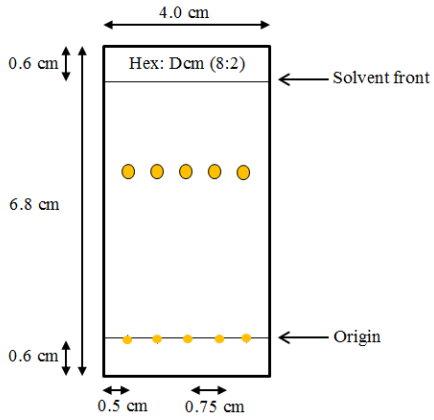


Figure 3.2: Developed TLC plate

The identity of an unknown compound can be determined by comparing the retention factor, R_f of the unknown compound with the retention factor, R_f of the reference compound. The R_f value is determined by following equation:

$$R_f = \frac{\text{Distance travelled by the compound (cm)}}{\text{Distance travelled by solvent front (cm)}}$$

3.4.3 TLC Detection Methods

3.4.3.1 Natural Colour

Coloured compound can be clearly observed with naked eyes under light. For example, xanthophyll and chlorophyll compounds possess natural colour will show distinct yellow and green spot on the TLC plate. However, most of the organic compounds appeared to be colourless on TLC plate which needed further visualization aids.

3.4.3.2 UV Detection

This method effectively detects the presence of UV active compounds which contain aromatic ring or conjugated system. UV lamp with long (365 nm) and short (254 nm) wavelengths were used to visualize the sample spots on a developed TLC plate. TLC plates are usually impregnated with indicator such as manganese-activated zinc silicate or fluorophore. Hence, compounds will appear as dark grey spots on a bright green background under the short wavelength, meanwhile at long wavelength on a pale purple background, sample spots will appear in fluorescence colours.

3.4.3.3 Iodine Vapour Detection

The iodine vapour chamber was prepared by placing some iodine crystals into a closed chamber. The developed TLC plate was placed into the iodine chamber which was saturated with iodine vapour for detection of chemical compounds. The TLC plate was removed from the chamber after a few minutes, and some yellow or brown colour spots were observed. The staining effect is useful for detection of colourless compound such as non-conjugated compounds or terpenoids. Iodine forms dark-brown complex with chemical compounds and the brown spots were marked down using a pencil immediately after removal from the iodine chamber as the spots might dissipate over time.

3.4.3.5 Ferric Chloride Solution

Ferric chloride solution was prepared by dissolving 1.0 g of ferric chloride in 100 mL of methanol. TLC plate was sprayed with ferric chloride solution for detection of phenolic compounds. Formation of dark blue and red colour spots indicates the presence of phenols and hydroxamic acids, respectively.

3.5 Extraction and Isolation of Chemical Constituents from *Garcinia parvifolia*

Solvent extractions on the stem bark of *G. parvifolia* (2.0 kg) yielded a total of 53.9, 52.3 and 51.9 g crude dichloromethane, ethyl acetate and methanol extracts,

respectively. Phytochemical studies on the crude extracts using chromatographic methods have afforded four pure compounds. The ethyl acetate extract gave brasixanthone B (29.7 mg), 1,3,7-trihydroxy-2,4-*bis*(3-methylbut-2-enyl)xanthone (167.6 mg), rubraxanthone (3.3906 g) and tetraprenyltoluquinone (172.8 mg). About 2 g of each crude extract were kept for antioxidant assay.

3.5.1 Isolation of Chemical Compounds from Ethyl Acetate Extract

Approximately 52.3 g of crude ethyl acetate extract was subject to silica gel column chromatography (CC) (40-63 µm, 8.5 x 50 cm) packed in n-hexane and eluted with solvent mixture of hexane-ethyl acetate, and ethyl acetate-acetone in increasing polarity (90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100) followed by increasing concentration of methanol in acetone (10:90, 20:80. 30:70) to give 16 fractions (LCA1-16). Fraction LCA12 was further fractionated via silica gel CC (40-63 µm, 3.5 x 50 cm) to afford 25 subfractions (LCB1-25). Fractions LCB10-11 were combined and separated by Si gel CC (40-63 µm, 3.5 x 50 cm) to give 26 subfractions (LCC1-26). Subfractions LCC12 and LCC17 afforded brasixanthone B **(67,** 29.7 mg) and 1,3,7-trihydroxy-2,4-*bis*(3-methylbut-2-enyl)xanthone **(68**, 172.8 mg), respectively. Meanwhile, subfractions LCB13-16 were pooled and subjected to Si gel CC (40-63 µm, 3.5 x 50 cm) to yield 16 subfractions (LCD1-16). Subfractions LCD12 yielded rubraxanthone (**69**, 3.3906 g). Apart from that, fractions LCA14-16 were combined and purified by Si gel CC (40-63 µm, 3.5 x 50 cm) to give 33 subfractions (LCE1-33). Subfractions LCE12-14 were later combined and subjected to Si gel CC (40-63 µm, 3.5 x 50 cm) to

give 17 subfractions (LCF1-17). From subfraction LCF9, tetraprenyltoluquinone (TPTQ) (**70**, 156.6 mg) was obtained.

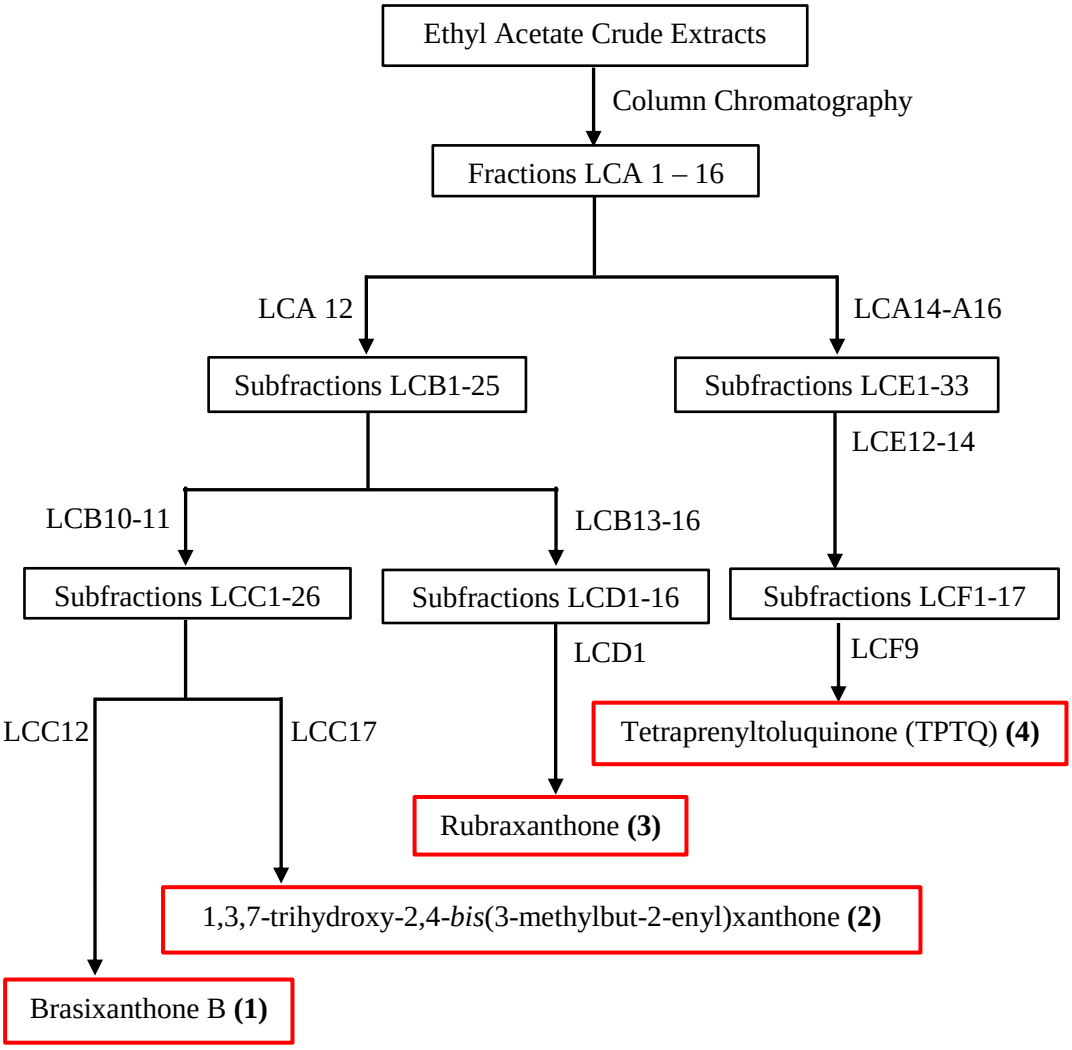


Figure 3.3: Isolation Pathway of Pure Compounds

3.6 Antioxidant Assay

The crude extracts, isolated compounds and standard compounds (kaempferol and ascorbic acid) were separately dissolved in methanol for preparation of master stocks at concentration of 1 mg/mL. The prepared master stocks were sonicated for 5 minutes to homogenize the solutions. DPPH solution was prepared at 2 mg/mL by dissolving DPPH powder in methanol. The DPPH solution and the prepared master stocks were kept in a 4^{o}C chiller in dark condition. This is to prevent samples exposure to light which may cause chemical decomposition.

Kaempferol and ascorbic acid served as positive control in this assay whereas negative control was prepared, which consists only the DPPH solution in methanol. The assay was conducted in a 96-well plate where the test compounds were prepared at concentrations of 240, 120, 60, 30, 15, 7.5 and 3.75 μg/mL from the master stocks through serial dilution, followed by addition of 10 μL DPPH solution and 90 μL methanol.

The 96-well plate was wrapped with aluminium foil immediately after the addition of reagents and stored in dark at room temperature for 30 minutes. Light is avoided because DPPH reagent is a light sensitive chemical. After 30 minutes, the absorbance of the mixtures was measured using a microplate reader, at wavelength of 520 nm. The assay was performed in triplicate and hence, the average absorbance value for each concentration was taken to calculate the inhibition rates of the test samples, using the following equation:

$$\text{Inhibition Rate (\%)} = \frac{A_0 - A_1}{A_0} \times 100\%$$

where A_0 = Absorbance of the negative control

A_1 = Absorbance of the test sample

IC_{50} value of sample can be obtained from the plotted graph of inhibition rate against concentration of sample. IC_{50} is defined as the concentration of sample that required to inhibit 50% of DPPH radical scavenging activity.

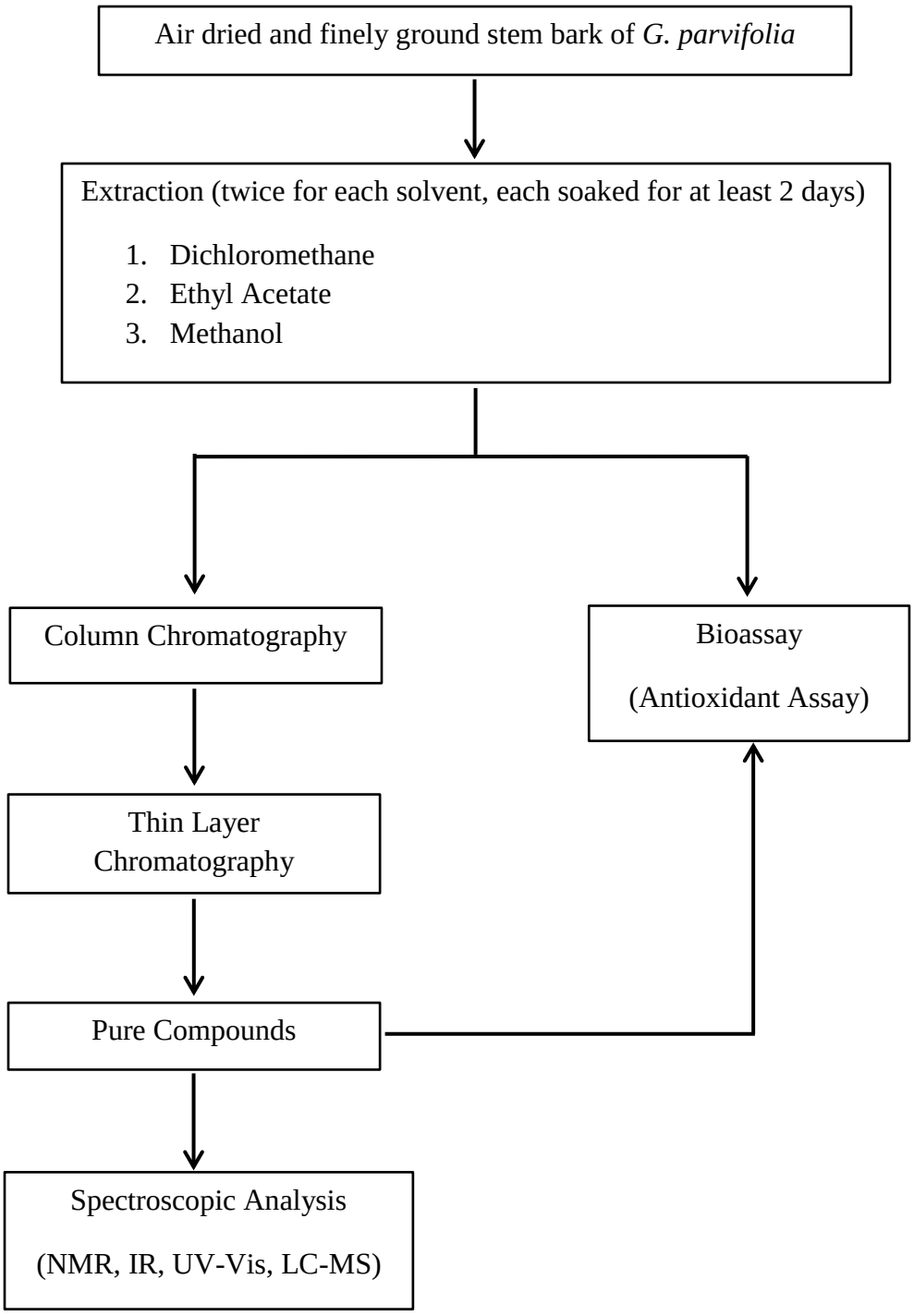


Figure 3.4: Summary of Methodology

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Isolation of Chemical Constituents from *Garcinia parvifolia*

Extensive column chromatography on the crude ethyl acetate extract of *G. parvifolia* led to the isolation of three xanthone derivatives, namely brasixanthone B **[67]**, 1,3,7-trihydroxy-2,4-*bis*(3-methylbut-2-enyl)xanthone **[68]** and rubraxanthone **[69]**, along with a terpenoid, tetraprenyltoluquinone (TPTQ) **[70]**. Compounds **69** (3.39 g) was isolated as a major compound. Meanwhile, compounds **67** (29.7 mg), **68** (172.8 mg) and **70** (156.6 mg) gave a much lesser yield than compound **69**. All these isolated compounds were found to be known as they have been previously reported from other *Garcinia* species along with their biological activities.

Isolation of brasixanthone B **[67]** from the stem bark of *Calophyllum soulattri* (Mah, *et al*, 2012) and *Calophyllum brasiliensis* (Chihiro, et al., 2002). It was found to exhibit significant cytotoxic activities against several cancer cell lines, such as Hep G2, HeLa, LS174T and IMR-32. Besides, this compound also showed strong inhibitory effect on Epstein-Barr virus (EBV) with an IC_{50} value of 120 mol ratio/32 pmol TPA.

1,3,7-Trihydroxy-2,4-*bis*(3-methylbut-2-enyl) xanthone **[68]** was reported for its isolation from the stem bark of *Garcinia dioica* and *Garcinia nitida* (Munekazu, et al, 1995). This compound was reported to show strong cytotoxic activity against the MDA-MB-231 human breast cancer cell line with an IC_{50} of 2.2 µg/ml (Ee, Lim and Rahmat, 2005).

Isolation of rubraxanthone **[69]** has been reported, from a few *Garcinia* species including *G. parvifolia* (Ee and Cheow, 2007), *G. eugenifolia* (Taher, Idris and Arbain, 2007), *G. griffithii* (Khaled, *et al*., 2013) and *G. cowa* (Fatma, *et al.*, 2015b). Compound **69** was found to exhibit cytotoxicity activities against HL-60 cell line (IC_{50} = 7.5 µg/ml) and CEM-SS cell line (IC_{50} = 5.0 µg/ml). Besides, this compound also showed antioxidant activity (IC_{50} = 0.89 mM) in DPPH assay and larvicidal activity (LC_{50} = 15.49 µg/ml) against larvae of *Aedes aegypti*.

Tetraprenyltoluquinone (TPTQ) **[70]** was previously reported for its isolation from the stem bark of *G. cowa* Roxb. Compound **70** was reported to exhibit strong cytotoxic activity towards H-460 cell line (lung cancer cells) with an IC_{50} value of 16.3 µg/ml (Fatma, *et al*., 2014).

In this study, the structural elucidation of isolated compounds **67**-**70** from the stem bark of *G. parvifolia* was accomplished based on the spectral analyses (NMR, UV, IR and LC-MS) and comparison with literature evidence.

4.2 Characterization and Structural Elucidation of Brasixanthone B [67]

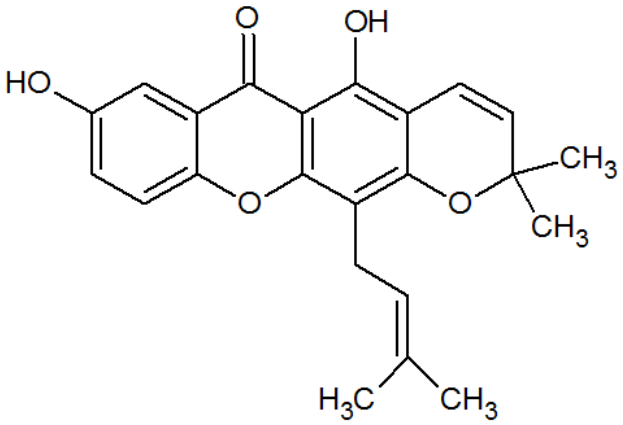


Figure 4.1: Chemical structure of brasixanthone B [67]

Brasixanthone B **[67]** was isolated from the crude ethyl acetate extract as pale yellow crystals, mp 225 – 228 ^{o}C (Lit. 227 – 229 ^{o}C, Mah, et al., 2012). This compound showed a single spot on TLC with an R_f value of 0.62 when developed using the solvent system of 20% hexane and 80% dichloromethane. It appeared as reddish-brown spot under 254 nm wavelength UV light, and as stained single brown spot when placed in iodine chamber. Compound **67** reacted positively to ferric chloride test, indicating it to be a phenolic compound.

The HRESI mass spectrum (Figure 4.2) indicated a pseudomolecular ion peak, $[M+H]^+$ at m/z 379.1540. The calculated accurate mass of compound **67** was 378.1484 g/mol which is consistent with the measured molecular weight, 378.1467 g/mol, and hence suggested compound **67** to have a molecular formula of $C_{23}H_{22}O_{5.}$

In the UV spectrum (Figure 4.3), compound **67** showed absorption maxima at wavelength of 245 and 294 nm indicating the presence of chromophores group with extended conjugation in the xanthone skeleton.

The proposed structure was further confirmed by FTIR analysis revealing the presence of hydroxyl (3235 cm^{-1}), carbonyl (1619 cm^{-1}), C=C (1466 cm^{-1}) and C-O (1229 cm^{-1}) functionalities in brasixanthone B **[67]**. The IR spectrum of compound **67** was showed in Figure 4.4.

The ^{1}H NMR (Figure 4.5) displayed signals suggesting a prenylated pyranoxanthone structure. The chelated hydroxyl proton, 1-OH gave a low field resonance at δ_H 13.28 (s, 1-OH). This hydroxyl proton is highly deshielded because of the intramolecular hydrogen bonding with the *peri* keto group. Meanwhile, the free hydroxy proton 7-OH displayed as sharp singlet at δ_H 8.95. The presence of 3-methylbut-2-enyl group was evident by the signals at δ_H 5.23 (1H, t, *J* = 6.73 Hz, H-2'), 3.46 (2H, d, *J* = 7.3 Hz, H-1'), 1.86 (3H, s, H-4'), 1.63 (3H, s, H-5'), and the existence of a dimethylchromene ring was indicated by the resonances of a pair of *cis*-olefinic protons at δ_H 6.68 and 5.72 (each *J* = 9.8 Hz) and the germinal dimethyl group at δ_H 1.48. The remaining three relatively deshielded proton signals at δ_H 7.54 (1H, d, *J* = 3.0 Hz), 7.50 (1H, d, *J* = 9.2 Hz) and 7.36 (1H, dd, *J* = 9.2, 3.0 Hz) were assigned to the three aromatic protons H-8, H-5 and H-6 in the ring B of xanthone.

The ^{13}C-NMR spectrum (Figure 4.6) revealed a total of 23 carbon signals assignable to a keto carbon, five oxygenated aromatic carbons, three protonated aromatic carbons, four substituted aromatic carbons, two pairs of olefinic carbons, one sp^3 quaternary carbon, one sp^3 methylene carbon and four methyl carbons. Carbon resonances in the region δ_C 100.0 to 200.0 were assigned to the sp^2 hybridized carbons in the proposed structure. Meanwhile, carbon signals in the region below δ_C 80.0 were due to the presence of sp^3 hybridized carbons. The most deshielded resonance at δ_C 180.9 was attributed to the keto carbon, C-9. Besides, the oxygenated aromatic carbons C-1, C-3, C-4a, C-7 and C-10a gave relatively more deshielded signals at δ_C 155.7, 158.0, 154.4, 154.1 and 150.0, respectively, then non-oxygenated aromatic carbons C-2, C-4, C-5, C-6, C-8, C-8a and C-9a gave signals at δ_C 104.0, 107.2, 119.1, 124.4, 108.4, 120.8 and 103.0, respectively. The sp^3 hybridized carbons C-13, C-14 & 15, C-1', C-4' and C-5' gave signals in the upfield region at δ_C 78.2, 27.6, 21.1, 17.3, 25.1 respectively.

The HMQC spectrum (Figure 4.7) provided details about $^1J_{CH}$ coupling between protons and their respective carbons revealing that all protonated carbons were found to give correlations in this spectrum. On the basis of integration of proton signals and their correlations to carbon signals in HMQC spectrum, compound **67** was established to have twelve quaternary, six methine, one methylene and four methyl carbons which were in agreement with the proposed structure.

In the HMBC spectrum (Figures 4.8 to 4.11), the chelated hydroxyl proton, 1-OH gave cross-peaks to an oxygenated carbon C-1 (δ_C 155.7) and two substituted aromatic carbons, C-2 (δ_C 104.0) and C-9a (δ_C 103.0); free hydroxyl proton, 7-OH was correlated to two protonated aromatic carbons, C-6 (δ_C 124.4) and C-8 (δ_C 108.4), revealing the two hydroxyl groups were linked to carbons C-1 and C-7, respectively. The presence of 3-methylbut-2-enyl group with its characteristic methylene protons H-1' showed correlations with carbons, C-3 (δ_C 158.0), C-4 (δ_C 107.2) and C-4a (δ_C 154.4) indicating the prenyl group to be attached to carbon C-4. Besides, the dimethylchromene ring was found to linearly fused to the ring A of xanthone at carbon positions C-2 and C-3, and this was evident from the correlations shown by the *cis*-olefinic proton, H-11 (δ_H 6.68) with carbon C-3 (δ_C 158.0), and proton H-12 (δ_H 5.72) with the carbon C-2 (δ_C 104.0). Based on the all the spectral evidence, compound **67** was deduced to be brasixanthone B and the spectral data are summarized in Table 4.1.

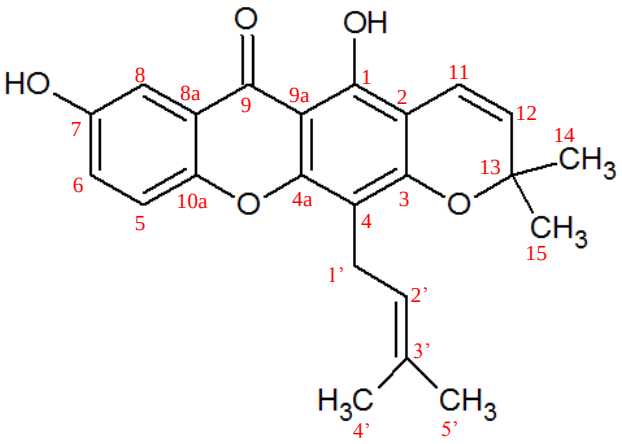
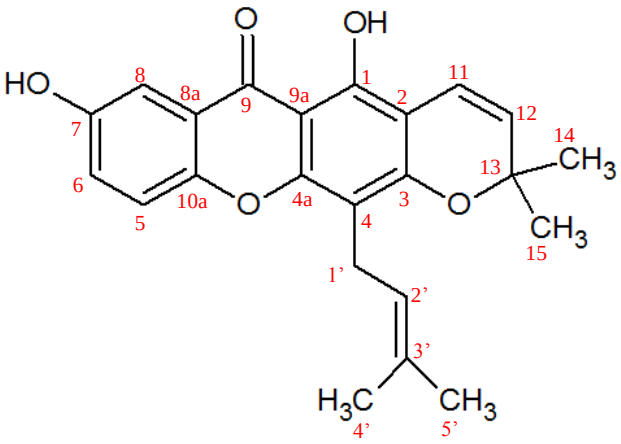
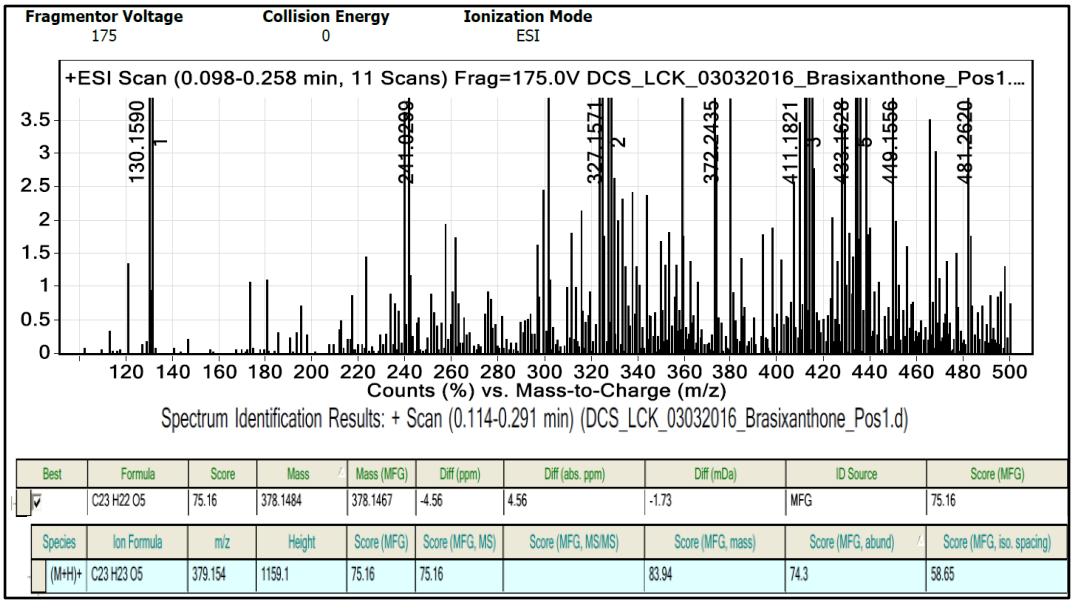


Table 4.1: Summary of NMR spectral data for brasixanthone B [67]

Position	δ_H (ppm)	δ_C (ppm)	HMBC
1	-	155.7	-
2	-	104.0	-
3	-	158.0	-
4	-	107.2	-
4a	-	154.4	-
5	7.50 (1H, d, *J* = 9.2 Hz)	119.1	C-7, 8a
6	7.36 (1H, dd, *J* = 9.2, 3.0 Hz)	124.4	C-8, 10a
7	-	154.1	-
8	7.54 (1H, d, *J* = 3.0 Hz)	108.4	C-6, 10a
8a	-	120.8	-
9	-	180.9	-
9a	-	103.0	-
10a	-	150.0	-
11	6.68 (1H, d, *J* = 9.8 Hz)	115.3	C-3, 13
12	5.72 (1H, d, *J* = 9.8 Hz)	127.8	C-2, 13
13	-	78.2	-
14	1.48 (3H, s)	27.6	C-12, 13, 15
15	1.48 (3H, s)	27.6	C-12, 13, 14
1’	3.46 (2H, d, *J* = 7.3 Hz)	21.1	C-2’, 3, 3’, 4, 4a
2’	5.23 (1H, t, *J* = 7.3 Hz)	122.3	-
3’	-	131.0	-
4’	1.86 (3H, s)	17.3	C-2’, 3’, 5’
5’	1.63 (3H, s)	25.1	C-2’, 3’, 4’
1 - OH	13.28 (1H, s)	-	C-1, 2, 9a
7 - OH	8.95 (1H, s)	-	C-6, 8





Best	Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)	ID Source	Score (MFG)
✓	C23 H22 O5	75.16	378.1484	378.1467	-4.56	4.56	-1.73	MFG	75.16

Species	Ion Formula	m/z	Height	Score (MFG)	Score (MFG, MS)	Score (MFG, MS/MS)	Score (MFG, mass)	Score (MFG, abund)	Score (MFG, iso. spacing)
(M+H)+	C23 H23 O5	379.154	1159.1	75.16	75.16		83.94	74.3	58.65

Figure 4.2: HRESI mass spectrum of brasixanthone B [67]

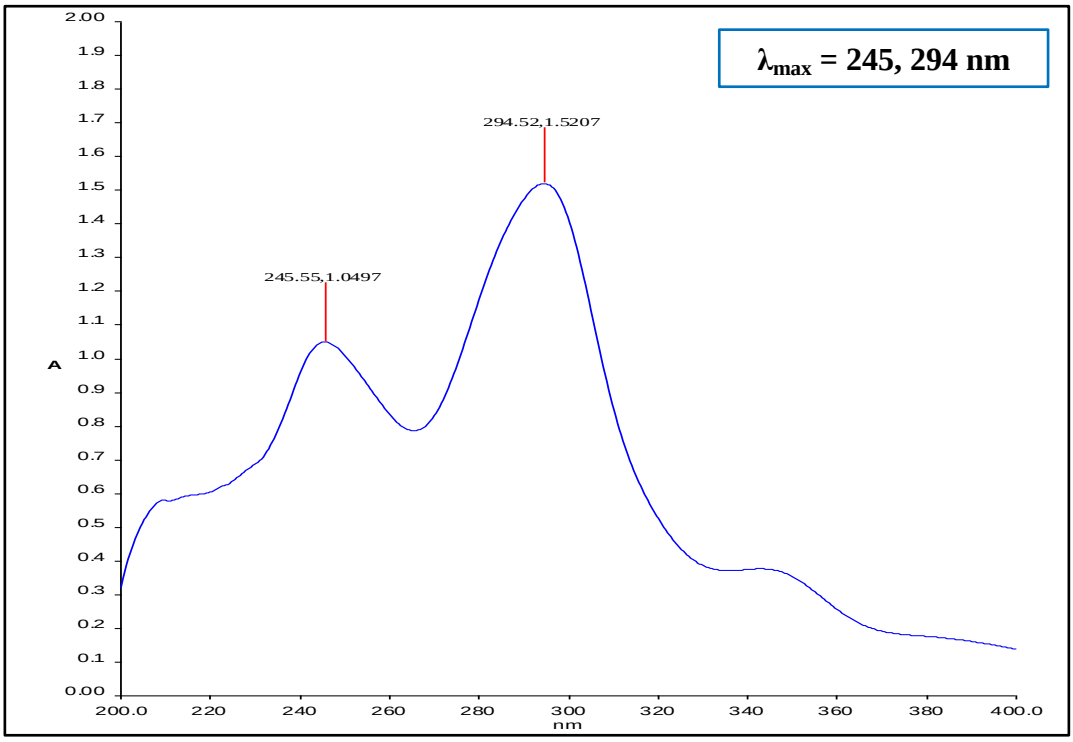


Figure 4.3: UV-Vis spectrum of brasixanthone B [67]

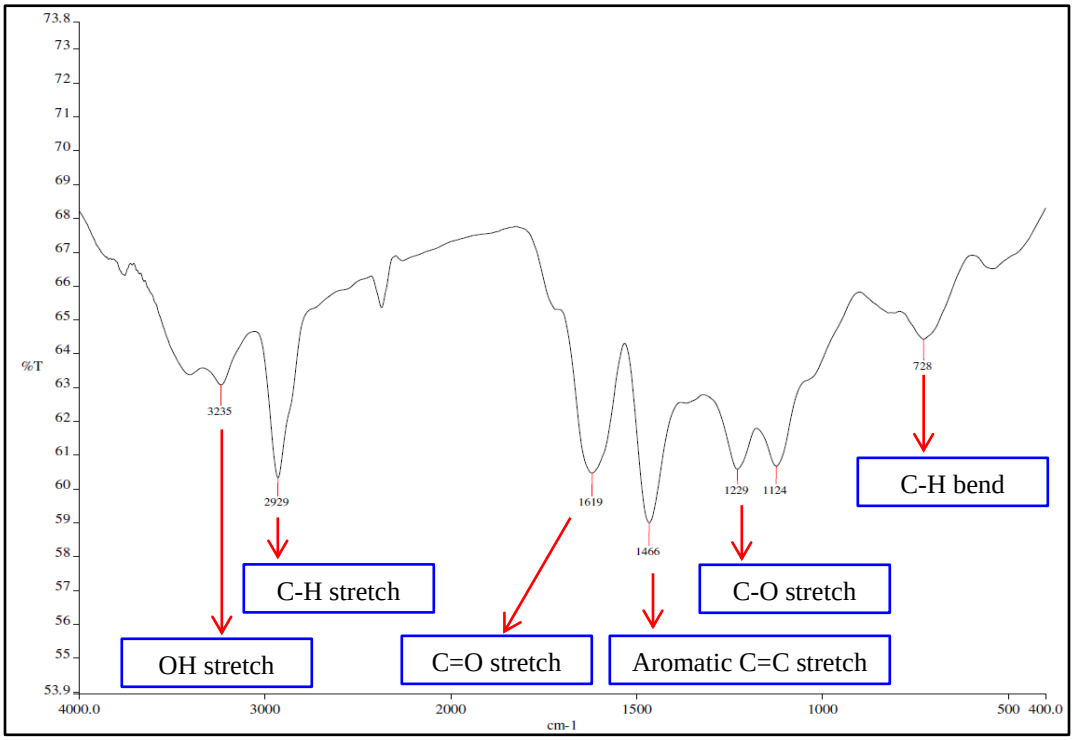


Figure 4.4: IR spectrum of brasixanthone B [67]

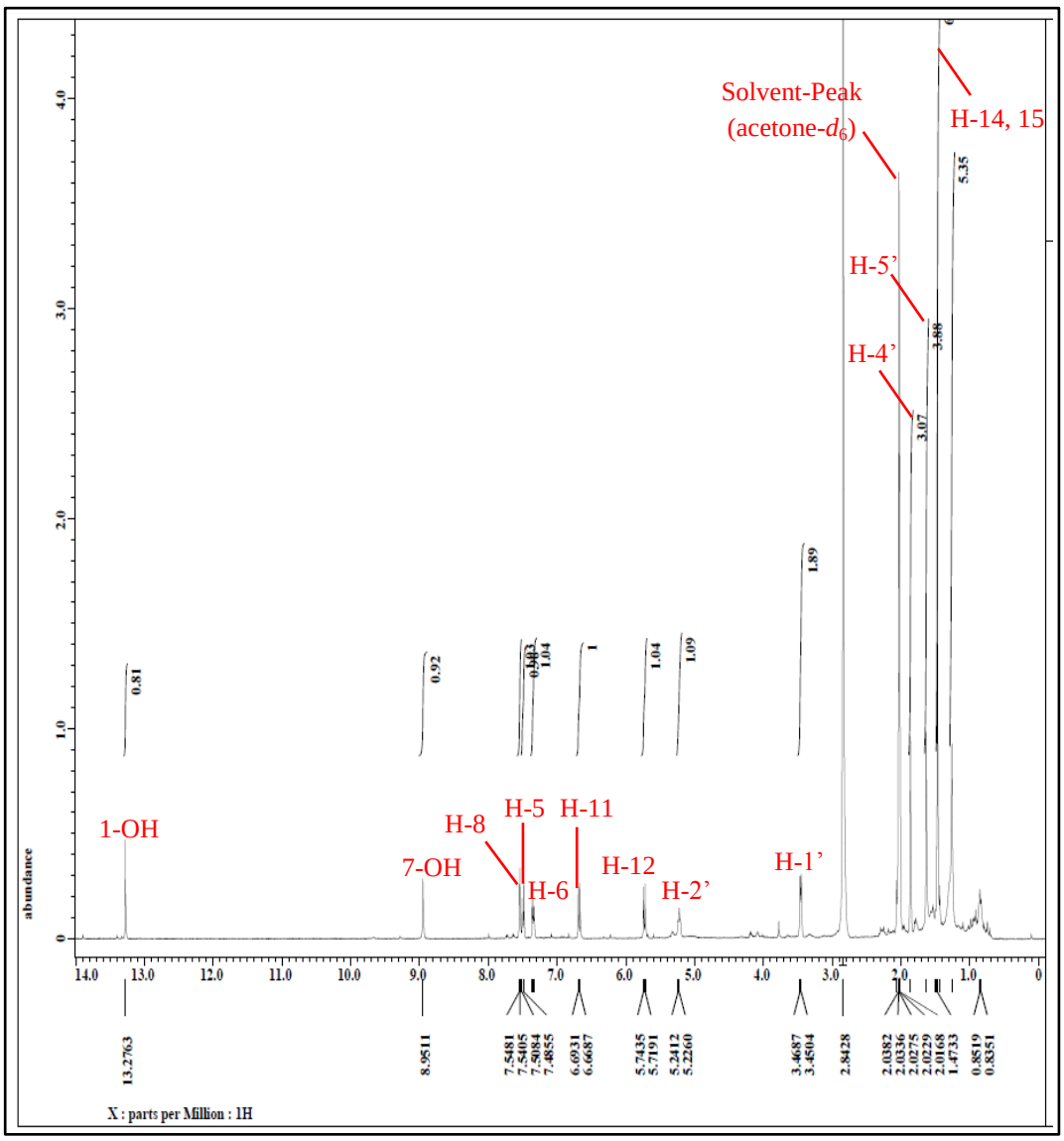
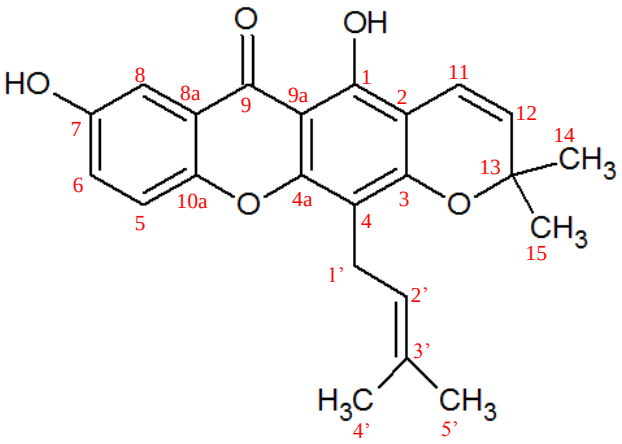


Figure 4.5: ^{1}H NMR spectrum of brasixanthone B [67] (400 MHz, acetone-d_6)



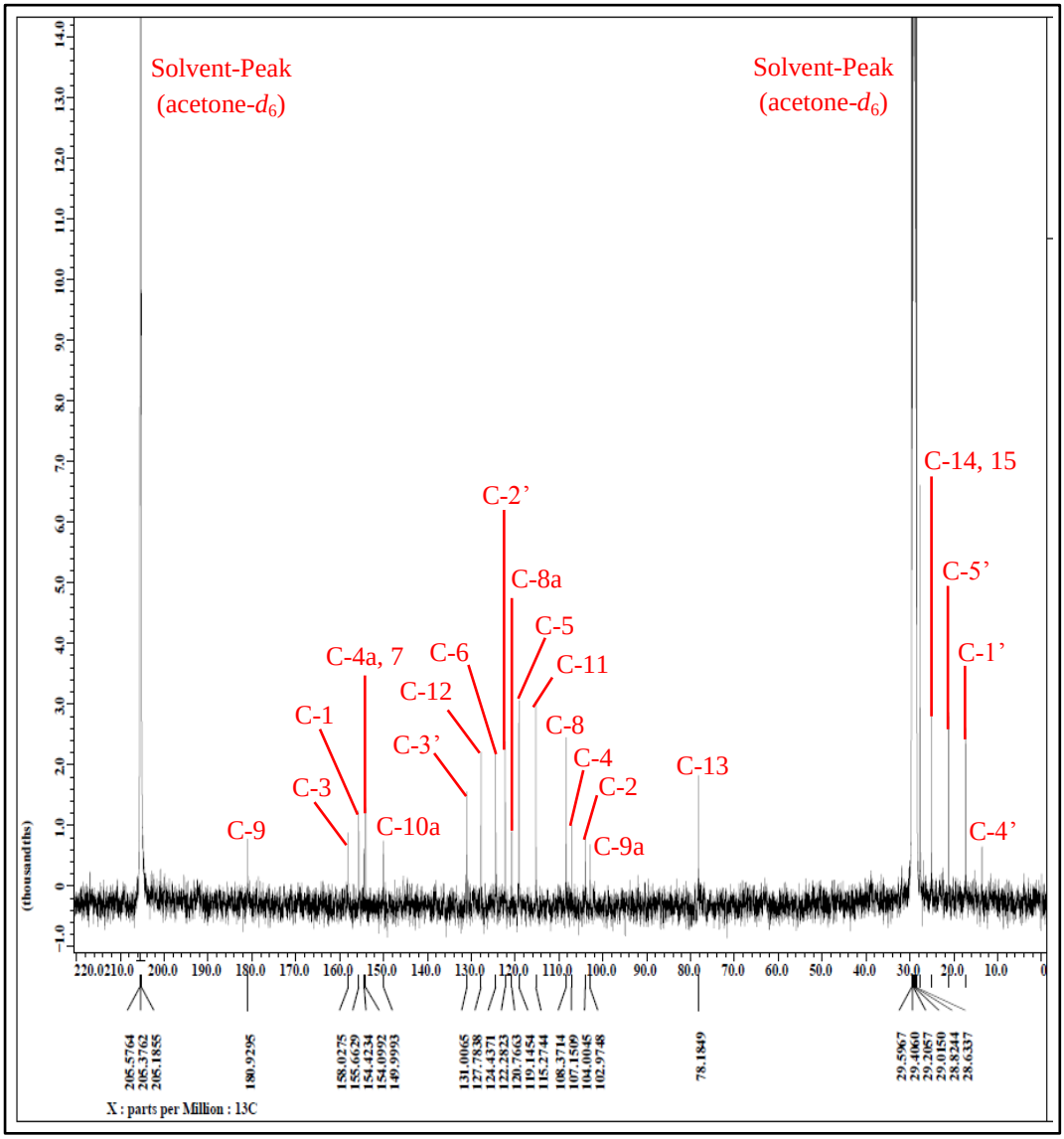


Figure 4.6: ^{13}C NMR spectrum of brasixanthone B [67] (100 MHz, acetone-d_6)

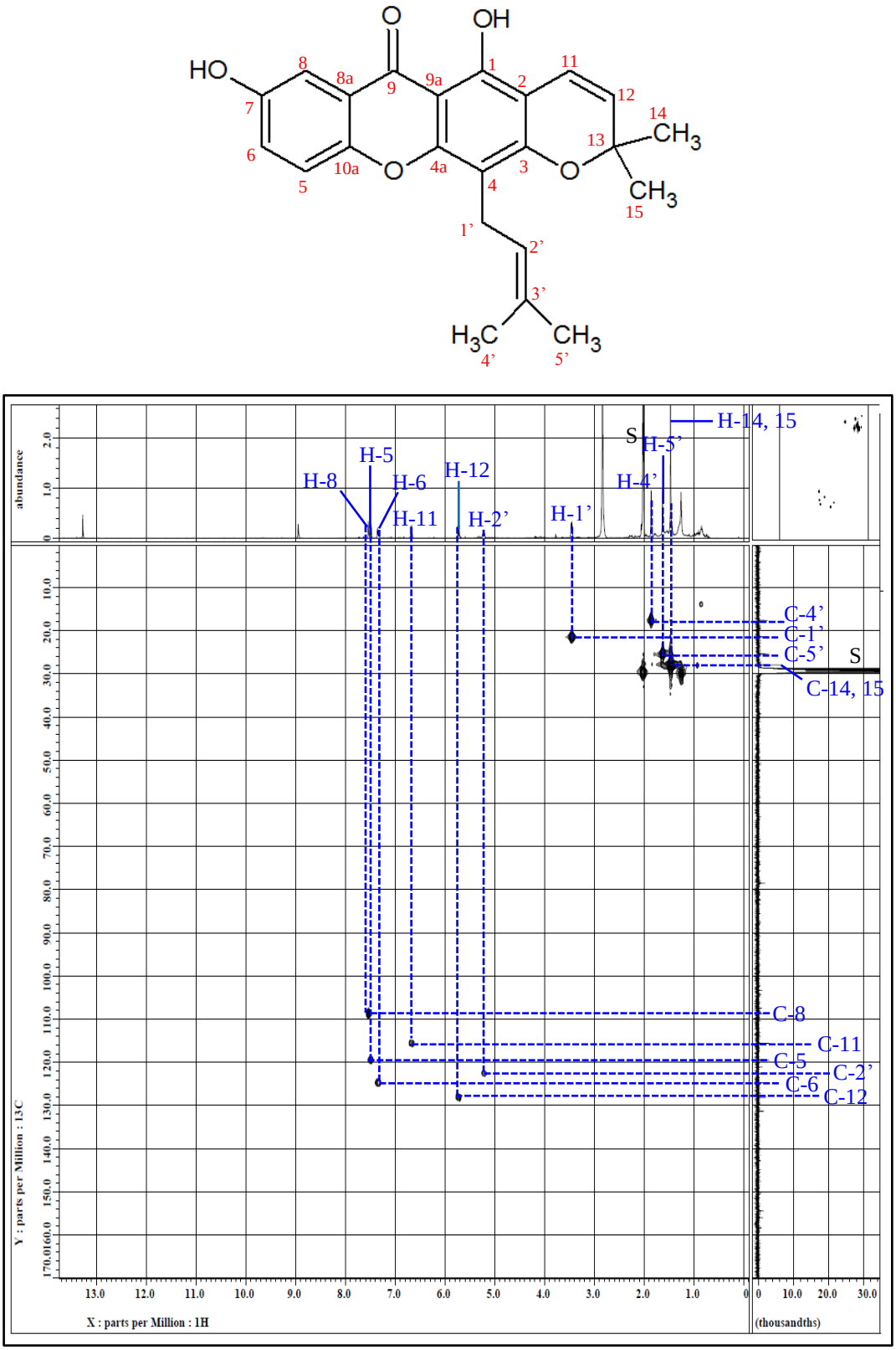


Figure 4.7: HMQC spectrum of brasixanthone B [67]

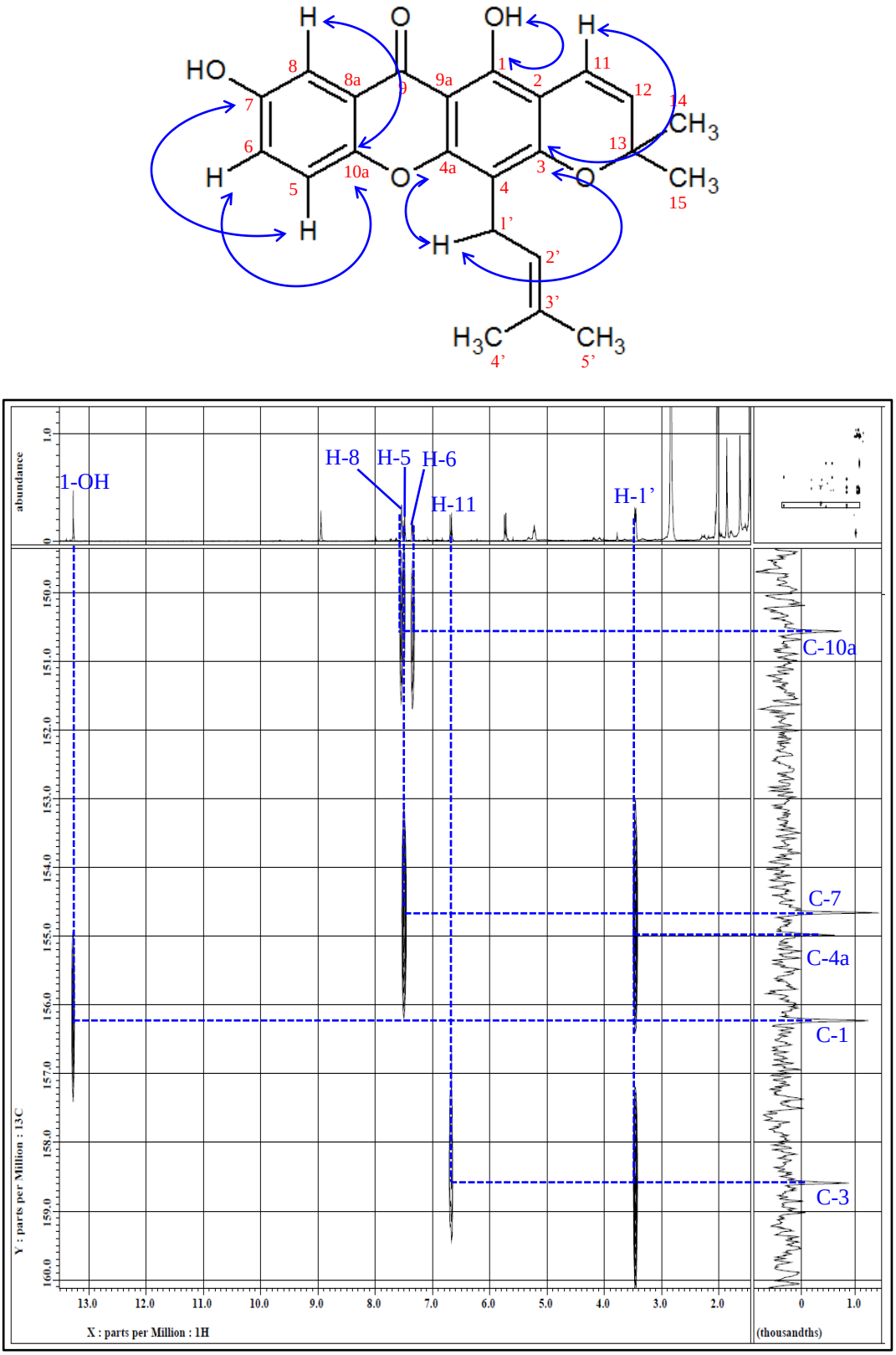


Figure 4.8: HMBC spectrum of brasixanthone B [67] (expanded)

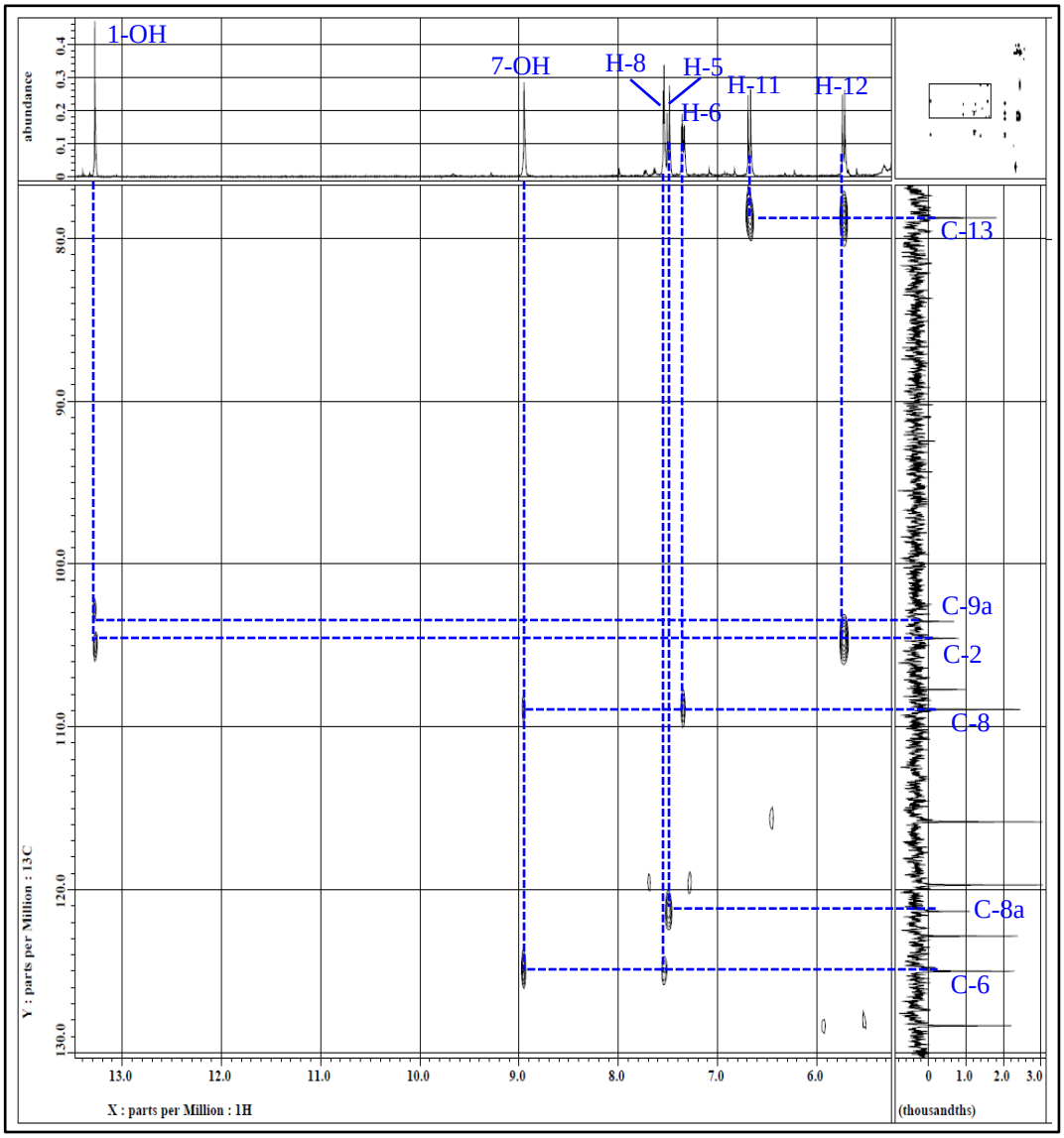


Figure 4.9: HMBC spectrum of brasixanthone B [67] (expanded)

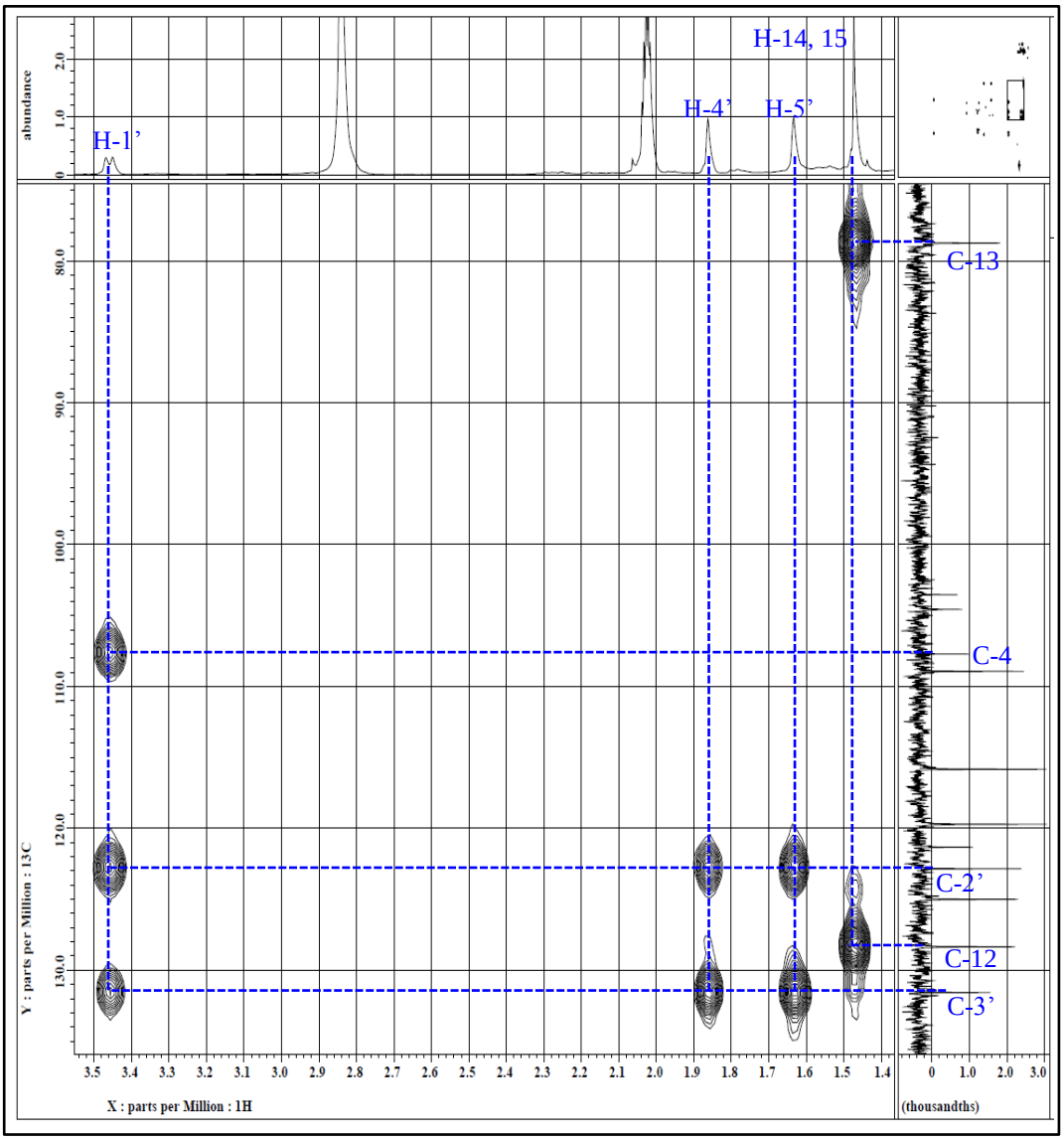


Figure 4.10: HMBC spectrum of brasixanthone B [67] (expanded)

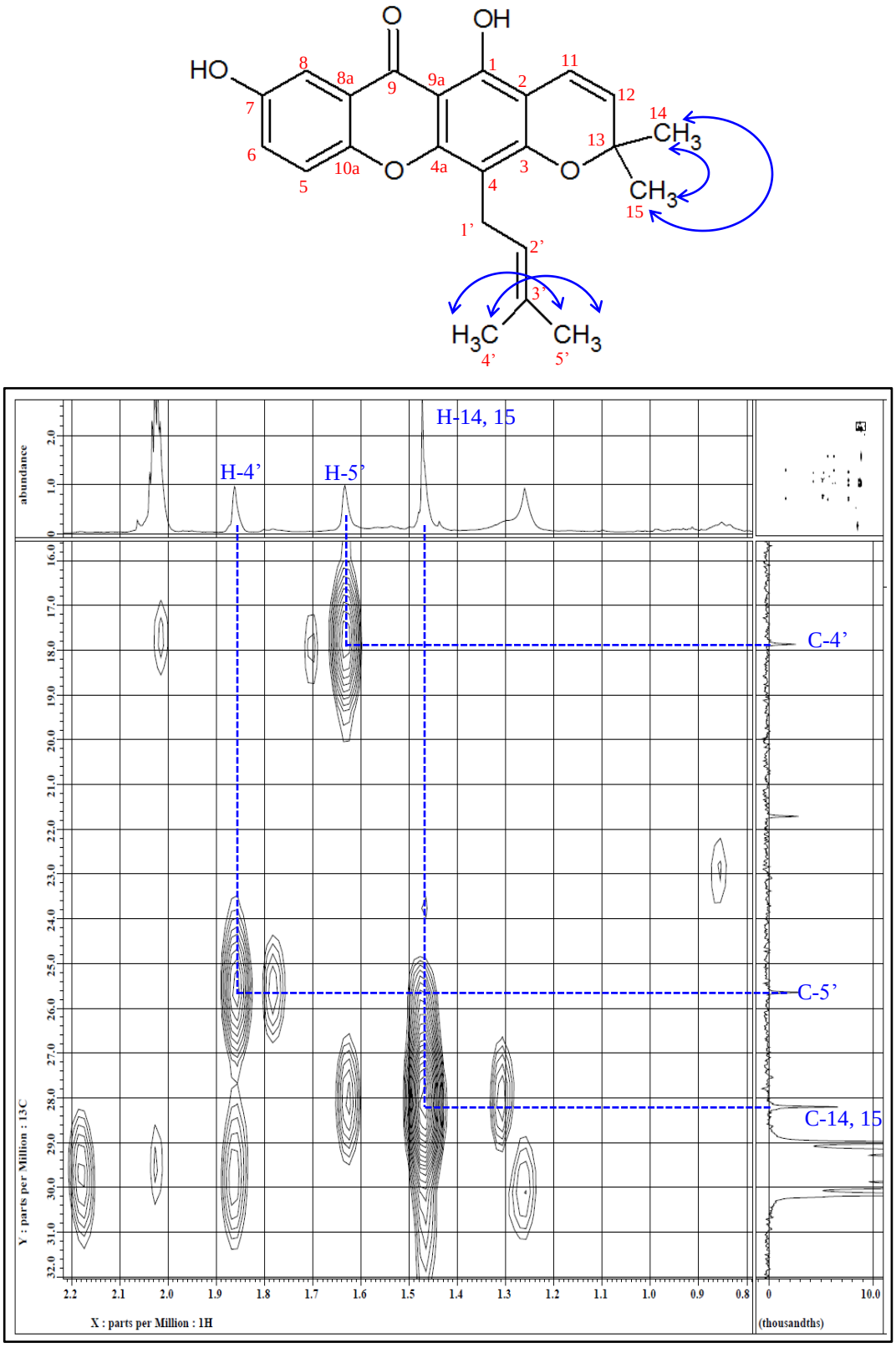


Figure 4.11: HMBC spectrum of brasixanthone B [67] (expanded)

4.3 Characterization and Structural Elucidation of 1,3,7-Trihydroxy-2,4-*bis*(3-methylbut-2-enyl)xanthone [68]

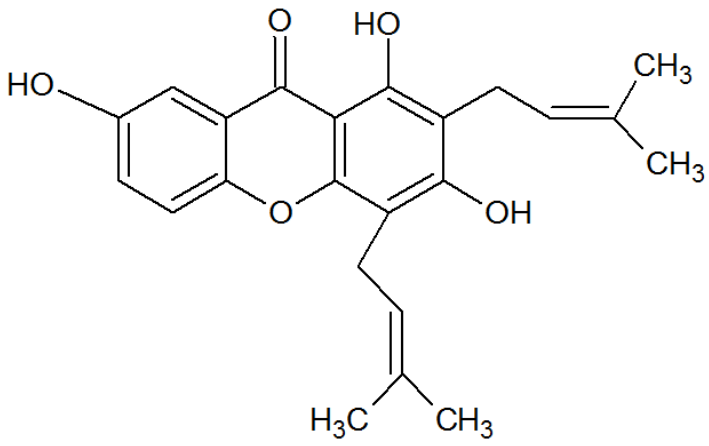


Figure 4.12: Chemical Structure of compound 68

Compound **68** was obtained as pale yellow crystals from crude ethyl acetate extract, mp 127 -130 ^{o}C (Lit. 128 – 129 ^{o}C, Ee, Lim and Rahmat, 2005). It gave a single spot on TLC with an R_f value of 0.65 when developed using the solvent system of dichloromethane and acetone in the ratio of 8:2. Compound **68** appeared as reddish-brown spot under 254 nm wavelength UV light, and as stained brown spot when placed in iodine chamber. It reacted positively to ferric chloride reagent to yield greenish-black spot indicating the compound to have a phenolic nature.

The HRESI mass spectrum (Figure 4.13) indicated a pseudomolecular ion peak, $[M+H]^+$ at m/z 381.1697. The calculated accurate mass of compound **68** was 380.1633 g/mol which is in correspondence with the measured molecular weight, 380.1624 g/mol. Thus, compound **68** was deduced to have a molecular formula of $C_{23}H_{24}O_5$.

In the UV-Vis spectrum (Figure 4.14), compound **68** gave absorption maxima (λ_{max}) at wavelength of 243, 266 and 298 nm. The results of spectrum indicating the presence of highly conjugated double bonds in the xanthone chromophores. The UV absorption bands was deduced to be the $\pi \longrightarrow \pi^*$ transitions.

The proposed structure was further characterized by FTIR analysis revealing the presence of OH stretch (3389 cm^{-1}), C-H stretch (2927 cm^{-1}), C=O (1630 cm^{-1}), sp^2 C=C stretch (1472 cm^{-1}), C-O stretch (1231 cm^{-1}) and C-H bends (803 cm^{-1}) functionalities in 1,3,7-trihydroxy-2,4-*bis*(3-methyl-but-2-enyl)xanthone **[68]**. Figure 4.15 illustrated the IR spectrum of compound **68**.

The ^1H NMR spectrum (Figure 4.16) revealed resonances indicating a diprenylated xanthone. The chelated hydroxy proton, 1-OH gave the most deshielded signal at δ_H 13.25 (s, 1-OH). The presence of two 3-methylbut-2-enyl groups in the proposed structure was evidenced by the signals δ_H 5.21 (2H, t, *J* = 6.7 Hz, H-12, H-17), 3.55 (2H, d, *J* = 6.7 Hz, H-16), 3.41 (2H, d, *J* = 6.7 Hz, H-11), 1.86 (3H, s, H-19), 1.76 (3H, s, H-14) and 1.63 (6H, s, H-15, H-20). Meanwhile, the free hydroxyl groups (7-OH / 3-OH) displayed a broad singlet at δ_H 8.68. The remaining three relatively deshielded proton signals at δ_H 7.54 (1H, d, *J* = 3.1 Hz), 7.44 (1H, d, *J* = 9.2 Hz), 7.31 (1H, dd, *J* = 9.2, 3.1 Hz) were assigned to the three aromatic protons, H-8, H-5 and H-6 in the ring B.

The ^{13}C-NMR spectrum (Figure 4.17) revealed a total of 23 carbon signals assignable to a keto carbon, five oxygenated aromatic carbons, three protonated aromatic carbons, four substituted aromatic carbons, two pairs of olefinic carbons, two sp^3 hybridized methylene carbons and four methyl carbons. The most deshielded resonance at δ_C 180.8 was attributed to the keto carbon, C-9. Besides, the oxygenated aromatic carbons C-1, C-3, C-4a, C-7 and C-10a gave relatively more deshielded signals at δ_C 158.4, 160.4, 153.1, 153.9 and 150.0, respectively. On the other hand, non-oxygenated aromatic carbons C-2, C-4, C-5, C-6, C-8, C-8a and C-9a exhibited signals at δ_C 110.0, 105.9, 119.0, 124.2, 108.4, 120.8 and 102.8, respectively. The sp^3 hybridized carbons C-11, C-14, C-15, C-16, C-19 and C-20 gave signals in the upfield region at δ_C 21.3, 17.2, 25.1, 21.6, 17.3 and 25.1, respectively. Besides, two pairs of olefinic carbons C-12 & 13 and C-17 & 18 showed carbon signals at δ_C 122.3, 131.7, 122.3 and 131.5, respectively.

The proposed structure was further confirmed by DEPT (Figure 4.18) revealing the presence of twelve quaternary carbons (C), five methine groups (CH), two methylene groups (CH_2) and four methyl groups (CH_3). The DEPT-90 spectrum showed only the methine carbon signals. Meanwhile, in the DEPT-135 spectrum, the upper trace signals were assigned either to methine or methyl carbons whereas lower trace signals (negative peaks) indicated the presence of methylene carbons. The result obtained was in agreement with the proposed structure.

The HMQC spectrum (Figure 4.19) provided information about $^{1}J_{CH}$ coupling between protons and their respective carbons. All the protonated carbons in the proposed structure gave correlations in this spectrum. For instance, the proton signal at δ_H 7.54 (1H, d, J = 3.1 Hz) was correlated to carbon signal at δ_C 108.4 (C-8) through $^{1}J_{CH}$ coupling.

In the HMBC spectrum (Figure 4.20), the presence of two 3-methylbut-2-enyl groups with their characteristic methylene protons, H-11 (δ_H 3.41) showed correlation with carbon, C-2 (δ_C 110.0), and H-16 (δ_H 3.55) gave cross peaks to carbons C-3 (δ_C 160.4), C-4 (δ_C 115.9) and C-4a (δ_C 153.1) indicating the two prenyl groups to be attached to carbons C-2 and C-4 in the ring B of xanthone. Compound **68** was thus established to be 1,3,7-trihydroxy-2,4-*bis*(3-methylbut-2-enyl)xanthone. The NMR spectral data of compound **68** are summarized in Table 4.2.

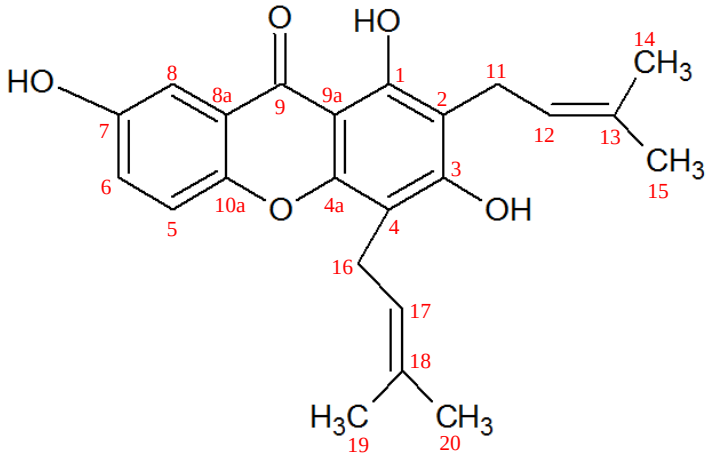
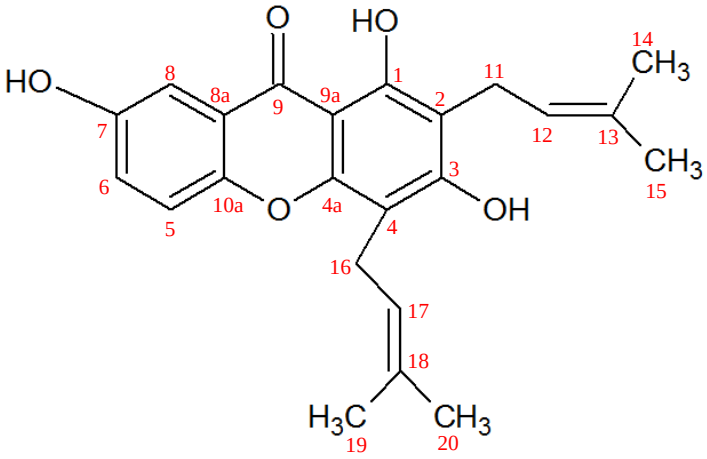


Table 4.2: Summary of NMR spectral data for compound 68

Position	δ_H (ppm	δ_C (ppm)	HMBC	DEPT
1	-	158.4	-	C
2	-	110.0	-	C
3	-	160.4	-	C
4	-	105.9	-	C
4a	-	153.1	-	C
5	7.44 (1H, d, *J* = 9.2 Hz)	119.0	C-7, 8a, 10a	CH
6	7.31 (1H, dd, *J* = 9.2, 3.1 Hz)	124.2	C-7, 8, 10a	CH
7	-	153.9	-	C
8	7.54 (1H, d, *J* = 3.1 Hz)	108.4	C-6, 7, 10a	CH
8a	-	120.8	-	C
9	-	180.8	-	C
9a	-	102.8	-	C
10a	-	150.0	-	C
11	3.41 (2H, d, *J* = 6.7 Hz)	21.3	C-2, 12, 13	CH_2
12	5.21 (1H, t, *J* = 6.7 Hz)	122.3	C-11, 14, 15	CH
13	-	131.7	-	C
14	1.76 (3H, s)	17.2	C-12, 13, 15	CH_3
15	1.63 (3H, s)	25.1	C-12, 13, 14	CH_3
16	3.55 (2H, d, *J* = 6.7 Hz)	21.6	C-3, 4, 4a, 17, 18	CH_2
17	5.21 (1H, t, *J* = 6.7 Hz)	122.3	C-16, 19, 20	CH
18	-	131.5	-	C
19	1.86 (3H, s)	17.3	C-17, 18, 20	CH_3
20	1.63 (3H, s)	25.1	C-17, 18, 19	CH_3
1 - OH	13.24 (1H, s)	-	C-1, 2, 9a	-
3 - OH	8.68 (1H, brs)	-	-	-
7 - OH	8.68 (1H, brs)	-	-	-



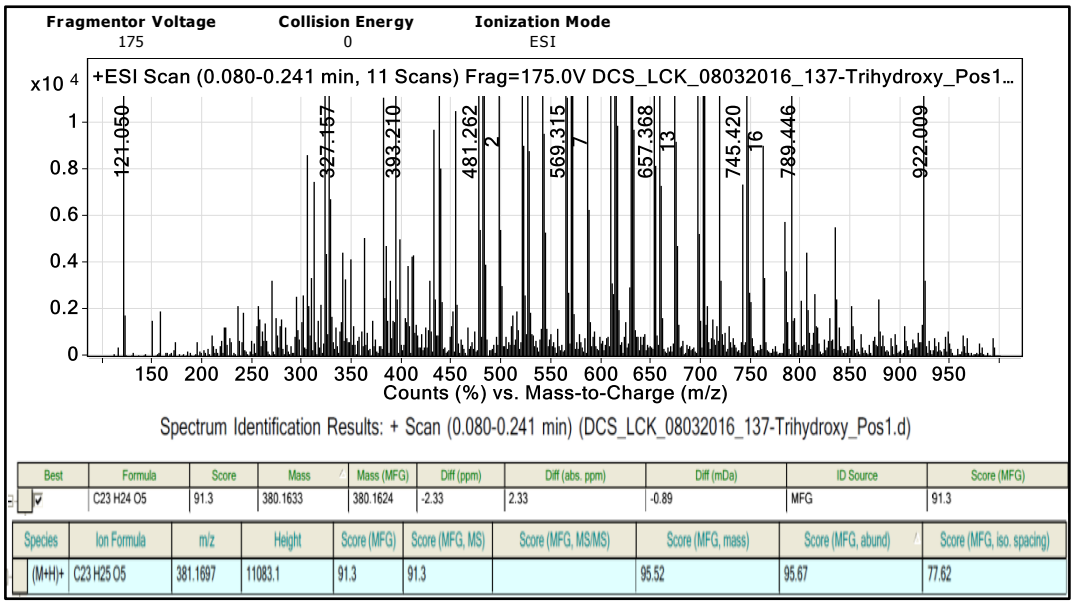


Figure 4.13: HRESI mass spectrum of compound 68

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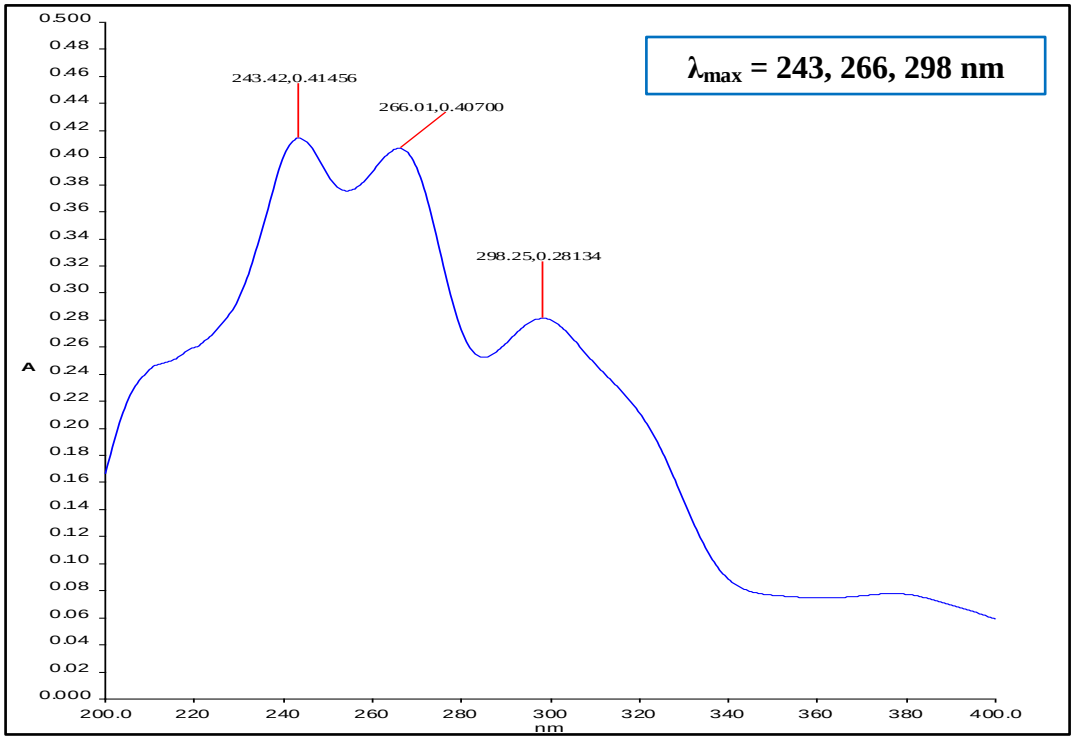


Figure 4.14: UV-Vis spectrum of compound 68

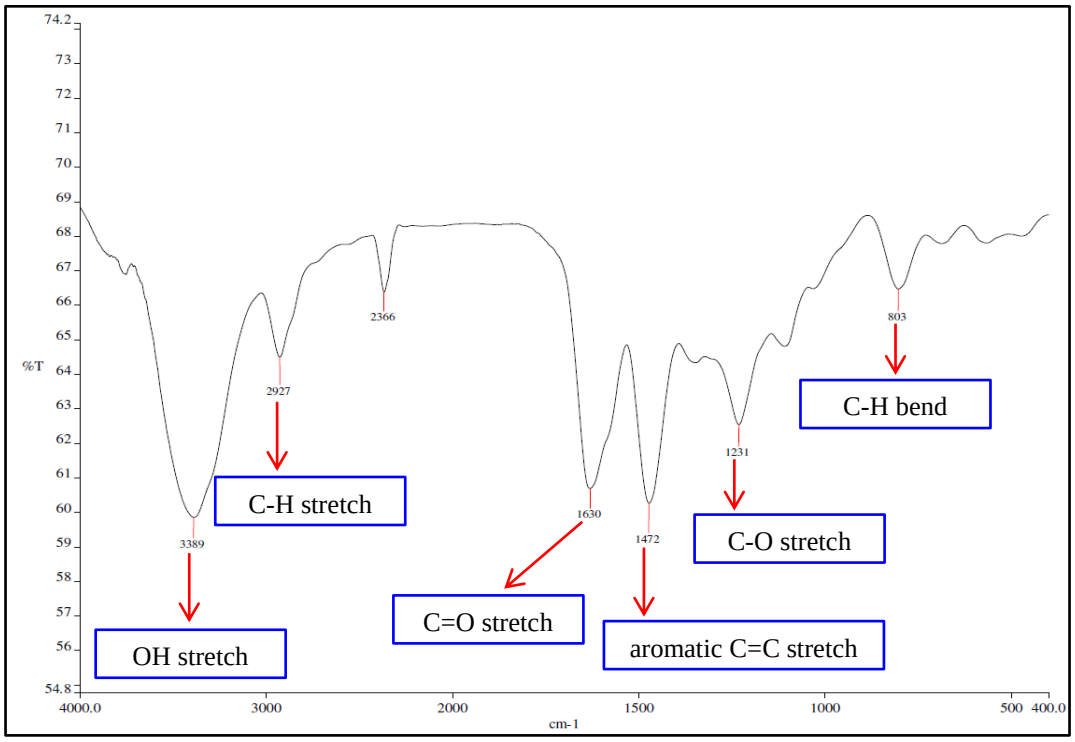


Figure 4.15: IR spectrum of compound 68

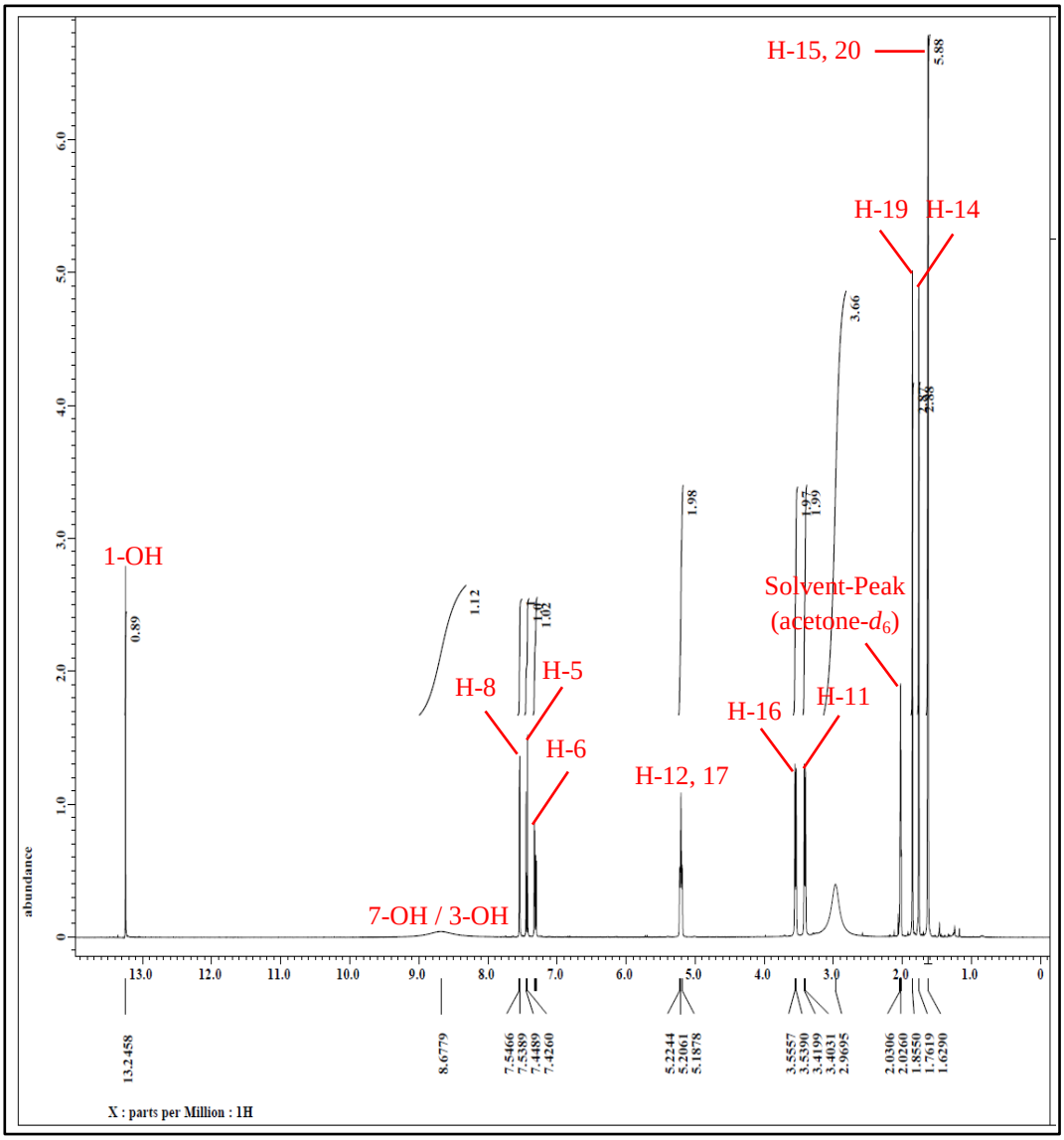
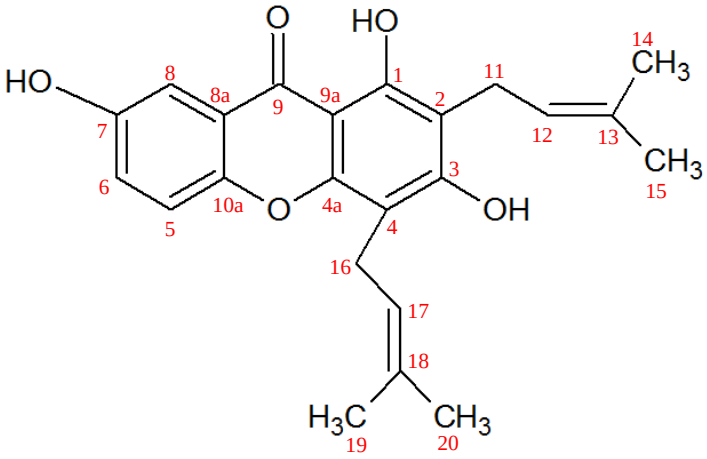


Figure 4.16: ^{1}H NMR spectrum of compound 68 (400 MHz, acetone-d_6)



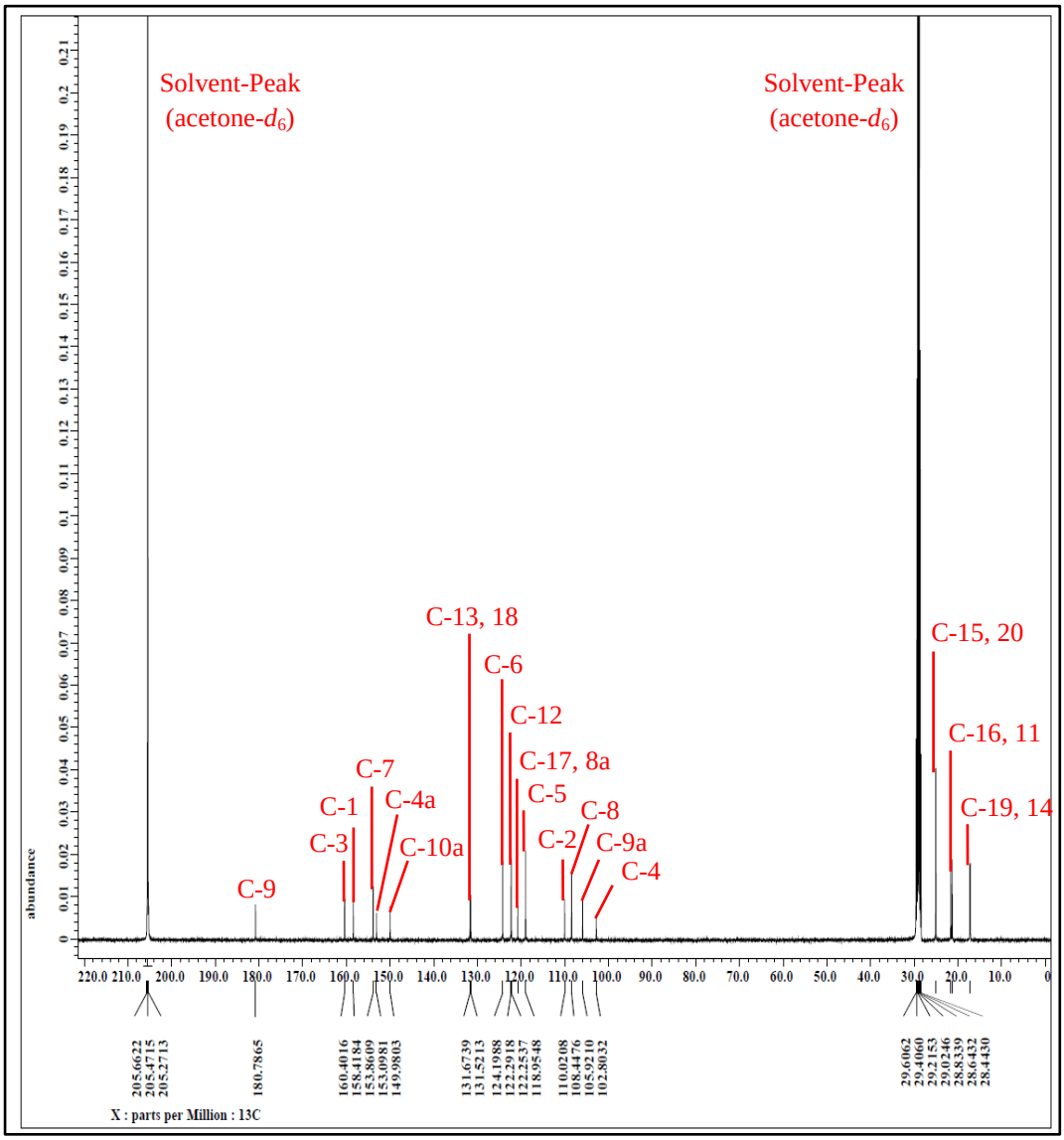


Figure 4.17: ^{13}C NMR spectrum of compound 68 (100 MHz, acetone-d_6)

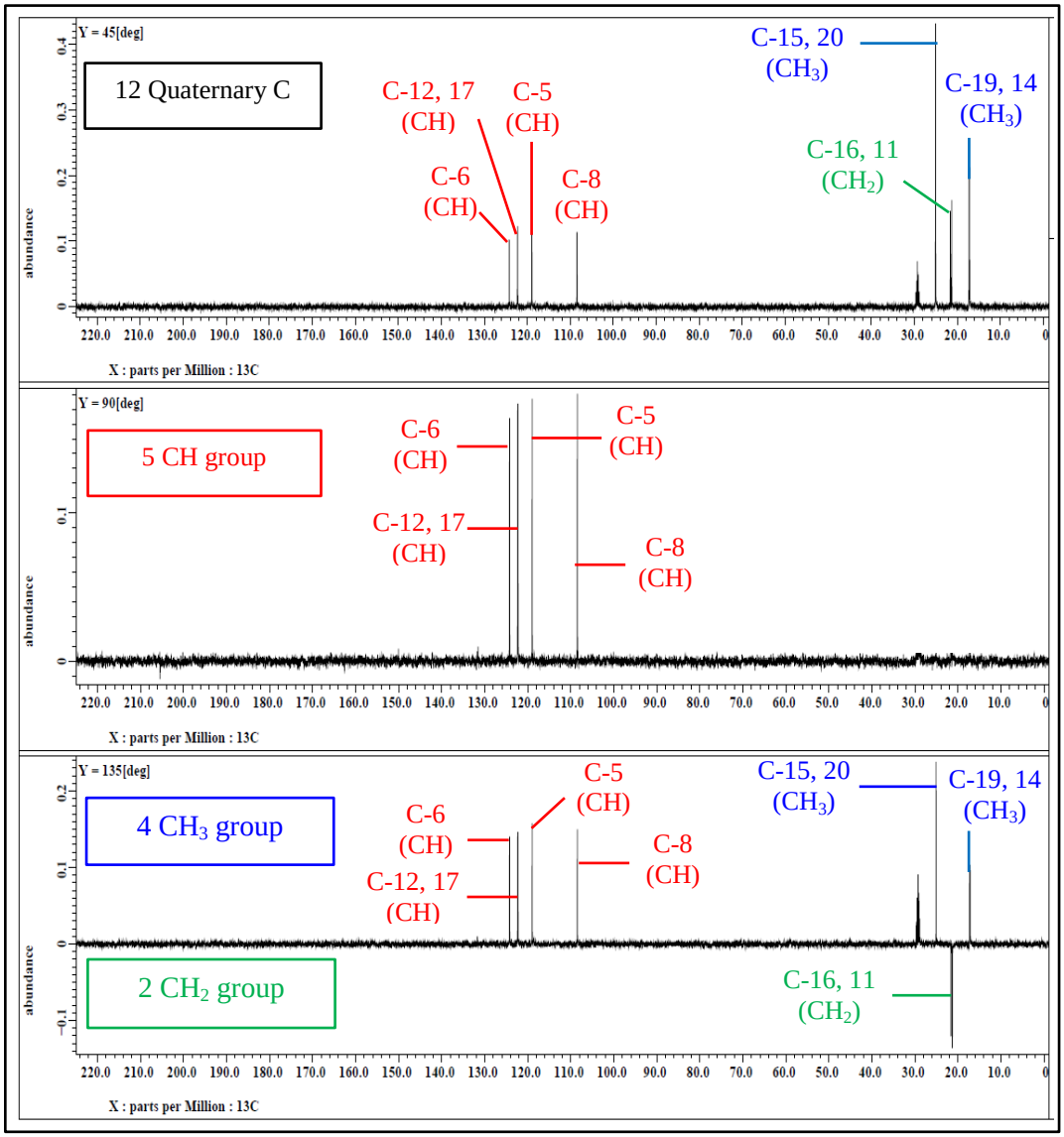


Figure 4.18: DEPT spectrum of compound 68

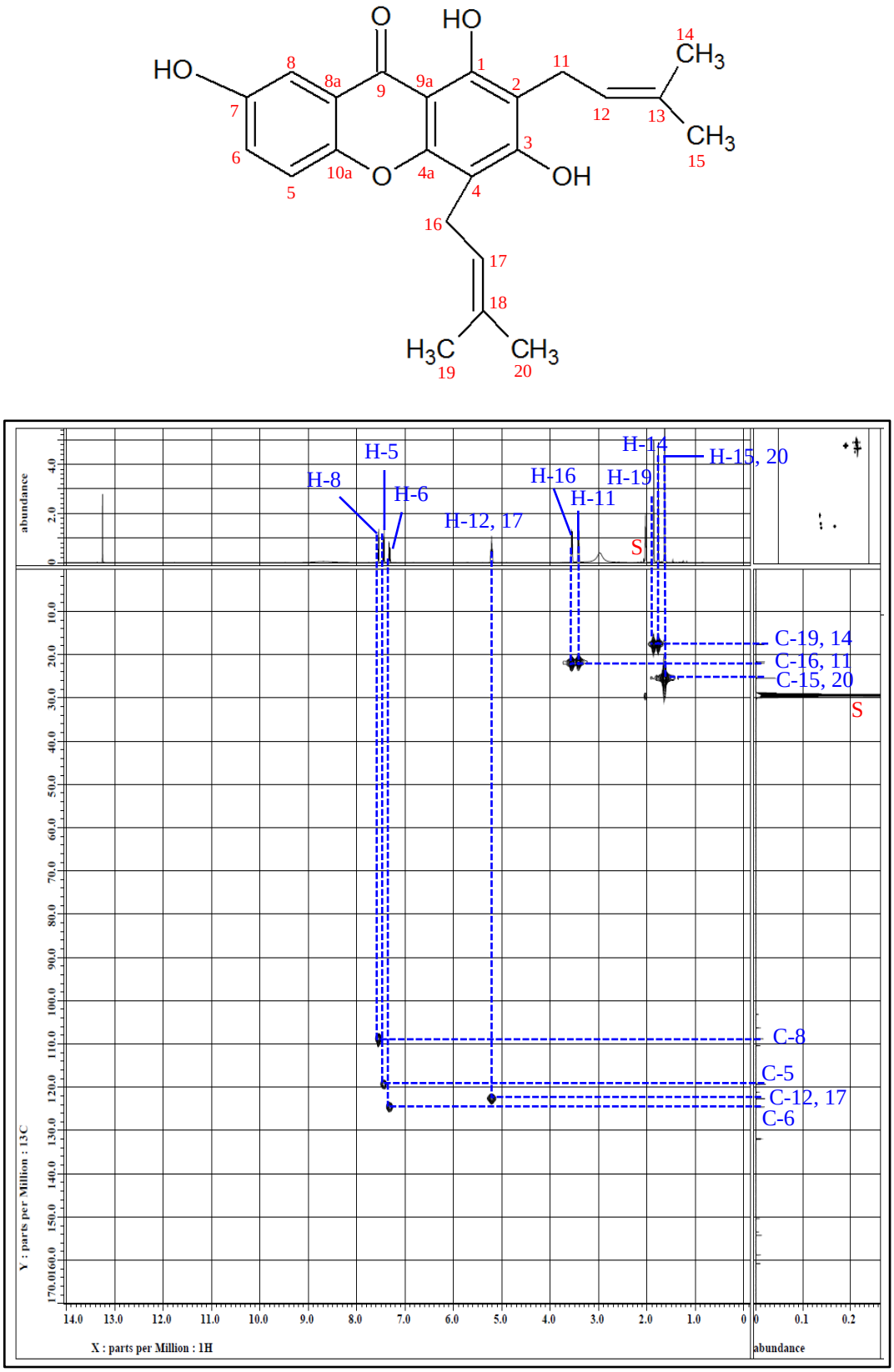
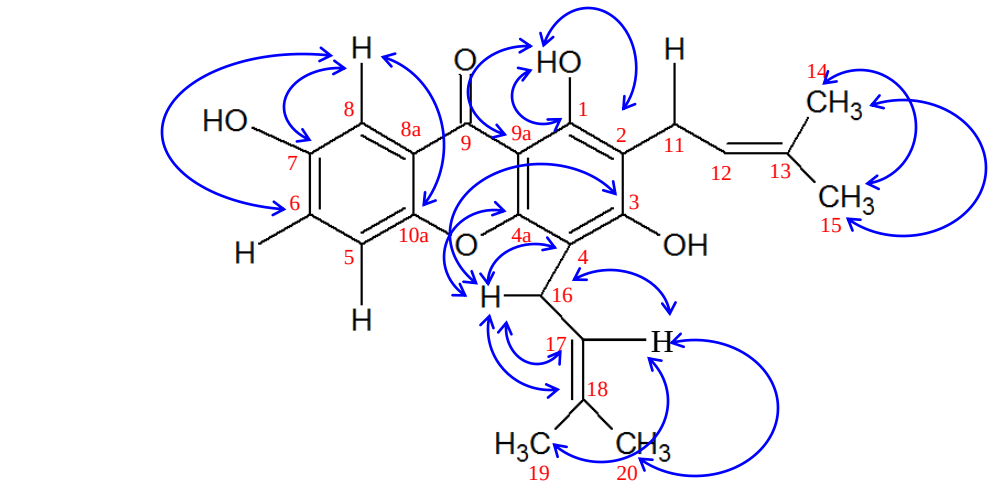


Figure 4.19: HMQC spectrum of compound 68



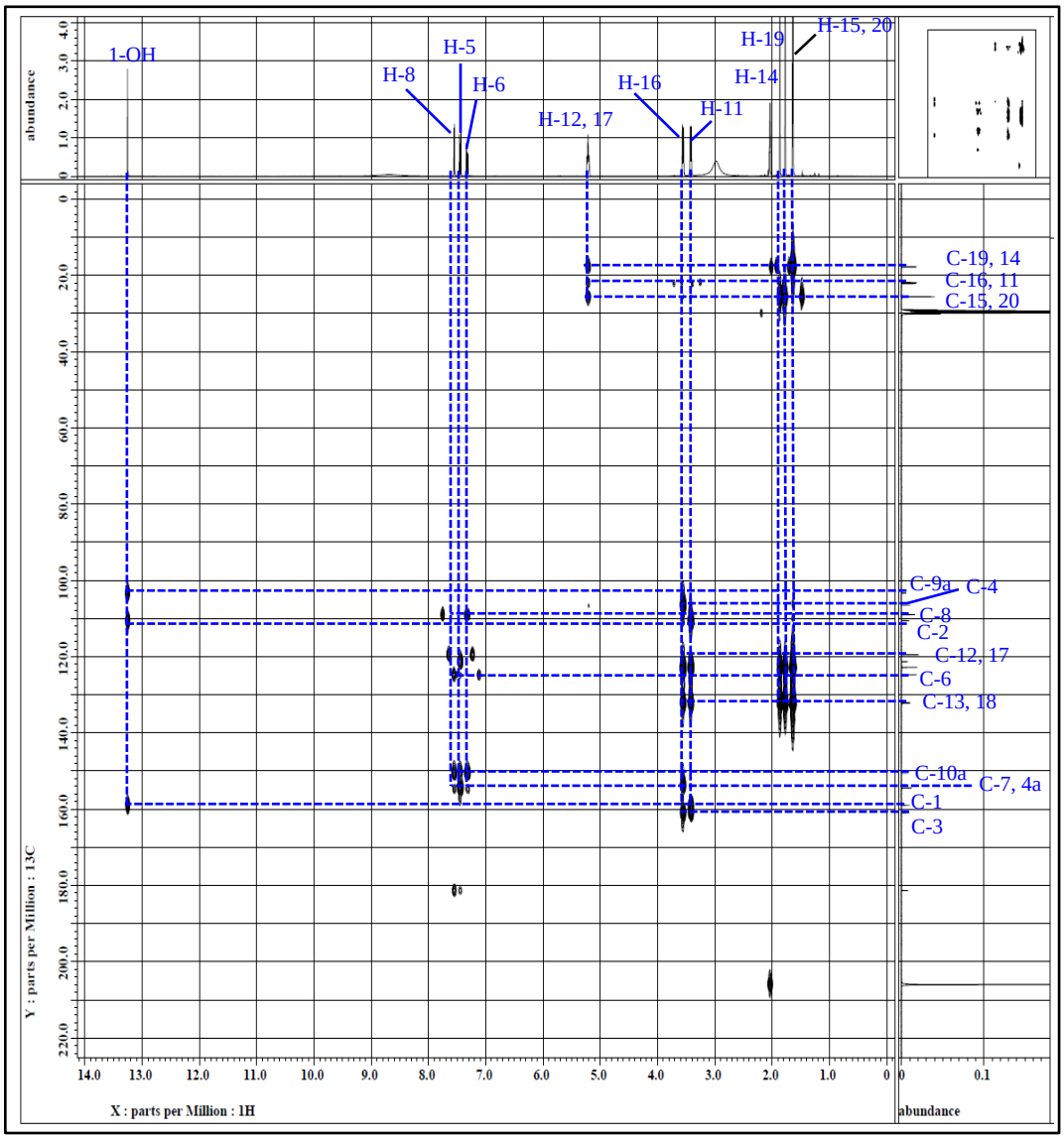


Figure 4.20: HMBC spectrum of compound 68

4.4 Characterization and Structural Elucidation of Rubraxanthone (69)

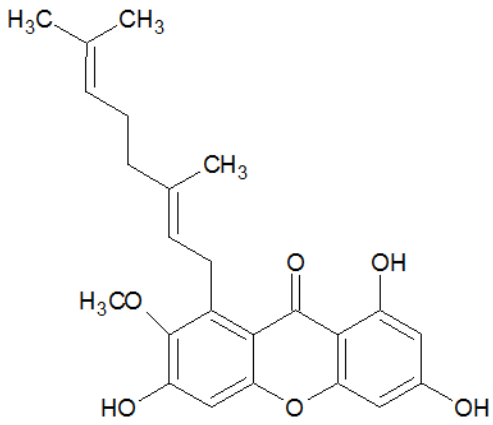


Figure 4.21: Chemical structure of compound 69

Rubraxanthone **[69]** was isolated from stem bark of *G. parvifolia* as orange solid, mp 199 – 201 ^{o}C (Lit. 201 – 203 ^{o}C, Fatma, et al., 2015b). This compound displayed a single spot on TLC with an R_f value of 0.85 when developed using the solvent system of 80 % dichloromethane and 20 % acetone. It appeared as reddish-brown spot under 254 nm wavelength UV light, and as stained single brown spot when placed in iodine chamber. Compound **69** reacted positively with $FeCl_3$ reagent indicating it to be a phenolic compound.

The HRESI mass spectrum (Figure 4.22) showed a pseudomolecular ion peak, $[M+H]^+$ at m/z 411.1802. The calculated accurate mass of rubraxanthone **[69]** was 410.1744 g/mol which is in agreement with the measured molecular weight, 410.1729 g/mol and suggested compound **69** to have a molecular formula of $C_{24}H_{26}O_6$.

In UV-Vis spectrum (Figure 4.23), compound **69** was found to exhibit absorption maxima at wavelengths of 248, 299 and 344 nm indicating the presence of chromophores group with extended conjugation in benzene and pyrone moieties. Absorption peaks present in the range of 150 -250 nm are corresponding to $n \rightarrow \sigma^*$ transitions, whereas $\pi \longrightarrow \pi^*$ transitions are revealed by absorption bands in the range of 270 -350 nm.

The proposed structure was further supported by FTIR (Figure 4.24) analysis showing the presence of different chemical groups in this compound including hydroxyl (3424 cm^{-1}), C-H stretch (2922 cm^{-1}), carbonyl (1612 cm^{-1}**),** C=C stretch (1457 cm^{-1}) and C-O stretch (1274 cm^{-1}).

The ^1H NMR spectrum (Figure 4.25) exhibited signals revealing a geranylated xanthone structure. The chelated hydroxyl proton, 1-OH displayed at the most deshielded signal at δ_H 13.45 (s, 1-OH). The presence of geranyl moiety in the structure was indicated by the characteristic proton signals at δ_H 5.25 (1H, t, *J* = 6.1 Hz, H-2'), 4.99 (1H, d, *J* = 6.1 Hz, H-6'), 4.07 (2H, d, *J* = 6.1 Hz, H-1'), 2.02 (3H, m, H-5'), 1.94 (2H, t, *J* = 6.7 Hz, H-4'), 1.79 (3H, s), 1.52 (3H, s) and 1.48 (3H, s). Meanwhile, both the free hydroxyl groups 7-OH and 3-OH gave a broad singlet at δ_H 9.55. The remaining three aromatic protons H-5, H-4 and H-2 gave signals at δ_H 6.77 (1H, s), 6.25 (1H, d, *J* = 1.8 Hz), 6.15 (1H, d, *J* = 1.8 Hz), respectively. Lastly, the methoxy protons, 7-OCH_3 revealed signal at δ_H 3.76 (3H, s).

The ^{13}C-NMR spectrum (Figure 4.26) showed a total of 24 carbon signals assignable to a keto carbon, six oxygenated aromatic carbons, three protonated aromatic carbons, three substituted aromatic carbons, two pairs of olefinic carbons, three sp^3 hybridized methylene carbons and four methyl carbons. The most deshielded resonance at δ_C 180.8 was attributed to the keto carbon, C-9. Meanwhile, the relatively deshielded signals at δ_C 164.5, 164.0, 157.2, 156.6, 155.4 and 143.7 were assigned to the six oxygenated aromatic carbons C-3, C-1, C-6, C-10a, C-4a and C-7, respectively. On the other hand, six non-oxygenated aromatic carbons C-4, C-2, C-5, C-9a, C-8a and C-8 gave resonances at δ_C 93.0, 97.9, 102.0, 102.9, 111.1 and 137.4, respectively. The six sp^3 hybridized carbons C-4', C-5', C-1', C-9', C-8' and C-10' gave signals in the upfield region at δ_C 39.6, 26.5, 26.0, 25.0, 16.9 and 15.8, respectively. The presence of methoxy carbon, 7-OCH_3 gave signal at δ_C 60.6, and two pairs of olefinic carbons, C-2' & 3' and C-6' & 7' in the geranyl moiety displayed signals at δ_C 124.0, 134.2, 124.4 and 130.7.

The proposed structure was confirmed by DEPT (Figure 4.27) analysis to have twelve quaternary carbons (C), five methine (CH), three methylene (CH_2) and four methyl (CH_3) groups. In the HMBC spectrum (Figure 4.29), the chelated hydroxyl proton, 1-OH showed correlations to an oxygenated carbon C-1 (δ_C 164.0), and two aromatic carbons, C-2 (δ_C 97.9) and C-9a (δ_C 102.9). Meanwhile, the presence of geranyl moiety with its characteristic methylene protons H-1' (δ_H 4.07) gave cross-peaks to three quaternary carbon C-7 (δ_C 143.7), C-8 (δ_C 137.4) and C-8a (δ_C 111.1), revealing the geranyl group to be linked to carbon C-8. The NMR spectral data are summarized in Table 4.3.

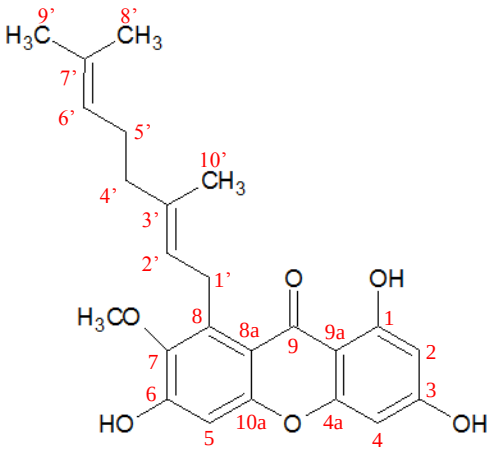
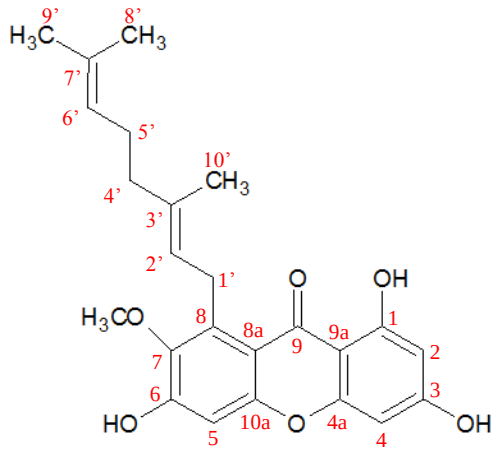
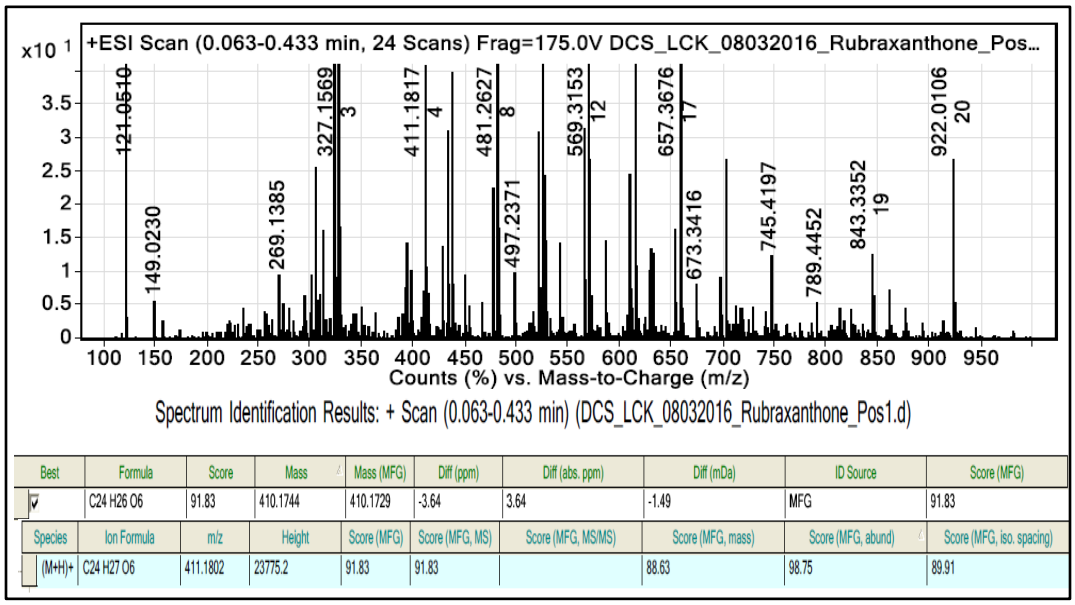


Table 4.3: Summary of NMR spectral data for compound 69

Position	δ_H (ppm)	δ_C (ppm)	HMBC	DEPT
1	-	164.0	-	C
2	6.15 (1H, d, *J* = 1.8 Hz)	97.9	C-3, 4, 9a	CH
3	-	164.5	-	C
4	6.25 (1H, d, *J* = 1.8 Hz)	93.0	C-2, 9a	CH
4a	-	155.4	-	C
5	6.77 (1H, s)	102.0	C-7, 8a, 10a	CH
6	-	157.1	-	C
7	-	143.7	-	C
8	-	137.4	-	C
8a	-	111.1	-	C
9	-	180.8	-	C
9a	-	102.9	-	C
10a	-	156.6	-	C
1’	4.07 (2H, d, *J* = 6.1 Hz)	26.0	C-3’, 7, 8, 8a	CH_2
2’	5.25 (1H, t, *J* = 6.1 Hz)	124.0	C-4’, 8	CH
3’	-	134.2	-	C
4’	1.94 (2H, t, *J* = 6.7 Hz)	39.6	C-3’, 5’, 6’, 10’	CH_2
5’	2.02 (3H, m)	26.5	C-3’, 4’, 7’	CH_2
6’	4.99 (1H, t, *J* = 6.1 Hz)	124.4	C-4’, 8’, 9’	CH
7’	-	130.7	-	C
8’	1.48 (3H, s)	16.9	C-9’	CH_3
9’	1.52 (3H, s)	25.0	C-7’, 8’	CH_3
10’	1.79 (3H, s)	15.8	C-3’, 4’	CH_3
1 - OH	13.45 (1H, s)	-	C-1, 2, 9a	-
3 – OH	9.55 (1H, s)	-	-	-
6 – OH	9.55 (1H, s)	-	-	-
7 – OCH_3	3.76 (3H, s)	60.6	C-7	CH_3





Best	Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)	ID Source	Score (MFG)
☑	C24 H26 O6	91.83	410.1744	410.1729	-3.64	3.64	-1.49	MFG	91.83

Species	Ion Formula	m/z	Height	Score (MFG)	Score (MFG, MS)	Score (MFG, MS/MS)	Score (MFG, mass)	Score (MFG, abund)	Score (MFG, iso. spacing)
(M+H)+	C24 H27 O6	411.1802	23775.2	91.83	91.83		88.63	98.75	89.91

Figure 4.22: HRESI mass spectrum of compound 69

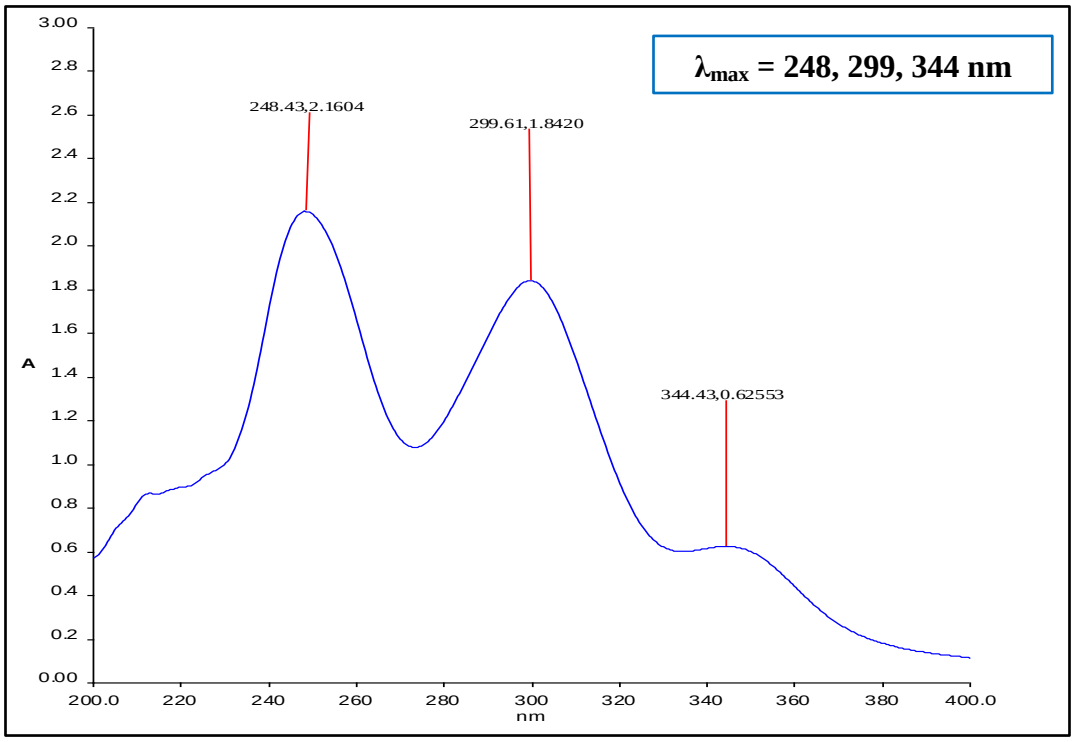


Figure 4.23: UV-Vis spectrum of compound 69

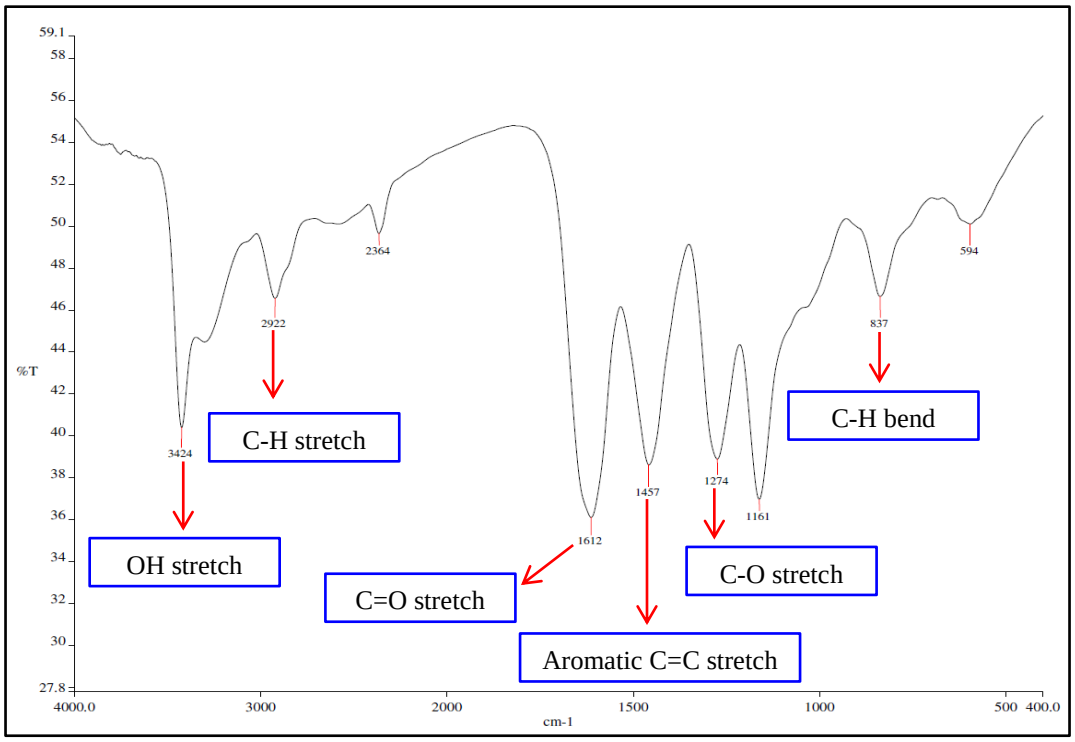


Figure 4.24: IR spectrum of compound 69

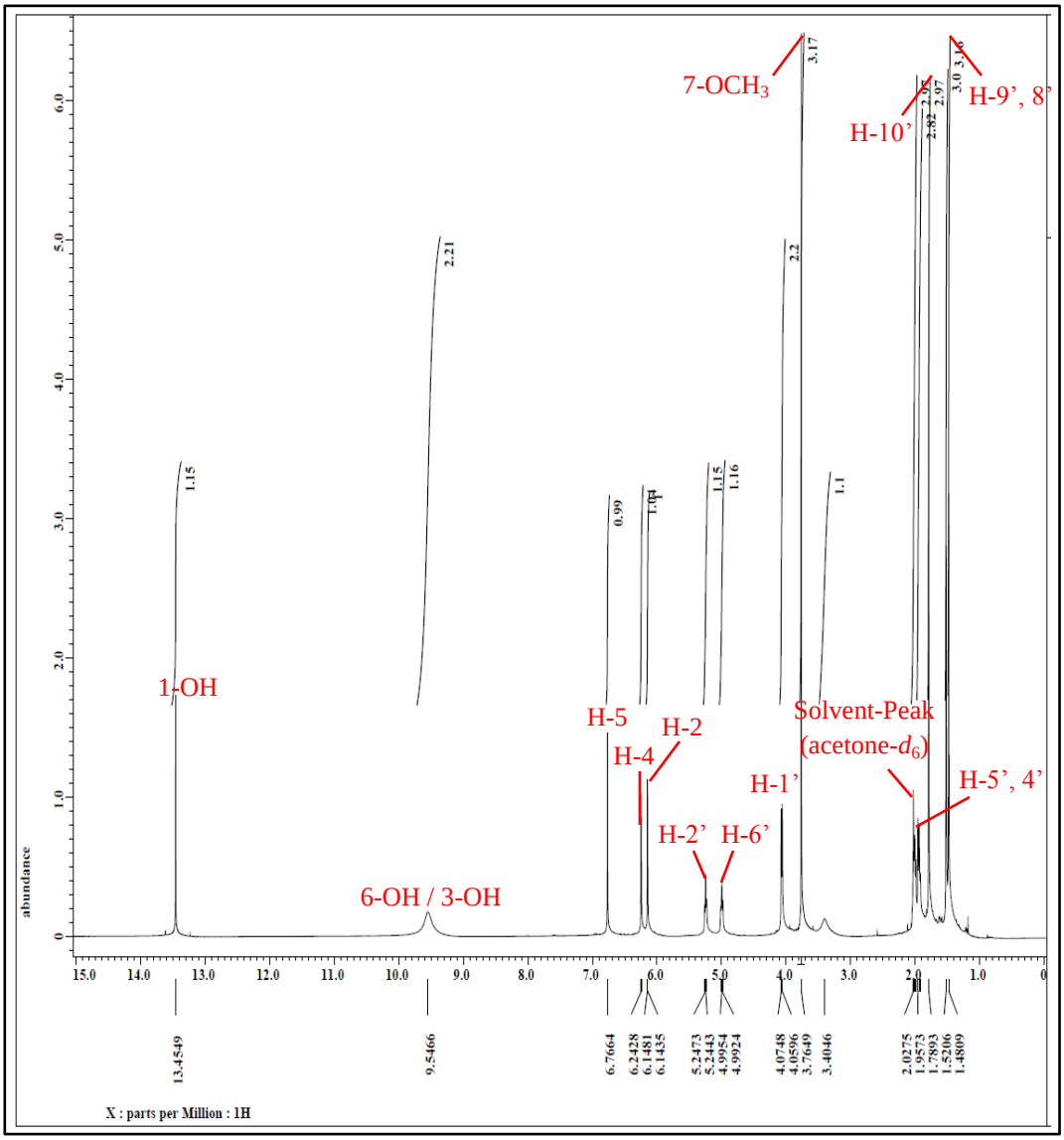


Figure 4.25: ^{1}H NMR spectrum of compound 69 (400 MHz, acetone-d_6)

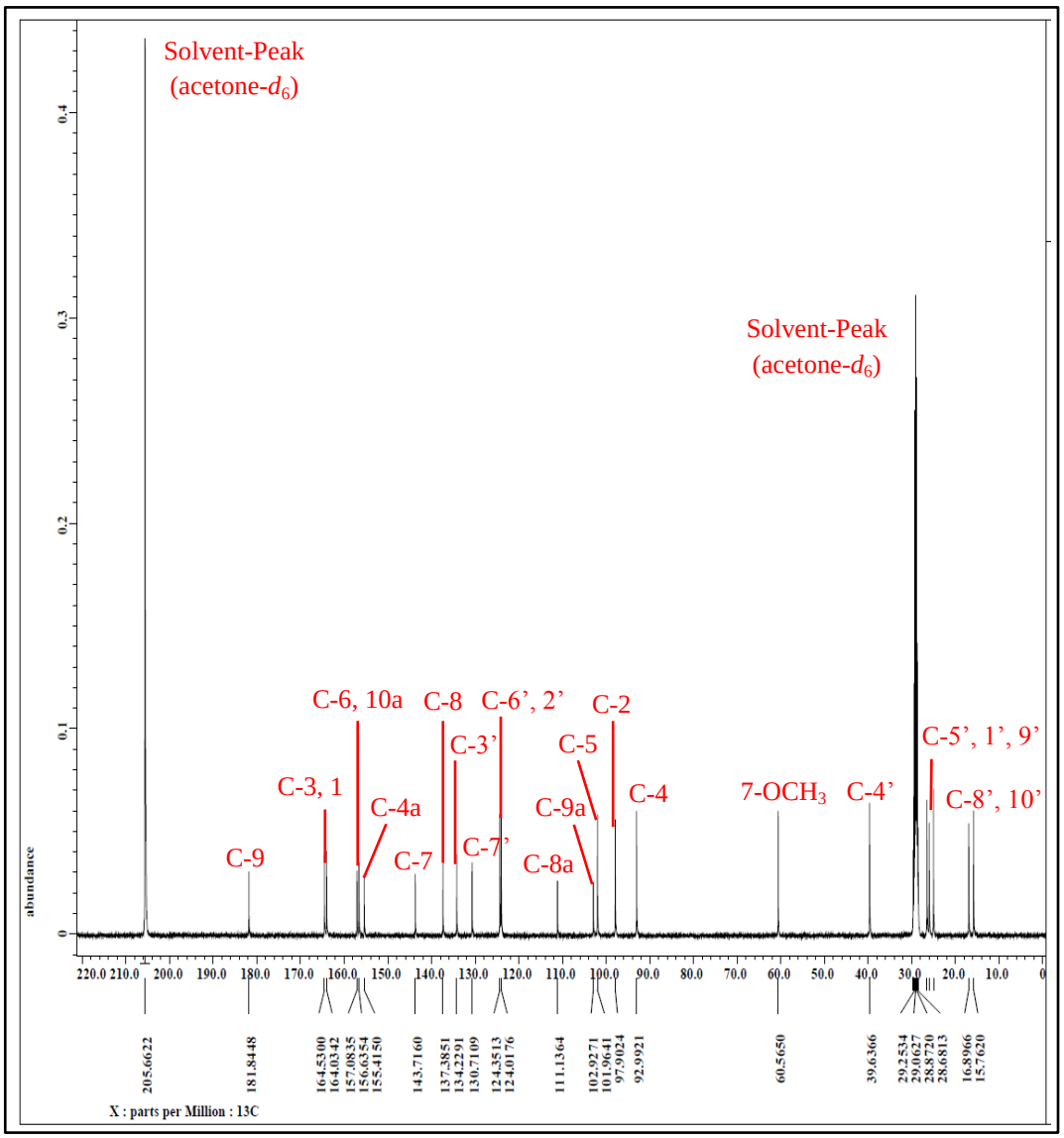


Figure 4.26: ^{13}C NMR spectrum of compound 69 (100 MHz, acetone-d_6)

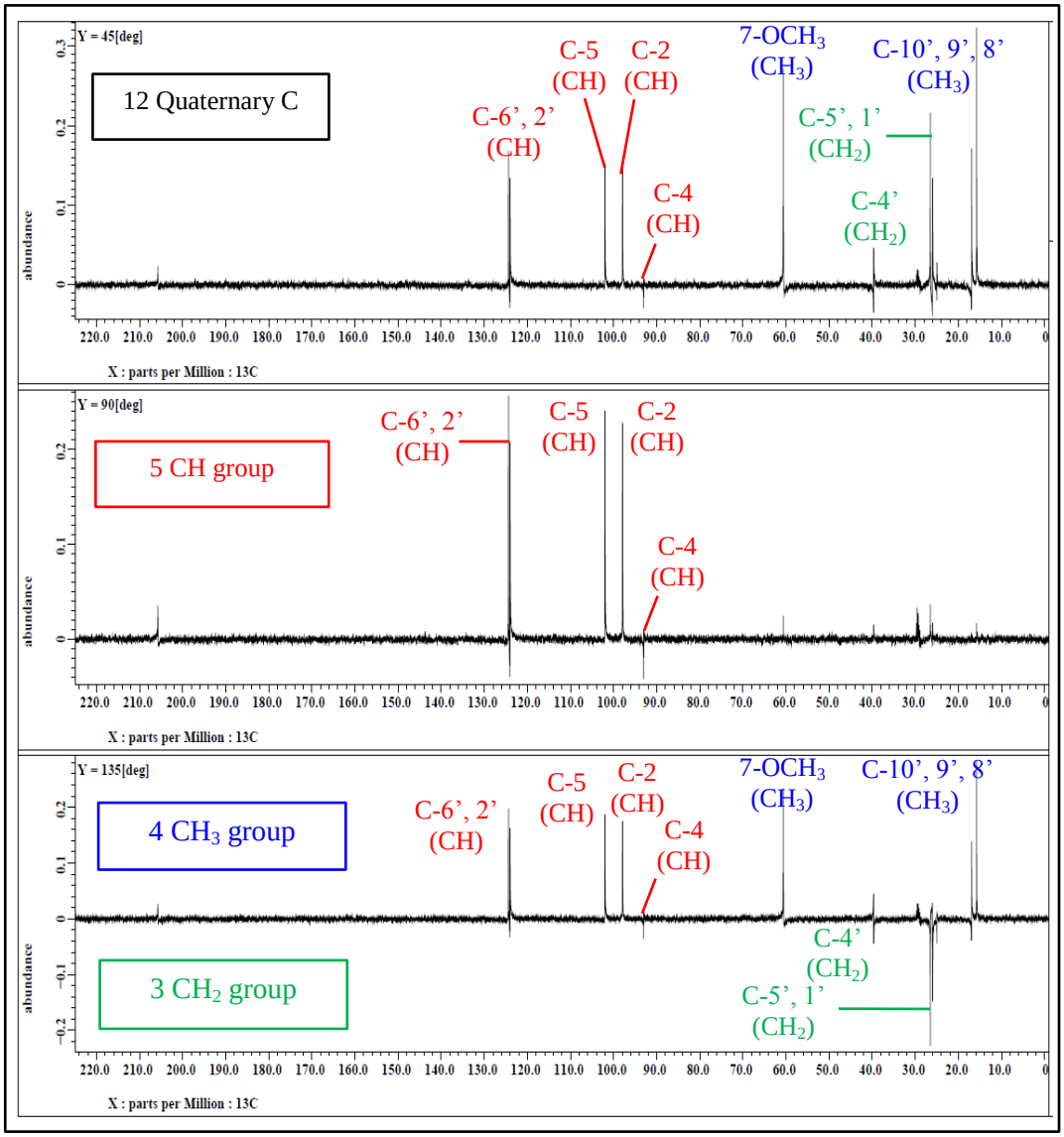
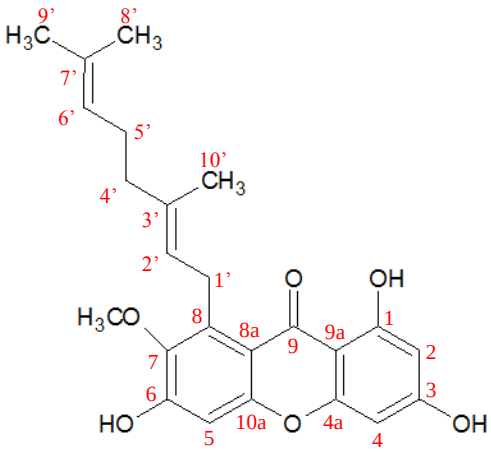


Figure 4.27: DEPT spectrum of compound 69



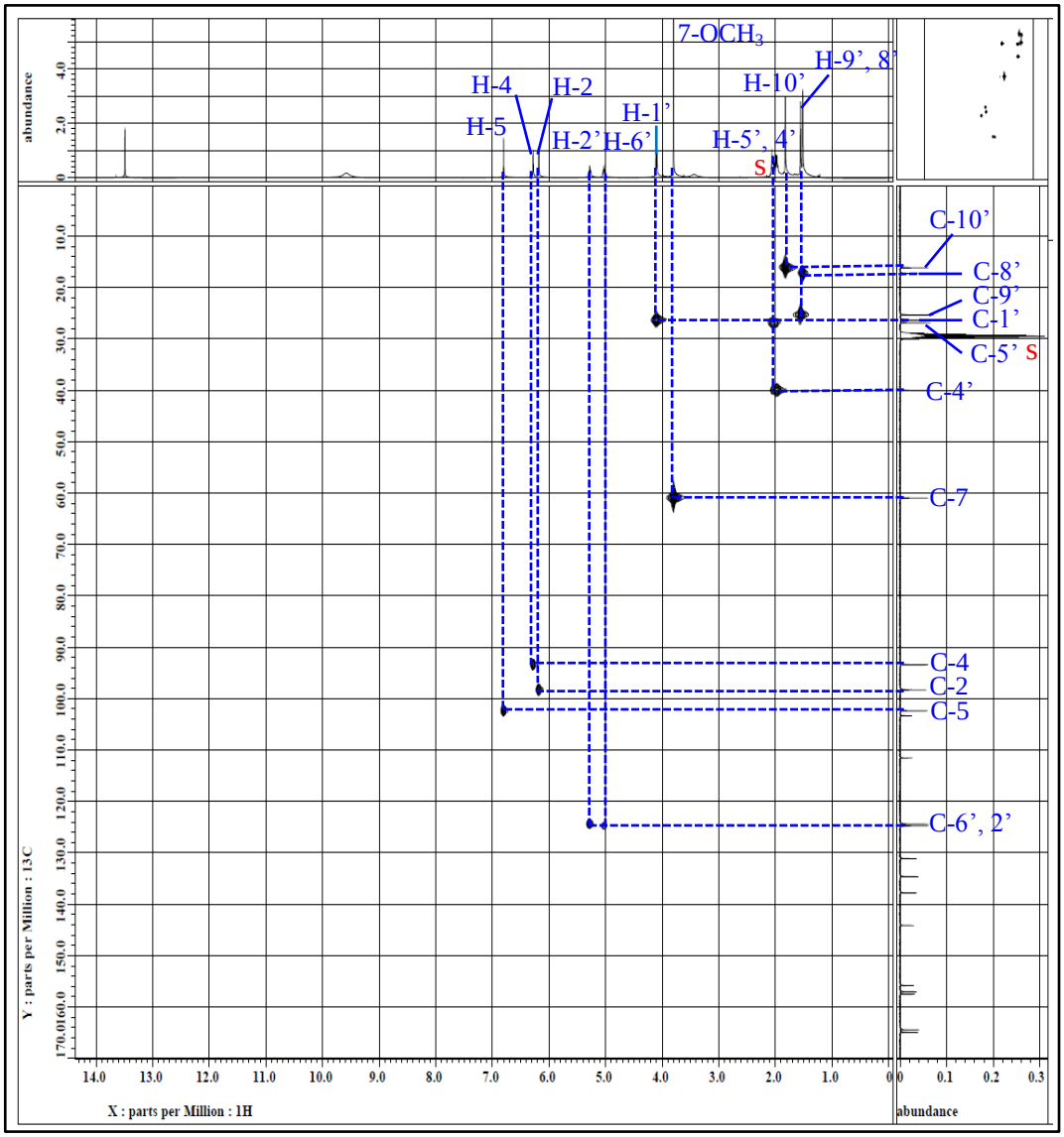


Figure 4.28: HMQC spectrum of compound 69

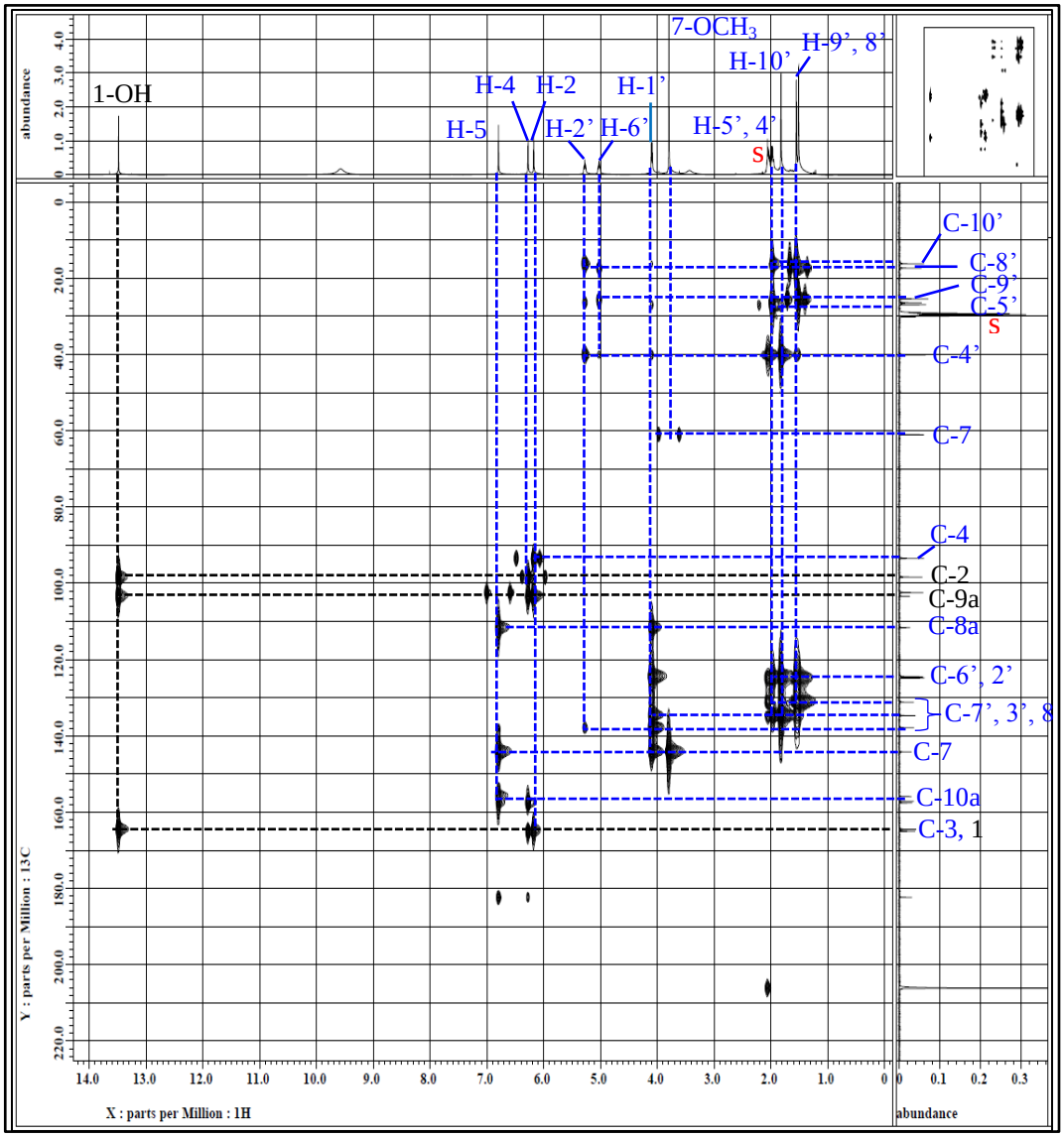


Figure 4.29: HMBC spectrum of compound 69

4.5 Characterization and Structural Elucidation of Tetraprenyltoluquinone (TPTQ) [70]

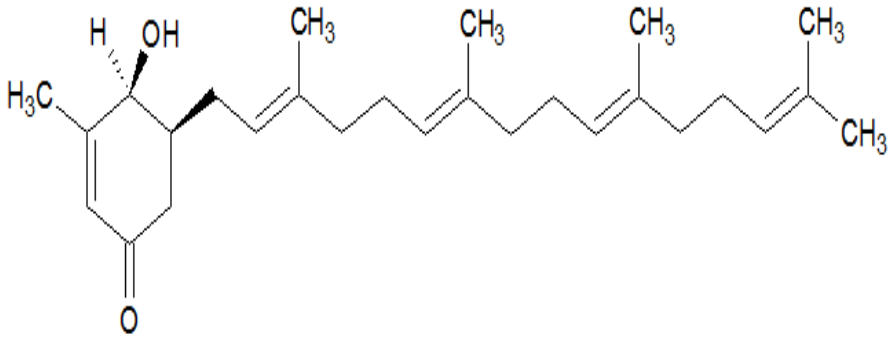


Figure 4.30: Chemical structure of compound 70

Compound **70** was identified as [2*E*,6*E*,10*E*]-(+)-4*β*-hydroxy-3-methyl-5*β*-(3,7,11,15-tetramethyl-2,6-10,14-hexadecatetraenyl-2-cyclohexen-1-one (TPTQ). It was isolated from the crude ethyl acetate extract as dark brown viscous liquid. This compound showed a single spot on TLC with an R_f value of 0.55 when developed using the solvent system of 90 % dichloromethane and 10 % acetone. It appeared as reddish-brown spot under 254 nm wavelength UV light, and as stained single brown spot when placed in iodine chamber.

The HRESI mass spectrum (Figure 4.31) reported a pseudomolecular ion peak, $[M+H]^+$ at m/z 399.3258. The calculated accurate mass of compound **70** was 398.3187 g/mol which is in agreement with the measured molecular weight, 398.3185 g/mol. Hence, the spectral data proposed compound **70** to have a molecular formula of $C_{27}H_{42}O_2$. In the UV-Vis spectrum (Figure 4.32), compound

70 showed absorption maxima at wavelengths of 204, 251 and 313 nm indicating the presence of chromophores with highly conjugated system in the compound.

The suggested structure was further confirmed by FTIR analysis revealing the presence of hydroxyl (3424 cm^{-1}), C-H stretch (2924 cm^{-1}), α, β-unsaturated ketone (1662 cm^{-1}), C=C (1439 cm^{-1}) and C-O stretch (1289 cm^{-1}) functionalities in compound **70**. The IR spectrum of compound **70** is shown in Figure 4.33.

The ^{1}H NMR spectrum (Figure 4.34) displayed resonances suggesting the presence of a cyclohex-2-enone ring and a geranyl-geranyl side chain in the proposed structure. The ring moiety gave signals at δ_H 5.82 (1H, s, H-2), 4.12 (1H, d, *J* = 6.1 Hz, H-4), 2.11 (1H, m, H-5), 2.51 (1H, d, *J* = 12.2 Hz, H-6), 2.08 (1H, m, H-6) and 2.02 (3H, s, 3-CH_3). On the other hand, the geranyl-geranyl moiety showed characteristic olefinic, methylene and methyl proton signals at δ_H 5.18 (1H, t, *J* = 7.0 Hz, H-2'), 5.07 (3H, d, *J* = 5.5 Hz, H-6', 10', 14'), 2.34 (1H, m, H-1'), 2.15 (1H, d, *J* = 1.2 Hz, H-1'), 2.05 (6H, m, H-5', 9', 13'), 1.96 (6H, m, H-4', 8', 12'), 1.66 (3H, s, 15'-CH_3), 1.61 (3H, s, H-16') and 1.58 (9H, s, 3'-CH_3, 7'-CH_3, 11'-CH_3).

A total of 27 carbon signals were observed in the ^{13}C NMR spectrum (Figure 4.35) revealing compound **70** to consist of 27 carbons. The most deshielded resonance at δ_C 198.9 was attributed to the keto carbon, C-1. Besides, the presence of five pairs of olefinic carbons C-2/C-3, C-2'/C-3', C6'/C-7', C-10'/C-11' and C-14'/C-15' gave proton signals at δ_C 126.8/163.6, 120.8/138.5, 124.5/135.5, 124.2/135.0 and

123.9/131.4, respectively. Meanwhile, the oxygenated sp^3 hybridized carbon C-4 gave relatively a more deshielded resonance at δ_C 73.6 as compared to other sp^3 hybridized carbon. The remaining carbon signals at δ_C 43.6, 41.4, 39.9, 39.8, 39.8, 30.9, 26.8, 26.7, 26.6, 25.8, 20.5, 17.8, 16.4, 16.1, 16.1 were assigned to the sp^3 hybridized methylene and methyl carbons, C-5, C-6, C-4', C-8', C-12', C-1', C-9', C-5', C-13', 15'-CH_3, 3-CH_3, C-16', 3'-CH_3, 7'-CH_3 and 11'-CH_3, respectively. The assignment of carbon signals was in agreement with the literature values reported by Fatma, et al., in year 2014.

In the DEPT spectrum (Figure 4.36), compound **70** was established to have six quaternary, seven methine, eight methylene and six methyl carbons which were in consistent with the proposed structure. On the basis of HMQC spectrum (Figure 4.37), the direct coupling between protons and their respective carbon signals were assigned. In the HMBC spectrum (Figure 4.38), the geranyl-geranyl group with its characteristic methylene protons, H-1' showed correlations with carbons C-5 (δ_C 43.7) and C-6 (δ_C 41.5) indicating the geranyl-geranyl group was attached to the cyclohex-2-enone ring at carbon C-5. The remaining assignment and NMR spectral data were summarized in Table 4.4.

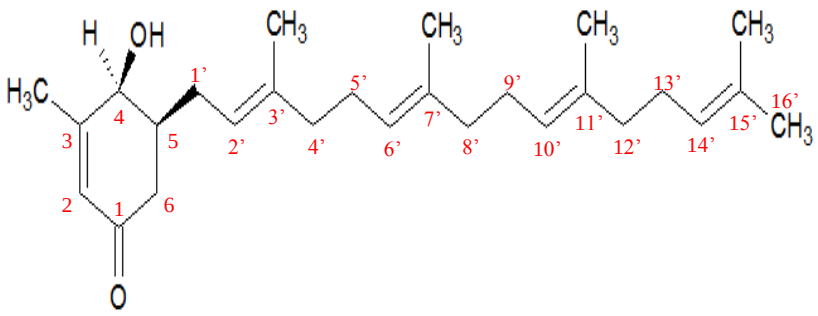


Table 4.4: Summary of NMR spectral data for compound 70 in comparison with literature values

Position	δ_H (ppm), Integration and Multiplicity	*δ_H (ppm) Integration and Multiplicity	δ_C (ppm)	*δ_C (ppm)	HMBC	DEPT
1	-	-	198.9	199.0	-	C
2	5.82 (1H, s)	5.80 (1H, s)	126.8	126.4	C-4, 6, 3-CH_3	CH
3	-	-	163.6	164.1	-	C
4	4.12 (1H, d, *J* = 6.1 Hz)	4.09 (1H, d, *J* = 6.0 Hz)	73.6	73.1	C-3, 5	CH
5	2.11 (1H, m)	2.10 (1H, m)	43.7	43.5	C-1	CH
6	2.51 (1H, d, *J* = 12.2 Hz) 2.08 (1H, m)	2.51 (2H, m)	41.4	41.1	C-1, 2, 4, 5	CH_2
3-CH_3	2.02 (3H, s)	2.03 (3H,s)	20.5	20.4	C-2, 3 ,4	CH_3
1’	2.34 (1H, m) 2.15 (1H, d, *J* = 1.2 Hz)	2.35 (1H, d, *J* = 8.0 Hz) 2.1 (1H, d, *J* = 8.0 Hz)	30.9	30.5	C-2’, 5, 6	CH_2
2’	5.16 (1H, t, *J* = 7.0 Hz)	5.2 (1H, t, *J* = 6.0 Hz)	120.8	120.5	C-1’, 4’, 3-CH_3	CH
3’	-	-	138.5	138.2	-	C
4’	1.96 (2H, m)	1.96 (2H, m)	39.9	39.8	C-5’, 6’, 3-CH_3	CH_2
5’	2.05 (2H, m)	2.14 (2H, m)	26.7	39.6	C-3’, 4’, 6’, 7’	CH_2
6’	5.07 (1H, d, *J* = 5.5 Hz)	5.09 (1H, d, *J* = 6.0 Hz)	124.5	124.3	C-4’, 5’, 7’-CH_3	CH
7’	-	-	135.5	135.1	-	C
8’	1.96 (2H, m)	1.96 (2H, m)	39.8	39.6	C-9’, 10’, 7’-CH_3	CH_2
9’	2.05 (2H, m)	2.14 (2H, m)	26.8	26.6	C-7’, 8’, 10’, 11’	CH_2
10’	5.07 (1H, d, *J* = 5.5 Hz)	5.09 (1H, d, *J* = 6.0 Hz)	124.2	124.1	C-8’, 9’, 11’-CH_3	CH
11’	-	-	135.0	134.8	-	C
12’	1.96 (2H, m)	1.96 (2H, m)	39.8	26.5	C-13’, 14’,	CH_2
13’	2.05 (2H, m)	2.14 (2H, m)	26.6	26.4	C-11’, 12’, 14’, 15’	CH_2
14’	5.07 (1H, d, *J* = 5.5 Hz)	5.09 (1H, d, *J* = 6.0 Hz)	123.9	123.8	C-12’, 13’	CH
15’	-	-	131.4	131.1	-	C
16’	1.61 (3H, s)	1.61 (3H, s)	17.8	17.5	C-15’-CH_3	CH_3
3’-CH_3	1.58 (3H, s)	1.58 (3H, s)	16.4	16.1	C-4’	CH_3
7’-CH_3	1.58 (3H, s)	1.58 (3H, s)	16.1	15.8	C-6’, 7’	CH_3
11’-CH_3	1.58 (3H, s)	1.58 (3H, s)	16.1	15.8	C-10’	CH_3
15’-CH_3	1.66 (3H, s)	1.66 (3H, s)	25.8	25.5	C-14’, 15’, 16’	CH_3

(*Literature source: Fatma, et al., 2014)

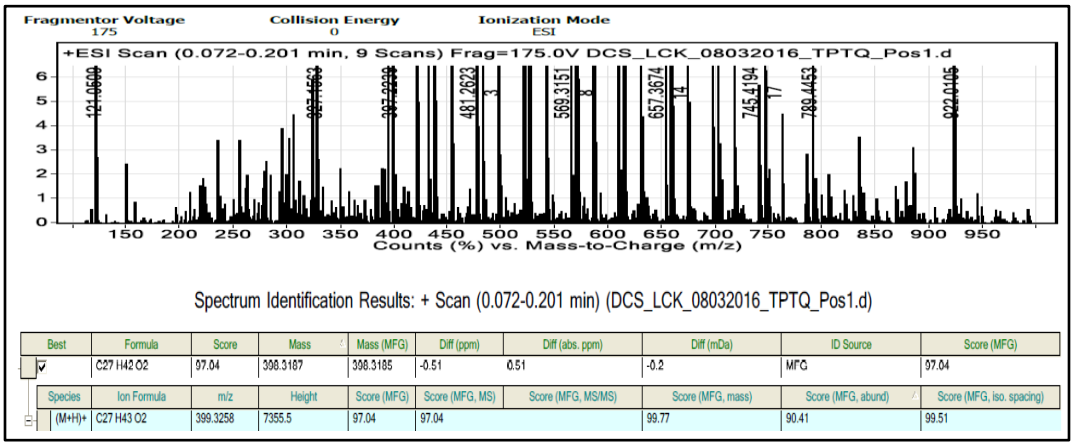


Figure 4.31: HRESI Mass Spectrum of compound 70

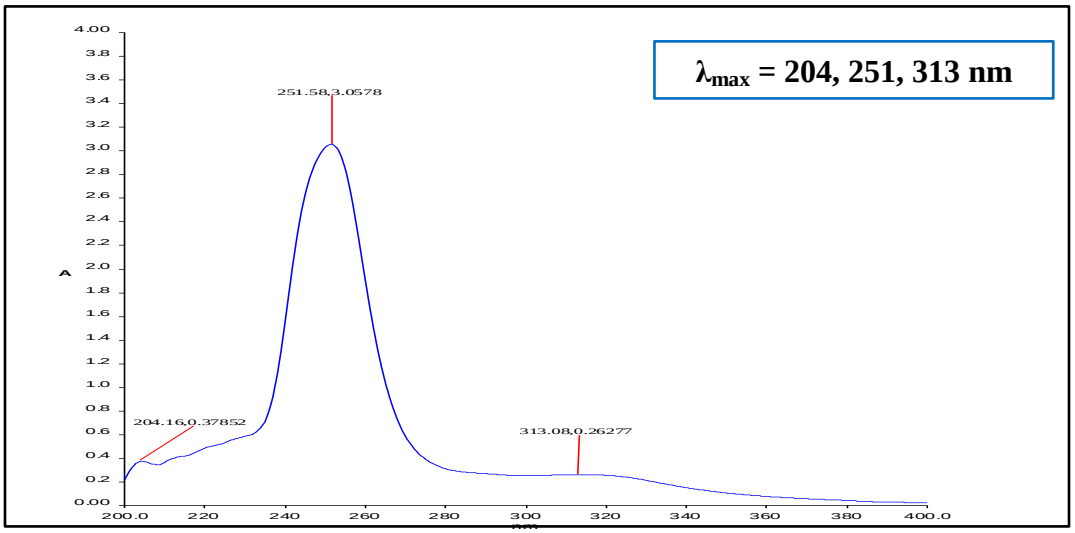


Figure 4.32: UV-Vis spectrum of compound 70

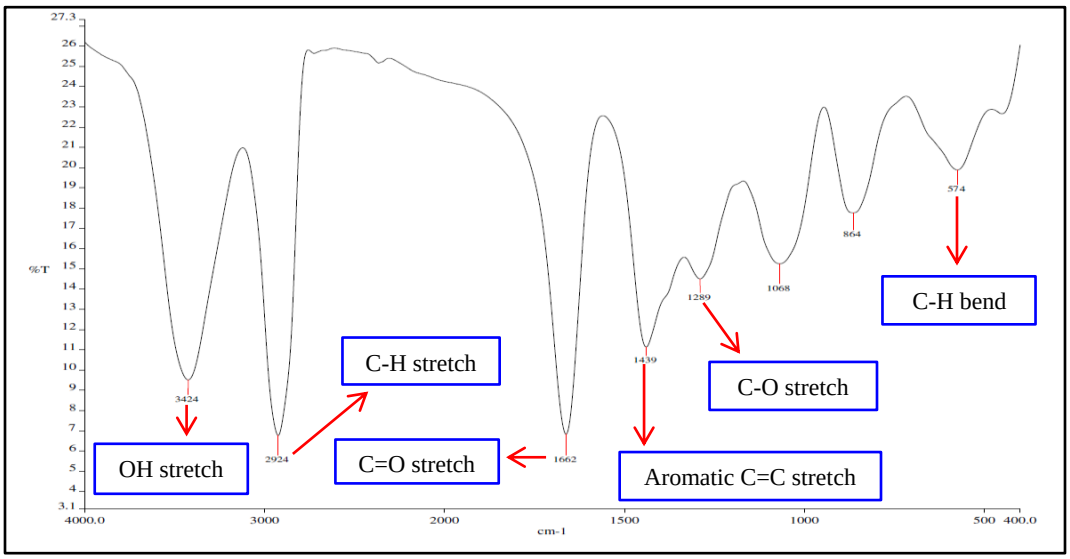


Figure 4.33: IR spectrum of compound 70

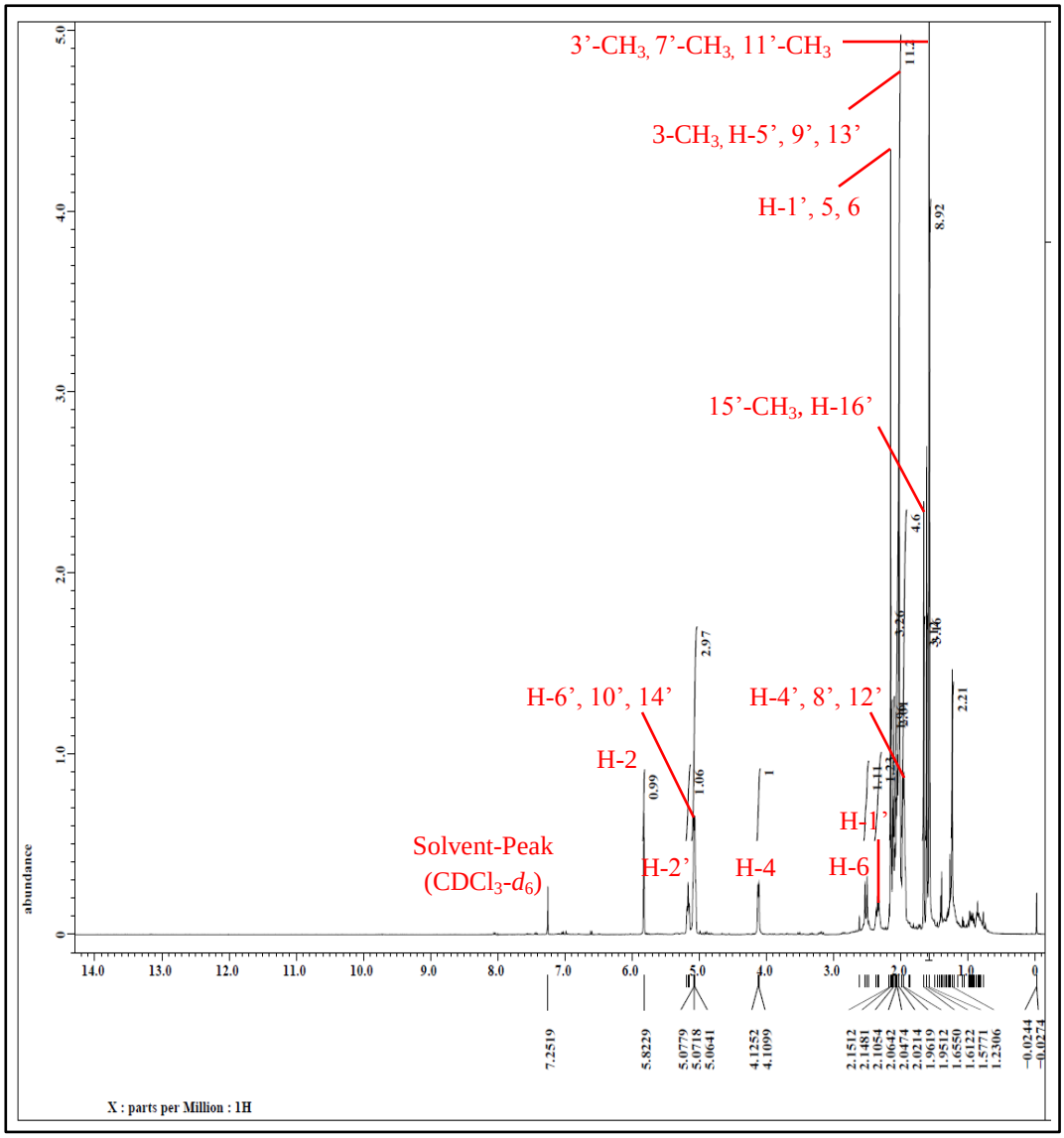


Figure 4.34: ^{1}H NMR spectrum of compound 70 (400 MHz, $CDCl_3$-d_6)

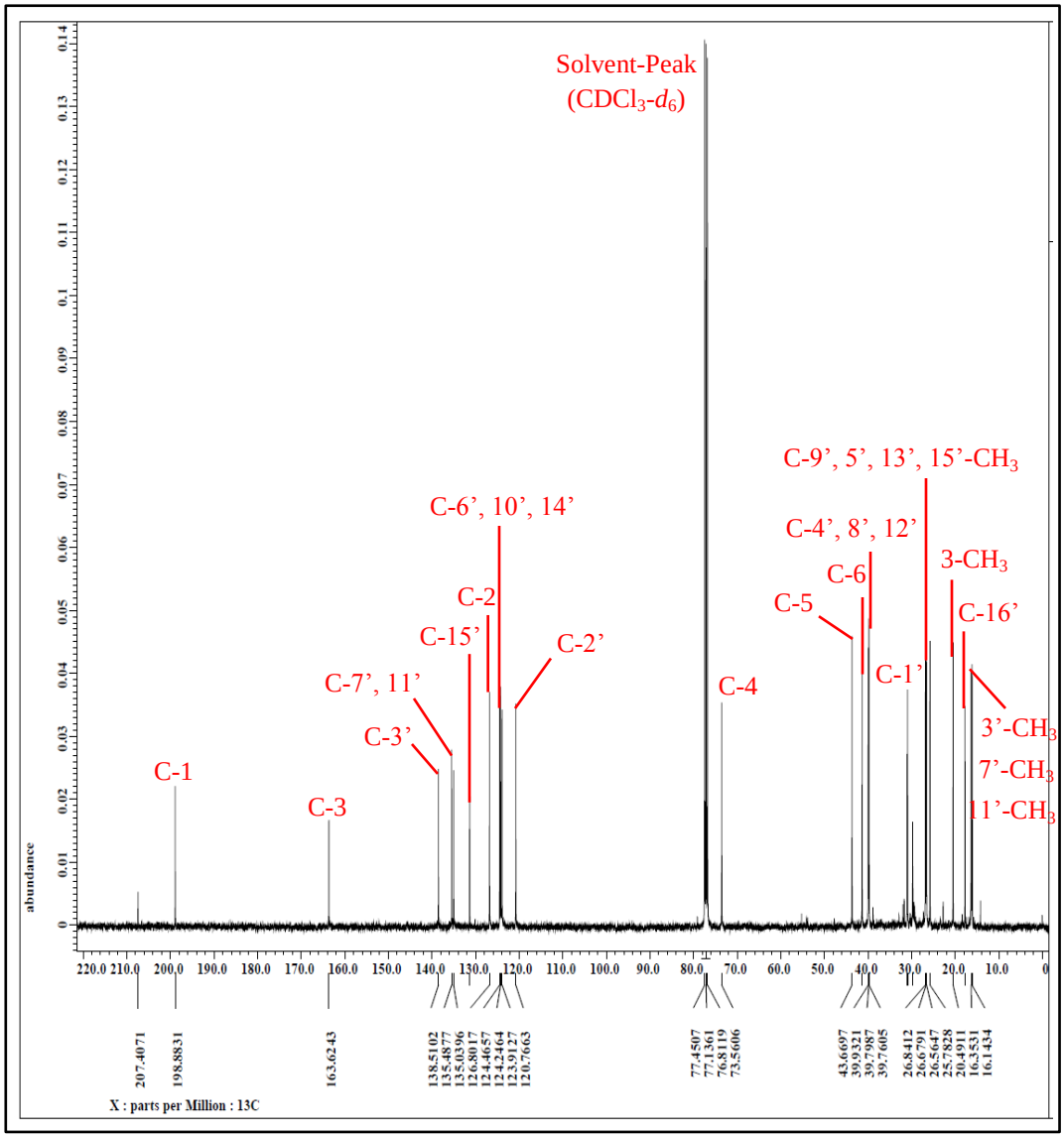


Figure 4.35: ^{13}C NMR spectrum of compound 70 (100 MHz, $CDCl_3$-d_6)

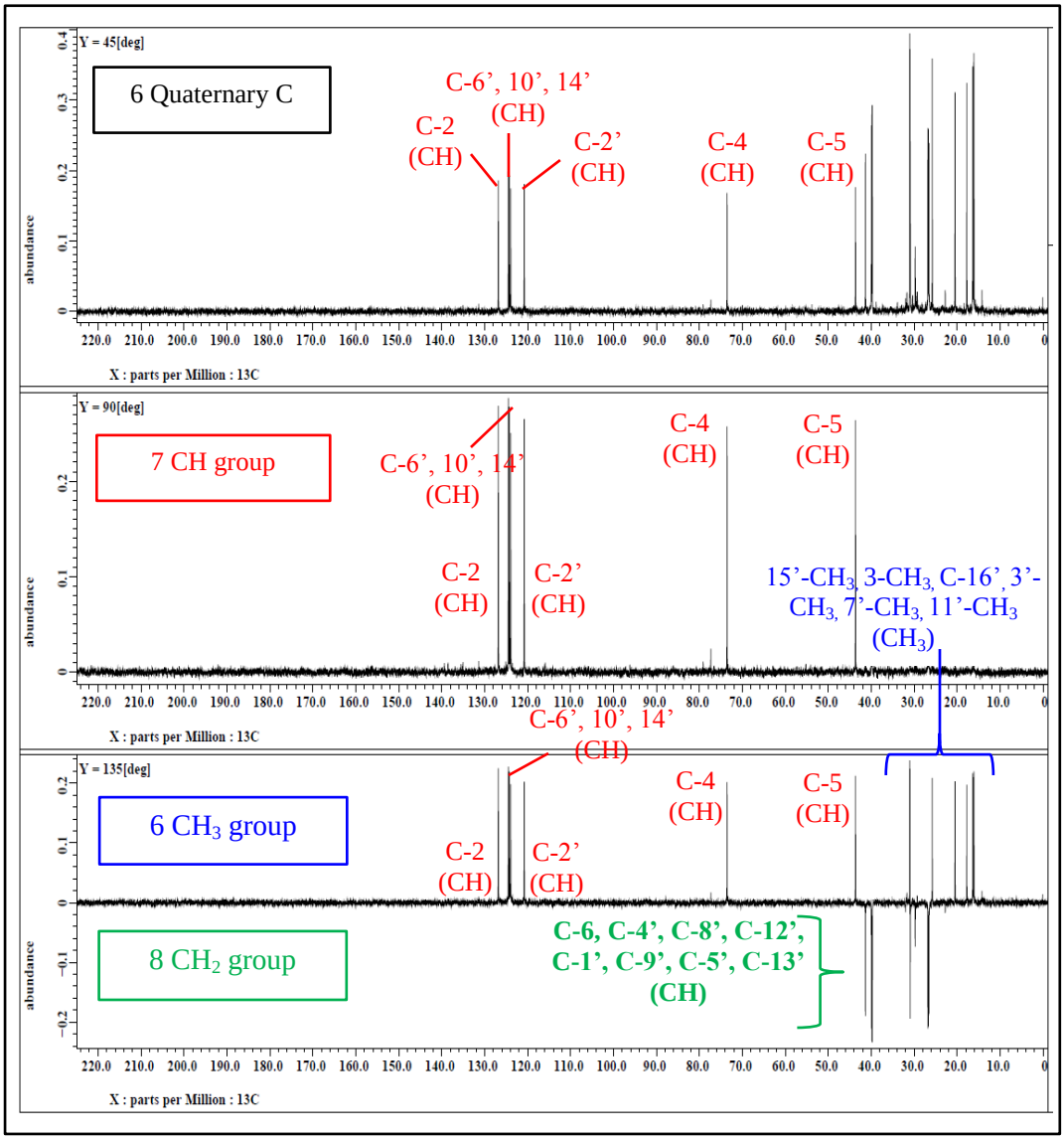


Figure 4.36: DEPT spectrum of compound 70

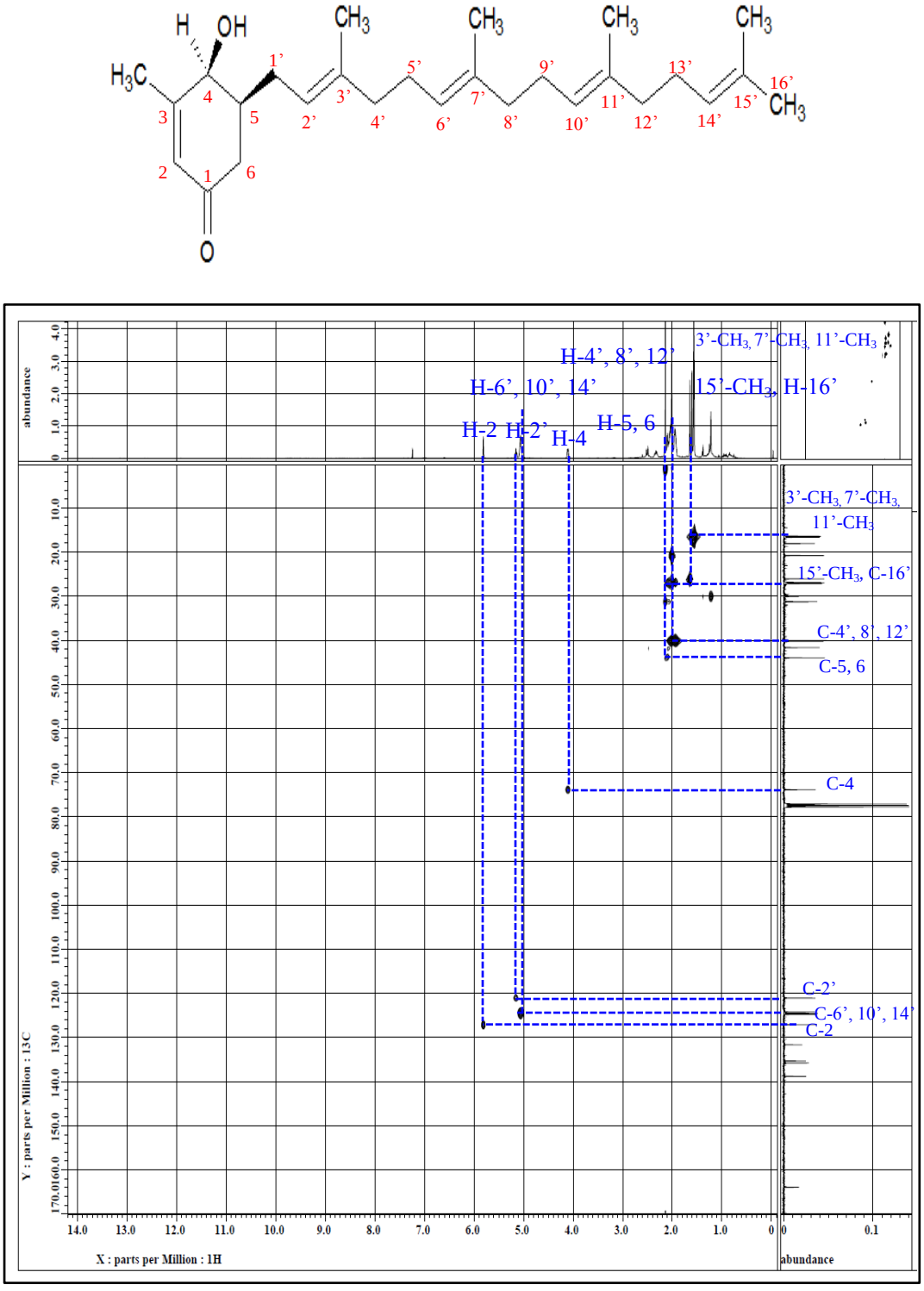


Figure 4.37: HMQC spectrum of compound 70

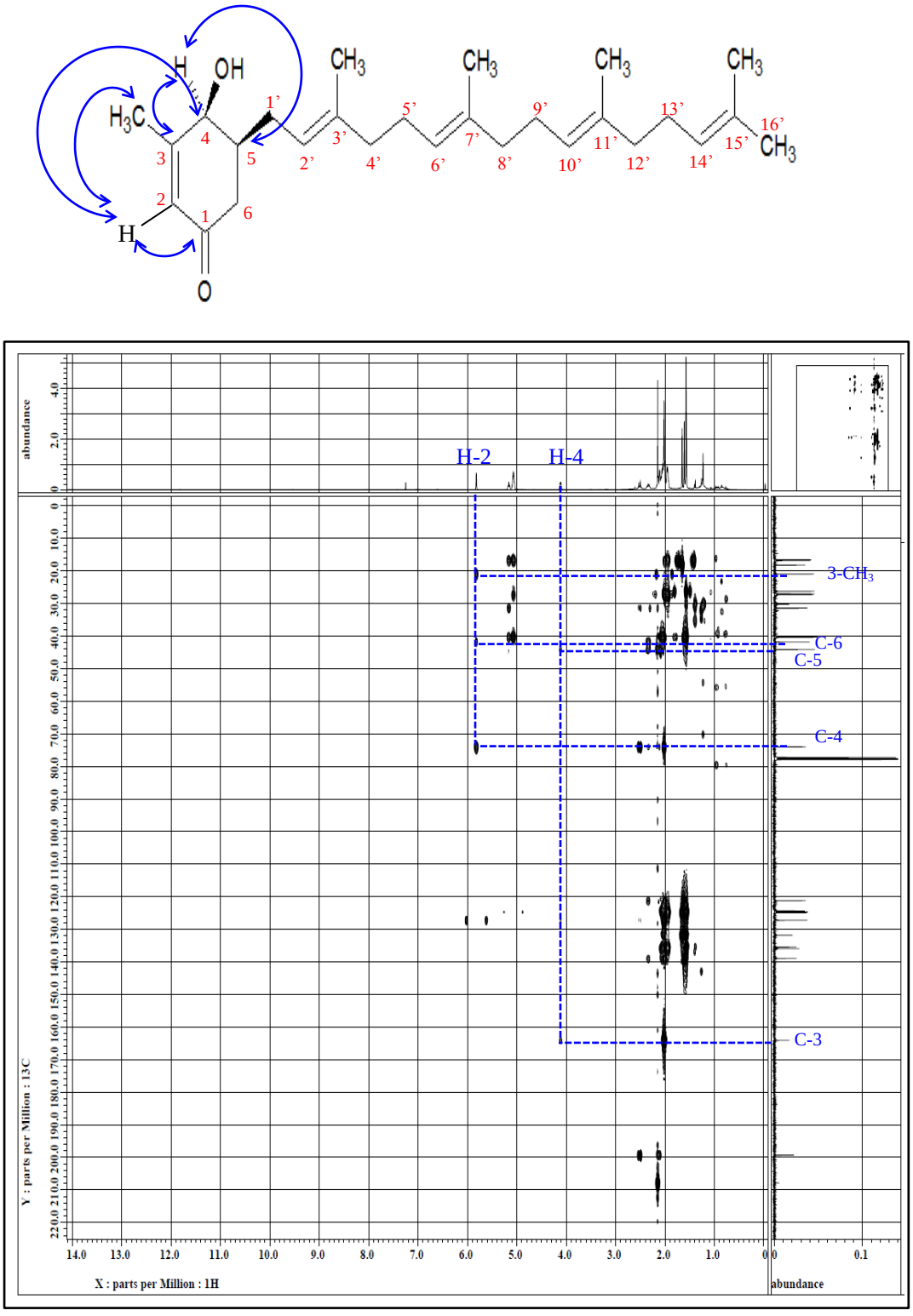


Figure 4.38: HMBC spectrum of compound 70

4.6 Antioxidant Assay

Oxidation reactions are known to produce free radicals, known as reactive oxygen species (ROS), such as superoxide ($O2^{\cdot-}$), hydroxyl ($HO^{\cdot}$), peroxyl ($ROO^{\cdot}$), alkoxyl ($RO^{\cdot}$). However, a high concentration of free radicals can cause oxidative cell damage, neurological disorder such as Parkinson's dementia and Alzheimer disease, and cancer (Ames, Shigeneaga and Hagen, 1993). Antioxidant is a compound which inhibits oxidation by either reacting with reactive oxygen species (ROS) to form harmless product or by disrupting free radical chain reaction. It reacts with these free radicals and terminates the chain reaction by removing free radical intermediates and inhibits other oxidation reactions by oxidizing themselves. Generally, polyphenolic or phenolic compounds are served as antioxidant in biological system.

In this study, crude dichloromethane, ethyl acetate extracts, as well as the four isolated compounds, namely brasixanthone B **[67]**, 1,3,7-trihydroxy-2,4-*bis*(3-methylbut-2-enyl)xanthone **[68]**, rubraxanthone **[69]** and TPTQ **[70]** were subjected to DPPH radical scavenging capacity assay in order to evaluate for their antioxidant activities.

DPPH radical is a stable organic nitrogen centered free radical, present in a dark purple colour that when it is reduced to its non-radical form by antioxidants, it becomes colourless or pale yellow. This assay measured the radical scavenging capacity of the samples at different solution concentrations, and then the sample

concentration required to reduce the initial DPPH concentration by 50% (IC_{50}) at steady state was obtained from the plotted graph of inhibition rate of DPPH radical against concentration of sample. A lower IC_{50} value indicates a stronger DPPH radical scavenging capacity, which means more potent antioxidant effect. Table 4.5 shows the summary of the antioxidant results (IC_{50}) of the test samples.

Table 4.5: Antioxidant results of test samples in DPPH assay

Test samples	IC_{50} (µg/mL)
Positive controls:	
Ascorbic acid	5.0
Kaempferol	7.0
Crude extracts:	
Dichloromethane	44.0
Ethyl acetate	44.5
Isolated compounds:	
Brasixanthone B **[67]**	>240
1,3,7-Trihydroxy-2,4-bis(3-methylbut-2-enyl)xanthone **[68]**	185
Rubraxanthone **[69]**	195
Tetraprenyltoluquinone (TPTQ) **[70]**	>240

From the results obtained from the graph of percent inhibition rate versus concentration of sample (Figures 4.39 and 4.40), crude dichloromethane and ethyl acetate extracts showed relatively weaker antioxidant activities with IC_{50} values of 44.0 and 44.5 µg/mL, respectively as compared to the positive controls ascorbic acid (IC_{50} of 5.0 µg/mL) and kaempferol (IC_{50} of 7.0 µg/mL). However, both of the crude extracts exhibited much stronger antioxidant activity as compared to all

the four isolated compounds. This might due to the reason that crude extracts contained more variety of phenolic compounds which were potential antioxidants.

Meanwhile, compounds **67** and **70** were found to be inactive with both the compounds gave IC_{50} values more than 240 μg/mL. On the other hand, compounds **68** and **69** showed significant but weak activity with IC_{50} of 185 and 195 μg/mL, respectively in DPPH assay. The antioxidant activity exhibited by compounds **68** and **69** may be attributed to the presence of free hydroxyl groups in both the compounds. Compound **68** was found to have two free hydroxyl groups at carbon C-2 and C-7, while compound **69** bearing the two hydroxyl groups at carbon C-2 and C-6. These free hydroxyl groups can act as proton donor to deactivate the DPPH free radicals.

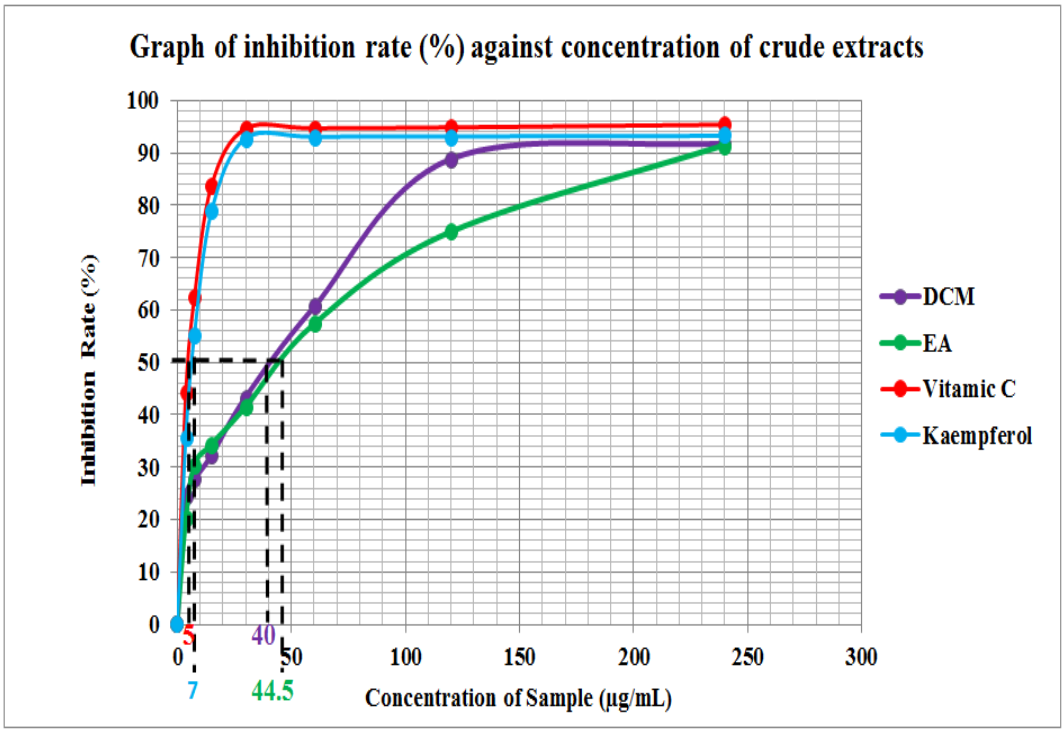


Figure 4.39: Graph of inhibition rate (%) against concentration of crude extracts

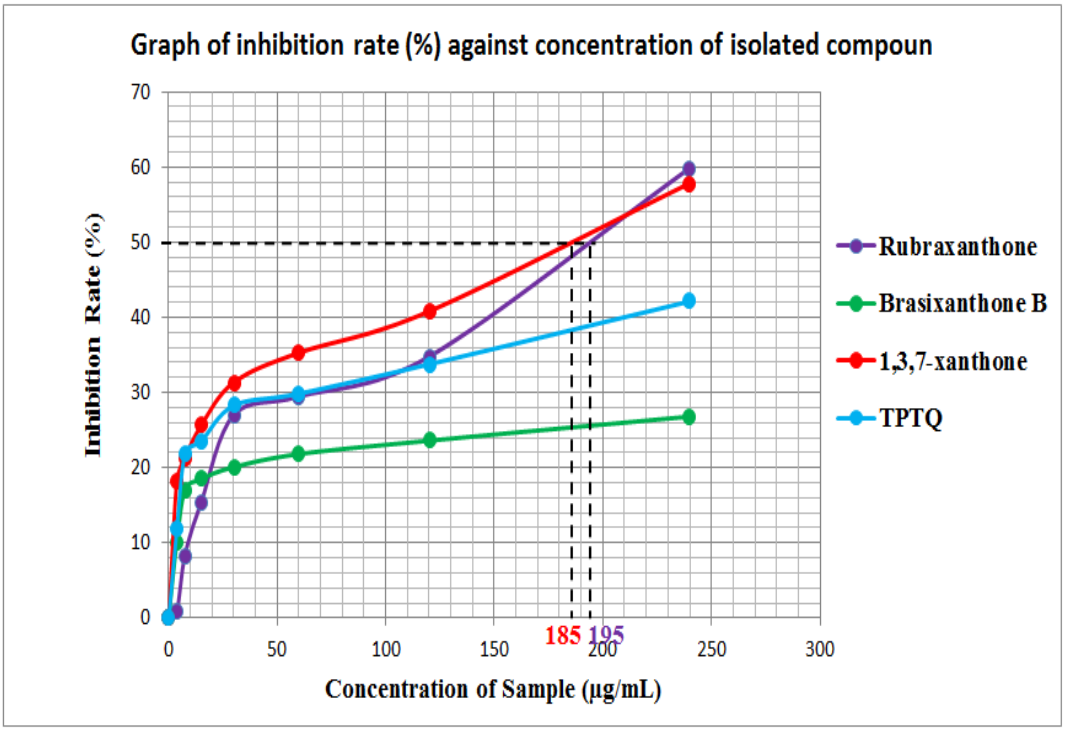


Figure 4.40: Graph of inhibition rate (%) against concentration of isolated compounds

CHAPTER 5

CONCLUSION

5.1 Conclusion

In this project, a total of four chemical compound were isolated from the crude ethyl acetate extract of *Garcinia parvifolia*. The structures of isolated compounds were elucidated and characterized via various modern spectroscopic techniques including 1D- and 2D-NMR, LC-MS, UV-Vis and IR analyses. They were identified as brasixanthone B **[67]**, 1,3,6-trihydroxy-2,4-*bis*(3-methylbut-2-enyl)xanthone **[68]**, rubraxanthone **[69]** and tetraprenyltoluquinone (TPTQ) **[70]**.

The crude dichloromethane and ethyl acetate extracts along with four isolated compounds were evaluated for their antioxidant activity via DPPH assay. In this assay, dichloromethane and ethyl acetate extracts were found to exhibit relatively weak antioxidant activity with IC_{50} values of 40.0 and 44.5 μg/ml, respectively as compared to positive controls of ascorbic acid (IC_{50} = 5.0 μg/ml) and kaempferol (IC_{50} = 7.0 μg/ml). On the other hand, isolated compounds **67** and **70** were inactive showing IC_{50} values of more than 240 μg/ml, whereas compounds **68** and **69** were found to exhibit weak antioxidant activities with their IC_{50} values of 185 and 195 μg/ml, respectively.

5.2 Future Perspectives

For future studies, it is recommended that more advance instrumental techniques including high performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC) should be used to increase the separation efficiency for isolation of minor compounds from the stem bark of *Garcinia parvifolia*. Furthermore, it was suggested the crude extracts and isolated compounds in this to be subjected to further investigation for other biological activities. Apart from antioxidant property, such as cytotoxic and antimicrobial activities. In addition, the potential pure compounds discovered in this study could be investigated for their chemical derivatives via organic synthesis.

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