CHEMICAL CONSTITUENTS ISOLATED FROM THE ETHYL ACETATE EXTRACT OF THE ROOTS OF *GARCINIA ATROVIRIDIS*

NUR SALSABILA BINTI AHMAD ROSLAN

MASTER OF SCIENCE

UNIVERSITI MALAYSIA PAHANG

UNIVERSITI MALAYSIA PAHANG

DECLARATION OF THESIS AND COPYRIGHT

Author's Full Name : NUR SALSABILA BINTI AHMAD ROSLAN

Date of Birth : 25 MAY 1993

Title : CHEMICAL CONSTITUENTS ISOLATED FROM THE ETHYL ACETATE EXTRACT OF THE ROOTS OF *GARCINIA ATROVIRIDIS*

Academic Session : SEM II 2019/2020

I declare that this thesis is classified as:

☐ CONFIDENTIAL (Contains confidential information under the Official Secret Act 1997)*

☐ RESTRICTED (Contains restricted information as specified by the organization where research was done)*

☐ OPEN ACCESS I agree that my thesis to be published as online open access (Full Text)

I acknowledge that Universiti Malaysia Pahang reserves the following rights:

1. The Thesis is the Property of Universiti Malaysia Pahang
2. The Library of Universiti Malaysia Pahang has the right to make copies of the thesis for the purpose of research only.
3. The Library has the right to make copies of the thesis for academic exchange.

Certified by:

_____ _____

(Student's Signature) (Supervisor's Signature)

930525-06-5722

New IC/Passport Number Name of Supervisor

Date: Date:

NOTE : * If the thesis is CONFIDENTIAL or RESTRICTED, please attach a thesis declaration letter.

SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree Master of Science.

_____

(Supervisor's Signature)

Full Name : NORMAIZA BINTI ZAMRI

Position : SENIOR LECTURER

Date :

_____

(Co-supervisor's Signature)

Full Name : MOHD FADHLIZIL FASIHI BIN MOHD ALUWI

Position : SENIOR LECTURER

Date :

STUDENT'S DECLARATION

I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

_____

(Student's Signature)

Full Name : NUR SALSABILA BINTI AHMAD ROSLAN

ID Number : MKT17002

Date :

CHEMICAL CONSTITUENTS ISOLATED FROM THE ETHYL ACETATE EXTRACT OF THE ROOTS OF *GARCINIA ATROVIRIDIS*

NUR SALSABILA BINTI AHMAD ROSLAN

Thesis submitted in fulfillment of the requirements
for the award of the degree of
Master of Science

Faculty of Industrial Sciences and Technology
UNIVERSITI MALAYSIA PAHANG

SEPTEMBER 2020

ACKNOWLEDGEMENTS

I would like to express my gratitude towards my supervisor, Dr. Normaiza binti Zamri for her continuous support, guidance and insightful advice throughout the time of the research and writing of this thesis. Her words of encouragement and her response towards my questions gave me confidence to finish this research.

I also appreciate the supervision that I received from my co-supervisor, Dr. Mohd Fadhlizil Fasihi bin Mohd Aluwi. I am also thankful towards Dr. Seema Zareen and Dr. Muhammad Nadeem Akhtar. I am grateful to the science officers and laboratory assistants of Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang for the help and technical assistance that they offered, in addition of providing good working environment. Special thanks to Institute of Postgraduate Studies and faculty's administration staff for their accommodating services. To my fellow friends, I thank them for their companionship when I needed it.

Deepest acknowledgement goes to my loving family especially my parents, Mr. Ahmad Roslan bin Ismail and Mdm. Noor Hashimah binti Mohd Sidek for their understanding and reassurance. This accomplishment would not have been possible without them. Thank you.

ABSTRAK

Kajian awal menunjukkan bahawa bahagian akar pokok *Garcinia atroviridis* (asam gelugur) mempunyai aktiviti antibakteria dan antioksida yang tinggi, berkemungkinan disumbangkan oleh pelbagai bioaktif metabolit sekunder. Sebatian daripada ekstrak etil asetat akan diasingkan dengan lebih lanjut memandangkan pelarut etil asetat sangat efektif dalam mengasingkan kumpulan fenolik. Oleh itu, kajian ini dijalankan untuk mengasingkan, menulenkan, mengenalpasti sebatian fenolik, dan menilai sifat-sifat biologi bagi ekstrak etil asetat dan sebatian yang diasingkan. Sebagai tambahan, kajian biologi awal terhadap pelbagai ekstrak daripada akar *G. atroviridis* turut dijalankan. Akar *G. atroviridis* di dapati dari Maran, Pahang, telah diekstrak menggunakan pelarut yang mempunyai kekutuban yang berbeza. Kajian biologi awal iaitu antibakteria dan antioksida telah dijalankan terhadap ekstrak etil asetat. Ekstrak etil asetat telah dipilih kerana penyelidikan terdahulu telah berjaya mengasingkan sebatian fenolik yang mempunyai pelbagai aktiviti biologi. Ekstrak ini menunjukkan aktiviti antibakteria terhadap bakteria Gram-positif iaitu *Bacillus cereus* dan *Staphylococcus aureus* melalui kaedah penyebaran cakera. Ekstrak ini juga menunjukkan jumlah kandungan fenolik (TPC) yang siknifikan melalui kaedah *Folin- Ciocalteau* (FC), dengan nilai 568.6 ± 2.724 mg GAE/g. Seterusnya, aktiviti antioksida ekstrak etil asetat telah di uji dengan lebih lanjut melalui kaedah *α, α-diphenyl-β-picrylhydrazyl* (DPPH) dan *ferric reducing antioxidant power* (FRAP) yang menunjukkan aktiviti antioksida yang memuaskan dengan nilai IC_{50} 51.7 ± 2.180 µg/mL (DPPH) dan 294.0 ± 20.173 mg AAE/g (FRAP). Memandangkan ekstrak etil asetat menunjukkan aktiviti antibakteria dan antioksida yang bagus, pengasingan sebatian fenolik melalui teknik *silica gel column chromatography* (Si gel CC) dan *preparative thin layer chromatography* (PTLC) telah dijalankan. Sebanyak lima sebatian telah berjaya diasingkan iaitu, *atrovirisidone* (**7**), *morelloflavone* (**12**), *GB1a* (**18**), 1,*3,5 - trihydroxy-2-methoxyxantone* (**29**), termasuk satu sebatian baru iaitu *volkensiflavone* (**33**), dimana sebatian ini berjaya diasingkan daripada pokok *G. atroviridis* buat pertama kali. Pelbagai teknik spektroskopi digunakan untuk mengenalpasti struktur sebatian tersebut termasuklah melalui teknik *mass spectrometer* (MS), *ultraviolet-visible* (UV-Vis), *fourier-transform infrared* (FTIR), dan 1D- *nuclear magnetic resonance* (NMR). Seterusnya, kajian biologi dilakukan terhadap sebatian yang telah diasingkan. Bagi aktiviti antibakteria, *GB1a* (**18**) dan *volkensiflavone* (**33**) menunjukkan perencatan terhadap *S. aureus*. Sementara itu, *morelloflavone* (**12**) menunjukkan aktiviti DPPH tertinggi dengan nilai IC_{50} 20.3 ± 1.667 µg/mL, setanding dengan asid askorbik (AA) dengan nilai IC_{50} 13.2 ± 0.021 µg/mL. Secara umum, semua sebatian menunjukkan aktiviti FRAP yang memuaskan dengan aktiviti tertinggi ditunjukkan oleh *1,3,5- trihydroxy-2-methoxyxantone* (**29**), 1643.3 ± 44623 mg AAE/g. Di samping itu, kajian biologi awal yang dilakukan terhadap ekstrak heksana, diklorometana, butanol dan metanol yang diekstrak daripada akar *G. atroviridis* mendedahkan bahawa ekstrak butanol mempunyai nilai farmaseutikal yang berpotensi tinggi kerana ekstrak ini menunjukkan aktiviti antibakteria dan antioksida yang paling tinggi. Selain itu, hubungan antara ekstrak akar *G. atroviridis* dan aktiviti antioksida menunjukkan korelasi positif yang kuat antara TPC dengan DPPH (r = 0.8760) dan FRAP (r = 0.9385), menunjukkan peranan sebatian fenolik dalam aktiviti antioksida. Kesimpulannya, aktiviti antibakteria dan antioksida oleh akar *G. atroviridis* berkemungkinan disumbangkan oleh kehadiran sebatian fenolik.

ABSTRACT

Several preliminary studies on the roots of *Garcinia atroviridis* have reported on their antibacterial and antioxidant properties, which might be contributed by various bioactive secondary metabolites. Chemical compounds in the roots of *G. atroviridis* were isolated from ethyl acetate extract as the solvent was reportedly efficient for extraction of phenolic compounds with intermediate polarity. Thus, this study aims to isolate, purify, elucidate the phenolic compounds, and evaluate the biological properties of ethyl acetate extract and its isolated compounds. In addition, preliminary investigation on biological activity of other extracts extracted with different polarities of solvent was also conducted. The roots of *G. atroviridis* collected from Maran, Pahang were subjected to solvent extraction with different polarities of solvents. Preliminary biological screening was done on ethyl acetate extract to investigate the antibacterial and antioxidant activities. This extract was chosen because, the chemical investigation on this extract leads to the isolation of various phenolic compounds with various bioactivities as proven in previous studies. Ethyl acetate extract showed antibacterial activity against Gram-positive bacteria, *Bacillus cereus* and *Staphylococcus aureus*, in disc diffusion experiment. Significant total phenolic content (TPC) was identified in ethyl acetate extract using Folin-Ciocalteu (FC) reagent method, with a value of 568.6 ± 25.724 mg GAE/g. Upon further investigation with *α, α*-diphenyl-*β*-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays, ethyl acetate extract showed moderate antioxidant activities with IC_{50} of 51.7 ± 2.180 µg/mL and 294.0 ± 20.173 mg AAE/g, respectively. Based on antibacterial and antioxidant activities exhibited by ethyl acetate extract, further chemical investigation was carried out to isolate the phenolic compounds in the extract using silica gel column chromatography (Si gel CC) and preparative thin layer chromatography (PTLC). Five compounds were isolated namely atrovirisidone (**7**), morelloflavone (**12**), GB1a (**18**), 1,3,5- trihydroxy-2-methoxyxantone (**29**), and volkensiflavone (**33**). Volkensiflavone (**33**) was reported for the first time in *G. atroviridis*. Several spectroscopy techniques were used to elucidate the structure of isolated compounds, such as mass spectrometer (MS), ultraviolet-visible (UV-Vis), fourier-transform infrared (FTIR), and 1D- nuclear magnetic resonance (NMR) spectroscopy. Biological assays were conducted to all isolated compounds except atrovirisidone (**7**) due to limited yield. GB1a (**18**) and volkensiflavone (**33**) inhibited the growth of *S. aureus* as observed in antibacterial test. Morelloflavone (**12**) showed the highest DPPH scavenging activity with IC_{50} of 20.3 ± 1.667 µg/mL which was comparable to ascorbic acid (AA) with IC_{50} of 13.2 ± 0.021 µg/mL. In general, all compounds showed significant reducing power, of which 1,3,5-trihydroxy-2-methoxyxantone (**29**) demonstrated the highest FRAP activity with a value of 1643.3 ± 44.623 mg AAE/g. In addition, preliminary biological screening of different solvent extracts from the roots of *G. atroviridis* revealed the pharmaceutical potential of butanol extract, which showed the highest antibacterial and antioxidant activities. Correlation between phenolic content in root extracts of *G. atroviridis* and antioxidant activity was evaluated. Strong positive correlation between TPC and the measured capacity in DPPH (r = 0.8760) and FRAP (r = 0.9385) assays signified the role of phenolic compounds in antioxidant activity in root extract of *G. atroviridis*. In conclusion, antibacterial and antioxidant activities in root extract of *G. atroviridis* may be attributable to the presence of phenolic compounds.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

cm	Centimeter
δ	Chemical shift
ºC	Degree Celsius
g	Gram
Hz	Hertz
kg	Kilogram
kHz	Kilohertz
L	Liter
λ_{max}	Maximum wavelength
ν_{max}	Maximum velocity
MHz	Megahertz
µg	Microgram
µg/mL	Microgram per milliliter
µL	Microliter
µm	Micrometer
µM	Micromolar
mg/mL	Milligram per milliliter
mg AAE/g	Milligram ascorbic acid equivalent per gram
mg GAE/g	Milligram gallic acid equivalent per gram
mm	Millimeter
mM	Millimolar
min	Minute
nm	Nanometer
ppm	Part per million
%	Percentage
cm^{-1}	Per centimeter

LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
ATP	Adenosine triphosphate
DPPH	*α, α*-diphenyl-*β*-picrylhydrazyl
AA	Ascorbic acid
AAE	Ascorbic acid equivalent
brd	Broad
BChE	Butyrylcholinesterase
^{13}C NMR	Carbon nuclear magnetic resonance spectroscopy
CFU	Colony forming units
J	Coupling constant
Acetone-d_6	Deuterated acetone
DCM	Dichloromethane
d	Doublet
dd	Doublet of doublet
EBV	Epstein Barr virus
EtOH	Ethanol
EtOAc	Ethyl acetate
ET	Electron transfer
ESI	Electrospray ionization
FRAP	Ferric reducing antioxidant power
FTC	Ferric thiocyanate
FC	Folin- Ciocalteau
FTIR	Fourier-transform infrared spectrometer
GA	Gallic acid
GAE	Gallic acid equivalent
IC_{50}	Half maximal inhibitory concentration
EC_{50}	Half maximal response concentration
HPLC	High performance liquid chromatography
HAT	Hydrogen atom transfer
HCA	Hydroxycitric acid
IR	Infrared spectroscopy

IMS	Ion-mobility mass spectrometry
LC/MS	Liquid chromatography/mass spectrometry
LC-Q/TOF-MS	Liquid chromatography quadrupole time-of-flight mass spectrometer
LDL	Low-density lipoprotein
LPLC	Low-pressure liquid chromatography
MS	Mass spectrometer
m/z	Mass to charge ratio
MeOH	Methanol
MAE	Microwave-assisted extraction
MIC	Minimum inhibitory concentration
MID	Minimum inhibitory dose
m	Multiplet
NA	Nutrient agar
NMR	Nuclear magnetic resonance spectroscopy
ORAC	Oxygen radical absorbance capacity
r	Pearson's correlation coefficients
KBr	Potassium bromide
PTLC	Preparative thin layer chromatography
^{1}H NMR	Proton nuclear magnetic resonance spectroscopy
ROS	Reactive oxygen species
RP	Reverse phase
Si gel CC	Silica gel column chromatography
s	Singlet
SD	Standard deviation
SFE	Supercritical fluid extraction
TLC	Thin layer chromatography
TBA	Thiobarbituric acid
TPC	Total phenolic content
TRAP	Total radical-trapping antioxidant parameter
t	Triplet
TEAC	Trolox equivalent antioxidant capacity
UPLC	Ultra-performance liquid chromatography

UAE	Ultrasound-assisted extraction
UV	Ultraviolet
UV-Vis	Ultraviolet–visible spectrometer
H_2O	Water
TPTZ	2,4,6-tripyridyl-s-triazine

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Garcinia is the largest genus of the family Clusiaceae (Lim, 2012). *Garcinia atroviridis* or locally known as 'asam gelugur' tree is a huge tropical rain forest tree that can be found mostly in Peninsular Malaysia (Mackeen et al., 2000). Yaacob and Tindall (1995) reported that *Garcinia* consists of 35 genera with over 800 species in the tropics of both hemispheres. About 80 *Garcinia* species grow in Malaysia's forests across wide range of habitats (Nordin et al., 2007). *Garcinia*, which has been used in folk treatment, consists of an array of medicinal plant species with potential therapeutic agents (Nguyen, 2015). It has been widely reported that *Garcinia* extracts particularly from *G. mangostana* and *G. kola* displayed diverse biological activities such as anti-HIV, antimicrobial, antihepatotoxic, antioxidant, anti-inflammatory, and anti-ulcerogenic activities (Jantan et al., 2011; Tadtong et al., 2009; Tewtrakul et al., 2009; Ee et al., 2008; Hu et al., 2005; Suksamrarn et al., 2003).

Although *G. atroviridis* grows in the wild, it is also cultivated by the locals for its medicinal and market value (Mackeen et al., 2000). Previous phytochemical studies of *G. atroviridis* revealed that this plant is a rich source of secondary metabolites, especially phenolic compounds such as xanthones and flavonoids (Tan et al., 2016; Permana et al., 2005; Permana et al., 2003; Dweck, 1999). This plant has medicinal values as it has been traditionally used to treat cough, earache, and in postpartum treatment (Tisdale et al., 2003; Burkill, 1966). Mackeen et al. (2000) found that crude methanol (MeOH) extracts from the fruits, roots, leaves, stem and trunk barks of *G. atroviridis* possessed antibacterial, antifungal, antioxidant, and antitumor-promoting properties, which suggests their potential as therapeutic agent. It was revealed that MeOH root extract of *G. atroviridis* exhibited strong antibacterial and antioxidant activities (Mackeen et al., 2000).

To date, few studies have reported on phytochemical investigation of *G. atroviridis*, of which three reported specifically on the root extracts (Permana et al., 2005; Permana et al., 2003; Permana et al., 2001). Permana et al. (2005) reported on successful purification of bioactive chemical constituents from the ethyl acetate (EtOAc) root extract and thus, the study reported in this thesis was first conducted in expectation to reveal new bioactive chemical constituents from the extract. Characterization of the compounds structure was determined based on spectral analysis and comparison with published data. Antibacterial and antioxidant activities of the extracts and isolated compounds from the roots of *G. atroviridis* were evaluated.

1.2 Problem Statement

Root extract of *G. atroviridis* showed strong antibacterial and antioxidant properties based on its preliminary biological studies done by Mackeen et al., 2000, as compared to other parts of the plant. The detailed studies regarding the chemical constituents and its biological properties of the root part was later reported by Permana et al. (2001; 2003; 2005). The results showed that and phenolic compounds isolated from the EtOAc root extract of *G. atroviridis* exhibited strong antibacterial and antioxidant properties (Permana et al., 2001; 2003; 2005). However, after more than decades, study on the root extract and its isolated compounds have not been extensively conducted. Hence, current study was conducted in accordance to the studies done by Permana et al. (2001; 2003; 2005) to identify new bioactive chemical constituents from the EtOAc extract of *G. atroviridis* root.

In recent years, microbial resistance towards antibiotics and undesirable side effects of using synthetic antibiotics have been a challenge. The use of phytochemicals with identified antibacterial properties in combination with existing antibiotics can potentially enhance antimicrobial activity. Besides the pharmaceutical industry, the antibacterial compounds particularly from phenolic group offer a natural alternative to synthetic preservatives in food and cosmetic applications.

Phenolic compounds are also known to exhibit good antioxidant properties. Antioxidants help to fight oxidation, a normal chemical process in the body. When there is a disruption to this process, free radicals are created. At high concentration, these free radicals causes oxidative stress, which involved in the development of chronic diseases

such as cancer, hypertension, cardiovascular and neurodegenerative diseases. Consuming antioxidants may be necessary if there is insufficient of antioxidants produced naturally in the body. There seems to be a high interest to find safe and inexpensive antioxidants from natural sources due to adverse effects produced by its synthetic counterparts. Furthermore, the commercial production of plants as sources of antioxidants can be used to improve the properties of foods, both for nutritional and preservation purposes. Therefore, based on the published biological potential of *G. atroviridis*, current study will lead to the discovery of potential antibacterial and antioxidant compounds from EtOAc root extract of the plant.

1.3 Research Objectives

Based on the published antibacterial and antioxidant potential of the root extract from *G. atroviridis*, the objectives of this study are outlined as follows;

1. To identify the active chemical constituents in the root extract of *G. atroviridis* EtOAc by isolation and purification using chromatography techniques namely silica gel column chromatography (Si gel CC), preparative thin layer chromatography (PTLC), and thin layer chromatography (TLC).
2. To elucidate the structure of isolated compounds from EtOAc root extract of *G. atroviridis* via spectroscopy techniques that include mass spectrometer (MS), infrared (IR) spectroscopy, ultraviolet-visible (UV-Vis) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy.
3. To evaluate antibacterial and antioxidant properties of EtOAc root extract of *G. atroviridis* roots and its isolated compounds via disc diffusion test, and α, α-diphenyl-β-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays, respectively.

1.4 Scope of the Study

Root part of *G. atroviridis* from Clusiaceae family was selected for this study based on its strong preliminary antibacterial and antioxidant activities of MeOH crude extract that was published by Mackeen and co-authors (2000). Permana et al. (2005; 2003; 2001) further investigate the chemical compositions of the root extract. According to Permana et al. (2005; 2003; 2001), various phenolic compounds isolated from EtOAc

root extract of *G. atroviridis* possessed biological properties such as antibacterial, antioxidant and cytotoxicity, which serves as the basis of focusing on EtOAc extract in this study. Furthermore, it was reported that EtOAc was a good solvent to recover polyphenols of intermediate polarity (Chung et al., 1999). Flavonoid aglycones such as isoflavones, flavanones, flavones, and flavonols usually soluble in solvents like EtOAc, chloroform and diethyl ether (Susanto et al., 2019; Santos-Buelga et al., 2012). In order to isolate other phenolic compounds from EtOAc extract of *G. atroviridis* roots, current study was conducted according to reports by Permana and co-authors (2005; 2003; 2001).

Isolation of phenolic compounds present in the EtOAc root extract of *G. atroviridis* was carried out via chromatography techniques such as silica gel column chromatography (Si gel CC), preparative thin layer chromatography (PTLC), and thin layer chromatography (TLC). Structures of the compounds were elucidated using mass spectrometer (MS), infrared (IR) spectroscopy, ultraviolet-visible (UV-Vis) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy. Spectral analysis of the compounds and its comparison with previous reports were used to characterize the compounds. Antibacterial and antioxidant activities of the EtOAc extract and its isolated compounds were also evaluated. In addition, preliminary screening of antibacterial and antioxidant activities of hexane, dichloromethane (DCM), butanol and MeOH extracts from the roots of *G. atroviridis* was evaluated. Antibacterial activity was observed via disc diffusion method, whereas antioxidant activity was evaluated via *α, α*-diphenyl-*β*-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays.

1.5 Significance of Research

Purification and isolation of bioactive compounds enable the discovery of new natural products. Chemical constituents will be screened for biological activities thus revealing therapeutic potential of plant constituents and possibly new discovery of drug sourced from plant. Research trends clearly demonstrate that in future, natural products will be one of the essential components in drugs. This study was conducted to investigate the potential antibacterial and antioxidant agents from the roots of *G. atroviridis*.

CHAPTER 2

LITERATURE REVIEW

2.1 Genus of *Garcinia*

Garcinia genus consists of over 250 species belongs to the Clusiaceae or Guttiferae family (Lim et al., 2012). *Garcinia* is rich in secondary metabolites, where four major classes known as xanthones, coumarins, biflavonoids, and benzophenones were identified in the plant (Acuna et al., 2009). Production of phytochemicals, primary or secondary metabolites, in medicinal plants has been used to prevent and treat diseases in human (Nostro et al., 2000). Thanks to its enormous remedial qualities, this genus has received considerable attention from pharmaceutical industries (Hemshekhar et al., 2011).

G. mangostana or locally known as mangosteen tree is among the well-known species of this genus, which anti-HIV, antitumor, anti-inflammatory, anti-tuberculosis, and antimicrobial properties have been extensively published (Tadtong et al., 2009; Tewtrakul et al., 2009; Ee et al., 2008; Hu et al., 2005; Suksamrarn et al., 2003). It was reported that the presence of xanthone derivatives, identified as α- and γ-mangostin contributed to diverse biological activities of the plant (Jung et al., 2006; Suksamrarn et al., 2002). It was suggested that antioxidant and anti-platelet properties of *Garcinia* species were partially due to its total phenolic content (Jantan et al., 2011). Same study revealed that oxidation of low-density lipoprotein (LDL) was significantly inhibited by the extracts from *G. cantleyana*, *G. hombroniana*, *G. prainiana*, *G. eugenifolia*, and *G. atroviridis*.

Biological activities of EtOAc extract could be associated with the presence of phenolic compounds as EtOAc was efficient to extract flavonoid aglycones such as isoflavones, flavanones, flavones, and flavonols (Susanto et al., 2019; Santos-Buelga et al., 2012). The EtOAc extract of the young fruit of *G. mangostana*, displayed strong cytotoxicity against epidermoid carcinoma of the mouth (KB) and breast cancer (BC-1)

cell lines. Extensive study on the chemical compositions of the extract able to isolate 19 xanthones that might contributed to the cytotoxic activity of the extract (Suksamrarn et al., 2006). EtOAc root extract of *G. parvifolia* was reported to show strong antibacterial and antioxidant activities, and high toxicity towards brine shrimp (Syamsudin et al., 2007). Antioxidant activity of EtOAc extract from *G. mangostana* pericarp was demonstrated via various assays (Zhou et al., 2011; Zarena and Sankar, 2009a; Zarena and Sankar, 2009b). Significant anticholinesterase activity against both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes was displayed by EtOAc extract of *G. hombroniana* bark (Jamila et al., 2013). Meanwhile, EtOAc extract of *G. mangostana* pericarp and *G. xanthocymus* bark showed potent α-glucosidase inhibitory properties (Godavari and Amutha, 2017; Nguyen et al., 2017).

Phytochemicals investigation of EtOAc extract of *Garcinia* enable identification of phenolic compounds, which may contribute to the therapeutic properties of the plant. For instance, compounds exhibiting antibacterial, anti-inflammatory and cytotoxic activities were reportedly isolated from EtOAc root extract of *G. atroviridis* (Permana et al., 2001; Permana et al., 2005). In addition, study by Meng et al. (2012) showed that the roots of *G. xanthochymus* are good sources of bioactive components, and isolation of the EtOAc fraction yielded large amount of biflavonoids. EtOAc extract of *G. madruno* twigs and leaves, able to purify several biflavonoids (Osorio et al., 2013). Extensive phytochemical study on EtOAc extract of *G. xanthocymus* bark revealed that the extract was rich with xanthones (Nguyen et al., 2017). Previous studies serve as concrete reference for further purification of bioactive compounds from EtOAc root extract of *G. atroviridis*.

2.2 *G. atroviridis*

G. atroviridis Griff ex T. Anders is a fruit tree of medium-sized widely distributed in Peninsular Malaysia, Myanmar, Thailand and India (Lim, 2012). It can grow in tropical, mixed lowland forest on the plains up to 600 meters, in the highlands of Southeast Asia with high rainfall (Lim, 2012). In Malaysia, it is usually known as ‘asam gelugur’ or ‘asam keping’ tree.

According to Whitmore (1973), *G. atroviridis* can grow up to 25 meters tall. This plant has trunk fluted at the base with dull grey, splintered and fissured bark with drooping branches. It produces a little translucent or washed out sap from the internal bark. Young shoots are reddish at first but when mature, it changes to dark glossy green (Figure 2.1). This species is a dioecious tree, which means that this tree either male or female reproductive organs. It has unisexual flowers, whereby the female flowers are solitary, reddish, bulky and occasionally slightly fragrant; male flowers are borne in terminal clusters. Male flowers are rarely found making female flowers readily selectable for planting by the locals. The fruits are juicy and round in shape, which color turns to yellowish orange upon ripen, with huge brownish disc-like stigma importunate at the tip (Figure 2.12). The seeds are vivid orange in color.

Preliminary phytochemical studies on the roots of different *Garcinia* species have reported on diverse biological activities (Kaennakam et al., 2015; Meng et al., 2012; Syamsudin et al., 2007). Methanolic crude extracts from the roots of *G. atroviridis* exhibited strong antibacterial and antioxidant properties (Mackeen et al., 2000). Bioactive chemical constituents in the roots of *G. atroviridis*, which potentially contribute to its biological properties including antibacterial, anti-inflammatory and cytotoxic activity, have been successfully purified (Permana et al., 2005; 2003; 2001).

Figure 2.1 *G. atroviridis* tree
Source: Lim, (2012)

Figure 2.2 *G. atroviridis* fruit
Source: Lim, (2012)

2.3 Chemical Compositions of *G. atroviridis*

Medicinal properties of plant is usually contributed by its various secondary metabolites. Various bioactive constituents from different parts of *G. atroviridis* were successfully isolated and identified. Kosin et al. (1998), who was able to characterize newly identified tetraoxygenated xanthone, atroviridin (**1**), from chloroform extract of the stem bark, did the first study on isolation of chemical compound from *G. atroviridis*. Dweck (1999) reported the presence of fruiting acid namely citric acid (**2**), tartaric acid (**3**), malic acid (**4**) and ascorbic acid (**5**) in the fruit of *G. atroviridis*, which showed antioxidant activity; atrovirinone (**6**) and atrovirisidone (**7**) were reportedly isolated from the roots of *G. atroviridis* (Permana et al., 2001); Jena et al. (2002) reported the presence of (-)- hydroxycitric acid (HCA) (**8**) in the fruit rind of *G. atroviridis*; the same year, Mackeen et al. (2002) was able to isolate 2-(butoxycarbonylmethyl)-3-butoxycarbonyl-2-hydroxy-3-propanolide (**9**) and 1′,1″-dibutylmethylhydroxycitrate (**10**).

Isolation of compounds namely 4-methylhydroatrovirinone (**11**), morelloflavone (**12**), 7-*O*-*β*-D-glucopyranoside (**13**), fukugiside (**14**), and 14-*cis*-docosenoic acid (**15**) from the EtOAc root extract of *G. atroviridis* was published by Permana et al. (2003). A new prenylated depsidone identified as atrovirisidone B (**16**) was later purified from the roots along with known compounds, naringenin (**17**) and GB1a (**18**) (Permana et al., 2005). Isolation of *β*-caryolanol (**19**), ginsenol (**20**) and clovanol (**21**) from the fruit of *G. atroviridis* was reported by Tan et al. (2013).

New compounds, triflavanone, garcineflavanone (**22**) and biflavonol, garcineflavonol (**23**) were isolated and tested for their cholinesterase inhibitor properties (Tan et al., 2014). Characterization of new xanthone namely garcinexanthone G (**24**), together with stigmasta-5,22-dien-3*β*-ol (**25**), stigmasta-5,22- dien-3-*O*-*β*-glucopyranoside (**26**), 3*β*-acetoxy-11*α*,12*α*-epoxyoleanan-28,13*β*-olide (**27**), 2,6-dimethoxy-*p*-benzoquinone (**28**), 1,3,5-trihydroxy-2-methoxyxanthone (**29**), 1,3,7-trihydroxyxanthone (**30**), kaempferol (**31**) and quercetin (**32**) from stem bark extract of *G. atroviridis* was extensively published (Tan et al., 2016). Details about the isolated compounds from various extracts of *G. atroviridis* are summarized in Table 2.1.

Table 2.1 Isolated compounds of *G. atroviridis*

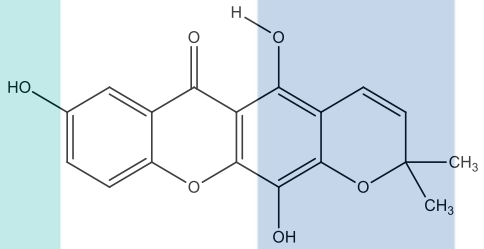
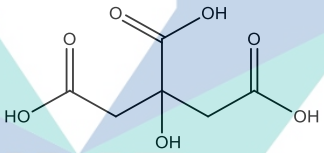
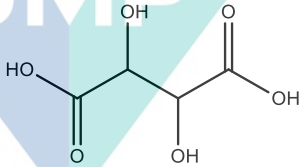
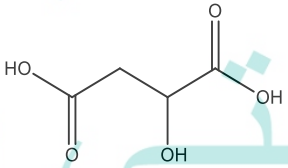
No	Name of compound	Structure of compound	Part of plant	Reference
1	Atroviridin		Stem bark	Kosin et al., 1998
2	Citric acid		Fruit	Dweck, 1999
3	Tartaric acid		Fruit	Dweck, 1999
4	Malic acid		Fruit	Dweck, 1999

Table 2.1 Continued

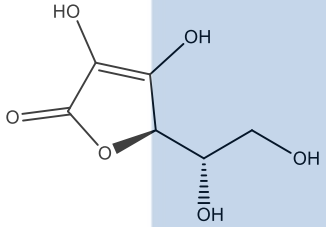
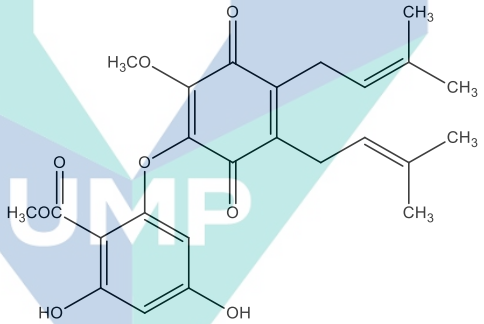
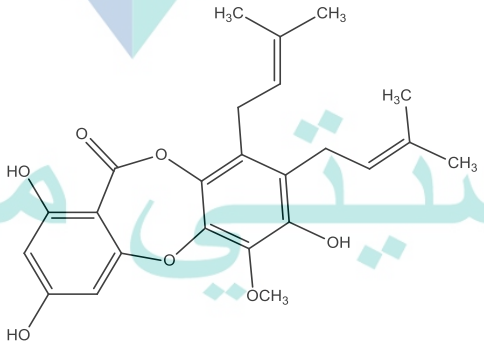
No	Name of compound	Structure of compound	Part of plant	Reference
5	Ascorbic acid		Fruit	Dweck, 1999
6	Atrovirinone		Root	Permana et al., 2001
7	Atrovirisidone		Root	Permana et al., 2001

Table 2.1 Continued

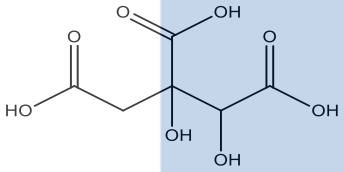
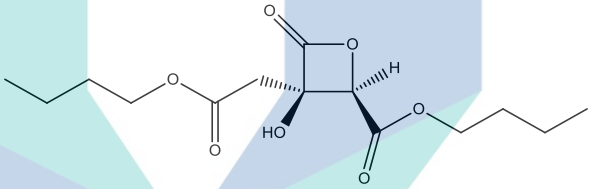
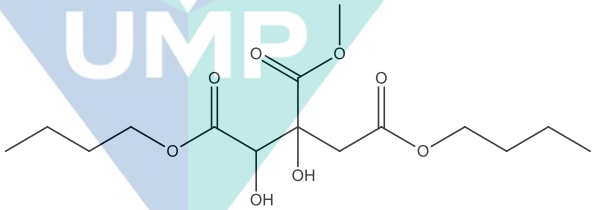
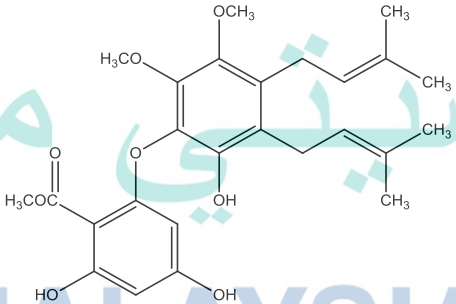
No	Name of compound	Structure of compound	Part of plant	Reference
8	Hydroxycitric acid (HCA)		Fruit rind	Jena et al., 2002
9	2-(butoxycarbonyl methyl)-3-butoxy carbonyl-2-hydroxy-3-propanolide		Fruit	Mackeen et al., 2002
10	1′,1″-dibutylmethyl hydroxycitrate		Fruit	Mackeen et al., 2002
11	4-methylhydroatrovirinone		Root	Permana et al., 2003

Table 2.1 Continued

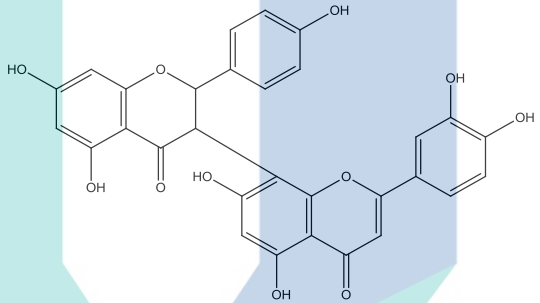
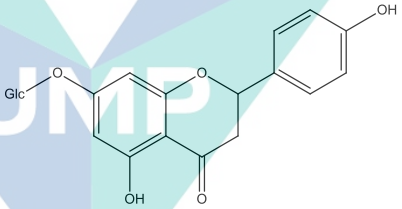
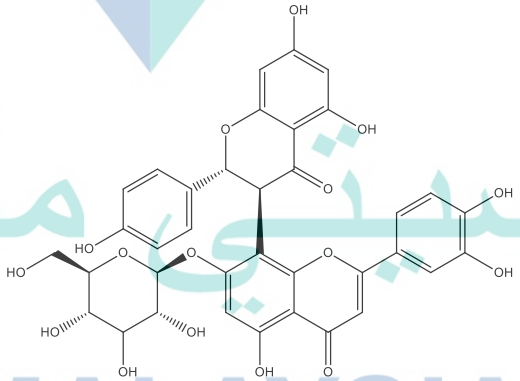
No	Name of compound	Structure of compound	Part of plant	Reference
12	Morelloflavone		Root	Permana et al., 2003
13	7-*O*-*β*-D-glucopyranoside		Root	Permana et al., 2003
14	Fukugiside		Root	Permana et al., 2003

Table 2.1 Continued

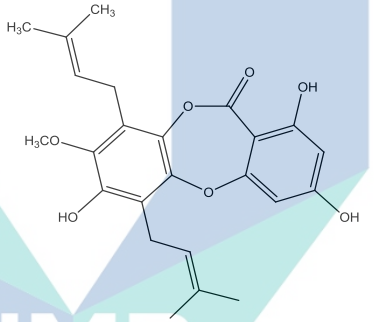
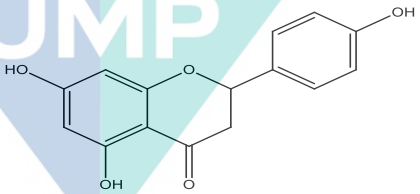
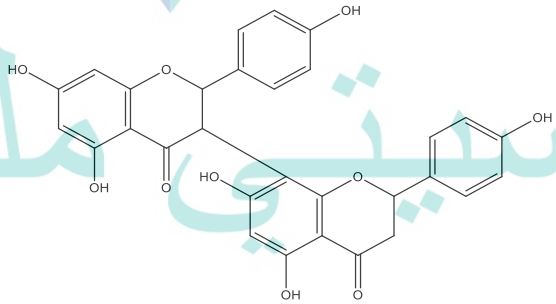
No	Name of compound	Structure of compound	Part of plant	Reference
15	14-cis-docosenoic acid		Root	Permana et al., 2003
16	Atrovirisidone B		Root	Permana et al., 2005
17	Naringenin		Root	Permana et al., 2005
18	GB1a		Root	Permana et al., 2005

Table 2.1 Continued

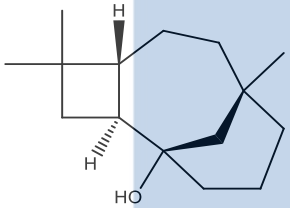
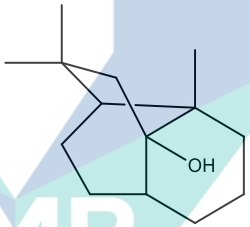
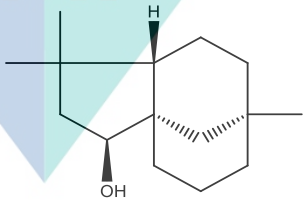
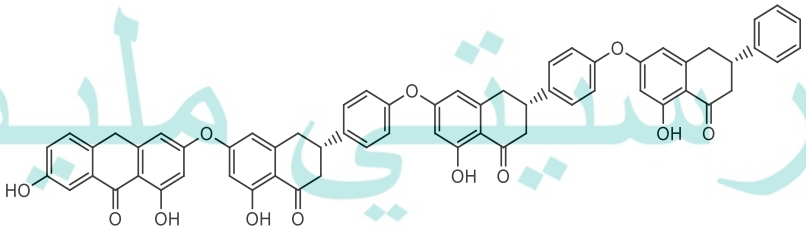
No	Name of compound	Structure of compound	Part of plant	Reference
19	*β*-caryolanol		Fruit	Tan et al., 2013
20	Ginsenol		Fruit	Tan et al., 2013
21	Clovanol		Fruit	Tan et al., 2013
22	Garcineflavanone		Stem bark	Tan et al., 2014

Table 2.1 Continued

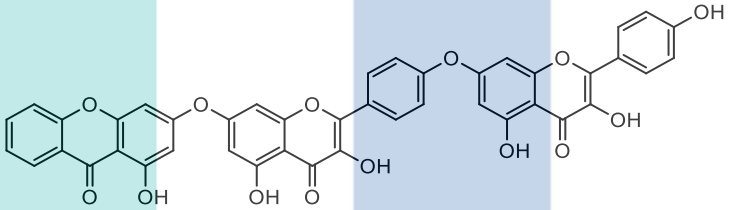
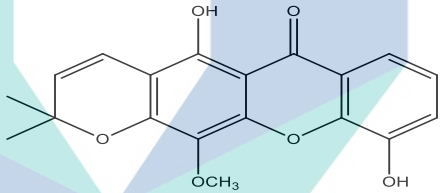
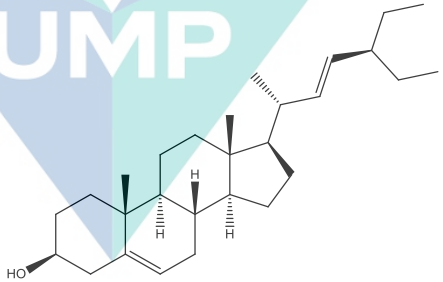
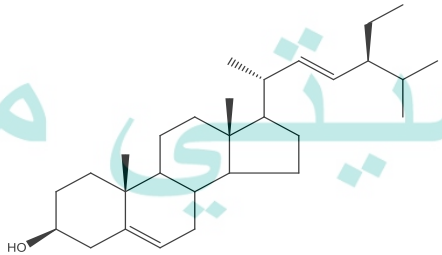
No	Name of compound	Structure of compound	Part of plant	Reference
23	Garcineflavonol		Stem bark	Tan et al., 2014
24	Garcinexanthone G		Stem bark	Tan et al., 2016
25	Stigmasta-5,22-dien-3β-ol		Stem bark	Tan et al., 2016
26	Stigmasta-5,22- dien-3-*O*-β-glucopyranoside (stigmasterol)		Stem bark	Tan et al., 2016

Table 2.1 Continued

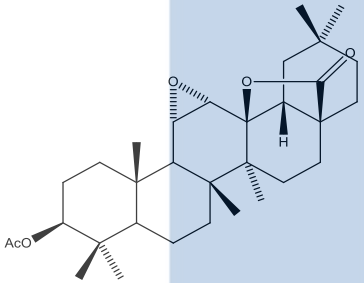
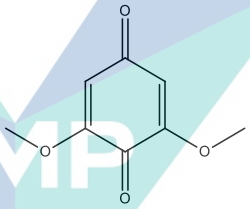
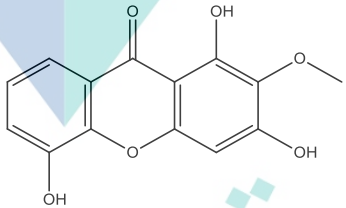
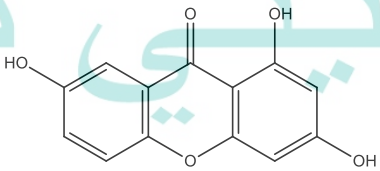
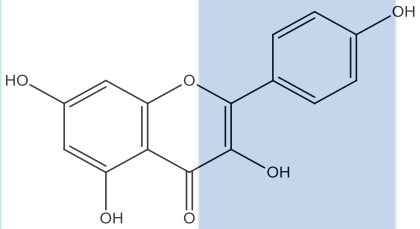
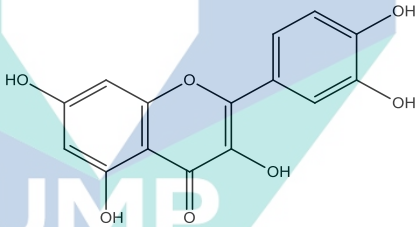
No	Name of compound	Structure of compound	Part of plant	Reference
27	3β-acetoxy-11α,12α-epoxyoleanan-28,13β-olide		Stem bark	Tan et al., 2016
28	2,6-dimethoxy-*p*-benzoquinone		Stem bark	Tan et al., 2016
29	1,3,5-trihydroxy-2-methoxyxanthone		Stem bark	Tan et al., 2016
30	1,3,7-trihydroxyxanthone		Stem bark	Tan et al., 2016

Table 2.1 Continued

No	Name of compound	Structure of compound	Part of plant	Reference
31	Kaempferol		Stem bark	Tan et al., 2016
32	Quercetin		Stem bark	Tan et al., 2016

2.4 Biological Properties of *G. atroviridis*

Plants are usually considered as a source of active agents, traditionally used in folk remedies (Siddiqui et al., 2014). *G. atroviridis* has been used as one of the active ingredients in folk medicine. In recent years, biological properties of *G. atroviridis* has been extensively investigated in pre-clinical and clinical studies, which led to discovery of potential active compounds that may contribute to the diverse medicinal properties of this plant.

2.4.1 Traditional Uses

Mixture of leaves and roots of *G. atroviridis* were used in traditional preparation of decoction used as pre-and post-partum medication to treat earache, throat irritation, cough, dandruff, and stomachache due to pregnancy (Lim, 2012). The fruit with the addition of vinegar have traditionally been used by the locals in Peninsular Malaysia for topical application over the abdomen of women after confinement. Meanwhile in Thailand, the dried fruits have been used in treating coughs, improving blood circulation, and as expectorant and laxative (Lim, 2012; Tisdale et al., 2003).

2.4.2 *In-vitro* Evaluation of Biological Activities

2.4.2.1 Antibacterial Activity

Mackeen et al. (2000) reported on antibacterial activity of methanolic extract from various parts of *G. atroviridis*. Root extract exhibited the strongest inhibition on the growth of bacteria namely *B. subtilis* B28, *B. subtilis* B29, methicillin-resistant *S. aureus*, *E. coli* and *P. aeruginosa* with minimum inhibitory dose (MID) of 15.6 µg/disc which was at least eight-fold stronger than the next most active extract. Basri et al. (2005) published on significant antibacterial activity of EtOAc and ethanol (EtOH) crude extract from the *G. atroviridis* fruit against *S. aureus*, *S. epidemidis*, *B. subtilis*, *E. coli*, *S. typhimurium*, *S. enteritidis* and *P. aeruginosa*. On the other hand, the growth of *S. aureus* and *E. coli* was inhibited in the presence of aqueous extract from *G. atroviridis* leaves (Zakaria et al., 2011).

Strong inhibitory activity of atrovirinone (**6**) and atrovirisidone (**7**) isolated from EtOAc root extract towards *S. aureus* and *B. cereus* was observed in a study by Permana

et al. (2001). Also reported was antibacterial activity of *β*-caryolanol (**19**), ginsenol (**20**) and clovanol (**21**) isolated from the fruits of *G. atroviridis* in a study conducted by Tan et al. (2013). The minimum inhibitory concentration (MIC) test using micro dilution assay showed that these three compounds demonstrated stronger activity against Gram-positive bacteria (*B. subtilis* and *S. aureus*) than the Gram-negative bacteria (*E. coli, P. stutzeri* and *S. typhimurium*).

2.4.2.2 Antioxidant Activity

Plants with high antioxidant phytochemicals may offer protection against various diseases caused by free radicals (Sofowora, 1993). The leaves and fruits of *G. atroviridis* can potentially be used as a source of natural antioxidants and nutrients that may help in mediating health conditions associated with free radicals, as they contains high phenolic compound. Furthermore, the absence of heavy metal elements such as arsenic and lead in the extracts make them safe for consumption (Nursakinah et al., 2012).

In a published work by Mackeen et al. (2000), MeOH crude extract from the leaves, roots, stem and trunk bark of *G. atroviridis* showed strong antioxidant activity in Ferric Thiocyanate (FTC) and Thiobarbituric Acid (TBA) assays. At the early stage of lipid oxidation, FTC method was used to calculate the amount of peroxide produced, while TBA method is used to calculate carbonyl compounds produced from decomposition of peroxide at the later stage of lipid oxidation (Aris et al., 2009). In the same study, except for the fruit extract, all extracts exhibited pronounced antioxidant activity by 64 to 90 % in FTC assay and 87 to 93 % in TBA assay, exceeding the capacity of commercial antioxidant, *α*-tocopherol. In both assays, trunk bark extract exhibited the strongest antioxidant activity while stem bark extract showed the weakest activity. The color of pigment in trunk bark extract was different to that of stem bark extract, whereby the former was yellow and the latter was reddish orange, signifying the different chemical compositions in the two plant parts (Mackeen et al., 2000).

One of the most common compound in *Garcinia*, morelloflavone (**12**) was present in the EtOAc extract from the root of *G. atroviridis* (Permana et al., 2001). This compound was also isolated from methanolic extract from the seeds of *G. dulcis* showed to have significant antioxidant activity tested via DPPH assay (Deachathai et al., 2008). This compound also purified from MeOH extract of *G. prainiana* leaves, exhibited strong

antioxidant activity via DPPH method with IC_{50} value of 72 µmol/L compared to the positive control, butylated hydroxyanisole (BHA) with IC_{50} value of 167 µmol/L (Klaiklay et al., 2011). GB1a (**18**) also demonstrated good antioxidant activity comparable to the standard with IC_{50} of 148 µmol/L in the same study. Potent lipid peroxidation inhibition activity of biflavonoids; amentoflavone, morelloflavone (**12**) and volkensiflavone (**33**), from EtOAc extract of twigs and leaves of *G. madruno* was displayed via thiobarbituric acid reactive substances assay (TBARS) (Osorio et al., 2013). Significant reduction of lipid peroxidation in biflavonoid fraction was associated with the presence of morelloflavone (**12**) in the fraction, although it might due to synergy processes (Osorio et al., 2013). Apart from that, compounds 1,3,7-trihydroxyxanthone (**30**) and quercetin (**32**) isolated from DCM extract of *G. atroviridis* stem bark demonstrated strong DPPH scavenging activity with EC_{50} values of 16.20 and 12.68 µg/mL, respectively, as compared to the ascorbic acid (AA) with a value of 7.4 µg/mL (Tan et al., 2016).

2.4.2.3 Cytotoxic and Antitumor Activity

G. atroviridis methanolic extract from trunk and stem bark exhibited pronounced antitumor-promoting activity, inhibiting activation of Epstein Barr virus (EBV) by about 90 %. Fruit and leaves extract also showed significant antitumor-promoting activity through successful inhibition of tumor-promotion in Raji cells by 67.5 and 64.7 %, respectively (Mackeen et al., 2000). Preliminary biological study of MeOH root extract from the roots of *G. atroviridis* revealed strong cytotoxicity of the extract towards Raji cells at the tested concentration of 200 µg/mL; also discovered was the slight lethal effect of the root extract on brine shrimp nauplii (Mackeen et al., 2000). A compound isolated from the EtOAc extract of the root, atrovirinone (**6**) exhibited cytotoxicity towards HeLa cells with IC_{50} of 15 µg/mL, which was comparable to doxorubicin (IC_{50} 11 µg/mL) and colchine (IC_{50} 21 µg/mL) (Permana et al., 2001). In addition, same researcher also isolated other compounds from EtOAc root extract namely, atrovirisidone (**7**) and atrovirisidone B (**16**) which demonstrated cytotoxic effects on the cell lines of human breast cancer (MCF-7), human prostate cancer (DU-145) and lung cancer (H-460) (Permana et al., 2005).

2.4.2.4 Anti-inflammatory Activity

Inflammation is a complicated disease involving production of prostaglandins that causes the sensation of pain. It was concluded that atrovirinone (**6**) from EtOAc extract of *G. atroviridis* roots able to inhibit several major pro-inflammatory mediators of inflammation (Syahida et al., 2006; Israf et al., 2010). On the other hand, strong anti-inflammatory activity of several volatile constituents isolated from the fruit extract of *G. atroviridis* namely *β*-caryolanol (**19**), ginsenol (**20**) and clovanol (**21**), was reported (Tan et al., 2013).

Morelloflavone (**12**) act as an inhibitor of secretory phospholipase A_2, selective to enzyme group II and III, while also demonstrated potent anti-inflammatory effects in mice (Gil et al., 1997). The anti-inflammatory activity of this compound might be due to the capability of morelloflavone (**12**) to scavenge reactive oxygen species (ROS). Morelloflavone (**12**) purified from MeOH extract of the stem bark of *G. prainiana* suppressed the level of nitric oxide production in lipopolysaccharides (LPS)-stimulated RAW 264.7 macrophages as compared to the LPS-stimulated cells group without any treatment (Taher et al., 2016).

2.4.2.5 Hypolipidaemic Activity

HCA (**8**) is the main acid in the fruit rinds of *G. atroviridis* and other *Garcinia* species (Dweck, 1999). Hypolipidaemic activity of methanolic fruit extract from *G. atroviridis* was investigated, which found that consumption of *G. atroviridis* reduced lipid content in the serum, and eliminated fat in the aorta of animals fed with high-cholesterol diet (Amran et al., 2009). A broad animal study revealed that HCA (**8**) represses fatty acid production in lipogenesis and induces weight loss, by inhibiting adenosine triphosphate (ATP)-citrate lyase (Jena et al., 2002). This shows that even by using different animal model, fruit extract of *G. atroviridis* displayed lipid modulating properties hence, have high potential as antihyperdaemic agent. Meanwhile, compound morelloflavone (**12**) isolated from EtOAc extract of *G. xanthochymus* leaves, showed good inhibitory activity against α-amylase, more effective than the acarbose which was used as the standard (Li et al., 2014).

2.4.2.6 Other Biological Activities

Inhibition of AChE and BChE by garcineflavanone (**22**) and garcineflavonol (**23**), of which garcineflavanone (**22**) acts as dual inhibitor was reported, demonstrating their potential as cholinesterase inhibitor (Tan et al., 2014). Cholinesterase inhibitors are essential for Alzheimer's disease treatment. Morelloflavone (**12**) and volkensiflavone (**33**) from stem bark of *G. prainiana* MeOH extract showed strong anti-tyrosinase inhibitory activity (On et al., 2016). Tyrosinase inhibitors are chemical agents capable of decreasing the enzymatic reactions, such as food browning and melanisation of human skin (Salleh et al., 2017). Morelloflavone (**12**) obtained from heartwood of *G. multiflora* exhibited anti-HIV activity by inhibiting both HIV-1 reverse transcriptase (HIV-1 RT) *in vitro* and HIV-1 (strain LAV-1) in phytohemagglutinin-stimulated primary human peripheral blood mononuclear cells at EC_{50} value of 6.9 µM (Lin et al., 1997).

2.5 Extraction of Phenolic Compounds

The target active constituents in this study are phenolic compounds. Phenolic compounds belong to one of the main groups of active compounds in plants, which consists of one aromatic ring (phenolic acids) or more (polyphenols), with hydroxyl groups attached to its structure (Minatel et al., 2017; Perveen and Al-Taweel, 2017). Phenolic compounds are classified into several classes such as xanthones, flavanones, flavones, tannins, and lignins according to their structures (Santos-Buelga et al., 2012). Physical and chemical characteristics of phenolic compounds are determined by their structure, which information is critical in the selection of method and solvent for extraction process (Ignat et al., 2011; Dai and Mumper, 2010).

Typical extraction process usually starts with preparation of plant sample. The plant material is collected from parts of the plant subjected to research interest. The plant sample are washed under running tap water to remove soil and unwanted debris. Next is to dry the sample either by air-drying or using microwave at suitable temperature. Temperatures under 30 °C should be used and in addition, exposure to direct sunlight should be avoided as it might degrade the heat-labile or UV-sensitive compounds (Jones and Kinghorn, 2012). To ensure efficient extraction, the sample is either pulverized or ground using mortar and pestle to produce coarse powder, thus increasing the surface area of the sample, enabling diffusion of solvent into the cells (Seidel, 2012).

2.5.1 Solvent Extraction

In general, solvent extraction is a technique that uses solvent to separate compounds from complex sample matrices such as plant materials (Susanto et al., 2019). Solvent extraction, otherwise known as liquid-liquid extraction, is the most common method used for extraction of phenolic compounds. Soxhlet extraction, maceration and hydro-distillation are among other techniques in solvent extraction (Jahromi, 2019). Several parameters that may influence the yield of phenolic compound include extraction time and temperature, solvent-to-sample ratio, solvent type and polarity, and number of repetition in extraction (Fan et al., 2015; Garcia-Salas et al., 2010).

Different polarity of organic solvent allows for extraction of phenolic compounds with different polarity. A solvent of similar polarity to the solute will properly dissolves the solute. Usually, different polar solvent are used successively to extract active components from the plant sample, starting from low polarity solvent such as hexane, to high polarity solvent such as butanol to separate the lipophilic compounds (Jones and Kinghorn, 2012). Solvents with different polarity of the solvent do not mix and form layers, which can easily be separated as shown in Figure 2.3. The solvents are removed by using rotary evaporator at a temperature of 40 °C prior to collecting and storing the sample for further investigation (Seidel, 2012).

Figure 2.3 Solvent extraction

It was reported that both polar and non-polar compounds could be extracted using MeOH and the solvent worked efficiently in recovering polyphenols from plant sample (Altemimi et al., 2017). MeOH has low boiling point, which ease the process of extracting and concentrating of the extract. Phenolic compounds of different classes including three biflavonoids namely amentoflavone, morelloflavone (**12**), and GB1a (**18**) was purified from the extraction of *G. prainiana* leaves with MeOH three times over a period of seven days at room temperature (Klaiklay et al., 2011). Meanwhile, the isolation of MeOH extract from mangosteen pericarps afforded five xanthones known as mangostanaxanthones I and II, gartanin, γ-mangostin, and mangostanaxanthone VII (Ibrahim et al., 2018).

Other common solvent used to extract phenolic compounds particularly the low polarity aglycone, such as isoflavones, flavanones, flavones, and flavonols is EtOAc (Susanto et al., 2019; Santos-Buelga et al., 2012). Chung and co-authors (1999) reported that EtOAc had worked efficiently to extract most of the phenolic substances with intermediate polarity. Other advantages of using EtOAc is its insolubility in water and the relatively low boiling point, which allows for easy solvent removal. Extraction using this solvent at 50 °C for 48 h lead to the isolation of 19 xanthones including α-mangostin and gartanin, which exhibited potent cytotoxic activity (Suksamrarn et al., 2006). Meanwhile, the purification of EtOAc fraction of *G.nervosa* leaves afforded one biflavonoid known as II-3,I-5,II-5,II-7,I-4′,II-4′-hexahydroxy-(I-3,II-8)-flavonylflavanonol together with two flavonoids, 6-methyl-4′-methoxyflavone and acacetin (Jalil et al., 2012). EtOAc extract of aerial parts (bark, twigs, and leaves) of *Acacia hydaspica* showed significant antioxidant potential in various *in vitro* antioxidant enzyme assays and it was probably due to the high phenolic content in the extract. The isolation of this extract yielded three polyphenols known as of 7-*O*-galloyl catechins, catechins and methyl gallate (Afsar et al., 2018).

Since phenolic compounds are generally hydrophilic, other polar solvents such as EtOH, chloroform, dietyl ether, acetonitrile and acetone, or mixture of these solvents with water can also be used to extract polyphenols (Ignat et al., 2011). These conventional extractions are still widely used to extract phytochemicals due to simple extraction procedure as it only requires basic apparatus set-up and allows for extraction of various

phenolic compounds using organic solvents with varying polarity; it is also convenient for initial and bulk extraction (Seidel, 2012; Garcia-Salas et al., 2010).

However, there are few shortcomings of using these methods: the extraction process requires high solvent consumption; the risk of using hazardous organic solvents; it is a time consuming process that usually results in low extraction yield (Garcia-Salas et al., 2010). Unconventional or assisted extraction techniques that use ultrasounds, and microwaves have been reportedly adopted for extraction of phenolic compounds to overcome the limitations in conventional extraction method (Santos-Buelga et al., 2012).

2.5.2 Ultrasound-assisted Extraction (UAE)

Sonication is the method that uses sound waves with frequencies ranging from 20 kHz to 10 MHz to produce cavitation bubbles near the sample tissue, which will eventually disrupt the plant cell wall to release the contents (Cheng et al., 2015). The method facilitates penetration of the solvents in the cells, resulting in improved extraction yield (Altemimi et al., 2017). Effectiveness of this extraction method depends on sonication time, frequency, temperature, and sample-to-solvent ratio; it is essential to optimize these parameters (Santos-Buelga et al., 2012). The technique employs the use of ultrasonic bath and probe systems (Altemimi et al., 2017).

UAE also can be used with any solvent just like conventional extraction, hence able to extract various compounds (Wang and Weller, 2006). The optimization of UAE able to extract lignan and other phenolic compounds such as herbacetin diglucoside, *p*-couramic and ferulic acid glucoside from *Linum usitatissimum* L. seed (Corbin et al., 2015). Additionally, the used of UAE technique optimized the extraction of phenolic content in the extract as compared to conventional maceration technique for fresh olives and mandarin peels (Nipornram et al., 2018; Deng et al., 2017). UAE exhibited more amount of all phenolic compounds including hyroxytyrosol, oleuropein, and rutin than maceration extraction of *Olea europaea* L. (Deng et al., 2017). Hesperidin (flavonoid) and other phenolic compounds in *Citrus reticulata* Blanco cv. Sainampueng peel using UAE showed 1.77 times higher yield than maceration extraction (Nipornram et al., 2018).

Due to its simplicity, ease of handling, cost and time-efficient, and relatively low solvent consumption, UAE is preferred as an alternative to conventional extraction method (Cheng et al., 2015). Furthermore, UAE was capable to reduce the degradation

of phenolics (Luque-García and Luque de Castro, 2003). However, the additional filtration step results in considerably a long time for overall process to complete, which is the major drawback of using this method. Additionally, there is risk of compounds degradation at high frequency (Jahromi, 2019).

2.5.3 Microwave-assisted Extraction (MAE)

MAE uses electromagnetic waves in its heating system, which allows for efficient disruption of plant cell wall and thus accelerates the extraction process. Two MAE systems that have been used to extract phenolic compounds are closed vessel system (high pressure) and open vessel system (atmospheric pressure) (Vlaisavljević et al., 2017). In this method, polar solvent such as MeOH, EtOH and water are commonly used since high polar solvents are able to absorb microwave energy thus increases the speed and efficiency in extraction of phenolic compounds (Sookjitsumran et al., 2016; Fang et al., 2015). Important parameters influencing the extraction efficiency include the solubility, microwave power, dielectric constant, solvent property, and extraction time. (Fang et al., 2015).

It was reported that this technique was more suitable to extract polar phenolic compounds such as oleuropein derivatives, apigenin rutinoside, and luteolin glucoside isomer 3 from Tunisian olive leaves (Taamalli et al., 2012). The yield of total phenolic compounds extracted from *Vaccinium corymbosum* leaves was found to be higher using MAE as compared to solvent extraction and UAE (Routray and Orsat, 2014). Meanwhile, Fang and co-authors (2015) able to identify 13 phenolic compounds including caffeic acid, 5-*O*-caffeoylquinic acid, quercetin-7-*O*-glucoside, 4, 5-dicaffeoylquinic acid, and 3, 5-dicaffeoylquinic acid, from *Eclipta prostrata* by employing 50 % EtOH as solvent, microwave power 400 W, temperature 70 °C, ratio of liquid/solid 30 mL/g, and extraction time of 2 min.

MAE renders high rate of heat transfer during extraction, lowering the use of solvents, as well as extraction time as compared to conventional solvent extraction method (Eskilsson and Bjorklund, 2000). Disadvantages of using MAE include the limited range of chemical compositions it can work on; phenolic compounds with higher number of hydroxyl-type substituents and temperature-sensitive compounds can degrade and oxidize in MAE (Biesaga and Pyrzynska, 2013; Liazid et al., 2007).

2.6 Isolation and Purification of Compounds

Plants extracts can be further fractionated to isolate pure compound which can be done using various chromatography techniques. Chromatography is a technique used to separate individual component or compound in mixture of chemical substances (Roy and Calvin, 2016). All chromatography techniques have two phases, which are stationary phase and mobile phase (Nguyen, 2015). Stationary phase can typically be solid, dense liquid, or bonded coating that stays static in one position; mobile phase or eluent, usually liquid or gas, moves through or across the stationary phase.

2.6.1 Low-pressure Liquid Chromatography (LPLC)

Column chromatography (CC) is commonly used to isolate phenolic compounds (Santos-Buelga et al., 2012). There are few mechanisms in separation that include adsorption, liquid-liquid partition (cellulose), ion exchange, bioaffinity or molecular sieving (Bucar et al., 2013). Phenolic compounds separation is mostly carried out on adsorption: separation involved formation of hydrogen bonds between phenolic proton donors and acceptor groups in stationary phase (resin) and hydrophobic interaction of phenolic compounds (Santos-Buelga et al., 2012). Affinity towards stationary phase is expected to increase with increased content of hydroxyl phenolic groups and aromatic rings (Santos-Buelga et al., 2012). Other factors such as physical properties of the stationary phase (material, surface, particle size, porosity) and conditions of the mobile phase (solvent, pH, temperature), also influence the separation process, which make it hard to predict the adsorption capacity (Santos-Buelga et al., 2012).

Low-pressure liquid chromatography (LPLC) is one of CC techniques that enables the flow of mobile phase at atmospheric pressure without extra forces (vacuum or pressure), which is still a common tool to isolate natural product (Bucar et al., 2013). It is a preferred method due to its simplicity, economical and availability of various stationary phases (Zhang et al., 2005). Silica gel is usually used to isolate natural products as it is inert against labile compounds and commercially available in various forms (Reid and Sarker, 2012). The size of silica gel for LPLC usually ranges from 40 to 200 µm.

The purification of EtOAc extract of young branches with leaves from *Clusia columnaris* by LPLC afforded flavonoid C-glucoside: isovitexin, and six biflavonoids: morelloflavone (**12**) GB1a (**18**), GB2a, GB2a glucoside, volkensiflavone (**33**) and

volkensiflavone glucoside (Compagnone et al., 2008). Meanwhile, the isolation of stem barks and roots of *Pentadesma grandifolia* by CC on silica gel yielded two xanthones: cowagarcinone B and *α*-mangostin, and two biflavanones: 3,8″-binaringenin (GB1a, **18**) and 3,6″-binaringenin (Djoufack et al., 2010). Additionally, *G. dulcis* leaves extract that was subjected to CC on Sephadex LH-20 and silica gel afforded the purification of dulcisbiflavonoid A, a prenylated biflavonoid and other phenolic compounds such as GB2a, amentoflavone, morelloflavone (**12**), morelloflavone-7′-sulfate and volkensiflavone (**33**) (Saelee et al., 2015).

2.7 Structural Elucidation of Isolated Compounds

Individual compounds isolated from a sample matrix are usually subjected to various spectroscopy techniques for structural elucidation purposes. Since phenolic compounds are diverse in structures and chemical properties, different spectroscopy protocols such as mass spectrometer (MS), UV-visible (UV-Vis), infrared (IR), and nuclear magnetic resonance (NMR) are commonly used to characterize the compounds with high precision (Jahromi, 2019; Altemimi et al., 2017; Sarker and Nahar, 2012). Spectroscopy is the study of absorption and emission of light and radiations. The amount of absorption of electromagnetic radiation is measured, from which a spectrum can be produced. The molecular structure can be identified as the spectra produced are specific to certain bonds in a molecule (Altemimi et al., 2017).

2.7.1 Mass Spectrometer (MS)

Electrons or lasers are used to bombard organic molecules in mass spectrometer (MS), which are later changed into high-energy, charged ions. Mass spectrum is a plot of the fragmented ion's relative abundance against the mass/charge ratio of these ions, which provides information on molecular mass, molecular formula, and fragmentation patterns (Sarker and Nahar, 2012; Christophoridou et al., 2005). When tandem MS is applied, information regarding the structure of the compounds can be obtained. Recently, analysis of phenolic compounds using liquid chromatography/mass spectrometry (LC/MS) is widely used. Due to high ionization efficiency of phenolic compounds, electrospray ionization (ESI) is usually a preferred source of electrons (Altemimi et al., 2017).

2.7.2 Ultraviolet-Visible (UV-Vis) Spectroscopy

UV-Vis spectroscopy is another method used for compounds identification. Chemical classification and structural determination by UV-Vis spectroscopy are achieved based on the fact that electronic ground and excited molecular state heavily rely on the number of electrons, their composition and geometry, as well as symmetry (Perkampus, 1992). Electron transfers between orbitals or bands of atoms, ions, and molecules can be studied in the gas, liquid and solid phase (Hunger and Weitkamp, 2001). Through this, information on chromophores that are present in the molecules can be obtained (Sarker and Nahar, 2012). Phenolic compounds are powerful chromophore due to its aromatic structure which can be detected by UV-Vis: flavones at 320 nm, phenolic acids at 360 nm and anthocyanins at 520 nm (Bucar et al., 2013). The ability of UV-Vis spectroscopy to detect individual electron transfer without superimposition by neighboring vibrational bands provides major advantage in chemical compounds identification (Hunger and Weitkamp, 2001).

2.7.3 Infrared (IR) Spectroscopy

IR spectroscopy is vibrational spectroscopy based on interaction of electromagnetic radiation with species with permanent or induced dipole moment and excitation of various vibrational states (Hunger and Weitkamp, 2001). IR spectrometer records electromagnetic radiation energy that is transmitted as a function of the wavenumber or frequency through a sample, allowing detection of functional groups or aromatic molecules present in phenolic compounds (Hunger and Weitkamp, 2001; Sarker and Nahar, 2012). Different vibrational frequencies transmitted from single bond, double bond, triple bond, carboxyl, hydroxyl and amino groups in functional groups can be detected using spectroscopy technique (Jahromi, 2019; Abbas et al., 2017). Currently, non-dispersive spectrometers are widely used, where the total spectrum is examined by an interference process and subjected to Fourier transform as in FTIR spectroscopy, which is translated into frequency or wavenumber (Hunger and Weitkamp, 2001). FTIR is a high-resolution analytical tool for structural elucidation of isolated compounds, which allows for a prompt and non-destructive analysis for chemical fingerprinting of plant extracts or powders (Altemimi et al., 2017).

2.7.4 Nuclear Magnetic Resonance (NMR) Spectroscopy

Another essential tool to analyze and identify phenolic compounds is NMR spectroscopy (Jahromi, 2019; Günther, 2013). The number and types of protons and carbon present in a molecule can be determined using NMR spectroscopy (Sarker and Nahar, 2012). Physical analysis in NMR spectroscopy is carried out based on magnetic properties of the nuclei (Günther, 2013; Atta Ur-Rahman, 1989). Chemical nature of the nucleus is determined by the chemical shift, which happens due to the shielding effect of electron shell (Günther, 2013; Hunger and Weitkamp, 2001). Relative number of nuclei present in a molecule can be determined by measuring the integration of the spectrum, as the area under resonance signal is proportional to number of proton (Günther, 2013).

The most common NMR analysis was by using 1D NMR: ^{1}H NMR and ^{13}C NMR. ^{1}H NMR specifically provides information on the substitution pattern of each ring, while ^{13}C NMR produces data on the total number of carbon atom present in the molecule, as the signals produced show the number of different types of carbon atoms (Negi et al., 2013). It is possible to outline the positional relationship between the nuclei since the spin-spin coupling resulting from magnetic interaction between different nuclei, also known as *J*-coupling, depends on the number and type of bonds separating them (Günther, 2013). Correlation between all parameters in NMR make it possible to determine the structure of unknown chemical constituents.

CHAPTER 3

RESEARCH DESIGN AND METHODOLOGY

3.1 Preparation of Extracts from *G. atroviridis* Roots

Roots of *G. atroviridis* was chosen as its strong biological activities was previously established (Mackeen et al., 2000). Approximately, 3 kg of *G. atroviridis* roots were collected from Maran, Pahang, in 2016. The roots were cleansed and air-dried for two weeks. The dried roots were then chopped into small pieces and ground into powder. The ground roots (1.76 kg) were subjected to extraction process.

3.1.1 Solvent Extraction

The ground roots of *G. atroviridis* (1.76 kg) were extracted three times in 1L MeOH, by soaking the root powder for three days at room temperature each time. The extract was filtered by using a sieve and the filtrate was concentrated using rotary evaporator to remove solvent under reduced pressure at temperature below 45 °C, which yielded 45.45 g (2.58 %) of gum-like brown residue. The MeOH extract was suspended in 1 L H_2O and successively extracted for three times with 1 L of different organic solvents, starting with lipophilic solvent *n*-hexane, DCM, EtOAc to a more hydrophilic solvent, *n*-butanol. The extracts of *n*-hexane (8.72 g, 0.5 %), DCM (4.45 g, 0.25 %, EtOAc (9.79 g, 0.56 %) and *n*-butanol (1.08 g, 0.06 %), were filtered and concentrated under reduced pressure using rotary evaporator to yield respective residue of the extracts (Figure 3.1). All extracts were stored at 4 °C until further analysis.

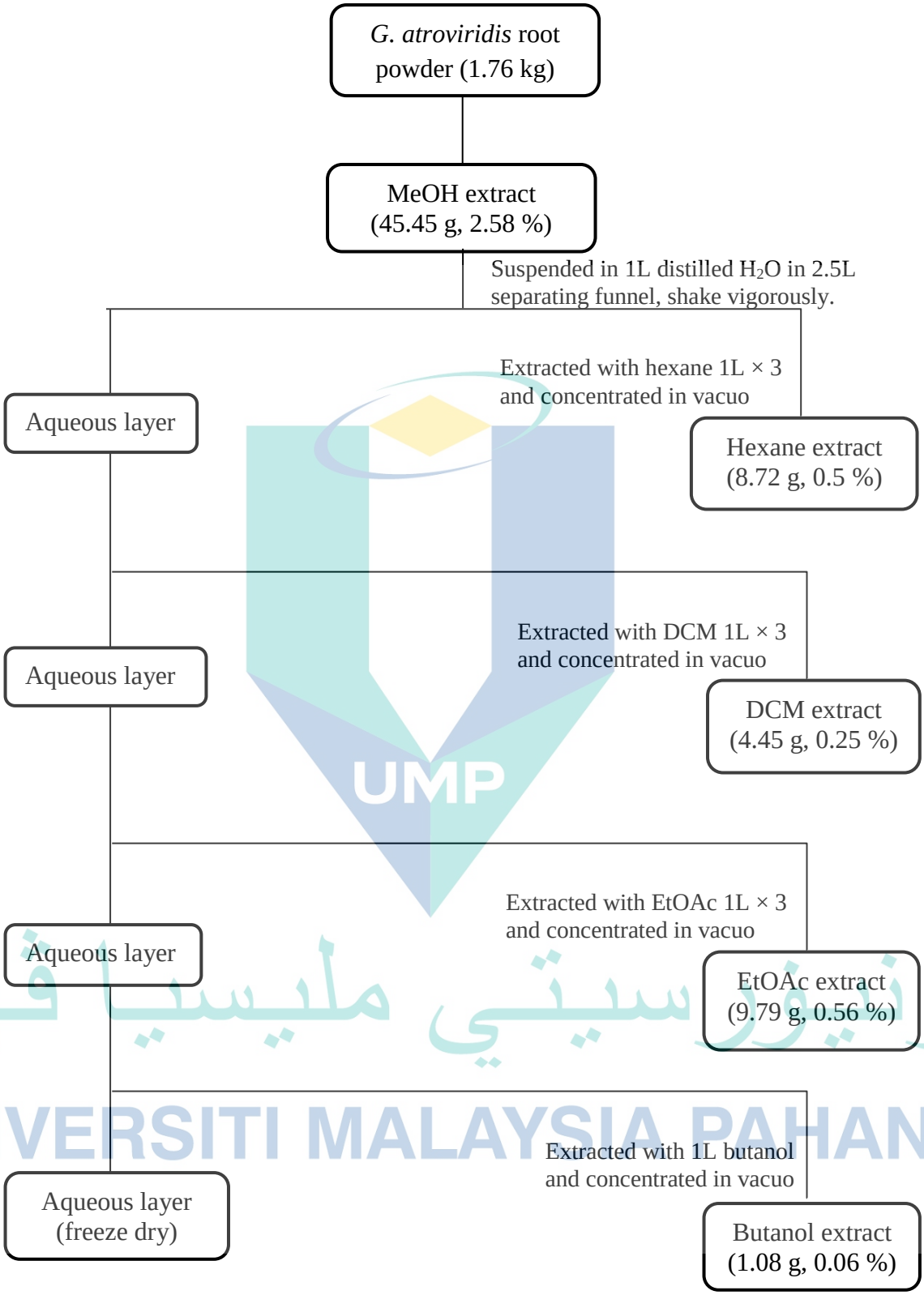


Figure 3.1 Solvent extraction scheme

3.2 Isolation of Compounds from EtOAc Extract

Solvent extraction of *G. atroviridis* roots yielded 8.72 g (0.5 %) of hexane, 4.45 g (0.25 %) of DCM, 9.79 g (0.56 %) of EtOAc, 1.08 g (0.06 %) of butanol and 45.45 g (2.58 %) of MeOH extracts, of which only the selected extract was subjected to isolation and purification process. The desired bioactive compounds in this study, phenolic compounds of intermediate polarity such as isoflavones, flavanones, flavones and flavonols were extracted efficiently in EtOAc solvent (Susanto et al., 2019; Santos-Buelga et al., 2012; Chung et al., 1999). Various bioactive compounds were also recovered in EtOAc root extract of *G. atroviridis* as reported by Permana et al. (2005; 2001). Thus, the brownish, gum-like EtOAc extract was further subjected to isolation process to isolate individual compounds.

3.2.1 Silica gel Column Chromatography (Si gel CC)

The EtOAc extract of *G. atroviridis* root was subjected to polyamide or Si gel CC, and eluted with solvents, in order to purify the compound that present in the sample. The first step in preparing Si gel CC was by selecting the suitable column of various sizes depending on the amount of materials. The wet-column packing was used. Cotton was put at the bottom of the column to avoid silica gel from leaking out. Silica gel was soaked in *n*-hexane and poured into the column. The silica gel was allowed to settle at the bottom of the column by opening the stopcock (Braithwaite and Smith, 1999; Marconi, 1975). The steps were repeated until 1/3 of the column was filled with silica gel. The column was packed as tightly as possible to ensure no bubbles were trapped in silica gel.

The EtOAc extract was prepared by slurry technique. EtOAc extract was diluted with acetone, mix with silica gel and the mixture was let to dry. The slurry sample was added into the packed column. The column was run by allowing the selected solvent system to pass through. Suitable solvent system was pre-determined via TLC. Approximately, 5 mL of eluate were collected in vials. The fractions collected was monitored by TLC and the vials containing similar TLC profiles were combined to be re-chromatographed or subjected to PTLC to purify individual compounds.

Initially, the EtOAc extract (9.79 g, 0.56 %) was chromatographed over silica gel (0.040-0.060 mm, Merck, 5 × 15 cm). The extract was successively eluted with different ratio of acetone/ *n*-hexane with increasing polarity: 0 : 100, 10 : 90, 20 : 80, 25 : 75, 30 :

70, 35 : 65, 40 : 60, 50 : 50, 60 : 40, 70 : 30, 80 : 20, 90 :10 to 100 : 0; the extract was finally eluted with MeOH to give twenty-eight fractions (1A-28A, 150 mL). Fraction 10A were re-chromatographed on Si gel column (0.040-0.060 mm, Merck, 2.5 × 15 cm) using solvent of increasing polarity, with acetone/ *n*-hexane and MeOH as eluent to give 92 fractions (1B-92B, 450 mL). Fractions 26B-35B (10 mg, 0.57×10^{-3} %) were pooled based on similarity in TLC profiles. The pooled fractions were then subjected to PTLC, which yielded 7.1 mg (0.4×10^{-3} %) of brown amorphous solid that later identified as compound **7**.

Fraction 11A from initial column were re-chromatographed over silica gel (0.040-0.060 mm, Merck, 2.5 × 15 cm) and eluted using acetone/ *n*-hexane in gradient system and finally with MeOH to give out a total of 123 fractions (1C-123C, 600 mL). The combined fractions of 47C-70C (20 mg, 1.14×10^{-3} %) were washed with chloroform. Yellow amorphous powder of 15.4 mg (0.88×10^{-3} %) was obtained, which was later identified as compound **29** based on its spectral data.

The combined fractions of 17A and 18A were re-chromatographed (0.040-0.060 mm, Merck, 2.5 × 15 cm). The fractions were eluted with isocratic solvent system, acetone/ *n*-hexane (15 : 85) before final elution with MeOH to give out a total of 16 fractions (1D-16D, 80 mL), of which fractions 8D-12D were pooled. The combined fractions were subsequently chromatographed on silica gel (0.040-0.060 mm, Merck, 2.5 × 15 cm) and eluted with increasing polarity of EtOAc/ *n*-hexane, and MeOH, to obtain 300 fractions (1E-300E, 1.5 L). Fractions 43E-73E (25 mg, 1.42×10^{-3} %) were pooled and subjected to PTLC, which yielded 17.3 mg (0.98×10^{-3} %) of brown amorphous solid that later identified as compound **18**. PTLC of pooled 112E-116E (24 mg, 1.36×10^{-3} %) fractions yielded 17.4 mg (0.99×10^{-3} %) of yellow amorphous solid, which later characterized as compound **33**, a new compound isolated from this species.

Fraction 19A was re-chromatographed (0.040-0.060 mm, Merck, 2.5 × 15 cm) and eluted with increasing polarity of EtOAc/ *n*-hexane that gave out 305 fractions (1F-305F, 1.5 L). The combined fractions of 118F-300F (22 mg, 1.25×10^{-3} %) were then subjected to PTLC to obtain 15 mg (0.85×10^{-3} %) of yellow amorphous solid, which was later identified as compound **12**. Flowchart of the isolation process was outlined in Appendix A.

Atrovirisidone (**7**): brown amorphous solid (7.1 mg, 0.4 × 10^{-3} %); ESI-LCMS *m/z* 427 ([M]$^+$ calcd for $C_{24}H_{26}O_7$, 426); UV-Vis λ_{max} 333 nm (2.547); IR (KBr) ν_{max} = 3466, 3419, and 1635 cm^{-1}; ^1H and ^{13}C NMR, see Table 4.1.

Morelloflavone, (**12**): yellow amorphous solid (15 mg, 0.85 × 10^{-3} %); ESI-LCMS *m/z* 557 ([M]$^+$ calcd for $C_{30}H_{20}O_{11}$, 556); UV-Vis λ_{max} 345 nm (3.422); IR (KBr) ν_{max} = 3420 and 1634 cm^{-1}; ^1H and ^{13}C NMR, see Table 4.2.

GB1a (**18**): brown amorphous solid (17.3 mg, 0.98 × 10^{-3} %); ESI-LCMS *m/z* 543 ([M]$^+$ calcd for $C_{30}H_{22}O_{10,}$ 542); UV-Vis λ_{max} 331 nm (1.767); IR (KBr) ν_{max} = 3458 and 1638 cm^{-1}; ^1H and ^{13}C NMR, see Table 4.3.

1,3,5- Trihydroxy-2-methoxyxanthone (**29**): yellow amorphous powder (15.4 mg, 0.88 × 10^{-3} %); ESI-LCMS *m/z* 275 ([M]$^+$ calcd for $C_{14}H_{10}O_{6,}$ 274); UV-Vis λ_{max} 311 nm (3.305); IR (KBr) ν_{max} = 3455 and 1636 cm^{-1}; ^1H and ^{13}C NMR, see Table 4.4.

Volkensiflavone (**33**): yellow amorphous solid (17.4 mg, 0.99 × 10^{-3} %); ESI-LCMS *m/z* 541 ([M]$^+$ calcd for $C_{30}H_{20}O_{10}$, 540); UV-Vis λ_{max} 340 nm (3.403); IR (KBr) ν_{max} = 3451, 1641 and 1097 cm^{-1}; ^1H and ^{13}C NMR, see Table 4.5.

3.2.2 Preparative Thin Layer Chromatography (PTLC)

PTLC was carried out to eliminate impurities or to separate compounds that are harder to separate using Si gel CC. Combined fractions of similar TLC profiles (26B-35B, 43E-73E, 112E-116E and 118F-300F) were subjected to PTLC. The concentrated extracts were dissolved in approximately 2 to 5 mL of acetone. The dissolved fractions were streaked onto silica plate (10 cm × 10 cm) using capillary tube. The fractions were applied uniformly and in narrow band to obtain good resolution of the compounds separation (Hostettmann et al., 1998). The edge of the plate was immersed in solvent system, EtOAc/ *n*-hexane in the TLC tank. The plate was dried after the development was complete. The plate was observed under UV light (254 nm and 365 nm) and the targeted band was marked. The marked compound was scraped off using spatula and dissolved in acetone. This is an important step to remove the compound that binds to the silica. It was then filtered to remove the silica and collect the compound. TLC was performed to verify purity of the compound, by which only one spot on the TLC plate should be observed under UV light.

3.2.3 Thin Layer Chromatography (TLC)

The aluminum coated with silica was cut accordingly. The baseline of 1.0 cm from the bottom and the solvent front of 1.0 cm from the top of the TLC plate was marked with pencil. Approximately, 2 to 5 mg of fractions were diluted with 5 mL of acetone and spotted on the baseline of the TLC plate using capillary tube. The plate was placed in the TLC tank with selected solvent system. Once the spot reached the solvent front, the plate was removed. The band appeared under the UV light (254 nm and 365 nm) was marked with pencil. The TLC plate was then sprayed with spraying reagent (diluted sulfuric acid) before heated on the hot plate for few minutes, or until the color of the compounds appeared. TLC was also used to monitor the purification process of compounds as the collected fractions from Si gel CC were combined according to similar profiles observed on TLC plate. The fractions were re-chromatographed or purified using PTLC until only one spot was observed on TLC plate.

3.3 Structural Elucidation of Compounds Isolated from EtOAc Extract

The isolated compounds were subjected to several spectroscopy techniques for structural elucidation purposes. The spectral data was analyzed and the comparison with established data was used to identify the compounds.

3.3.1 Liquid Chromatography Quadrupole Time-Of-Flight Mass Spectroscopy (LC-Q/TOF-MS) Analysis

The MS analysis of isolated compounds was carried out using LC-Q/TOF-MS at Central Laboratory, Universiti Malaysia Pahang. The high resolution mass spectra were measured on Vion IMS QTOF Mass Spectrometer (Waters Corp.) equipped with UPLC system, ACQUITY UPLC I-Class System and QTOF detector. Separation was done on ACQUITY UPLC BEH C18 (2.1 × 50 mm, 1.7 µm) column. Mobile phase composed of water + 0.1 % formic acid (solvent A) and acetonitrile (solvent B) was used and the flow rate was set to 0.6 mL/min at 40 °C. The initial mobile phase, 99 % of solvent A and 1 % of solvent B was held for 5 min, followed by 65 % of A at 16 min, 0 % of A at 18 min and was reset to 99 % of A at 20 min. Scans were performed in positive mode and injection volume was 20 µL. The software used for data acquisition, processing and reporting was UNIFI Scientific Information System with Traditional Library (Waters Corp.).

3.3.2 Ultraviolet-Visible Spectrometer (UV-Vis) Analysis

The isolated compound was quantitatively determined using Genesys 10S UV-Vis spectrophotometer (ThermoFisher Scientific). The wavelength was set from 200 to 700 nm. Approximately, 1 mg of the isolated compounds was diluted in 5 mL acetone and was transferred to the cuvette. Acetone was used as a blank and the absorbance of the compounds was read.

3.3.3 Fourier-Transform Infrared Spectrometer (FTIR) Analysis

Potassium bromide (KBr) discs of the compounds were prepared for IR analysis. Approximately, 1 mg of compounds was added to 5 mg of KBr. The mixture was ground into fine powder. The powder was compressed to form thin pellet, which was then placed in the instrument. Spectrum 100 FTIR Spectrometer by PerkinElmer at Faculty of Industrial Sciences and Technology Laboratory, Universiti Malaysia Pahang was used to obtain the spectrum. The infrared spectrum of the sample was taken from 400 to 4000 cm^{-1}. The band observed or the wavenumber of the functional group present in the compounds was obtained from the literature.

3.3.4 Nuclear Magnetic Resonance Spectrometer (NMR) Analysis

Bruker Ultra Shield Plus instrument was used to obtain NMR spectra of morelloflavone (**12**) and GB1a (**18**) at 500 MHz in ^{1}H NMR, and 125 MHz in ^{13}C NMR. NMR experiment of atrovirisidone (**7**), 1,3,5- trihydroxy-2-methoxyxantone (**29**), and volkensiflavone (**33**), was performed by using JEOL instrument at 600 MHz in ^{1}H NMR and 150 MHz in ^{13}C NMR. All samples were prepared in deuterated acetone (Acetone-d_6). Chemical shifts (δ) was expressed in ppm and coupling constant (J) in Hz. Coupling constant (J) was described with abbreviations: singlet, s; doublet, d; triplet, t; doublet of doublet, dd.

3.4 Biological Activities of *G. atroviridis* Roots

Preliminary screening of antibacterial and antioxidant activities of EtOAc extract was conducted. Following the positive results of EtOAc extract, bioactivities of its isolates namely morelloflavone (**12**), GB1a (**18**), 1,3,5- trihydroxy-2-methoxyxantone (**29**), and one new biflavonoid isolated from this species, volkensiflavone (**33**) was further

evaluated. Bioactivities of atrovirisidone (**7**) could not be tested due to limited yield. Additionally, preliminary biological studies on root extracts of *G. atroviridis* in different solvents with different polarity namely hexane, DCM, butanol and MeOH were investigated using the same protocol.

3.4.1 Antibacterial Assay of Plant Samples

3.4.1.1 Microorganism

The procedure in antibacterial assay was carried out according to Mackeen et al., (2000). The bacterial cultures were maintained on nutrient agar (NA) (Oxoid, UK) slant and stored at 4 °C. Four bacterial strains i.e. *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 10536) and *Proteus vulgaris* (ATCC 33420) were obtained from Faculty of Industrial Sciences and Technology Laboratory, Universiti Malaysia Pahang and used in antibacterial assay.

The microorganisms were cultured in nutrient broth and incubated in incubator shaker at 30 °C overnight. The concentration of the cultures were adjusted to 10^8 colony forming units per milliliter (CFU/mL) and read at a wavelength of 600 nm.

3.4.2 Disc Diffusion Method

Petri plates containing NA were inoculated with bacteria culture (10^8 CFU/mL). 15 µl of EtOAc extract with a concentration of 20 mg/mL was loaded onto filter paper discs (diameter: 6 mm) and the discs were placed on the inoculated agar. The plates were inverted and incubated for 24 hours at 30 °C. The zone of inhibition was measured by using a ruler. Tests were performed in triplicate (n=3) and the average measurements were calculated. Chloramphenicol (30 µg/disc) and gentamicin (120 µg/disc) were used as positive controls, while acetone (15 µl) and hexane (15 µl) were used as the negative control. Antibacterial activity of isolated compounds and the extracts of different polarity (hexane, DCM, butanol and MeOH) were also evaluated in the same manner.

3.4.3 Antioxidant Assay of Plant Samples

3.4.3.1 Determination of Total Phenolic Content (TPC) using Folin–Ciocalteu (FC) Method

The TPC of EtOAc, hexane, DCM, butanol and MeOH root extracts was determined according to the method described by Wang and co-authors (2011) with some modifications to suit the use of microplate technique (Li et al., 2012). In this assay, the calibration curve of gallic acid (GA) was first established. The curve was obtained by using different concentration of GA ranging from 5 to 40 µg/mL (3.1). 125 µL FC reagent (diluted at 1:10 with distilled water) was added to 25 µL of gallic acid with different concentration or root extracts in 96-well microplates. The mixtures were left to react for 10 min at room temperature. 125 µL of 7.5 % sodium carbonate (Na_2CO_3) solution was then added to the mixture and was allowed to stand at room temperature for 30 min. The absorbance of the reaction mixture was read at 765 nm using TECAN infinite M200 PRO microplate reader. Methanol was used instead of root extracts for blank. The test was done in triplicate. The calculation was made based on the value obtained from the calibration curve of GA as shown in 3.1.

$$y = 0.0035x - 0.0166,\ R^2 = 0.9852 \qquad 3.1$$

The TPC in all samples was calculated as described in 3.2 (Genwali et al., 2013). The results were expressed as milligram gallic acid equivalent/ gram dry weight (mg GAE/g).

$$C = c\ V/m \qquad 3.2$$

where,
C = total phenolic content mg GAE/g dry extract
c = concentration obtained from GA calibration curve in mg/mL
V = volume of extract in mL
m = mass of extract in gram

3.4.3.2 *α, α*-diphenyl-*β*-picrylhydrazyl (DPPH) radical scavenging Method

The scavenging activity of samples on DPPH radical was tested according to the method by Hui et al. (2017), with slight modification. 96-well microplate was used in this assay. DPPH stock solution of 0.2 mM was prepared. DPPH solution was kept from the light throughout the experiment. Ascorbic acid (AA) was used as a standard. AA, *G. atroviridis* root extracts and the isolated compounds from EtOAc extract were dissolved in analytical grade MeOH with initial concentration of 1 mg/mL. Serial dilutions were performed; the wells were occupied with 100 µL of AA, root extracts or isolates of different concentrations ranging from 7.8125 to 1000 µg/mL. Next, 100 µL of 0.2 mM DPPH was added to the wells.

The negative control containing MeOH instead of tested samples was used. The microplate was incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using TECAN infinite M200 PRO microplate reader. Experiment was done in triplicate (*n*=3). The ability to scavenge the DPPH radical was calculated by using 3.3.

$$\frac{Abs\ (C - S)}{Abs\ C} \times 100 \qquad 3.3$$

where,

Abs C= Absorbance of control at *t*, 30 minutes

Abs S= Absorbance of sample *t*, 30 minutes

Percentage of radical scavenging activity was plotted against the sample concentration to obtain the IC_{50} value. The lower the IC_{50} value, the higher the antioxidant potential of the sample (Jadid et al., 2017; Muthumperumal et al., 2016; Molyneux, 2004).

3.4.3.3 Ferric Reducing Antioxidant Power (FRAP) Method

Microplate analysis of FRAP assay was conducted according to procedure published in Henderson et al. (2015). AA was used as a standard and calibration curve was constructed by using AA with different concentrations ranging from 5 to 40 µg/mL. FRAP reagent was prepared freshly by mixing 10 mM 2,4,6-tris[2-pyridyl)-s-triazine (TPTZ), 20 mM ferric chloride and 300 mM sodium acetate buffer (pH 3.6) at a ratio of 1:1:10 (Benzie and Strain, 1996). The mixture was then heated at 37 °C in water bath for 10 min. Next, 20 µL of AA, *G. atroviridis* root extracts and isolated compounds from EtOAc extract with varying concentrations were added to the respective well in flat-bottomed 96-well microplates. This was followed by addition of 280 µl of FRAP reagent. The blank composed of MeOH instead of tested sample was used. The reaction mixtures were incubated in the dark at 37 °C for 30 min. The absorbance was read at 593 nm using TECAN infinite M200 PRO microplate reader. The test were conducted in three replicates (n=3). The FRAP values were reported as milligram of ascorbic acid equivalents (AAE) per gram of sample (mg AAE/g), which was calculated using the value obtained from the calibration curve as shown in 3.4.

$$y = 0.0142x - 0.0209,\ R^2 = 0.9828 \qquad 3.4$$

where,

y = the absorbance at 593 nm

x = the amount of ascorbic acid equivalent

Statistical Analysis

All tests were conducted at least three times (n=3). The data was expressed as mean ± standard deviation (SD) using Microsoft Excel 2016. GraphPad Prism version 7 was used to compute IC_{50} and Microsoft Excel 2016 was used to obtain the standard curve equation and calculate the Pearson's correlation coefficient.

3.5 Flowchart of Research Activity

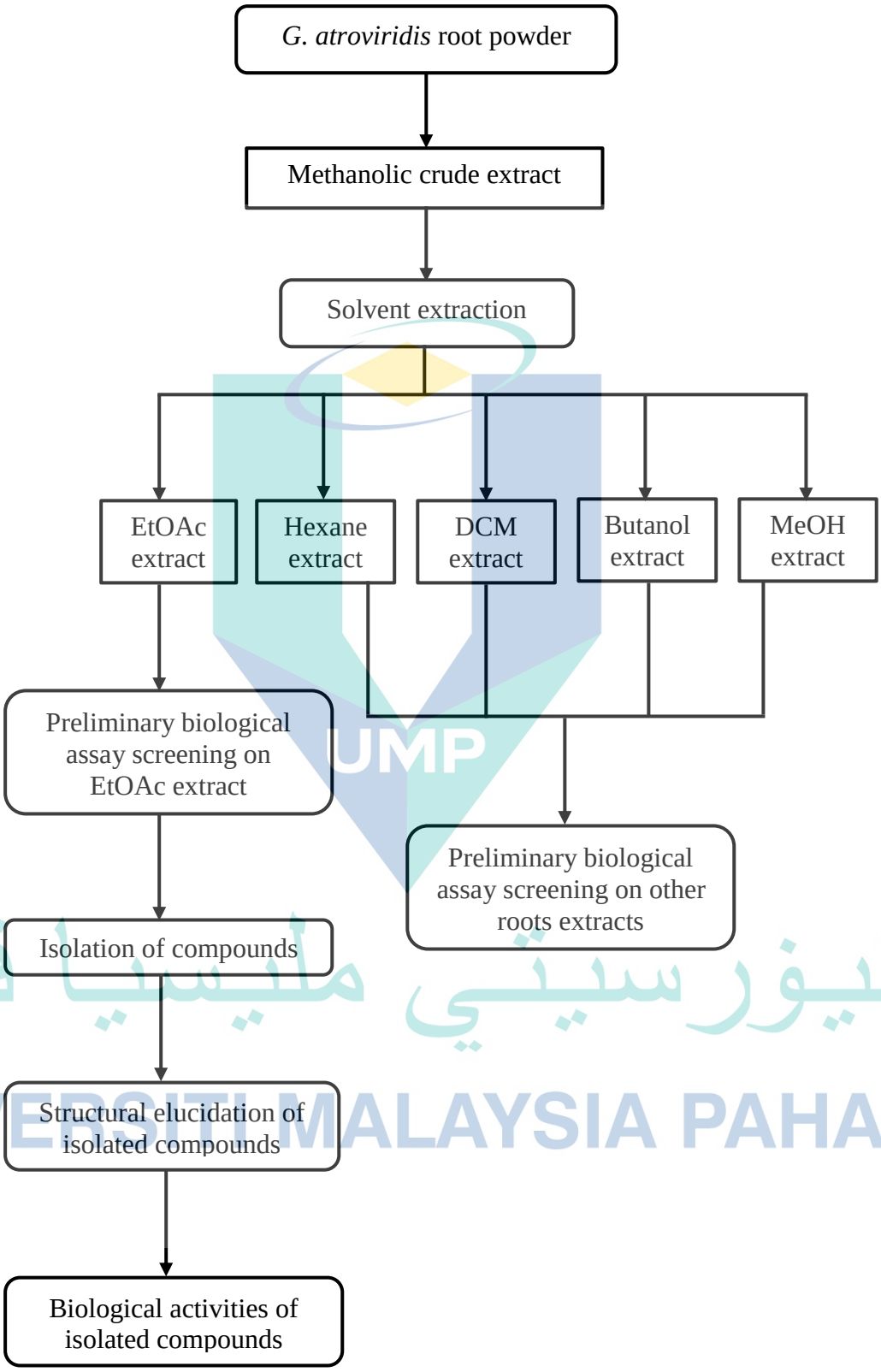


Figure 3.2 Flow chart of research activity

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This study was conducted based on the preliminary biological study of *G. atroviridis* reported by Mackeen et al. (2000), which found that extract from the roots of *G. atroviridis* exhibited strong antibacterial and antioxidant activities. The finding was further supported by Permana et al. (2005; 2001), who reported on the strong bioactivities of EtOAc root extract of *G. atroviridis* which could be contributed by its phenolic contents.

The study began with solvent extraction from the root of *G. atroviridis* using solvent with different polarity namely hexane, DCM, EtOAc, butanol and MeOH. Since EtOAc; the solvents have been reported effective for isolation of phenolic compounds with intermediate polarity (Susanto et al., 2019; Santos-Buelga et al., 2012; Chung et al., 1999), the study reported in this thesis focused on EtOAc extract. Preliminary screening revealed positive antibacterial and antioxidant activities in the extract, which agreed with the previous report.

To identify the chemical constituents contributing to bioactivities, isolation of individual compounds from EtOAc root extract of *G. atroviridis* was carried out. Further investigation found that the positive bioactivity in the extract might have been due to the presence of phenolic compounds, which were successfully isolated and elucidated in this study. In addition, among all extracts, butanol extract exhibited the strongest antibacterial and antioxidant activities.

4.2 Extraction and Preliminary Biological Assay Screening of EtOAc Extract from *G. atroviridis* Roots

Extracts from the root powder of *G. atroviridis* obtained using solvents with different polarity namely, hexane, DCM, EtOAc, butanol, and MeOH yielded 8.72 g (0.5 %), 4.45 g (0.25 %), 9.79 g, (0.56 %), 1.08 g (0.06 %), and 45.45 g (2.58 %) of extracts

respectively. EtOAc extract, which was the focus of this study was subjected to preliminary biological assay screening for further investigation on antibacterial and antioxidant activities.

4.2.1 Antibacterial Activity

Disc diffusion method was used to investigate the antibacterial activity of EtOAc root extract of *G. atroviridis*. A total of four microorganisms consists of two Gram-positive bacteria: *B. cereus* and *S. aureus*; and two Gram-negative bacteria: *E. coli* and *P. vulgaris*, were used to evaluate antibacterial activity of EtOAc extract. Disc diffusion is a qualitative method used for observation of antibacterial activity in the tested sample. The blank disc with a diameter of 6 mm was filled with 20 mg/mL of sample, diluted using acetone. Hence, acetone was used as the negative control. The zone of inhibition was determined after incubation at 37 °C for 24 hours. The results demonstrated that EtOAc root extract of *G. atroviridis* showed positive antibacterial activity with inhibition zone of 7 mm against Gram-positive bacteria, *B. cereus* and *S. aureus*. However, the antibacterial activity of this extract was lower compared to commercial antibiotics (positive control) which displayed inhibition zone more than 18 mm at lower concentration. This extract did not inhibit the growth of Gram-negative bacteria (Table 4.6).

4.2.2 Antioxidant Activity

4.2.2.1 TPC via FC Reagent Assay

In this method, the reaction was activated by the presence of phenolic compounds under alkaline condition, causing the reduction of FC reagent which was characterized by the intensity of the blue pigment produced in the solution (Huang et al., 2005). The TPC of EtOAc was calculated based on the equation obtained from the gallic acid (GA) standard curve ($y = 0.0035x - 0.0166$) with concentration ranging from 5 to 40 µg/mL. The TPC of the extract was expressed in milligram gallic acid equivalent per gram of dry weight sample (mg GAE/g).

TPC value of EtOAc root extract of *G. atroviridis* was 568.6 ± 25.724 mg GAE/g, as tabulated in Table 4.11. Due to the significant phenolic content in EtOAc extract, antioxidant activity in the extract was further evaluated via DPPH and FRAP assay. The

presence of phenolic compounds in EtOAc extract was later verified by the isolation of morelloflavone (**12**), GB1a (**18**), 1,3,5- trihydroxy-2-methoxyxantone (**29**), and volkensiflavone (**33**), which belong to different classes of phenolic compounds.

4.2.2.2 DPPH Assay

DPPH assay provides data on interaction of tested sample with free radicals, which correlates with the capacity of the sample to donate radical hydrogen (Younes et al., 2015). Reduction reaction causes the color to change from violet to yellow. The degree of changes in color can be measured using spectrophotometer, enabling the determination of percentage of activity and the corresponded IC_{50} value (Prior et al., 2005). A low IC_{50} value indicates high radical scavenging activity in the tested sample as it only requires low amount of sample to reduce DPPH.

The results showed that as the concentration of EtOAc increased, the antioxidant activity in the extract also increased, with the exception of minor inconsistencies as displayed in Table 4.7. EtOAc extract showed moderate free radical scavenging activity with IC_{50} of 51.7 ± 2.180 µg/mL as compared to ascorbic acid (AA) with IC_{50} value of 13.2 ± 0.021 µg/mL. The IC_{50} data was tabulated in Table 4.8.

4.2.2.3 FRAP Assay

Since antioxidants are usually involved in several mechanism of actions, it is necessary to evaluate the antioxidant activity using multiple assays (Antolovich et al., 2002). Methods namely DPPH and FRAP assays were used in this study to estimate antioxidant capacity of the extract. In this assay, the antioxidants cause the reduction of a ferroin analog, the Fe^{3+} complex of tripyridyltriazine $Fe(TPTZ)^{3+}$ to Fe^{2+} complex $Fe(TPTZ)^{2+}$ under acidic condition to maintain iron solubility, turning the reaction solution into blue (Prior et al., 2005; Antolovich et al., 2002). Different concentrations of AA ranging from 5 to 40 µg/mL were used to generate a standard curve. Based on the standard curve equation ($y = 0.0142x - 0.0209$), the FRAP values of the sample were calculated and the results were expressed as milligram of ascorbic acid equivalents per gram of dry weight of respective samples (mg AAE/g). EtOAc root extract of *G. atroviridis* exhibited moderate FRAP activity, 294.0 ± 20.173 mg AAE/g, as tabulated in the Table 4.9.

4.3 Isolation and Structural Elucidation of Compounds Isolated from EtOAc Extract

Following the positive antibacterial and antioxidant activities observed in EtOAc root extract of *G. atroviridis*, isolation of the chemical constituents that may be responsible for bioactivities in the extract was carried out. Si gel CC, PTLC, and TLC techniques were used to purify the individual compounds.

The isolation procedures yielded 7.1 mg (0.4×10^{-3} %), 15 mg (0.85×10^{-3} %), 17.3 mg (0.98×10^{-3} %), 15.4 mg (0.88×10^{-3} %), and 17.4 mg (0.99×10^{-3} %) of compounds identified as atrovirisidone (**7**), morelloflavone (**12**), GB1a (**18**), 1,3,5-trihydroxy-2-methoxyxantone (**29**), and one new compound isolated for the first time in *G. atroviridis*, volkensiflavone (**33**), respectively.

Characterization of the compounds isolated from EtOAc root extract of *G. atroviridis* were carried out using spectroscopy techniques namely LC-Q/TOF-MS, UV-Vis, FTIR and NMR (^{1}H-NMR and ^{13}C-NMR), which are further elaborated in the following section. All compounds obtained were identified as polyphenols, of which atrovirisidone (**7**) is a depsidones, morelloflavone (**12**) and volkensiflavone (**33**) were biflavonoids with flavanone and flavone units, GB1a (**18**) were identified as biflavonoid with two flavanone units, whereas 1,3,5- trihydroxy-2-methoxyxantone (**29**) as xanthones. This finding verified the effectiveness of EtOAc as solvent for recovery of phenolic compounds from the plant.

4.3.1 Characterization of Atrovirisidone

Compound **7** was obtained as brown amorphous solid. The molecular formula was determined as $C_{24}H_{26}O_7$ (426) by using ESI-LCMS, which showed a $[M+1]^+$ peak at *m/z* 427. The maximum absorption of UV spectrum was at λ_{max} 333 nm. Mass spectrum and the UV absorption of **7** was similar to the novel depsidone, garcinisidone A, which information was useful for structural elucidation (Ito et al., 1997). The IR spectrum showed strong absorption at ν_{max} 3466 cm^{-1} and ν_{max} 3419 cm^{-1}, indicating the presence of hydroxyl group, while absorbance at ν_{max} 1635 cm^{-1} indicated the presence of lactone carbonyl group.

The NMR spectrum was tabulated in Table 4.1. From ^{1}H NMR spectrum, aromatic protons resonated at δ 6.46 (1H, d, 2.34 Hz) (H- 4) and δ 6.30 (1H, d, 2.4 Hz) (H- 2), suggesting the *meta*-substitution pattern in ring A. The singlet at δ 4.00 (3H, s) in ring B representing the three protons as metoxyl groups. Meanwhile, the presence of two set of signals at δ 3.46 (2H, d, J= 6.6 Hz), δ 5.07 (1H, t), δ 1.82 (3H, s), δ 1.68 (3H, s) and at δ 3.39 (2H, d, J= 6.6 Hz), δ 5.07 (1H, t), δ 1.75 (3H, s), δ 1.65, (3H, s) indicated the presence of prenyl side chains, located in adjacent to carbons in ring B. Although hydroxyl protons were not visible in ^{1}H NMR spectrum, the presence of this hydroxyl group was detected in FTIR. The ^{1}H NMR spectrum was displayed in Figure 4.2.

The expected 24 carbon signals were observed in ^{13}C NMR spectrum (Figure 4.3). Carbonyl lactone at δ 168.6 (C- 11) was identified. Hydroxyl group at C- 1 and C- 3 in ring A was also detected as the signals appeared at δ 166.45 and δ 166.7, respectively. The oxygen bearing carbon was detected at δ 147.0 (C- 7, -OH), δ 142.5 (C- 5a, -O-), δ 138.7 (C- 6, -O-), and δ 137.9 (C- 9a, -O-). Based on the study and comparison with spectral data of garcinisidone A-D (Ito et al., 2001; 1997), Permana and co-authors (2001) interpreted that the linkage between ring A and ring B as depsidone. Present study showed similar spectrum hence the structure was determined to be atrovirisidone (**7**) (Permana et al., 2001) (Figure 4.1).

Atrovirisidone (**7**) is a depsidone that is rarely reported in higher plants although it has been commonly isolated from lichens and fungi. Previous study showed that this compound exhibited moderate antibacterial activity against Gram-positive such as *S. aureus* and *B. cereus* (Permana et al., 2001).

Table 4.1 ^{1}H NMR (600 MHz, Acetone-d_6) and ^{13}C NMR (150 MHz, Acetone- d_6) of **7**

Position	**^{1}H (δ_H in ppm)**		**^{13}C (δ_C in ppm)**	
	Obtained reading	Ref reading	Obtained reading	Ref reading
1			166.45	166.6
2	6.30 (1H, d, J= 2.4 Hz)	6.18 (1H, d, J= 2.4 Hz)	100.98	101.1
3			166.1	166.7
4	6.46 (1H, d, J= 2.34 Hz)	6.31 (1H, d, J= 2.4 Hz)	101.4	101.5
4a			162.8	163.5
5a			142.5	143.0
6			138.7	138.4
CH_3O-6	4.00 (3H, s)	3.94 (3H, s)	62.8	62.9
7			147.0	147.5
8			128.5	129.0
9			131.4	126.0
9a			137.9	137.3
11			168.6	169.2
11a			98.8	99.0
1′	3.39 (2H, d, J= 6.6 Hz)	3.32 (2H, d, J= 6.4 Hz)	26.2	26.2
2′	5.07 (1H, t)	5.00 (1H, m)	123.9	124.0
3′			131.4	132.2
CH_3-4′k	1.75 (3H, s)	1.71 (3H, s)	25.8	25.8
CH_3-5′k	1.65 (3H, s)	1.64 (3H, s)	18.2	18.0
1″	3.46 (2H, d, J= 6.6 Hz)	3.39 (2H, d, J= 6.4 Hz)	25.9	26.5
2″	5.07 (1H, t)	4.96 (1H, m)	123.1	123.6
3″			131.9	132.9
CH_3-4″l	1.82 (3H, s)	1.76 (3H, s)	25.7	25.9
CH_3-5″l	1.68 (3H, s)	1.64 (3H, s)	18.0	18.3

Ref: reference; Permana et al., (2001)

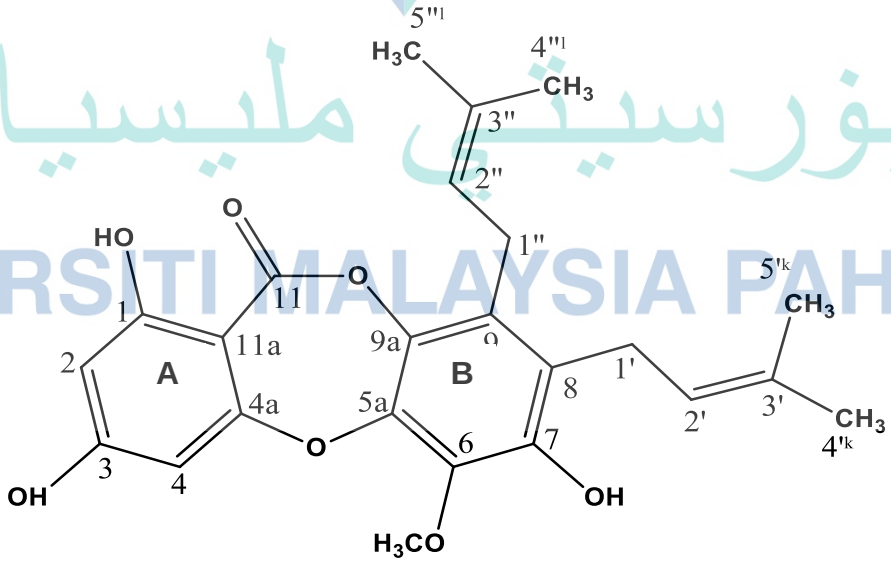


Figure 4.1 Structure of atrovirisidone (**7**)

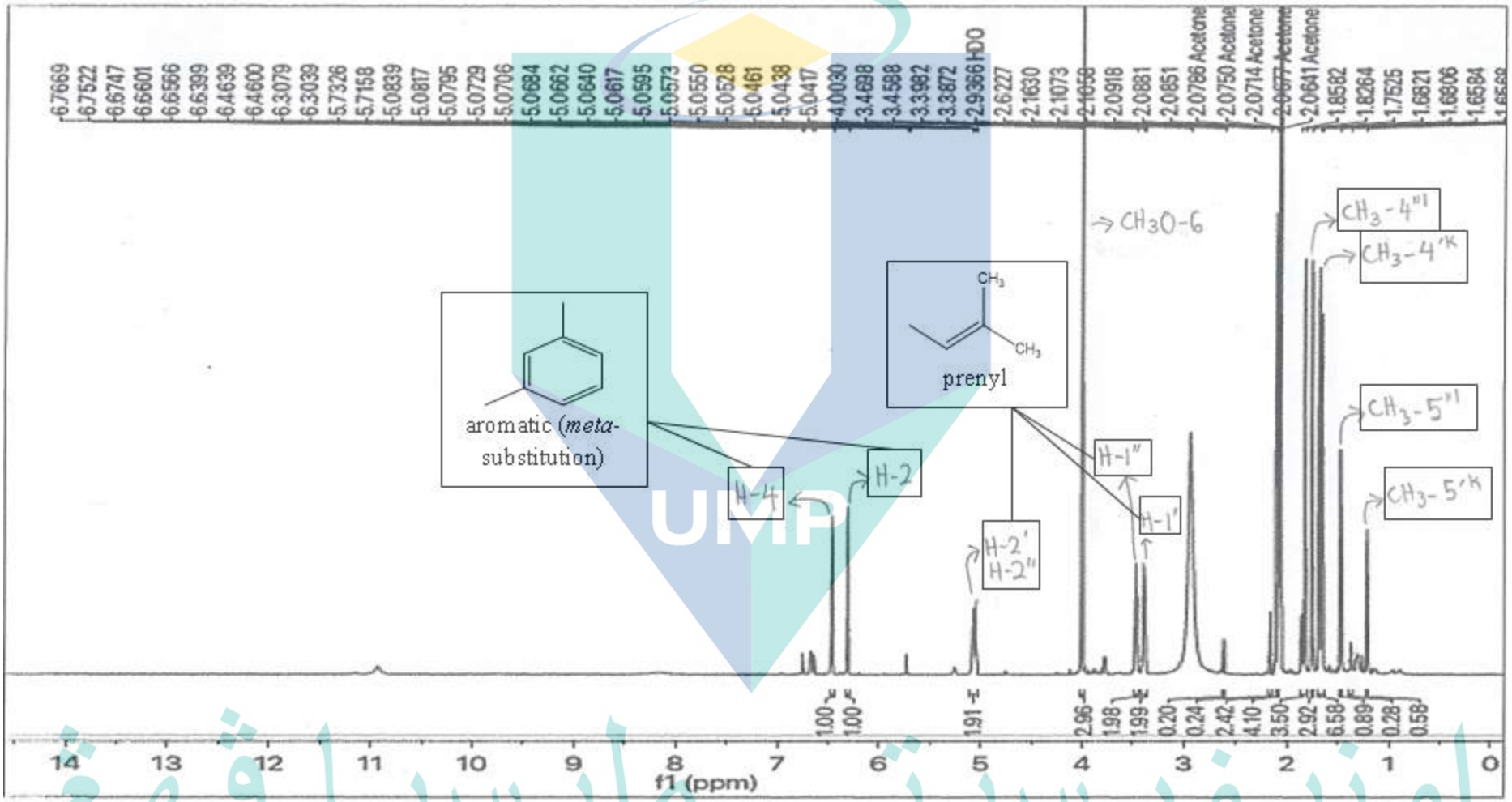


Figure 4.2 ^{1}H NMR spectrum of atrovirisidone (7)

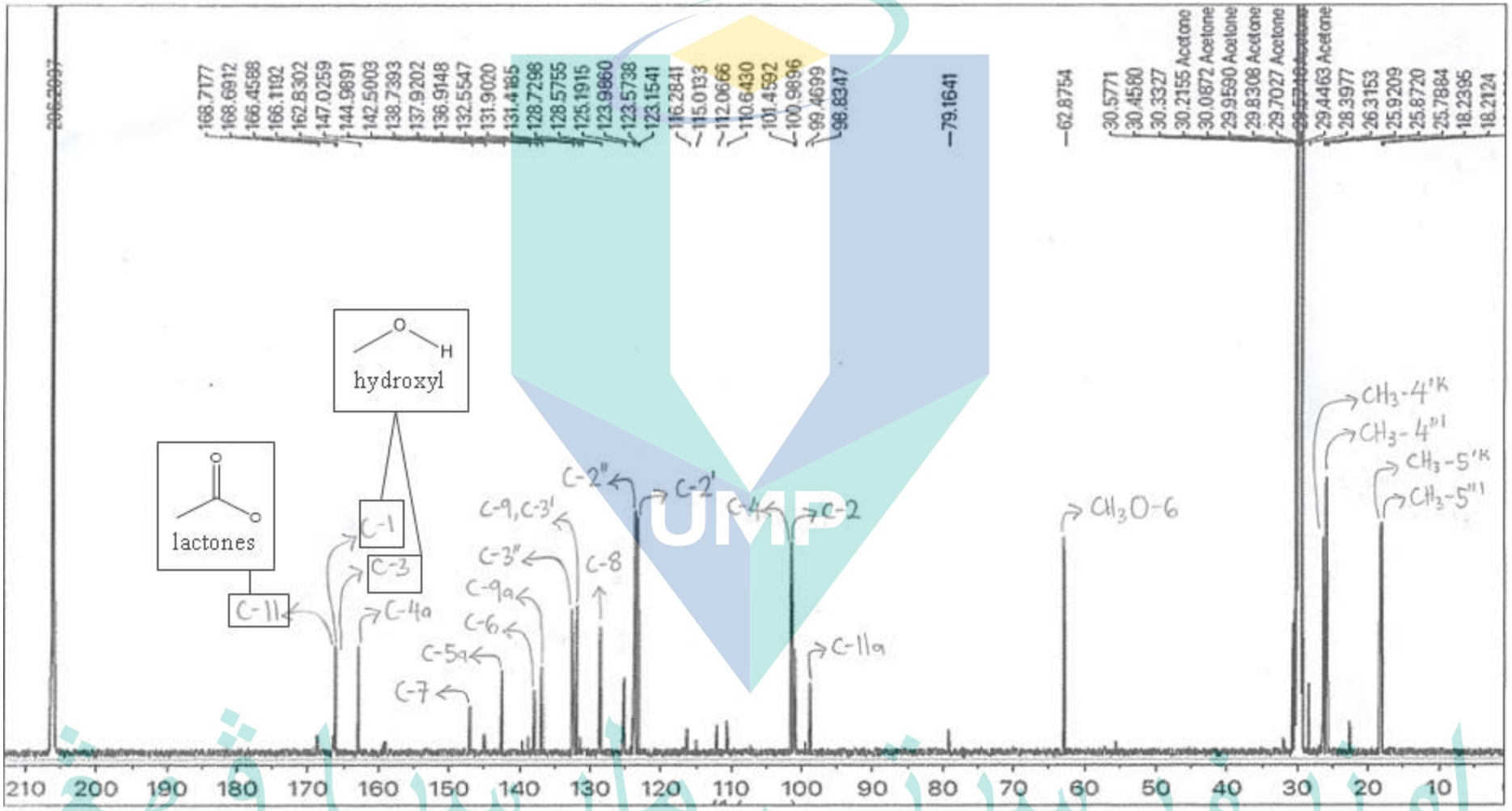


Figure 4.3 ^{13}C NMR spectrum of atrovirisidone (**7**)

4.3.2 Characterization of Morelloflavone

Compound **12** was obtained as yellow amorphous solid. The molecular formula was determined as $C_{30}H_{20}O_{11}$ (556) by using ESI-LCMS, which showed a peak of $[M+1]^+$ at *m/z* 557. The UV spectrum showed maximum absorption at λ_{max} 345 nm; the IR (KBr) spectrum observed at ν_{max} 3420 cm^{-1} and ν_{max} 1634 cm^{-1} indicated the presence of hydroxyl and carbonyl groups, respectively. The mass, UV, and IR spectra provide information about the presence of flavanone-flavone system (Herbin et al., 1970).

The NMR spectrum of compound **12** is shown in Table 4.2. The ^{1}H NMR of the compound showed the presence of three aromatic protons at ring E, assigned at position II- 6′, II- 2′ and II- 5′, based on the signals at δ 7.53 (d, J= 8.5 Hz), δ 7.50 (s), and δ 7.04 (d, J= 8.2 Hz), respectively. Four aromatic protons appeared as a set of doublets at δ 7.26 (I- 2′ and I- 6′) and δ 6.55 (I- 3′ and I- 5′). Singlet signal at δ 6.48 (II- 3) indicated the presence of one proton. Meanwhile, signals at δ 6.34 (II- 6), and δ 6.03 (I- 8 and I- 6) represented aromatic protons. The remaining protons were assigned to position I- 2 and I- 3 of ring C, following doublet signals at δ 5.89 (d, J= 12 Hz) and δ 5.02 (d, J= 11.95 Hz), respectively. The presence of two aliphatic protons and three aromatic protons showed there were a linkage between ring C and ring D (Herbin et al., 1970). According to Clark-Lewis (1968), the large coupling constant of more than 11.0 Hz showed the existence of *trans*-diaxial hydrogens.

Although hydroxyl protons were not visible in ^{1}H NMR spectrum (Figure 4.5), the presence of hydroxyl groups was observed in the data from FTIR analysis with ν_{max} 3420 cm^{-1}. As explained by Pavia and co-authors (2008), the absence of hydroxyl spin-spin splitting in proton NMR was due to the fact that the rate of proton exchanged between alcohol molecules is higher than the rate of which NMR spectrometer can react. Hence, it effectively decouples the spin interaction between hydroxyl proton and other proton. Other conditions might also effect the observation such as the temperature, the purity of the sample and the type of solvent. It was mentioned that the usage of deuterated chloroform might ease the detection of hydroxyl group.

Table 4.2 ^{1}H NMR (500 MHz, Acetone-d_6) and ^{13}C NMR (125 MHz, Acetone-d_6) of **12**

Position	**^{1}H (δH in ppm)**		**^{13}C (δC in ppm)**	
	Obtained reading	Ref reading	Obtained reading	Ref reading
I-2	5.89 (d, J= 12 Hz)	5.88 (1H, d, J= 12.4 Hz)	81.48	81.5
3	5.02 (d, J= 11.95 Hz)	5.00 (1H, d, J= 12.4 Hz)	49.17	49.2
4			205.51	196.5
5		12.34 (OH, s)	163.97	164.7
6	6.03 (s)	6.04 (2H, s)	96.26	96.3
7			164.72	166.4
8	6.03 (s)	6.04 (2H, s)	95.15	95.2
9			163.45	164.0
10			101.12	102.1
1′			127.01	129.2
2′	7.26 (d, J= 8.15 Hz)	7.25 (2H, d, J= 8 Hz)	128.38	128.4
3′	6.55 (d, J= 8.2 Hz)	6.54 (2H, d, J= 8 Hz)	114.61	114.6
4′			157.61	157.5
5′	6.55 (d, J= 8.2 Hz)	6.54 (2H, d, J= 8 Hz)	114.61	114.6
6′	7.26 (d, J= 8.15 Hz)	7.25 (2H, d, J= 8 Hz)	128.38	128.4
II-2			162.63	163.4
3	6.48 (s)	6.48 (1H, s)	102.86	102.9
4			182.26	182.3
5		13.17 (OH, s)	159.52	164.7
6	6.34 (s)	6.32 (1H, s)	98.71	98.7
7			161.63	161.7
8			100.87	104.0
9			155.89	155.9
10			103.55	100.8
1′			122.63	122.6
2′	7.50 (s)	7.52 (1H, s)	113.28	113.3
3′			145.52	145.5
4′			149.37	149.3
5′	7.04 (d, J= 8.2 Hz)	7.03 (2H, d, J= 8 Hz)	115.77	115.8
6′	7.53 (d, J= 8.5 Hz)	7.54 (1H, d, J= 8 Hz)	119.56	119.6

Ref: reference; Salleh et al., (2017); (Morelloflavone)

According to the results obtained from ^{13}C NMR as presented in Figure 4.6, a total of 30 signals representing 30 carbons were detected. Two carbonyl signals at δ 205.51 (I- 4, ring C) and δ 182.26 (II- 4, ring F) confirmed the presence of flavanone and flavone unit in the molecule (Duddeck et al., 1978). Signals that appeared between region δ 165 to δ 145 corresponded to nine oxygenated carbons at C- 5, C- 7, C- 9, C- 4′ of flavanone and at C- 5, C- 7, C- 9, C- 3′, C- 4′ of flavone unit. Signals at δ 98.71 (II- 6), δ 96.26 (I- 6) and δ 95.15 (I- 8) confirmed the presence of three methine carbons in the compound. Duddeck and co-authors (1978) discussed the ^{13}C NMR spectrum of this structure in details. The ^{13}C NMR spectral data of present study was in accordance to the data reported.

Based on previous literatures, the compound was identified as morelloflavone (**12**), also known as fukugetin. It is the first biflavonoid reported with a flavanone and flavone unit (naringenin- 3 → 8″ luteolin) as shown in Figure 4.4 (Salleh et al., 2017; Duddeck et al., 1978; Fa-Ching et al., 1975; Karanjgaokar et al., 1967). This compound is a common constituent in *Garcinia*, previously purified from the root of *G. atroviridis* (Permana et al., 2003).

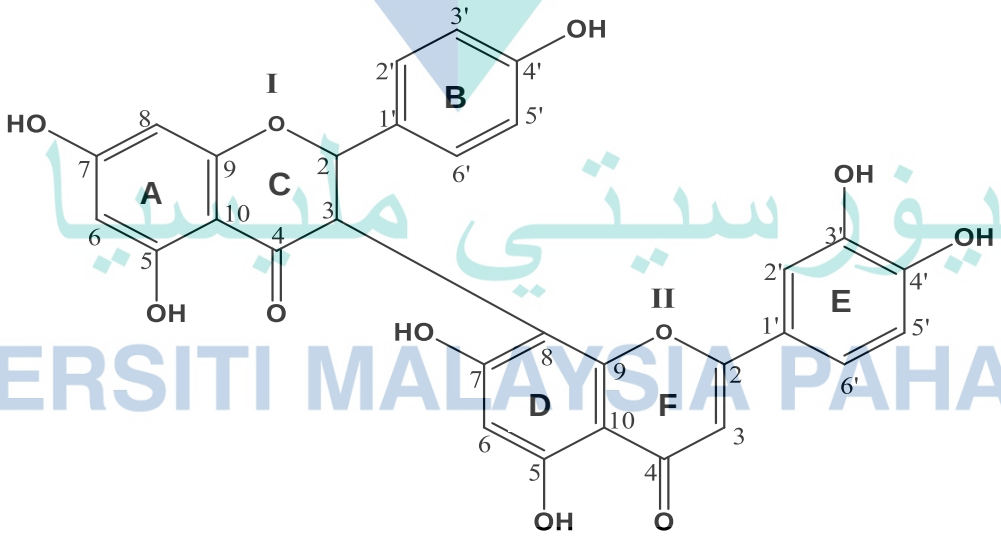


Figure 4.4 Structure of morelloflavone (**12**)

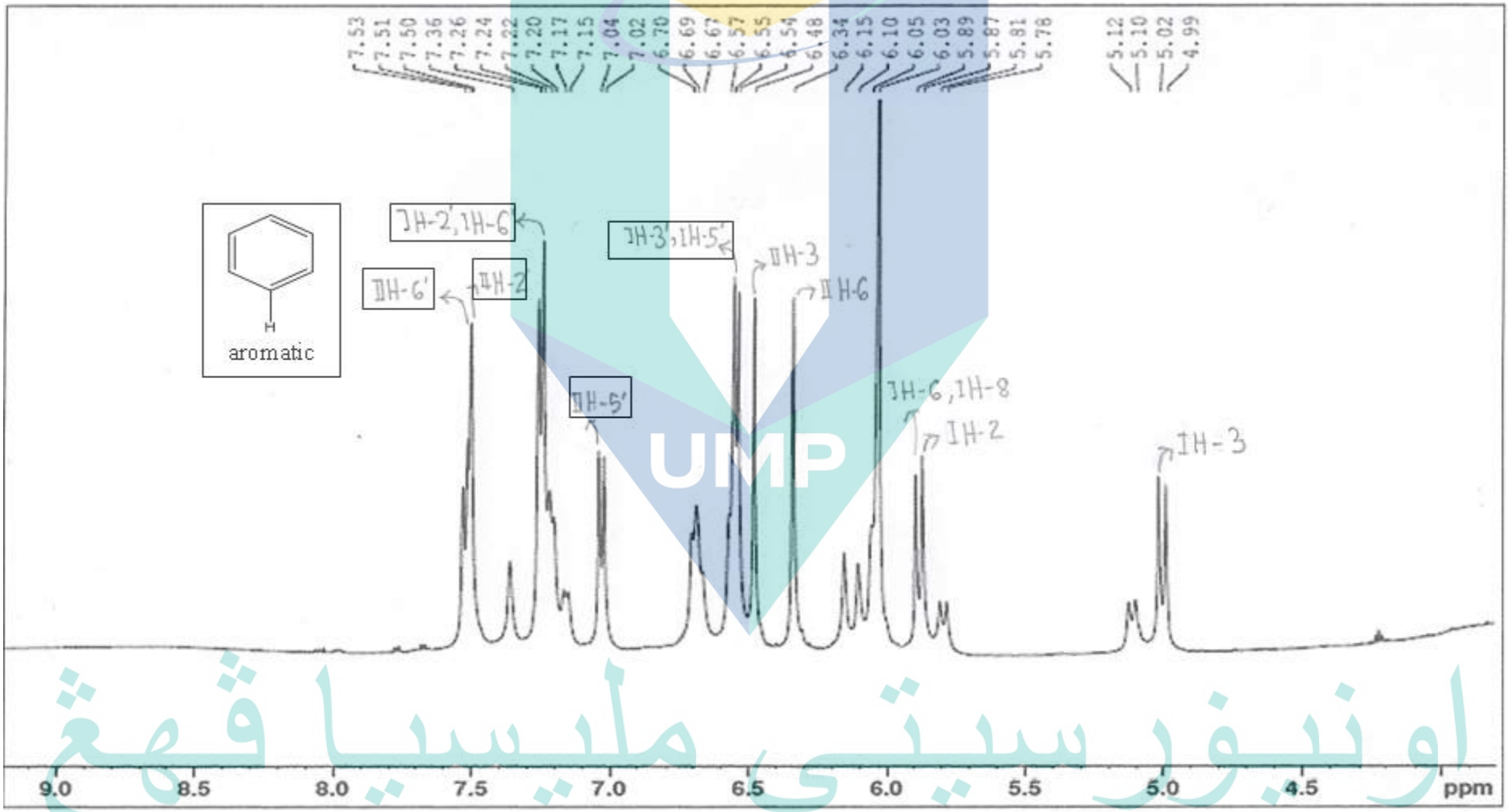


Figure 4.5 ^{1}H NMR spectrum of morelloflavone (**12**)

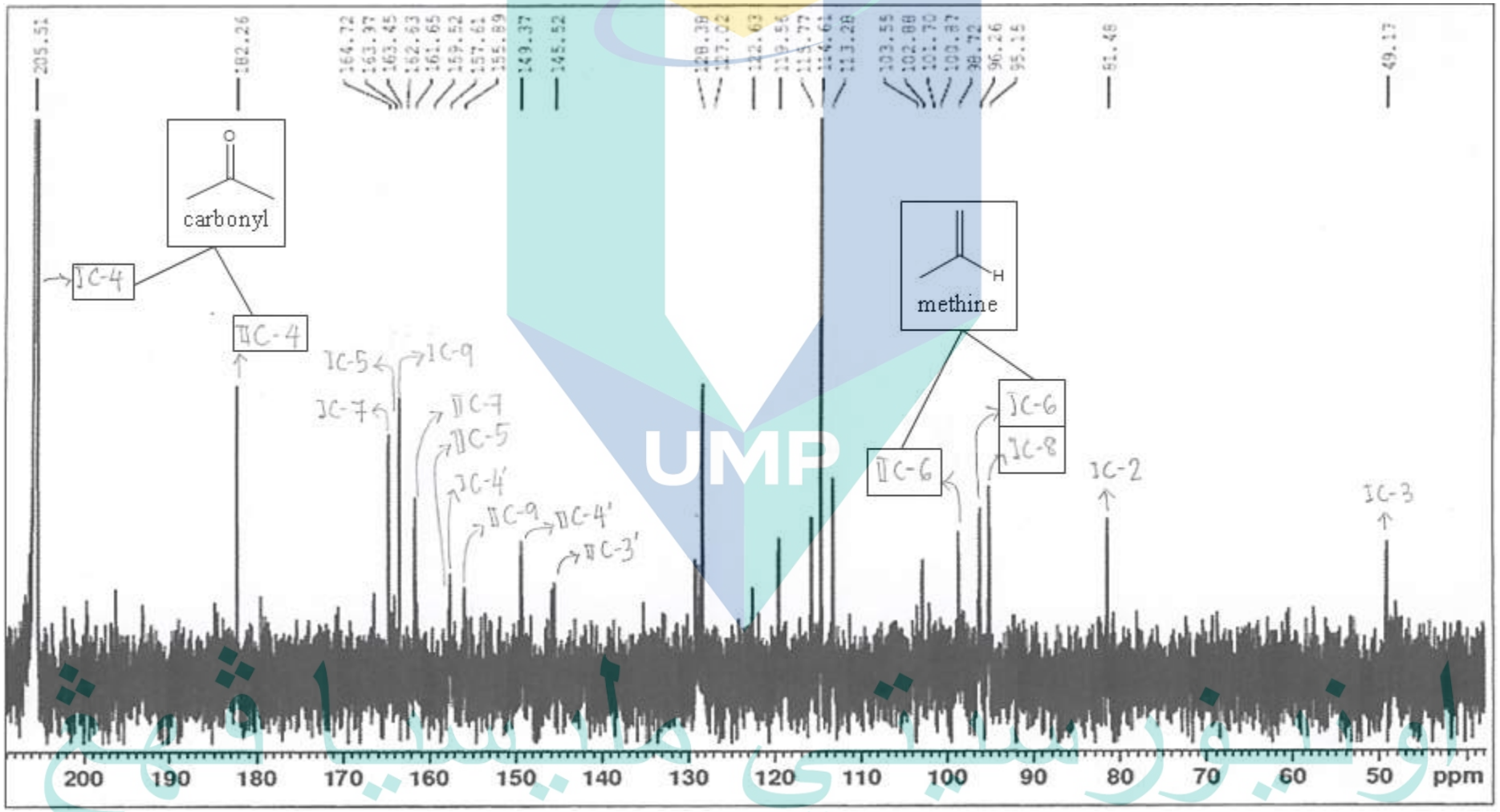


Figure 4.6 ^{13}C NMR spectrum of morelloflavone (**12**)

4.3.3 Characterization of GB1a

Compound **18** was obtained as brown amorphous solid. The molecular formula of $C_{30}H_{22}O_{10}$ (542) gave a $[M+1]^+$ ion peak at *m/z* 543 in the ESI-LCMS, signifying characteristics of biflavonoid. The UV spectrum with maximum absorption at λ_{max} 331 nm were typical for flavanone skeleton (Jackson et al., 1971). The IR (Kbr) spectrum confirmed the presence of aromatic structure, which contained hydroxyl and carbonyl groups at ν_{max} 3458 cm^{-1} and ν_{max} 1638 cm^{-1}, respectively.

As tabulated in Table 4.3, the proton NMR spectrum showed the presence of eight aromatic protons, which appeared as two sets of doublets at δ 7.18 (II- 2′, II- 6′), δ 6.82 (II- 3′, II- 5′) and at δ 7.1 (I- 2′, I- 6′) and δ 6.61 (I- 3′, I- 5′). Another three aromatic protons corresponded to signals at δ 5.81 (I- 6 and I- 8, ring A) and δ 5.75 (II- 6, ring D) were detected. The presence of two protons in ring C of the flavanone unit was deduced from the signals observed at δ 5.47 (I- 2) and δ 4.69 (I- 3). According to Duddeck et al. (1978), the lower field signal at δ 4.69 assigned at carbon I- 3 was shifted downfield due to flavanyl substitution. Meanwhile, the 3-substitution caused the downfield shift of carbon I- 2 signal. Additionally, the large coupling constant of more than 11.0 Hz showed that there were *trans*-diaxial disposition for the H- 2 and H- 3 of rings C and F (Clark-Lewis, 1968).

On the other hand, protons in another flavanone unit in ring F were detected at δ 5.2 (II- 2) and δ 2.62 (II- 3). The information suggested that there were a linkage between ring C and D of both flavanone units in this structure (Jackson et al., 1967). GB1a representing reduced biflavonoid derivatives since there are two flavanone units with 3, 8″ linkage, where the linkage might be due by oxidative coupling (Jackson et al., 1967). Hydroxyl groups were not detected, as hydroxyl proton signals are not visible in ^1H NMR spectrum (Figure 4.8) however, the presence of hydroxyl groups were evident in FTIR (ν_{max} 3458 cm^{-1}), verifying the existence of this functional group in this structure.

Table 4.3 ^{1}H NMR (500 MHz, Acetone-d_6) and ^{13}C NMR (125 MHz, Acetone-d_6) of **18**

Position	**^{1}H (δ_H in ppm)**		**^{13}C (δ_C in ppm)**	
	Obtained reading	Ref reading	Obtained reading	Ref reading
I-2	5.47	5.85 (1H, m)	82.68	82.0
3	4.69 (d, J= 11.6 Hz)	4.72 (1H, d, J= 12.0 Hz)	49.21	48.2
4			197.04	196.3
5		12.32 (OH)	165.49	164.6
6	5.81	5.98 (2H, s)	97.01	96.2
7			167.03	166.2
8	5.81	5.98 (2H, s)	95.7	95.8
9			164.2	163.4
10			102.94	102.0
1′			129.77	130.9
2′	7.1	7.24 (2H, s)	130.20	129.0
3′	6.61	6.73 (2H, m)	116.16	115.3
4′			158.0	157.7
5′	6.61	6.73 (2H, m)	116.16	115.3
6′	7.1	7.24 (2H, s)	130.20	129.0
II-2	5.2	5.33 (1H, dd, J= 12.0, 3.0 Hz)	79.93	79.3
3	2.62	2.61 (1H, dd, J= 13.2, 3.2 Hz)	44.57	43.3
4			197.04	196.9
5		12.20 (OH)	165.56	164.6
6	5.75	5.94 (1H, s)	95.7	94.9
7			167.03	164.6
8			102.42	102.0
9			164.24	163.4
10			102.47	101.5
1′			132.02	130.9
2′	7.18	6.92 (2H, m)	128.73	129.0
3′	6.82	6.83 (2H, m)	115.56	114.8
4′			158.45	145.2
5′	6.82	6.83 (2H, m)	115.56	114.8
6′	7.18	6.92 (2H, m)	128.75	129.0

Ref: reference; Salleh et al., (2017); (3,8″-Binaringenin)

Figure 4.9 shows the ^{13}C NMR spectrum of **18** with 30 signals representing 30 carbon atoms, similar to the ^{13}C NMR signal reported in the past study (Duddeck et al., 1978). The spectrum was composed of two carbonyl signals that appeared at δ 197.04 (I- 4, ring C and II- 4, ring F), corresponded to combination of two flavanone units present in the compound. Eight oxygenated carbons at δ 167 to δ 158 were identified from the signals produced at C- 5, C- 7, C- 9, and C- 4′ in both flavanone units. The signals detected at δ 97.01 (I- 6) and δ 95.7 (I- 8 and II- 6) signified the presence of three methine carbons in the structure.

The data conformed to the previous study which reported on compound **18** as GB1a, also known as 3-8″-binaringenin as shown in Figure 4.7 (Salleh et al., 2017; Fa-Ching et al., 1975; Jackson et al., 1967). GB1a (**18**) is a known 3-8″ linked biflavonoids, which was first isolated from the heartwood of *G. buchananii* from chloroform extract (Jackson et al., 1967). Previously, this biflavonoid was also isolated from the EtOAc root extract of *G. atroviridis* (Permana et al., 2005).

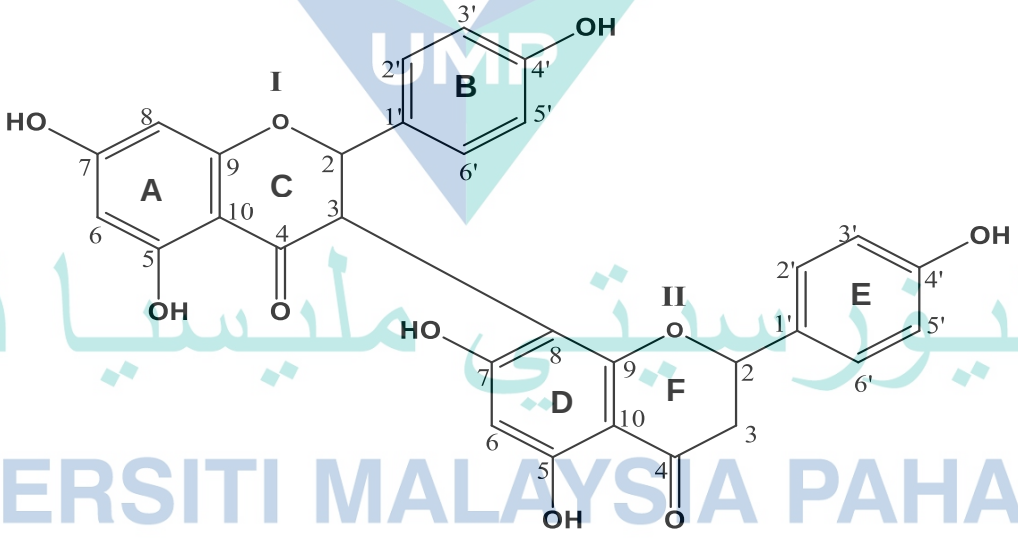


Figure 4.7 Structure of GB1a (**18**)

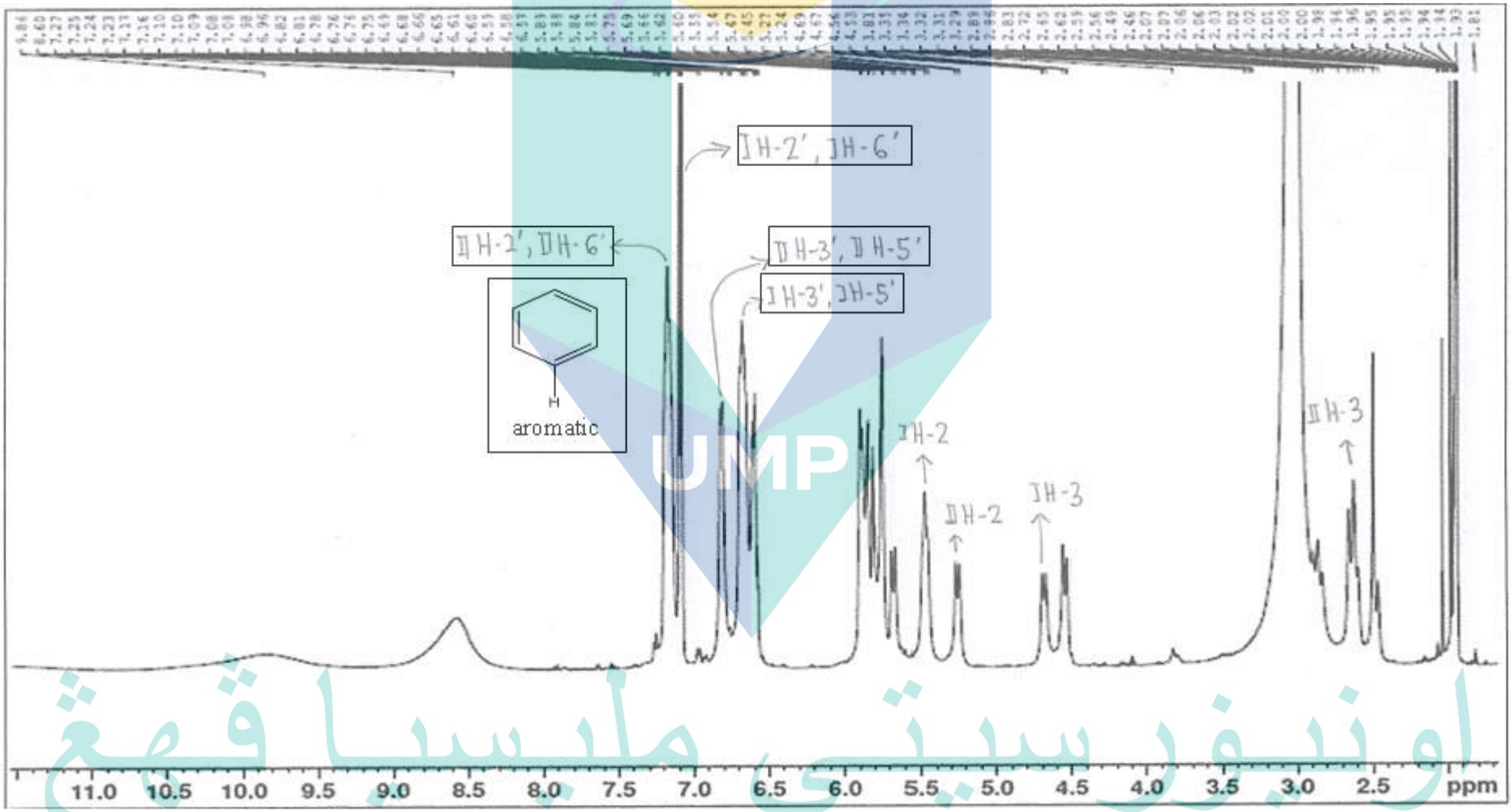


Figure 4.8 ^{1}H NMR spectrum of GB1a (**18**)

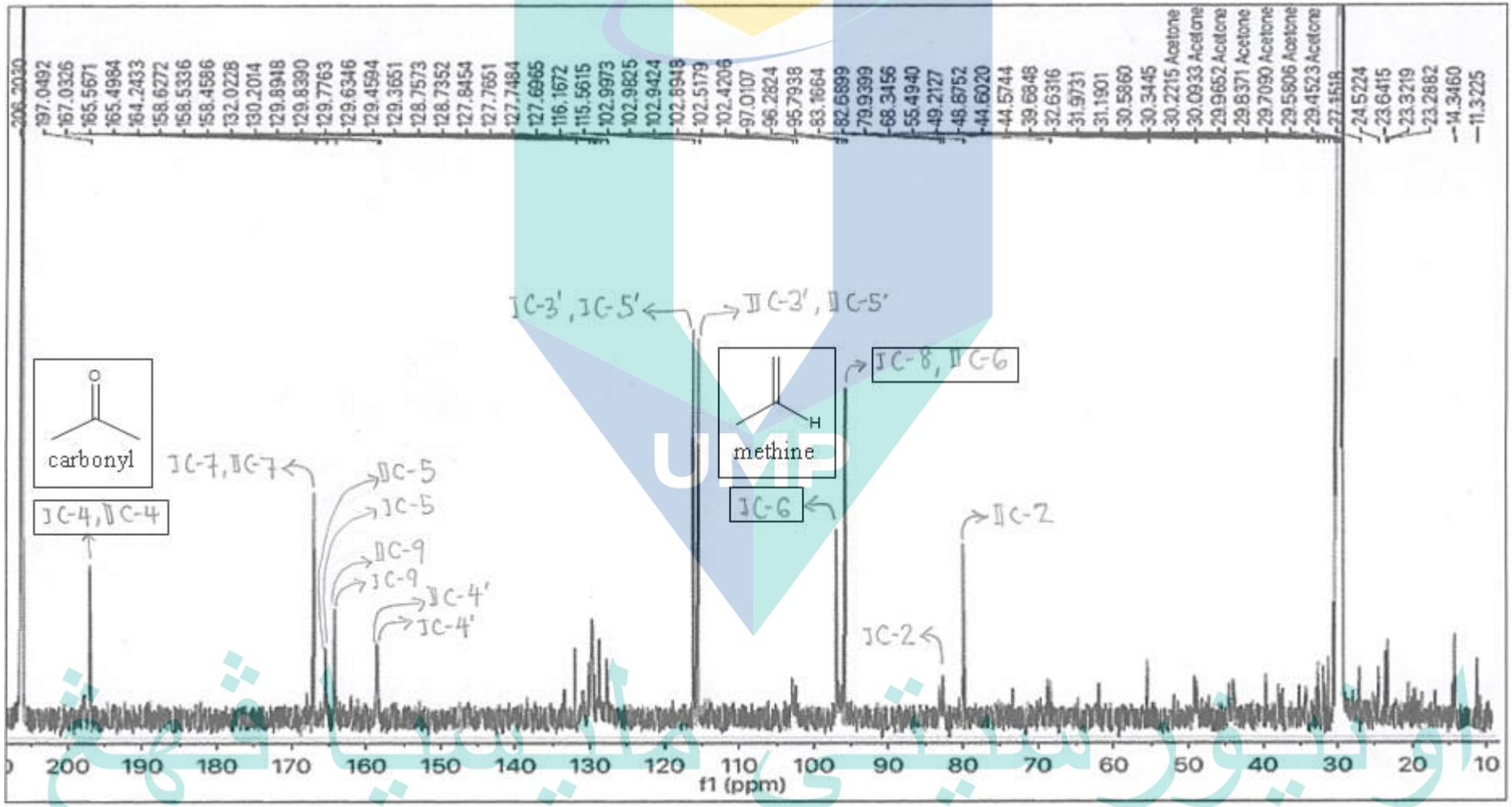


Figure 4.9 ^{13}C NMR spectrum of GB1a (**18**)

4.3.4 Characterization of 1,3,5- trihydroxy-2-methoxyxantone

Compound **29** was isolated as yellow amorphous powder. The mass spectrum of **29** consisted of a molecular ion $[M+1]^+$ at *m/z* 275, conforming with a chemical formula of $C_{14}H_{10}O_6$ (274). UV spectrum of **29** showed maximum absorption at λ_{max} 311 nm with band characteristics of a xanthone (Negi et al., 2013). Xanthones is a three-membered ring phenolic compounds composed of oxygen and basic skeleton typical for all xanthones containing dibenzo-γ-pyrone or diphenylene ketone, with a molecular formula $C_{13}H_8O_2$ (Na, 2009; Diderot et al., 2006). The IR (KBr) spectrum demonstrated broad absorption at ν_{max} 3455 cm^{-1} and appearance of prominent band at ν_{max} 1636 cm^{-1}, which revealed the presence of hydroxyl and carbonyl group, respectively.

As shown in ^1H NMR spectrum (Figure 4.11), a signal at δ 3.90 indicated the presence of one metoxyl group at H- 2. A chelated hydroxyl group at δ 13.15 (H- 1), one aromatic proton at δ 6.55 (H- 4) on ring A, and three aromatic protons at δ 7.68 (H- 8), δ 7.35 (H- 6), and δ 7.28 (H- 7) on ring B, suggested that at H- 5, oxygenated substituent was present. The analysis was supported by ^{13}C NMR spectrum (Figure 4.12), which showed the signals corresponded to three vicinal oxygenated carbons appeared at δ 159.23, δ 155.36, and δ 131.57 on ring A at C- 3, C- 1, and C- 2, respectively, indicating substitution. The spectrum data were consistent with the one reported by Valentão and co-authors (2000). Apart from that, one carbonyl group was detected at δ 182.13 from the signal produced at C- 9. A total of 14 signals were detected in ^{13}C NMR spectrum representing 14 carbon atoms in the structure. The NMR spectrum data is outlined in Table 4.4.

The data obtained supported the previous finding which identified the structure as 1,3,5-trihydroxy-2- methoxyxanthone (**29**) (Valentão et al., 2000; Pinto et al., 1994) (Figure 4.10). 1,3,5-trihydroxy-2- methoxyxanthone (**29**), a xanthone, was previously purified from the stem bark of *G. atroviridis* (Tan et al., 2016). This compound exhibited antifungal activity against *Trichophyton mentagmphytes* and *Cladosporium cucumerinum* at 50 µg/mL (Pinto et al., 1994).

Table 4.4 ^{1}H NMR (600 MHz, Acetone-d_6) and ^{13}C NMR (150 MHz, Acetone-d_6) of **29**

Position	**^{1}H (δ_H in ppm)**		**^{13}C (δ_C in ppm)**	
	Obtained reading	Ref reading	Obtained reading	Ref reading
1	13.15 (1H, s, OH)	12.91 (1H, s, OH)	155.36	153.9
2			131.57	130.7
CH_3O-2	3.90 (3H, s)	3.75 (3H, s)	60.75	59.8
3			159.23	159.3
4	6.55 (1H, s)	6.5 (1H, s)	94.75	94.1
4a			153.84	152.3
4b			146.12	144.8
5			146.90	146.0
6	7.35 (1H, dd, J= 1.5, 7.8 Hz)	7.28 (1H, dd, J= 2.25, 7.9 Hz)	121.31	120.3
7	7.28 (1H, J=7.86, 7.92 Hz)	7.21 (1H, dd, J= 7.9, 7.4 Hz)	124.79	123.9
8	7.68 (1H, dd, J= 1.5, 7.92 Hz)	7.5 (1H, dd, J= 2.25, 7.4 Hz)	116.17	114.3
8a			121.76	120.4
8b			104.02	102.2
9			182.13	180.4

Ref: reference; Pinto et al., (1994)

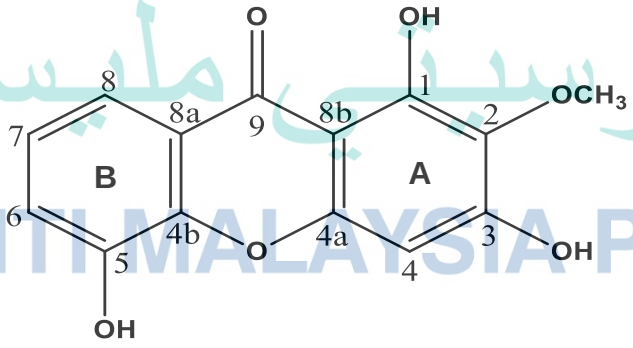


Figure 4.10 Structure of 1,3,5-trihydroxy-2- methoxyxanthone (**29**)

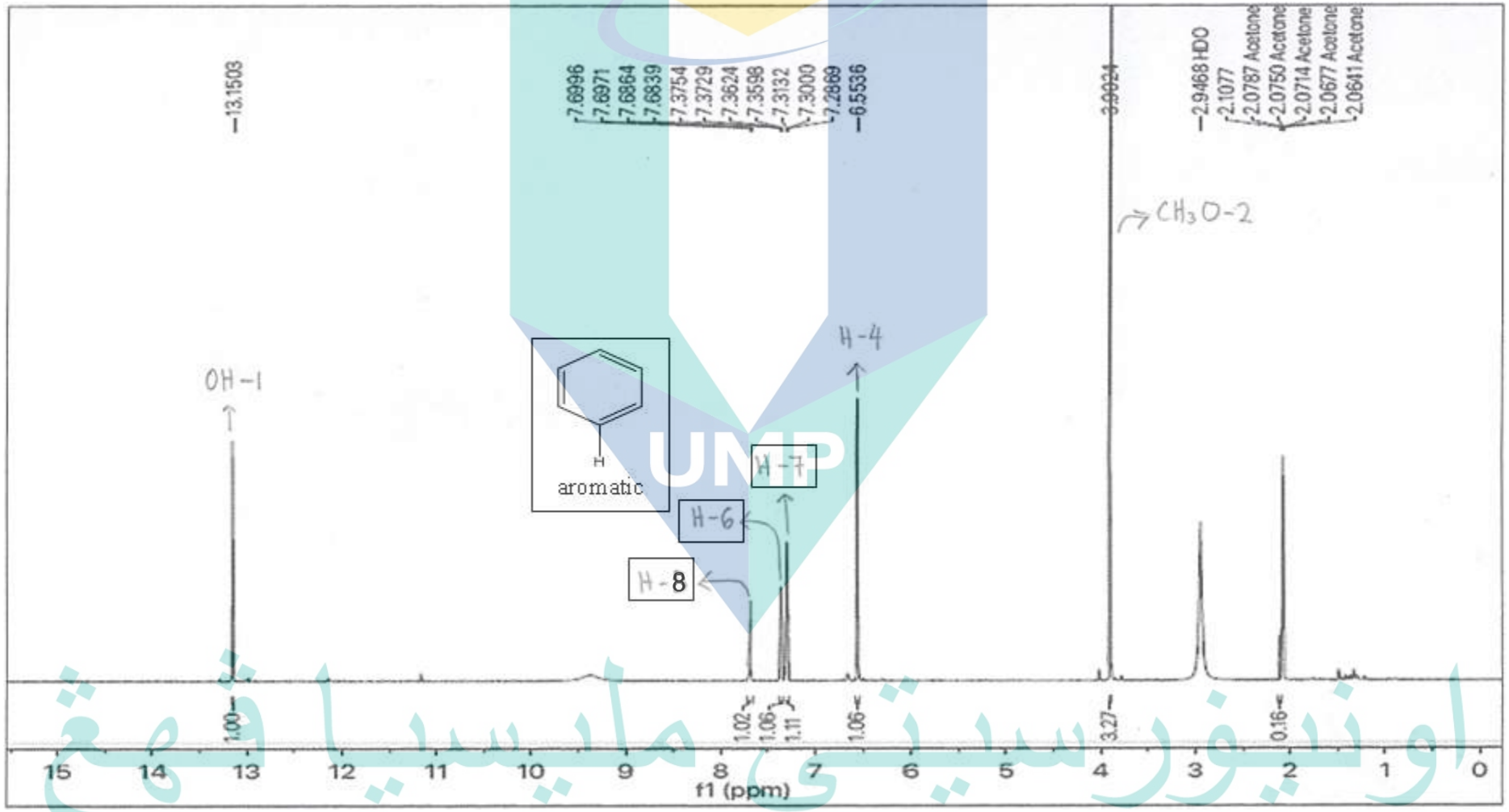


Figure 4.11 ^{1}H NMR spectrum of 1,3,5-trihydroxy-2- methoxyxanthone (**29**)

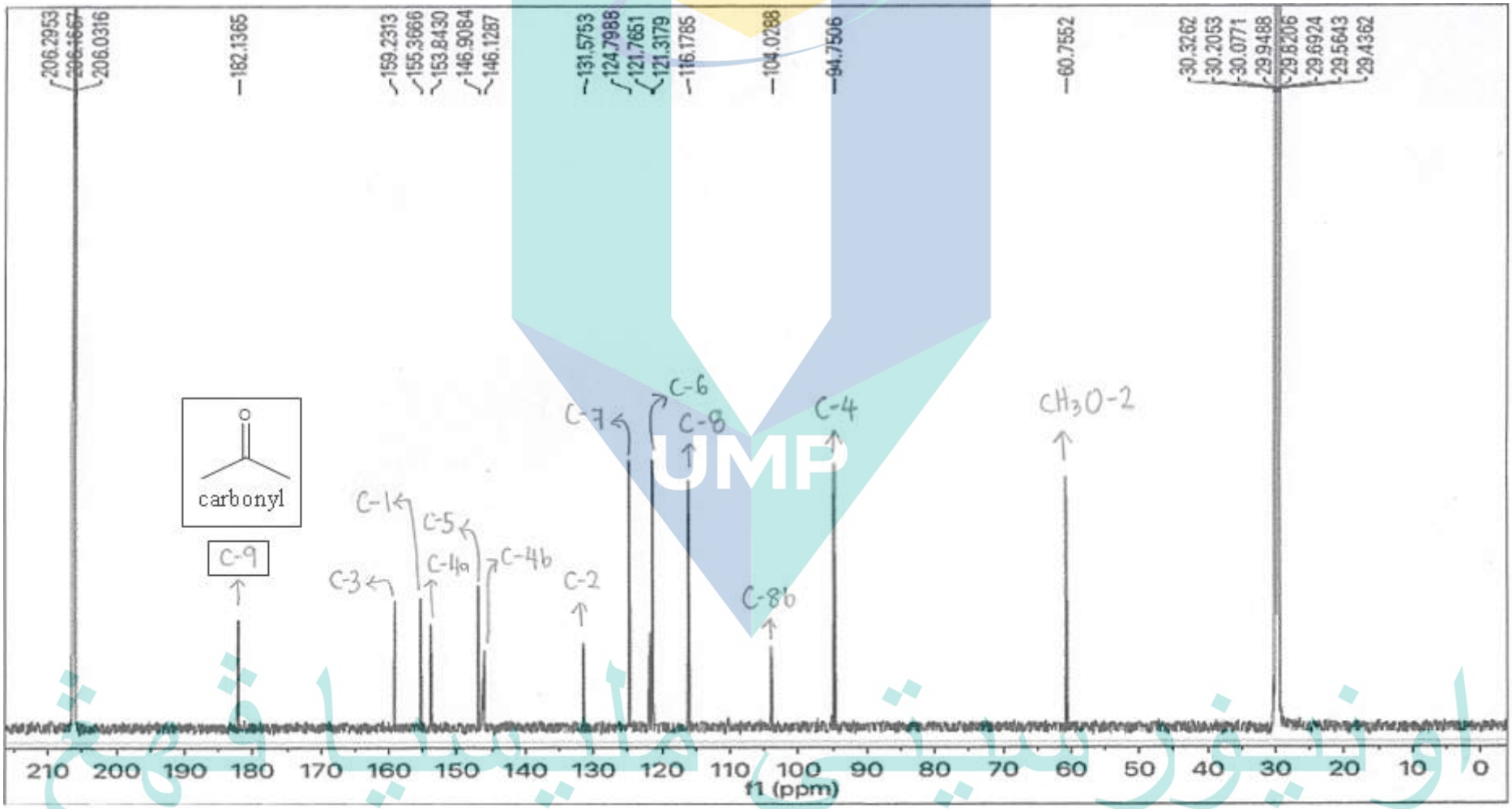


Figure 4.12 ^{13}C NMR spectrum of 1,3,5-trihydroxy-2- methoxyxanthone (**29**)

4.3.5 Characterization of Volkensiflavone

Compound **33** was obtained as yellow amorphous solid. The mass spectrum measured via ESI-LCMS showed $[M+1]^+$ peak at *m/z* 541, corresponding to the molecular formula of $C_{30}H_{20}O_{10}$ (540). The maximum absorption of UV spectrum was at λ_{max} 340 nm. The IR (KBr) spectrum showed broad hydroxyl absorption at ν_{max} 3451 cm^{-1}, carbonyl absorption at ν_{max} 1641 cm^{-1} and aromatic group at ν_{max} 1097 cm^{-1}. The mass, UV, and IR spectra were identical to that of biflavonoids (Joshi et al., 1970).

The NMR data is tabulated in Table 4.5. Based on the proton NMR spectrum (Figure 4.14), there are eight aromatic protons indicated by two set of doublets at δ 7.98 (II- 2′, II- 6′), δ 7.06 (II- 3′, II- 5′), δ 7.72 (I- 2′, I- 6′) and δ 6.79 (I- 3′, I- 5′). One proton in pyranone of flavone unit was detected as singlet at δ 6.33 (II- 3, ring F). Three aromatic protons corresponded to the signals at δ 6.33 (II- 6, ring D) and δ 6.03 (I- 6 and I- 8, ring A) were discovered. Two aliphatic protons were detected as doublets in the heterocyclic ring C of flavanone unit at δ 5.87 (J= 12.3 Hz) and δ 5.05 (J= 12 Hz), at position I- 2 and I- 3, respectively. The aliphatic protons with coupling constant of more than 11.0 Hz represented *trans*-diaxial hydrogens (Clark-Lewis, 1968). Herbin and co-authors (1970) stated that since there are two aliphatic hydrogens and three aromatics signals indicated a linkage between flavanone and flavone unit between ring C and ring D as shown in Figure 4.13. Hydroxyl groups were detected following the singlet signals at δ 13.15 and δ 12.35, which were assigned to C- 5 of ring A and ring D, respectively.

There are 30 carbon atoms observed in the ^{13}C NMR spectra of the structure (Figure 4.15). Signals appeared at δ 83.2 (I- 2) and δ 49.9 (I- 3) represented naringenin, which belongs to flavanone group (Chari et al., 1977). Two resonances from the structure of carbonyl at δ 197.24 (I- 4) and δ 183.17 (II- 4) signified the level of oxidation in two rings (ring C and F) thus suggesting the presence of flavanone and flavone units in this compound. A total of eight oxygenated carbons were discovered, which corresponded to signals at δ 164.34 (C- 5), δ 167.14 (C- 7), δ 163.56 (C- 9), δ 162.29 (C- 4′) of unit I and at δ 161.80 (C- 5), δ 167.78 (C- 7), δ 158.36 (C- 9), δ 156.86 (C- 4′) of unit II. Three methine carbons were observed at δ 99.61 (II- 6), δ 97.09 (I- 6) and δ 95.99 (I- 8).

Compound **33** was identified as volkensiflavone (**33**), also known as talbotaflavone (Compagnone et al., 2008; Fa-Ching et al., 1975; Herbin et al., 1970; Joshi et al., 1970). Volkensiflavone (**33**) is a combination of naringenin and apigenin (Herbin et al., 1970). The compound has been previously found in *G. volkensii*, *G. livingstonei*, *G. hombroniana* and *G. madruno*, and this study is the first to report on its isolation from *G. atroviridis* (Ramirez et al., 2018; Muriithi et al., 2016; Jamila et al., 2014; Herbin et al., 1970).

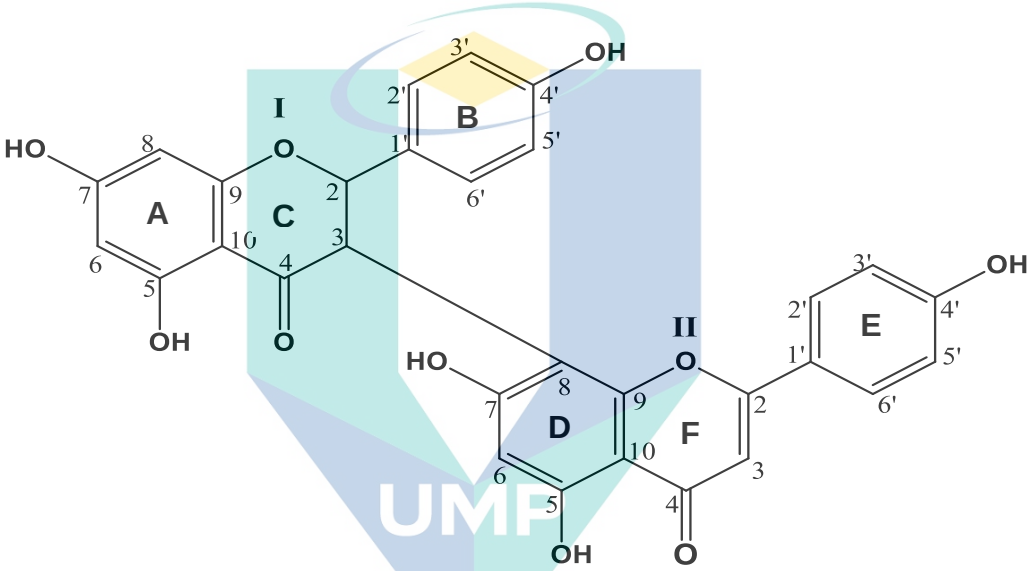


Figure 4.13 Structure of volkensiflavone (**33**)

Table 4.5 ^{1}H NMR (600 MHz, Acetone-d_6) and ^{13}C NMR (150 MHz, Acetone-d_6) of **33**

Position	**^{1}H (δ_H in ppm)**		**^{13}C (δ_C in ppm)**	
	Obtained reading	Ref reading	Obtained reading	Ref reading
I-2	5.87 (d, J= 12.3 Hz)	6.54	83.2	82.3
3	5.05 (d, J= 12 Hz)	5.56	49.9	50.2
4			197.24	197.9
5	13.15 (s, OH)	13.20 (OH)	164.34	165.5
6	6.03 (s)	6.55	97.09	97.5
7			167.14	168.3
8	6.03 (s)	6.50	95.99	96.4
9			163.56	164.4
10			101.41	102.9
1′			129.15	129.7
2′	7.72 (d, J= 7.5 Hz)	7.72	129.82	130.0
3′	6.79 (d, J= 7.5 Hz)	6.99	115.46	116.9
4′			162.29	159.3
5′	6.79 (d, J= 7.5 Hz)	6.99	115.46	116.9
6′	7.72 (d, J= 7.5 Hz)	7.72	129.88	130.0
II-2			163.56	164.2
3	6.33 (s)	6.70	103.69	103.6
4			183.17	182.8
5	12.35 (s, OH)	12.87 (OH)	161.80	162.7
6	6.33 (s)	6.82	99.61	98.9
7			167.78	168.3
8			102.29	102.1
9			158.36	162.4
10			104.22	104.7
1′			120.65	122.3
2′	7.98 (d, J= 8.16 Hz)	8.12	129.29	129.2
3′	7.06 (d, J= 8.10 Hz)	7.34	116.83	115.8
4′			156.86	156.8
5′	7.06 (d, J= 8.10 Hz)	7.34	116.86	115.8
6′	7.98 (d, J= 8.16 Hz)	8.12	129.23	129.2

Ref: reference; Compagnone et al., (2008); (Volkensiflavone)

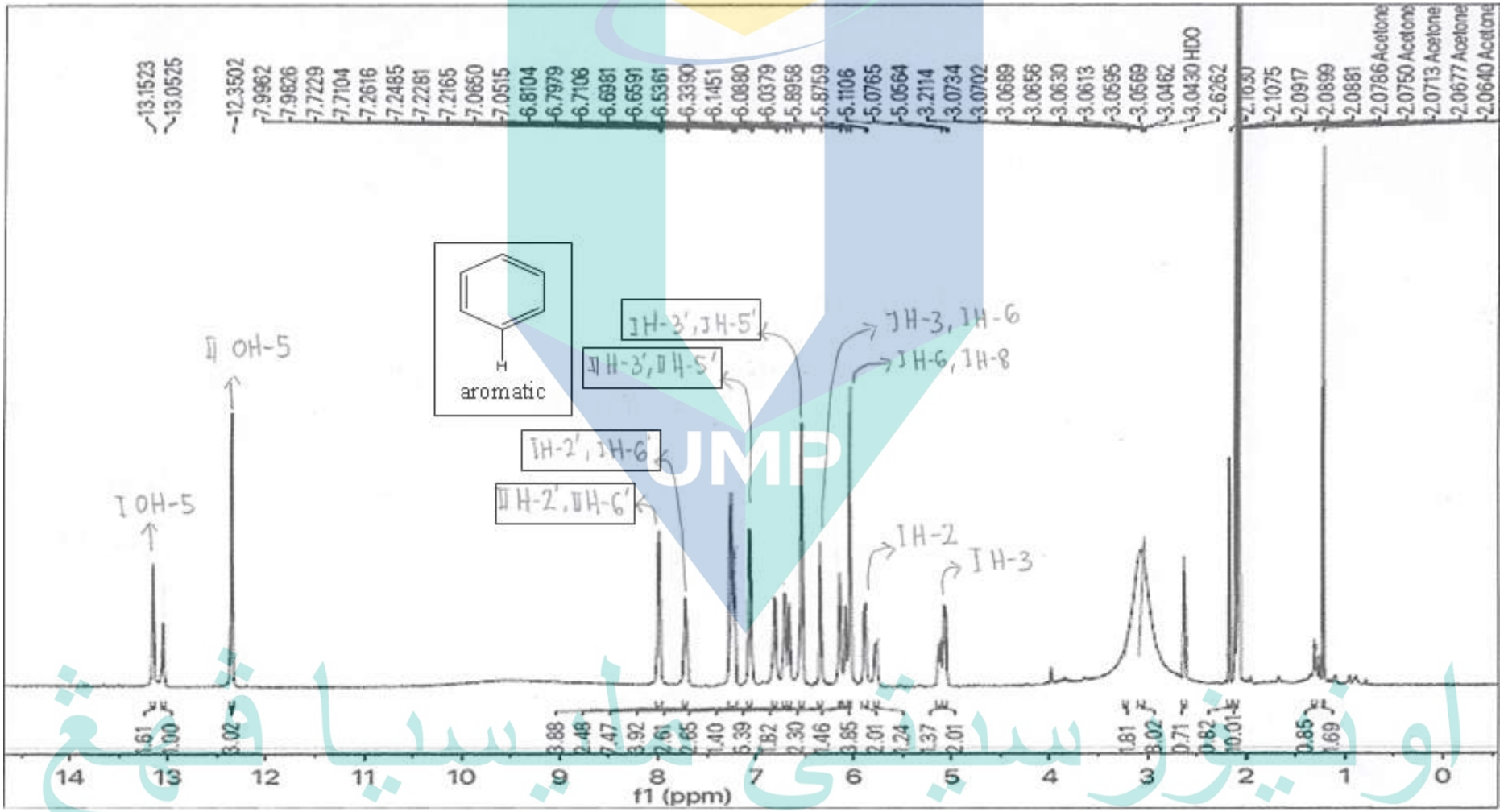


Figure 4.14 ^{1}H NMR spectrum of volkensiflavone (**33**)

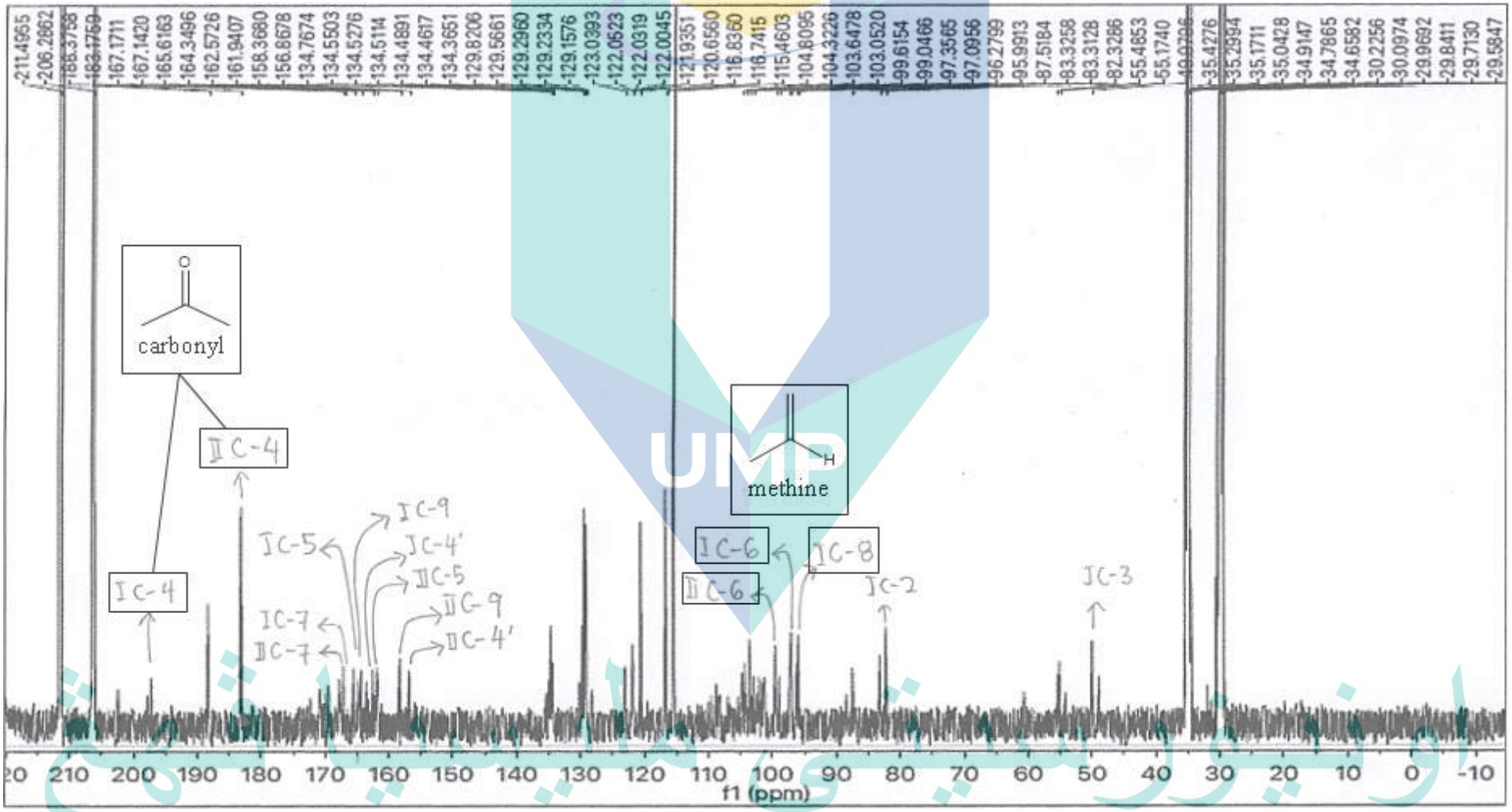


Figure 4.15 ^{13}C NMR spectrum of volkensiflavone (**33**)

4.4 Biological Activities of Chemical Constituents Isolated from EtOAc Extract

Following the positive antibacterial and antioxidant activities observed in the EtOAc root extract of *G. atroviridis*, the investigation was extended to study the biological activities of the individual compounds isolated from the extract; morelloflavone (**12**), GB1a (**18**), 1,3,5- trihydroxy-2-methoxyxantone (**29**), and volkensiflavone (**33**) were evaluated. Bioactivities of atrovirisidone (**7**) was not evaluated due to inadequate materials available.

4.4.1 Antibacterial Activity

To further investigate the antibacterial properties of *G. atroviridis*, the isolated compounds namely morelloflavone (**12**), GB1a (**18**), 1,3,5- trihydroxy-2-methoxyxantone (**29**), and volkensiflavone (**33**) were tested against *S. aureus* and *E. coli*. Only GB1a (**18**) and volkensiflavone (**33**) showed significant antibacterial activity towards *S. aureus* with 10.3 mm and 9.0 mm of inhibition zone, respectively. However, the antibacterial effects in all samples were less potent than that of commercial antibiotics that were used as standard in this study. Similar level of antibacterial activity were observed in the individual compounds, whereby none of the isolates was active against Gram-negative bacteria. All data are presented in Table 4.6.

In previous study, both GB1a (**18**) and volkensiflavone (**33**) purified from the twigs of *G. merguensis* showed weak antibacterial activity against *S. aureus* and methicillin-resistant *S. aureus*, with MIC value of more than 128 µg/mL (Trisuwan et al., 2013). On the other hand, inactivity observed in morelloflavone (**12**) against microorganisms tested in this study contradicted the previous findings, which have extensively reported on broad antibacterial activities of morelloflavone (**12**). Jamila et al. (2014) reported that morelloflavone (**12**) showed the highest antibacterial activity with MIC of 62.5 µM against *S. aureus*, *B. subtilis* and *E. coli*. The contradicting findings obtained from this study may be due to diffusion of sample into agar in disc diffusion assay that might have affected the potency of sample, which may not happen in MIC assay using dilution method (Marimuthu et al., 2014). Inability to inhibit Gram-negative bacteria may also be due to complexity of the bacterial outer membrane, which could have slowed down the flow of chemicals across the cell, making the bacteria resilient to plant secondary metabolites including phenolic compounds (Inouye et al., 2001).

Table 4.6 Inhibition zones of *G. atroviridis* EtOAc extract and its isolates

Samples (20 mg/mL)	**Inhibition zone (mm)**			
	B. cereus	*S. aureus*	*E. coli*	*P. vulgaris*
EtOAc extract	7.0 ± 0	7.0 ± 0	-	-
Morelloflavone (**12**)	NT	-	-	NT
GB1a (**18**)	NT	10.3 ± 1.528	-	NT
1,3,5- trihydroxy-2-methoxyxantone (**29**)	NT	-	-	NT
Volkensiflavone (**33**)	NT	9.0 ± 1	-	NT
(+) Chloramphenicol (30 µg)	24.7 ± 0.577	NT	20.0 ± 1	NT
(+) Gentamicin (120 µg)	NT	18.7 ± 0.577	NT	33.3 ± 1.528
(-) Acetone	-	-	-	-

Data was expressed as mean (*n*=3) ± SD; -: no inhibition zone; NT: not tested; (+): positive control; (-): negative control

4.4.2 Antioxidant Activity

4.4.2.1 DPPH Assay

Antioxidant activity of isolated compounds was determined using the same protocol used on EtOAc extract. DPPH scavenging activity of morelloflavone (**12**), GB1a (**18**), 1,3,5- trihydroxy-2-methoxyxantone (**29**) and volkensiflavone (**33**) was evaluated. The IC_{50} results are tabulated in Table 4.8. Among all compounds tested, morelloflavone (**12**) showed the highest DPPH scavenging activity with IC_{50} of 20.3 ± 1.667 µg/mL, which was comparable to AA (IC_{50} 13.2 ± 0.021 µg/mL). Morelloflavone (**12**) has been reported as an effective DPPH radical scavenger (Klaiklay et al., 2011; Deachathai et al., 2005). The results supported a study published by Salleh et al., 2017, which reported on the strong DPPH scavenging activity in morelloflavone (**12**) with IC_{50} of 57.5 µg/mL as compared to AA with IC_{50} of 17.4 µg/mL.

On the other hand, GB1a (**18**), 1,3,5- trihydroxy-2-methoxyxantone (**29**), and volkensiflavone (**33**) showed low antioxidant activity with IC_{50} of more than 200 µg/mL. Previously, 1,3,5- trihydroxy-2-methoxyxantone (**29**) exhibited low DPPH scavenging activities with EC_{50} of 50.81 µg/mL as compared to AA, 7.4 µg/mL (Tan et al., 2016). The previous finding is consistent with the present study, which showed low DPPH

radical scavenging activity of 1,3,5- trihydroxy-2-methoxyxantone (**29**) with IC_{50} value of 205.7 ± 5.877 µg/mL as compared to AA with $IC_{50\ of}$ 13.2 ± 0.021 µg/mL.

The study done by Ramirez et al. (2018), Muriithi et al. (2016), and Jamila et al. (2014), demonstrated that scavenging activity on DPPH by volkensiflavone (**33**) was low as compared to morelloflavone (**12**). The result is consistent with current study. On the other hand, GB1a (**18**) was reported to have low DPPH scavenging activity with IC_{50} of 31.98 µg/mL as compared to AA with IC_{50} of 3.2 µg/mL (Aravind and Rameshkumar, 2016). The report was further supported by Salleh et al. (2017), whom published on the low scavenging activity of GB1a (**18**) with IC_{50} value of 112.4 µg/mL, compared to AA with IC_{50} 17.4 µg/mL. Similar results were obtained in the current study, which showed that 1,3,5- trihydroxy-2-methoxyxantone (**29**), volkensiflavone (**33**) and GB1a (**18**) exhibited low DPPH scavenging activity.

The differences of the value from this study with previous studies might attributed by the different methodology adapted during the experiment. Although the same assay was used in both current and previous studies, the volume, the concentration of the solvent among few others were different. Direct comparison of data is not suggested as for the various differences. There were also some minor inconsistency observed in inhibition activity in EtOAc extract and morelloflavone (**12**), where at 1000 µg/mL the percentage of inhibition was slightly reduced. This could be attributed to some errors that occurred while running the experiment. As all samples and reagents used in this test were diluted in solvent, evaporation might take place due to prolonged storage. This could cause alteration in the concentration of samples thus interfering with the results.

Based on the results, it could be deduced that the radical scavenging activity observed in the isolated compounds may have contributed to the total antioxidant activity of EtOAc extract from the root of *G. atroviridis*.

Table 4.7 Effect of *G. atroviridis* EtOAc extract and its isolates on DPPH radicals

Conc. (µg/mL)	**Percentage of inhibition (%)**					
	EtOAc extract	(**12**)	(**18**)	(**29**)	(**33**)	AA
7.8125	13.6±0.030	38.4±0.055	18.7±0.085	30.9±0.007	19.3±0.094	51.7±0.041
15.625	18.8±0.012	53.0±0.042	19.9±0.096	31.6±0.015	20.3±0.108	86.1±0.007
31.25	31.7±0.016	76.4±0.035	19.3±0.083	34.8±0.015	21.3±0.099	86.6±0.005
62.5	50.3±0.024	85.5±0.01	24.9±0.067	39.3±0.013	27.4±0.051	86.8±0.006
125	78.9±0.011	85.9±0.005	29.2±0.052	46.5±0.007	34.2±0.048	87.3±0.007
250	80.9±0.013	85.6±0.006	33.1±0.046	60.4±0.001	43.0±0.008	86.7±0.003
500	80.9±0.003	86.0±0.003	40.1±0.028	81.5±0.025	54.7±0.006	87.3±0.005
1000	74.3±0.023	85.3±0.002	52.7±0.006	87.0±0.003	72.0±0.028	87.6±0.004

Data was expressed as mean (*n*=3) ± SD

Table 4.8 IC_{50} (µg/mL) of *G. atroviridis* EtOAc extract and its isolates

Samples	**IC_{50} (µg/mL)**
EtOAc extract	51.7 ± 2.180
Morelloflavone (**12**)	20.3 ± 1.667
GB1a (**18**)	263.3 ± 71.707
1,3,5- trihydroxy-2-methoxyxantone (**29**)	205.7 ± 5.877
Volkensiflavone (**33**)	256.7 ± 76.869
AA (control)	13.2 ± 0.021

Data was expressed as mean (*n*=3) ± SD

4.4.2.2 FRAP Assay

Generally, all compounds exhibited good reducing power as outlined in Table 4.9. 1,3,5- trihydroxy-2-methoxyxantone (**29**) exhibited the highest reducing power with a value of 1643.3 ± 44.623 mg AAE/g, followed by morelloflavone (**12**), GB1a (**18**), and volkensiflavone (**33**) with a FRAP value of 380.3 ± 42.78, 121.2 ± 4.321 and 102.1 ± 12.648 mg AAE/g, respectively.

It was also found that morelloflavone (**12**) exhibited higher reducing ability than volkensiflavone (**33**), supported the previous study by Jamila et al. (2014), which reported on morelloflavone (**12**) with FRAP value of 298.8 µM trolox equivalent (TE), while volkensiflavone (**33**) with 10.9 µM TE (Jamila et al., 2014). To our best knowledge, this thesis is the first to report on FRAP activity of GB1a (**18**) and 1,3,5- trihydroxy-2-methoxyxantone (**29**).

Antioxidant assays of the individual isolated compounds correlated with their antioxidant activities. Morelloflavone (**12**) always showed the best activity among biflavonoids and 1,3,5- trihydroxy-2-methoxyxantone (**29**) demonstrated good antioxidant activity. Although the reaction mechanisms in DPPH and FRAP techniques are distinct from each other and the methods do not evaluate the same activity, it was evident in the findings from both methods that the roots of *G. atroviridis* showed significant antioxidant and anti-radical activities (Prior et al., 2005). Based on the results, morelloflavone (**12**) and 1,3,5- trihydroxy-2-methoxyxantone (**29**) demonstrated the strongest antioxidant activities compared to other isolates with moderate activities, which collectively may contribute to antioxidant activity of EtOAc extract from the roots of *G. atroviridis*.

Table 4.9 FRAP activity of *G. atroviridis* EtOAc extract and its isolates

Samples	FRAP value (mg AAE/g)
EtOAc extract	294.0 ± 20.173
Morelloflavone (**12**)	380.3 ± 42.780
GB1a (**18**)	121.2 ± 4.321
1,3,5- trihydroxy-2-methoxyxantone (**29**)	1643.3 ± 44.623
Volkensiflavone (**33**)	102.1 ± 12.648

Data was expressed as mean (n=3) ± SD

4.4.3 Structure-Antioxidant Relationship

Previous studies have reported the structure-activity relationship of the flavonoids in relation to antioxidant activity (Miller and Rice-Evans, 1997; Bors et al., 1995; Sichel et al., 1991; Bors et al., 1990; Hudson and Lewis, 1983). Similar antioxidant activities in biflavonoids were observed in this study. Morelloflavone (**12**) was the most active compared to volkensiflavone (**33**) and GB1a (**18**). There is an additional hydroxyl group in the structure of morelloflavone (**12**) (Figure 4.4) as compared to GB1a (**18**) (Figure 4.7) and volkensiflavone (**33**) (Figure 4.13).

The presence of hydroxyl group in morelloflavone (**12**) may have contributed to the strong antioxidant activity in the compound as discovered in this study. Vice versa, the lower antioxidant activity in both GB1a (**18**) and volkensiflavone (**33**) may have been due to the lack of hydroxyl group. Previously, it was revealed that the strong radical scavenging activity in morelloflavone (**12**) is due to the presence of the ortho 3′,4′-dihydroxyl groups, meta 5,7-dihydroxy moiety and 2,3-double bond with 4-keto group at

II as they are the bases of scavenging free radical (Rice-Evans et al., 1997; Rice-Evans et al., 1996). Other reports have also shown that the number and location of the hydroxyl group in the structure may influence the free radical scavenging activity of the compound (Zhao et al., 2010; Cao et al., 1997).

In this study, volkensiflavone (**33**) showed better activity than GB1a (**18**) in DPPH assay, although the opposite was observed in FRAP assay albeit with insignificant differences. Antioxidant activity of morelloflavone (**12**), GB1a (**18**), and volkensiflavone (**33**) may be explained by the presence of flavanone and flavone in the structure of these compounds. As reported by Muriithi and co-authors, biflavonoids with both flavanone and flavone units such as morelloflavone (**12**) and volkensiflavone (**33**) showed better activity than those with two units of flavanones, such as GB1a (**18**) (Muriithi et al., 2016).

While the structure-activity relationship of various flavonoids particularly in relation to their antioxidant activity has been extensively studied, reports on similar research on xanthones are few (Panda et al., 2013). Scavenging activity in xanthones on several oxidizing species such as superoxide anion, hydroxyl and peroxyl radicals have been reported (Panda et al., 2013). The current study found that 1,3,5- trihydroxy-2-methoxyxantone (**29**), isolated from the roots of *G. atroviridis* exhibited good antioxidant activity in DPPH and FRAP assays.

4.5 Preliminary Biological Assay Screening of *Garcinia atroviridis* roots

Apart from EtOAc extract, the roots of *G. atroviridis* were also extracted using hexane, DCM, butanol and MeOH. A preliminary biological screening of these root extracts with different polarity was conducted. Similar procedures used on EtOAc extract were applied in evaluating the biological properties of the root extracts in different solvents with different polarity. Antibacterial study was carried out using disc diffusion method. TPC of the root extracts was evaluated using FC reagent method, whereas their antioxidant activity was assessed through DPPH and FRAP assays.

4.5.1 Antibacterial Activity

The data indicated that all extracts except for MeOH inhibited the growth of Gram-positive bacteria, namely *B. cereus* and *S. aureus* tested in this study. None of the extracts exhibited antibacterial activity towards Gram-negative bacteria namely *E.coli*

and *P. vulgaris*. Butanol extract showed the highest activity against *B. cereus* and *S. aureus*, creating zone of inhibition with a diameter of 10.3 and 10.7 mm, respectively; this was followed by DCM and hexane extracts. However, antibacterial activity of these extracts were lower than the standard antibiotics used in this study (Table 4.10).

There is no prior study that compares the antibacterial activities of root extract from *G. atroviridis* in different solvents, although the MeOH extract was evaluated by Mackeen et al. (2000). It was found in this study that the MeOH extract showed no antibacterial activity against all microorganisms tested, which contradicted the findings reported by Mackeen et al. (2000). In the study, strong inhibition was observed against both Gram-positive and Gram-negative bacteria namely *Bacillus subtilis* B28, *B. subtilis* B29, methicillin-resistant *S. aureus*, *E. coli* and *P. aeruginosa* at the MID of 15.6 µg/disc.

Table 4.10 Inhibition zones of *G. atroviridis* roots extracts

Extract (20 mg/mL)	**Inhibition zone (mm)**			
	B. cereus	*S. aureus*	*E. coli*	*P. vulgaris*
Hexane extract	9.0 ± 0	9.3 ± 0.577	-	-
DCM extract	10.3 ± 1.155	9.7 ± 0.577	-	-
Butanol extract	10.3 ± 2.309	10.7 ± 1.528	-	-
MeOH extract	-	-	-	-
(+) Chloramphenicol (30 µg)	24.7 ± 0.577	NT	20.0 ± 1	NT
(+) Gentamicin (120 µg)	NT	18.7 ± 0.577	NT	33.3 ± 1.528
(-) Acetone	-	-	-	-
(-) Hexane	-	-	-	-

Data was expressed as mean (*n*=3) ± SD; -: no inhibition zone; NT: not tested; (+): positive control; (-): negative control

The contradictory results obtained from the assessment of antibacterial activity in the extracts of *G. atroviridis* may have been due to several factors. According to Kokate et al. (2004), environmental factors such as climate, altitude, rainfall and other conditions may affect the growth of the plant, which alter the chemical compositions in the plant despite being in the same species or from the same country. These factors may have caused major variations in the constituents present in the plants, which influence its bioactivities. Method of extraction used, the harvesting time and chemical characteristics of the compounds i.e. polarity, concentration and pH, may also influence the antibacterial activity of the extracts (Radulovic et al., 2013).

Inability of the plant extracts to inhibit the growth of Gram-negative bacteria may be due to the cell wall structure in the bacteria. The cell wall of Gram-positive bacteria consists of numerous layer of peptidoglycan, which is usually permeable to various compounds. On the other hand, cell wall of Gram-negative bacteria is more complex, as it is composed of single layer of peptidoglycan and a layer of lipoproteins and lipopolysaccharides that act as an outer membrane, which protect the cell from of penetration of foreign compounds (Stefanović, 2018).

4.5.2 Antioxidant Activity

4.5.2.1 TPC via FC reagent Assay

Generally, all extracts contained significant phenolic content as presented in Table 4.11. As shown in the result, butanol extract had the highest TPC with a value of 1071.4 ± 35.286 mg GAE/g, followed by DCM extract with 791.9 ± 48.901 mg GAE/g, hexane with 262.1 ± 18.028 mg GAE/g and MeOH extract with 238.3 ± 7.835 mg GAE/g. Low phenolic content in MeOH extract may be due to high recovery of polar non-phenolic compounds such as carbohydrates (Ramirez et al., 2018). Low TPC was also obtained from hexane extract, as the extract might have largely recovered non-phenolic compounds including waxes, terpenes, or steroids (Ramirez et al., 2018).

Table 4.11 TPC activity of *G. atroviridis* roots extracts

Extracts	TPC value (mg GAE/g)
Hexane	262.1 ± 18.028
DCM	791.9 ± 48.901
EtOAc	568.6 ± 25.724
Butanol	1071.4 ± 35.286
MeOH	238.3 ± 7.835

Data was expressed as mean (n=3) ± SD

4.5.2.2 DPPH Assay

Following the determination of significant phenolic content in the hexane, DCM, butanol, and MeOH extracts, further evaluation on antioxidant activities in all extracts was carried out. The results demonstrated that all extracts showed similar increasing trend in antioxidant activity with increase in concentration, with the exception of some irregularities as shown in Table 4.12.

Further evaluation via DPPH assay showed that butanol extract exhibited the highest free radical scavenging activity followed by DCM extract with IC_{50} of 50.6 ± 13.213 and 53.17 ± 8.761 µg/mL, respectively. Meanwhile, MeOH and hexane extracts demonstrated low radical scavenging activity with IC_{50} of more than 100 µg/mL. However, none of the extracts outperformed the radical scavenging activity of ascorbic acid, AA (IC_{50} of 13.2 ± 0.021 µg/mL), which was used as a standard in this assay. The results demonstrated that the radical scavenging activity of the extracts did not correlate with TPC, with the exception of butanol extract. Data collected on IC_{50} of extracts from the roots of *G. atroviridis* in different solvents with different polarity are summarized in Table 4.13.

As mentioned earlier, there were inconsistency observed in inhibition activity of the extracts. This inconsistency could be due to some errors that occurred while running the experiment. As all samples and reagents used in this test were diluted in solvent, evaporation might take place due to prolonged storage. This would have altered the concentration of samples thus interfering with the results.

Table 4.12 Effect of *G. atroviridis* roots extracts on DPPH radicals

Conc. (µg/mL)	**Percentage of inhibition (%)**				
	Hexane	DCM	Butanol	MeOH	AA
7.8125	5.9±0.006	14.7±0.011	11.0±0.033	6.0±0.009	51.7±0.041
15.625	10.1±0.017	19.7±0.017	206±0.022	11.4±0.010	86.1±0.007
31.25	15.2±0.011	31.1±0.005	30.8±0.011	17.5±0.032	86.6±0.005
62.5	24.1±0.014	52.0±0.020	50.8±0.024	26.6±0.031	86.8±0.006
125	39.1±0.011	80.0±0.012	77.8±0.015	41.4±0.014	87.3±0.007
250	61.6±0.013	83.0±0.008	82.7±0.008	60.8±0.027	86.7±0.003
500	82.4±0.005	83.0±0.003	83.0±0.002	81.7±0.004	87.3±0.005
1000	81.5±0.005	81.4±0.004	82.0±0.004	83.0±0.005	87.6±0.004

Data was expressed as mean (*n*=3) ± SD

Table 4.13 IC_{50} (µg/mL) of *G. atroviridis* roots extracts

Extracts	**IC_{50} (µg/mL)**
Hexane	131.8 ± 3.112
DCM	53.2 ± 8.761
Butanol	50.6 ± 13.213
MeOH	124.7 ± 11.112
AA (control)	13.2 ± 0.021

Data was expressed as mean (*n*=3) ± SD

4.5.2.3 FRAP Assay

Currently, there is no study reported on FRAP activity of root extracts from *G. atroviridis* in solvents with different polarity. As part of our continuing efforts to search for new antioxidant agents, this study reported the first FRAP activity of root extracts from *G. atroviridis* in hexane, DCM, butanol, and MeOH. Table 4.14 summarizes the FRAP activity of different root extracts from *G. atroviridis*. The higher the FRAP value, the greater the antioxidant activity. All extracts demonstrated similar increasing trend in activity with increase in concentration. Significant FRAP values were obtained for all extracts. It was found that butanol extract exhibited the highest reducing power followed by DCM, hexane and MeOH extracts, with FRAP values of 566.02 ± 36.119, 370.30 ± 15.358, 265.2 ± 25.433 and 122.07 ± 7.321 mg AAE/g, respectively. The differences in the values were significant, which correlated with TPC in the extract (Table 4.11).

Table 4.14 FRAP activity of *G. atroviridis* roots extracts

Extracts	FRAP value (mg AAE/g)
Hexane	265.2 ± 25.433
DCM	370.3 ± 15.358
Butanol	566.0 ± 36.119
MeOH	122.1 ± 7.321

Data was expressed as mean (*n*=3) ± SD

4.5.2.4 Correlation between TPC and Antioxidant Assays

Quantitative analysis using Pearson's correlation coefficients (r) was carried out to investigate the correlation between phenolic content and the antioxidant activities in the root extracts of *G. atroviridis*. The TPC determined in all extracts from *G. atroviridis*, and values obtained from DPPH and FRAP assays were used to calculate the correlation. For determination of correlation between TPC and DPPH, $1/IC_{50}$ was calculated since it indicates parallelism with antioxidant activity (Li et al., 2009). The correlation was higher between TPC and FRAP (r = 0.9385) compared to TPC and DPPH (r = 0.8760) as shown in Figure 4.16 and Figure 4.17, respectively. It is well established that the antioxidant activity of plant sample usually correlate with TPC due to their redox characteristics, which include roles as hydrogen donors, singlet oxygen quenchers and reducing agents

(Chang et al., 2001). The positive strong correlations between TPC with both assays suggested the role phenolic compounds in antioxidant activities of the root extracts from *G. atroviridis*.

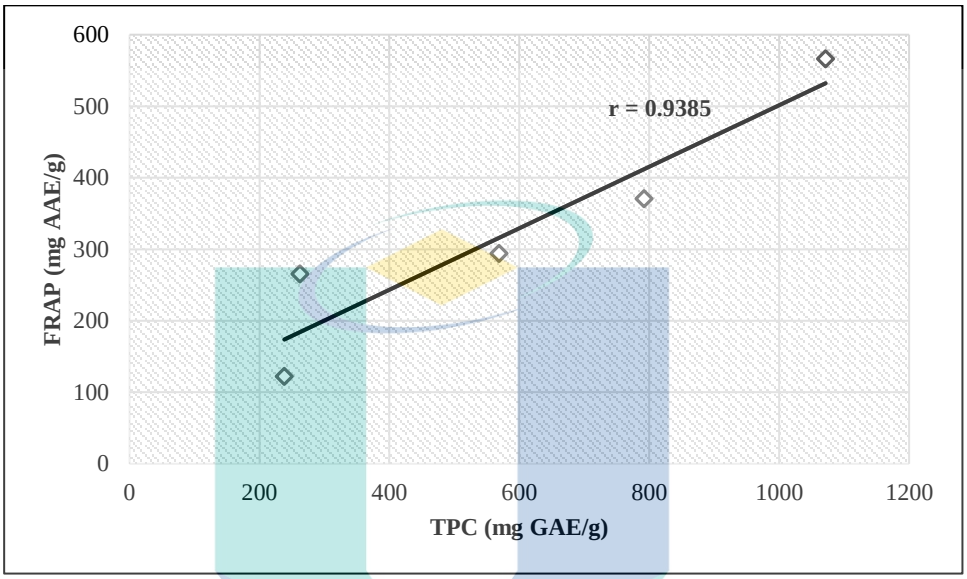


Figure 4.16 Correlation between TPC and FRAP assay

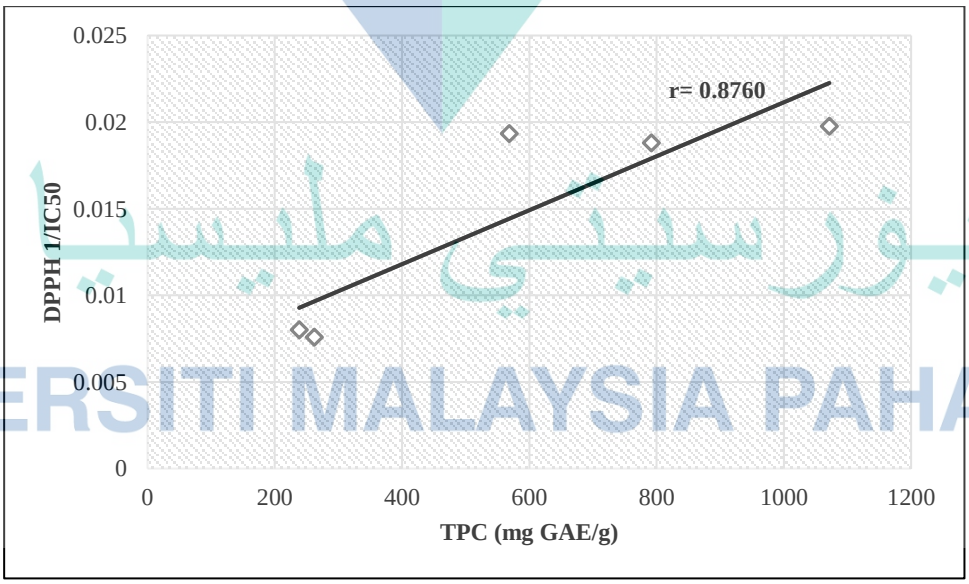


Figure 4.17 Correlation between TPC and DPPH assay

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study focused on the root extracts of *G. atroviridis* following the previous study which reported that the highest antibacterial and antioxidant activities were observed in the root extract of *G. atroviridis* as compared to extracts from other parts of the plant (Mackeen et al., 2000). Root extracts from *G. atroviridis* were prepared via solvent extraction using different solvents with different polarity, which yielded hexane (8.72 g, 0.5 %), DCM (4.45 g, 0.25 %), EtOAc (9.79 g, 0.56 %), butanol (1.08 g, 0.06 %), and MeOH (45.45 g, 2.58 %) extracts.

Preliminary biological studies carried out on EtOAc extract revealed the positive antibacterial activity against Gram-positive bacteria namely *B. cereus* and *S. aureus* with inhibition zone of 7.0 mm in diameter, determined via disc diffusion method. EtOAc extract recovered significant phenolic content with calculated value of 568.6 ± 25.724 mg GAE/g; further evaluation on its antioxidant activity was carried out, which revealed moderate DPPH and FRAP activities with IC_{50} of 51.7 ± 2.180 µg/mL and 294.0 ± 20.173 mg AAE/g, respectively. The positive antibacterial and antioxidant activities of EtOAc extract from the roots of *G. atroviridis* observed in this study supported the previous findings, which demonstrated strong biological activities in the root extracts from *G. atroviridis*.

Isolation of individual compounds from EtOAc extract was conducted to identify the compounds which may contribute to bioactivities observed in the extract. Five individual compounds with a yield of 7.1 mg (0.4×10^{-3} %), 15 mg (0.85×10^{-3} %), 17.3 mg (0.98×10^{-3} %), 15.4 mg (0.88×10^{-3} %), and 17.4 mg (0.99×10^{-3} %) were obtained using several chromatography techniques, which were later identified and characterized as atrovirisidone (**7**), morelloflavone (**12**), GB1a (**18**), 1,3,5- trihydroxy-2-methoxyxantone (**29**), and volkensiflavone (**33**), respectively. The presence of volkensiflavone (**33**) in *G. atroviridis* was reported for the first time. All individual compounds isolated in this study belong to phenolic group thus supporting the previous

report on the capacity of EtOAc for effective recovery of phenolic compounds. The isolated constituents were further subjected to biological assays using the similar protocol as used on EtOAc extract.

Antibacterial assay on isolates from EtOAc showed that GB1a (**18**) and volkensiflavone (**33**) were active against *S. aureus* with inhibition zone of 10.3 mm and 9.0 mm in diameter, respectively. All compounds showed no inhibition towards Gram-negative bacteria tested in this study. The inability to inhibit the Gram-negative bacteria might due to the complexity of the cell wall structure of the bacteria. In antioxidant assay, morelloflavone (**12**) showed the highest DPPH scavenging activity compared to other compounds with IC_{50} of 20.3 ± 1.667 µg/mL, which was comparable to AA, with IC_{50} of 13.2 ± 0.021 µg/mL. The result was in accordance with previous studies that stated the efficiency of this compound to scavenge DPPH radicals. Other compounds showed low antioxidant activity with IC_{50} of more than 200 µg/mL. Generally, all compounds exhibited significant FRAP activity with the highest reducing power shown by 1,3,5-trihydroxy-2-methoxyxantone (**29**), with a value of 1643.3 ± 44.623 mg AAE/g.

The presence of additional hydroxyl group in morelloflavone (**12**) compared to other biflavonoids: GB1a (**18**) and volkensiflavone (**33**), attributed to its strong antioxidant properties. Meanwhile, xanthones was reported to scavenge several oxidizing species hence displayed significant antioxidant activity. Overall, the results demonstrated that the isolated compounds may contribute to the antibacterial and antioxidant properties of EtOAc extract from the roots of *G. atroviridis*.

Preliminary biological screening of hexane, DCM, butanol and MeOH extracts from the roots of *G. atroviridis* was also conducted. Antibacterial activity was observed in all extracts except for MeOH extract. Butanol extract showed the highest activity against *B. cereus* and *S. aureus*, with inhibition zone of 10.3 and 10.7 mm in diameter, respectively. Significant phenolic content was recovered in all extracts, of which butanol extract contained the highest TPC with calculated value of 1071.4 ± 35.286 mg GAE/g. Antioxidant properties of these root extracts were further evaluated. The DPPH radical scavenging activity was the highest in butanol extract with IC_{50} of 50.6 ± 13.213 µg/mL. Butanol extract also exhibited the highest FRAP activity with a value of 566.02 ± 36.119 mg AAE/g. Correlation between phenolic content in hexane, DCM, EtOAc, butanol and MeOH extracts with antioxidant activities was calculated using Pearson's correlation coefficients (r). Strong positive correlation between TPC with both DPPH (r = 0.8760)

and FRAP (r = 0.9385) assays was obtained, suggesting significant role of phenolic compounds in antioxidant activity of root extracts from *G. atroviridis*.

In conclusion, chemical investigation carried out on EtOAc extract from the roots of *G. atroviridis* yielded five phenolic compounds of which one new compound, volkensiflavone (**33**) was isolated. Taken together, the antibacterial and antioxidant activities may be attributed to the presence of biflavonoids and xanthones in the root extracts of *G. atroviridis*.

5.2 Recommendation

There are some recommendations that can be considered for future works based on the findings from this study. This study revealed the potential antioxidant properties of some isolates from EtOAc extract. Further evaluation on antioxidant activities can be carried out using techniques such as trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC), and total radical-trapping antioxidant parameter (TRAP), to investigate the mechanisms in antioxidant activity of the isolates. Detailed antibacterial study to determine the MIC of the extracts will provide comprehensive information about antibacterial activity of root extracts and the individual isolated compounds from *G. atroviridis*. In addition, other biological properties of the extracts and compounds can be investigated to determine potential biological activities such as anti-inflammatory, anti-diabetic, and anti-tumor.

Preliminary biological screening of root extracts from *G. atroviridis* in different solvents revealed the potential antibacterial and antioxidant properties of butanol extract, which may be due to the presence of biologically valuable constituents in the extract. Further isolation of individual compound from the extract should be carried to gain comprehensive understanding on its potential bioactivities. Other isolation techniques can be used to isolate bioactive compounds from butanol extract to improve efficiency in purification process.

Although results obtained from *in vitro* study cannot be extrapolated to represent *in vivo* applications, the finding does provide preliminary understanding and room for improvement in regard to potential antibacterial and antioxidant activities of the extract *in vivo*. Further *in vivo* assessment, particularly on antioxidant potential of the chemical constituents from the extract should be carried out to investigate the biological effects on human.

REFERENCES

Abbas, O., Compère, G., Larondelle, Y., Pompeu, D., Rogez, H., & Baeten V. (2017). Phenolic compound explorer: A mid-infrared spectroscopy database. *Vibrational Spectroscopy*, *92*, 111-118.

Acuna, U.M., Jancovski, N., & Kennelly, E.J. (2009). Polyisoprenylated benzophenones from Clusiaceae: potential drugs and lead compounds. *Current Topics in Medicinal Chemistry*, *9*(16), 1560-1580.

Afsar, T., Razak, S., Shabbir, M., & Khan, M. R. (2018). Antioxidant activity of polyphenolic compounds isolated from ethyl-acetate fraction of *Acacia hydaspica* R. Parker. *Chemistry Central Journal*, *12*(1), 5.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightdoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, *6*(4), 42.

Amran, A. A., Zaiton, Z., Faizah, O., & Morat, P. (2009). Effects of *Garcinia atroviridis* on serum profiles and atherosclerotic lesions in the aorta of guinea pigs fed a high cholesterol diet. *Singapore Medical Journal*. *50*(3), 295-299.

Antolovich, M., Prenzler, P. D., Patsalides, E., McDonald, S., & Robards, K. (2002). Methods for testing antioxidant activity. *Analyst*, *127*(1), 183-198.

Aravind, A. A., & Rameshkumar, K. B. (2016). Antioxidant and cytotoxic activities of Fukugiside-The major biflavonoid from *Garcinia travancorica* Bedd. *Diversity of Garcinia species in the Western Ghats: Phytochemical Perspective*, 187-195.

Aris, S. R. S., Mustafa, S., Ahmat, N., Jaafar, F. M., & Ahmad, R. (2009). Phenolic content and antioxidant activity of fruits of *Ficus deltoidea var angustifolia* sp. *Malaysian Journal of Analytical Sciences*, *13*(2), 146-150.

Atta-ur-Rahman. (1989). *Basic Principles of Modern NMR Spectroscopy* in One and two dimensional NMR spectroscopy (pp 1-75). Elsevier Science.

Basri, D. F., Sharif, R., Morat, P., & Latip, J. (2005). Evaluation of antimicrobial activities of the crude extracts from *Garcinia atroviridis* and *Solanum torvum*. *Malaysian Journal of Science*, *24*(1), 233-238.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, *239*(1), 70-76.

Biesaga, M., & Pyrzynska, K. (2013). Stability of bioactive polyphenols from honey during different extraction methods. *Food Chemistry*, *136*(1), 46-54.

Bors, W., Heller, W., Michel, C., & Saran, M. (1990). Flavonoids as antioxidants: Determination of radical scavenging efficiencies. *Methods in Enzymology*. *186*, 343-355.

Bors, W., Michel, C., & Schikora, S. (1995). Interaction of flavonoids with ascorbate and determination of their univalent redox potentials: A pulse radiolysis study. *Free Radical Biology and Medicine, 19,* 45-52.

Braithwaite, A., & Smith, F. J. Chromatographic Methods. 1999. Blackie Academic & Professional, New York. Chicago

Bucar, F., Wube, A., & Schmid, M. (2013). Natural product isolation- How to get from biological material to pure compounds. *Natural Product Reports*.*30*(4), 525-545.

Burkill, I.H. (1966). A Dictionary of the Economic Products of the Malay Peninsula. Crown Agent, London.

Cao, G., Sofic, E., & Prior, R. L. (1997). Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free Radical Biology and Medicine*, *22*(5), 749-760.

Chang, S. T., Wu, J. H., Wang, S. Y., Kang, P. L., Yang, N. S., & Shyur, L. F. (2001). Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. *Journal of Agricultural and Food Chemistry*, *49*(7), 3420-3424.

Chari, V. M., Ilyas, M., Wagner, H., Neszmelyi, A., Fa-Ching, C., Li-Kuang, C., Yu-Chin, L., & Yuh-Meei, L. (1977). ^{13}C-NMR spectroscopy of biflavanoids. *Phytochemistry*, *16* (8), 1273-1278.

Cheng, X., Zhang, M., Xu, B., Adhikari, B., & Sun, J. (2015). The principles of ultrasound and its application in freezing related processes of food materials: A review. *Ultrasonics Sonochemistry*, *27*, 576-585.

Christophoridou, S., Dais, P., Tseng, L. H., & Spraul, M. (2005). Separation and identification of phenolic compounds in olive oil by coupling high-performance liquid chromatography with postcolumn solid-phase extraction to nuclear magnetic resonance spectroscopy (LC-SPE-NMR). *Journal of Agricultural and Food Chemistry*, *53*, 4667-4679.

Chung, H. S., Chang, L. C., Lee, S. K., Shamon, L. A., van Breeman, R. B., Mehta, R. G., Farnsworth, N. R., Pezzuto, J. M., & Kinghorn, A. D. (1999). Flavonoids constituents of *Chorizanthe diffusa* with potential cancer chemopreventive activity. *Journal of agricultural and Food Chemistry*, *47*(1), 36-41.

Clark-Lewis, J. W. (1968). Flavan derivatives. XXI. Nuclear magnetic resonance spectra, configuration, and conformation of flavan derivatives. *Australian Journal of Chemistry*, *21*(8), 2059-2075.

Compagnone, R. S., Suarez, A. C., Leitao, S. G., & Delle Monache, F. (2008). Flavonoids, benzophenones and a new euphane derivative from *Clusia columnaris* Engl. *Revista Brasileira de Farmacognosia*, *18*(1), 6-10.

Corbin, C., Fidel, T., Leclerc, E. A., Barakzoy, E., Sagot, N., Falguiéres, A., Renouard, S., Blondeau, J.P., Ferroud, C., Doussot, J., & Lainé, E. (2015). Development and validation of an efficient ultrasound assisted extraction of phenolic compounds from flax (*Linum usitatissimum* L.) seeds. *Ultrasonics Sonochemistry*, *26*, 176-

185.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules* , *15*(10), 7313-7352

Deachathai, S., Mahabusarakam, W., Phongpaichit, S., & Taylor, W. C. (2005). Phenolic compounds from the fruit of *Garcinia dulcis*. *Phytochemistry*, *66*(19), 2368-2375.

Deachathai, S., Phongpaichit, S., & Mahabusarakam, W. (2008). Phenolic compounds from the seeds of *Garcinia dulcis*. *Natural product research*, *22*(15), 1327-1332.

Deng, J., Xu, Z., Xiang, C., Liu, J., Zhou, L., Li, T., Yang, Z., & Ding, C. (2017). Comparative evaluation of maceration and ultrasonic-assisted extraction of phenolic compounds from fresh olives. *Ultrasonics Sonochemistry*, *37*, 328-334.

Diderot, N.T., Silvere, N., & Etienne, T. (2006). Xanthones as therapeutic agents, chemistry and pharmacology. *Advances in Phytomedicine*, *2*, 273–298.

Djoufack, G. L. N., Valant-Vetschera, K. M., Schinnerl, J., Brecker, L., Lorbeer, E., & Robien, W. (2010). Xanthones, biflavanones and triterpenes from *Pentadesma grandifolia* (Clusiaceae): structural determination and bioactivity. *Natural Product Communications*, *5*(7), 1934578X1000500714.

Duddeck, H., Snatzke, G., & Yemul, S. S. (1978). ^{13}C NMR and CD of some 3,8″-biflavanoids from *Garcinia* species and of related flavanones. *Phytochemistry*, *17*(8), 1369-1373.

Dweck, A.C. (1999). A Review of Asam Gelugur (*Garcinia atroviridis* Griff. ex. T. Anders). Available in: http://www.dweckdata.com/research_files/garcinia_atroviridis.pdf

Ee, G. C. L., Daud, S., Izzaddin, S. A., & Rahmani, M. (2008). *Garcinia mangostana*: a source of potential anti-cancer lead compounds against CEM-SS cell line. *Journal of Asian Natural Products Research*, *10*(5), 475-479.

Eskilsson, C. S., & Bjorklund, E. (2000). Analytical-scale microwave-assisted extraction. *Journal of Chromatography*, *902*, 227–250.

Fa-Ching, C., Yuh-Meei, L., & Jeng-Ching, H. (1975). Phenolic compounds from the heartwood of *Garcinia multiflora*. *Phytochemistry*, *14* (1), 300-303.

Fan, R., Yuan, F., Wang, N., Gao, Y., & Huang, Y. (2015). Extraction and analysis of antioxidant compounds from the residues of *Asparagus officinalis* L. *Journal of Food Science and Technology*, *52*(5), 2690-2700.

Fang, X., Wang, J., Hao, J., Hao, J., Li, X., & Guo, N. (2015). Simultaneous extraction, identification and quantification of phenolic compounds in *Eclipta prostrata* using microwave-assisted extraction combined with HPLC-DAD-ESI-MS/MS. *Food Chemistry*, *188*, 527-536. Garcia-Salas, P., Morales-Soto, A., Segura-Carretero, A., & Fernández-Gutiérrez, A.(2010). Phenolic-compound extraction systems for fruit and vegetable samples. *Molecules, 15(12), 8813-8826.*

Garcia-Salas, P., Morales-Soto, A., Segura-Carretero, A., & Fernández-Gutiérrez, A.(2010). Phenolic-compound extraction systems for fruit and vegetable samples. *Molecules, 15(12), 8813-8826.*

Genwali, G. R., Acharya, P. P., & Rajbhandari, M. (2013). Isolation of gallic acid and estimation of total phenolic content in some medicinal plants and their antioxidant activity. *Nepal Journal of Science and Technology*, *14*(1), 95-102.

Gil, B., Sanz, M. J., Terencio, M. C., Gunasegaran, R., Payá, M., & Alcaraz, M. J. (1997). Morelloflavone, a novel biflavonoid inhibitor of human secretory phospholipase A2 with anti-inflammatory activity. *Biochemical pharmacology*, *53*(5), 733-740.

Godavari, A., & Amutha, K. (2017). In vitro Antidiabetic Activity of *Garcinia mangostana* by Enzymatic Inhibition Assay. *Research Journal of Pharmacy and Technology*, *10*(2), 508-512.

Günther, H. (2013). NMR spectroscopy: basic principles, concepts and applications in chemistry. John Wiley & Sons.

Hemshekhar, M., Sunitha, K., Santhosh, M.S., Devaraja, S., Kemparaju, K., Vishwanath, B.S., Niranjana, S.R. & Girish, K.S. (2011). An overview on genus Garcinia: phytochemical and therapeutical aspects. *Phytochemistry Reviews*, *10*(3), 325-351.

Henderson, T., Nigam, P. S., & Owusu-Apenten, R. K. (2015). A universally calibrated microplate ferric reducing antioxidant power (FRAP) assay for foods and applications to Manuka honey. *Food chemistry*, *174*, 119-123.

Herbin, G. A., Jackson, B., Locksley, H. D., Scheinmann, F., & Wolstenholme, W. A. (1970). The biflavonoids of *Garcinia volkensii* (Guttiferae). *Phytochemistry*, *9*(1), 221-226.

Hostettmann, K., Marston, A., & Hostettmann, M. (1998). *Preparative chromatography techniques*. Berlin: Springer. (revised version)

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, *53*(6), 1841-1856.

Hudson, B. J. F., & Lewis, L. I. (1983). Polyhydroxy flavonoid antioxidants for edible oils. Structural criteria for activity. *Food Chemistry*, *10*, 47-55.

Hu, J., Chen, J., Zhao, Y., Wang, R., Zheng, Y., & Zhou, J. (2005). Chemical constituents from fruit hulls of *Garcinia mangostana* (Guttiferae). *Acta Botanica Yunnanica*, *28*(3), 319-322.

Hui, A. C., Foon, C. S., & Hock, C. C. (2017). Antioxidant Activities of *Elaeis Guineensis* Leaves. *Journal of Oil Palm Research*, *29*(3), 343-351.

Hunger, M., & Weitkamp, J. (2001). In situ IR, NMR, EPR, and UV/Vis spectroscopy: Tools for new insight into the mechanisms of heterogeneous catalysis. *Angewandte Chemie International Edition*, *40*(16), 2954-2971.

Ibrahim, S. R., Mohamed, G. A., Elfaky, M. A., Zayed, M. F., El-Kholy, A. A., Abdelmageed, O. H., & Ross, S. A. (2018). Mangostanaxanthone VII, a new cytotoxic xanthone from *Garcinia mangostana*. *Zeitschrift für Naturforschung C*, *73*(5-6), 185-189.

Ignat, I., Volf, I., & Popa, V. I. (2011). A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chemistry*, *126*, 1821-1835.

Inouye, S., Yamaguchi, H., & Takizawa, T. (2001). Screening of the antibacterial effects of a variety of essential oils on respiratory tract pathogens, using a modified dilution assay method. *Journal of Infection and Chemotherapy*, *7*(4), 251-254.

Israf, D. A., Tham, C. L., Syahida, A., Lajis, N. H., Sulaiman, M. R., Mohamad, A. S., & Zakaria, Z. A. (2010). Atrovirinone inhibits proinflammatory mediator synthesis through disruption of NF-κB nuclear translocation and MAPK phosphorylation in the murine monocytic macrophage RAW 264.7. *Phytomedicine*, *17*(10), 732-739.

Ito, C., Itoigawa, M., Mishina, Y., Tomiyasu, H., Litaudon, M., Cosson, J. P.,Mukainaka, T., Tokuda, H., Nishino, H., & Furukawa, H. (2001). Cancer chemopreventive agents. New depsidones from *Garcinia* plants. *Journal of Natural Products*, *64*(2), 147-150.

Ito, C., Miyamoto, Y., Nakayama, M., Kawai, Y., Rao, K. S., & Furukawa, H. (1997). A novel depsidone and some new xanthones from *Garcinia* species. *Chemical and Pharmaceutical Bulletin*, *45*(9), 1403-1413.

Jackson, B., Locksley, H. D., Scheinmann, F., & Wolstenholme, W. A. (1967). The isolation of a new series of biflavanones from the heartwood of *Garcinia buchananii*. *Tetrahedron Letters*, *8*(9), 787–792.

Jackson, B., Locksley, H. D., Scheinmann, F., & Wolstenholme, W. A. (1971). Extractives from Guttiferae. Part XXII. The isolation and structure of four novel biflavanones from the heartwoods of *Garcinia buchananii* Baker and *G. eugeniifolia* Wall. *Journal of the Chemical Society C: Organic*, 3791-3804.

Jadid, N., Hidayati, D., Hartanti, S. R., Arraniry, B. A., Rachman, R. Y., & Wikanta, W. (2017). Antioxidant activities of different solvent extracts of *Piper retrofractum* Vahl. using DPPH assay. In *AIP Conference Proceedings* (Vol. 1854, No. 1, p. 020019). AIP Publishing.

Jahromi, S. G. (2019). Extraction Techniques of Phenolics Compounds from Plants. In *Plant Physiological Aspects of Phenolic Compounds*. IntechOpen.

Jalil, J., Jantan, I., Ghani, A. A., & Murad, S. (2012). Platelet-activating factor (PAF) antagonistic activity of a new biflavonoid from *Garcinia nervosa* var. pubescens king. *Molecules*, *17*(9), 10893-10901.

Jamila, N., Khairuddean, M., Khan, S.N. and Khan, N. (2014). Complete NMR assignments of bioactive rotameric (3→ 8) biflavonoids from the bark of *Garcinia hombroniana*. *Magnetic Resonance in Chemistry*, 52(7), 345-352.

Jamila, N., Khairuddean, M., Lai, C.S., Osman, H., Wong, K.C., Vikneswaran, M., and Khaw, K.Y. (2013). Anti-oxidant, anti-cholinesterase and anti bacterial activities of the bark extracts of *Garcinia hombroniana. African Journal of Pharmacy and Pharmacology*, 7(8), 454-459.

Jantan, I., Jumuddin, F.A., Saputri, F. C., and Rahman, K. (2011). Inhibitory effects of the extracts of *Garcinia* species on human low-density lipoprotein peroxidation and platelet aggregation in relation to their total phenolic contents. *Journal of Medicinal Plants Research*, *5*(13), 2699-2709.

Jena, B. S., Jayaprakasha, G. K., Singh, R. P., & Sakariah, K. K. (2002). Chemistry and biochemistry of (−)-hydroxycitric acid from *Garcinia*. *Journal of Agricultural and Food Chemistry*, *50*(1), 10-22.

Jones, W. P., & Kinghorn, A., D. (2012). Extraction of Plant Secondary Metabolites. In *Natural Products Isolation* (pp. 341-366). Humana Press.

Joshi, B. S., Kamat, V. N., & Viswanathan, N. (1970). The isolation and structure of two biflavones from *Garcinia talbotii*. *Phytochemistry*, *9*(4), 881-8.

Jung, H. A., Su, B. N., Keller, W. J., Mehta, R. G., & Kinghorn, A. D. (2006). Antioxidant xanthones from the pericarp of *Garcinia mangostana* (Mangosteen). *Journal of Agricultural and Food Chemistry*, *54*(6), 2077-2082.

Kaennakam, S., Siripong, P., & Tip-pyang, S. (2015). Kaennacowanols A-C, three new xanthones and their cytotoxicity from the roots of *Garcinia cowa*. *Fitoterapia*, *102*, 171-176.

Karanjgaokar, C. G., Radhakrishnan, P. V., & Venkataraman, K. (1967). Morelloflavone, a 3-(8)flavonylflavanone, from the heartwood of *Garcinia morella*. *Tetrahedron Letters*, *8*(33), 3195-3198.

Klaiklay, S., Sukpondma, Y., Rukachaisirikul, V., Hutadilok-Towatana, N. & Chareonrat, K. (2011). Flavanone glucuronides from the leaves of *Garcinia prainiana*. *Canadian Journal of Chemistry*, 89(4), 461-464.

Kokate, C. K., Purohit, A. P., & Gokhale, S. B. (2004). *Practical Pharmacognosy*; 2nd edition. Vallabh Prakashan, New Delhi, 466-470.

Kosin, J., Ruangrungsi, N., Ito, C., & Furukawa, H. (1998). A Xanthone from *Garcinia atroviridis*. *Phytochemistry*. *47* (6), 1167-1168.

Liazid, A., Palma, M., Brigui, J., & Barroso, C. G. (2007). Investigation on phenolic compounds stability during microwave-assisted extraction. *Journal of Chromatography A*, *1140*(1-2), 29-34.

Li, Y., Chen, Y., Xiao, C., Chen, D., Xiao, Y., & Mei, Z. (2014). Rapid screening and identification of α-amylase inhibitors from *Garcinia xanthochymus* using enzyme-immobilized magnetic nanoparticles coupled with HPLC and MS. *Journal of Chromatography B*, *960*, 166-173.

Li, H., Deng, Z., Zhu, H., Hu, C., Liu, R., Young, J. C., & Tsao, R. (2012). Highly pigmented vegetables: Anthocyanin compositions and their role in antioxidant activities. *Food Research International*, *46*(1), 250-259.

Li, X., Wu, X., & Huang, L. (2009). Correlation between antioxidant activities and phenolic contents of radix Angelicae sinensis (Danggui), *Molecules*, *14*(12), 5349-5361.

Lim, T. K. (2012). Edible medicinal and non-medicinal plants. Springer. 2, 21-28.

Lin, Y. M., Anderson, H., Flavin, M. T., Pai, Y. H. S., Mata-Greenwood, E., Pengsuparp, T., Pezzuto, J.M., Schinazi, R.F., Hughes, S.H., & Chen, F. C. (1997). In vitro anti-HIV activity of biflavonoids isolated from *Rhus succedanea* and *Garcinia multiflora*. *Journal of Natural Products*, *60*(9), 884-888.

Luque-Garcıa, J. L., & De Castro, M. L. (2003). Ultrasound: a powerful tool for leaching. TrAC *Trends in Analytical Chemistry*, *22*(1), 41-47.

Mackeen, M.M., Ali, A.M., Lajis, N.H., Kawazu, K., Kikuzaki, H., & Nakatani, N. (2002). Antifungal garcinia acid esters from the fruits of *Garcinia atroviridis*. *Zeitschrift fur Naturforschung C. 57*(3/4), 291-295.

Mackeen, M.M., Ali, A.M., Lajis, N.H., Kawazu, K., Hassan Z., Mohamed H., Mohidin A., Lim Y.M., & Mariam, S. (2000). Antimicrobial, antioxidant, antitumour-promoting and cytotoxic activities of different plant part extracts of *Garcinia atroviridis* griff. Ex T. anders. *Journal of Ethnopharmacol*. *72*, 395-402.

Marconi, G. G. (1975). [13] Silica gel chromatography of antibiotics. *Methods in enzymology*, *43*, 291-296.

Marimuthu, M. M., Aruldass, C. A., Sandrasagaran, U. M., Mohamad, S., Ramanathan, S., Mansor, S. M., & Murugaiyah, V. (2014). Antimicrobial activity and phytochemical screening of various parts of *Ixora coccinea*. *Journal of Medicinal Plants Research*, *8*(10), 423-429.

Meng, F. U., Hui-Jin, F. E. N. G., Yu, C. H. E. N., De-Bin, W. A. N. G., & Guang-Zhong, Y. A. N. G. (2012). Antioxidant activity of *Garcinia xanthochymus* leaf, rootsand fruit extracts in vitro. *Chinese Journal of Natural Medicines*, *10*(2), 129-134.

Miller, N. J. & Rice-Evans, C. (1997). Factors infuencing the antioxidant activity determined by the ABTS radical cation assay, *Free Radical Research*, *26*(3), 195-199.

Minatel, I. O., Borges, C. V., Ferreira, M. I., Gomez, H. A. G., Chen, C. Y. O., & Lima, G. P. P. (2017). Phenolic compounds: Functional properties, impact of processing and bioavailability. *Phenolic Compounds Biological Activity*. *Ed. InTech. Rijeka, Croatia*, 1-24.

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, *26*(2), 211-219.

Muriithi, E., Bojase-Moleta, G., & Majinda, R. R. (2016). Benzophenone derivatives from *Garcinia livingstonei* and their antioxidant activities. *Phytochemistry letters*, *18*, 29-34.

Muthumperumal, C., Stalin, N., Das, A., & Swamy, P. S. (2016). Chemical profiling of leaf essential oil, Antioxidant potential and Antibacterial activity of *Syzygium lanceolatum* (Lam.) Wt. & Arn.(Myrtaceae). *Free Radicals & Antioxidants*, *6*(1), 13-22.

Na, Y. (2009). Recent cancer drug development with xanthone structures. *Journal of Pharmacy and Pharmacology*, *61*(6), 707-712.

Negi, J. S., Bisht, V. K., Singh, P., Rawat, M. S. M., & Joshi, G. P. (2013). Naturally occurring xanthones: chemistry and biology. *Journal of Applied Chemistry*, *2013*.

Nguyen, C. N., Trinh, B. T., Tran, T. B., Nguyen, L. T. T., Jäger, A. K., & Nguyen, L. H. D. (2017). Anti-diabetic xanthones from the bark of *Garcinia xanthochymus*. *Bioorganic & medicinal chemistry letters*, *27*(15), 3301-3304.

Nguyen, T. H. (2015). Phytochemical and biological investigation of the bark of *Garcinia fusca* Pierre (Doctoral dissertation).

Nipornram, S., Tochampa, W., Rattanatraiwong, P., & Singanusong, R. (2018). Optimization of low power ultrasound-assisted extraction of phenolic compounds from mandarin (*Citrus reticulata* Blanco cv. Sainampueng) peel. *Food chemistry*, *241*, 338-345.

Nordin, S.M., Ariffin, Z., Jajuli, R., Abdullah, W.D.W., & Denis, M.G. (2007). Country Report on the State of Plant Genetic Resources for Food and Agriculture in Malaysia (1997-2007). FAO, Rome, Italy.

Nostro, A., Germano, M.P., D'angelo, V., Marino, A., & Cannatelli, M. A. (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Letters in applied microbiology*, *30*(5), 379-384.

Nursakinah, I., Zulkhairi, H.A., Norhafizah, M., Hasnah, B., Zamree Md, S., Farrah Shafeera, I., Razif, D., & Hamzah Fansuri, H. (2012). Nutritional Content and in vitro Antioxidant Potential of *Garcinia atroviridis* (Asam gelugor) Leaves and Fruits. *Malaysian Journal of Nutrition*. *18*(3), 363-71.

On, S., Aminudin, N.I., Ahmad, F., Mohd Sirat, H. and Bakhtiar, M.T. (2016). Chemical constituents from stem bark of *Garcinia prainiana* and their bioactivities. *International Journal of Pharmacognosy and Phytochemical Research*, 8(5), 756-760.

Osorio, E., Londoño, J., & Bastida, J. (2013). Low-density lipoprotein (LDL)-antioxidant biflavonoids from *Garcinia madruno*. *Molecules*, *18*(5), 6092-6100.

Panda, S. S., Chand, M., Sakhuja, R., & Jain, S. C. (2013). Xanthones as potential antioxidants. *Current medicinal chemistry*, *20* (36), 4481-4507.

Pavia, D. L., Lampman, G. M., Kriz, G. S., & Vyvyan, J. A. (2008). Introduction to spectroscopy. Cengage Learning.

Perkampus, H. H. (1992). Analytical Applications of UV-VIS Spectroscopy in *UV-VIS atlas of organic compounds*. pp. 68-75. VCH.

Permana, D., Abas, F., Maulidiani, F., Shaari, K., Stanslas, J., Ali, A. M., & Lajis, N. H. (2005). Atrovirisidone B, a new prenylated depsidone with cytotoxic property from the roots of *Garcinia atroviridis*. *Zeitschrift fur Naturforschung C-Journal of Biosciences*. *60*(7-8), 523-526.

Permana, D., Lajis, N.H., Mackeen, M.M., Ali, A.M., Aimi, N., Kitajima, M., & Takayama, H. (2001). Isolation and Bioactivities of Constitutents of the Roots of *Garcinia atroviridis*. *Journal of Natural Products*. *64*(7), 976-979.

Permana, D., Lajis, N.H., Shaari, K., Ali, A.M., Mackeen, M.M., Kitajima, M., Takayama, H., and Aimi, N. (2003). A new prenylated hydroquinone from the roots of *Garcinia atroviridis* Griff ex T. Anders (Guttiferae). *Zeitschrift fur Naturforschung B. 58*(4), 332-335.

Perveen, S., & Al-Taweel, A. M. (2017). Phenolic Compounds from the Natural Sources and Their Cytotoxicity. *Phenolic Compounds: Natural Sources, Importance and Applications*, 29.

Pinto, D. C., Fuzzati, N., Pazmino, X. C., & Hostettmann, K. (1994). Xanthone and antifungal constituents from *Monnina obtusifolia*. *Phytochemistry*, *37*(3), 875-878.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of agricultural and food chemistry*, *53*(10), 4290-4302.

Radulovic, N. S., Blagojevic, P. D., Stojanovic-Radic, Z. Z., & Stojanovic, N. M. (2013). Antimicrobial plant metabolites: structural diversity and mechanism of action. *Current medicinal chemistry*, *20*(7), 932-952.

Ramirez, C., Gil, J. H., Marín-Loaiza, J. C., Rojano, B., & Durango, D. (2018). Chemical constituents and antioxidant activity of *Garcinia madruno* (Kunth) Hammel. *Journal of King Saud University-Science.*

Reid, R. G., & Sarker, S. D. (2012). Isolation of Natural Products by Low-Pressure Column Chromatography. In *Natural Products Isolation* (pp. 155-187). Humana Press.

Rice-Evans, C., Miller, N., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology & Medicine*, *20*(7), 933-956.

Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in plant science*, *2*(4), 152-159.

Routray, W., & Orsat, V. (2014). MAE of phenolic compounds from blueberry leaves and comparison with other extraction methods. *Industrial Crops and Products*, *58*, 36-45.

Roy, A. K. and Calvin, G. (2016). Chromatography. Encyclopædia Britannica. https://www.britannica.com/science/chromatography (Access Date: December 14, 2017).

Saelee, A., Phongpaichit, S., & Mahabusarakam, W. (2015). A new prenylated biflavonoid from the leaves of *Garcinia dulcis*. *Natural product research*, *29*(20), 1884-1888.

Salleh, W. M. N. H. W., Sazali, N. S. A. N., Ahmad, F., & Taher, M. (2017). Biflavonoids from the leaves and stem bark of *Garcinia griffithii* and their biological activities. *Marmara Pharmaceutical Journal*, *21*(4), 889-897.

Santos-Buelga, C., Gonzalez-Manzano, S., Dueñas, M., & Gonzalez-Paramas, A. M. (2012). Extraction and Isolation of Phenolic Compounds. In *Natural products isolation* (pp. 427-473). Humana Press.

Sarker, S. D., & Nahar, L. (2012). An introduction to natural products isolation. In *Natural products isolation* (pp. 1-25). Humana Press.

Seidel, V. (2012). Initial and bulk extraction of natural products isolation. In *Natural Products Isolation* (pp. 27-41). Humana Press.

Sichel, G., Corsaro, C., Scalia, M., Di Bilio, A. J., & Bonomo, R. P. (1991). *In vitro* scavenger activity of some flavonoids and melanins against O_2. *Free Radical Biology and Medicine*, *11*, 1-8.

Siddiqui, A. A., Iram, F., Siddiqui, S., & Sahu, K. (2014). Role of natural products on drug discovery process. *International Journal of Drug Development and Research*, *6*(2), 172-204. Simmler, C., Napolitano, J. G., McAlpine, J. B., Chen, S. N., & Pauli, G. F. (2014). Universal quantitative NMR analysis of complex natural samples. *Current opinion in biotechnology*, *25*, 51-59.

Sofowora A. (1993). Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd (Pub.), Ibadan.

Sookjitsumran, W., Devahastin, S., Mujumdar, A. S., & Chiewchan, N. (2016). Comparative evaluation of microwave-assisted extraction and preheated solvent extraction of bioactive compounds from a plant material: A case study with cabbages. *International Journal of Food Science and Technology*, *51*(11), 2440-2449.

Stefanović, O.D. (2018). Synergistic Activity of Antibiotics and Bioactive Plant Extracts: A Study Against Gram-Positive and Gram-Negative Bacteria. *Bacterial Phatogenesisand Antibacterial Control*, *23*.

Suksamrarn, S., Komutiban, O., Ratananukul, P., Chimnoi, N., Lartpornmatulee, N., & Suksamrarn, A. (2006). Cytotoxic prenylated xanthones from the young fruit of *Garcinia mangostana*. *Chemical and pharmaceutical bulletin*, *54*(3), 301-305.

Suksamrarn, S., Suwannapoch, N., Phakhodee, W., Thanuhiranlert, J., Ratananukul, P., Chimnoi, N., & Suksamrarn, A. (2003). Antimycobacterial activity of prenylated xanthones from the fruits of *Garcinia mangostana*. *Chemical and pharmaceutical bulletin*, *51*(7), 857-859.

Suksamrarn, S., Suwannapoch, N., Ratananukul, P., Aroonlerk, N., & Suksamrarn, A. (2002). Xanthones from the Green Fruit Hulls of *Garcinia mangostana*. *Journal of Natural Products*, *65*(5), 761-763.

Susanto, D. F., Aparamarta, H. W., Widjaja, A., & Gunawan, S. (2019). *Calophyllum inophyllum*: Beneficial Phytochemicals, Their Uses, and Identification. In *Phytochemicals in Human Health*. IntechOpen.

Syahida, A., Israf, D. A., Permana, D., Lajis, N. H., Khozirah, S., Afiza, A. W., Khaizurin, T. A., Somchit, M. N., Sulaiman, M. R., & Nasaruddin, A. A. (2006). Atrovirinone inhibits pro-inflammatory mediator release from murine macrophages and human whole blood. *Immunology and cell biology*, *84*(3), 250-258.

Syamsudin, Shirly Kumala and Broto Sutaryo (2007). Screening of some Extracts from *Garcinia parvifolia* Miq. (Guttiferae) for Antiplasmodial, Antioxidant, Cytotoxic, and Antibacteria Activities. *Asian Journal of Plant Sciences*, 6(6), 972-976.

Taamalli, A., Arráez-Román, D., Barrajón-Catalán, E., Ruiz-Torres, V., Pérez-Sánchez, A., Herrero, M., Ibañez, E., Micol, V., Zarrouk, M., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2012). Use of advanced techniques for the extraction of phenolic compounds from Tunisian olive leaves: phenolic composition and cytotoxicity against human breast cancer cells. *Food and chemical toxicology*, *50*(6), 1817-1825.

Tadtong, S., Viriyaroj, A., Vorarat, S., Nimkulrat, S., & Suksamrarn, S. (2009). Antityrosinase and antibacterial activities of mangosteen pericarp extract. *Journal of Health Research*, *23*(2), 99-102.

Taher, M., Aminuddin, A., Susanti, D., Aminudin, N.I., On, S., Ahmad, F. and Hamidon, H. (2016). Cytotoxic, anti inflammatory and adipogenic effects of inophyllum D, calanone, isocordato-oblongic acid, and morelloflavone on cell lines. *Natural Product Sciences*, *22*(2), 122-128.

Tan, W.N., Khairuddean, M., Wong, K.C., Khaw, K.Y, & Vikneswaran, M. (2014). New cholinesterase inhibitors from *Garcinia atroviridis*. *Fitoterapia*. *97*, 261-267.

Tan, W.N., Khairuddean, M., Wong, K.C., Tong, W.Y., & Ibrahim, D. (2016). Antioxidant compounds from the stem bark of *Garcinia atroviridis*. *Journal of Asian Natural Products Research*. *18*(8), 804-810.

Tan, W.N., Wong, K.C., Khairuddean, M., Eldeen, I.M., Asmawi, M., & Sulaiman, B. (2013). Volatile constituents of the fruit of *Garcinia atroviridis* and their antibacterial and anti-inflammatory activities. *Flavour and Fragrance Journal*. *28*(1), 2-9.

Tewtrakul, S., Wattanapiromsakul, C., & Mahabusarakam, W. (2009). Effects of compounds from *Garcinia mangostana* on inflammatory mediators in RAW264. 7 macrophage cells. *Journal of Ethnopharmacology*, *121*(3), 379-382.

Tisdale, E. J, Kochman, D. A., & Theodorakis, E. A. (2003). Total synthesis of atroviridin. *Tetrahedron Letters*. (44), 3281-3284.

Trisuwan, K., Rukachaisirikul, V., Phongpaichit, S., & Hutadilok-Towatana, N. (2013). Tetraoxygenated xanthones and biflavanoids from the twigs of *Garcinia merguensis*. *Phytochemistry letters*, *6*(4), 511-513.

Valentão, P., Areias, F., Amaral, J., Andrade, P., & Seabra, R. (2000). Tetraoxygenated Xanthones from *Centaurium erythraea*. *Natural Product Letters*, 14(5), 319-323.

Vlaisavljević, S., Kaurinović, B., Popović, M., & Vasiljević, S. (2017). Profile of phenolic compounds in *Trifolium pratense* L. extracts at different growth stages and their biological activities. *International Journal of Food Properties*. *20*(12), 3090-3101.

Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology*, *17*(6), 300-312.

Wang, S., Meckling, K. A., Marcone, M. F., Kakuda, Y., & Tsao, R. (2011). Synergistic, additive, and antagonistic effects of food mixtures on total antioxidant capacities. *Journal of Agricultural and Food Chemistry*, *59*(3), 960-968.

Whitmore, T.C. (1973). Garcinia L. Tree flora of Malaya: A Manual For Foresters, 2, 196-225.

Yaacob, O., & Tindall, H.D. (1995). Mangosteen cultivation. FAO Plant Production and Protection Paper No. 129:100.

Younes, K., Merghache, S., Djabou, N., Selles, C., Museli, A., Tabti, B., & Costa, J. (2015). Chemical Composition and Free Radical Scavenging Activity of Essential Oils and Extracts of Algerian *Cardaria draba* (L.) Desv. *Journal of Essential Oil Bearing Plants*, *18*(6), 1448-1458.

Zakaria, Z.A., Zakaria, M.L., Amom, Z., Desa, M., & Nasir, M. (2011). Antimicrobial activity of the aqueous extract of selected Malaysian herbs. *African Journal of Microbiology Research*. *5*(30): 5379-5383.

Zarena, A. S., & Sankar, K. U. (2009a). A study of antioxidant properties from *Garcinia mangostana* L. pericarp extract. *Acta Scientiarum Polonorum Technologia Alimentaria*, *8*(1), 23-34. (a)

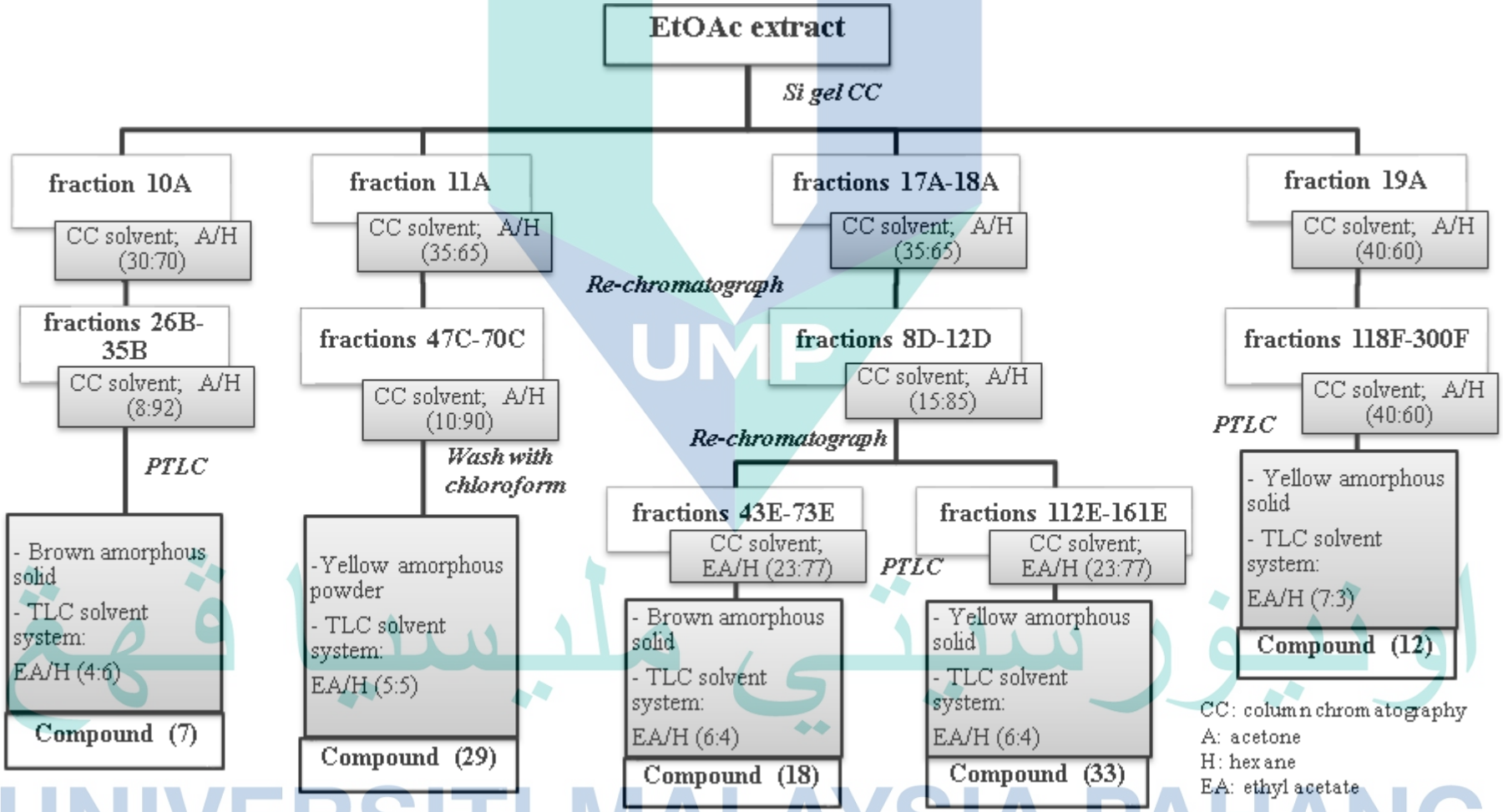
Zarena, A. S., & Sankar, K. U. (2009b). Screening of xanthone from mangosteen (*Garcinia mangostana* L.) peels and their effect on cytochrome c reductase and phosphomolybdenum activity. *Journal of Natural Products (India)*, *2*, 23-30. (b)

Zhang, Z.,Pang, X., Xuewu, D., Ji, Z., & Jiang, Y. (2005). Role of peroxidase in anthocyanin degradation in litchi fruit pericarp. *Food Chemistry*, *90*, 47-52.

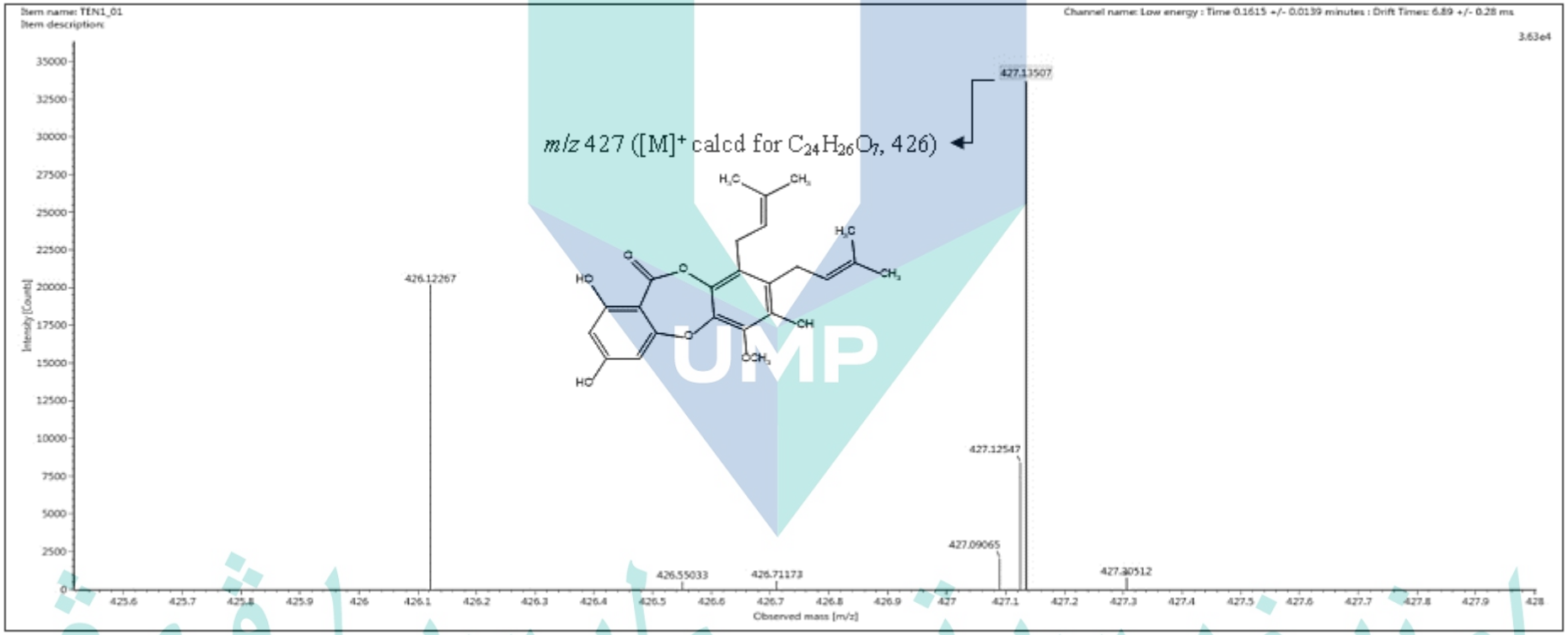
Zhao, Y., Liu, J. P., Lu, D., Li, P. Y., & Zhang, L. X. (2010). A new antioxidant xanthone from the pericarp of *Garcinia mangostana* Linn. *Natural Product Research*, *24*(17), 1664-1670.

Zhou, X., Huang, R., Hao, J., Huang, H., Fu, M., Xu, Z., Zhou, Y., Li, X.E., Qiu, S.X. & Wang, B. (2011). Two new prenylated xanthones from the pericarp of *Garcinia mangostana* (mangosteen). *Helvetica Chimica Acta*, *94*(11), 2092-2098.

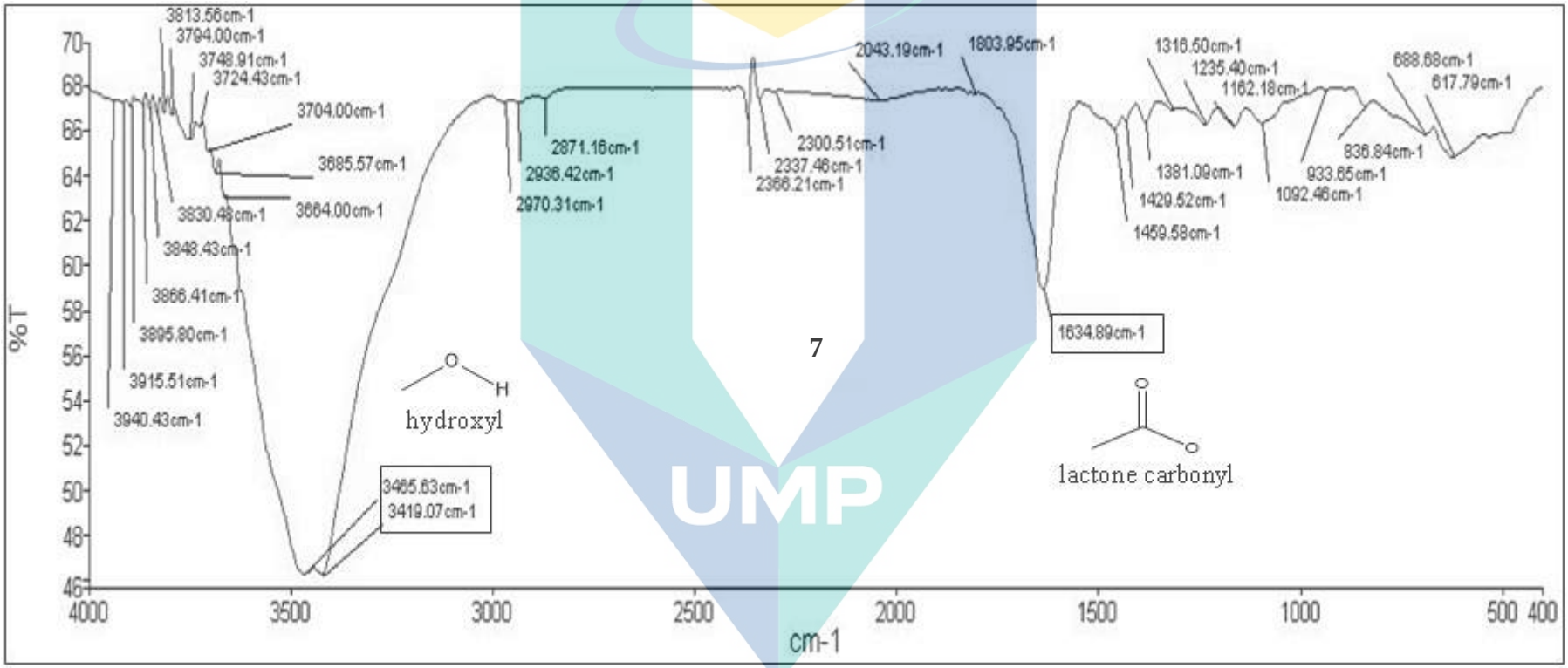
APPENDIX A
FLOWCHART OF ISOLATION PROCESS



APPENDIX B
SUPPLEMENTARY SPECTROSCOPY DATA OF ATROVIRISIDONE (7)

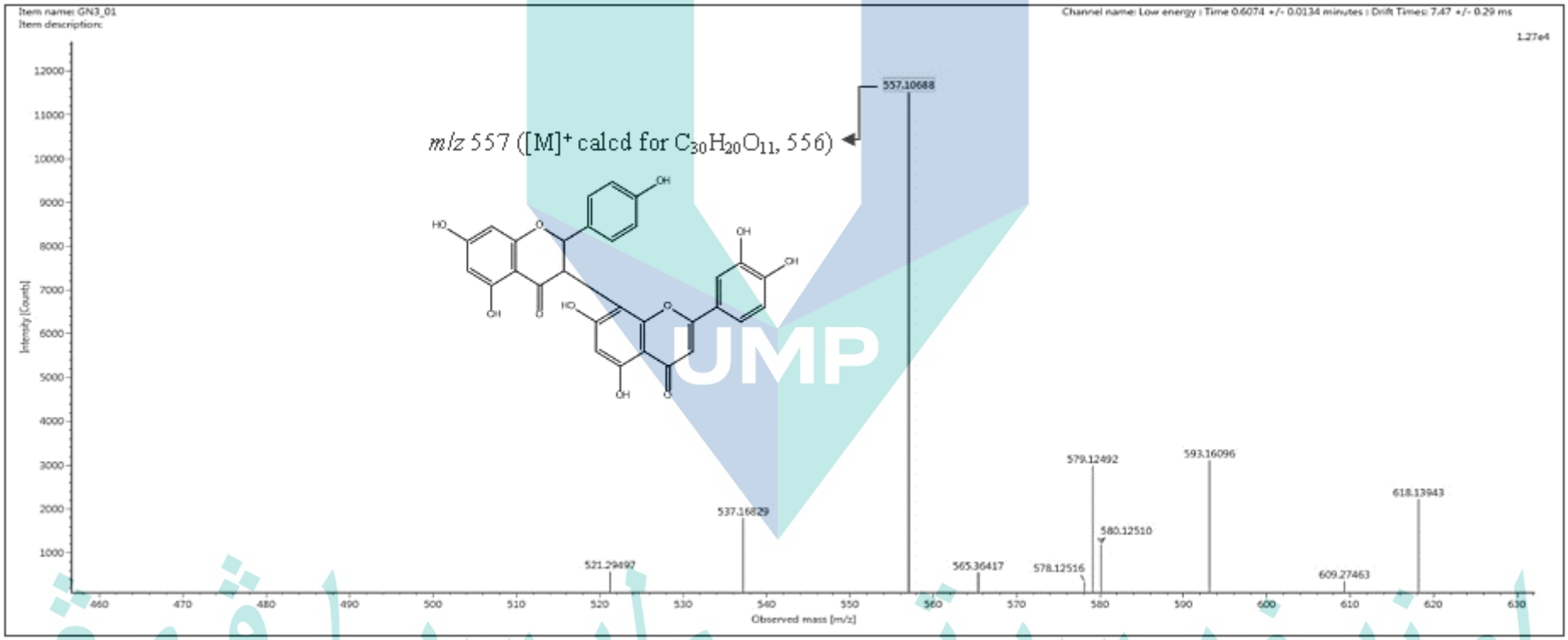


LC-Q/TOF-MS spectrum of **7**

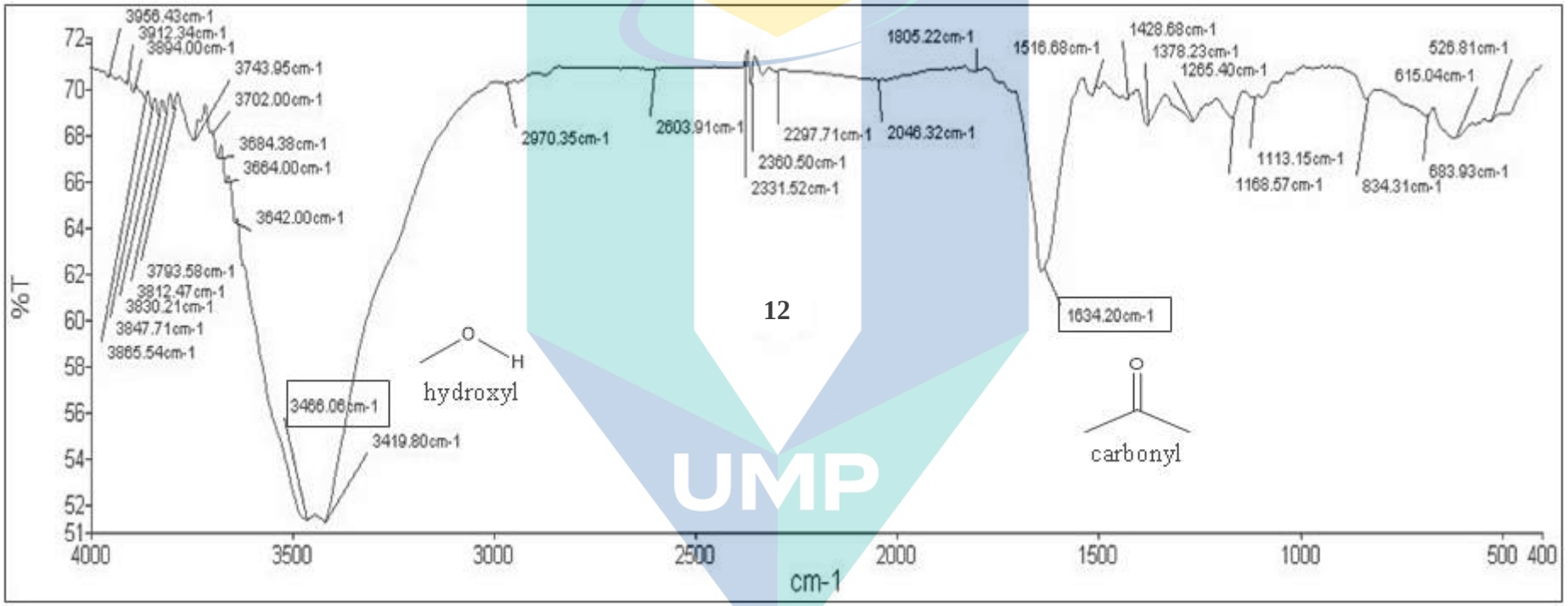


FTIR spectrum of **7**

APPENDIX C
SUPPLEMENTARY SPECTROSCOPY DATA OF MORELLOFLAVONE (12)



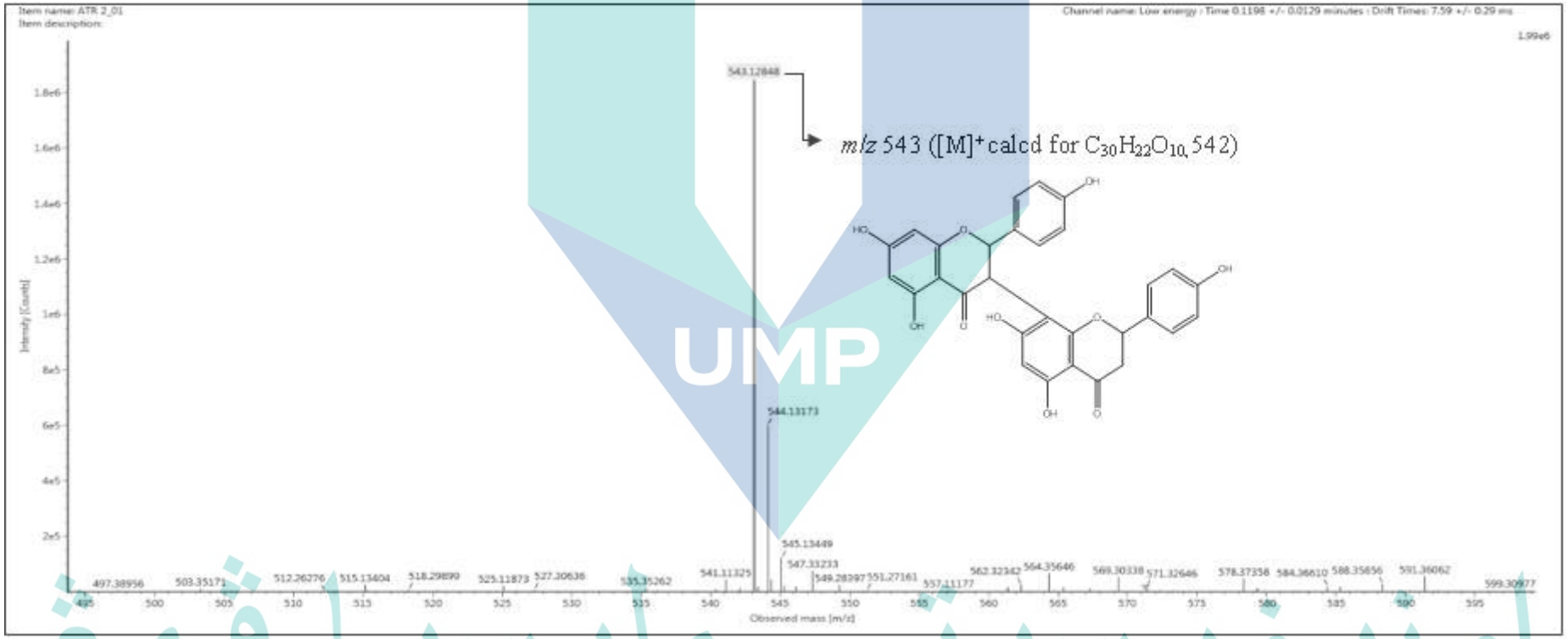
LC-Q/TOF-MS spectrum of **12**



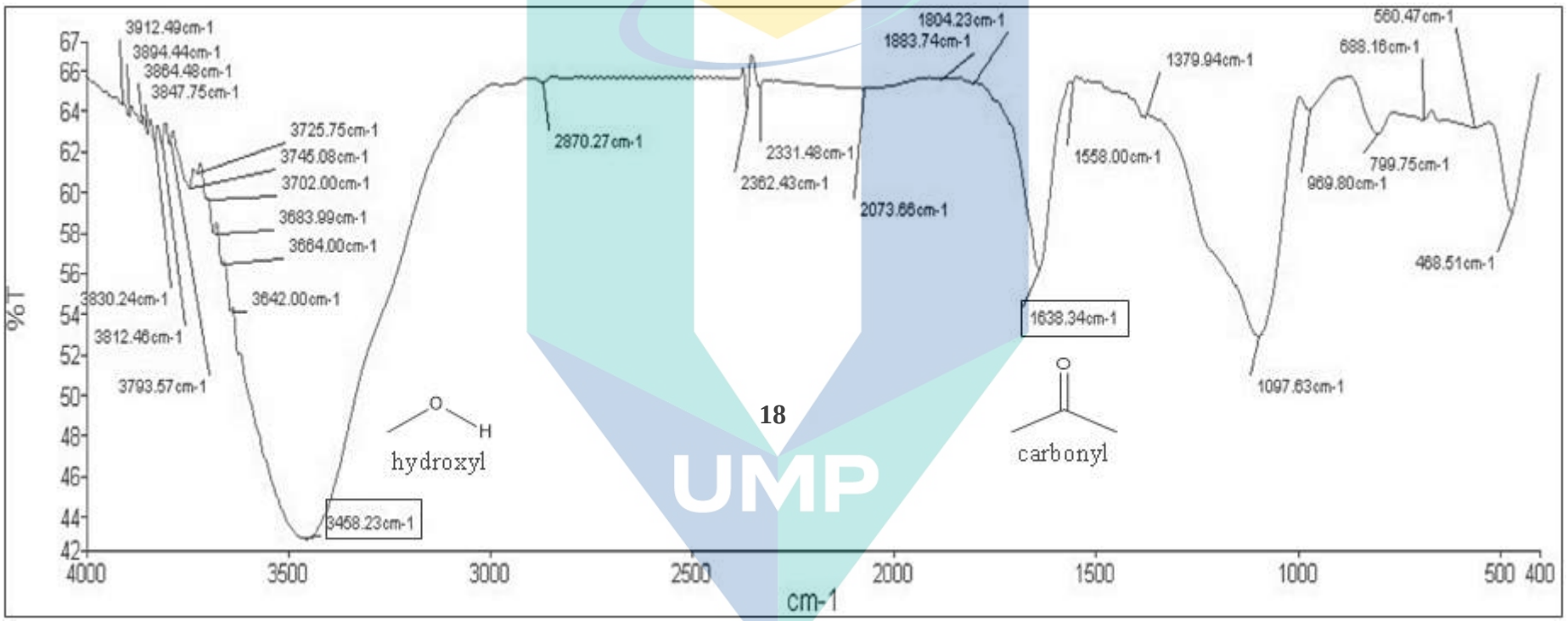
12

FTIR spectrum of **12**

APPENDIX D
SUPPLEMENTARY SPECTROSCOPY DATA OF GB1A (18)



LC-Q/TOF-MS spectrum of **18**

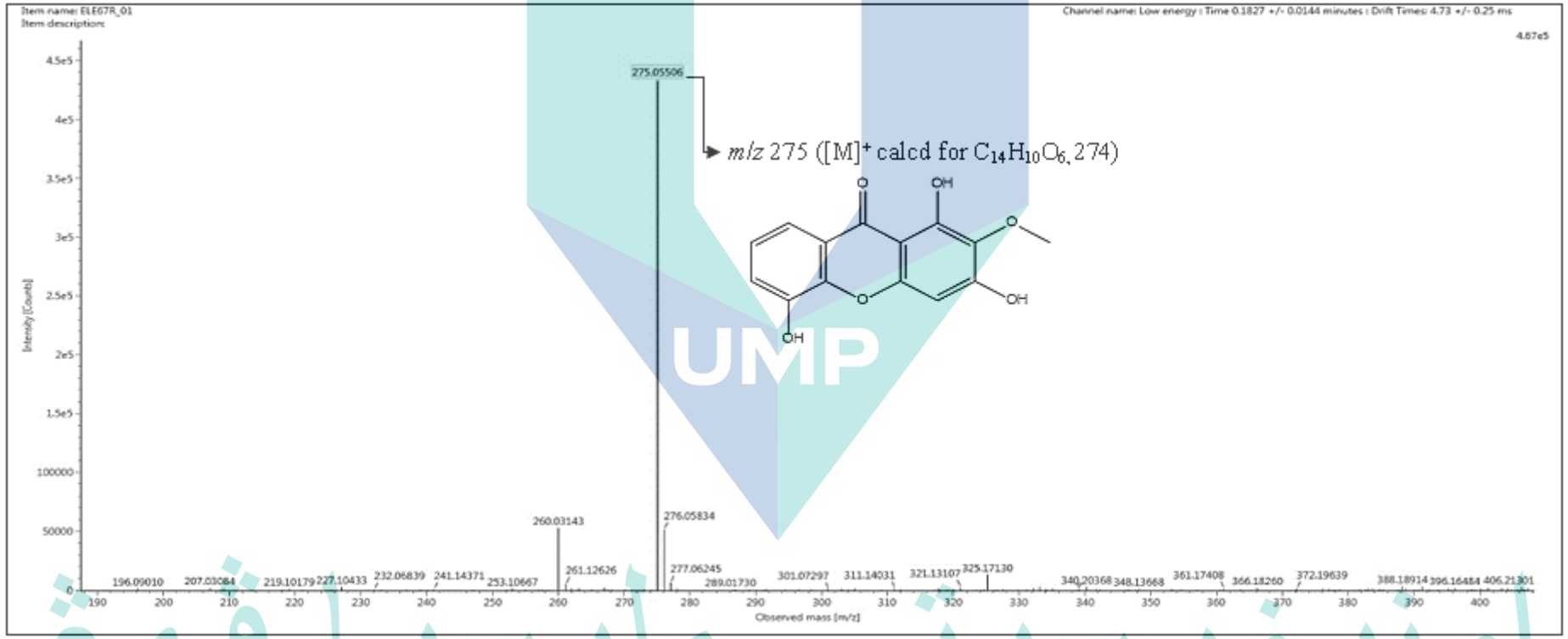


18

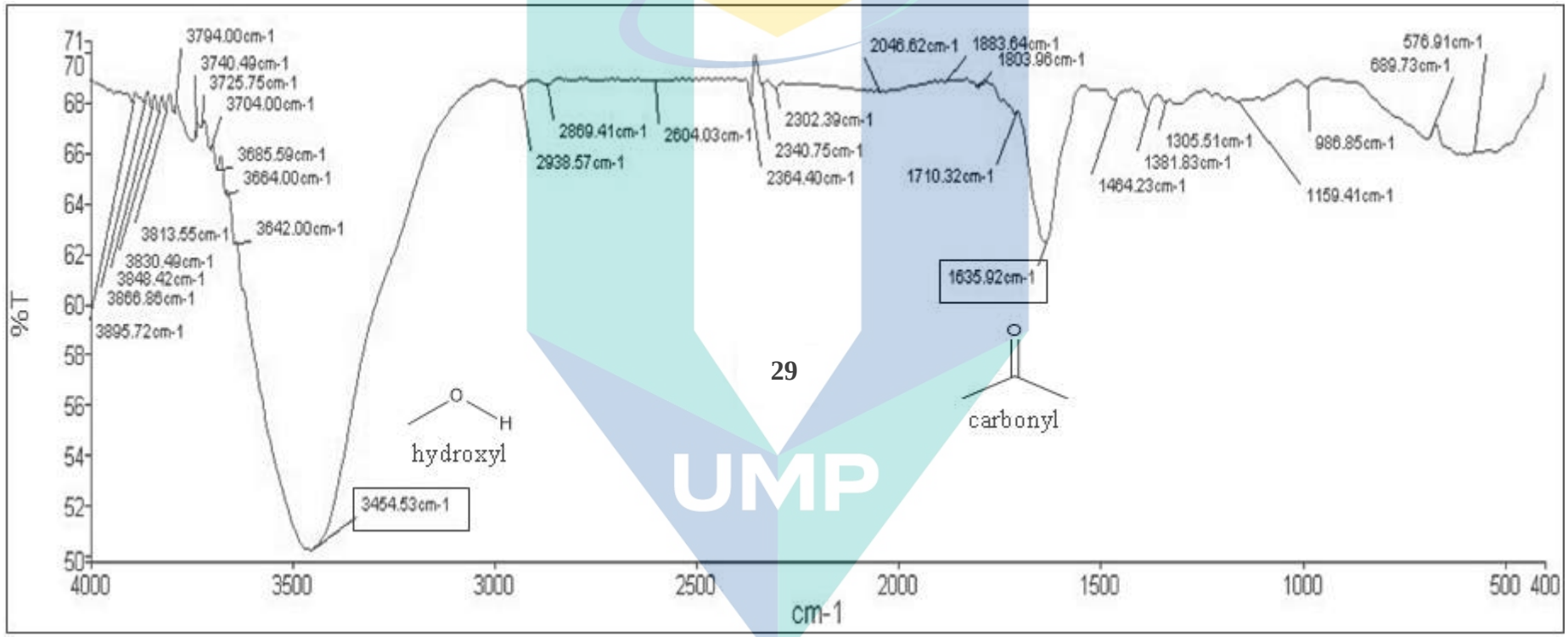
FTIR spectrum of **18**

APPENDIX E

SUPPLEMENTARY SPECTROSCOPY DATA OF 1,3,5-TRIHYDROXY-2- METHOXYXANTHONE (29)



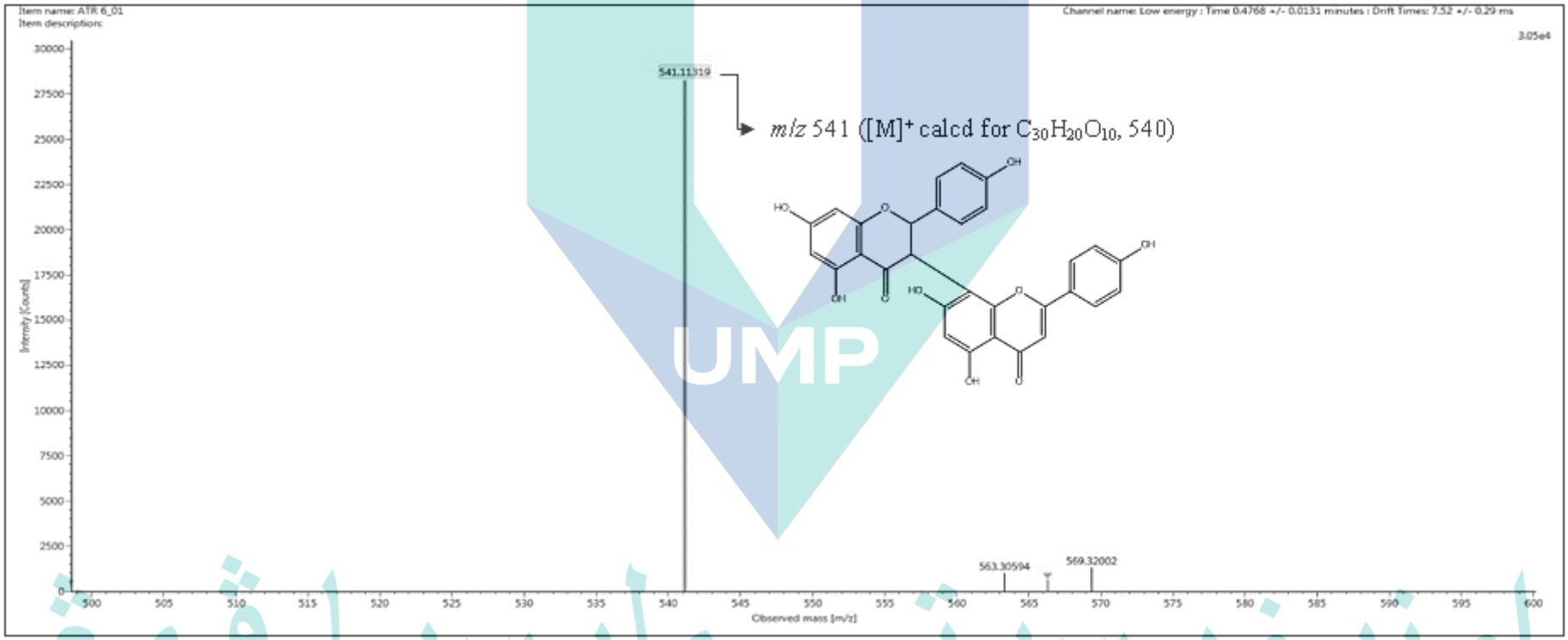
LC-Q/TOF-MS spectrum of **29**



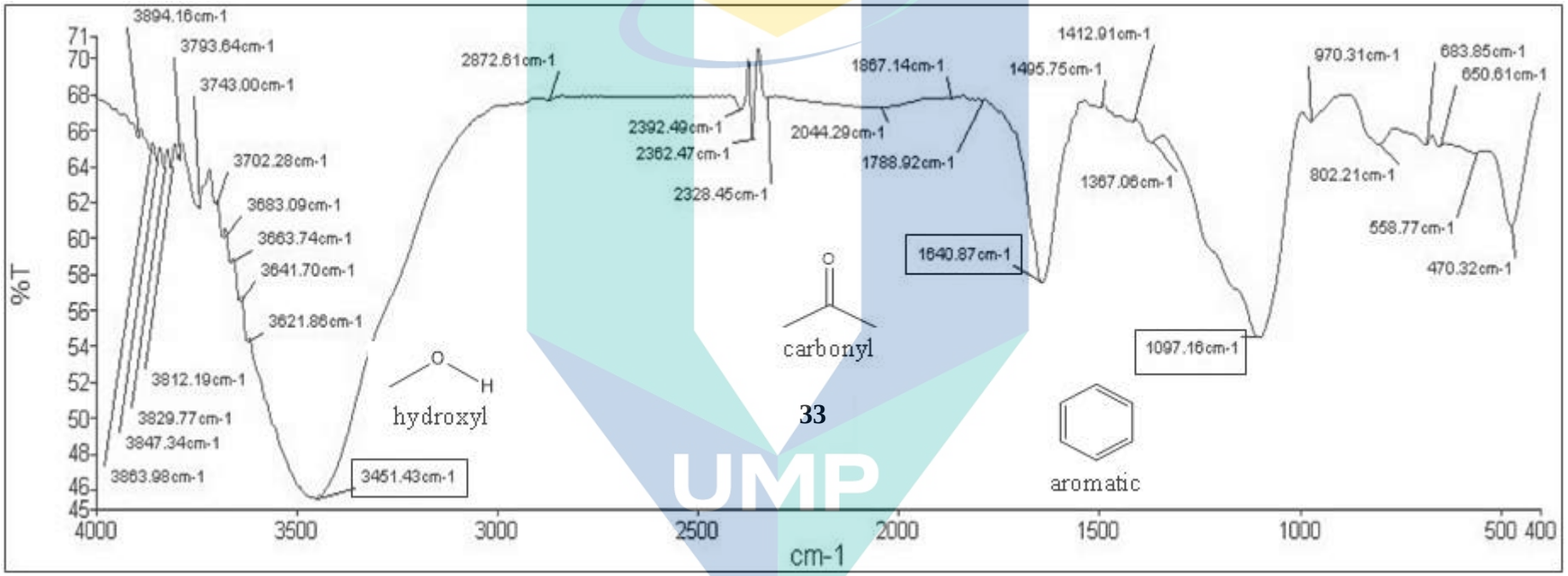
29

FTIR spectrum of **29**

APPENDIX F
SUPPLEMENTARY SPECTROSCOPY DATA OF VOLKENSIFLAVONE (33)



LC-Q/TOF-MS spectrum of **33**



33

FTIR spectrum of **33**

APPENDIX G
TPC CALCULATION OF *G. ATROVIRIDIS* ROOT EXTRACTS

Absorbance reading (593 nm) of extracts for TPC assay. Measurements were carried out in triplicate.

	Mean Abs (Abs sample - Abs blank)									
Sample	Hexane		DCM		EtOAc		Butanol		MeOH	
Conc (µg/mL)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
5	0.004267	0.000954	0.0072	0.000945	0.006833	0.001724	0.0154	0.002098	0.004367	0.001992
10	0.0072	0.001328	0.0202	0.00315	0.017833	0.002214	0.024	0.000551	0.005033	0.000208
15	0.012067	0.002524	0.037633	0.016214	0.021367	0.001916	0.039167	0.003119	0.006033	0.000513
20	0.0151	0.001358	0.0409	0.001804	0.028967	0.003118	0.0543	0.002139	0.007167	0.001473
25	0.0188	0.002023	0.058233	0.001704	0.042667	0.002646	0.0726	0.000208	0.008967	0.0003
30	0.022033	0.001955	0.061733	0.00106	0.045367	0.004766	0.098867	0.001709	0.012867	0.000608
35	0.026333	0.001201	0.073967	0.000265	0.053833	0.002754	0.1146	0.003819	0.015833	0.001069
40	0.0351	0.00125	0.094267	0.005717	0.063	0.002444	0.1334	0.004162	0.016767	0.000265

TPC Value Calculations of *Garcinia atroviridis* Extracts

Based on GA standard curve equation: $x = (y + 0.0166)/ 0.0035$

TPC was calculated using following formula;

$$C = c\ V/m$$

where,

C = total phenolic content mg GAE/g dry extract

c = concentration obtained from GA calibration curve in mg/mL

V = volume of extract in mL (1 mL)

m = mass of extract in gram (0.00004 g)

DCM extract

When $y = 0.094267$

$x = (0.094267 + 0.0166)/ 0.0035$

$x = 31.67628571$ µg/mL

so c= 0.031676286 mg/mL

C = 0.031676286 × (1/0.00004)

C = 791.9071429

1 g of DCM extract = 791.91 mg GA

Hexane extract

When $y = 0.0351$

$x = (0.0351 + 0.0166)/ 0.0035$

$x = 10.48571429$ µg/mL

so c= 0.010485714 mg/mL

C = 0.010485714 × (1/0.00004)

C = 262.1428571

1 g of hexane extract = 262.14 mg GA

EtOAc extract

When $y = 0.063$

$x = (0.063 + 0.0166)/ 0.0035$

$x = 22.74285714$ µg/mL

so c= 0.022742857 mg/mL

C = 0.022742857 × (1/0.00004)

C = 568.5714286

1 g of EtOAc extract = 568.57 mg GA

Butanol extract

When $y = 0.1334$

$x = (0.1334 + 0.0166)/ 0.0035$

$x = 42.85714286$ µg/mL

so c= 0.042857143 mg/mL

C = 0.042857143 × (1/0.00004)

C = 1071.428571

1 g of butanol extract = 1071.43 mg GA

MeOH extract

When $y = 0.016767$

$x = (0.016767 + 0.0166)/ 0.0035$

$x = 9.533428571$ µg/mL

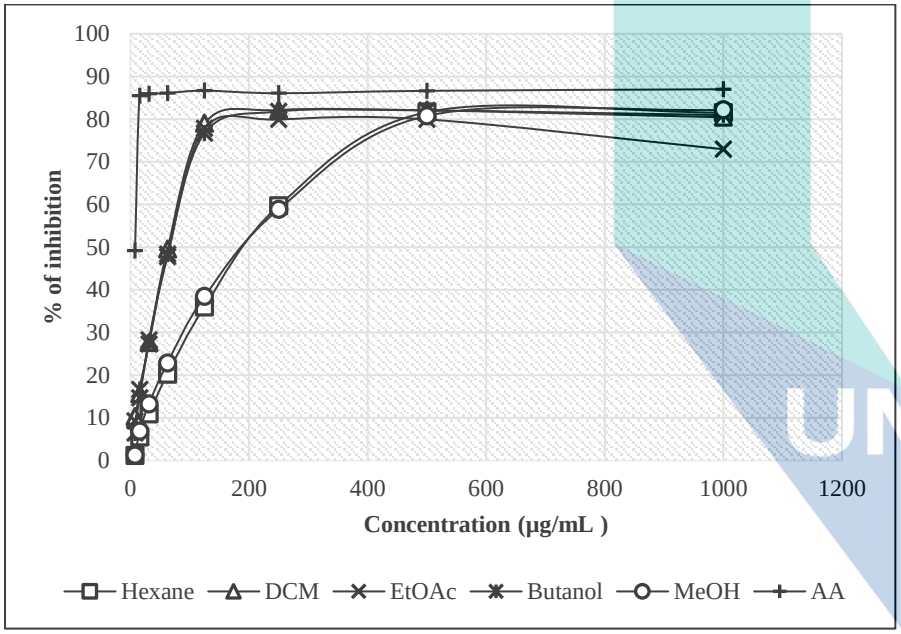
so c= 0.009533429 mg/mL

C = 0.009533429 × (1/0.00004)

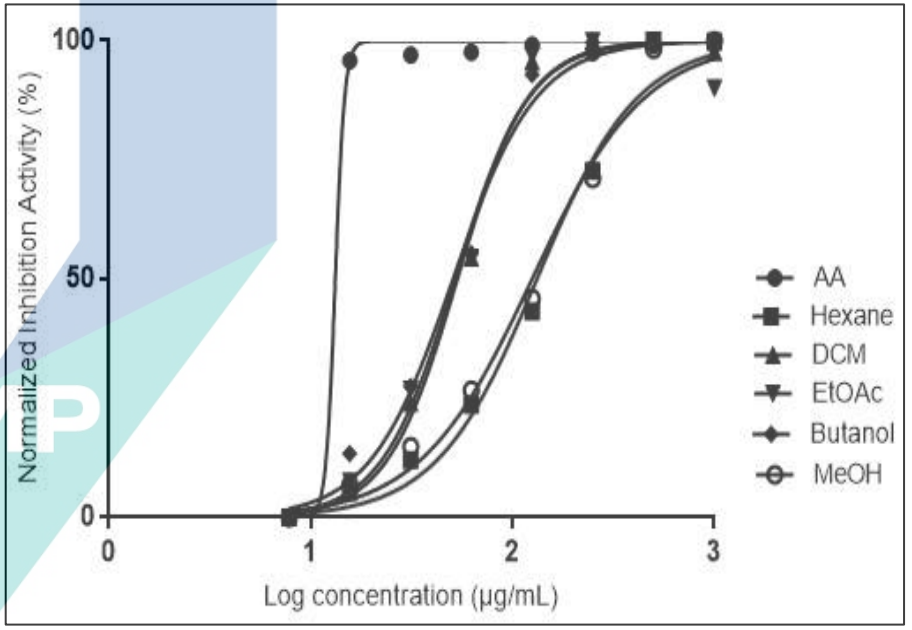
C = 238.3357143

1 g of MeOH extract = 238.34 mg GA

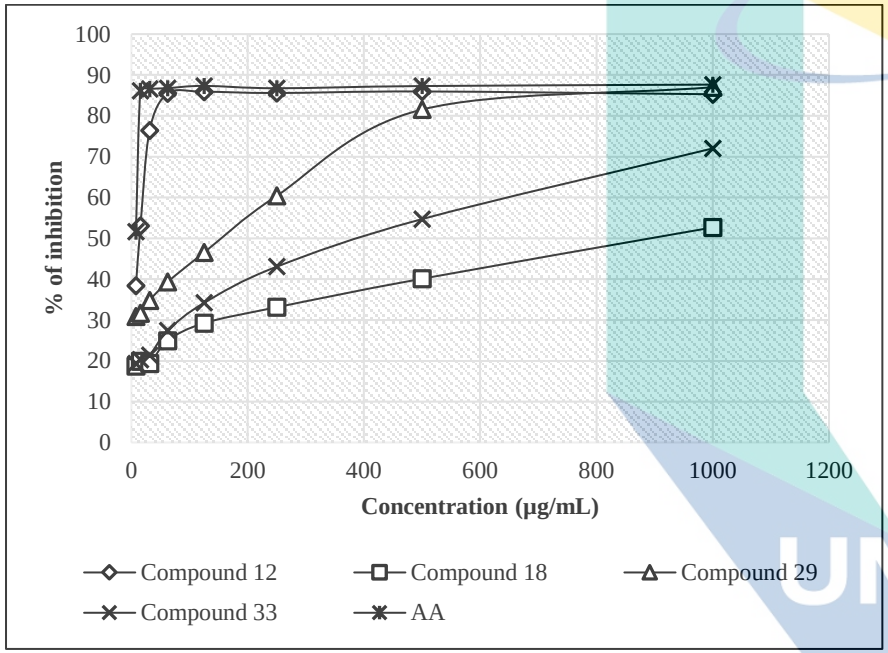
APPENDIX H
SUPPLEMENTARY DATA OF DPPH ASSAY



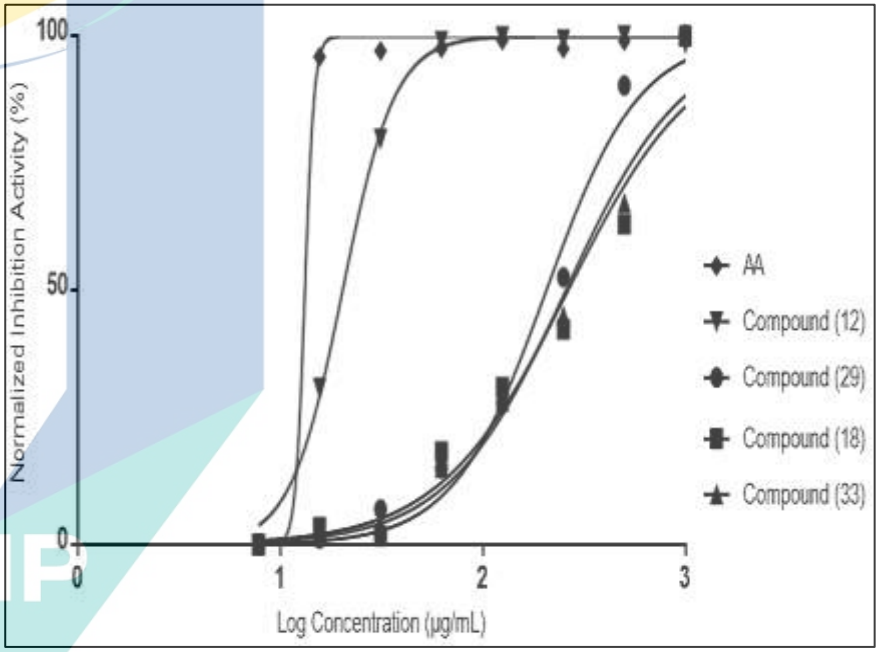
Graph chart of the percentage activity of the of *G. atroviridis* roots extracts



IC_{50} (µg/mL) graph of the *G. atroviridis* root extracts



Graph chart of the percentage activity of *G. atroviridis* isolated compounds



IC_{50} (µg/mL) graph of the *G. atroviridis* isolated compounds

APPENDIX I
CALCULATION OF FRAP VALUES

Absorbance reading (593 nm) (*n*=3) of extracts for FRAP assay. Means (*n*=3) and standard deviation are indicated.

Mean Abs (Abs sample-Abs blank)										
Sample	Hexane		DCM		EtOAC		Butanol		MeOH	
Conc (µg/mL)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
5	0.0884	0.013336	0.1003	0.004769	0.127067	0.011002	0.139333	0.004351	0.047667	0.002627
10	0.0931	0.003453	0.1061	0.001012	0.132866	0.008208	0.140766	0.003821	0.0598	0.001795
15	0.1035	0.002325	0.12	0.002318	0.140766	0.002411	0.165533	0.008927	0.0569	0.003288
20	0.1139	0.005056	0.1378	0.00215	0.156166	0.003863	0.185466	0.00465	0.067033	0.003483
25	0.129533	0.017811	0.158666	0.00851	0.164566	0.006038	0.220733	0.004747	0.076566	0.004751
30	0.135766	0.011238	0.181033	0.013061	0.1744	0.010997	0.2828	0.015224	0.0808	0.004306
35	0.151233	0.001706	0.195366	0.001041	0.189533	0.017122	0.322566	0.009403	0.086933	0.000755
40	0.171533	0.013016	0.231233	0.009403	0.1879	0.008864	0.3424	0.017556	0.090233	0.002771

FRAP Value Calculations of *Garcinia atroviridis* Extracts

Based on AA standard curve equation: $x = (y - 0.0209)/ 0.0142$

When $y = 0.171533$

$x = (0.171533 - 0.0209)/ 0.0142$

$x = 10.60795775$

40 µg of hexane extract = 10.60795775 µg AA

If, 1 µg of hexane extract = 0.265198944 µg AA

1000 µg of hexane extract = 265.1989437 µg AA

1 mg of hexane extract = 265.1989437 µg AA

So, **1 g of hexane extract = 265.1989437 mg AA**

When $y = 0.231233$

$x = (0.231233 - 0.0209)/ 0.0142$

$x = 14.8121831$

40 µg of DCM extract = 14.8121831 µg AA

If, 1 µg of DCM extract = 0.370304577 µg AA

1000 µg of DCM extract = 370.3045775 µg AA

1 mg of DCM extract = 370.3045775 µg AA

So, **1 g of DCM extract = 370.3045775 mg AA**

When $y = 0.1879$

$x = (0.1879 - 0.0209)/ 0.0142$

$x = 11.76056338$

40 µg of EtOAc extract = 11.76056338 µg AA

If, 1 µg of EtOAc extract = 0.294014085 µg AA

1000 µg of EtOAc extract = 294.0140845 µg AA

1 mg of EtOAc extract = 294.0140845 µg AA

So, **1 g of EtOAc extract = 294.0140845 mg AA**

When $y = 0.3424$

$x = (0.3424 - 0.0209)/ 0.0142$

$x = 22.64084507$

40 µg of butanol extract = 22.64084507µg AA

If, 1 µg of butanol extract = 0.566021127 µg AA

1000 µg of butanol extract = 566.0211268 µg AA

1 mg of butanol extract = 566.0211268 µg AA

So, **1 g of butanol extract = 566.0211268 mg AA**

When y = 0.090233

x= (0.090233 – 0.0209)/ 0.0142

x= 4.882605634

40 µg of MeOH extract = 4.882605634 µg AA

If, 1 µg of MeOH extract = 0.122065141 µg AA

1000 µg of MeOH extract = 122.0651408 µg AA

1 mg of MeOH extract = 122.0651408 µg AA

So, **1 g of MeOH extract = 122.0651408 mg AA**

Absorbance reading (593 nm) of isolated compounds for FRAP assay. Means (n=3) and standard deviation are indicated.

	Mean Abs (Abs sample-Abs blank)							
Sample	(**12**)		(**18**)		(**29**)		(**33**)	
Conc (µg/mL)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
5	0.026233	0.001054	0.038667	0.00121	0.103333	0.007808	0.037133	0.004924
10	0.069833	0.002893	0.042233	0.001852	0.2017	0.011245	0.040566	0.003403
15	0.0658	0.007223	0.050533	0.005856	0.254	0.005729	0.046066	0.004934
20	0.1036	0.003134	0.0565	0.002859	0.448733	0.021979	0.050666	0.001305
25	0.131933	0.006851	0.058166	0.001501	0.503833	0.022924	0.055566	0.001102
30	0.141766	0.012966	0.081266	0.009016	0.608733	0.00694	0.057133	0.000781
35	0.182533	0.032165	0.0889	0.001704	0.8759	0.168025	0.073266	0.004646
40	0.236933	0.027507	0.089766	0.001861	0.954266	0.027227	0.0789	0.004274

FRAP Value Calculations of *Garcinia atroviridis* Compounds

Based on AA standard curve equation: $x = (y - 0.0209)/ 0.0142$

When $y = 0.236933$

$x = (0.236933 - 0.0209)/ 0.0142$

40 µg of **(12)** = 15.21359155 µg AA

If, 1 µg of **(12)** = 0.380339789 µg AA

1000 µg of **(12)** = 380.3397887 µg AA

1 mg of **(12)** = 380.3397887 µg AA

So, **1 g of (12) = 380.3397887 mg AA**

When $y = 0.089766$

$x = (0.089766 - 0.0209)/ 0.0142$

40 µg of **(18)** = 4.84971831 µg AA

If, 1 µg of **(18)** = 0.121242958 µg AA

1000 µg of **(18)** = 121.2429577 µg AA

1 mg of **(18)** = 121.2429577 µg AA

So, **1 g of (18) = 121.2429577 mg AA**

When $y = 0.954266$

$x = (0.954266 - 0.0209)/ 0.0142$

40 µg of **(29)** = 65.73 µg AA

If, 1 µg of **(29)** = 1.64325 µg AA

1000 µg of **(29)** = 1643.25 µg AA

1 mg of **(29)** = 1643.25 µg AA

So, **1 g of (29) = 1643.25 mg AA**

When $y = 0.0789$

$x = (0.0789 - 0.0209)/ 0.0142$

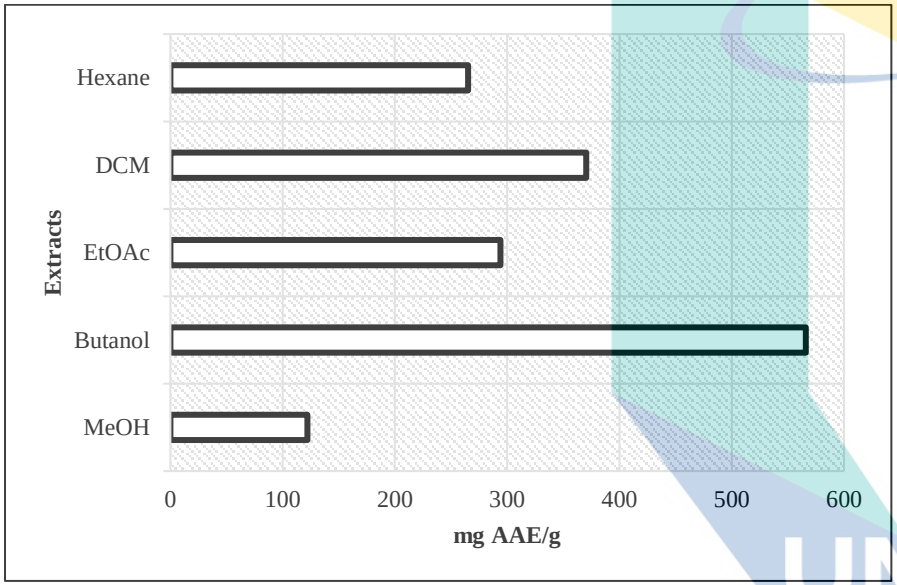
40 µg of **(33)** = 4.084507042 µg AA

If, 1 µg of **(33)** = 0.102112676 µg AA

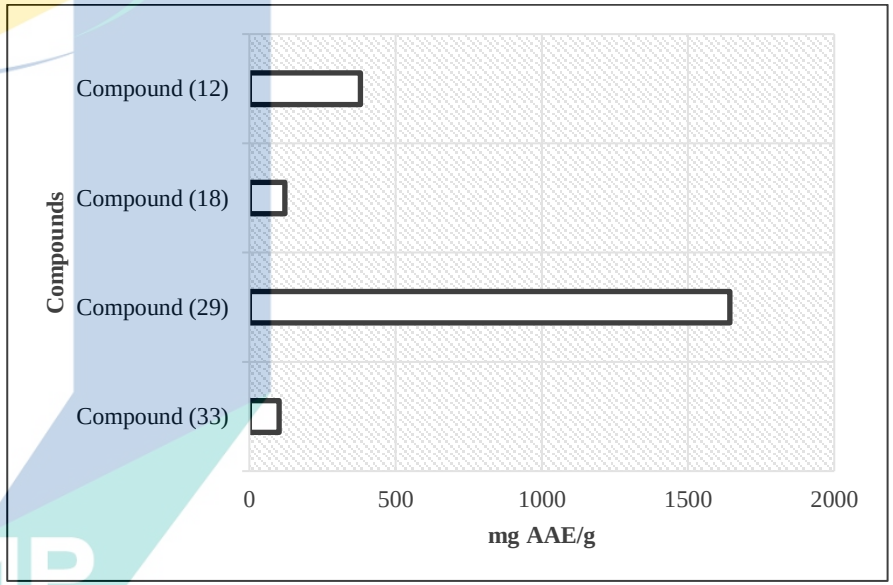
1000 µg of **(33)** = 102.112676140 µg AA

1 mg of **(33)** = 102.112676140 µg AA

So, **1 g of (33) = 102.112676140 mg AA**



FRAP values of *G. atroviridis* roots extracts



FRAP values of *G. atroviridis* isolated compounds

APPENDIX J
PUBLICATION

ISSN: 2320-5407 Int. J. Adv. Res. 7(1), 53-61

Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/xxx
DOI URL: http://dx.doi.org/10.21474/IJAR01/8295
ISSN NO. 2320-5407

RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL SCREENING, GC-MS PROFILING AND IN VITRO EVALUATION OF BIOLOGICAL ACTIVITIES OF GARCINIA ATROVIRIDIS ROOT EXTRACTS.

Nur Salsabila Ahmad Roslan, Seema Zareen, Normaiza Zamri and Muhammad Nadeem Akhtar
Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, 26300 Gambang, Malaysia.

Manuscript Info

Manuscript History
Received: 01 November 2018
Final Accepted: 03 December 2018
Published: January 2019

Keywords:
Garcinia atroviridis; solvent-solvent extraction; preliminary phytochemical screening; GC-MS; antibacterial activity; antioxidant.

Abstract

Therapeutic properties of the medicinal plant are due to the presence of phytochemical constituents. *Garcinia atroviridis* is locally known as 'asam gelugur' belongs to the Guttiferae family. Bioassay-guided solvent-solvent extraction method and yielded, hexane, dichloromethane, ethyl acetate, butanol, and methanolic extracts. These extracts were used to investigate the presence of phytochemicals. The preliminary phytochemical screening showed the existence of fixed oils and fats, carbohydrate, saponins, phenolic, flavonoids and anthraquinone glycosides in *G. atroviridis* roots. The chemical compositions were investigated by using Gas Chromatography-Mass Spectroscopy (GC-MS). Major compound identified in hexane, DCM, EA, BuOH and methanolic extracts was (*Z*)-13-docosenoic acid methyl ester (24.32%), ethyl-9-hexadecenoate (6.36%), *bis*(1,3-diisopropylcyclopentadienyl) (12.09%), 2-methyl-2-phenyl- 1,3-dioxolane (2.34%) and furfural (33.55%) respectively. The antibacterial and antioxidant activities of the extracts were investigated. The methanolic crude extract exhibited resistance towards both bacteria tested; *Bacillus subtilis* and *Escherichia coli*, thus suggesting its antibacterial activity.

Introduction:-

Garcinia atroviridis is commonly known as 'asam gelugur' in Malaysia, India, Myanmar, and Indo-China. This plant species specifically belongs to the Guttiferae. This plant is endemic species in Peninsular Malaysia. It grows wildly in lowland and hill forest up to 600-meter altitude. It was also planted by the locals for its economic and medicinal purpose (MacKeen et al., 2000). The dried *G. atroviridis* fruits known as 'asam keping' are sold commercially as seasoning. It is sour and is used to season curry, dressing fish and others. *G. atroviridis* have medicinal values as traditionally, it has been used to treat a cough, the decoction of its leaf with roots can be used to treat an earache, and the juice of the leaf is given to female after delivery (Tisdale et al., 2003; Burkill, 1966). In the previous study (MacKeen et al., 2000), it was reported that the crude methanolic extract of the different parts of the *G. atroviridis* such as fruit, leaf, stem, and trunk barks showed it possessed antibacterial, antifungal, antioxidant, and antitumor-promoting properties. This study analyses the chemical compositions of the different extracts of *G. atroviridis* roots via preliminary phytochemical screening and GC-MS. The antibacterial activities of all extracts were determined by the disc diffusion method while antioxidant activities were evaluated based on the DPPH radical scavenging activities.