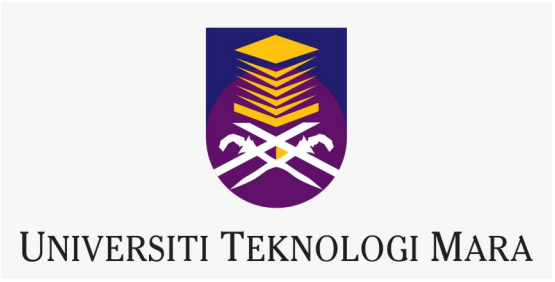


CHEMICAL COMPOSITIONS AND ANTIOXIDANT CAPACITIES OF ESSENTIAL OILS FROM *Aquilaria sinensis* STEM BARK

MUHAMMAD IRSYAD 'IMADUDDIN BIN ISKANDAR

Final Year Project Report Submitted in
Partial Fulfilment of the Requirements for the
Degree of Bachelor of Science (Hons.) Chemistry with Management
in the Faculty of Applied Sciences
Universiti Teknologi MARA

JULY 2023

DECLARATION

This Final Year Report Project entitled "Chemical Compositions and Antioxidant Capacities of Essential Oils from *Aquilaria sinensis* Stem Bark" was submitted by Muhammad Irsyad 'Imaduddin Bin Iskandar, in partial fulfillment of the requirement for Degree of Bachelor of Science (Hons.) Chemistry with Management, in the Faculty of Applied Science, and was approved by

Professor Ts. Dr. Khong Heng Yen
Supervisor
B. Sc. (Hons.) Chemistry with Management
Center for Applied Sciences Studies
Universiti Teknologi MARA Cawangan Sarawak
93400 Kota Samarahan
Sarawak

Nurhafizah Binti Mohd Selihin
Project Coordinator
B. Sc. (Hons.) Chemistry
with Management
Center for Applied Sciences Studies
Universiti Teknologi MARA
Cawangan Sarawak
93400 Kota

Ts. Dr. Siti Kartina Binti Abdul Karim
Head
Center for Applied Sciences Studies
Universiti Teknologi MARA
Cawangan Sarawak
93400 Kota Samarahan

Date: 8th August 2023

ABSTRACT

CHEMICAL COMPOSITIONS AND ANTIOXIDANT CAPACITIES OF ESSENTIAL OILS FROM *Aquilaria sinensis* STEM BARK

In recent years, *Aquilaria* essential oil (AEO) has gained much attention due to its potential medicinal properties. *Aquilaria,* also known as "gaharu," is a highly sought-after tree species in Southeast Asia, mainly Malaysia and Indonesia, due to its fragrant resin (Agarwood) containing EO. Agarwood is considered a natural treasure and is extensively used in traditional and modern medicines. AEO has many potential medicinal benefits due to its chemical composition. However, source materials from different geographical regions and extraction techniques can produce different chemical compositions in essential oils. This study aims to acquire data on AEO from Sarawak, which is expected to have different chemical compositions and antioxidant capacities due to varying environmental factors. Fresh *Aquilaria sinensis* stem bark samples that employed a stimulating inoculation method were collected, cut into small pieces, and grinded to a fine powder. The samples underwent hydro-distillation using the Clevenger apparatus to extract essential oils. Gas chromatography-mass spectroscopy (GC-MS) analysis revealed sesquiterpenes as the major chemical group, and 10-epi- γ -eudesmol was one of the major compounds identified in AEO. The EO contained high total phenolic content (1121.04±0.15 mg GAE/g extract) and high total flavonoid content (23.66±10.39 mg QE/g extract), which led to strong antioxidant activities with an IC_{50} value of 11.37±1.71 µg/mL. The data obtained serves as additional information for commercialising *Aquilaria* in Sarawak. It is expected to increase farmers' income and contribute to the future technology-based agro-food sector in line with the National Agrofood Policy 2030.

ABSTRAK

KOMPOSISI KIMIA DAN KAPASITI ANTIOKSIDA MINYAK PATI DARIPADA BATANG *AQUILARIA*

Sejak kebelakangan ini, penggunaan minyak pati *Aquilaria* (AEO) telah mendapat banyak perhatian kerana potensi nilai perubatannya. *Aquilaria*, juga dikenali sebagai "gaharu", merupakan spesies pokok yang sangat terkenal di Asia Tenggara, terutamanya Malaysia dan Indonesia, kerana resin wanginya (Agarwood) yang mengandungi EO. Gaharu dianggap sebagai khazanah semula jadi dan digunakan secara meluas dalam perubatan tradisional dan moden. AEO mempunyai banyak potensi manfaat perubatan kerana komposisi kimianya. Walau bagaimanapun, bahan sumber dari kawasan geografi yang berbeza dan teknik pengekstrakan boleh menghasilkan komposisi kimia yang berbeza dalam minyak pati. Kajian ini bertujuan untuk memperoleh data AEO dari Sarawak, yang dijangka mempunyai komposisi kimia dan kapasiti antioksida yang berbeza disebabkan oleh faktor persekitaran yang berbeza-beza. Sampel mentah kulit *Aquilaria sinensis* yang telah melakukan kaedah simulasi inokulasi telah dikutip, dipotong menjadi kepingan kecil dan mengisarnya menjadi serbuk halus. Sampel telah menjalani penyulingan hidro menggunakan alat *Clevenger* untuk mendapatkan EO yang diekstrak. Analisis Kromatografi gas-spektroskopi jisim (GC-MS) mendedahkan sesquiterpenes sebagai kelas kimia utama dan 10-epi-γ-eudesmol ialah salah satu sebatian utama yang dikenal pasti dalam AEO. EO mengandungi jumlah kandungan fenolik yang tinggi (1121.04±0.15 mg ekstrak GAE), dan jumlah kandungan flavonoid yang tinggi (23.66±10.39 mg ekstrak QE/g) yang menyebabkan aktiviti antioksidan yang kuat dengan nilai IC_{50} 11.37±1.71 µg/mL. Data kajian yang diperolehi berfungsi sebagai maklumat asas untuk mengkomersialkan *Aquilaria* di Sarawak. Ia dijangka untuk meningkatkan pendapatan petani dan menyumbang kepada sektor agromakanan berasaskan teknologi masa depan selaras dengan Dasar Agromakanan Negara 2030.

ACKNOWLEDGEMENT

First of all, praise and glory be to God. Through Him, all things are possible, Alhamdulillah, I could complete this thesis in good health at my fullest strength, knowledge, and capability.

I want to acknowledge and express my most sincere thanks and appreciation to the following people and institutions for their significant role in enabling me to complete my bachelor's degree in chemistry and management.

My dearest supervisor, Professor Ts. Dr. Khong Heng Yen for providing me with thoughtful advice, continuous supervision, constant encouragement, and guidance throughout this study. She is truly the backbone of this project which brought this study to success. I am truly thankful for all the time spared and her willingness to accept me as her supervisee.

I want to express my sincerest gratitude to Hope BioTech Sdn Bhd for providing raw samples of *Aquilaria sinensis* for my final year project. Providing these samples has contributed significantly to realizing my research objectives and helped me achieve meaningful conclusions.

Not forget, I would like to express my sincere appreciation to the laboratory staff, Siti Suhana Binti Hassan, Siti Khuzaifah Binti Joha, Mas Atikah Lizazman, and Ding Fredrick for their encouragement and guidance, that are very helpful throughout this semester for me to finish my project.

Also, my classmates, for their support and encouragement throughout this year. I struggled academically and emotionally for a few semesters, but I was blessed to have them help me through this semester.

Finally, I am grateful to my parents for their endless support financially and spiritually throughout my study period at UiTM.

Muhammad Irsyad 'Imaduddin Bin Iskandar

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviations

ABTS	2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
DNA	Deoxyribonucleic acid
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High-Performance Liquid Chromatography
IC_{50}	Half-Maximal Inhibitory Concentration
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
UV-VIS	Ultraviolet - Visible

CHAPTER 1

INTRODUCTION

1.1 Background of study

Aquilaria is a *Thymelaeaceae* (Malvales) family member and a member of the *Thymelaeoideae* subfamily (previously Aquilariodeae). The aromatic agarwood produced by the *Aquilaria* genus is well-recognised as a non-wood product. Numerous diverse civilizations across the world hold agarwood in high respect, which accounts for its many names, including agar (Hindi), agaru (Tibetans), akil (Tamil), chenxiang (Chinese), eaglewood (Papua New Guinea), gaharu (Malay), jinkoh (Japanese), oud (Arabic), mai Ketsana (Laos), mai kritsan (Vietnamese). Agarwood has several historical names in Europe, including Lignum aquila (eaglewood), Lignum aloes, and aloeswood (Adam *et al*., 2017). The highest grade, kyara, is called jinkoh in Japanese, which translates to "sinking incense." Many countries highly demand agarwood to make perfumes, incense, cosmetic products, and traditional and modern medicines. (Ali *et al.*, 2016).

Aquilaria trees are found in Southeast Asian jungles. This plant has a medium to considerable height range of 10 meters with light to dark grey bark. The leaves are smooth, glossy, green, and acute to acuminate in shape. The petals may have greenish tones. The major drivers of this expansion are the expensive heartwood and potential essential oil yields from the

resinous wood of the *Aquilaria* species. However, it takes at least ten years for *Aquilaria* trees to reach maturity and a quality level that allows for essential oil extraction. (Wongwad *et al.*, 2019)

The Chinese Pharmacopoeia includes the well-known fragrant traditional Chinese medicine agarwood. It is also a valuable traditional medicinal substance in nations like Japan, India, and others. Agarwood is utilized as incense in Buddhism, Hinduism, Islam, and other religious rites, because of its potent aroma. It is referred to as "the king of all incense, the first of all incense" because of this. According to contemporary pharmacology, agarwood essential oil (AEO) contains antioxidant, antibacterial, anti-inflammatory, air-purifying, relaxing, and soothing qualities. (Chen *et al.*, 2022). Essential oils are often used in aromatherapy to treat anxiety, depression, sleeplessness, and other mental diseases (Chen *et al.*, 2022).

The essential oils of *Aquilaria* species have been reported in a few scientific investigations to be effective in treatment, including antioxidant, antibacterial, cytotoxic, laxative, digestive, analgesic, and anti-ischemic properties (Dahham *et al.*, 2015; Chen *et al.*, 2022). Agarwood is utilized in clinics for the effective treatment of anxiety and depression with little toxicity and maximum safety, according to contemporary pharmacological investigations. Additionally, the agarwood had shown significant bioactivities in gastrointestinal control, antibacterial, anti-inflammatory,

cytotoxicity, and antioxidant characteristics. Because of this, agarwood is in high demand as a raw material for incense, perfume, and medical uses worldwide. (Tian *et al.*, 2021)

The effectiveness of *Aquilaria* essential oil is due to its chemical compositions. In addition, varied source materials and extraction techniques provide different chemical compositions in essential oils. (Chen *et al.*, 2022). By identifying the chemical compositions found in *Aquilaria* essential oil, the substances that contribute to this antioxidant capacity can be identified.

1.2 Problem statement

Agarwood essential oil (AEO) is reported to have many medicinal benefits. Its therapeutic uses are attributed to its chemical compositions. In addition, source materials from different geographical regions and extraction techniques provide different chemical compositions in essential oils. Furthermore, there still needs to be a scientifically published report on *Aquilaria* from Sarawak. It is expected that *Aquilaria* contains different chemical compositions and antioxidant capacities. Hence, this study is to obtain data on the chemical compositions and antioxidant capacities of *Aquilaria* essential oil from Sarawak.

1.3 Significance of study

The findings from this study could contribute to the additional information on *Aquilaria*'s chemical compositions and antioxidant capacity that serve as basic information for developing new natural resources-based products. This other data is essential to establish medicinal plants' therapeutic characteristics, traditional knowledge, and applications. Furthermore, the data acquired will aid in increasing *Aquilaria*'s commercialization values. As a result, it will increase farmers' income and the future development of the technology-based agro-food sector, which aligns with the National Agrofood Policy 2030 and indirectly contributes to the country's economic growth.

1.4 Objectives of study

This study aims to identify the chemical compositions in *Aquilaria* essential oil and their antioxidant capacities to provide basic scientific information for potential natural product development. The objectives of this study are:

1. To identify the chemical compositions in the *Aquilaria sinensis* essential oil from Sarawak based on Gas Chromatography-Mass Spectroscopy (GC-MS) analysis.

2. To determine the antioxidant capacity in the *Aquilaria sinensis* crude extracts based on total phenolic contents (TPC) and total flavonoid contents (TFC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

CHAPTER 2

LITERATURE REVIEW

2.1 Stimulating method applied to *Aquilaria*

Previous studies in Haikou City, Hainan Province, revealed that cultivated *A. sinensis* trees grown in the wild reported that different stimulating methods contribute to different extracted essential oil products (Bhuiyan *et al.*, 2009; Chen *et al.*, 2011). According to Chen *et al.* (2011), *A. sinensis* plant with different stimulating methods (Table 2.1) resulted in other properties and displayed various stages and hues (Figure 2.1). To encourage the production of resins in *Aquilaria*, chemical treatment was applied to *A. sinensis* trees. A gradual injection of a chemical reagent, sodium chloride, a specific concentration, was made into a tree's xylem. The chemical agent was spread throughout the whole body of the tree through water transport, causing general damage to the tree. The entire tree would be stimulated to create resinous substances to protect itself from harm. (Chen *et al.*, 2011)

Table 2.1 Materials used in the study

Sample	Stimulating Method	Characterization	Age	Plant Origin
S1	Chemical method	Agarwood	6 years	*A, sinensis*
S2	Unknown natural factor	Agarwood	Unknown	*A. sinensis*
S3	No healthy damage	Healthy trees	6 years	*A. sinensis*

Source: Chen *et al.* (2011)

2.2 Extraction methods of *Aquilaria* essential oil

Aquilaria essential oil is often extracted using a variety of techniques, including hydro-distillation (HD), supercritical fluid carbon dioxide extraction (SFE), subcritical fluid extraction, cellulase-assisted extraction, microwave extraction method (MWE), and others (Chen *et al.*, 2022).

The essential oil of S1 was liquid, yellow, fragrant, and distinct from that of S3 but comparable to S2 at room temperature. S2's essential oil was liquid, fragrant, and green. The S3 essential oil was solid at room temperature and had an acidic odor (Figure 2.1) (Chen *et al*., 2011)

Note: S1: Stems Sample from six-year-old and chemically stimulated plants;
S2: stems from wild agarwood; S3: Stems from six-year-old, heathy trees

Figure 2.1 Extracted *Aquilaria* essential oil

2.3 Crude extract of *Aquilaria*

According to Prissilla *et al.* (2022), soxhlation extraction has its advantages, including the ability to minimize damage to extract compounds due to the low boiling point of the solvent and the ability to extract more compounds with a small amount of solvent because the circulation of immersion took more place and was faster (Prissilla *et al.*, 2022). The number of extracts obtained varied by the type of solvents employed during extraction. The polar solvent is superior for *A. malaccensis* extraction since the yield extract was higher than other solvents, such as chloroform, which is a nonpolar solvent (Prissilla *et al.*, 2022).

The yield with oven dried was greater than with air dried for all extracts. This is consistent with references cited by Wan-Adilah *et al.* (2020), who observed that oven dried extracts yielded a greater extraction yield than air-dried extracts in cauliflower extract. Furthermore, in both drying techniques, *Aquilaria* extracted with Ethanol (70%) extract produced much more than Ethanol (100%) extract, water extract, and hexane extract, indicating that yield increases with increasing polarity of solvent employed, *Aquilaria sunilasi* > *Aquilaria sinensis* > *Aquilaria malessenisis* is the yield percentage per species (Wan-Adilah *et al.* 2020). As summarized in Table 2.2, the drying process substantially influenced the extract yield.

Table 2.2 Percentage of yield for each drying method and different solvents

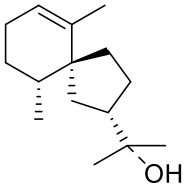
Sample	Part Used	Drying Method	Yield %			Reference
			AMA	ASU	ASI	
n-Hexane	Leaf	AD	5.23	-	-	Prissilla. A *et al.* (2022)
Ethyl Acetate	Leaf	AD	8.07	-	-	
Ethanol	Leaf	AD	10.7	-	-	
Ethanol (100%) Extract	Leaf	AD	8.59	18.53	5.29	Wan-Adilah *et al*., (2020)
	Leaf	OD	14.66	18.54	13.00	
Ethanol (70%) Extract	Leaf	AD	16.93	14.70	10.51	
	Leaf	OD	21.32	30.82	22.35	
Aqueous Extract	Leaf	AD	11.24	12.71	13.83	
	Leaf	OD	13.50	16.21	17.72	
Hexane Extract	Leaf	AD	1.18	0.82	1.04	
	Leaf	OD	1.30	1.42	2.39	
Distilled Water	Leaf	AD	21.70	-	-	Wan-Nadilah, *et al*. (2019)
	Leaf	OD	21.73	-	-	
Ethanol Extract	Leaf	AD	18.61	-	-	
	Leaf	OD	26.94	-	-	
Methanol Extract	Leaf	AD	22.98	-	-	
	Leaf	OD	16.82	-	-	
Chloroform Extract	Leaf	AD	4.03	-	-	
	Leaf	OD	5.95	-	-	
Hexane Extract	Leaf	AD	1.48	-	-	
	Leaf	OD	2.44	-	-	

Wan-Nadilah claims that oven-dried samples yielded a more significant percentage of air-dried samples in prior research except for methanol. According to Yunus *et al.* (2015), oven drying is efficient since it produces high quality goods with minimal nutritional loss, more flavor preservation, and the ability to efficiently to remove moisture quickly and efficiently, OD-ETOH had the most significant percentage yield (26.94%), followed by AD-MEOH (22.98%) and OD-DW (21.73%). Non-polar solvents like AD-CHL and AD-HEX yielded the lowest percentage yields of 4.03% and 1.48%, respectively. This study's findings are like those of Wan-Nadilah *et al.* (2018), who discovered a high percentage yield of polar

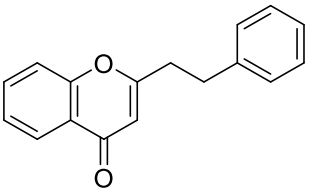
solvent (EOTH with 15.83% and DW with 14.8%) and la ow yield non-polar (HEX with 6.5%) solvent from air dry of *Cosmos caudatus* sample.

2.4 Chemical compositions of *Aquilaria* essential oil

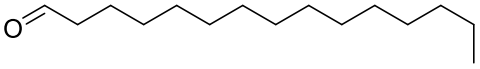
In Gogoi *et al.* (2023) study, among the identified major compounds, agarospirol **(1)** is one of the compounds responsible for agarwood fragrance among the identified major compounds. The team also reported that the subcritical water extraction method could be utilized to produce good quality agarwood essential oil (Gogoi *et al*., 2023), compared crude extract of juvenile and matured *A. malaccensis*, where they have reported 2-(2-phenyl ethyl) chromone derivative **(2)** as the major compound. In leaves of the *A. malaccensis* essential oil were composed of approximately 42 compounds, where pentadecanal **(3)** 32.082% was the major compound. From India, the chemical profile of different grades of agarwood essential oil investigated where reported that highly infected agarwood constitutes- aromadendrene 2 **(4)** (24.76%), valencene 2 **(5)** (17.53%); moderately infected wood constitutes- τ- cadinol **(6)** (16.90%), valencene 2 (**5**) (1.73%); on the other hand, less infected agarwood essential oil constitutes, 1-methyl-1-caprolactone **(7)** (39.10%), 7-hydroxymethyl-2-methoxy-xanthone **(8)** (32.06%).



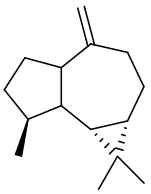
(1)



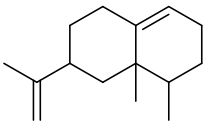
(2)



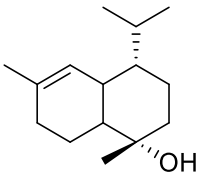
(3)



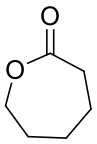
(4)



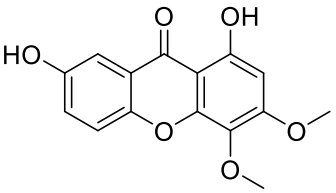
(5)



(6)



(7)



(8)

Previous studies have revealed that different species of Agarwood from other places contained different chemical compositions in extracted essential oils (Table 2.3). Besides, different artificial methods used to stimulate agarwood formation in *Aquilaria* also resulted in different agarwood.

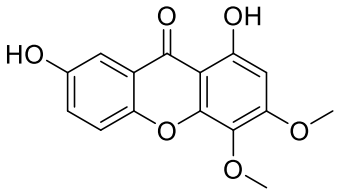
Table 2.3 shows different chemical compositions in *Aquilaria* essential oil obtained from *Aquilaria* trees located at other places, and different extraction methods (Hydrodistillation, supercritical fluid extraction, Soxhlet extraction, and microwave-assisted extraction) were employed. These findings indicated that different *Aquilaria* species, geographical regions, stimulating methods, and extraction methods give different chemical compositions. The previously reported data also show that the *Aquilaria* consists of various chemical classes: aromatics, sesquiterpenes, chromones, and fatty acids. Sesquiterpenes usually have a higher percentage among these classes of chemical composition in the *Aquilaria* essential oil.

Table 2.4 Chemical compositions in different *Aquilaria* essential oil.

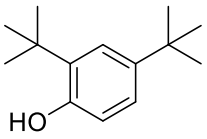
Agarwood Species	Origin	Agarwood Induction Method	Extraction Method	Chemical Compositions (%)				Reference
				Aromatics	Sesquiterpenes	Fatty Acids	Chromones	
A. sinensis	China	Agar-Wit (6m)	HD	47.43 ~ 52.78	0.5 ~ 0.98	47.43 ~ 52.78	-	Liu *et al.* (2013)
A. sinensis	China	FI (12m)	HD	2.70	34.84	0.16	-	Liu *et al.* (2013)
A. sinensis	China	BCD (20m)	HD	2.10	57.58	0.36	-	Liu *et al.* (2013)
A. sinensis	China	PTP (28m)	HD	1.51	52.03	0.69	-	Liu *et al.* (2013)
A. sinensis	China	Wild (unknown)	HD	2.34 ~ 2.39	62.35 ~ 71.48	0.06 ~ 0.35	-	Liu *et al.* (2013)
A. crass	Vietnam	FI (3 years)	HD	2.54 ~ 12.47	61.06 ~ 72.37	6.57 ~ 8.46	-	Thuy *et al.* (2019)
A. crassna	Thailand	Hammering trunks	HD	1.79	92.86	2.84	-	Wetwitayaklung *et al*., (2009)
A. crassna	Thailand	Hammering trunks	SFE	0.8	34.52	5.91	-	Wetwitayaklung *et al*., (2009)
A. sinensis	China	Insect infestation	HD*	5.12	12.34	23.8	31.62	Chen *et al.*, (2022)
A. sinensis	China	Insect infestation	SE*	11.6	7.72	19.72	21.68	Chen *et al.*, (2022)
A. sinensis	China	Insect infestation	SFE*	9.64	13.58	28	34.48	Chen *et al.*, (2022)
A. sinensis	China	Insect infestation	MAE*	11.02	21.6	25.77	30.81	Chen *et al.*, (2022)
A. sinensis	China	Hole drilling (18m)	HD	0.98	90.28	-	0.93	Chen *et al.*, (2022)
A. sinensis	China	BCD (unknown)	HD	10.63	68.68	-	-	Chen *et al.* (2022)
A. sinensis	China	BCD (unknown)	SFE	12.46	23.78	-	29.42	Chen *et al.*, (2022)
A. sinensis	China	unknown	SFE	0.22 ~ 1.15	8.93 ~ 26.10	0 ~ 0.38	23.95 ~ 53.27	Chen *et al.*, (2022)
A. sinensis	China	unknown	HD	-	54.97 ~ 57.53	1.79 ~ 2.42	-	Chen *et al.* (2022)
A . malaccensisz	Malaysia	unknown	HD	2.81 ~ 6.26	37.66 ~ 75.43	0 ~ 16.376	0 ~ 0.38	Chen *et al.*, (2022)

i. Aromatics (volatile compounds)

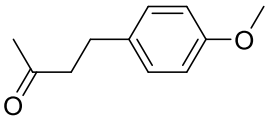
Aromatic compounds are a crucial component of agarwood. For instance, the extensively researched chemical benzyl acetone **(9)** is recognized as a landmark material for aromatic components (Chen *et al.*, 2012). Many aromatic compounds that extracted from the volatile oil, in which the mass fraction of chemical **9** was the highest, reaching 19.51%. Significant differences exist in the mass fraction of aromatic compounds in different species. Tajuddin and Yusoff (2010) discovered that Malaysian agarwood volatile oil has more aromatic chemicals than other species, with benzyl acetone **(9)** accounting for 32.1% of the volatile oil. According to Mei *et al.* (2007), benzyl acetone **(9)**, 2,4-di-tert-butylphenol **(10)**, 4-me4-methoxyphenyl acetone **(11),** and 3,5-di-tert-butylphenol **(12)** are a few identified aromatic chemicals from agarwood. These chemical structures were discovered to have no cytotoxic effects on several cell types (Mei *et al.*, 2007).



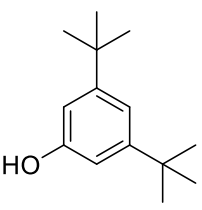
(9)



(10)



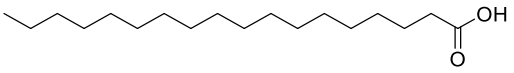
(11)



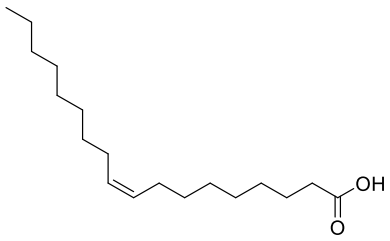
(12)

ii. Fatty acids (Non-volatile compounds)

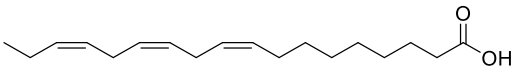
Based on Wang *et al.* (2021), the cutting method's agarwood mainly showed high fatty acids based on GC-MS spectral data. The agarwood extract generated by the nailing, drilling, and cutting techniques with ether is discovered to contain stearic acid **(13)**, oleic acid **(14)**, linoleic acid **(15)**, and palmitic acid **(16)** in the volatile agarwood oil. Besides, a variety of isolated fatty acids were included in hexadecenoic **(17)**, tridecanoic **(18)**, octadecenoic **(19)**, and other types.



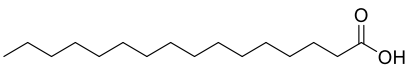
(13)



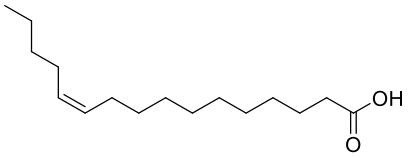
(14)



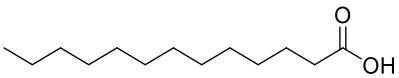
(15)



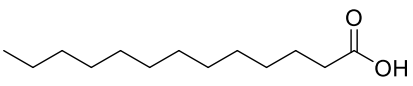
(16)



(17)



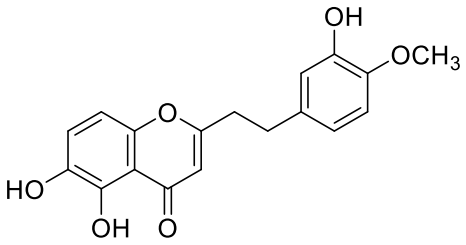
(18)



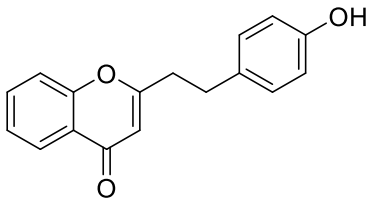
(19)

iii. Chromones

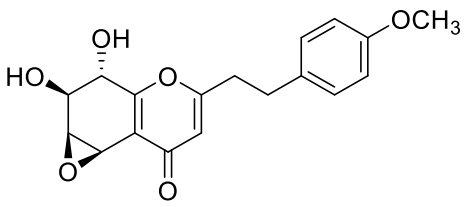
Chromones are mostly found in the *Aquilaria*. The structure-based identification of agarwood was made possible by the dimeric 2-(2-phenyl ethyl) chromone **(20-28).** High-quality agarwood has a 60% relative percentage of 2-(2-phenyl ethyl) chromone compounds in ether extract. A spectrophotometer assesses the agarwood's quality and measures the absorbance of 2-(2-phenyl ethyl) chromones at 230 and 250 nm. Different 2-(2-phenyl ethyl) chromone compounds are produced by co-fermenting endophytic bacteria with agarwood. After ethanol extraction, isolation, and purification, thirteen 2-(2-phenyl ethyl) chromone derivatives were produced (Wang *et al.*, 2021).



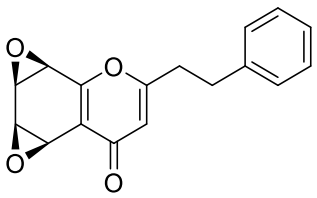
(20)
5,6-Tetrahydroxy-2-[2- (3-hydroxy-4-methoxyphenyl) ethyl]-chromone



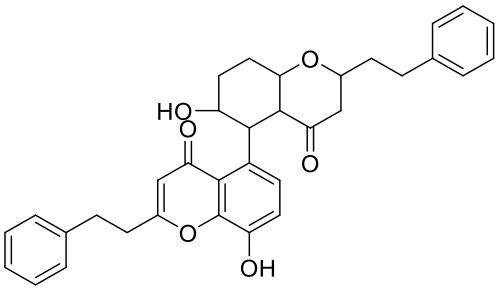
(21)
2-[2-(4-Hydroxyphenyl) ethyl] chromone



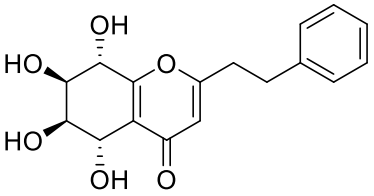
(22)
Rel-(1*AR*,2*R*,3*R*,7*bS*)-1a,2,3,7bTetrahydro-2,3- hidydroxy-5[2-(4-methoxyphenyl) ethy]-7*H* oxireno[f][1] benzophran-7-one



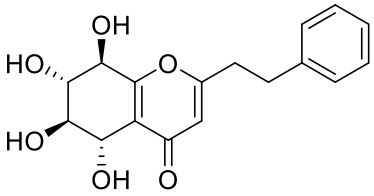
(23)
Oxidoagarchromones A



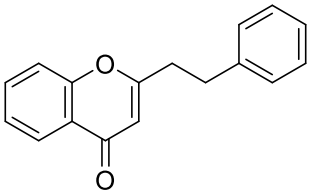
(24)
6,8*0* -Dihydroxy-2-2*0* -bis(2- phenyl ethyl)- 4*H*,4*0 H*-5,5*0* - bichromone-4,40 -dione



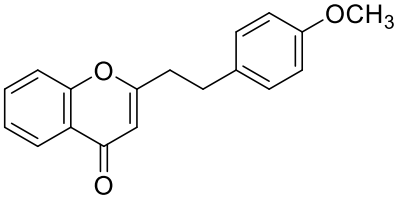
(25)
Agarotetrol



(26)
Isoagarotetrol



(27)
2-(2-phenyl ethyl) chromone

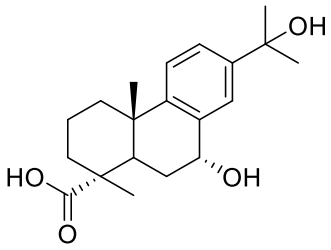


(28)
2-[2-(4-methoxyphenyl) ethyl] chromone

iv. Sesquiterpenes

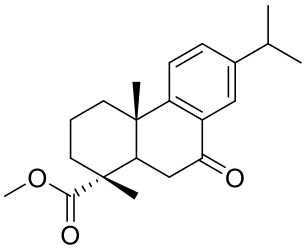
Based on the National Library of Medicine, sesquiterpenoids, specifically sesquiterpene, may play a highly significant role in human health, as part of a balanced diet and as pharmaceutical agents, due to their potential for treating cardiovascular disease and cancer. Sesquiterpenes are C-15 terpenoids that are built

from three isoprene units. One of the criteria used to evaluate the quality of agarwood is the content of triterpenes and sesquiterpenes **(29-49)**. This chemical group is responsible for giving medical advantages such as antimicrobial and antioxidant to the *Aquilaria*. Sesquiterpenes isolated from agarwood are classified into several groups based on their chemical structures. These groups include agarofurans, eudesmanes, eremophilanes, guaianes, agarospirols, and cadinanes. Sesquiterpenes were extracted from "whole-tree agarwood-inducing" technology in artificial agarwood (Wang *et al.*, 2021).



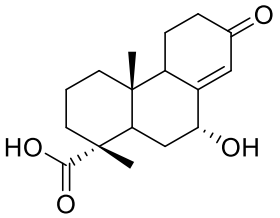
(29)

7α,15-Dihydroxydehydroabietic acid



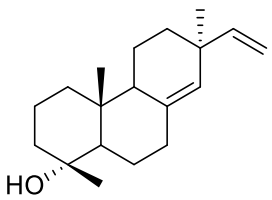
(30)

Methyl 7-oxodehydroabietate



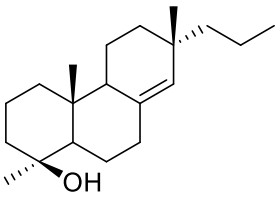
(31)

7α-hydroxypodocarpen-8(14)-en-13-on-18- oic acid



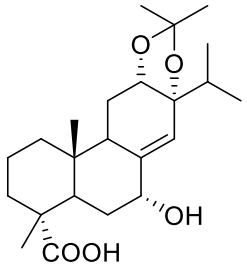
(32)

18-norpimara-8(14),15-dien-4αα-ol



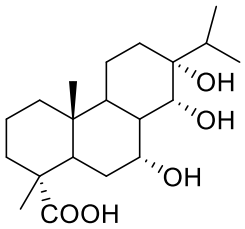
(33)

18-norpimara-8(14), and 15-dien-4α-ol



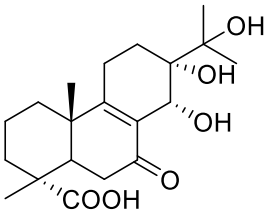
(34)

7α, 12α, 13α-trihydroxyabiet-8(14)-en-18-oic acid acetonide



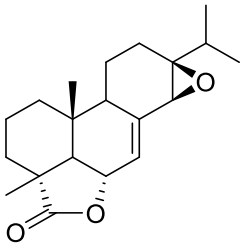
(35)

6α, 13α, 14α-trihydroxyabiet-7-en-18-oic acid



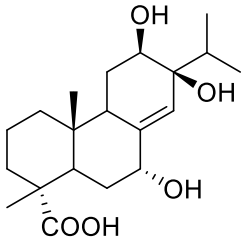
(36)

13α, 14α, 15-trihydroxy-7-oxoabiet-8-en-18-oic acid



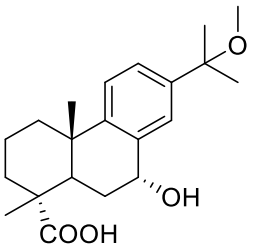
(37)

13β, 14β-epoxyabiet-7-en-18, 6α-olide



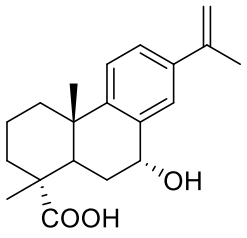
(38)

7α, 12β, 13β-trihydroxyabiet-8(14)-en-18-oic acid



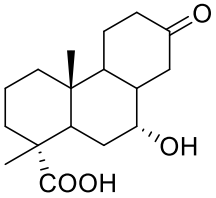
(39)

7α-hydroxyabieta-15-methoxy-8,11,13- trien18-oic acid



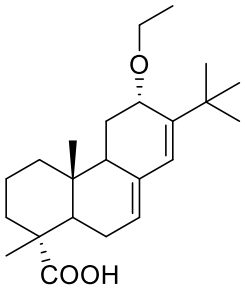
(40)

7α-hydroxyabieta-15-methoxy-8,11,13- trien18-oic acid



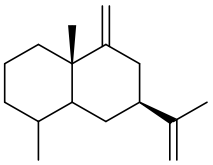
(41)

7,13-dioxopodocarpan-18-oic acid



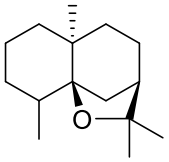
(42)

12α-ethoxyabieta-7,13-dien-18-oic acid



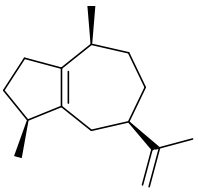
(43)

(5S,7S,10S) -(-) Selina-3,11-dien-9-one



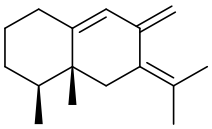
(44)

α-Agarofuran



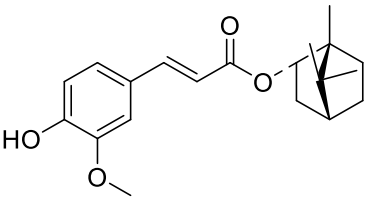
(45)

α-Guaiene



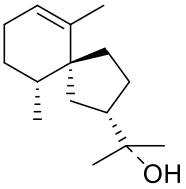
(46)

(+) -(4*S*,5*R*)-Dihydrokaranone



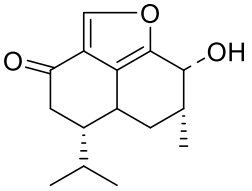
(47)

(-)-bornyl ferulate



(48)

Agarospirol

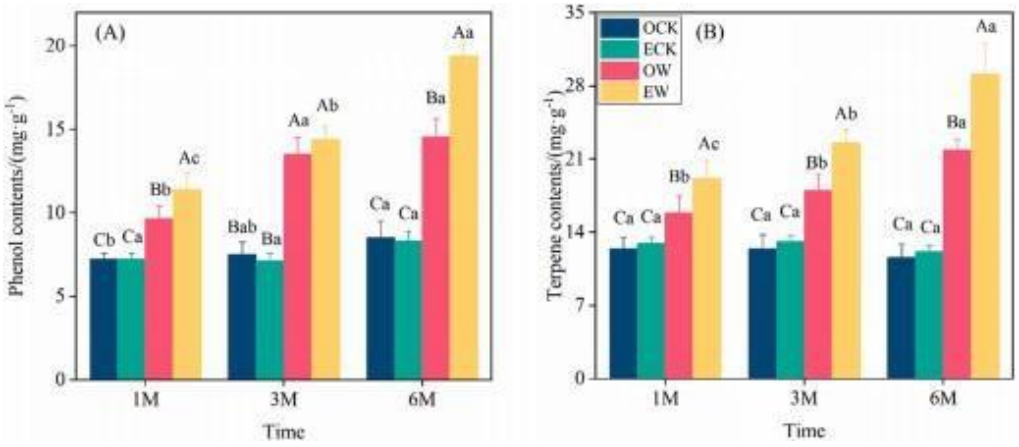


(49)

8-β-*H*-Dihydrogmelofuran

2.5 Antioxidant capacities of *Aquilaria species*

The data in Figure 2.4 shows the results of total phenols and terpenes of secondary metabolites. Based on Zhang *et al.* (2022), EW was consistently greater than OW throughout the trial, and total phenols and terpenes of the wounding stress treatments were considerably higher than the control. The overall phenol and terpene concentrations for the wounded treatments rose steadily during the therapy. Following the six-month treatment, OW and EW had phenol contents that were 1.71 and 2.27 times greater than those of OCK and ECK, respectively. Terpene content was 1.89 and 2.51 times greater in OW and EW than in OCK and ECK, respectively. Total phenol and terpene concentrations in EW were substantially higher than in OW, increasing by 4.83 and 7.25 mg g-1, respectively. (Zhang *et al*., 2022).



Note: **OCK**: ordinary *A. sinensis* control group
ECK: more accessible induced agarwood *A. sinensis* control group
OW: ordinary *A. sinensis* wounded experimental group
EW: more accessible induced agarwood *A. sinensis* impaired experimental group.
The time on the x-axis indicates the duration: one month, three months, and six months.

Figure 2.2 Effect of wounding stress on total phenols and terpenes in *A. sinensis*
Source: Zhang *et al.* (2022)

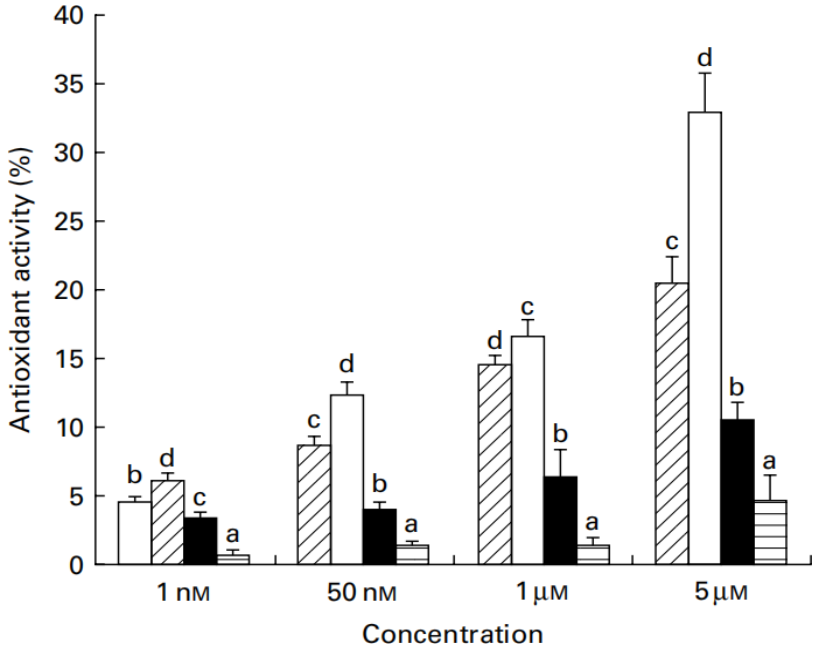
The findings show that the *Aquilaria* tree that has been induced with wounding treatment (experimental group) and the *Aquilaria* tree that has been exposed to an ordinary nature environment (control group) show positive results presence of antioxidant capacities that increase in the *Aquilaria*. This indicates that there is an antioxidant activity that occurs in the *Aquilaria*; Due to more damage treatment that has been introduced to the experimental *Aquilaria* tree, it produces more antioxidant capacities compared to ordinary *Aquilaria* tree, also increasing in time of damage stimulating treatment to the *Aquilaria* tree, increasing antioxidant capacities of *Aquilaria* essential oil (Zhang *et al.*, 2022).

Another study by Dahham *et al*. (2015) reported that the sesquiterpene, β-caryophyllene in the essential oil of *Aquilaria* showed antibacterial, antioxidant, and selective antiproliferative actions against colorectal cancer. This result underlined sesquiterpenes' tremendous potential medicinal value from agarwood (Adam *et al*., 2017). In addition, *Aquilaria*'s essential oil has antioxidant properties that may effectively scavenge DPPH and 2,20-amino-bis(3-ethylbenzthiazoline)-6-sulphonic acid (ABTS) radicals. Besides, another study by Adam *et al*. (2017) reported that luteolin and genkwanin could guard against DNA damage from hydroxyl. It works by the absorption of metals, the scavenging of free radicals, and the donation of hydrogen atoms or electrons. Additionally, the *in vitro* antioxidant activity of the ethanolic extract of agarwood tea was outstanding, although the shape of the tea leaves may have an impact on the activity. Compared to two other forms, whole and chopped leaves, antioxidant activity testing

revealed that the most potent antioxidant activity is generated from fine particles of agarwood tea leaves (Adam *et al*., 2017).

The DPPH and FRAP scavenging assays showed that *β*-caryophyllene has potent antioxidant properties (Wang *et al.* 2018). In PC12 cells (cells that are types of catecholamine cells that synthesize, store, and release norepinephrine and dopamine), hydrogen peroxide (H_2O_2)-induced oxidative damage was decreased by the essential oil of agarwood (Wang *et al.*, 2018). The aqueous extract of *A. crassna* leaves had radical scavenging capacities determined by ABTS, ferric reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) scavenging assays. A methanol extract of *A. crassna* leaves also had anti-oxidative activities. The 100% (v/v) ethanol extract exhibited the highest DPPH radical scavenging activity among the 0% to 100% (v/v) ethanol extracts isolated from *A. crassna* young leaves. *β*-Caryophyllene displayed strong antioxidant effects determined by the DPPH and FRAP scavenging methods.

Based on Dahham *et al.* (2015), the radical scavenging capability of *β*-caryophyllene, determined by the DPPH and FRAP scavenging methods, is depicted. The results of the current study agree with a previous study on the antioxidant efficacy of *β*-caryophyllene. Calleja *et al.* (2013) reported that at all concentrations, *α*-humulene, probucol, and vitamin E did not substantially inhibit lipid peroxidation as did *β*-caryophyllene (Figure 2.3).



Note: Antioxidant activity of probucol (c), *β*-caryophyllene (d), *α*-tocopherol (b), and *α*-humulene (a) on the Fe2þ/ascorbate-induced lipid peroxidation of liver microsomes, expressed as the percentage inhibition of the production of thiobarbituric acid-reactive substances. Values are means with standard errors represented by vertical bars (n 6). a,b,c,d Mean values with unlike letters were significantly different (P, 0·05).

Figure 2.3 Antioxidant activity *Aquilaria malaccensis*
Source: Calleja *et al.* (2013)

Positive controls for manufactured and natural antioxidant substances were probucol and vitamin E. In a concentration-dependent manner, *β*-caryophyllene demonstrated scavenging efficacy against the hydroxyl radical (Fenton reaction) and superoxide anion (Riboflavin test) (Table 2.4).

Table 2.4 Antioxidant efficacy of *β*-Caryophyllene

Sample	**IC_{50} Values in μM**	
	DPPH	**FRAP**
β-Caryophyllene	1.25 ± 0.06	3.23 ± 0.07
Ascorbic acid	1. 5 ± 0.03	3.8 ± 0.4

Source: Dahham *et al.* (2015)

Additionally, the hypoxanthine/xanthine oxidase system's superoxide anion-dependent cytochrome c reduction was blocked by *β*-caryophyllene.

Like α-tocopherol, β-caryophyllene prevented the lipoxygenase-dependent oxidation of linoleic acid. Compared to probucol, β-caryophyllene had higher superoxide anion and equivalent hydroxyl radical scavenging capabilities, whereas α-tocopherol had lower levels.

These findings indicate that most of the chemical compositions were sesquiterpenes, and their presence in the *Aquilaria* is responsible for the medical advantages that *Aquilaria* possesses.

According to Huda *et al.* (2009), the DPPH experiment shows that each crude extract of *A. malaccensis* has the best potential for antioxidant activities, particularly the methanol crude extract. Figure 2.4 displays the IC_{50} value of each crude extract and quercetin's standard employed in this investigation. The IC_{50} value of each crude extract is the concentration required to achieve 50% of maximal scavenging capability.

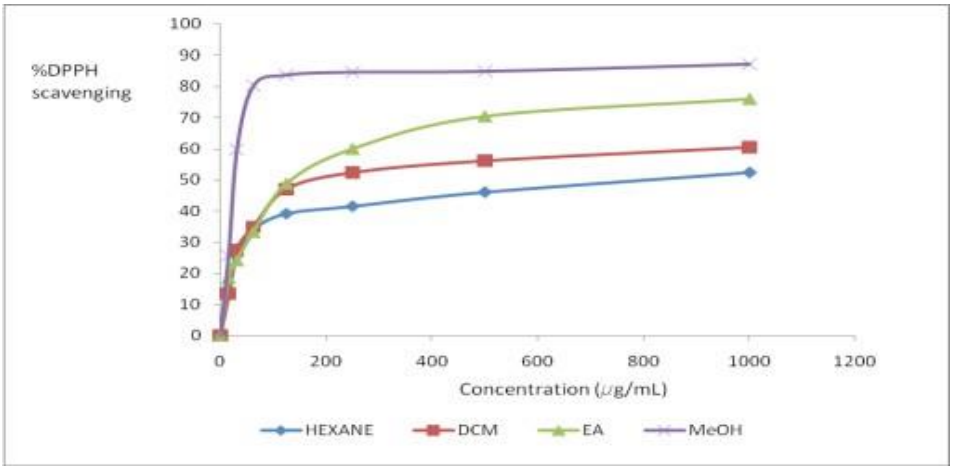


Figure 2.4 Percentage of inhibition H-donor activity of crude extract as measured using DPPH assay.
Source: Huda *et al.* (2009)

As demonstrated in Figure 2.4, the DPPH free radical scavenging activity of each crude extract may be simplified. It was discovered that all the crude extracts had positive DPPH free radical scavenging capabilities. The IC_{50} values of DPPH free radical scavenging activity were in decreasing order:

MeOH > DCM > EA > Hexane

Methanol crude extract has the most potent antioxidant activity compared to DCM, EA, and Hexane crude extract (Huda *et al.*, 2009). The most potent DPPH scavengers were methanol extracts. Quercetin is an effective free radical scavenger. Compared to such pure compounds, the IC_{50} value of the various crude extracts is high, indicating that they are effective DPPH free radical scavengers. This can be related to the extract's presence of flavonoids.

Table 2.5 TPC, TFC and DPPH values of different *Aquilaria* species

Sample/ Standard	Part Used	Drying Method	TPC (mg GAE/gm)				TFC (que ppm)				DPPH (IC_{50})(µg/mL)				Reference
			AMA	ASU	ASI	AC	AMA	ASU	ASI	AC	AMA	ASU	ASI	AC	
Ethanol (100%) Extract	Leaf	AD	82.57 ± 0.64^{a}	18.78 ± 0.14^{b}	36.72 ± 1.63^{b}	-	164.77 ± 3.04^{a}	773.92 ± 4.33^{e}	-	-	161.58 ± 1.16^{a}	364.60 ± 1.64^{d}	377.77 ± 4.55^{d}	-	
		OD	85.15 ± 1.38^{a}	20.02 ± 0.18^{b}	17.3 ± 0.28^{b}	-	268.92 ± 5.28^{c}	-	-	-	145.80 ± 1.44^{a}	290.41 ± 0.69^{c}	191.81 ± 3.94^{ae}	-	
Ethanol (70%) Extract	Leaf	AD	41.09 ± 0.45^{c}	39.97 ± 0.40^{c}	36.85 ± 0.60^{b}	-	2180.97 ± 5.48^{b}	3116.06 ± 2.14^{f}	2954.44 ± 5.77^{h}	-	291.68 ± 0.85^{c}	224.60 ± 0.85^{e}	315.98 ± 1.10^{cd}	-	Wan-Adilah, *et al.*, (2020)
		OD	64.27 ± 0.89^{d}	42.42 ± 0.17^{b}	42.56 ± 0.86^{b}	-	3733.45 ± 1.77^{d}	435.68 ± 3.58^{g}	1325.52 ± 4.19^{i}	-	33.60 ± 4.17^{b}	189.41 ± 0.79^{ae}	257.04 ± 1.67^{ce}	-	
Aqueous Extract	Leaf	AD	36.09 ± 0.95^{c}	39.29 ± 0.11^{c}	27.05 ± 0.63^{b}	-	-	-	-	-	297.37 ± 3.69^{c}	270.47 ± 0.67^{c}		-	
		OD	52.98 ± 0.95^{b}	48.99 ± 0.58^{b}	46.31 ± 0.89^{b}	-	-	-	-	-	344.17 ± 3.35^{d}	310.28 ± 3.70^{cd}	341.10 ± 1.11^{cd}	-	
Ethanol (95%) Extract	wood	-	-	-	-	11.00 ± 1.26	-	-	-	67.15 ± 6.74	-	-	-	>100	Duangsari *et al.*, (2022)
N-hexane	Leaf	AD	-	-	-	-	-	-	-	-	206.25	-	-	-	Prissilla *et al.* (2022)
Ethyl Acetate	Leaf	AD	-	-	-	-	-	-	-	-	177.41	-	-	-	
Ethanol	Leaf	AD	-	-	-	-	-	-	-	-	37.22	-	-	-	
Hexane	Leaf	-	-	-	-	-	-	-	-	-	800	-	-	-	Huda *et al.* (2009)
Dichloromethane	Leaf	-	-	-	-	-	-	-	-	-	160	-	-	-	
Ethyl Acetate	Leaf	-	-	-	-	-	-	-	-	-	140	-	-	-	
Methanol	Leaf	-	-	-	-	-	-	-	-	-	30	-	-	-	

Aqueous Extract	Leaf	-	61.16 ± 2.42	32.33 ± 3.05	29.32 ± 3.15	-	-	-	-	-	84.00 ± 93.00	286.00 ± 49.00	490.00 ± 75.00	-	Zalilawati Mat *et al*. (2020)
Polysaccharides	Leaf	-	-	-	-	-	-	-	-	-	377 ± 93.00	437.00 ± 33.00	179.00 ± 19.00	-	
Distilled Water	Leaf	AD	-	-	-	-	-	-	-	-	303.57 ± 5.11	-	-	-	Ahmad *et al*. (2019)
Ethanol	Leaf	AD	-	-	-	-	-	-	-	-	201.09 ± 4.54	-	-	-	
	Leaf	OD	-	-	-	-	-	-	-	-	152.17 ± 3.55	-	-	-	
Methanol	Leaf	AD	-	-	-	-	-	-	-	-	84.55 ± 2.93	-	-	-	
	Leaf	OD	-	-	-	-	-	-	-	-	77.21 ± 4.88	-	-	-	

Note: AD is air drying, and OD is oven drying. AMA is *Aquilaria malaccensis*, ASU is *Aquilaria subintegra*, ASI is *Aquilaria sinensis*, AC is *Aquilaria crassna*.
Data are expressed as mean ± standard deviation with three replicates.
The superscripts a, b, c, d, e, f, g, h, i represented the significance of the sample in each activity.

CHAPTER 3

METHODOLOGY

3.1 General experimental procedures

To identify its antioxidant activities, cold extraction techniques were performed to extract the phytochemicals from the dried sample using different polarity solvents, such as hexane, chloroform, and methanol (Analytical grade). Chemicals used for antioxidant, total phenolic, and total flavonoid contents, including Folin-Ciocalteau reagent, gallic acid, aluminium chlorine, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich, USA. The chemical components in essential oil from *A. sinensis* were identified using Gas Chromatography-Mass spectroscopy (GC-MS) spectroscopy. The flow chart below shows the experimental works involved (Figure 3.1).

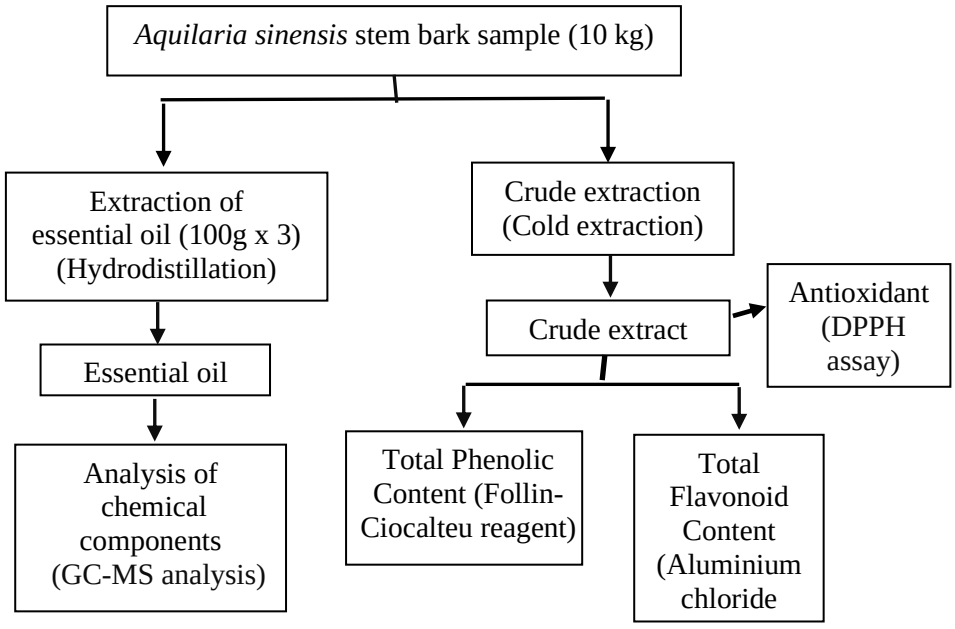


Figure 3.1 Flow chart of experimental work

3.2 Plant Materials

About 10 kg of three years old fresh *Aquilaria sinensis* stem bark (Figure 3.2) were collected from Batu Kawa Rantau Panjang. The plant employed a stimulating inoculation method to produce the resins.

Figure 3.2 *Aquilaria sinensis* stem bark

3.3 Essential oil extraction

The essential oil was extracted from *Aquilaria sinensis* stem bark using hydrodistillation in a Pyrex glass Clevenger-type apparatus for 6-8 hours. The fresh and dried *Aquilaria* wood sample was cut into small pieces (100g) and grinded to a fine powder. The fresh fine powdered samples were soaked in water in a separate beaker for about three days in a round bottom flask 2L before hydrodistillation (Figure 3.3). The complete hydrodistillation apparatus was set up and underwent the process for 6 hours. The essential oil was collected using a pipette dropper, extracted with diethyl ether (3 x 10 mL), dried over anhydrous sodium sulphate, and filtered. The experiments were performed in triplicates. The percentage yields of the oils

(w/w) were calculated based on the fresh weight of the sample using the formula as shown below:

$$\% \text{ yields of essential oil} = \frac{\text{weight of essential oils (g)}}{\text{weight of fresh sample (g)}} * 100$$

The oils were then stored in a sealed container under refrigeration at about 4 °C before being analyzed.

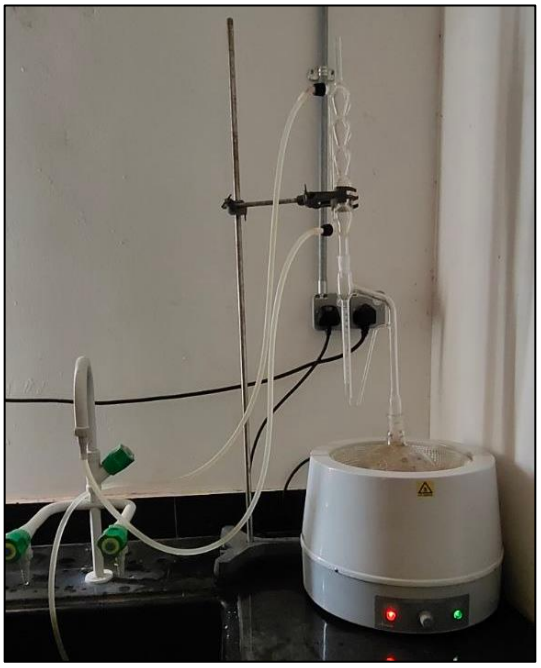


Figure 3.3 Essential oil extraction using hydrodistillation in Clevenger-type apparatus

3.4 Crude sample extraction

Three samples of 100g powdered *Aquilaria sinensis* stem bark were soaked with hexane overnight in a separate beaker and labeled as A, B, and C. The solvent was filtered and removed using a rotavapor. Using the same wood sample, the extraction process was repeated with solvent in increasing polarity, which was chloroform and followed by methanol. The powdered samples were dried entirely before being soaked with different solvents.

3.5 Identification of chemical compositions using GC-MS analysis

Chemical analysis of *Aquilaria sinensis* stem bark was undertaken by gas chromatography-mass spectrometry (GC-MS), Perkin Elmer Clarus 680 coupled to MS. An electron ionization system was set with an ionization energy of 70eV. Helium gas was the carrier gas at a 1.0 mL/min flow rate.

Approximately 1 μL essential oil in hexane (HPLC grade) was injected into the column, which held initially at 50˚C for one minute and then increased to 230˚C at a rate of 6˚C/min. The temperature was then ramped to 270˚C and held for 10 minutes. The injection was performed at 250˚C in a spitless ratio of 20:1.

The single component was identified utilizing Wiley and NIST mass spectral library based on each component's mass fragments and e/Z values (Dhara, Bhattacharyya, and Gosh, 2010).

3.6 Total Phenolic Content (TPC)

It was used with Follin-Ciocalteu reagent to determine total phenolic content. The crude sample was diluted with ethanol producing a 1 mg/mL sample solution. 200 µL of each sample was added into the microtube, and 1500 µL of FC reagent was added and vortexed thoroughly. After that, 1500 µL of 6% sodium carbonate and the mixture were incubated at room temperature for 2 hours. After the incubation process, the sample was transferred into a glass vial. The absorbance was read at 725 nm using a UV-VIS spectrophotometer (Perkin Elmer, Lambda 25) with gallic acid as the standard reference. The prepared standard solutions' concentration ranged from 200 to 1000 µg/mL. After TPC values were obtained and the calibration curve was established, the result was expressed as mg of gallic acid equivalent (GAE) per g of the extract.

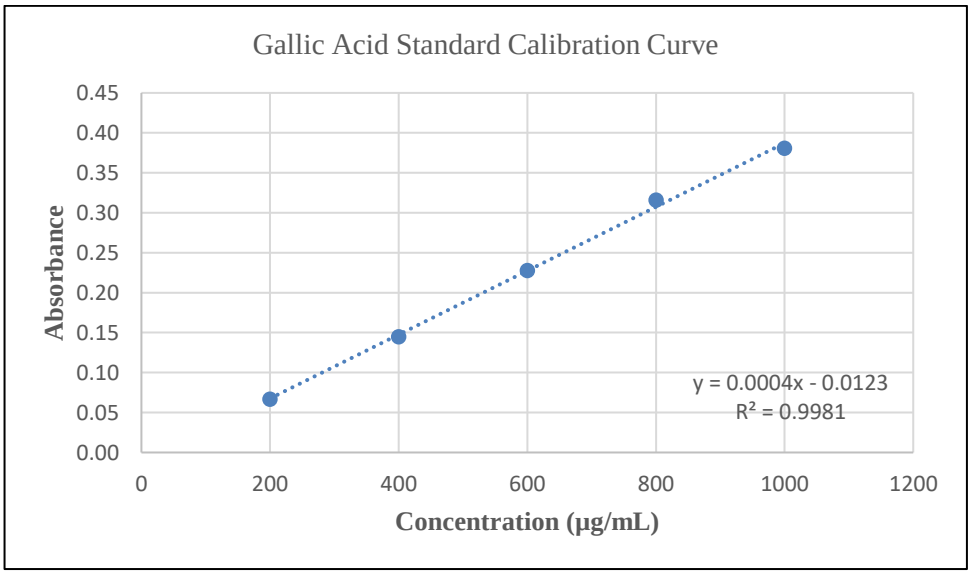


Figure 3.4 Calibration curve of gallic acid

3.7 Total Flavonoid Content (TFC)

The total flavonoid content was determined using aluminum chloride. 2 mL of sample extract, 100 µL of 2% aluminum chloride, and 900 µL of distilled water were added and mixed well. Next, the mixture was incubated for 30 minutes at room temperature and was transferred into a glass vial. The absorbance was read at 420 nm using a UV-VIS spectrophotometer (Perkin Elmer, Lambda 26), and quercetin was used as a standard. After the readings were obtained using samples prepared with serial dilution of a concentration of 100 ppm, which ranged from 50 µg/L to 3.125 µg/L, a calibration curve was established based on these data. Results were expressed as mg of quercetin (QUE) per gram (g) of extract.

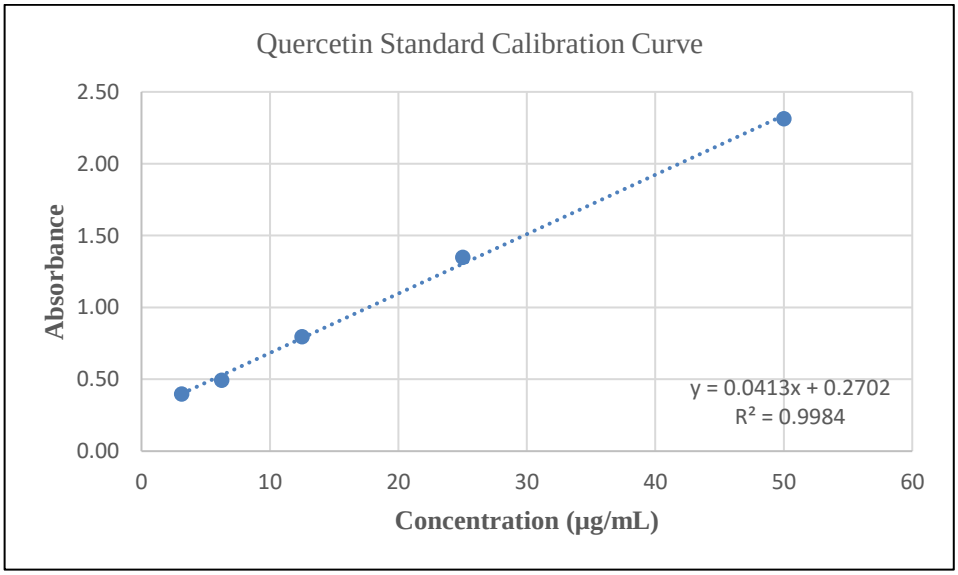


Figure 3.5 Standard curve of quercetin

3.8 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

DPPH free radical scavenging assay protocol was employed to determine the antioxidant activity of the *Aquilaria* s*inensis* crude samples. The results were expressed in inhibition percentage.

Quercetin was used as the standard. The sample was prepared by dissolving the sample with ethanol producing 1mg/mL sample solution, and the control test was added with an equal volume of ethanol. 1 mL of DPPH was dissolved in ethanol. After that, 3 mL of ethanol was *Aquilaria* extract crude sample at different concentrations using a series of dilutions of 1000 ppm, 500 ppm, 250 ppm, 125 ppm, and 62.5 ppm. The mixture was shaken vigorously and homogenously and incubated in the dark for 30 minutes at room temperature before analysis. After incubation, the absorbance was measured using a UV-VIS spectrophotometer at 517 nm. The free radical scavenging activity was calculated below.

$$\boldsymbol{RSA}\ (\%) = \frac{(\boldsymbol{Abs\ control} - \boldsymbol{Abs\ sample})}{\boldsymbol{Abs\ control}} * \mathbf{100}$$

After calculating the inhibition percentage, a graph of inhibition against the concentration was plotted.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 *Aquilaria sinensis* essential oil extraction

The essential oil extracted from *Aquilaria sinensis* was yellow-dark (Figure 4.1). The oils have a fragrant and solid form at room temperature. The sample from the *Aquilaria* tree has undergone a stimulating inoculation method, which can stimulate a whole tree and produce high quality resin. Thus, it has been expected that the sample could produce high quality essential oil. The extraction process was repeated three times. The volume of essential oil obtained was 0.1 mL ± 0.05, while the weight of essential oil was 0.039 g ± 0.01.

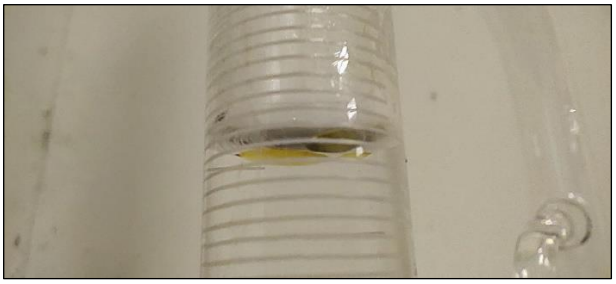


Figure 4.1 *Aquilaria sinensis* essential oil

4.2 *Aquilaria sinensis* crude extract

Aquilaria sinensis crude extracts have obtained 0.21-0.61 % yield (Table 4,1). The methanol crude extract produces a higher yield than other solvent crude (Table 4.1). This is because of the solubility of different

solvents that have been used. Several previous reports by Prissilla *et al.* (2022), Wan-Adilah *et al.* (2020), Wan Ahmad *et al.* (2019) support this production, in which yield increases with increasing solvent polarity employed where methanol > chloroform > hexane. The drying method also contributes to this production, where oven drying is efficient since it produces high-quality goods with minimal nutritional loss, more flavor preservation, and the ability to remove moisture quickly and efficiently (Wan Ahmad *et al*., 2019).

Table 4.1 Percentage (%) yield of different solvent crude extracts

Samples	Yield % ± SD
Hexane Extract	0.21 ± 0.02
Chloroform Extract	0.39 ± 0.03
Methanol Extract	0.61 ± 0.05

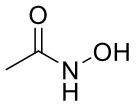
4.3 Chemical composition of *Aquilaria sinensis* essential oil

The volatile chemical compounds in the studied samples were analysed using GC-MS data based on the NIST library data. The results revealed significant amounts of volatile compounds in *Aquilaria sinensis* essential oil. The class of compound that is majorly presented is sesquiterpene. 10-epi-γ-Eudesmol (**55**) is the most abundant volatile compound (24.70%) identified in *Aquilaria sinensis* essential oil. Among the identified major compounds, agarospirol (**48**) (11.97%) is one of the compounds responsible for agarwood fragrance. Due to their potential for treating cardiovascular disease and antioxidant abilities, sesquiterpene class in the essential oil led to a highly significant role in human health, both as part of a balanced diet and as a pharmaceutical agent.

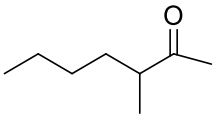
Table 4.2 Chemical compositions of *Aquilaria sinensis* essential oil

Compound	Retention Time (min)	Molecular Weight (g/mol)	Molecular Formula	Peak Area (%)
Acetohydroxamic acid **(50)**	3.9	75.06	$C_2H_5NO_2$	13.15
2-Heptanone, 3-methyl- **(51)**	4.12	128.21	$C_8H_{16}O$	8.94
Acetamide, N-2-propenyl **(52)**	4.36	99.13	C_5H_9NO	9.36
Benzene, 1,2,3,5-tetramethyl- **(53)**	7.13	134.21	$C_{10}H_{14}$	12.06
Hexadecane **(54)**	14.58	226.44	$C_{16}H_{34}$	5.96
10-epi- γ -Eudesmol **(55)**	15.19	222.36	$C_{15}H_{26}O$	24.70
Agarospirol **(48)**	15.66	222.36	$C_{15}H_{26}O$	11.97
4,4-Dimethyladamantan-2-ol **(56)**	15.98	116.20	$C_7H_{16}O$	10.37
α-Agarofuran **(44)**	16.98	220.35	$C_{15}H_{24}O$	1.74
γ-Eudesmol **(57)**	17.79	222.36	$C_{15}H_{26}O$	1.75
	Total Peak Area (%)			100.00

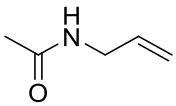
Compared to another study by Gogoi *et al.* (2023), agarospirol (**48**), *α*-agarofuran (**44**), and *γ*-eudesmol (**57**) also can be found in *Aquilaria malaccensis*. Despite different *Aquilaria species* and other stimulating methods, both species obtained sesquiterpenes that give antioxidant abilities to both *Aquilaria species*.



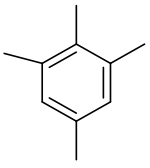
(50)



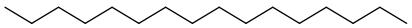
(51)



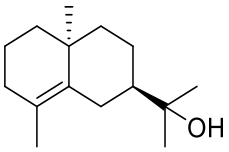
(52)



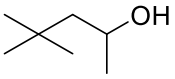
(53)



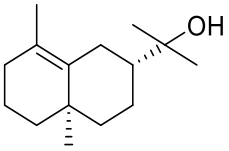
(54)



(55)



(56)



(57)

4.4 Determination of Total Phenolic Contents (TPC)

Phenolic compounds in all plants have brought many benefits that have been tested and clinically testified. The TPC was carried out using the method from the Folin-Ciocalteu reagent. The extracts were determined by extrapolation from the calibration curve prepared from the various gallic acid concentrations (Figure 3.5). The presence of phenolic anions from the extract reduces the Folin compounds, thus changing the colour of the solution from yellow to blue colour. The results were expressed in mg of gallic acid equivalence per gram (mg GAE/g).

From the study that was conducted, it was found that (Table 4.3) the TPC values of three different solvent extracts varied from 290.84 to 1121.04 mg GAE/g. This study shows that the methanol extract of *A. sinensis* has the highest phenolic contents (1121.04 mg GAE/g) compared to the other extracts.

However, the results obtained were higher compared to studies that have been referred to. The TPC found from the same species is *A. sinensis*, which is 17.3 to 46.31 mg GAE/g, using ethanol (100%) extract, ethanol (70%) extract, and aqueous extract. This study focused more on the drying method than the stimulating process. Hence the stimulating method used for the tree to produce the resin still needs to be discovered. The significant differences in the values also result from the different parts of the *Aquilaria* and the prepared solvent.

Table 4.3 TPC of different solvent extracts of *Aquilaria sinensis*

Samples	TPC ± SD (mg GAE/g)
Hexane Extract	290.84 ± 0.06
Chloroform Extract	951.66 ± 0.08
Methanol Extract	1121.04 ± 0.15

4.5 Determination of Total Flavonoid Contents (TFC)

Flavonoids are one of the most studied subclasses of polyphenols, which can be found in various plants. They possess unique properties such as anti-inflammatory, antiviral, restraining pathogenic bacteria, and protecting against ultraviolet radiation damage (Hwang *et al.*, 2015; Han *et al.*, 2016). The TFC of the extracted samples was determined by Aluminium trichloride ($AlCl_3$). The presence of flavonoids in the extract stabilized $AlCl_3$ forming acid-stable complexes. The result was expressed in mg of quercetin equivalence per gram (mg QE/g). The finding reveals that methanol extract has the highest TFC values, followed by Chloroform and hexane extract.

The finding was in opposition to a study conducted by Wan-Adilah *et al.* (2020) showed that the TFC values were higher than TPC values when using ethanol (70%) extract that gave TFC values of 1325.5 and 2954.4 mg QE/g. Because of a different part of *Aquilaria* and different solvents that have been used, it gave different values that can be expected. The flavonoid content in other *Aquilaria sinensis* extracts is shown in Table 4.4. The trend observed in TFC was tallied with TPC, in which the TFC of the extracts increases with the solvents' polarity. The polarity of extracting the solvent used is crucial as it greatly enhances the solubility of various antioxidant compounds (Muhammad *et al.*, 2014; Do *et al.*, 2015).

Table 4.4 TFC of different solvent extracts of *Aquilaria sinensis*

Samples	TFC ± SD (mg QE/g)
Hexane Extract	5.16 ± 0.43
Chloroform Extract	8.74 ± 3.60
Methanol Extract	23.66 ± 10.39

4.6 DPPH Radical Scavenging Activity

DPPH is a stable free radical with a solid purple intensity, which is widely used for the spectrophotometric measurement of antioxidant activity in plant extracts. Upon reduction by compounds through the transfer of electrons or hydrogen, it undergoes discoloration and acquires a yellowish hue. DPPH is preferred mainly due to its ability to detect the presence of active compounds even at low concentrations and short analysis time (Do *et al.*, 2014). Figure 4.2 shows DPPH scavenging activity by the *Aquilaria sinensis* with different solvent extracts., which range between

29% to 87.67%. Among these the extracted samples (Figure 4.2), methanol extract gave the maximum antioxidant potential, which was 87.67 %, followed by chloroform extract (83.33%) and hexane extract (29%).

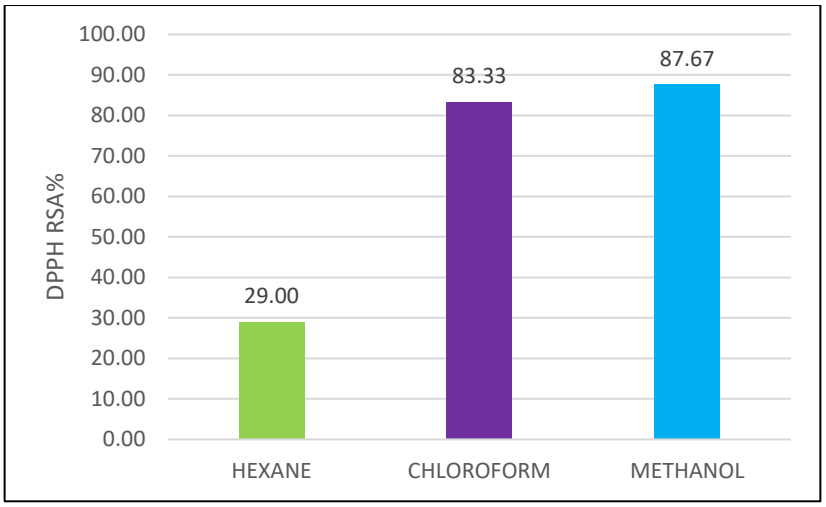


Figure 4.2 Antioxidant activities of different extracts of *Aquilaria sinensis*

The antioxidative property showed a positive linear correlation with the phenolic content in the extracts (R^2= 0.9837), in which increasing phenolic content contributed to better antioxidant capacity (Figure 4.3). The lack of polyphenolic compounds in the extract may contribute to hexane's low free radical scavenging activity. Plant phenolics are known to exhibit potent antioxidant activity towards harmful free radicals due to their reducing character, which gives them hydrogen-donating properties as well as singlet oxygen scavengers (Rice-Evans *et al.*, 1995; Rice-Evans, Miller and Paganga, 1996; Chandra *et al.*, 2014).

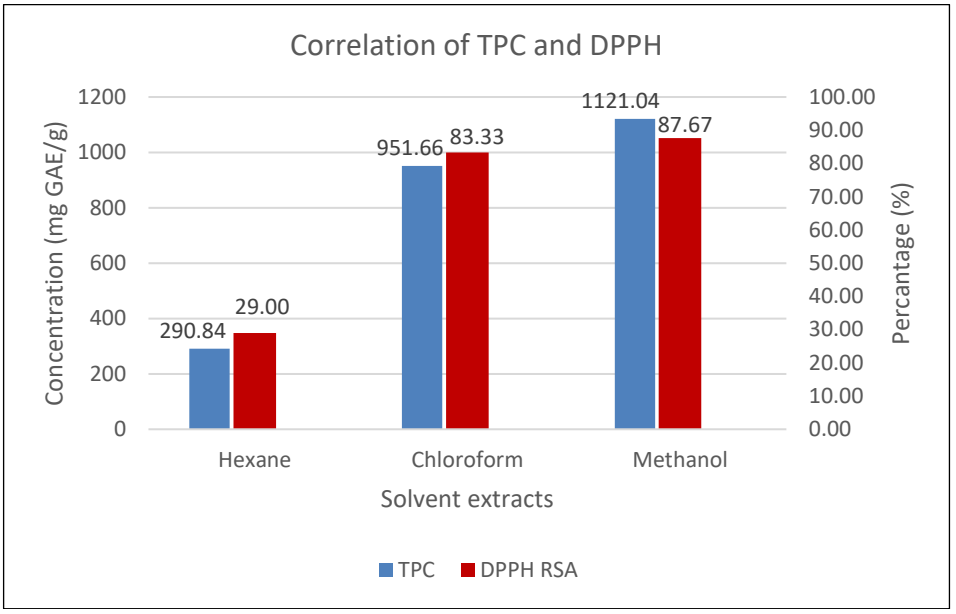


Figure 4.3 Correlation between TPC and antioxidant activity by DPPH assay of *Aquilaria sinensis.*

The extract's potency in scavenging the artificial free radical (DPPH) is more effective at higher concentrations. The half maximal inhibitory concentration, $IC_{50,}$ is the concentration of an antioxidant-containing substance required to scavenge 50% of the initial DPPH free radicals. The lower the IC_{50} value, the more potent the substance is at scavenging DPPH, which implies a higher antioxidant activity. The IC_{50} value is inversely proportional to the sample's free radical scavenging activity/ antioxidant property of the sample. That means the sample will require less amount in scavenging the free radical if the IC_{50} value is less or vice versa. The scavenging activity of free radicals in the sample is due to the presence of molecules known as antioxidants.

Figure 4.4 shows the percentages of inhibition H-donor activity of the quercetin, hexane, chloroform, and methanol as measured using DPPH

assay with different concentration. DPPH assay clearly shows the highest potential of antioxidant properties in each crude extract of *Aquilaria sinensis*, especially the methanol crude extract.

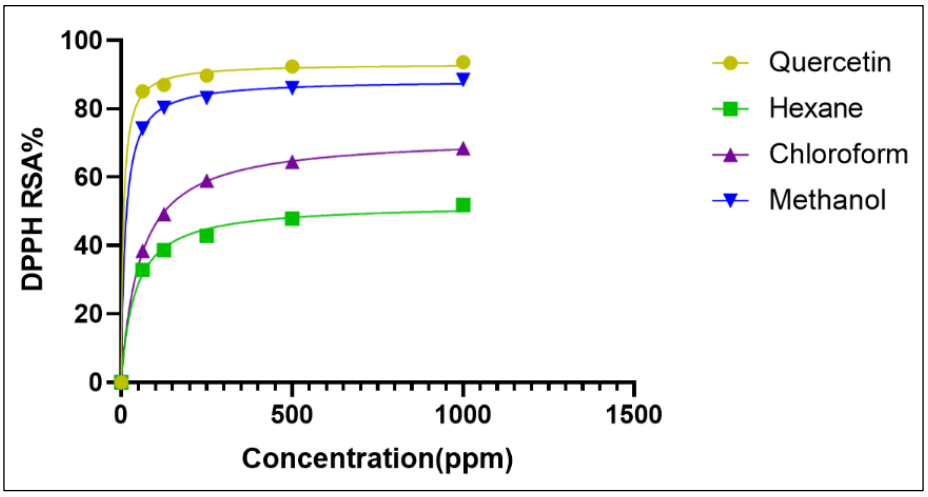


Figure 4.4 Percentage of inhibition H-donor activity of different crude extract as measured using DPPH assay with different concentration.

Table 4.5 shows the data of DPPH IC_{50} of each crude extract and the quercetin as the standard used in this study. It could be observed that all the crude extract exhibited a positive DPPH free radical scavenging activity. The IC_{50} values of DPPH free radical scavenging activity were in decreasing order:

Methanol >Chloroform > Hexane

The methanol crude possessed the highest antioxidant activity than chloroform and hexane. The methanol extract was the most effective DPPH scavenger. Quercetin is a potent free radical scavenger. So, when compared to such pure compounds, the IC_{50} value of the different crude extracts is quite good, proving that they are potent DPPH free radical scavengers.

Table 4.5 IC_{50} Values of DPPH free radical scavenging

Samples	IC_{50} (DPPH) ± SD (µg/mL)	Antioxidant activity
Hexane Extract	55.85 ± 4.92	Strong
Chloroform Extract	40.85 ± 3.91	Very strong
Methanol Extract	12.37 ± 1.71	Very strong
Quercetin	6.70 ± 0.50	Very strong

Remember that, by referring to Table 4.6, these three extracted samples show a strong antioxidant activity since three of them IC_{50} values are <100 ppm, and methanol extract and chloroform extract have very strong antioxidant characteristics since the values are <50 ppm.

Table 4.6 Antioxidant Characteristics Based on IC_{50} values.

IC_{50} Value (ppm)	Antioxidant Characteristic
200 - 150	Less
150 - 100	Moderate
100 - 50	Strong
<50	Very Strong

Source: Priska *et al.* (2019)

Compared to the findings reported by previous studies, the IC_{50} values obtained from this study are relatively low (Huda *et al.*, 2009; Wan Ahmad *et al.*, 2019; Wan-Adilah *et al.*, 2020; Zalilawati Mat *et al.*, 2020; Prissilla *et al.*, 2022; Duangsari *et al.*, 2022) which shows these samples are more potent. This might be due to the simulating inoculation method and chemicals used to simulate the entire tree of the studied samples.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

This study has achieved its objectives successfully. Essential oils and different solvent crude extracts from *Aquilaria sinensis* stem bark have yielded 0.039% for *Aquilaria sinensis* essential oil and 0.21- 0.61 % yield of *A. sinensis* crude extracts. The chemical compositions present in its essential oil are mainly from the sesquiterpenes group, which is responsible for *Aquilaria's* antioxidant abilities.

Additionally, a few chemicals screenings, including TPC, TFC as well as the DPPH antioxidant activity on the crude extracts and essential oils, have revealed that the *A. sinensis* stem bark contained high TPC, which was observed from methanol extract (23.66 ± 10.39), followed by chloroform extract (8.74 ± 3.60), and hexane extract (5.16 ± 0.43). TFC data also shows that methanol extract (1121.04 ± 0.15) contributes a high value, followed by chloroform extract (1121.04 ± 0.15) and hexane extract (1121.04 ± 0.15). In antioxidant activity using the DPPH assay, the maximum antioxidant potential was observed in methanol extract (87.67%), followed by chloroform extract (83.33%) and hexane extract (29%). In IC_{50} DPPH assay also shows that methanol extract (12.37 ± 1.71) has the lowest value compared to chloroform extract (40.85 ± 3.91) and hexane extract (55.85 ± 4.92).

The results from this study on the stem bark of *A. sinensis* provide additional data to the chemotaxonomy significance. It also provides data for further product

development derived from the stem bark of *A. sinensis* and has the potential to be marketed as a pharmaceutical and health product. As such, this finding provides valuable information and contributes to the importance of the chemotaxonomic study of this genus from the Sarawak region. The results from this study suggest that *A. sinensis* can be integrated as an antioxidant agent against illnesses associated with free radical damage.

Moreover, this study needs to be extended to assess the *in vivo* biological activity and isolate and identify bioactive compounds from the methanol fraction and more solvents. It is hoped that different species of *Aquilaria* with the same ages and stimulation methods can be done to compare the antioxidant abilities present in Sarawak. Further investigation into its chemical and biological profiles is expected to benefit humankind for its critical medicinal uses.

.

CITED REFERENCES

Adam, A. Z., Lee, S. Y., & Mohamed, R. (2017). The pharmacological properties of agarwood tea derived from *Aquilaria* (Thymelaeaceae) leaves is an emerging contemporary herbal drink. *Journal of Herbal Medicine, 10*, 37-44.

Alam, J., Mujahid, M., Badruddeen, Jahan, Y., Bagga, P., & Rahman, M. A. (2017). Hepatoprotective potential of ethanolic extract of *Aquilaria agallocha* leaves against paracetamol induced hepatotoxicity in SD rats. *Journal of Traditional and Complementary Medicine*, *7*(1), 9-13.

Alamil, J. M., Paudel, K. R., Chan, Y., Xenaki, D., Panneerselvam, J., Singh, S. K., Gulati, M., Jha, N. K., Kumar, D., Prasher, P., Gupta, G., Malik, R., Oliver, B. G., Hansbro, P. M., Dua, K., & Chellappan, D. K. (2022). Rediscovering the therapeutic potential of Agarwood in the management of chronic inflammatory diseases. *Molecules*, *27*(9), 3038.

Ali, N. A. M., Jin, C. B., & Jamil, M. (2016). Chapter 18 - Agarwood (*Aquilaria malaccensis*) Oils. In *Essential Oils in Food Preservation, Flavor and Safety*. V. R. Preedy (ed.), *Academic Press.* 173-180.

Alizadeh, Z., & Fattahi, M. (2021). Essential oil, total phenolic, flavonoids, anthocyanins, carotenoids and antioxidant activity of cultivated Damask Rose (*Rosa damascena*) from Iran: With chemotyping approach concerning morphology and composition. *Scientia Horticulturae*, *288*, 110341.

Prissilla. A, H., Nastiti, W., Lisna, H., & Tri Rini, N. (2022). Antioxidant Activity Evaluation of Agarwood *Aquilaria malaccensis* Lamk. Leaves Extract Using DPPH, FRAP, and ABTS Assays. *Proceedings of the 7th International Conference on Biological Science* (ICBS 2021), *Atlantis Press*, 2022, 18-25

Bajalan, I., Zand, M., Goodarzi, M., & Darabi, M. (2017). Antioxidant activity and total phenolic and flavonoid content of the extract and chemical composition of the essential oil of *Eremostachys laciniata* collected from Zagros. *Asian Pacific Journal of Tropical Biomedicine*, *7*(2), 144-146.

Bhardwaj, I., & Sharma, M. (2019). Herbal anesthetic agents: An overview on sources, uses and future perspectives. *Asian Journal of Pharmacy and Pharmacology*, *5*(S1), 21-27.

Bhuiyan, N. I., Begum, J., & Bhuiyan, N. H. (2009). Analysis of essential oil of eaglewood tree (*Aquilaria agallocha* Roxb.) by gas chromatography-mass spectrometry. *Bangladesh Journal of Pharmacology*, *4*(1), 24-28.

Calleja, M. A., Vieites, J. M., Montero-Meterdez, T., Torres, M. I., Faus, M. J., Gil, A., & Suárez, A. (2013). The antioxidant effect of *β*-caryophyllene protects rat liver from carbon tetrachloride-induced fibrosis by inhibiting hepatic stellate cell activation. *British Journal of Nutrition*, *109*(3), 394-401.

Chaiyawathanananthn, P., Duangsri, W., Itharat, A., Pipatrattanaseree, W., & Ooraikul, B. (2022). *In vitro α*-amylase, *α*-glucosidase inhibition, and anti-oxidant activities of plant components in Ya-Hom Teppajid. *Asian Medical Journal and Alternative Medicine, 22*(-), S22-S32.

Chen, H., Yang, Y., Xue, J., Wei, J., Zhang, Z., & Chen, H. (2011). Comparison of Compositions and Antimicrobial Activities of Essential Oils from Chemically Stimulated Agarwood, Wild Agarwood and Healthy *Aquilaria sinensis* (Lour.) Gilg Trees. *Molecules*, *16*(6), 4884-4896.

Chen, X., Wang, C., He, Q., Feng, J., Chen, D., Wei, J., & Liu, Y. (2022). Chemical composition and potential properties in mental illness (anxiety, depression and insomnia) of agarwood essential oil: A review. *Molecules*, *27*(14), 4528.

Dahham, S. S., Tabana, Y. M., Ahmed Hassan, L. E., Khadeer Ahamed, M. B., Abdul Majid, A. S., & Abdul Majid, A. M. S. (2016). *In vitro* antimetastatic activity of Agarwood (*Aquilaria crassna*) essential oils against pancreatic cancer cells. *Alexandria Journal of Medicine*, *52*(2), 141-150.

Dahham, S. S., Tabana, Y. M., Iqbal, M. A., Ahamed, M. B. K., Ezzat, M. O., Majid, A. S. A., & Majid, A. M. S. A. (2015). The anticancer, antioxidant, and antimicrobial properties of the sesquiterpene *β*-caryophyllene from the essential oil of *Aquilaria crassna*. *Molecules*, *20*(7), 11808-11829.

Eroglu, E., & Girgin, S. N. (2021). A unique phenolic extraction method from olive oil macerate of Hypericum perforatum using DMSO: Assessment of *in vitro* anticancer activity, LC-MS/MS profile, total phenolic content, and antioxidant capacity. *South African Journal of Botany*, *139*, 6-11.

Farhadi, N., Babaei, K., Farsaraei, S., Moghaddam, M., & Ghasemi Pirbalouti, A. (2020). Changes in essential oil compositions, total phenol, flavonoids, and antioxidant capacity of *Achillea millefolium* at different growth stages. *Industrial Crops and Products*, *152*, 112570.

Gogoi, R., Sarma, N., Begum, T., Chanda, S. K., Lekhak, H., Sastry, G. N., & Lal, M. (2023). Agarwood (*Aquilaria malaccensis* L.) is a quality fragrant and medicinally significant plant-based essential oil with pharmacological potentials and genotoxicity. *Industrial Crops and Products*, *197*, 116535.

Huda, A. W. N., Munira, M. A. S., Fitrya, S. D., & Salmah, M. (2009). Antioxidant activity of *Aquilaria malaccensis* (Thymelaeaceae) leaves. *Pharmacognosy Research*, *1*(5), 270-273.

Ibrahim, A. H., Al-Rawi, S. S., Majid, A. M. S. A., Rahman, N. N. A., Salah, K. M. A., & Kadir, M. O. A. (2011). Separation and fractionation of *Aquilaria malaccensis* oil using supercritical fluid extraction and the cytotoxic properties of the extracted oil. *Procedia Food Science*, *1*, 1953-1959.

Jong, P. L., Tsan, P., & Mohamed, R. (2014). Gas chromatography-mass spectrometry analysis of agarwood extracts from mature and juvenile *Aquilaria malaccensis*. *International Journal of Agriculture and Biology*, *16*(3), 644-648.

Kandi, S., & Charles, A. L. (2019). Statistical comparative study between the conventional DPPH spectrophotometric and dropping DPPH analytical method without spectrophotometer: Evaluation for the advancement of antioxidant activity analysis. *Food Chemistry*, *287*, 338-345.

Liu, Y., Chen, H., Yang, Y., Zhang, Z., Wei, J., Meng, H., Chen, W., Feng, J., Gan, B., Chen, X., Gao, Z., Huang, J., Chen, B., & Chen, H. (2013). Whole-tree agarwood-inducing technique: an efficient novel technique for producing high-quality agarwood in cultivated *Aquilaria sinensis* trees. *Molecules*, *18*(3), 3086-3106.

Mbopi, P. Y., Fozeng, H. D. S., Nguekeu, Y. M. M., Bitchagno, G. T. M., Ngantchouko, C. B. N., Awouafack, M. D., Opatz, T., Ngouela, S. A., Morita, H., & Tene, M. (2021). Chemical constituents, total phenolic content, antioxidant activity, and bactericidal effect of *Dicliptera verticillate* (Acanthaceae). *South African Journal of Botany*, *142*, 216-221.

Mei, W.-L., Yang, D.-L., Wang, H., Yang, J.-L., Zeng, Y.-B., Guo, Z.-K., Dong, W.-H., Li, W., & Dai, H.-F. (2013). Characterization and Determination of 2-(2-Phenylethyl)chromones in Agarwood by GC-MS. *Molecules, 18*(10), 12324-12345.

Mei, W.-L., Zeng, Y.-B., Wu, J., Cui, H.-B., & Dai, H.-F. (2008). Chemical composition and anti-MRSA activity of the essential oil from Chinese eaglewood. *Journal of Chinese Pharmaceutical Sciences*, *17*(5), 225-229.

Nurcholis, W., Sya'bani Putri, D. N., Husnawati, H., Aisyah, S. I., & Priosoeryanto, B. P. (2021). Total flavonoid content and antioxidant activity of ethanol and ethyl acetate extracts from accessions of *Amomum compactum* fruits. *Annals of Agricultural Sciences*, *66*(1), 58-62.

Polatoğlu, K., Karakoç, Ö. C., & Gören, N. (2013). Phytotoxic, DPPH scavenging, insecticidal activities and essential oil composition of *Achillea vermicularis*, *A. teretifolia* and proposed chemotypes of *A. biebersteinii* (Asteraceae). *Industrial Crops and Products*, *51*, 35-45.

Priska, M., Peni, N., & Carvallo, L. (2019). Phytochemical screening and antioxidant effectiveness of garlic (*Allium sativum*) from Timor Island. *Biosaintifika: Journal of Biology & Biology Education*, *11*, 1-7.

Rojas-Ocampo, E., Torrejón-Valqui, L., Muñóz-Astecker, L. D., Medina-Mendoza, M., Mori-Mestanza, D., & Castro-Alayo, E. M. (2021). Antioxidant capacity, total phenolic content, and phenolic compounds of pulp and bagasse of four Peruvian berries. *Heliyon*, *7*(8), e07787.

Tajuddin, S. N., & Yusoff, M. M. (2010). Chemical composition of volatile oils of *Aquilaria malaccensis* (Thymelaeaceae) from Malaysia. *Natural Product Communications*, *5*(12), 1965-1968.

Takatsuka, M., Goto, S., Kobayashi, K., Otsuka, Y., & Shimada, Y. (2022). Evaluation of pure antioxidative capacity of antioxidants: ESR spectroscopy of stable radicals by DPPH and ABTS assays with singular value decomposition. *Food Bioscience*, *48*, 101714.

Thuy, D. T., Tuyen, T. T., Thuy, T. T., Minh, P. T., Tran, Q. T., Long, P. Q., Nguyen, D. C., Bach, L. G., & Chien, N. Q. (2019). Isolation process and compound identification of agarwood essential oils from *Aquilaria crassna* cultivated at three different locations in Vietnam. *Processes*, *7*(7), 432.

Tian, C., Wu, A., Yao, C., Song, Z., Shen, L., & He, L. (2021). UHPLC−QTOF−MS based metabolite profiling analysis and the correlation with biological properties of wild and artificial agarwood. *Journal of Pharmaceutical and Biomedical Analysis*, *194*, 113782.

Wan-Nadilah, W. A., Ghani, D. A., Abd Jalil, M. S., Ali, A. M., Mamat, W. N. A. W., & Mahmod, N. H. (2020). Effects of drying methods and solvents on phenolic and flavonoid content, dpph radical scavenging and α-glucosidase activities of *Aquilaria* Leaves Extract. *Malaysian Applied Biology*, *49*(4), 201-205.

Wan-Nadilah, A.W.A., Abdul Manaf, A., Wan Nur Amalina Wan, M., & Nor Hasima, M. (2019). Evaluation of dpph free radical scavenging, α-glucosidase inhibitory, and antimicrobial activities of *Aquilaria malaccensis* leaf extracts. *Journal of Agrobiotechnology*, *10*(1), 36–45.

Wang, S., Yu, Z., Wang, C., Wu, C., Guo, P., & Wei, J. (2018). Chemical constituents and pharmacological activity of agarwood and *Aquilaria* Plants. *Molecules*, *23*(2), 342.

Wang, Y., Hussain, M., Jiang, Z., Wang, Z., Gao, J., Ye, F., Mao, R., & Li, H. (2021). *Aquilaria* species (Thymelaeaceae) distribution, volatile and non-volatile phytochemicals, pharmacological uses, agarwood grading system, and induction methods. *Molecules*, *26*(24), 7708.

Wetwitayaklung, P., Thavanapong, N., & Charoenteeraboon, J. (2009). Chemical constituents and antimicrobial activity of essential oil and extracts of heartwood of *Aquilaria crassna* obtained from water distillation and supercritical fluid carbon dioxide extraction. *Science, Engineering and Health Studies*, *3*, 25-33.

Wongwad, E., Pingyod, C., Saesong, T., Waranuch, N., Wisuitiprot, W., Sritularak, B., Temkitthawon, P., & Ingkaninan, K. (2019). Assessment of the bioactive components, antioxidant, antiglycation, and anti-inflammatory properties of *Aquilaria crassna* Pierre ex Lecomte leaves. *Industrial Crops and Products*, *138*, 111448.

Yan, T., Yang, S., Chen, Y., Wang, Q., & Li, G. (2019). Chemical profiles of cultivated agarwood induced by different techniques. *Molecules*, *24*(10), 1990.

Yang, J., Chen, J., Hao, Y., & Liu, Y. (2021). Identification of the DPPH radical scavenging reaction adducts of ferulic acid and sinapic acid and their structure-antioxidant activity relationship. *LWT*, *146*, 111411.

Yang, L., Yang, J.L., Dong, W.H., Wang, Y.L., Zeng, J., Yuan, J.Z., Wang, H., Mei, W.L., & Dai, H.F. (2021). The characteristic fragrant sesquiterpenes and 2-(2-phenylethyl)chromones in wild and cultivated "Qi-Nan" agarwood. *Molecules*, *26*(2), 436.

Yao, C., Qi, L., Zhong, F., Li, N., & Ma, Y. (2022). An integrated chemical characterization based on FT-NIR, GC–MS, and LC-MS for the comparative metabolite profiling of wild and cultivated agarwood. *Journal of Chromatography B, 1188,* 123056.

Yousefi, M., Rahimi-Nasrabadi, M., Pourmortazavi, S. M., Wysokowski, M., Jesionowski, T., Ehrlich, H., & Mirsadeghi, S. (2019). Supercritical fluid extraction of essential oils. *TrAC Trends in Analytical Chemistry*, *118*, 182-193.

Zalilawati Mat, R., Nur Nasyitah Mohd, N., Wan Nadilah Wan, A., & Nor Hasima, M. (2020). α-Glucosidase inhibition, DPPH scavenging, and chemical analysis of polysaccharide extracts of *Aquilaria sp*. leaves. *Journal of Agrobiotechnology*, *11*(2), 59-69.

Zhang, P., Li, X., Cui, Z., & Xu, D. (2022). Morphological, physiological, biochemical, and molecular analyses reveal wounding-induced agarwood formation mechanism in two types of *Aquilaria sinensis* (Lour.) Spreng. *Industrial Crops and Products*, 178, 114603.

Zhang, Z., Han, X.M., Wei, J.H., Xue, J., Yang, Y., Liang, L., Li, X.J., Guo, Q.M., Xu, Y.H., & Gao, Z.H. (2014). Compositions and antifungal activities of essential oils from agarwood of *Aquilaria sinensis* (Lour.) Gilg induced by *Lasiodiplodia theobromae* (Pat.) Griffon. & Maubl. *Journal of the Brazilian Chemical Society*, *25*, 20-26.

***CURRICULUM* VITAE**

A. Personal profile

Full name	Muhammad Irsyad 'Imaduddin Bin Iskandar
National IC no	990409 13 5599
Birth date	09th April 1999
Citizenship	MALAYSIA
Place of birth	Sarawak Public Hospital
Gender	Male
Correspondence address	No 151 Kampung Semera Tengah, Sadong Jaya, 94600, Asajaya, Sarawak
Telephone no. (H)	-
Telephone no. (HP)	016-4354944
Email adress	irsy4d09@gmail.com

B. Hobbies and interests

I enjoy doing many things since I am not really into doing something that is the same as my daily routine. Through that, I have many hobbies such as playing games, reading books, cooking, playing guitars, editing videos, and photoshopping. I am eager to learn new things since we live only once; I would like to experience many things that can increase my life values.

C. Academic qualifications

Degree	Area	Institution	Year Awarded
B.Sc. (Hons)	Chemistry with Management	Universiti Teknologi Mara Kampus Samarahan 2	2023
Diploma	Applied Science	Universiti Teknologi Mara Kampus Samarahan 2	2020
Foundation	Life Science	Pre-University Malaysia Sarawak	2017
S.P.M	Pure Science	SMK Sadong Hilir	2016

D. Work Experience

Post	Place	Year
Research Assistant (SKP)	Universiti Teknologi Mara Kampus Samarahan 2	2023
Tutor	Home	2016-2020
Labour	Freelance	2020-present