

IDENTIFICATION OF CHEMICAL CONSTITUENTS OF AGARWOOD
(*AQUILARIA MALACCENCIS*) OIL EXTRACTED BY SUPERCRITICAL FLUID
AND HYDRODISTILLATION METHOD

SITI NOOR FAHIMAH BT JUSOH

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UNIVERSITI MALAYSIA PAHANG

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ABSTRACT

Agarwood oil is regarded as the one of the most valuable essential oil due to its odours and applications in the perfumery industry. The essential oil was extracted by different extraction methods: i.e; hydrodistillation (HD) and supercritical fluid extraction (SFE), and analysed via gas chromatography flame-ionization detector (GC-FID) and gas chromatography mass spectrometer (GC-MS). This research aims to identify the optimum extraction condition for isolating agarwood essential oil and to profile chemical constituents via SFE. The effects of different parameters such as pressure, temperature, volume of modifier and static time were investigated. The optimum parameter; pressure of 41.37 MPa, temperature of 60 °C, 48 mL of modifier and 30 minutes of static time. The recovery of essential oils were as follows; 0.20 % for HD, 0.65 % for SFE without modifier and 1.73 % with modifier. Fourty three compounds were identified in the hydrodistilled oil with agarospirol (13.57 %), tetradecanal (6.63 %) and pentadecanal (4.9 %) as major compounds. However for SFE, it composed by fourty seven compounds with eudesmol (13.68 %), oxo-agarospirol (4.54 %) and 2-hydroxyquaiia-1(10),11,15-oic acid (3.24 %) for non-modifier meanwhile with modifier ; eudesmol (11.53-13.75 %), hexadecanol (4.58-5.00 %) and dehydrojinkoh-eremol (2.42 -2.91 %). Data shows that SFE with 41.37 MPa, 60 °C and 48 mL ethanol, gives higher recovery meanwhile nonmodified SFE and conventional extraction yields were comparable.

ABSTRAK

Minyak gaharu dianggap sebagai salah satu daripada minyak yang paling berharga kerana bau dan aplikasinya dalam industri minyak wangi. Minyak diekstrak dengan kaedah pengekstrakan yang berbeza: iaitu ; penyulingan (HD) dan pengekstrakan cecair genting lampau (SFE) , dan dianalisis melalui kromatografi gas pengesan api-pengionan (GC- FID) dan kromatografi gas spektrometer jisim (GC- MS). Kajian ini bertujuan untuk mengenal pasti keadaan pengekstrakan optimum untuk mengasingkan minyak pati gaharu dan ke profil kimia melalui SFE . Kesan parameter yang berbeza seperti tekanan , suhu, jumlah pengubahsuai dan masa statik yang diasasat. Parameter optimum; tekanan 41.37 MPa, suhu 60 ° C , 48 mL pengubahsuai dan 30 minit masa statik. Pemulihan minyak pati adalah seperti berikut ; 0.2% untuk HD, 0.65% untuk SFE tanpa pengubahsuai dan 1.73 % dengan pengubahsuai . Empat puluh tiga komponen telah dikenal pasti dalam minyak penyulingan dengan agarospirol (13.57 %), tetradecanal (6.63 %) dan pentadecanal (4.9%) sebagai sebatian utama. Walau bagaimanapun untuk SFE , ia terdiri oleh empat puluh tujuh komponen dengan eudesmol (13.68 %), Oxo - agarospirol (4.54 %) dan 2- hydroxyquaia -1 (10) asid ,11,15 oic (3.24 %) bagi bukan pengubahsuai, sementara itu dengan pengubahsuai ; eudesmol (11.53-13.75 %), hexadecanol (4.58-5 %) dan dehydrojinkoh - eremol (2.42 - 2.91 %). Data menunjukkan bahawa SFE dengan 41.37 MPa , 60 ° C dan 48 mL etanol, memberikan pemulihan yang lebih tinggi sementara itu tanpa pengubahsuaian SFE dan konvensional hasil pengekstrakan adalah sama.

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LIST OF SYMBOL

$^{\circ}\text{C}$	Celsius
α	Alpha
β	Beta
δ	Delta
γ	Gamma
μ	Micro
%	Percentage
$[\alpha]_D^{25}$	Optical rotation at (25°C)
Psi	Pounds per square inch
kPa	Kilopascal
bar	Unit of pressure
T	Temperature
Min	Minute
L	Litre
g	Gram
m	Metre
cm	Centimetre
Kg	Kilogram
RM	Ringgit Malaysia
mL	Millilitre
nm	Nanometre
λ	Lambda

eV	Electronvolt
C _n	Number of carbon
i.d	Internal diameter
kV	kilovolt
m/z	Mass-to-charge-ratio

LIST OF ABBREVIATIONS

b.p	Boiling Point
HD	Hydrodistillation
SFE	Supercritical Fluid Extraction
MAE	Microwave Assistant Extraction
GC	Gas Chromatography
GC-FID	Gas Chromatography-Flame Ionization Detector
GC-MS	Gas Chromatography-Mass Spectrometry
KI	Kovats Index
NIST	National Institute Of Standards Technology
CO ₂	Carbon Dioxide
DCM	Dichloromethane
RT	Retention Time
HS-SPME	Headspace-Solid Phase Microextraction
DPMS	100% Dimethylpolysiloxane
MSD	Mass Selective Detector
PDMS	Polydimethylsiloxane
PVB	Polyvinyl Butyral
DVB	Divinylbenzene
FPP	Farnesyl Diphosphahate
IPP	Isopentenylpyrophosphate
DMAPP	Dimethylallyldiphosphate
MEP	Methylerythritol Phosphate

GPP	Geranyl Diphosphate
GGPP	Geranylgeranyl Diphosphate
HPLC	High Performance Liquid Chromatography
SEM	Scanning Electron Microscopy
PAHs	Polycyclic Aromatic Hydrocarbons
FID	Flame Ionization Detector
ECD	Electron Capture Detector
FPD	Flame Photometric Detector
PID	Photo Ionization Detector
ANOVA	Analysis Of Variance

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Essential oil represents a small fraction of a plant's composition but confers the characteristics for which aromatic plants are used in the pharmaceutical, food and fragrance industries. Malaysia's tropical forest contains many plants with important chemical compounds. Of the estimated 12 000 species in Malaysia, more than 1 000 species are said to have therapeutic property and used in the local traditional medicine system (Ikram, 1995). Plants have gained prominence due to their long term uses by societies in traditional healthcare.

Essential oil composes a complex composition, containing from a few dozen to several hundred constituents, especially hydrocarbons terpenes and sesiterpenes and oxygenated compounds (alcohols, aldehydes, ketones, acids, oxides, lactones, acetals, ethers and esters). Both hydrocarbons and oxygenated compounds are responsible for the aroma and odour characteristics of the oils.

Agarwood is the resinous, fragrant and highly valuable heartwood produced by *Aquilaria malaccensis* and other species of the Indomalaysian tree genus *Aquilaria*, from the family of Thymelaeaceae. There are fifteen species in the *Aquilaria* genus and eight are known to produce Agarwood (Gaharu). In Malaysia, agarwood is primarily produced from *A. malaccensis*, *A. hirta*, *A. microcarpa*, *A. rostrata* and *A. beccariana* (Chang et al., 2002) and they are large evergreen trees growing over 15-30 m tall and 1.5-2.5 m in diameter, and has white flowers (Chakrabarty et al., 1994).

In this study, the ground agarwood is used to produce essential oil by using hydrodistillation (HD) and supercritical fluid extraction (SFE) method. Agarwood essential oil is highly prized for the scent produced and the oil is many used in industries. Generally, its oils are mixture of sesquiterpenes, sesquiterpene alcohols, oxygenated compounds, chromone derivatives and resin (Chang et al., 2002). Essential oils are complex mixtures of fragrance and flavor compounds originating in plants. They are generally used as odorants, flavorings and pharmaceutical ingredients in hundreds of consumer products (Burt, 2004).

Hydrodistillation has traditionally been applied for essential oil recovery from plant materials since past 1000 years ago. The HD method is different with SFE extraction which is created by heating any substance above its critical temperature and raising its pressure above its critical limit as well. Critical temperature refers to the highest temperature at which a gas can be converted to a liquid through an increase in pressure. Similarly, critical pressure is the highest pressure a liquid can be converted to a gas by an increase in temperature. Parameters such as the density, diffusivity and viscosity of SFE are therefore intermediary of liquids and gases. Disadvantages of the HD method are essential oil will undergo chemical alteration and heat sensitive compounds will be degraded (Pourmortazavi and Hajimirsadeghi, 2007). Therefore, the quality of essential oil will decrease.

Supercritical fluid extraction (SFE) have been works for essential oil extracts by for flavor, fragrance, cosmetics and pharmaceutical industries whereas shown attractive technology compared to conventional process with respect to the product quality. In practice, more than 90 % of supercritical fluid extraction work on by using carbon dioxide (CO₂) due several practical reasons: having low critical pressure (74 bar), and temperature (32 °C). Apart from that, CO₂ also have non-toxic, non-flammable, available in high quality with low cost and easily removed from the extract.

Indeed, the high demand for agarwood product makes it becomes good choice for collection and investment. Most researches now work on agarwood extraction of essential oils and also determination of quality oil have been done actively at research

institutes and labs in order to produce the best method of extraction with high quality and productivity.

The aim of the present work is to investigate the optimum parameters, such as pressure, temperature, and effect of modifier on the supercritical fluid extraction of agarwood. The essential oil obtained by HD was used for comparison. Quality oil agarwood were analysis using GC-FID and GC-MS techniques.

1.2 PROBLEM STATEMENT

Generally, traditional method of agarwood extraction needs to be revised in order to get a better quality of oils due to the important of essential oil in perfumery industry. Moreover, the oil's odor is the result after the combination of the odor of all components called trace components. Trace components are important since they will give the oil characteristics and natural odor. Thus, there are very important things to maintain all trace components during extraction essential oil process.

In order to maintain important compounds in agarwood essential oil, the traditional method needs to be upgraded with new technology due to time consuming. Nowadays, the most popular method to extract agarwood essential oil is the hydrodistillation method with low yield and time consuming. All of this will result in higher operating cost because of the process is slow and the distillation time is much longer. Prolonged heating in contact with water can also lead to hydrolysis of esters, polymerization of aldehydes or decomposition (e.g. dehydration) of other components (Stewart, 2005). Another problem is the current method also includes the extraction using solvent. The disadvantages of all these techniques are: low yield, loss of volatile compounds, long extraction time, toxic solvent residues and degradation of unsaturated compounds, giving undesirable off-flavors compounds due to heat (Pourmortazavi and Hajimirsadeghi, 2007).

The oils isolated under various SFE and HD conditions were analyzed by headspace solid micro-extraction (HS-SPME), gas chromatography flame ionization

detector (GC-FID), and gas chromatography mass spectroscopy (GC-MS). Sensory analysis was used to determine the optimum oil composition that was compared with that of essential oil isolated by hydro distillation. The problem is when analyzed their chemical compound by GC-FID and GC-MS that do not provide the full identification of the components and consequently do not give a guarantee of authenticity.

1.3 OBJECTIVES

The main objectives of this research are:

- 1) To investigate optimum parameters of *Aquilaria malaccensis* (agarwood) essential oil extraction using SFE and hydrodistillation.
- 2) To identify the chemical compound present in the essential oil of agarwood using Gas Chromatography - Flame Ionization Detector (GC-FID) and Gas Chromatography- Mass Spectrometer (GC-MS).
- 3) Comparison of chemical composition of agarwood oil processed with supercritical fluid technology to the conventional method.

1.4 SCOPE OF STUDY

The scope of this study is to compare essential oil from agarwood extracted by using supercritical fluid extraction (SFE) and hydro distillation (HD). In order to achieve the objective, the scope of study is about the investigation of optimum parameter of SFE in term of temperature, pressure and modifier. Lastly, the obtained essential oil will be analysed using gas chromatography (GC) on DB-1MS column and headspace method.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Thymelaeaceae are a cosmopolitan family of flowering plants and perennial herbs. It composed of 46-50 genera with 891 species. The family distribution widely in tropical areas such as Africa and Australia. Commonly, members of Thymelaeaceae were shrubs or small trees. Their bark were tough and fibrous with leaves opposite or alternate. Plants mostly bisexual and sometimes dioecious. *Aquilaria* spp. is the one from this family. It is the principal source of heartwood (Soehartono and Newton, 2001), a resin-impregnated heartwood that is fragrant and highly valuable.

2.1.1 Genus *Aquilaria*

The genus *Aquilaria* is best known as the principal producer of the dark brown resin impregnated agarwood or eaglewood, especially *A. crassna*, *A. malaccensis* and *A. sinensis*. It widely distributed in South and South-East Asia (Bhutan, Cambodia, China, India, Laos, Malaysia, Myanmar, Thailand and Vietnam). Trees have been over-harvested throughout the range of the genus, and most species are of conservation concern. The genus as a whole is included in Category II of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

Aquilaria is a large evergreen tree growing over 15 – 30 m tall and 1.5- 2.5 m in diameter and has white flowers (Chakrabarty et al., 1994). This species have adapted to live in various habitats, including rocky, sandy or calcareous, well drained slopes and ridges and land near swamps. They typically grow between altitudes of 0 – 850 m, in locations with average daily temperatures of 20 - 22 °C (Figure 2.1).



Figure 2.1 : Wild tree of *Aquilaria malaccensis*

Source : Forest Research Institute Malaysia (2011)

In Malaysia, the tree of *Aquilaria* is called karas and its fragrant wood is known as agarwood. Other names used by both collectors and traders of the fragrant wood are agar, aloeswood, eaglewood, kalambak or gaharu depending on the country and generally encompass the fragrant wood produced by most species of *Aquilaria* (Ng et al., 1997). Meanwhile, the resin is commonly called Jinkoh, Aloeswood, Agarwood or Oud and is valued in many cultures for its distinctive fragrance, thus it is used for incense and perfumes (Chakrabarty et al., 1994).

Economically for its aromatic, fumigatory, and medicinal properties, agarwood was found in approximately 17 species of sub-canopy trees of the genus *Aquilaria* and 5 species were commonly available in Malaysia such as *A. malaccensis*, *A. microcarpa*, *A. hirta*, *A. rostrata* and *A. beccariana*. In nature, agarwood hunting has been done aggressively and imprudent. Agarwood, producing trees which were found with small holes named as ant-holes were cut down and were harvested. By high demand for medicine, incense and perfume across Asia and the middle east, agarwood hunting threatened the preservation of agarwood in its natural habitats. Table 2.1 was summarized the major species of *Aquilaria* and their distribution in the world.

Table 2.1 : Summarized of following species of *Aquilaria* and their distributions

Species	Location
<i>Aquilaria hirta</i>	Peninsular Malaysia, East Sumatra, Riau, Lingga.
<i>Aquilaria malaccensis</i>	India, Myanmar, Sumatra, Peninsular Malaysia, Singapore, Borneo (Sabah and Kalimantan), Philippines.
<i>Aquilaria rostrata</i>	Peninsular Malaysia, upper hill Dipterocarp forest.
<i>Aquilaria microcarpa</i>	Sumatra, Singapore, Peninsular Malaysia, Borneo (Sabah, Sarawak, Brunei).

Source : Naef.(2011)

2.1.2 *Aquilaria malaccensis* in Malaysia

Five species of *Aquilaria* are recorded for Peninsular Malaysia and all are believed to be able to produce oleoresins. The most popular species generally associated with agar is *A. malaccensis* (Chang et al., 2002). The grade of agarwood essential oil is divided by 5 types, which are Grade Super A, A, B, C, and D. The Grade Super A is the most expensive compared to the others grade. The grade (and hence value) of agarwood and agarwood derivatives such as oil is determined by a complex set of factors including country of origin, fragrance strength and longevity, wood density, product purity, resin content, colour, and size of the form traded.

Malaysia has a long history in the trade in agarwood, which has long been collected by the indigenous people of the interior of Peninsular Malaysia, Sabah and Sarawak to supplement their income. In Peninsular Malaysia, *A. malaccensis* products in domestic trade are in the form of woodchips and powder or sawdust (Burkill, 1966; Nor Azah et al., 2002; Nor Azah et al., 2009; Yaacob and Joulian, 2000). Some uses have been recorded locally for medicinal purposes, but it appears that the majority of *A. malaccensis* harvested is exported (Barden et al., 2000).

Agarwood has been recognized by the local Malaysian since a long time and its valuable oil has been collected and extracted traditionally as a ‘backyard industry’ by the local people. Nowadays, Malaysian extracting the essential oil of agarwood by using distillation unit made from stainless steel as a container that contains ground-up agarwood that will undergo a 96 hour distillation process to get its essence. High quality agarwood can fetch RM 10, 000 per kg depending to the grade of the resin.

Malaysia has been known as a country that produce agarwood. According to research in year 2000, it is estimated that nearly 700 tonnes of agarwood were produced in the international market mostly came from the jungle of Malaysia and Indonesia. The price is estimated at least RM 3.5 billion. It has been Malaysian natural treasure because of its rarity and its high value. In Malaysia, there is a report that agarwood can be found

in heart of Kelantan, Perak, Pahang and Terengganu jungle even though it is a rare species (Barden et al., 2007) (Figures 2.2 and 2.3).



Figure 2.2: Tree of *Aquilaria* growth (6 months).



Figure 2.3 : *Aquilaria* farm at Jerantut, Pahang.