COMPARISON OF SUPERCRITICAL FLUID EXTRACTION AND HYDRODISTILLATION METHOD FOR DETERMINATION OF AGARWOOD ESSENTIAL OIL

NOR ATIKAH BT MAT YUSOFF

UNIVERSITI MALAYSIA PAHANG

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Report submitted in partial fulfillment of the requirements for the award of the degree of Bachelor of Applied Science (Honours) - Industrial Chemistry

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SUPERVISOR'S DECLARATION

We hereby declare that we have checked this project report and in our opinion this project is satisfactory in terms of scope and quality for the award of the degree of Bachelor of Applied Sciences (Hons) in Industrial Chemistry.

Signature:
Name of Supervisor: Dr.Saiful Nizam B. Tajuddin
Position: Senior Lecturer
Date:

STUDENT'S DECLARATION

I hereby declare that the work in this report in my own except for quotations and summaries which have been duly acknowledged. The report has not been accepted for any degree and is not concurrently submitted for award of other degree.

Signature:
Name: Nor Atikah bt Mat Yusoff
ID Number: SA08047
Date:

DEDICATION

Special Dedication to my beloved mother (Meriam Awang) and my father (Mat Yusoff Selimin) for their love and encouragement. And, special thanks to my friends, my fellow course mates and all faculty members. This is for you Nik Noor Asma, Ahmad Khairulddin and Noor Asiah.

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The final year project has been both a challenge and an experience to cherish for my life time. Although a lot of hard work and sacrifice did come from my part, there are many without whom this project would not have even lifted off the ground, let alone come to completion.

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ABSTRACT

Gaharu or agarwood is a tree in the family *Thymelaeceae*. Gaharu is the occasional product of two to four spesies in the family *Thymelaeaceae*. Mature trees will grow up to 40 meter in height and 40 centimeter in diameter. Gaharu trees in natural forests began to produce agarwood resin at the age of 20 to 45 years, depending on the resistance of trees and tree injury response. It is also one of the most expensive natural products existing today. Gaharu is many used in perfumes, medicines, and toiletery product. The objective of the experiment is to identify the compounds of the gaharu using supercritical fluid extraction (SFE) and hydrodistillation extraction methods. Results obtained from the different instrument method were then compared to analyze the compounds. The essentials oil of gaharu was obtained by supercritical extraction of components from solid materials. This is a relatively new process. From previous experiment this process gives a better quality extract but the capital costs are high. Carbon dioxide is usually used for solvent in this process. Then, a common conventional method used to extract the essential oil from gaharu is hydrodistillation. The problems of this technique are low efficiency and acquire high and continuous heating and required long extraction time. The essential oil was extract will be analyzed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The results from GC-MS and GS-FID were compared to produce composition of compound from gaharu with different method of extractions.

ABSTRAK

Gaharu terdiri daripada pokok di dalam keluarga Thymelaeceae. Kadang-kadang terdapat dua hingga empat jenis spesies produk gaharu dalam keluarga Thymelaeaceae. Pokok dewasa akan tumbuh hingga 40 meter tingginya dan 40 sentimeter untuk diameternya. Pokok gaharu dalam hutan semulajadi mula menghasilkan gaharu resin pada usia 20 hingga 45 tahun bergantung pada ketahanan pokok dan tindakbalas kecederaan pokok. Ia juga salah satu bahan semulajadi yang mahal pada ketika ini. Gaharu banyak digunakan dalam produk minyak wangi, perubatan dan alatan tandas. Tujuan kajian ini adalah untuk menentukan komponen gaharu dengan menggunakan ekstraksi bendalir kritikal dan kaedah destilasi air. Dengan menggunakan kedua-dua alatan ini, perbezaan keputusan dapat dibandingkan dalam menanalisis sebatian. Pati minyak gaharu dapat dihasilkan melalui komponen interaksi bendalir kritikal indeks daripada bahan pepejal. Ini adalah proses baru. Daripada kajian yang lepas, proses ini memberi ekstrak kualiti yang baik tetapi memerlukan cos yang tinggi. Dalam proses ini, karbon dioksida biasanya digunakan sebagai bahan pelarut. Kemudian, kaedah lama digunakan untuk ekstrak pati minyak daripada gaharu ialah destilasi air. Masalah daripada teknik ini ialah rendah dalam kecekapannya, sentiasa dipanaskan dan memerlukan masa pengekstrakan yang lama. Pati minyak yang sudah diekstrak akan dianalaisis dengan menggunakan GC-FID dan GC-MS. Keputusan sebatian daripada GC-FID dan GC-MS daripada komposisi sebatian gaharu dapat dibandingkan dengan kaedah pengekstrakan yang berbeza.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

α	Alpha
β	Beta
°C	Celsius
δ	Delta
γ	Gamma
μ	Micro
%	Percentage

LIST OF ABBREVIATIONS

b.p	Boiling Point
CAS	Chemical Abstracts Service
dbh	Diameter at breast height
GC	Gas chromatography
GC-FID	Gas Chromatography-Flame Ionization Detector
GC-MS	Gas Chromatography-Mass Spectrometry
HD	Hydrodistillation
KI	Kovats Index
Min	Minutes
NIST	National Institute of Standards Technology
Psi	Pound per square inch
RT	Retention time
Ref	References
SFE	Supercritical Fluid Extraction

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Gaharu is the resinous, fragrant and highly valuable heartwood produced by *Aquilaria malaccensis* and other species of the Indomalesian tree genus *Aquilaria*, from the family of Thymelaeceae. There are fifteen species in the *Aquilaria g*enus and eight are known to produce gaharu. In Malaysia, gaharu 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 gaharu chipwood used to produce essential oil and obtained from different instrument which is by using hydro distillation (HD) and supercritical fluid extraction (SFE) method. Gaharu essential oil is highly prized for the scent produced and the oil is widely used in industries. Generally, gaharu oils are mixture of sesquiterpenes, sesquiterpene alcohols, oxygenated compounds, chromone derivatives and resin (Chang *et al.,* 2002). Plant extracts as seen as a way of meeting the demanding requirement of the modern industry for the past two decades (Simandi *et al.,* 1996).

Besides that, hydrodistillation (HD) method is different with supercritical fluid extraction (SFE) 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 increasing in temperature. Parameters such as the density, diffusivity and viscosity of SFE are therefore intermediary of liquids and gases.

The aim of the present work is to investigate of the effect of different parameters, such as pressure, temperature, modifier volume and dynamic and static extraction time on the supercritical fluid extraction of *agarwood* (gaharu). The essential oil obtained by hydrodistillation was used for comparison. These extraction methods will be further discussed in the literature review.

1.2 Problem statement

The main problem in this research is lack information of gaharu and its essential oil. Other than that, understanding operation of different instruments is important to determine essential oil from gaharu. Nowadays, the most popular method to extract gaharu essential oil is the traditional hydrodistillation method. The efficiency of this method is relatively low and it is too time consuming. All of this will result in higher operating cost because of the process is not efficient in cost and processing period time is much longer. Furthermore, prolong action of hot water can cause hydrolysis of some constituents of the essential oils such as ester, (Mohammad, 2008).

Another problem is current method also including the extraction using solvent. Even though it take shorter time than the hydrodistillation, the oil produced by this method is not suitable for skin use (Wilson, 1995). Besides that, traditional method different with a supercritical fluid extraction (SFE) method which is large number of compounds that can be used as a fluid in supercritical techniques, but by far the most widely used is carbon dioxide. The solubility of polar compounds and the selectivity of the process can be increased by adding small quantities of other solvents, such as ethanol, in the fluid that named as co-solvent or modifier.

The oils isolated under various SFE and HD conditions were analyzed by GC-FID and 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 compare extraction of essential oil from gaharu using different method namely supercritical fluid (SFE) method and hydro distillation (HD) method.

2. To identify the chemical compound present in the essential oil of gaharu using Gas Chromatography -Flame Ionization Detector (GC-FID) Gas Chromatography-Mass Spectrometer (GC-MS).

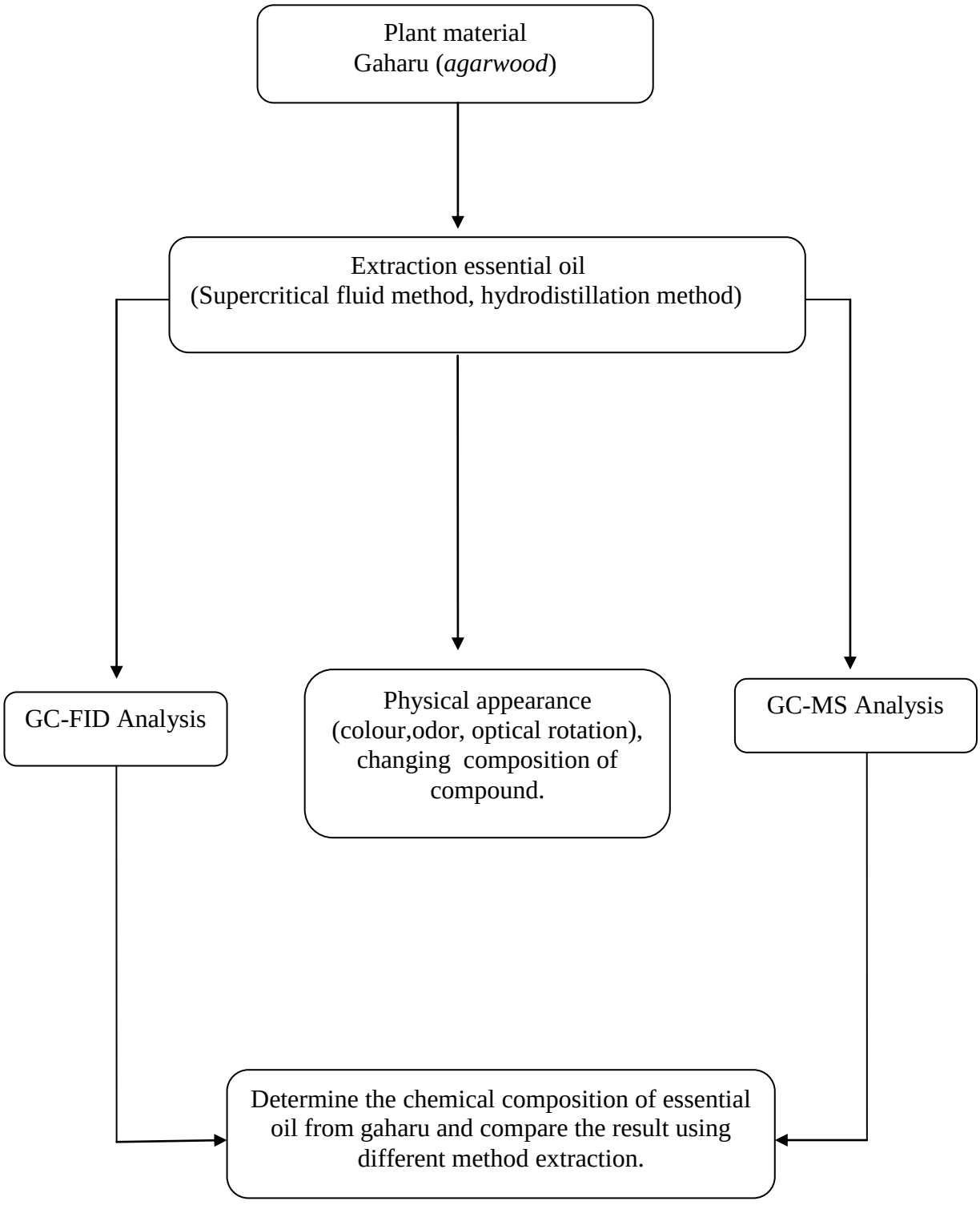


Figure 1.1: The flow chart research of analysis essential oil from gaharu using different method extraction.

1.4 Scope of study

The scope of this study is to compare essential oil from gaharu between two method by using supercritical fluid extraction (SFE) and hydro distillation (HD) . In order to achieve the objective the scope of study is about the effect of different instrument on essential oil from gaharu and influent result to determine composition essential oil using gas chromatography (GC-FID and GC-MS).

CHAPTER 2

LITERATURE REVIEW

2.1 Background of Aloeswood (Gaharu)

In Malaysia, the tree of *Aquilaria* is called as karas and its fragrant wood is known as gaharu. Five species of *Aquilaria* are recorded in Peninsular of Malaysia and all are believed to be able to produce oleoresins. The most popular species generally associated with gaharu is *A. Malaccensis* (Chang *et al.*, 2002). The grade of gaharu essential oil are divided by five types, which are Grade Super A, A, B, C, and D. The Grade Super A is the most expensive compared to the other grades. 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.

Aquilaria spp. (Thymelaeaceae) are the principal source of Gaharu (Soehartono and Newton, 2001), a resin-impregnated heartwood that is fragrant and highly valuable. 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). Agarwood is a fast growing, evergreen tree, that normally grows to 18-21 m but sometimes up to 40 m in height. The trees occasionally become infected with a parasite mould and begin to produce an aromatic resin in response to this attack. As the fungus grows, the tree produces a very rich, dark resin within the heartwood. The resin is commonly called Jinko, Aloeswood, Agarwood or Oud and is valued in many cultures for its distinctive fragrance, thus it is used for incense and perfumes (Fauzi, 2008).

Gaharu is a resinous wood that sometimes occurs in trees belong to the *Aquilaria* genus, *Thymelaeceae* family. Gaharu producing species are found from India eastwards throughout Southeast Asia (Indonesia, Thailand, Cambodia, Laos, Vietnam, and Malaysia). *Aquilaria* is a fast-growing, archaic tropical forest tree. There are different names for gaharu such as ch'en hsiang, eagle wood, jin-koh, oud and others. There are 15 species of *Aquilaria*. In Malaysia, there are five species of *Aquilaria* which are *Aquilaria Malaccensis, Aquilaria Microcorpa, Aquilaria HIrta, Aquilaria Rostrata* and *Aquilaria Becanana.* Agarwood contains more than 12 chemical components that can be extracted.

Figure 2.1: Aloeswood (gaharu).

(Source: Mohd Rosli Bin Ramly, 2006)

The use of gaharu for perfumery extends back several thousands of years, and is referenced, for example, in the Old Testament several times using the term ‘aloes’. Both gaharu smoke and oil are customarily used as perfume in the Middle East (Chakrabarty et al, 1994). In India, various grades of gaharu are distilled separately before blending to produce final ‘attar’. Minyak attar is a water based perfume containing gaharu oil, which is traditionally used by Muslims to lace prayer clothes (Yaacob, 1999).

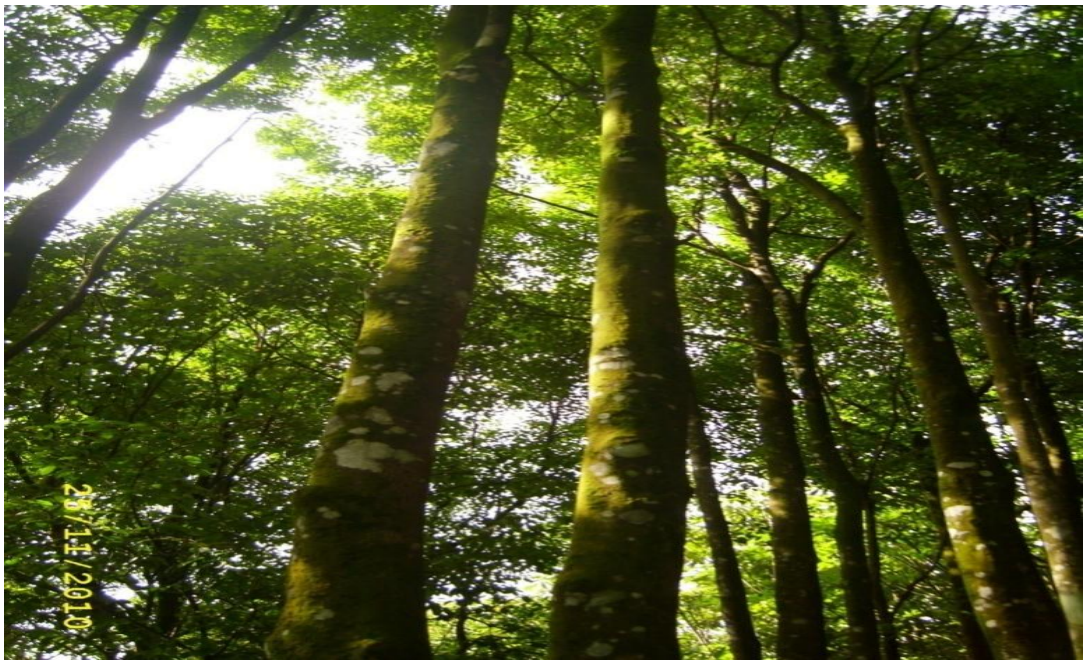


Figure 2.2: Tree of gaharu

2.1.1 Gaharu Species

Three species of *Aquilaria* are found in Malaysia: *A. hirta*, *A. malaccensis* and *A. rostrata*. *Aquilaria malaccensis* is well distributed throughout Peninsular Malaysia, except for the States of Kedah and Perlis. It is confined mainly to plains, hill slopes and ridges up to 750 m in both primary and secondary Malaysian lowland and hill dipterocarp forests (Jantan, 1990). The average diameter growth rate of *A. malaccensis* in native forests in Malaysia is rather low, e.g. a mean of 0.33 cm/ year, but the fastest-growing larger specimens are reported to grow at 0.8-1 cm/year (La Frankie, 1994). Although *A. malaccensis* enjoys good geographical coverage, its occurrence is rather rare.

The following species of *Aquilaria* produce agarwood:

1. AQUILARIA SUBINTEGRA, found in Thailand
2. AQUILARIA CRASSNA, found in Thailand, Cambodia, Loas, Vietnam
3. AQUILARIA MALACCENSIS, found in Thailand, India, Indonesia
4. AQUILARIA APICULATA, found in Philippines
5. AQUILARIA BAILLONIL, found in Thailand, Combodia, Loas, Vietnam
6. AQUILARIA BANEONSIS, found in Vietnam
7. AQUILARIA BECCARIAN, found in Indonesia
8. AQUILARIA BRACHYANTHA, found in Malaysia
9. AQUILARIA CUMINGIANA, found in Indonesia, Philippines
10. AQUILARIA FILARIA, found in Nuegini, China
11. AQUILARIA GRANDIFLORA, found in China
12. AQUILARIA HILATA, found in Indonesia, Malaysia
13. AQUILARIA KHASIANA, found in India
14. AQUILARIA MICROCAPA, found in Indonesia, Malaysia
15. AQUILARIA ROSTRATA, found in Malaysia
16. AQUILARIA SINENSIS, found in China

(Source: Ng, L.T., Chang Y.S. and Kadir, A.A., 1997)

Table 2.1: Comparison of four types of Gaharu based on agarwood oil yield and prices

	Type of gaharu	**Oil yield**	**Price**
1	SUBINTEGRA	Good	Good
2	CRASSNA	Medium	Medium
3	MALACCENSIS	Poor	Poor
4	BAILLONIL	Poor	Poor

(Source: www.gaharuonline.com/gaharu_species.htm)

Figure 2.3: Species of gaharu

2.1.2 Formation of Gaharu

Gaharu formation investigation was first initiated in 1926 by Bose but it is uncertain whether the fragrant wood result from fungal infection which brings about pathological conditions, certain chemical changes in the tree, or environmental factors. Research conducted so far focused mainly on pathological and non-pathological conditions (L.T. Ng, et al., 1997). Commonly, gaharu formation is caused by the tree response to mechanical or natural injury associated with the wood. In brief, the tree has two response mechanisms to injury. The first line of defense is for the phloem cells to produce callus growth over the injury. If the formation of callus prevented then the tree will produce resin as a chemical defense to the injury (Fatmawati, 2005).

Not all of Aquilaria trees produce the resin, (Gianno, 1986) suggested that only 10% of mature Aquilaria trees above 20 cm diameter at breast height (dbh) produce agarwood. (Chakrabarty et al, 1994) stated that infected trees produce resin from the age of 20 years onwards, while (Hooper, 1904) had noted that trees that were at least 50 years old yielded the largest amount of resin.

Figure 2.4 : Method of Producing Gaharu Resin

(Source: Yip and Lai, Hong Kong Herbarium, 2005)

Research conducted so far has focused mainly on the following three hypotheses exist regarding gaharu formation, namely that it is the result of diseased or pathological, wounding/pathological and or non-pathological processes (Ng *et al.*, 1997). The pathological condition was first hypothesized by fungal infections that lead to diseased wood. Moreover, the wounding/pathological condition considered that wounding has a primary effect on gaharu formation with fungal infection as a secondary influence (Gibson, 1977; Rahman and Basak, 1980). Ng *et al*. (1997) suggested the non-pathological condition is a defensive response of the tree towards wounding, therefore release the gaharu resin.

Infections may also occur due to mechanical or natural injuries on the stem, for example following wind or storm damage. Due to infections, oleoresins are accumulated in the infected wood and later become odoriferous. At the initial stage infections appear as brown streaks in the tissue. Accumulation of oleoresins goes on increasing with the increase of infection rate as well as aging of the infection. As more of oleoresins are deposited the intensity of colour of the infected wood increases and finally it becomes black due to increase in concentration. Figure 2.2 shows the cross section cut of the tree showing dark regions of gaharu formed in the heartwood.

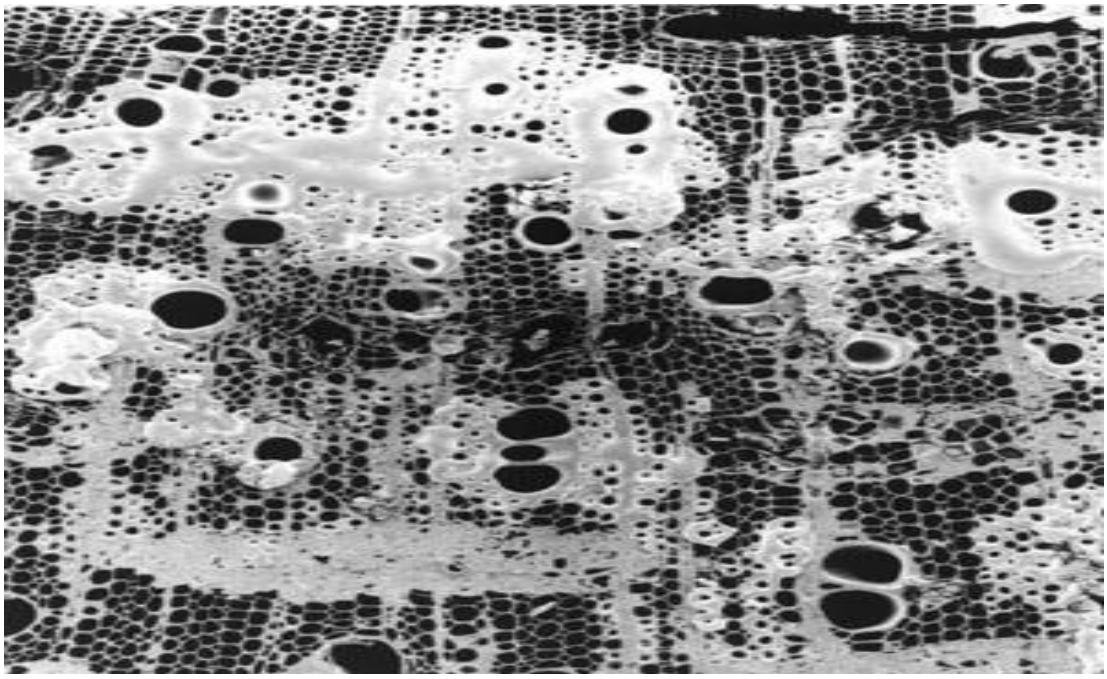


Figure 2.5 Abundant amounts of resin formed in the wood cells

(Source: Robert A. Blanchette)

2.1.3 Gaharu in Malaysia

Malaysia has a long history in the trade in agarwood, which has long been collected by the indigenous peoples of the interior of Peninsular Malaysia, Sabah and Sarawak to supplement their income. In Peninsular Malaysia, the *gaharu* products in domestic trade are woodchips and powder or sawdust (Chua, 2003). Some application has been recorded locally for medicinal purposes, but it appears that the majority of *A. Malaccensis* harvested is exported (Barden *et al*.2000). The wood is also used for making small boxes in Sabah (Sabah Forest Department, 2003).

Gaharu 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 gaharu by using distillation unit made from stainless steel as a container that contains ground-up gaharu that will undergo a 96 hour distillation process to get its essence. High quality gaharu can fetch RM10,000 per kg depending to the grade of the resin. A 12 g bottle of oil is sold at between RM50 and RM200 (Hilary, C., 2005).

Malaysia has been known as a country that produce gaharu. According to research in year 2000, it is estimated that nearly 700 tonnes of gaharu 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. Gaharu has been Malaysian natural treasure because of its rarity and its high value (Haikal, 2006). In Malaysia, there is a report that gaharu can be found in heart of Kelantan, Perak, Pahang and Terengganu jungle even though it is a rare species (Hilary, C., 2005). Figure 2.6 showing the distillation process of gaharu in Malaysia.

Figure 2.6: Gaharu oil extraction process

(Source: All Malaysia Info, The Star Online, 2005)

As it is a rare species, hard to found, and because of its high value, the federal Forestry Department has urged the state governments to regulate the collection, trade and processing of gaharu through a licensing system where the Gaharu collectors or buyers have to pay a royalty fee amounting to 10% of the raw material market price and an extraction permit is issued and this will facilitate the traders in obtaining export and CITES (Convention on International Trade in Endangered Species) permit (Hillary, C., 2005).

2.2 Essential oil of Gaharu

Essential oils are used widely as natural flavor additives for food, as fragrances in perfumery, and in traditional and alternative medicines such as aromatherapy. They are extracted from plants or plant parts by a variety of techniques. Terpenes and terpenoids are the primary constituents of essential oils and they are manufactured following systematic biosynthetic pathways in many types of plants.

An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as volatile oils, ethereal oils or aetherolea, or simply as the "oil of" the plant from which they were extracted, such as *oil of agarwood*. An oil is "essential" in the sense that it carries a distinctive scent, or essence, of the plant. While the unique chemical and molecular properties of essential oils are a topic of study, they are commonly defined by the fact that they convey characteristic fragrances. It follows that the common tendency to speak of essential oils as a category, as if that implied anything in particular about their medical, pharmacological, or culinary properties, is highly unreliable and often actually dangerous. Essential oils are generally extracted by distillation. Other processes include expression, or solvent extraction.

2.2.1 Advantages of Essential Oil

Essential oils is the primary ingredients in aromatherapy which are safe and simple, natural products. They can be used just for pleasure, or to help individual heal physical and emotional ailments. It can be a complete which is holistic and natural form of therapy, taking into account the effect of the treatment on the body, the mind and the emotions of the person receiving it. The effectiveness of essential oils usage has been proven by scientific analysis, confirming the intuitive link, understood by our ancestors, between nature and general well-being. The dynamics of aromatherapy enable us to bring the essence of nature into our everyday lives (Mohd, 2006).

In Malaysia, gaharu was used in various folk remedies for the treatment of weakness, stomach pains, in pregnancy, after delivery, fever, chest pains, body pains, rheumatism, women dieses and dropsy**.** It is reputed to be somatic and sedative, has antibiotic, anti-tumor and anti cancer effect (Shahrizal, 2006).

Other than that, both gaharu wood and oil are highly prized for the scent produced. The unique gaharu scent is released on burning the resinous wood. Many uses are recorded for gaharu.

I. Incense

II. Medicinal Uses

III. Aromatherapy

2.3 Extraction Method of Essential Oil from Gaharu.

Extraction is a separation process to separate solute or removed undesirable solute component from the solid where the solid is contacted with a liquid phase. Fragrance extraction is processes which involve extracting aromatic compounds from the raw materials using various methods such as distillation, solvent extraction and expression. The first and the most common method due to their simple construction, low cost and easy operation used for essential oil extraction is hydrodistillation.

In other definition, the two phases are in intimate contact and the solute can diffuse from the solid to the liquid phases, which cause a separation of the components originally in the solid. In this process, there will be the advantages and disadvantages. One of the advantages is extraction can be performed at ambient temperature. Thus, it is relatively energy efficient and can be applied to separations involving thermally unstable molecules (Fadzli, 2006).

2.3.1 Hydrodistillation (HD) Method

Hydro distillation is used in the manufacture and extraction of essential oils. The botanical material is immersed in the water then being boiled with the water. The hot water helps to release the aromatic molecules from the plant material since the hot water forces to break the pockets in which the oils are kept in the plant material. The molecules of these volatile oils then escape from the plant material and evaporate into the steam. The temperature of the process needs to be carefully controlled which is just enough to force the plant material to let go of the essential oil, yet not too hot as to burn the plant material or the essential oil. The steam which then contains the essential oil is passed through a cooling system to condense the steam, which form a liquid from which the essential oil and water is then separated, (Aizudin, 2006).

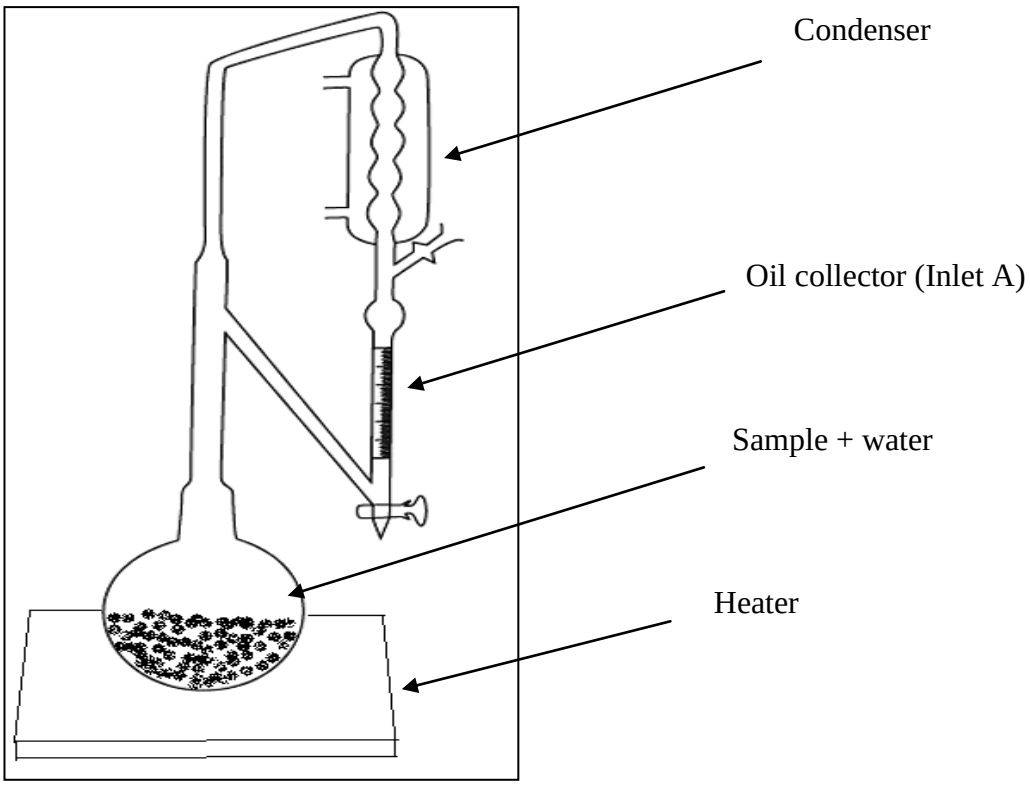


Figure 2.7: Hydrodistillation extraction diagram.

2.3.2 Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) is an interesting technique for the extraction of flavoring compounds from vegetable material. It can constitute an industrial alternative to Solvent extraction and steam distillation processes. SFE allows a continuous modification of solvent power and selectivity by changing the solvent density. Nevertheless, the simple SFE process, consisting of supercritical fluid extraction and a one-stage subcritical separation, in many cases does not allow a selective extraction because of the simultaneous extraction of many unwanted compounds. This situation is typical of CO2 supercritical fluid extraction of essential oils from herbaceous material in which, even when the process is conducted at conditions that produce the optimum oil composition, cuticular waxes are co-

extracted because of their lipophilic character and their localization on the leaf surface. SFE followed by fractional separation of the extract in multiple-stage separators overcomes these limitations and produces high-quality essential oil (Blvd. Elisabeta, Bucharest).

Advantages of SFE are having solvating powers similar to liquid organic solvents, but with higher diffusivities, lower viscosity, and lower surface tension. Besides that, since the solvating power can be adjusted by changing the pressure or temperature separation of analyses from solvent is fast and easy. In industrial processes involving food or pharmaceuticals, one does not have to worry about solvent residuals as we would if a "typical" organic solvent were used. Supercritical fluid extraction is generally cheap, simple and many are safe to using. Disposal costs are much less and in industrial processes, the fluids can be simple to recycle.

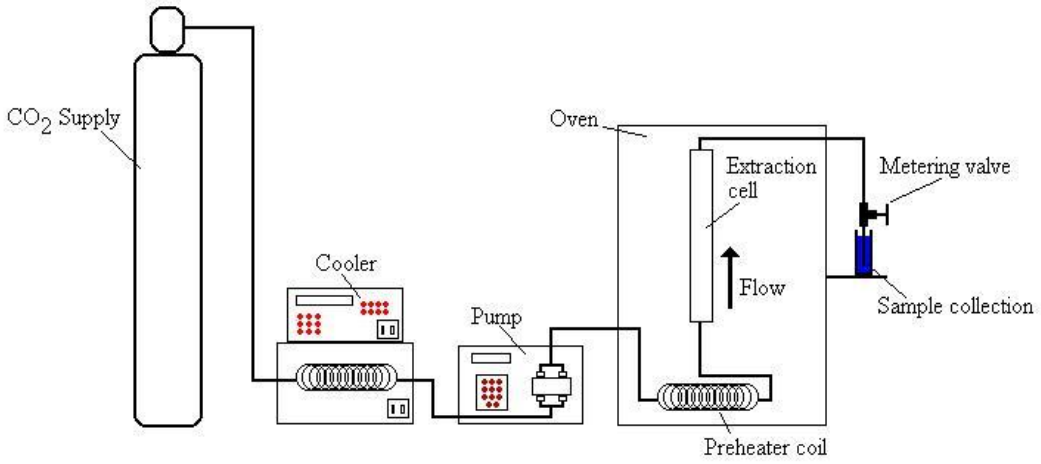


Figure 2.8: Schematic diagram of supercritical fluid extraction apparatus

2.4 Analysis volatile compound composition using (GC-MS).

Figure 2.9: Example of a GC-MS instrument

Gas chromatography–mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify the different substances within a test sample. Gas chromatography (GC) and mass spectrometry (MS) make an effective combination for chemical analysis. GC analysis separates all of the components in a sample and provides a representative spectral output. Foods and beverages analysis contain numerous aromatic compounds, some naturally present in the raw materials and some forming during processing. GC-MS is extensively used for the analysis of these compounds which include esters, fatty acids, alcohols, aldehydes, terpenes. It is also used to detect and measure contaminants from spoilage or adulteration which may be harmful. There often controlled by governmental agencies, for example pesticides.

The technician injects the sample into the injection port of the GC devices. The GC instrument vaporizes the sample and then separates and analyzes the various components. Each component ideally produces a specific spectral peak that may be recorded on a paper chart or electronically. The time elapsed between injection and elution is called the "retention time." The retention time can help to differentiate between some compounds. The size of the peaks is proportional to the quantity of the corresponding substances in the specimen analyzed. The peak is measured from the baseline to the tip of the peak. GC analysis depends on similar phenomena to separate chemical substances.

A mixture of chemicals present in a specimen can be separated in the GC column. Some chemical and physical characteristics of the molecules cause them to travel through the column at different speeds. If the molecule has low mass it may travel more swiftly. Also, the molecule's shape may affect the time needed to exit the column. Different substances might effect to each retention time due to time needed to travel the column to increase or decrease. Interactions between the sample's molecule and the column surface may cause the molecule to be retained inside the column for a different amount of time than similar molecules that interact with the column differently.

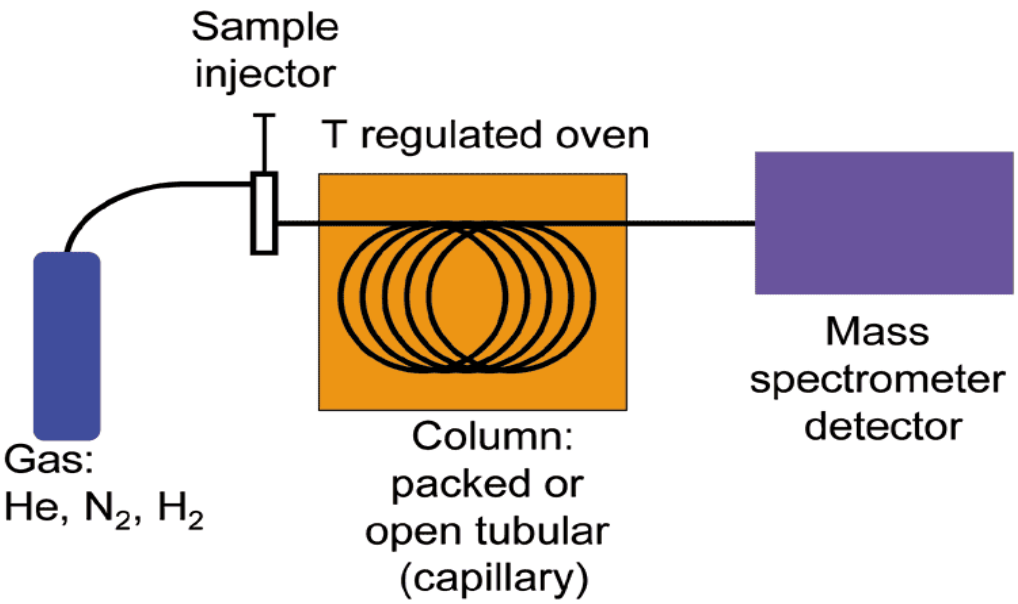


Figure 2.10: GC-MS process schematic

2.5 Analysis volatile compound composition using (GC-FID).

The essential oil from plant were extracted through hydro-distillation process using simultaneous distillation extraction (SDE) and analysis of the volatile compounds were performed by using gas chromatography equipped with flame ionization detector (GC-FID). A flame ionization detector (FID) is a type of gas detector used in gas chromatography.

FID analysis involves the detection of ions. The source of these ions is a small hydrogen-air flame. Sometimes hydrogen-oxygen flames are used due to an ability to increase detection sensitivity, however for most analysis, the use of compressed breathable air is sufficient. The resulting flame burns at such a temperature as to paralyze most organic compounds, producing positively charged ions and electrons. The current measured corresponds roughly to the proportion of

reduced carbon atoms in the flame. Specifically how the ions are produced is not necessarily understood, but the response of the detector is determined by the number of carbon atoms (ions) hitting the detector per unit time. This makes the detector sensitive to the mass rather than the concentration, which is useful because the response of the detector is not greatly affected by changes in the carrier gas flow rate.

2.6 Chemical Component of Gaharu

Gaharu contains a sesquiterpene alcohol which produces its characteristics aroma. The component in gaharu is depending on the types of species woods respectively. It was reported that 2-[2-(4-methoxyphenyl)ethyl]chromone and 2-(2-phenylethyl)chromone through pyrolysis at 150 °C produces 4-methoxybenzaldehyde and benzaldehyde respectively and this molecules are odourless at room temperature but produce a long lasting fragrance upon burning (Ng et al., 1996).

2.7 Physical Properties of Gaharu

2.7.1 Colour and Scent

Traders are look for blackened, resinous and aromatic gaharu that believe has a higher grade and resin content (Frank, Z. and James, C. 2001. The colour mention for gaharu is green, dark green, yellow, golden, red, black, brown and white. The scent of gaharu also affecting the grade of gaharu which a softer scent is consider as a higher grade and higer price than those with more intense scent.

2.7.2 Size and Form

For the pieces of the same level of grade of gaharu, the larger pieces carrying a higher value than the smaller pieces according to their respective weight.

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

Gaharu woods were bought from local entrepreneur in Malaysia. This research involves several major processes to form the small pieces of gaharu wood. Five steps are involved which are drying, grinding, soaking, hydrodistillation (HD) and Supercritical Fluid Extraction (SFE) as the lastly analysis process. There are three methods that have been developed before extraction process. They are also known as pretreatment process, which are drying, grinding and soaking. The extracting process will be run in different range of temperature cut and also different range heating rate to find the optimum condition to extract the oil.

3.2 SAMPLE PREPARATION

3.2.1 Sample Collection

Aquilaria malaccensis sp. from Kelantan Forest (Malaysia) was procured by the Forestry Department, Malaysia. Commercial samples obtained by hydrodistillation at the mini pilot plant scale were also procured.

3.2.2 Drying

Drying process defined as a process that removes a liquid (usually water) from a solid. Drying process need to be done so that the wood is completely dry from any moisture before goes to the next step of experiment. It is also to get rid of any substance that can distract the impurities of oil when it has been extracted (Norazlina, 2005). The drying process in completed when the humidity inside the tray drier is longer falling. The air flow speed will be set at 1.44 meter per second (m/s) and the temperature will be set at 60°C (Fadzli, 2006).

3.2.3 Grinding

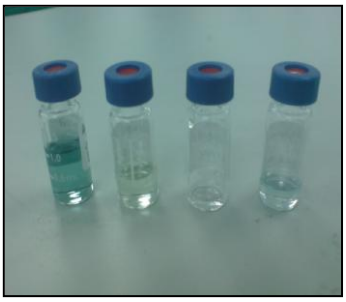
The pieces of dry agarwood was grinded into milled agarwood to give the maximum surface area for extraction process and to maximize the contact time between the solvent and gaharu particle. In this experiment, the size of gaharu particle is prepared at 1.00 mm. The large trunk of gaharu need to be choped to a smaller size and before it can be grinding. After finish the process, the fine sawdust of gaharu wood is packed into a big packed.

3.2.4 Soaking

Before the extraction process, grinded gaharu must be soaked in deionizer water. The ratio of gaharu to water is 1:8 for period of five to seven days in order to break down the parenchymatous and oil glands. For this experiment seven days was needed in order to maximize the soaking effect. The amount of gaharu sawdust used is 150g gram and water equal to 1200 mL. Figure 3.1 shown the steps of sample preparation of extraction essential oil.

AGARWOOD

GRINDER



ESSENTIAL OIL

SOAKING

EXTRACTION

Figure 3.1: General steps of extraction methods.

3.3 TYPES OF EXTRACTION METHODS

There are 2 types of extraction methods were applied in this project. Each of method has several different parameters for example time of extraction, temperature, pressure and preparation sample.

3.3.1 Hydrodistillation

150 g of agarwood are subjected to hydrodistillation in 2 L distilled water for 12 hours using Clevenger type B hydrodistillation Figure 3.2. 5 ml grade n-hexane (99%) was pipetted into the apparatus before distillation. The resulting essential oil was trapped in the hexane. The volatile distillate was collected over anhydrous sodium sulphate and refrigerated prior to analysis. Then it was filtered to remove the anhydrous sodium sulphate. After that, blown with a stream of industrial grade nitrogen gas to remove the hexane fraction. The essential oils were then stored at -4°C in an amber vial with Teflon sealed cap. The yields of the essential oils were calculated using Equation 3.1.

Equation (3.1)

$$\text{Weight percentage recovery} = \frac{\text{weight of essential oil}}{\text{weight of dried sample}} \times 100$$

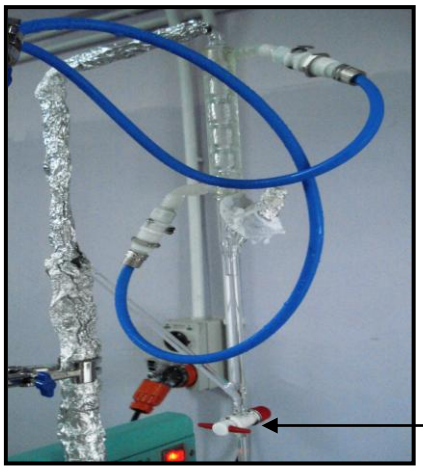


Figure 3.2: Clavenger type B hydrodistillation

3.3.2 Supercritical Fluid Extraction (SFE)

An SFT-150 system (SFT Company, USA) in SFE mode was used. A schematic of the extraction apparatus has been illustrated in Figure 3.3. The extraction vessel was a 100 ml stainless steel vessel. SFE were conducted at pressures of 3000, 4000, 5000, 6000 and 7000 psi and at a temperature of 60^{o}C for the duration of 4 hour. A duraflow manual variable restrictor was used in the SFE system to collect the extracted analytes. In order to prevent sample plugging, the restriction point was warmed electrically. The supercritical fluid extraction flow rate through the Duraflow restrictor was approximately 0.5–1.0 mL min^{-1} (compressed). The essential oil was extracted using supercritical fluid extraction under various conditions of pressure at constant temprature. The extracted oil was collected in a 10.0 mL volumetric flask and the final volume was adjusted to 5.0 mL with ethyl acetate at the end of the extraction. In order to improve the collection efficiency, the 10.0 mL collecting flask was placed in an ice bath during the dynamic extraction stage. Agarwood essential oil was dissolve in ethyl acetate, and bubbling of the solution was done by using nitrogen gas to evaporate the solution. Then, the weight of essential oil was measured. Finally the extraction yield was calculated.

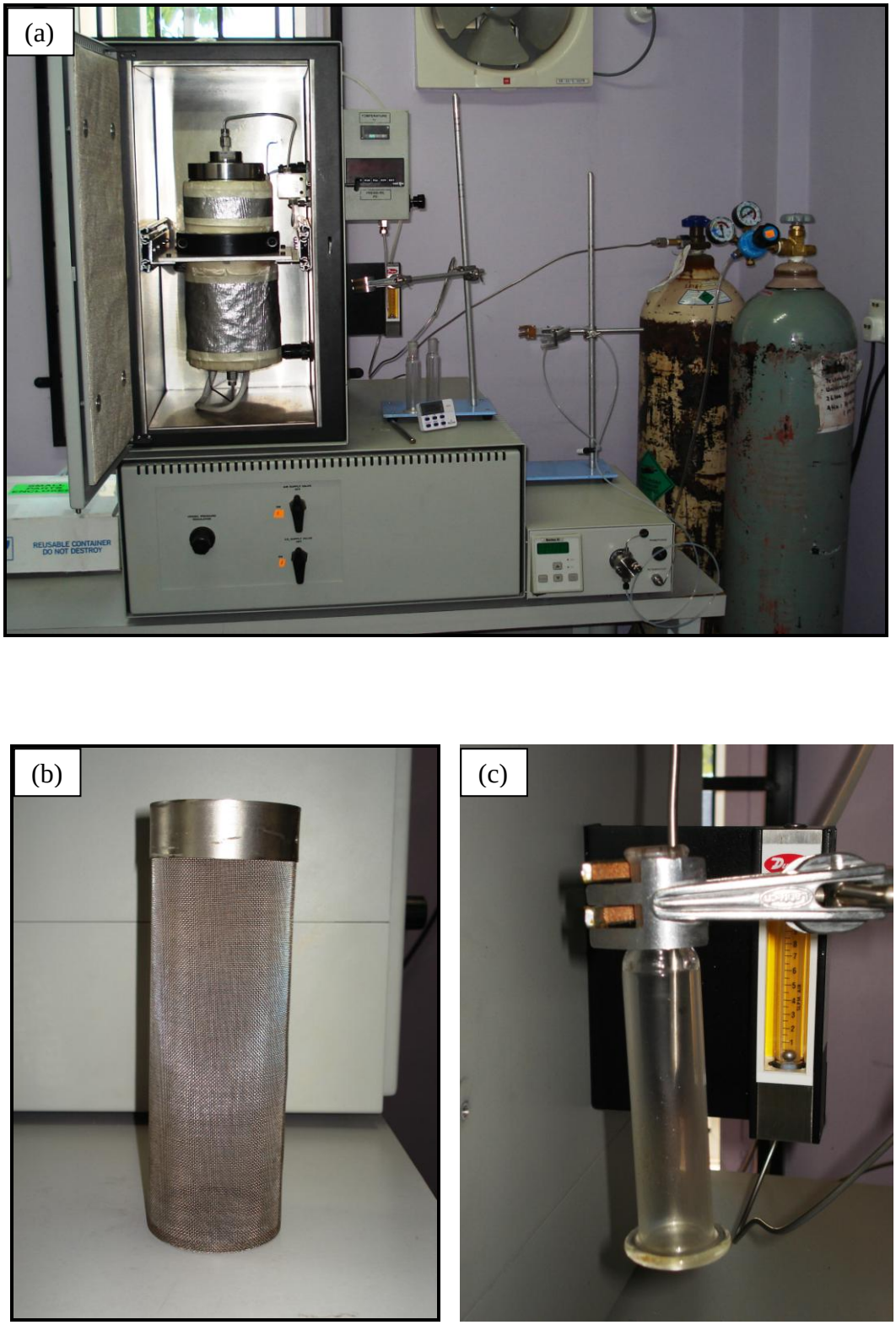


Figure 3.3: Pictures of supercritical fluid extraction. (a) Overview SFE system, (b) Vessel sample and (c) Oil collector flask.

3.4 METHODS OF ANALYSIS

3.4.1 Gas Chromatography-Flame Ionization Detector (GC-FID)

GC-FID for analytical purposes Agilent 7890A gas chromatograph, shown in **Figure 3.4 (a)** equipped with 30m long fused silica capillary columns (DB-1ms 0.25 mm I.D.; 0.25μm film thickness) was used. The instrument was equipped with a flame ionization detector (FID) and split injectors with a split ratio of 1:5. Helium (1.0 ml/min) was used as carrier gas. The injector and detector temperatures were arranged at 250°C and 250°C, respectively, for volatile oil. The oven temperature was programmed at 60°C for 1minutes, then ramped at 3°C/min to 230°C and held for 10 minutes (Table 3.1). The components were identified on the basis of comparison of their retention indices and mass spectra with published data (Joulian and Konig, 1999) and matching with National Institute of Standards Technology (NIST) libraries. Retention indices were calculated using a homologous series of n-alkanes (C_7-C_{20}).

Table 3.1: Experiment parameter for GC-FID

GC-FID analysis conditions	
Column	**DB-1MS**
Length (m)	30
Diameter (mm)	0.25
Film thickness (μm)	0.25
Temperature program	$60^oC \rightarrow 3^oC\ min^{-1} \rightarrow 230^oC$ (10min)
Carrier gas	Helium
	Flow rate: 1.0 ml/min
Injected sample	1μl at split ratio 1:5

3.4.2 Gas Chromatography-Mass Spectrometry Detector (GC-MSD)

GC-MS analysis was performed using an Agilent 7890A Network System gas chromatography, Figure 3.4 (b) was attached to a mass spectrometer (Agilent 5975C) with detector in full scan mode under electron impact ionization (EI, 70eV) and fitted with a capillary column (DB-1ms 30m × 0.25mm I.D.; 0.25μm film thickness). The oven temperature was programmed for 60°C for 1 min, then ramped at 3°C/min to 250°C and held for 10 min. Injector inlet and detector temperatures were set at 250°C. Each sample was diluted in hexane and then injected in 1 μl volume in the split mode (ratio 1:5) using helium as carrier gas (1 ml/min). Parameter analysis was summarized in Table 3.2.

Table 3.2: Experiment parameter for GC-MS

GC-MS analysis conditions	
Column	**DB-1MS**
Length (m)	30
Diameter (mm)	0.25
Film thickness (μm)	0.25
Temperature program	$60^oC \rightarrow 3^oC\ min^{-1} \rightarrow 230^oC$ (10min)
Carrier gas	Helium
	Flow rate: 1.0 ml/min
Injected sample	1 μl at split ratio 1:5
MS conditions	
EI	70 eV
Source temperature	200 ^oC
Scan range	20 - 500*u*
Scan speed (s/spectrum)	1/sec

Figure 3.4: Pictures of instruments; (**a**) GC-FID and (**b**) GC-MS

CHAPTER 4

RESULTS AND DISCUSSION

4.1 INTRODUCTION

The hydrodistillation process has been traditionally used in the extraction of essential oil at a laboratory scale. The aim work of this experiment was to compare two type extraction of volatile composition from *agarwood* by using hydrodistillation and SFE method. The results analyzed were compared with essential oil composition obtained through GC-FID and GC-MS. As the preliminary work, *agarwood* sample after grinded was bought at Gua Musang Kelantan in a big plastic. Samples were dividing for 150kg for different parameters analysis and after that, have been stored in laboratory. The procedure of doing this experiment is carefully followed to ensure the accuracy data obtained from each experiment run.

4.2 EXTRACTION OF VOLATILE OIL

Two types of extraction methods were studied, namely, hydrodistillation and supercritical fluid extraction in laboratory. Prior to extraction, milled *agarwood* was immersed in water for a period of seven days in order to break down the *agarwood* and oil glands (Chang, et al., 2002).

4.2.1 Hydrodistillation

Hydrodistillation was performed using the Clevenger-type apparatus at 12 hours. The optimum duration was found to be 12 hours when a yield of ~0.1% of greenish oil with woody aroma was obtained (Table 4.1).

Table 4.1: Parameter of product of hydrodistillation method

Time of extraction, h	Yield,%	Odor Characteristic	Color
12	0.1	Sweet woody, aromatic	Greenish

4.2.2 Supercritical fluid extraction

Supercritical fluid extraction was performed using a SFT Technologies model SFT-150 at 3000, 4000, 5000, 6000 and 7000 psi. The duration of operation was immersed in SFE for three hour (static) and oil collected over one hour (dynamic). All parameter for five different pressure in SFE method produced the same result in percentage of yield which is 0.2% as shown in (Table 4.2).

Table 4.2: Parameter of product of supercritical fluid extraction method

Time of extraction, h	Yield, %	Characteristic	Color
4 (static + dynamic)	0.2	Sweet, woody	Greenish,dark yellow

4.3 GC-FID Analyses Volatile Oil

The oil components ware identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices (RI). The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes (C9-C22) (Safaralie A, et al., 2007). The result of composition analysis in retention indices will be referred with DB-1 column references for identification of essential oil compound.

In comparing GC-FID chromatograms of oil samples, a pattern of similarity is observed as shown in Figures 4.1-4.6. This may be attributed to extraction based on an identical principle namely, hydrodistillation. In the case of SFE, the oil obtained exhibit entirely different chemical characteristics as evidenced by its chemical composition as shown in Table 4.3-4.8.

4.3.1 Hydrodistillation Extraction

Hydrodistillation of the agarwood material using hexane as collecting solvent yielded greenish oil. The chromatograms of volatile compounds of agarwood oil were analyzed by GC-FID are shown as Figure 4.1- 4.6.

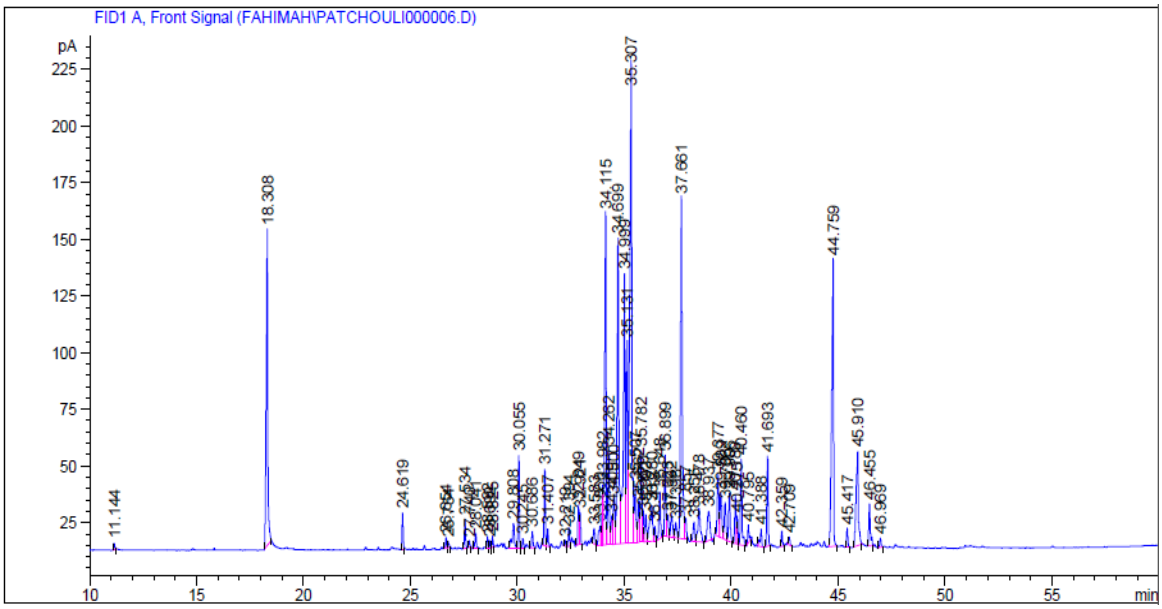


Figure 4.1: Chromatogram of essential oil from hydrodistillation method.

Table 4.3: Components present in the GC-FID of the extract using hydrodistillation method.

Retention Time (min)	Compound	Area %
32.394	α-agarofuran	0.325
34.115	10-epi- g-eudesmol	6.420
34.699	1,5-epoxy-nor-ketoguaiene	7.224
35.131	valerianol	3.744
35.307	agarospirol	13.006
35.782	jinkoh-eremol	1.744
36.899	dehydrojinkoh-eremol	1.539
37.661	pentadecanal	8.499
40.460	sinenofuranol	1.627
44.759	palmitic acid	6.974
45.910	9-hydroxyselina-4,11-dien-14-oic acid	2.867

4.3.2: Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction of the *agarwood* material using ethyl acetate as collecting solvent yielded greenish oil. The volatile compounds of *agarwood* oil were analyzed by GC-FID. The results using supercritical fluid extraction with variations of pressure (3000, 4000, 5000, 6000, 7000 psi) at constat temperature (60°C). Since various parameters potentially affect the extraction process, the optimization of the experimental conditions represents in different compound produced. All compounds are labeled in the chromatogram in Figure 4.2-4.6 for each parameter. The results of SFE experiments, based on different pressure used are given in Table 4.4-4.8.

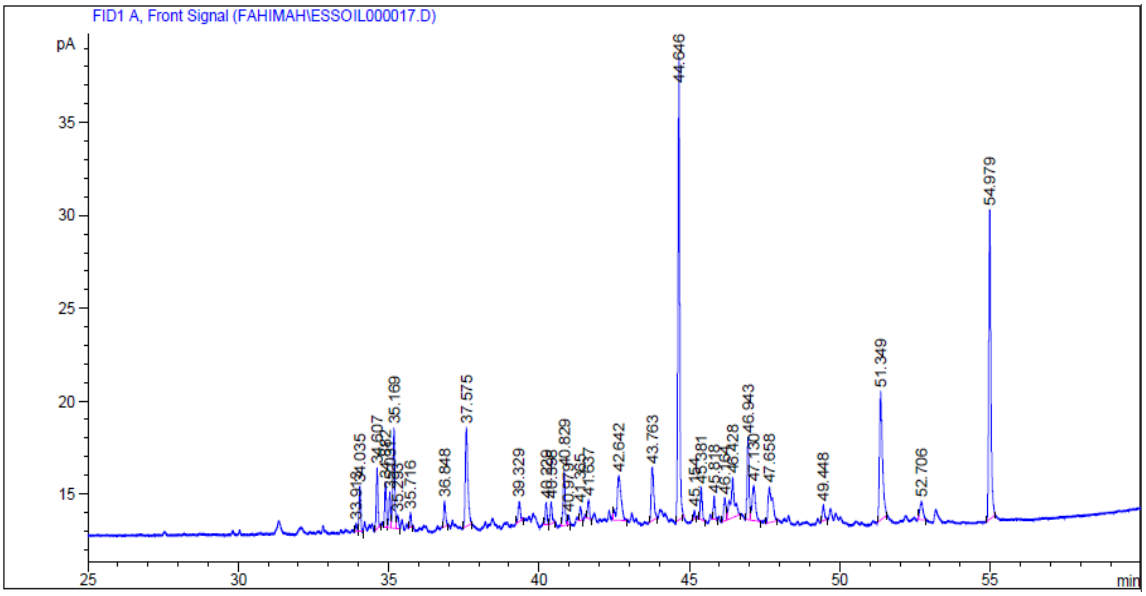


Figure 4.2: Chromatogram of essential oil from supercritical fluid extraction in pressure 3000 psi.

Table 4.4: Components present in the GC-FID of the extract using supercritical fluid extraction in pressure 3000 psi.

Retention Time (min)	Compound	Area %
34.035	10-epi- g-eudesmol	1.756
34.607	1,5-epoxy-nor-ketoguaiene	2.351
35.169	valerianol	4.434
35.293	agarospirol	0.595
35.716	jinkoh-eremol	0.506
36.848	dehydrojinkoh-eremol	1.205
37.575	pentadecanal	5.967
40.398	sinenofuranol	1.103
41.365	guaia-1(10),11-dien-15-al	0.595
47.658	1,5-diphenyl-2-pentene	3.557

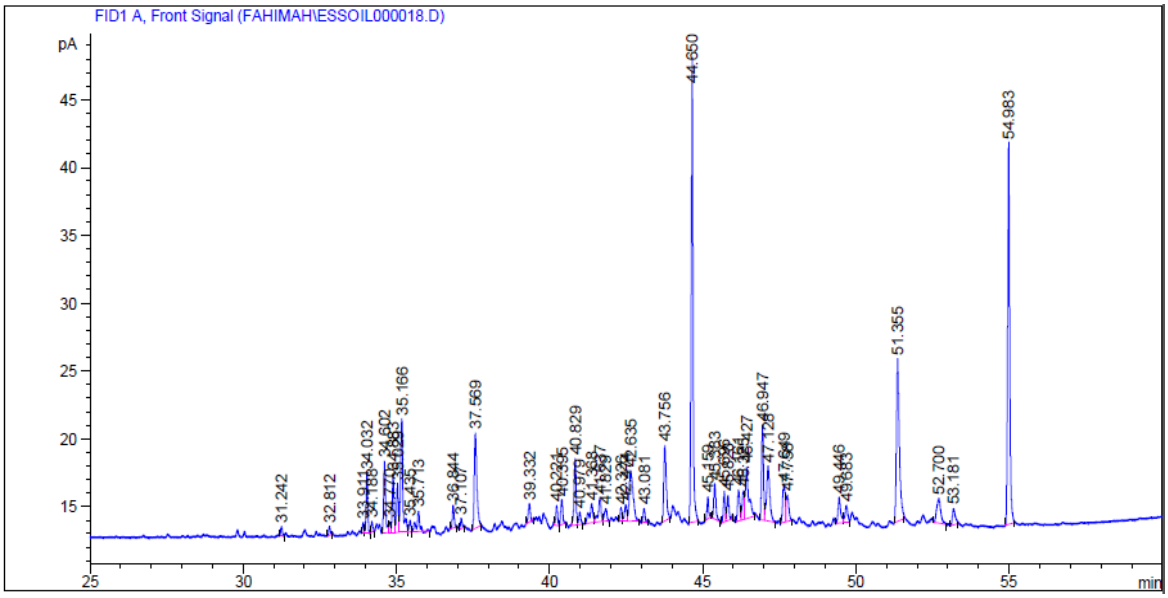


Figure 4.3: Chromatogram of essential oil from supercritical fluid extraction in pressure 4000 psi.

Table 4.5: Components present in the GC-FID of the extract using supercritical fluid extraction in pressure 4000 psi.

Retention Time (min)	Compound	Area %
33.911	tetradecanal	0.305
34.602	1,5-epoxy-nor-ketoguaiene	2.433
35.166	valerianol	4.658
35.713	jinkoh-eremol	1.100
36.844	dehydrojinkoh-eremol	0.747
37.569	pentadecanal	4.336
40.395	sinenofuranol	0.907
41.637	karanone	1.213
42.635	pentadecanoic acid	3.365
45.383	2-hydroxyquia-1(10),11-dien-15-oic acid	1.341

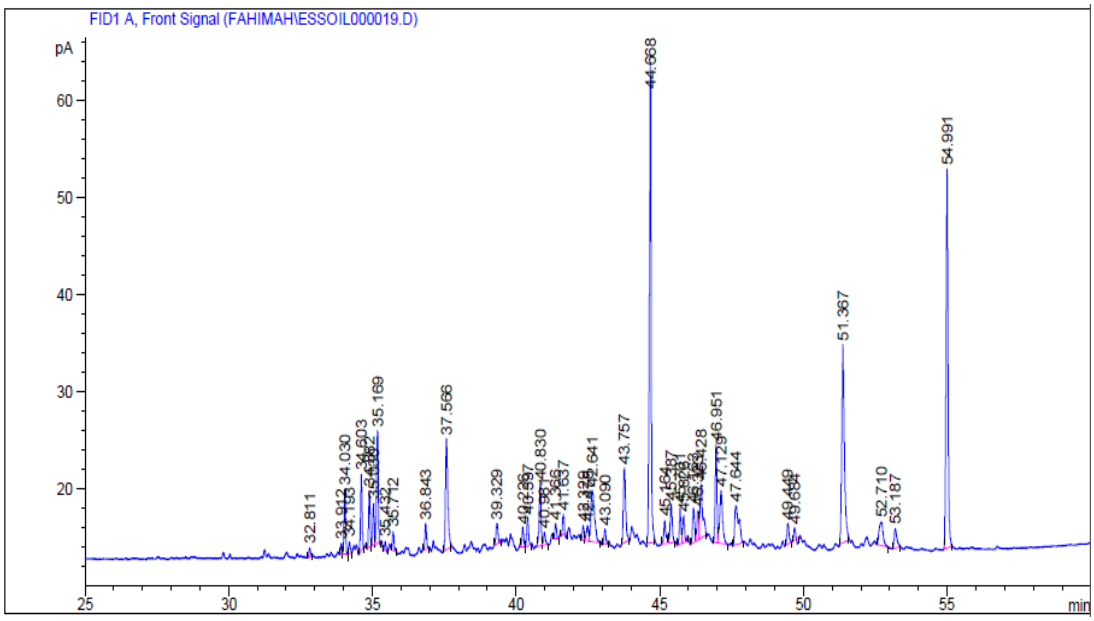


Figure 4.4: Chromatogram of essential oil from supercritical fluid extraction in pressure 5000 psi

Table 4.6: Components present in the GC-FID of the extract using supercritical fluid extraction extraction in pressure 5000 psi.

Retention Time (min)	Compound	Area %
34.603	1,5-epoxy-nor-ketoguaiene	2.294
35.169	valerianol	3.771
35.712	jinkoh-eremol	0.612
36.843	dehydrojinkoh-eremol	0.853
37.566	pentadecanal	4.959
40.397	sinenofuranol	0.976
45.387	2-hydroxyquia-1(10),11-dien-15-oic acid	1.553
47.644	1,5-diphenyl-2-pentene	3.153

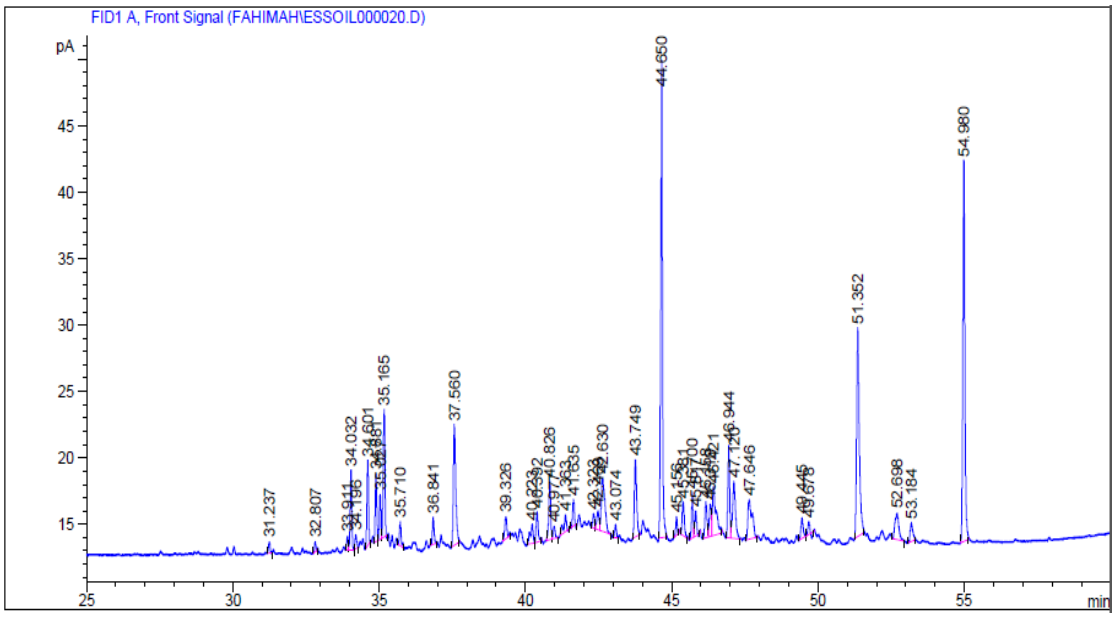


Figure 4.5: Chromatogram of essential oil from supercritical fluid extraction for 6000 psi.

Table 4.7: Components present in the GC-FID of the extract using supercritical fluid extraction in pressure 6000 psi.

Retention Time(min)	Compound	Area %
34.032	10-epi- g-eudesmol	2.339
34.601	1,5-epoxy-nor-ketoguaiene	2.551
35.165	valerianol	4.142
35.710	jinkoh-eremol	0.756
36.841	dehydrojinkoh-eremol	0.889
37.560	pentadecanal	5.220
40.392	sinenofuranol	1.165
45.700	hexadecanoic acid	1.425
47.646	1,5-diphenyl-2-pentene	3.063

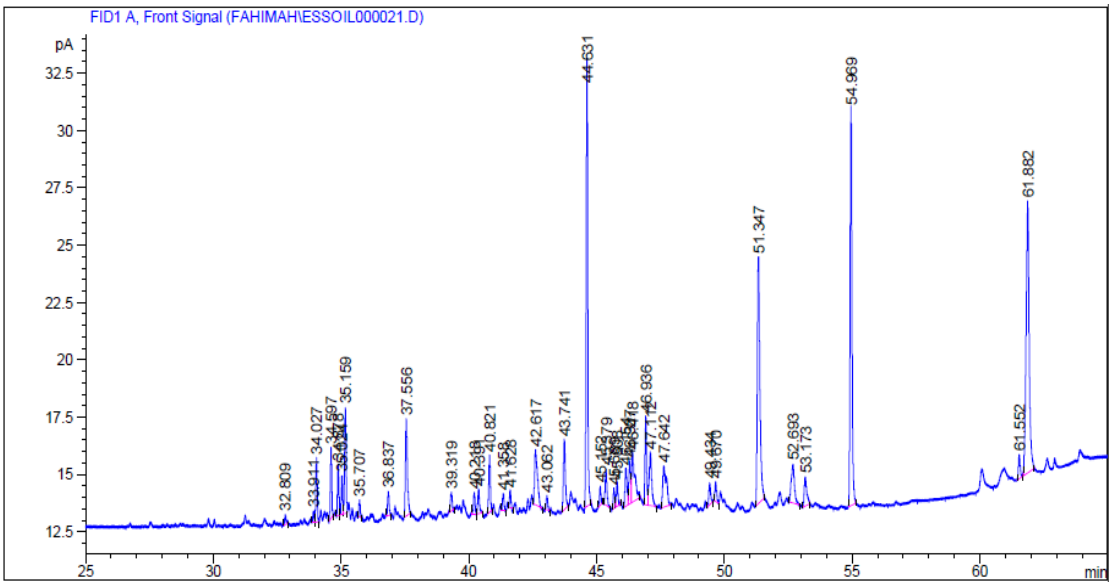


Figure 4.6: Chromatogram of essential oil from supercritical fluid extraction in pressure 7000 psi

Table 4.8:.Components present in the GC-FID of the extract using supercritical fluid extraction in pressure 7000 psi

Retention Time(min)	Compound	Area %
33.911	tetradecanal	0.263
34.597	ϒ-eudesmol	1.794
35.159	valerianol	3.025
35.707	jinkoh-eremol	0.383
36.837	dehydrojinkoh-eremol	0.657
40.218	guaia-1(10),11-dien-15-ol	0.598
40.391	sinenofuranol	0.765
41.628	karanone	0.371
42.617	pentadecanoic acid	2.822
47.642	1,5-diphenyl-2-pentene	2.750

Summary of Findings

As a result, nineteen compounds were identified in volatile oil products from 2 types of extraction methods and differences parameter for SFE method. Then, essential oil for each type extraction was analyzed by using GC-FID. Four compounds, namely, Valerianol, Jinkoh-eremol, Dehydrojinkoh-eremol and Sinenofuranol were present both extraction methods. While three compound α-agarofuran, Palmitic Acid and 9-Hydroxyselina-4, 11-Dien-14-oicacid was obtained by hydrodistillation extraction method and they were not at any experiment in supercritical fluid extraction method.

Besides that, ϒ-eudesmol and Guaia-1(10), 11-Dien-15-ol compound were obtained in supercritical fluid extraction at high pressure which is in 7000psi. Then only two compound Pentadecanal and 1,5-Epoxy- nor-ketoguaiene can't obtained in high pressure for SFE method. According to the Table 4.9 it can be observed that the extract obtained from lower pressure (3000psi) in supercritical fluid extraction have eleven compounds obtained. But, only two component 10-Epi- g-eudesmol and Guaia-1(10),11-Dien-15-Al were present only under pressure 3000 psi and not at any other pressure for SFE method.

For the complete recoveries of the main components of the assential oil, higher pressures are necessary. This is because rising the extraction pressure, at constant temperature, leads to higher fluid density, which increases the solubility of the analytes. To obtain quantitative recoveries of analytes, they must be efficiently partitioned from the sample matrix into the supercritical fluid (Khajeh M, et al., 2009). All another compounds identified using this method is summarized in Table 4.9.

Table 4.9: Compounds identified using GC-FID

No	Compound	KI		HD	SFE 3000 psi	SFE 4000 psi	SFE 5000 psi	SFE 6000 psi	SFE 7000 psi
		Ref	Sample						
1	α-agarofuran	**1553**	**1554**	/					
2	10-Epi- g-eudesmol	**1599**	**1600**	/	/		/	/	/
3	ϒ-eudesmol	**1608**	**1611**						/
4	10-Epi- g-eudesmol	**1599**	**1596**		/				
5	1,5-Epoxy- nor-ketoguaiene	**1614**	**1612**	/	/	/	/	/	
6	Valerianol	**1626**	**1628**	/	/	/	/	/	/
7	Agarospirol	**1631**	**1632**	/	/				
8	Jinkoh-eremol	**1643**	**1643**	/	/	/	/	/	/
9	Dehydrojinkoh-eremol	**1673**	**1674**	/	/	/	/	/	/
10	Guaia-1(10),11-Dien-15-ol	**1770**	**1771**						/
11	Sinenofuranol	**1776**	**1777**	/	/	/	/	/	/
12	Guaia-1(10),11-Dien-15-Al	**1806**	**1805**		/				
13	Karanone	**1812**	**1814**			/			/
14	Pentadecanoic Acid	**1842**	**1845**			/			/
15	Palmitic Acid	**1912**	**1910**	/					
16	2-Hydroxyquia-1(10),11-Dien-15-Oic Acid	**1932**	**1930**			/	/		
17	Hexadecanoic Acid	**1940**	**1940**					/	
18	9-Hydroxyselina-4,11-Dien-14-Oic Acid	**1948**	**1946**	/					
19	1,5-Diphenyl-2-Pentene	**2000**	**2000**		/		/	/	/

4.4 GC-MS ANALYSIS

The hydrodistillation and SFE extracts of *agarwood* showed a relatively simple by GC-MS chromatographic pattern. Selective analysis of sesquiterpene hydrocarbons was a accomplished using a DB-1 column and comparison of mass spectra data. Peak identification for these components was based on the NIST08 library. All components have similarities with NIST library spectra higher than 80% (a similarity of 99% represents a perfect match with the library) and the percentage content of the components was more than 0.1% per area.

4.4.1: Hydrodistillation Extraction

Hydrodistillation of the *agarwood* material using hexane as collecting solvent yielded a greenish oil. The volatile compounds were analyzed by GC-MS. Sixty six compounds of essential oil were identified from GC-MS, but the major compound was obtained only 16 compounds. Then fourty nine compounds are unknown compound. Detailed identification and quantization of the major compound found in *gaharu* assential oil, produced by hydrodistillation were showed in Table 4.10.

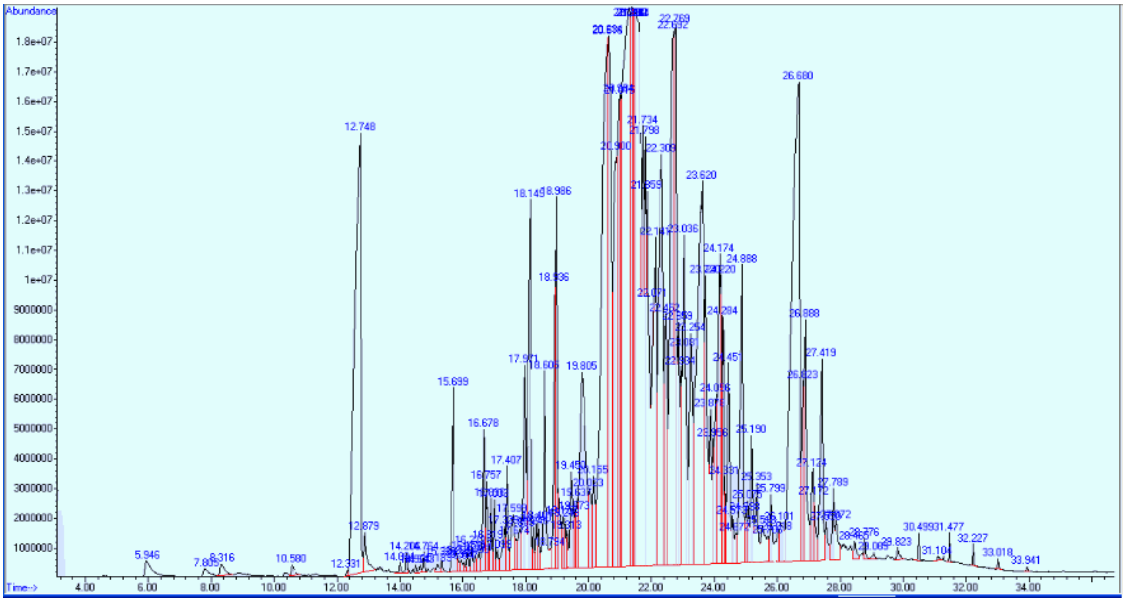


Figure 4.7: GC -MS chromatogram in product from hydrodistillation extraction

Table 4.10: Components present in the GC–MS of the extract using hydrodistillation.

No	Retention Time (min)	Compound	Area %	CAS number	Quality
1	5.948	Benzaldehyde	0.19	100-52-7	94
2	8.317	Acetophenone	0.10	98-86-2	87
3	12.746	2-Butanone, 4-phenyl-	4.71	2550-26-7	91
4	12.877	Benzenepropanol, .beta.-methyl-	0.22	7384-80-7	87
5	14.004	Unknown	-	-	-
6	14.206	Unknown	-	-	-
7	14.765	Unknown	-	-	-
8	15.698	Unknown	-	-	-
9	16.677	α -Guaiene	0.53	3691-12-1	99
10	16.757	Unknown	-	-	-
11	16.894	Unknown	-	-	-

Table 4.10: continued

No	Retention Time (min)	Compound	Area %	CAS number	Quality
12	17.329	*allo*-Aromadendrene	0.24	25246-27-9	95
13	17.409	unknown	-	-	-
14	17.598	unknown	-	-	-
15	17.970	unknown	-	-	-
16	18.147	α-Bulnesene	1.62	3691-11-0	99
17	18.244	Unknown	-	-	-
18	18.342	Unknown	-	-	-
19	18.399	Spathulenol	0.13	77171-55-2	86
20	18.605	Unknown	-	-	-
21	18.937	Unknown	-	-	-
22	18.988	Unknown	-	-	-
23	19.177	Unknown	-	-	-
24	19.246	Unknown	-	-	-
25	19.452	Unknown	-	-	-
26	19.572	Unknown	-	-	-
27	19.635	Unknown	-	-	
28	19.806	Unknown	-	-	-
29	20.024	Unknown	-	-	-
30	20.899	Agarospirol	2.82	1460-73-7	95
31	21.014	β-Guaiene	0.92	88-84-6	90
32	21.403	Unknown	-	-	-
33	21.735	Unknown	-	-	-
34	21.798	Unknown	-	-	-
35	21.861	Unknown	-	-	-
36	22.072	Unknown	-	-	-
37	22.141	Unknown	-	-	-
38	22.307	Unknown	-	-	-

Table 4.10: continued

No	Retention Time (min)	Compound	Area %	CAS number	Quality
39	22.690	6-Isopropenyl-4,8a-dimethyl-1,2,3, 5,6,7,8,8a-octahydro-naphthalen-2-ol	3.88	189-10-2	89
40	22.770	Unknown	-	-	-
41	22.936	Unknown	-	-	-
42	23.079	2(1H)Naphthalenone ,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	0.75	188-66-5	86
43	23.623	Unknown	-	-	-
44	23.720	Unknown	-	-	-
45	23.880	Unknown	-	-	-
46	23.955	Unknown	-	-	-
47	24.058	Unknown	-	-	-
48	24.172	Unknown	-	-	-
49	24.218	Unknown	-	-	-
50	24.287	Unknown	-	-	-
51	24.333	zierone	0.17	6754-66-1	91
52	24.453	Unknown	-	-	-
53	24.573	Unknown	-	-	-
54	24.888	Unknown	-	-	-
55	25.191	Unknown	-	-	-
56	25.517	Unknown	-	-	-
57	25.797	Unknown	-	-	-
58	26.101	Unknown	-	-	-

Table 4.10: continued

No	Retention Time (min)	Compound	Area %	CAS number	Quality
59	26.679	Unknown	-	-	-
60	26.822	n-Hexadecanoic acid	0.56	57-10-3	95
61	27.125	Unknown	-	-	-
62	27.417	3-Pentanone,1,5-diphenyl-	0.97	5396-91-8	91
63	27.789	Unknown	-	-	-
64	27.874	Unknown	-	-	-

Table 4.11: Structure for each component for hydrodistillation (GC-MS)

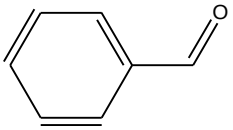
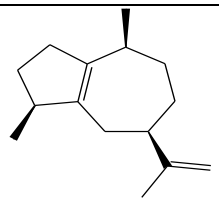
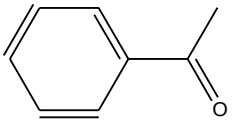
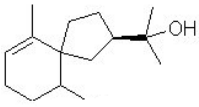
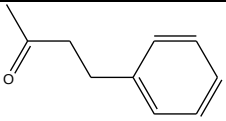
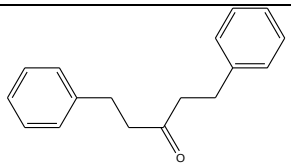
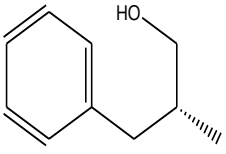
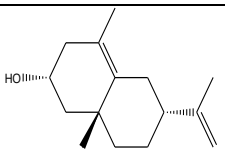
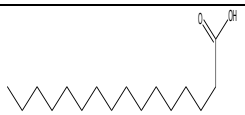
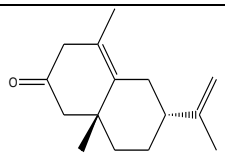
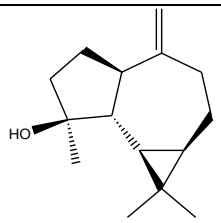
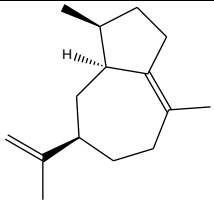
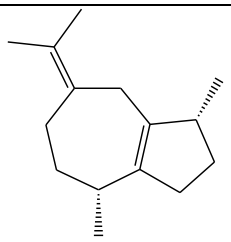
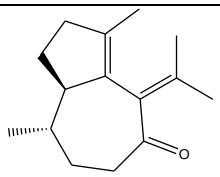
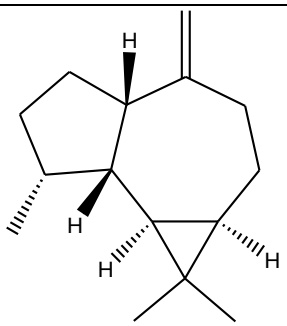
Benzaldehyde		α-Guaiene	
Acetophenone		Agarospirol	
2-Butanone, 4-phenyl-		3-Pentanone,1,5-diphenyl-	
Benzenepropanol,.beta.-methyl-		6-Isopropenyl-4,8a-dimethyl-1,2,3, 5,6,7,8,8a-octahydro-naphthalen-2-ol	

Table 4.11: continued

n-Hexadecanoic acid		2(1H)Naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	
Spathulenol		α –Bulnesene	
β -guaiene		zierone	
allo-Aromadendrene			

4.4.2: Supercritical Fluid Extraction

Supercritical fluid extraction of the agarwood material using hexane as collecting solvent yielded a greenish oil. The volatile compounds were analyzed by GC-MS. All compounds were identified under five different pressures (3000, 4000, 5000, 6000, 7000 psi). All the compounds are labeled in the chromatogram in Figure 4.8-4.12.

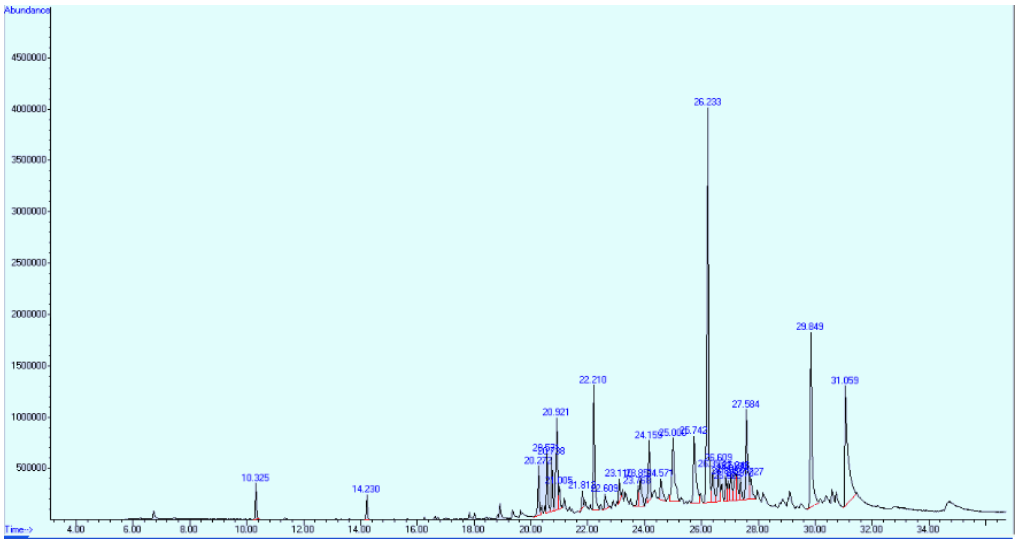


Figure 4.8: GC -MS chromatogram in product from SFE in pressure 3000psi

Table 4.12: Components present in the GC–MS of the extract using SFE in pressure 3000 psi

No	Retention Time(min)	Compound	Area %	CAS number	Quality
1	18.908	Unknown	-	-	-
2	19.354	Unknown	-	-	-
	20.373	*allo*-Aromadendrene	0.28	25246-27-9	96
3	20.573	Agarospirol	1.75	1460-73-7	94
4	20.739	Naphthalene, 1,2,4a,5,8,8a-hexahyd ro-4,7-dimethyl-1-(1-methylethyl)-	1.58	5951-61-1	93
5	20.922	γ-Gurjunene	3.90	22567-17-5	95
6	21.008	Unknown	-	-	-
7	21.185	Unknown	-	-	-
8	22.210	Unknown	-	-	-
9	22.610	α –Copaene	0.87	4586-22-5	86
10	23.766	Unknown	-	-	-
11	24.573	unknown	-	-	-
12	25.002	unknown	-	-	-
13	25.963	Unknown	-	-	-
14	26.232	Unknown	-	-	-
15	26.398	Unknown	-	-	-

Table 4.12: continued

No	Retention Time(min)	Compound	Area %	CAS number	Quality
16	27.371	Spathulenol	1.34	77171-55-2	81
17	27.583	Unknown	-	-	-
18	27.726	Unknown	-	-	-
19	27.972	Unknown	-	-	-
20	29.053	Unknown	-	-	-
21	29.110	Unknown	-	-	-
22	29.848	Unknown	-	-	-
23	30.169	Unknown	-	-	-
24	30.386	Unknown	-	-	-
25	30.592	Unknown	-	-	-
26	31.061	8-Naphthol, 1-(benzyloxy)-	10.06	326875-68-7	91
27	31.473	Unknown	-	-	-
28	34.712	Unknown	-	-	-

Table 4.13: Structure for each component for SFE 3000psi

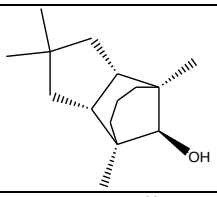
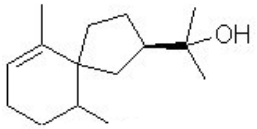
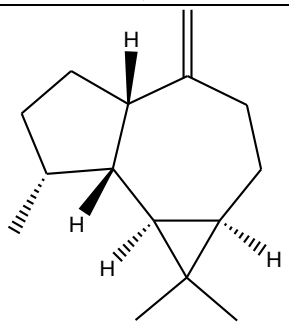
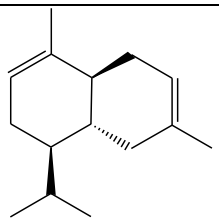
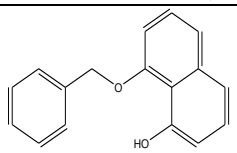
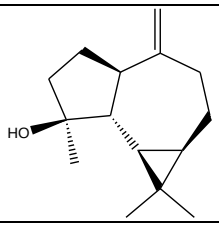
α-Copaene		Agarospirol	
allo-Aromadendrene		Naphthalene, 1,2,4a,5,8,8a-hexahyd ro-4,7-dimethyl-1-(1-methylethyl)-	
8-Naphthol, 1-(benzyloxy)-		Spathulenol	

Table 4.13: continued

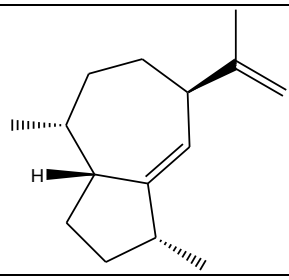
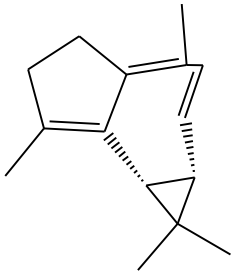
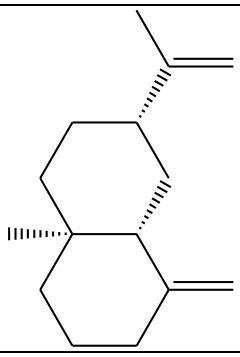
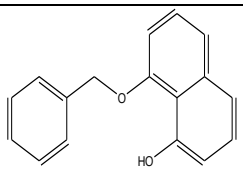
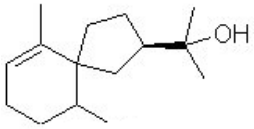
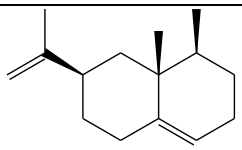
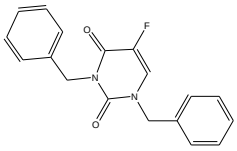
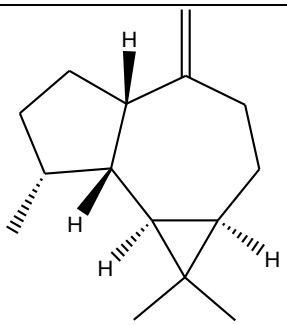
γ-Gurjunene	

Figure 4.9: GC -MS chromatogram in product from SFE extractionin pressure 4000 psi

Table 4.14: Components present in the GC–MS of the extract using SFE in pressure 4000 psi

No	Retention Time(min)	Compound	Area %	CAS number	Quality
1	18.834	Unknown	-	-	-
2	19.635	Neoisolongifolene, 8,9-dehydro-	0.19	67517-14-0	86
3	20.379	α-Gurjunene	0.29	489-40-7	97
4	20.579	Agarospirol	1.58	1460-73-7	93
5	20.928	β-Selinene	3.56	17066-67-0	95
6	21.186	Unknown	-	-	-
7	21.815	Unknown	-	-	-
8	21.912	*allo*-Aromadendrene	0.50	25246-27-9	80
9	22.215	Unknown	-	-	-
10	22.456	Unknown	-	-	-
11	23.113	Unknown	-	-	-
12	23.772	Unknown	-	-	-
13	24.172	Unknown	-	-	-
14	24.579	Unknown	-	-	-
15	25.019	Unknown	-	-	-
16	24.647	Unknown	-	-	-
17	25.069	Unknown	-	-	-
18	25.763	Unknown	-	-	-
19	25.981	Unknown	-	-	-
20	26.255	Unknown	-	-	-
21	26.415	Unknown	-	-	-
22	26.639	Unknown	-	-	-
23	26.873	Unknown	-	-	-
24	26.948	eremophilene	0.96	10219-75-7	93
25	27.056	Unknown	-	-	-
26	27.194	Unknown	-	-	-
27	27.268	Unknown	-	-	-
28	27.606	Unknown	-	-	-
29	29.881	Unknown	-	-	-
30	31.062	8-Naphthol, 1-(benzyloxy)-	8.47	326875-68-7	90
31	34.598	5-Fluoro-1,3-bis[phenylmethyl]-2,4(1H,3H)-pyrimidinedione	3.03	75500-02-6	83

Table 4.15: Structure for each component for SFE 4000 psi

Neoisolongifolene, 8,9-dehydro		α-Gurjunene	
β-Selinene		8-Naphthol, 1-(benzyloxy)-	
Agarospirol		eremophilene	
5-Fluoro-1,3-bis[phenylmethyl]-2,4(1H,3H)-pyrimidinedione		*allo*-Aromadendrene	

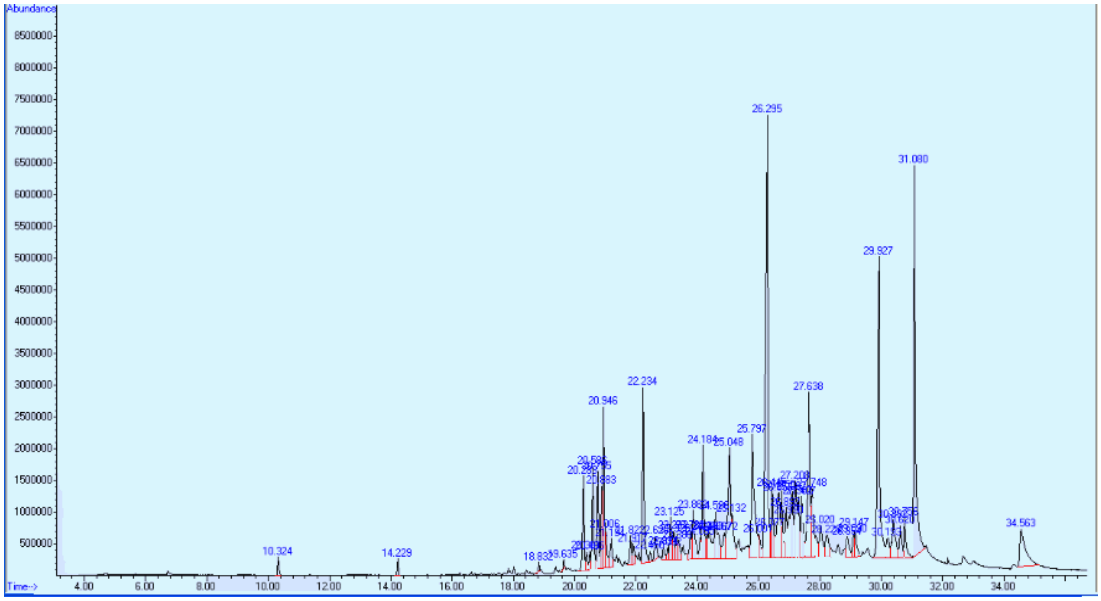


Figure 4.10: GC -MS chromatogram in product from SFE extraction in pressure 5000 psi

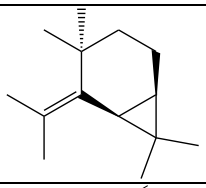
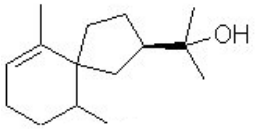
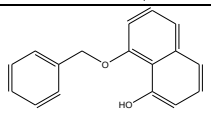
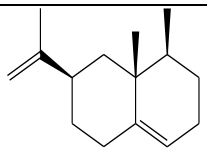
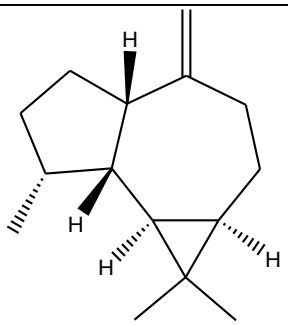
Table 4.16: Components present in the GC–MS of the extract using SFE in 5000 psi

No	Retention Time(min)	Compound	Area %	CAS number	Quality
1	20.280	α-gurjunene	1.33	489-29-2	95
2	20.586	Agarospirol	1.55	1460-73-7	96
	21.008	*allo*-Aromadendrene	0.72	25246-27-9	96
3	21.197	Unknown	-	-	-
4	21.821	Unknown	-	-	-
5	21.918	Unknown	-	-	-
6	22.101	Unknown	-	-	-
7	22.233	Unknown	-	-	-
8	23.125	unknown	-	-	-
9	24.098	eremophilene	0.58	10219-75-7	83
10	24.184	unknown	-	-	-
11	24.584	Unknown	-	-	-
12	25.048	Unknown	-	-	-
13	25.797	Unknown	-	-	-
14	26.003	Unknown	-	-	-
15	26.295	Unknown	-	-	-
16	27.091	Unknown	-	-	-
17	27.205	Unknown	-	-	-
18	27.638	Unknown	-	-	-

Table 4.16: continued

No	Retention time(min)	Compound	Area %	CAS number	Quality
19	28.018	Unknown	-	-	-
20	29.929	Unknown	-	-	-
21	31.080	8-Naphthol, 1-(benzyloxy)-	6.75	326875-68-7	91
22	34.563	unknown	-	-	-

Table 4.17: Structure for each component for SFE 5000 psi

Gurjunene.alpha.		Agarospirol	
8-Naphthol, 1-(benzyloxy)-		Eremophilene	
allo-Aromadendrene			

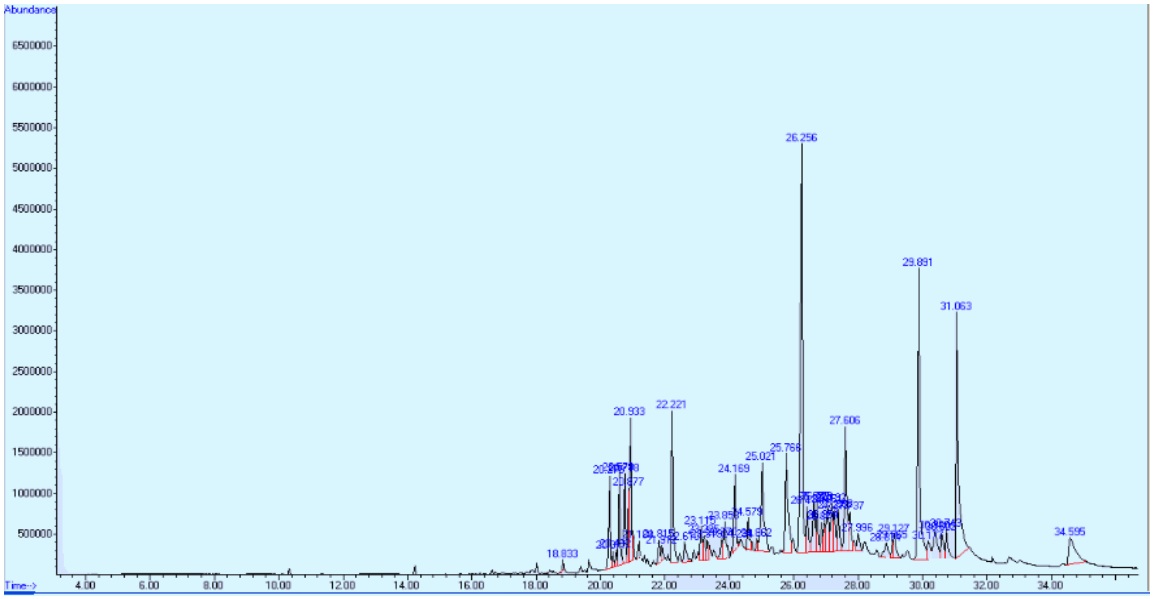


Figure 4.11: GC -MS chromatogram in product from SFE extraction in pressure 6000 psi

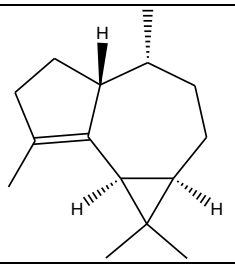
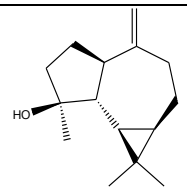
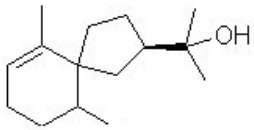
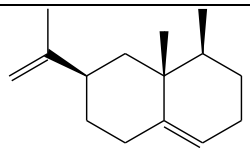
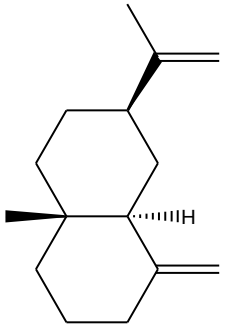
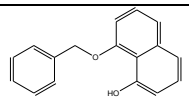
Table 4.18: Components present in the GC–MS of the extract using SFE in 6000 psi

No	Retention Time(min)	Compound	Area %	CAS number	Quality
1	18.834	Unknown	-	-	-
2	19.635	Unknown	-	-	-
3	20.379	α-gurjunene	0.44	489-40-7	96
4	20.579	Agarospirol	2.19	1460-73-7	98
5	20.877	β-Selinene	1.30	17066-67-0	91
6	21.002	eremophilene	0.99	10219-75-7	96
7	21.191	Unknown	-	-	-
8	22.221	Unknown	-	-	-
9	22.450	Unknown	-	-	-
10	23.114	*Allo*-aromadendrene	0.97	6750-60-3	83
11	24.166	Unknown	-	-	-
12	24.292	Unknown	-	-	-
13	24.367	Unknown	-	-	-
14	24.578	Unknown	-	-	-
15	24.865	Unknown	-	-	-
16	25.019	Unknown	-	-	-
17	25.763	Unknown	-	-	-
18	26.255	Unknown	-	-	-
19	26.730	Unknown	-	-	-

Table 4.18: continued

No	Retention Time	Compound	Area %	CAS number	Quality
20	27.193	Unknown	-	-	-
21	27.606	Unknown	-	-	-
22	27.994	Unknown	-	-	-
23	29.891	Unknown	-	-	-
24	31.061	8-Naphthol, 1-(benzyloxy)-	8.75	326875-68-7	90
25	34.592	unknown	-	-	-

Table 4.19: Structure for each component for SFE 6000 psi

α-Gurjunene		*Allo*-aromadendrene	
Agarospirol		Eremophilene	
β-Selinene		8-Naphthol, 1-(benzyloxy)-	

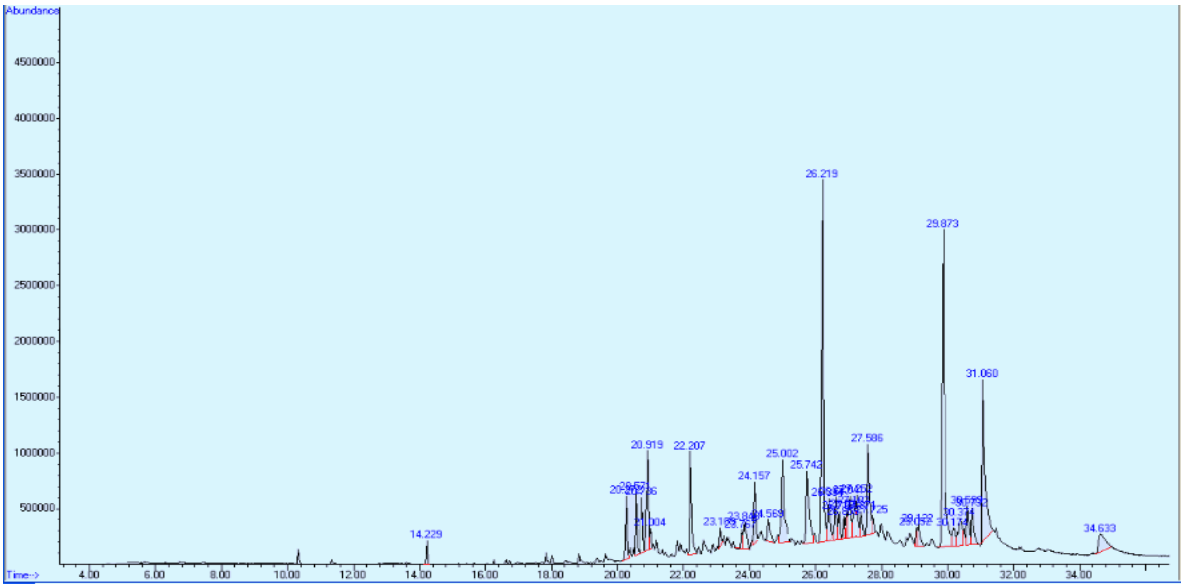


Figure 4.12: GC -MS chromatogram in product from SFE extractionin pressure 7000 psi

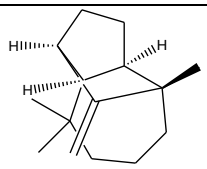
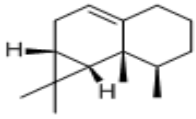
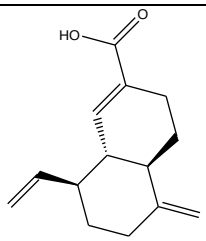
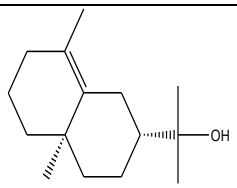
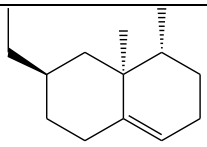
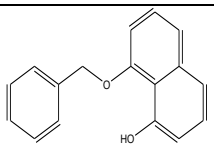
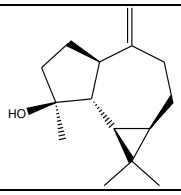
Table 4.20: Components present in the GC–MS of the extract using SFE in 7000 psi

No	Retention Time(min)	Compound	Area %	CAS number	Quality
1	20.270	Unknown	-	-	-
2	20.487	γ -eudesmol	0.25	1209-71-8	95
3	20.733	Aristolene	1.46	6831-16-9	90
4	20.916	*allo*-Aromadendrene	3.53	489-39-4	97
5	21.185	Unknown	-	-	-
6	21.809	Unknown	-	-	-
7	21.906	longifolene	0.44	475-20-7	91
8	22.210	Unknown	-	-	-
9	22.610	Unknown	-	-	-
10	23.108	2-Naphthalenecarboxylic acid, 8-ethenyl-3,4,4a,5,6,7,8,8a-octahydro-5-methylene-	0.56	1451-36-1	91
11	23.766	β-Neoclovene	0.51	56684-96-9	95
12	24.155	Unknown	-	-	-
13	25.740	Unknown	-	-	-
14	25.740	Unknown	-	-	-
15	26.221	Unknown	-	-	-
16	26.856	Unknown	-	-	-
17	27.039	Unknown	-	-	-
18	27.251	Unknown	-	-	-
19	27.588	Unknown	-	-	-

Table 4.20: continued

No	Retention time(min)	Compound	Area %	CAS number	Quality
20	27.726	Unknown	-	-	-
21	27.977	Unknown	-	-	-
22	28.756	Unknown	-	-	-
23	29.122	Unknown	-	-	-
24	29.871	Unknown	-	-	-
25	30.175	Unknown	-	-	-
26	30.489	Unknown	-	-	-
27	30.598	Unknown	-	-	-
28	30.735	Unknown	-	-	-
29	31.062	8-Naphthol, 1-(benzyloxy)-	9.14	326875-68-7	91
30	34.632	Unknown	-	-	-

Table 4.21: Structure for each component for SFE 7000 psi

longifolene		Aristolene	
2-Naphthalenecarboxylic acid, 8-ethenyl-3,4,4a,5,6,7,8,8a-octahydro-5-methylene-		γ -eudesmol	
β-Neoclovene		8-Naphthol, 1-(benzyloxy)-	
Allo-aromadendrene			

Summary of Findings

Twenty eight compounds of assential oil were identified in volatile oil products from 2 types of extraction methods, by analysis using GC-MS. Six compounds were identified only in hydrosistillation method as shown in Table 4.22. Only one compound namely, allo-aromadendrene were identified in both of the extraction methods. While, 4 compounds γ-eudesmol, Aristolene, 2-Naphthalenecarboxylic acid, 8-ethenyl-3,4,4a,5,6,7,8,8a-octahydro-5-methylene and longifolene were identified only in high pressure (7000 psi) for SFE method and not at any other parameter.

But the hydrodistillation method mostly sixty four compounds were produced. Hydrodistillation and SFE method have been different compounds produced due to the different of method using. This also might be because of trapping the volatile oil in two methods. In supercritical fluid extraction, a part of the extracted volatile components is escaping along with carbon dioxide gases from the vessel containing ethyl acetate. However, in hydrodistillation trapping the essential oil is simultaneously performed along with condensing the steam in a pipe (Safaralie, A. et al., 2007;). All the compounds identified using this method is summarized in Table 4.22.

Table 4.22: Total essential oil composition identified using GC-MS.

No	**Compounds**	HD	Sfe 3000	Sfe 4000	Sfe 5000	Sfe 6000	Sfe 7000
1	Benzaldehyde	/					
2	Acetophenone	/					
3	2-Butanone, 4-phenyl-	/					
4	6-Isopropenyl-4,8a-dimethyl-1,2,3, 5,6,7,8,8a-octahydro-naphthalen-2-ol	/					
5	4,6,6-Trimethyl-2-(3-methylbuta-1, 3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)]octane	/					
6	Spathulenol	/	/				
7	beta.-methyl-Benzenepropanol, .	/					
8	5(1H)-Azulenone, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene)-, (8S-cis)-	/					
9	n-Hexadecanoic acid	/					
10	1,5-diphenyl-3-Pentanone,	/					
11	*allo*-Aromadendrene	/	/	/	/	/	/
12	Naphthalene, 1,2,4a,5,8,8a-hexahyd ro-4,7-dimethyl-1-(1-methylethyl)-		/				
13	α-Copaene		/				
14	8,9-dehydro-Neoisolongifolene,			/			
15	Isoaromadendrene epoxide				/		
16	β-Selinene					/	
17	γ-Gurjunene		/				
18	β-Humulene					/	
19	α-Gurjunene		/	/	/	/	
20	α-Guaiene	/	/				
21	Agarospirol	/	/	/	/	/	
22	α-Bulnesene	/	/				
23	eremophilene		/	/	/	/	/
24	8-Naphthol, 1-(benzyloxy)-		/	/	/	/	/
25	γ -eudesmol						/
26	Aristolene						/
27	2-Naphthalenecarboxylic acid, 8-ethenyl-3,4,4a,5,6,7,8,8a-octahydro-5-methylene-						/
28	longifolene						/

Previously, 3 compounds were identified that were similar to this study, namely, α-guaiene (Ishihara et al., 1992(a), α-bulnesene (Ishihara et al., 1992(a) and Wetwitayaklung, et al., 2009) and β-gurjunene (Wetwitayaklung, et al., 2009). The other sesquiterpene compounds were identified and reported for the first time in agarwood oil. However, these compounds have previously been identified in other plant oils. α-Copaene, for example, has been reported in volatile oils from leaf-buds of *Populus nigra* L.(Salicaceae) (Jerkovic and Mestalic, 2003. All the compounds identified using this method is summarized in Table 4.20.

Table 4.23: Odor characteristic and major compounds identified (Terry, et al., 1984).

Compound	**Odor characteristic(s)**
Gurjunene, a-guaiene	wood, balsamic
α -humulene, α -selinene	wood
Aromadendrene	wood
α-Copaene	wood, spice

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

In this experiment, the composition of essential oil from hydrodistillation and SFE method were compared by using GC-FID and GC-MS. In these studies, twenty one compounds essential oil were obtained using GC-FID and thirty one were obtained by GC-MS. Composition compound from GC-MS higher than GC-FID due to the method of analysis. Only three compounds were found to be common in both analyses, namely, Hexadecanoic acid, agarospirol and γ–eudesmol.

Regarding the results achieved in this research, supercritical fluid extraction and hydrodistillation had both the advantages and disadvantages for isolating the essential oil and the volatile compounds. It was observed that the yield of oil extracted by supercritical fluid extraction was much more than the same obtained from hydrodistillation. The SFE method offers many important advantages over hydrodistillation. SFE requires shorter extraction times (4h vs. 12h for hydrodistillation). Energy cost is rather higher for performing hydrodistillation than that required for reaching SFE conditions.The possibility of manipulating the composition of the oil, by changing the parameters of the extraction (pressure) is more attainable in SFE. We obtained a higher selectivity in SFE than by the hydrodistillation method.

5.2 RECOMMENDATION FOR FUTURE STUDY

The result in this study focus to determined comparison essential oil from *agrawood* by using two different method extraction. The best result can identified if have several guideline and improvement in experiment. The repeated extraction of essential oil should be employed in order to get the good result. Future research should further explore the potential of extraction method and analysis method.

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APPENDICES

A.1 : Stadard carbon for GC-FID analysis

Standard Carbon C7-C20

	A(C7-C11)	B (C12-C16)	C (C17-C20)	MIN	LOG
C7	3.27			3.27	0.515
C8	4.543			4.543	0.657
C9	6.742			6.742	0.829
C10	9.928			9.928	0.997
C11	13.823			13.823	1.141
C12		18.051		18.051	1.257
C13		22.301		22.301	1.348
C14		26.438		26.438	1.422
C15		30.403		30.403	1.483
C16		34.179		34.179	1.534
C17			37.807	37.807	1.578
C18			41.23	41.23	1.615
C19			44.493	44.493	1.648
C20			47.616	47.616	1.678

A.2: Technique seperation in Supercritical Fluid Extraction.

TECHNIQUE OF SEPARATION	SUPERCRITICAL FLUID EXTRACTION (SFE)

Pressure

Pressure regulator-maintains pressure upstream of the regulator by means of a spring, compressed air, or electronically driven valve

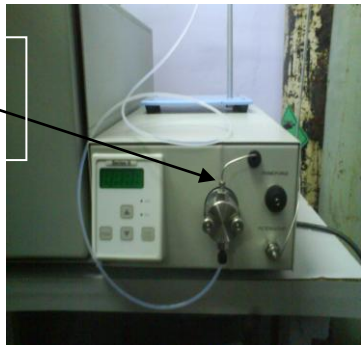
Vessel

Vessel-as place to put the sample gaharu

Gas Clynder

Clynder gas-supply carbon dioxide gases and compress air gases

Pump

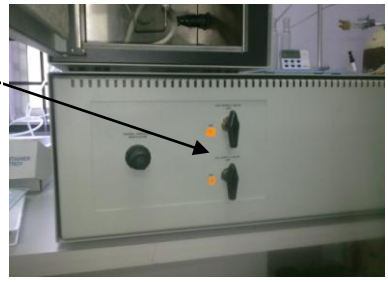


Pump supercritical-The solvent is pumped as a liquid as it is then almost incompressible**.**

oven

Oven- containing the extraction vessel, a restrictor to maintain a high pressure in the extraction line, and a trapping vessel.

CO_2 valve and air valve



To control CO_2 gases and compress air controller.

A.3 : Soaking of Gaharu

A.4 : Gaharu after grinding process