APPLICATION OF SOLID PHASE MICROEXTRACTION IN GAHARU ESSENTIAL OIL ANALYSIS

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APPLICATION OF SOLID PHASE MICROEXTRACTION IN GAHARU ESSENTIAL OIL ANALYSIS

PRAVINA ASHOK KUMAR

A thesis submitted in fulfillment of the
requirements for the award of the degree of
Bachelor of Chemical Engineering

Faculty of Chemical Engineering and Natural Resources
Universiti Malaysia Pahang

APRIL 2008

I declare that this thesis entitled "Application of Solid Phase Microextraction in Gaharu Essential Oil Analysis" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

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To my beloved parents, sisters and friends

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ABSTRAK

Minyak asli gaharu penting dalam mentafsirkan komponen utama yang menyebabkan minyak tersebut bernilai dan menganalisis kualiti kayu. Pengekstrakkan minyak dan penyediaan sampel amat mempengaruhi ketepatan dalam menganalisis minyak asli. Fasa pengektrakkan mikro pejal (SPME) adalah satu kaedah baru dalam penyediaan sampel yang menggunakan 'fiber' seperti span. Dalam kajian ini, suatu kaedah yang mudah, cepat dan tepat telah digunakan untuk mengetahui komponen di dalam kayu wangian *Aquilaria* spp dari famili Thymelaeaceae iaitu melalui 'headspace' fasa pengextrakkan mikro pejal (HS-SPME) dan diikuti oleh GC-MS. Minyak asli gaharu ini diektrakkan daripada kayu gred C melalui penyulingan air dan sampel minyak itu disediakan melalui dua jenis 'fiber' yang berlainan iaitu CAR/PDMS dan PDMS/DVB. Paramiter fiber tersebut juga dianalisiskan secara sistematik. Akhirnya, GC-MS diikuti oleh HS-SPME diaplikasikan di dalam kajian ini untuk mengetahui komponen di dalam minyak asli gaharu dengan dua jenis fiber yang berlainan. Kajian ini mecadangkan bahawa cara kerja eksperimen ini adalah cara yang amat berkesan untuk mengetahui komponen gaharu dan mengenal pasti kriteria fiber SPME.

ABSTRACT

The gaharu essential oil is important for the evaluation of the important compounds that makes it valuable and quality of the wood. Extraction of oil, sample preparation, concentration and isolation of analytes, greatly influences the reliable and accurate analysis of food. Solid phase microextraction (SPME) is a new sample preparation technique using fused silica fiber that is coated on the outside with an appropriate stationary phase. In this work, a simple, rapid and sensitive method was developed for the determination of compounds in fragrant wood *Aquilaria* spp. from the family of Thymelaeaceae, which was based on headspace solid phase microextraction (HS-SPME) followed by gas chromatography-mass spectrometry (GC-MS). The gaharu essential oil was extracted by hydroditillation and prepared by two different fibers, CAR/PDMS and PDMS/DVB. The extraction parameters of fiber coating were systemically analyzed. Finally, GC-MS following HS-SPME was applied to determination of compounds in gaharu essential oil with two different fibers. The experiment results suggest that the proposed method provided an alternative and novel approach to the study of components in gaharu and SPME fibers.

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Agarwood or gaharu is one of the rarest and precious woods on the planet, prized for its rich and wonderful fragrance. Gaharu is extremely rare and often difficult to obtain and its value is pegged at 1.5 times the worth of gold. It is sometimes referred to as 'liquid gold' or 'wood of the gods.' The genus, which belongs to the family of Thymelaeaceae, consists of 15 species (Chakrabarty *et al*. 1994). Gaharu is a resin deposited part of the trunk of two to four genera in the family of Thymelaeaceae which are *Aquilaria agallocha* and *Aquilaria malaccensis*. The wood is a production of the tree's immune response to fungal infection.

Table 1.1: Scientific Classification Of Gaharu (Source: www.wikipedia.org)

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Malvales
Family	Thymelaeacea
Genus	*Aquilaria*

As the international demand for gaharu increases, gaharu trees are becoming rare and difficult to find. According to the collectors, the non-infected trees are increasingly being felled to harvest just a few kilos of diseased wood and collection is taking place even in protected areas (http://www.ameinfo.com/46846.html).

Normal gaharu is nearly odorless until a fungus invades the wood. Gaharu is used primarily for medicine, perfume and incense. Gaharu woodchips are meant to be used as incense. When the gaharu is burned, it gives a heavenly incense smoke. It is beyond a pleasant smell and invades into lungs and entire body, taking total possession of the person. Smoke from the gaharu remedies nervous disorders such as neurosis and obsessive behaviour. The chemical composition of the gaharu scent depends not only on the *Aquilaria* species, but also on whether the essential oil or incense smoke is being considered. According to Roman Kaiser (2006), many investigations and enormous variety of sesquiterpenes are important to this scent.

Solid phase microextraction (SPME) , developed by Pawliszyn and co-workers in 1990, is a new sample preparation technique using fused silica fiber that is coated on the outside with an appropriate stationary phase (C. L. Arthur *et al*. 1990). Analyte in the sample is directly extracted and concentrated to the fiber coating. The method saves preparation time, solvent purchase and disposal cost, and can improve the detection limits (J. Pawliszyn, 1997). It has been routinely in combination with GC and GC-MS, and successfully applied to a wide variety of compounds, especially for the extraction of volatile and semi-volatile organic compounds from plants (H. L. Lord and J. Pawliszyn, 1998).

Grading gaharu is a subjective and complicated process based on size, colour, odor, shape and weight. The allocation of grades varies from country to country and from buyer to buyer. According to recent studies, it shows that gaharu are graded and valued based on their physical qualities only but not on their scientific criteria. Extraction of gaharu essential oil by varies fiber differs based on their characteristic of

their fiber. When a gaharu is extracted by SPME with different extraction method, the components in the oil vary for each method. Therefore a more extensive investigation should be initiated in order to thoroughly understand the compounds in the oil of different graded gaharu.

1.2 Objective

i. To study the components of the gaharu essential oil extracted from hydrodistillation.
ii. To identify the components in gaharu essential oil by two different solid phase microextraction fibers which are Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) and Carboxen/Polydimethylsiloxane (CAR/PDMS).

1.3 Problem Statements

i. Gaharu are graded into 5 different groups, super A, A, B, C and D. The wood is graded based on their physical properties. The physical properties of the wood vary from one country to another and from time to time. This gives the greatest impact on dividing the wood into the exact grade that it should belong to based on their physical properties. Supposedly, the wood should be graded based on their chemical properties to ensure that the analysis and researches on the wood are done with the same grade and gives accurate results.
ii. Components of the gaharu essential oil identified by solid phase microextraction (SPME) vary between each fiber. Each of the fibers has different properties based on their thickness and chemical properties. Followed by that, there are two types of method in extracting analytes with SPME such as headspace (HS-SPME) and direct immersion (DI-SPME).

Studies show that it is still unsure on the compounds that each fiber and method would identify.

1.4 Scopes of Research Work

i. To study the components gaharu grade C
ii. To study the effectiveness of solid phase microextraction (SPME)
iii. To compare the effectiveness of CAR/PDMS and PDMS/DVB fibers

CHAPTER 2

LITERATURE REVIEW

2.1 Essential Oil

Essential oil contains the true essence of the plant that was derived from. Essential oils are not the same as perfume oils or fragrance oils because artificially created fragrances contain artificial substances or are diluted with carrier oils and do not offer the caliber of therapeutic benefits that essential oils offer (Lu, K. C. 1983). Essential oils contain volatile aroma compounds from plants, which are called aromatic herbs or aromatic plants. Oils do not as a group needs to have any specific chemical properties in common.

Essential oils are generally extracted by distillation. Other processes include expression, or solvent extraction. They are used in perfumes, cosmetics and bath products, for flavoring food and drink, and for scenting incense and household cleaning products (www.wikipedia.com). Essential oils are derived from various sections of plants such as berries, seeds, wood, bark, rhizome, leaves, resin, flowers, peel and root. There are currently three types of plants which are derived from resin; myrrh, gaharu and frankincense.

2.2 Gaharu

Gaharu is the resinous wood that is derived from the *Aquilaria* tree. There are many names for this resinous wood, including agar, agarwood, aloeswood, kalamabak and eaglewood (Angela.B *et al*. 2003). It 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). Gaharu, from the family of Thymalaeaceae are well known for its unique fragrant and highly valuable non-timber products in Asian tropical forest. Odor of agarwood is complex and pleasing with few or no similar natural analogoes. Whereas the odor of the agarwood oil is extremely long lasting and this can be used to introduce a novel effect in men's fragrances.

As a results, gaharu and its essential oil holds a strong connection with religious and traditional significance around the world. The gaharu is traditionally used to produce incence in the Far East. Gaharu is also believed to have tonic and therapeutic properties (Burkill 1966, Okugawa *et al*., 1993) which can cure rheumatism, shortness of breath, general pains, diarrhea, asthma and a lot more. The essential oil extracted from lesser quality wood is used in the perfume industry. The uses gaharu has widened lately to include new products such as gaharu essence, soap and shampoo (Chakrabarty *et al*., 1994).

The genus *Aquilaria* of the family Thymelaeaceae consists of eight species which are distributed throughout India, China, Southeast Asia and the East Indies (Willis, 1955). Several species are known to produce gaharu and they are shown in Table 2.1. Of the 15 species in the genus *Aquilaria*, only *Aquilaria malaccensus* and *Aquilaria agallocha* have received significant attention in the last few decades (L.T.Ng *et al*., 1997).

Table 2.1: Aquilaria (Thymelaeaceae) and other species that form gaharu or agar (Source: A Review on Agar (Gaharu) Producing Aquilaria Species)

Species	**Country**	**Local Name for wood**	**Grade**
A. agallocha	India, Pakistan	Agaru, agar	High
A. crassna	Thailand	Aloeswood	High
A. malaccensis	Malaysia	Gaharu	Medium
A. hirta		Cendana	?
A. rostrala		?	?
A. moszkowskii	Indonesia (Sumatra)	?	Low
A.baillonii	Cambodia	Kalambak	High
A.crassna			
A. grandiflora	Hainan	Ch'end hsiang	High
Gonystylus banvanus	Borneo,Sumatra	Gaharu	Low
G. affinis	Malaysia	Gaharu	?
G. confusus			?

Works by Sunari (2002) have shown that gaharu is an aromatic olio-resin produced by tree when it is infected by fungus or mold, Phialophora parasitica. Formation of gaharu occurs in the trunk and roots of tree. As a response, the tree produces a resin high in volatile organic compounds that aids in suppressing or retarding fungal growth. In natural forest, only 7% of the trees are infected by the fungus. There are three different hypotheses regarding the gaharu formation. The formation is the result of pathological wounding or pathological and non-pathological processes (Ng *et al*., 1997). According to Ng *et al*. (1997), studies have not proven any of these hypotheses. Oldfield *et al*. (1998) stated that resin production is in response to fungal infection, whereas Heuveling van Beek concluded that it is in response to wounding. Another finding stated that the formation results from mechanical injury associated with the wood (Blanchette *pers. comm*. 2003). In brief, the tree has two response mechanisms to injury.

The first line of defence is for the phloem cells to produce callus growth over the injury. Next the formation of callus will be prevented when the tree produces resin as a chemical defence to the injury (B.Gunn *et al*., 2003). Other factors such as the age of the tree and differences in the tree caused by seasonal, environmental and genetic changes in *Aquilaria* species may also play an important role in gaharu formation (Ng *et al.*, 1997). The white spots or areas formed on the tree are the resins, which are shown in Figure 2.1.

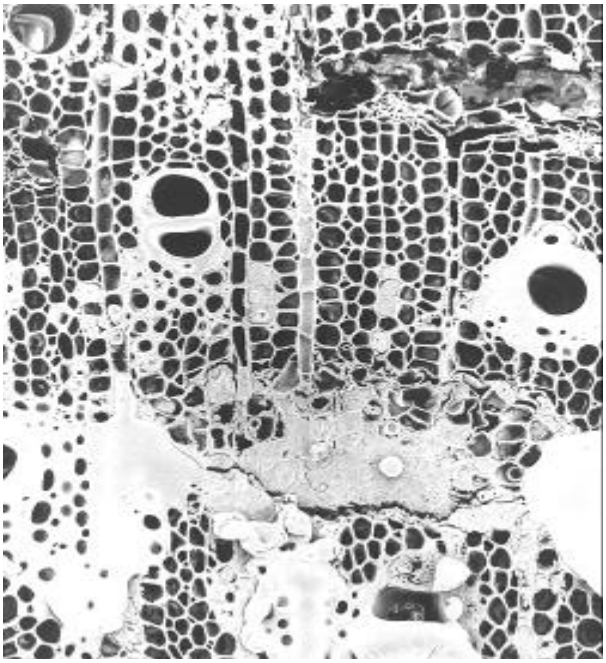


Figure 2.1: Cell structure within an *Aquilaria* tree (Source : Heart of the Matter : Agarwood use and trade and cites implementation for Aquilaria Malaccensis)

2.3 Grading and Prizing of Gaharu

Grading of gaharu is merely a complicated process because it is not a uniform product but instead possesses different characteristics. The gaharu derivatives such as oil are graded based on size, colour, fragrance strength and longevity, shape, weight, wood density, flammability, product purity and resin content. The grading differs from country

to country and buyers to buyers. Currently there are five grades of wood, super A, A, B, C and D as presented in Table 2.2.

Table 2.2: Guidelines for grading gaharu based on size, shape and weight of wood. (Source: Eaglewood in Papua New Guinea)

Grading on colour	Heavy irregular shape	Heavy regular shape	Light large pieces	Heavy thick chips
Black shiny	Super A	A	B	C
Mixture of dark black & chocolate brown	B	B	C	C
Mixed colour (pale black or chocolate brown)	C	C	C	C
Brown	D	D	D	D
Pale yellow or tan brown	D mostly rejected	D mostly rejected	D mostly rejected	D mostly rejected
White	Rejected	Rejected	Rejected	Rejected

Content of resin can be identified by burning the gaharu. Therefore, brown chips burns with strong flame, while the black gaharu for a shorter time before the flame dies and incense from the smoke lasts for a long period of time. Another way of separating high grade from low grade is to place the wood in water and the higher grades sinks, while the lower grades float (B.Gunn *et al*. (2003). Baruah *et al*. (1982) distinguished four grades of infected fragrant wood; the poorest grade being buff-colored and being exclusively used for distillation, better grades being used to make incense agar-battis.

According to Chaudhari (1993), grade A and B woods are mainly exported to Arab as incense; grade C extracted for superior oil and grade D are used for oil.

According to Heuveling van Beek and Philips (1999), gaharu oil which is obtained by the method of distillation is graded based on the quality of raw materials and the skill used in processing. Based on studies, it is said to be virtually impossible to find pure gaharu oil.

The retail prices of gaharu vary between countries depending on the quality. Chips a few centimeters long are likely to fetch a higher price per kg compared with larger pieces, even though their colour and odour may indicate a lower grade (B. Gunn *et al*. 2003). In Dubai, the lowest grade gaharu was traded at Dhs100 (about US$27) per kg in 1993, while the most expensive grade was reported to be priced at Dhs35000 (US$9598) per kg (L.T.Ng *et al*. 1997). In terms of gaharu oil, it is reported by Chakrabarty (1994) that the price ranges between Dhs50 and 800 per tola. The popular price is around Dhs600 per tola which is equal to US$14 per gram. Traders around the world have quoted prices for pure gaharu oil as high as USD30000/kg and grade two oil costs approximately USD 15000/kg (B.Gunn *et al*. 2003). In February 2001, the PNGFA introduced pricing guideline (Table 2.3).

Table 2.3: PNGFA guideline on the minimum prices (Source: RMAP Working Papers)

Grade of gaharu	**Value USD/kg**
Super A	560
A	420
B	280
C	140
D	14

2.4 Prospects of Gaharu in Malaysia

Today, gaharu is becoming more popular in Malaysia. *Aquilaria malaccensis* is well distributed throughout Peninsular Malaysia, except in Kedah and Perlis (Whitmore, 1972) and is known to produce medium quality grade gaharu (Burkill, 1966). There are three species of *Aquilaria* in Malaysia which are *A. malaccensis, A. hirta and A.rostrata* and the last two types are very less reported on (Whitmore, 1972).

Malaysia has a long history in the trade of gaharu. Majority of gaharu are exported and relatively small quantities of gaharu being used locally. The gaharu in the form of wood sections, flakes, chips, incense and occasionally powder are mainly traded in Malaysia (Yaacob, 1999). Research undertaken by the Forest Research Institute of Malaysia (FRIM) has identified five to seven grades of gaharu for Peninsular Malaysia (Angela, B. *et al*. 2003). There is at least one processing plant in Malaysia and owned by a Singaporean. The oil extracted through distillation is observed for sale in Singapore (Heaveling van Beek and Philips, 1999). Gaharu oil is being distilled illegally in Peninsular Malaysia and harvested and purchased by Cambodians (Dr C. Y. Shyun, 2000).

Chakrabarty *et al.* (1994) stated that the lowest grade of Malaysian agarwood could be obtained for USD19/kg in the Middle East. The high grades, normally reserved for exclusive buyers and are said to cost up to USD9589/kg. More expensive grades are also available and can sell for as much as USD27 400/kg. Ng *et al.* (1997) reported that, in 1991, south-east Asian countries exported approximately RM48.3million worth of gaharu to Saudi Arabia, of which approximately 26% was sourced from Malaysia.

According to James Compton and Akiko Ishihara (2007), 700 tonnes of gaharu in the international market are from the forestry of Malaysia and Indonesia. The 700 tonnes of gaharu are estimated to be about RM3.5 billion. As cited in Bernama.com (2007), currently the value of the wood in Malaysia is between RM5000 to RM100, 000 per

kilogram depending on the grade. Moreover, the value gaharu oil is ten times more than the value of wood.

Konsesi Utama Sdn. Bhd owns approximately 1.2 millions of Aquilaria tree between the age of 6 to 8 months and hundreds of trees at the age of 6 years and over. These trees are placed in an unoccupied wide land about 313 acre owned by the company in Pahang. Moreover, they have more than 3000 trees in a 33 acre of land that are being used for regeneration (Bernama.com 2007).

Villagers in Kelantan are into the business of collecting and processing gaharu for a long time. Distillation process to extract fragrant oil is carried out in the backyard under a zinc-roofed shack. Twenty one stainless steel vats are used for the distillation process that undergoes 96 hours of operation to extract oil. High quality gaharu costs RM10, 000 per kg and 12g bottle of oil is sold at between RM50 and RM200. Gaharu is supplied by native people and the remainders from the villagers. It costs from RM1 to RM50 per kg but there is no fixed price. Each vat that owned by the villagers produces 12gm of oil. It's quite hard to get the gaharu graded A and B because there are more buyers for it. In advance to the distillation process, gred C resin is chipped, dried, ground into powder and soaked for a week.

Gaharu collectors or buyers have to pay a royalty fee amounting to 10% of the raw material market price. 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 Chiew 2005).

2.5 Chemical Components of Gaharu Essential Oil

Based on a study, gaharu have been reported to contain sesquiterpenoids of eremophilane, spirovetivane, eudesmane, non-guaiane, guaiane and prezizaanetype, 2-(2-

phenylethyl)chromone derivatives and many more (Jun-ya Ueda *et al*. 2006). The first investigation on gaharu was done by Kafuku and Ichikawa (Shimada *et al.* 1982). Aroma from gaharu is produced by sesquiterpene alcohol.

According to Blanchette and Heavling van Beek (2006), three important naturally occurring aroma constituents of gaharu are –(+)- Jinkohol II, (+)- Karanone and (+)-Dihydrokaranone. According to Hashimoto *et al*. (1985), 2-[2-(4' methoxyphenyl)ethyl]chromone and 2-(2-phenylethyl)chromone, through pyrolysis at 150ºC produces 4-methoxybenzaldehyde and benzaldehyde respectively. These molecules are odorless at room temperature but produce a long lasting fragrance upon burning. In a structural investigation conducted on A. *malaccensis*, Shimada *et al*. (1986) identified six molecules, namely AH_1-AH_6. A. malaccensis is considered special because it has 2-phenylethyl which has not been detected in normal tissues (Ng, L. T. *et al*. 1996).

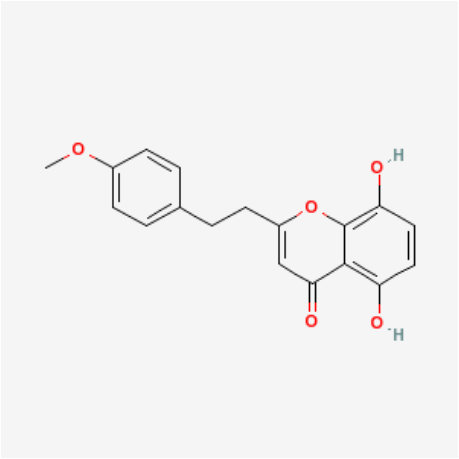
Differences in chemical components also noted between the best and lesser quality gaharu are shown in Table 2.4. Chemical profile for each grade is different. Gas chromatograms showed similar gas chromatography profile suggesting a region of peaks with retention times ranging from 28.0 to 42.0 min to be indicative of gaharu presence (Chang *et al.,* 2002).

Table 2.4: Differences in chemical components between best and lesser quality gaharu (Source: A Review on Agar (Gaharu) Producing *Aquilaria* Species)

Grade of gaharu	**Compounds indentified**	**Remark**
Best	Sesquiterpenes : (-)-guaia-1(10),11-dien-15-al (-)-selina-3,11-dien-9-one (+)-selina-3,11-dien-9-ol	Absent from lesser quality gaharu
Lesser	Kusunol Dihydrokaranone Karanone Oxo-agarospirol	Present in considerable amounts

2.5.1 Chemical Structure of Gaharu Components

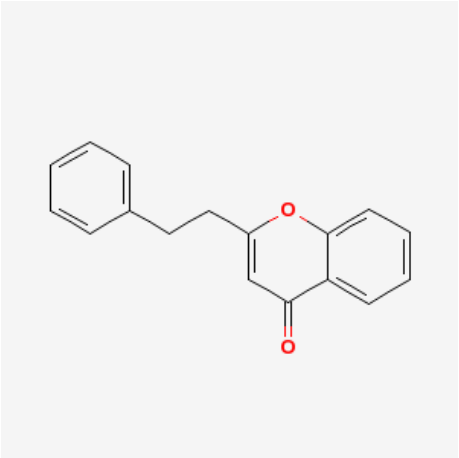
Chemical component in gaharu determines the characteristics or qualities of gaharu. Figures below show some chemical component structures in gaharu.



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

IUPAC: 5,8-dihydroxy-2-[2-(4-methoxyphenyl)ethyl]chromen-4-one

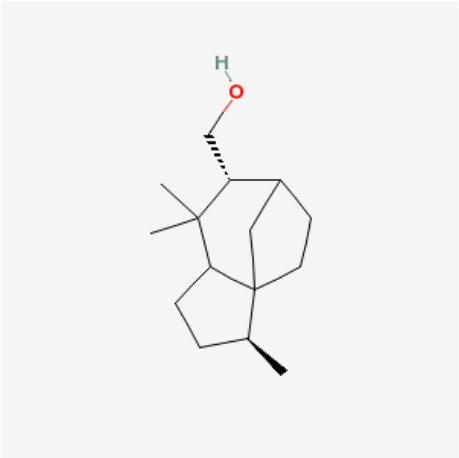
MW: 312.317



Flindersiachromone

IUPAC: 2-(2-cyclohexylethyl)chromen-4-one

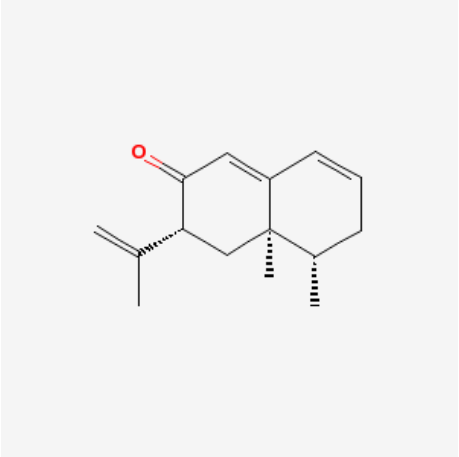
MW: 250.292



Jinkohol

MF : $C_{15}H_{26}O$

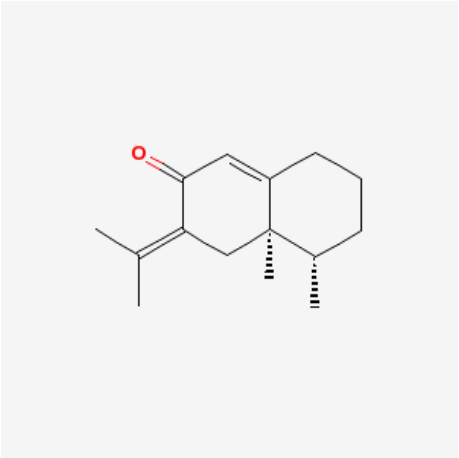
MW : 222.366



Karanone

IUPAC: (3R,4aR,5S)-4a,5-dimethyl-3-prop-1-en-2-yl-3,4,5,6 tetrahydronaphthalen-2-one

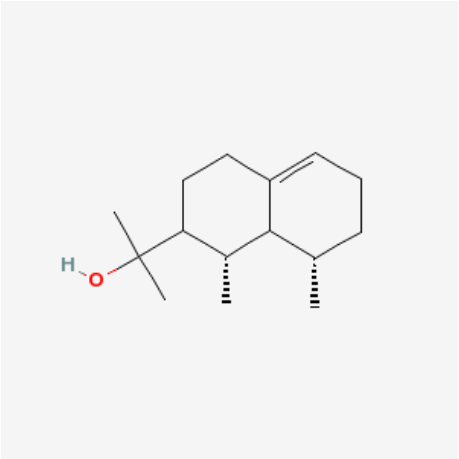
MW: 216.319



Dihydrokaranone

IUPAC: (4aR,5S)-4a,5-dimethyl-3-propan-2-ylidene-5,6,7,8-tetrahydro-4H-naphthalen-2-one

MW: 218.335



Kusunol

IUPAC: 2-[(1S,8S)-1,8-dimethyl-1,2,3,4,6,7,8,8a-octahydronaphthalen-2 yl]propan-2-ol

MW: 222.366

(Source:http://pubchem.ncbi.nlm.nih.gov)

2.6 Extraction of Essential Oil

Essential oil is extracted through various methods and the method used is normally dependant on the type of botanical material is being used (http://www.essentialoils.co.za/extraction-methods.htm). The type of plant material determines which method will be used to obtain the essential oil. But there are exceptions, for instance, carbon dioxide is a great way to extract most oils but the cost of it plays a role also.

Extraction of essential oil is one of the key points which determine the quality of the oil that is used. If a wrong or wrongly executed extraction takes place, it can damage the oil and alter the chemical signature of the essential oil (http://www.essentialoils.co.za/extraction-methods.htm). Based on research, there are few methods that are being used to extract essential oil which are distillation, expression and solvent extraction.

A majority of essential oils are produced by distillation. Distillation converts the volatile liquid into vapor and then condenses the vapor back into a liquid. There are different processes in distillation; however, in all of them, water is heated to produce steam which carries the most volatile chemicals of the aromatic material with it. The steam is then chilled in a condenser and the resulting distillate is collected. The essential oil will normally float on top of the hydrosol, the distilled water and may be separated off (Gilbert *et al*, .2002).

Followed by that, there are few types of distillation methods such steam distillation, hydrodistillation and water and steam distillation. But most popular method for extraction in old times is hydro distillation, but as technological advances is made more efficient and economical methods being developed.

2.6.1 Hydrodistillation

Hydro distillation is used in the manufacture and extraction of essential oil. This is the simplest and usually the cheapest process of distillation. Hydrodistillation works best for very though materials like roots, wood or nuts. From Ruthven, D. M. *et al*. (1997), the best available technique in extracting the gaharu essential oil is by using hydrodistillation. The main advantages of this method are that less steam is used; shorter processing time and higher oil yield (Riera *et al*., 2004).

The plant material is immersed in water and boiled. The steam and oil vapor is condensed and the oil is separated from the water. The hot water is helped to release the aromatic molecules from the plant material since the hot water forces to break the pockets in which the oil is kept in plant material.

The temperature should be about 100ºC. Care needs to be taken in preventing the plant material being damaged by contacting the overheated of still wall. The pressure in the still should be atmospheric. Time of distillation depends on the plant material being processed.

2.7 Sample Preparation Technique

2.7.1 Solid Phase Microextraction Device

Solid Phase Microextraction (SPME) is an innovative, solvent free technology that is fast, economical, and versatile. It consists of fiber holder and fiber assembly with built-in fiber inside the needle which looks like a modified syringe which can be moved between two positions, inside and outside the needle (Figure 2.2).

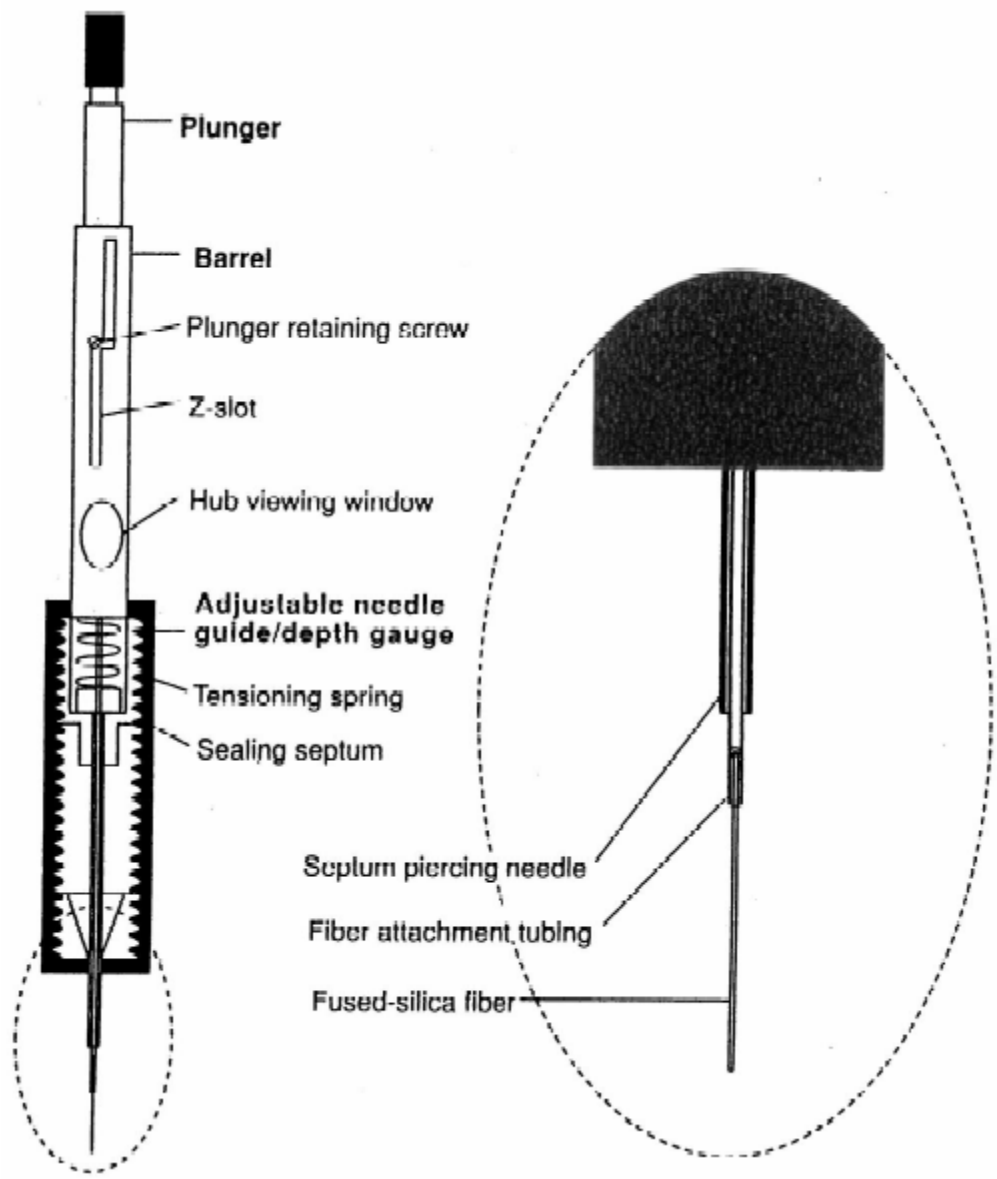


Figure 2.2: Commercial SPME (Source: Application Solid Phase Microextraction in Food Analysis)

The fiber holder consists of a spring-loaded plunger, a stainless steel barrel and an adjustable depth gauge with needle. According to Ulrich (2000), SPME is based on a modified syringe which contains stainless steel microtubing within its syringe needle. This microtubing has an about 1-cm fused-silica fiber tip which is coated with an organic polymer. It is designed to be used with reusable and replaceable fiber. Extraction and enrichment of the analyte is completed by the coating on the outside the syringe needle.

Desorption of the analyte and transfer to the capillary is performed after again moving the fiber to the position outside the syringe (S.Ulrich, 2000). This procedure can be repeated with one device several times (Figure 2.3). According to Katoaka (2000), the fused silica fiber is coated with a relatively thin film of several polymeric stationary phases. The organic analytes are concentrated on the surface of the film that acts like a sponge during absorption or adsorption from the sample matrix.

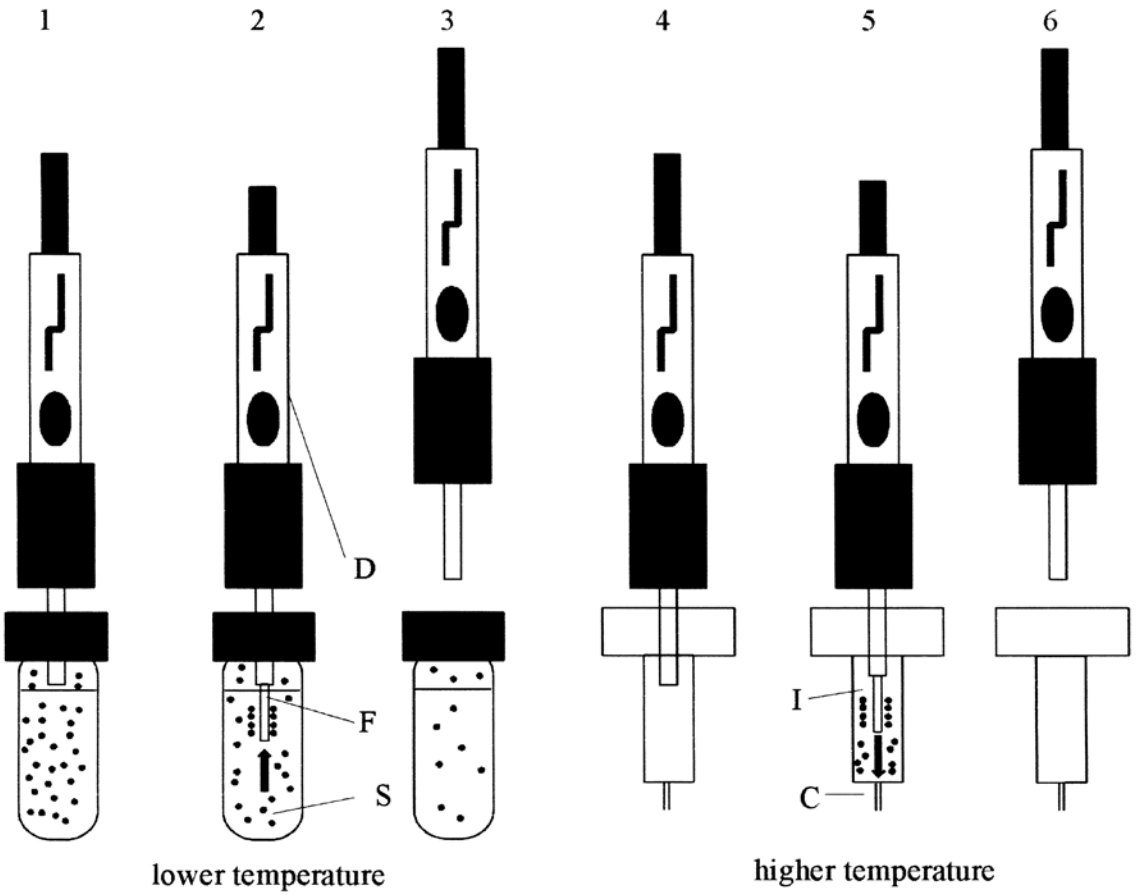


Figure 2.3: The principle of SPME: 1=introduction of syringe needle of the SPME device (D) into the sample vial and close to the sample (S), 2=moving the fiber (F) into the position outside the syringe and into the sample (extraction), 3=moving the fiber back into the syringe needle and subsequent transfer of the device to the GC injector port (I) and capillary head (C), 4=penetration of the septum with syringe needle, 5=moving the fiber into the position outside the syringe (desorption), 6=moving the fiber back into the syringe needle and withdrawing the syringe needle (S. Ulrich, 2000).

There are seven kinds of fibers available commercially. Figure 2.4 shows the seven types of fibers that immobilizes stationary phase by non-bonding, bonding, partial crosslinking or high crosslinking with various thickness. Non-bonded phases are stable with some water miscible organic solvents but slight swelling may occur when used with non-polar solvents. Bonded phases are stable with all organic solvents except for some non-polar solvents. Partially crosslinked phases are stable in most water miscible organic solvents and non-polar solvents. Highly crosslinked phases are equivalent to partially crosslinked phases except that some bonding to core has occurred (H. Kataoka *et al*. 2000).

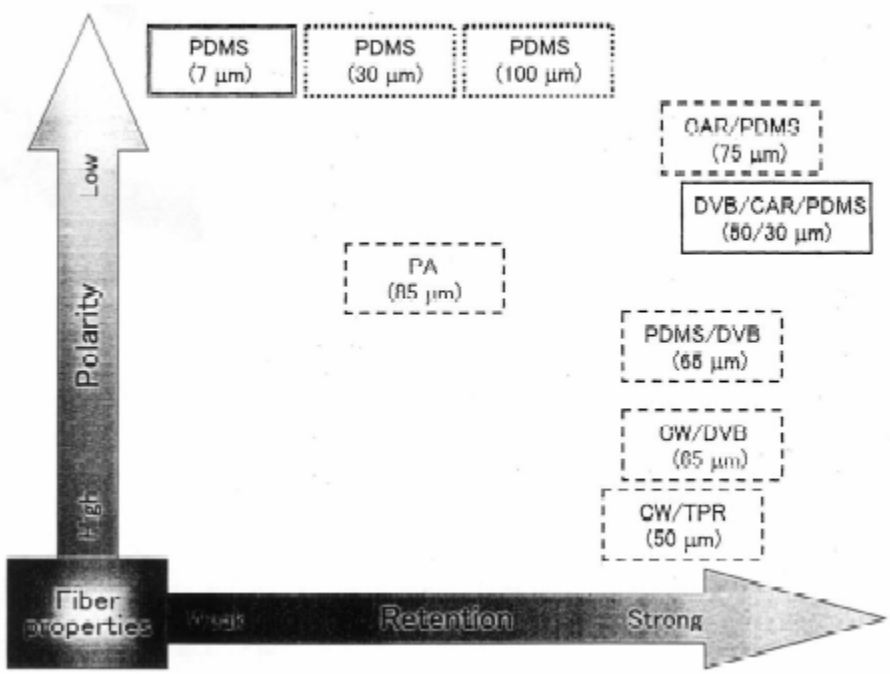


Figure 2.4: Properties of commercially available SPME fibers. Bonded, non-bonded, partially crosslinked, highly crosslinked. (Source: Application Solid Phase Microextraction in Food Analysis)

2.7.2 Solid Phase Microextraction Process

2.7.2.1 Fiber of Solid-Phase Microextraction

The sample is placed in a vial, which is sealed with a septum-type cap. The fiber should be cleaned before analyzing any sample in order to remove contaminants which give a high background in the chromatogram. Cleaning can be done by inserting the fiber in an auxiliary injection port or a syringe cleaner (H. Kataoka *et al*. 2000). When the SPME needle pierces the septum and the fiber is extended through the needle into the sample, the target analytes partition from the sample matrix into the stationary phase.

There are two types of fiber SPME techniques which can be used to extract analytes, headspace (HS)-SPME and direct immersion (DI)-SPME. In (HS)-SPME, the fiber is exposed in the vapor phase above gaseous, liquid or solid sample. In (DI)-SPME, the fiber is directly immersed in liquid samples. HS- and DI-SPME techniques can be used in combination with any GC-MS (H. Kataoka *et al*. 2000).

For the GC and GC-MS analysis of volatile compounds, fiber HS-SPME is a more appropriate sampling mode (H. Kataoka *et al*. 2000). A HS method should be applied whenever possible in SPME of body fluids (S. Ulrich, 2000). In this SPME technique, the fiber is placed in the vapor phase of the liquid and cannot have any contact with the sample. This gives a longer lifetime (H. Kataoka *et al*. 2000). The outstanding advantages HS-SPME the prevention of contamination of the surface of the fiber with organic polymers. There will be no diffusion barrier of clotted protein and no burning-in of adsorbed organic material is possible during desorption in the hot injector (S.Ulrich, 2000). Furthermore, the transfer of fibers to the gas chromatography and desorption should be performed immediately after extraction because of the high vapor pressure of analytes also in the coating and the risk of loss of analytes during storage of the loaded fiber (S. Ulrich, 2000).

On the other hand, in the DI-SPME sampling mode, the fiber is inserted into the sample directly and this decreases the lifetime of the fiber. This decrease is caused by the influence of the addition of salts with supersaturation, pH adjustment or coexisting compounds of the complex matix (H. Kataoka *et al*. 2000). Another problem that relates to the lifetime of both the techniques is the high concentration of the alcoholic beverages which interfere with the extraction of the analytes.

As a rule of thumb analytes with a molecular mass below 200 g/mol and without groups forming hydrogen bonds, i.e., NRH groups, NH groups and OH groups are suitable for HS- and at various SPME because they are likely to have a high vapor pressure. Direct SPME should only be tried if HS-SPME failed to give sufficient peak areas (S. Ulrich, 2000). The fibers should be carefully handled because they are fragile and can easily be broken, and the fiber coating can be damaged during insertion.

After a suitable extraction time, the fiber is withdrawn into the needle; the needle is removed from the septum and is then inserted directly into the injection port of the GC-MS. Desorption of analyte from the fiber coating is performed by heating the fiber in the injection port if a GC or GC-MS is used.

2.7.3 Selection of Fiber Coatings

As shown in Figure 2.4, several types of coating fibers are currently available for the extraction of analytes. They consist of one or two polymers; PDMS, PA, Carboxen–PDMS, PDMS–polydivinylbenzene and Carbowax–DVB, for example. The affinity of the fiber for an analyte depends on the principle of ‘like dissolves like’ and coating fibers having different properties or thickness are selected in accordance with different compounds.

Works by Kataoka (2000) have shown that non-polar polydimethylsiloxane (PDMS) fiber is preferred for the extraction of non-polar analytes such as many volatile flavor compounds. PDMS is able to withstand high injector temperatures, up to about 300ºC. The coatings with a phase of DVB consist of porous particles of DVB which are held together either by PDMS or Carbowax as glue (S.Ulrich, 2000). Alternatively, the DVB phase is a template resin in another coating (S. Ulrich, 2000).

In general, volatile compounds require a thick polymer coat and a thin coat is effective for semi-volatile compounds. Furthermore, fibers coated thick films of fiber take longer time to achieve equilibrium but might provide higher sensitivity due to the large amount of the analytes that can be extracted (H. Kataoka, 2000). The thicknesses of the usual coatings are 7µm, 30 µm and 100 µm for PDMS, 85 µm for PA, 75 µm for Carboxen–PDMS, 65 µm for PDMS–DVB and Carbowax–DVB. The 7-µm PDMS coating is a bonded phase and the 30-µm and 100-µm PDMS coatings are nonbonded phases (S. Ulrich, 2000). Non-polar compounds are detectable at the thickness of µg/L level, while polar compounds could be detected at the mg/L level only (F. Augusto, 2003).

Polyacrylate (PA) is a more polar fiber which is preferred for the extraction of more-polar analytes, especially phenols and alcohols. Mixed coating fibers, containing divinylbenzene (DVB) copolymers, templated resin (TPR) or Carboxen (CAR) increase retention capacity because the effect of adsorption and distribution to the stationary phase. PDMS-DVB fiber can be used for the extraction of volatile low molecular mass and polar analytes. CAR-PDMS fiber shows better extraction efficiency than a 100 µm PDMS fiber and similar fibers but it is a poorer and equilibrium more time consuming (H. Kataoka, 2000).

2.8 Analyzing Equipments

Oil analysis is important of the evaluation of components and their characteristics. Therefore, it is important to know the typical chromatographic pattern of the smoke and modified pattern during the changes in the method of experiment. Using highly efficient instruments such as gas chromatography (GC) and their combination with mass spectrometry (MS) have been developed for oil analysis.

In general, the analytical method involves processes such as sampling, sample preparation, separation, detection and data analysis. More than 80% of the analysis time is spent on sampling and sample preparation steps such as extraction, concentration, fractionation and isolation of analytes. Therefore, the choice of an appropriate sample preparation method greatly influences the reliable and accurate analysis of smoke.

GC and GC-MS are successfully applied to wide variety of compounds, especially for the extraction of volatile and semivolatile organic compounds from environmental, biological and chemical samples.

2.8.1 Gas Chromatography

Gas chromatography is specifically known as gas-liquid chromatography. Figure 2.5 shows the schematic diagram of gas chromatography. It involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.

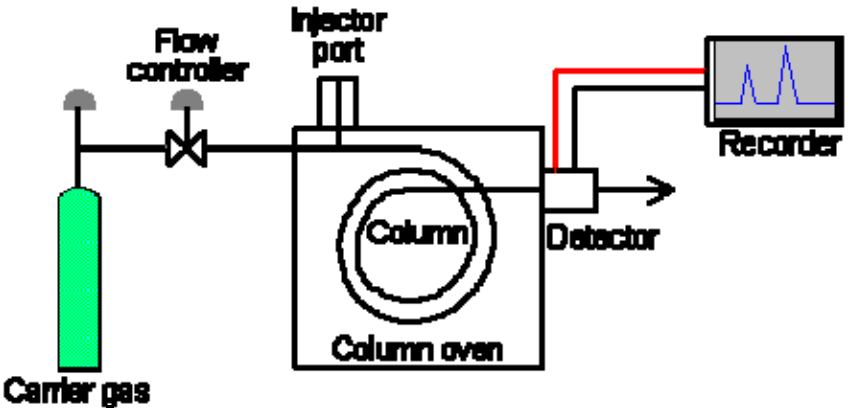


Figure 2.5 Schematic diagram of gas chromatography (Source: www.gaschromatography.com)

The carrier gas in the gas chromatography must be chemically inert. Gases such as nitrogen, helium, argon and carbon dioxide are used commonly as carrier gas. The carrier gasses are chosen based on the type pf detector used. The carrier gas system contains a molecular sieve to remove water and other impurities in the sample.

The sample that is being analyzed should not be too large for optimum column efficiency. Slow injection of large samples can cause band broadening and loss of resolution. Most commonly, micro syringe is used to inject sample through a rubber septum into the head of the column. The temperature of the sample port is usually about 50°C higher than the boiling point of the least volatile component of the sample. The sample size varies between different types of columns. For packed columns, sample size ranges from tenths of a micro liter up to 20 micro liters. Capillary columns, on the other hand, need much less sample, typically around 10^{-3} μL. For capillary GC, split or splitless injection is used.

There are two general types of column, packed and capillary. Cappilary column has two types of it, which are wall-coated open tubular (WCOT) and support coated open tubular (SCOT). SCOT columns are generally less effiecient than WCOT columns. Cappilary column are more efficient than packed columns.

There are many detectors which can be used in gas chromatography. Different detectors will give different types of selectivity. A non-selective detector responds to all compounds except the carrier gas, a selective detector responds to a range of compounds with a common physical or chemical property and a specific detector responds to a single chemical compound.

GC instrument uses a detector to measure the different compounds as they emerge from the column. Among the available detectors are the argon ionization detector, flame ionization detector, flame emission detector, cross section detector, thermal conductivity detector, and the electron capture detector. Choosing the proper detector depends upon the use.

The amount of time that a compound is retained in the GC column is known as the retention time. The retention time is measured from the sample injection until the compound elutes from the column. The retention time is helpful in differentiating the identity of a compound. If two samples do not have equal retention times, those samples are not the same substance. However, identical retention times for two samples only indicate a possibility that the samples are the same substance.

2.8.2 Gas Chromatography Mass Spectrometry

Gas chromatography and mass spectrometry make an effective combination for chemical analysis. The GC instrument is a reliable analytical instrument and effectively separates compounds into various components. Whereas, the MS instrument provides specific results but produces uncertain qualitative results. When an analyst uses the GC instrument to separate compounds before analysis with an MS instrument, a complementary relationship exists. Figure 2.6 shows the combination of GC and MS.

GC/MS analysis, where the effluent to the GC instrument is the feed to the MS instrument, is in wide use for confirmation testing of substances.

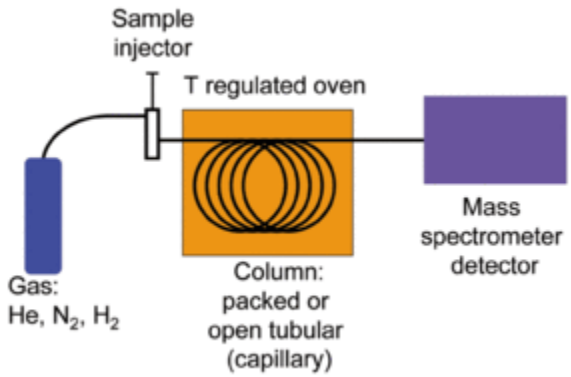


Figure 2.6: Schematic diagram of GC-MS (www.wikipedia.com)

The sample injected into gas chromatography goes under process and the molecules take different amounts of time or known as retention time to come out of the instrument. Elute from gas chromotography goes into the mass spectrometer and it captures, ionizes and detect the molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone.

The most common type of mass spectrometer (MS) associated with a gas chromatography (GC) is the quadruple mass spectrometer. Another relatively common detector is the ion trap mass spectrometer. After the molecules travel the length of the column, pass through the transfer line and enter into the mass spectrometer they are ionized by various methods such as electron and chemical ionization. GC-MS are used in various fields, medicine, astrochemistry, food, beverage and perfume analysis, environmental monitoring and cleanup, law enforcement and criminal forensics.

CHAPTER 3

METHODOLOGY

3.1 Introduction

The source of plant used in this study is gaharu or identified as *Aquilaria malaccensis*. Gaharu which are used in this experiment is from Kelantan and bought through local gaharu trade entrepreneur by middle man at the cost of RM100 per kilogram. Gaharu graded C will be used to extract as much as possible of essential oil with low cost of operating process. They are few processes that a gaharu should go through before its oil is being analyzed. Flow diagram 3.1 shows the flow of analyzing process.

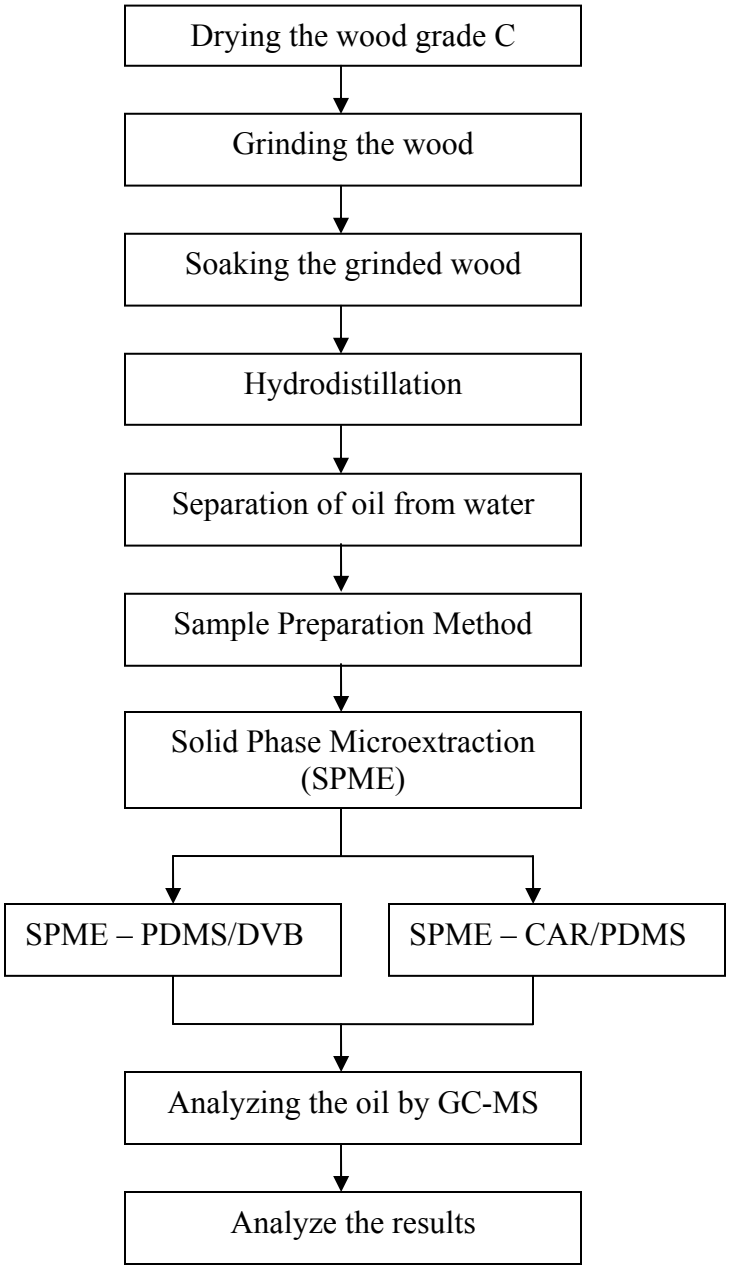


Figure 3.1 Flow diagram of the analyzing process

3.2 Drying

Gaharu wood has been cut into small pieces to increase surface area of gaharu chips for easier drying process. 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 wood was dried in oven at 60ºC until there is no changes on the humidity level. The purpose of this drying process is to avoid any blockade in grinding.

3.3 Grinding

The pieces of dry gaharu wood are grounded into sawdust with the size of 1mm. The gaharu woods which are bigger in size after grinding were grinded once again to achieve the size of 1mm. The large trunk of gaharu need to be chopped to a smaller size and before it can be grinded. In extraction process, the rate of extraction increases when the area of contact between the solvent and solid is high. So, higher the surface area of gaharu sawdust, more essential oil can be extracted. Once the grinding is completed, the sawdust is dried again in an oven at 60ºC for about two to three hours.

3.4 Soaking

Before the extraction process, grinded gaharu was soaked in water. The ratio of gaharu to water is 1:7 (Dong-ping et al., 1999) for a period of three to seven days in order to break down the parenchymatous and oil glands (Chang et al., 2002). Seven days was chosen in order to maximize the soaking effect. The amount of gaharu sawdust used in this experiment is 500 gram and amount of water which is about 3500 ml.

3.5 Hydrodistillation

The equipment of hydrodistillation process was set up. Then, the mixture of 500 gram sawdust of gaharu wood and 3500 ml of water was put into the flask of distillation unit. A boiling chip was inserted in the three neck flask. This is to ensure the bumping process that occurred when water is boiled did not affect the distillation process.

Followed by that, the heating mantel and re-circulating cooler were switched on. The temperature was set at boiling point of water which should be below than 100^0C for the whole experiment. Aluminium foil was wrapped all over the apparatus to make sure there is no heat loss occurs. The extraction process runs for three hours. The first sample of essential oil occurs after three hours of experiment. The essential oil present in the flask vaporizes.

Steam and essential oil vapors are passed through a condenser. The condensate, which has a mixture of water and essential oil, is collected in a receiving flask. At the receiving flask the layer of essential oil is decanted and collected into sample bottle.

3.6 Sample Preparation Method

3.6.1 Solid Phase Microextraction

In the headspace extraction (HS-SPME) process, the parameters of fiber coating can affect the extraction efficiency of SPME (L. Dong *et al*. 2007). Headspace extraction of gaharu essential oil was performed with two types of fiber which are Polydimethylsiloxane/Divinylbenzene(PDMS/DVB) and Carboxen/Polydimethylsiloxane (CAR/PDMS). The fiber thickness of PDMS/DVB is 65µm and CAR/PDMS is 75µm. The polarity and the retention of CAR/PDMS are higher than the PDMS/DVB. Both the

fiber used in this experiment is partially crosslinked and stable in most water-miscible organic solvents.

A 1.5 ± 0.01 g gaharu essential oil sample was weighted into a 4 ml vial. The SPME fiber with PDMS/DVB and CAR/PDMS were inserted into the headspace of the vial; the volatiles were then adsorbed on the fiber. The SPME fibers was placed in a manual fiber holder and exposed to the headspace of the sample for 15 minutes at a depth of 1 cm as shown in Figure 3.2.

Figure 3.2 Sample Preparation Method

3.7 Analyzing the sample

3.7.1 Gas Chromatography-Mass Spectroscopy

Sample in the coating of the SPME fiber are desorbed from the fiber in the heated gas chromatography – mass spectrometry (GC-MS) injection port. A GC-MS analysis of gaharu essential oil was performed employing a Agilent Technologies gas chromatography (Model 5975C), a printer plotter and an electronic integrator. It uses a bonded phase fused silica capillary column BP-1 with a 30m length, 250µm internal diameter and 0.25µm of film thickness coated with polydimethylsiloxane. Helium at a flow rate of 22.422 ml/min and vacuum inlet pressure is the carrier gas employed. The initial column temperature was 50°C and a inlet temperature increases of 10°C/min was programmed into each 22 minutes run with a final hold time of 3 minutes. Injector and detector are maintained at 250°C and 300°C, respectively.

3.7.2 Identification of Compounds

Essential oil components are identified by comparing the retention times of the chromatogram peaks with the reference compounds run under identical conditions, by comparison of retention indices with literature data, peak enrichment on co-injection of authentic compounds and comparison of mass spectra of the peaks with the standard compounds reported in literature. Peak area and retention times are measured by the electronic integrator.

CHAPTER 4

RESULTS AND DISCUSSIONS

Headspace (HS) SPME can avoid the interference with sample matrices and prevent from contamination of fiber in oil samples. So, the headspace mode of SPME was used in this work. In the proposed method, the gaharu essential oil samples were headspace extracted by two types of SPME fiber and then desorbed at GC-MS injector. The analytical step in this work is conventional GC/MS analysis of gaharu essential oil. Due to the large numbers of analytes was trapped on the fiber; the analytical column may either have insufficient resolving power or may be easily overloaded by larger abundance peaks resulting in serious peak asymmetry. This study is intended to provide qualitative comparisons of the compositions of the samples investigated.

4.1 Optimization of HS-SPME Parameters

Selection of the optimal coating is essential in the HS-SPME process; two different fibers of Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) and Carboxen/Polydimethylsiloxane (CAR/PDMS) were used for the headspace extraction of gaharu essential oil. Headspace extraction was performed for 15 minutes. The extracted analytes were desorbed and analyzed by GC-MS. The same amount of sample was extracted for both the fibers. The chromatograms of gaharu essential oil at different fibers are shown in Figure 4.1 and 4.2.

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Operator    :
Acquired    : 15 Jan 2008  16:17     using AcqMethod GAHARU.M
Instrument :    GCMSD
Sample Name:
Misc Info  :
Vial Number: 1
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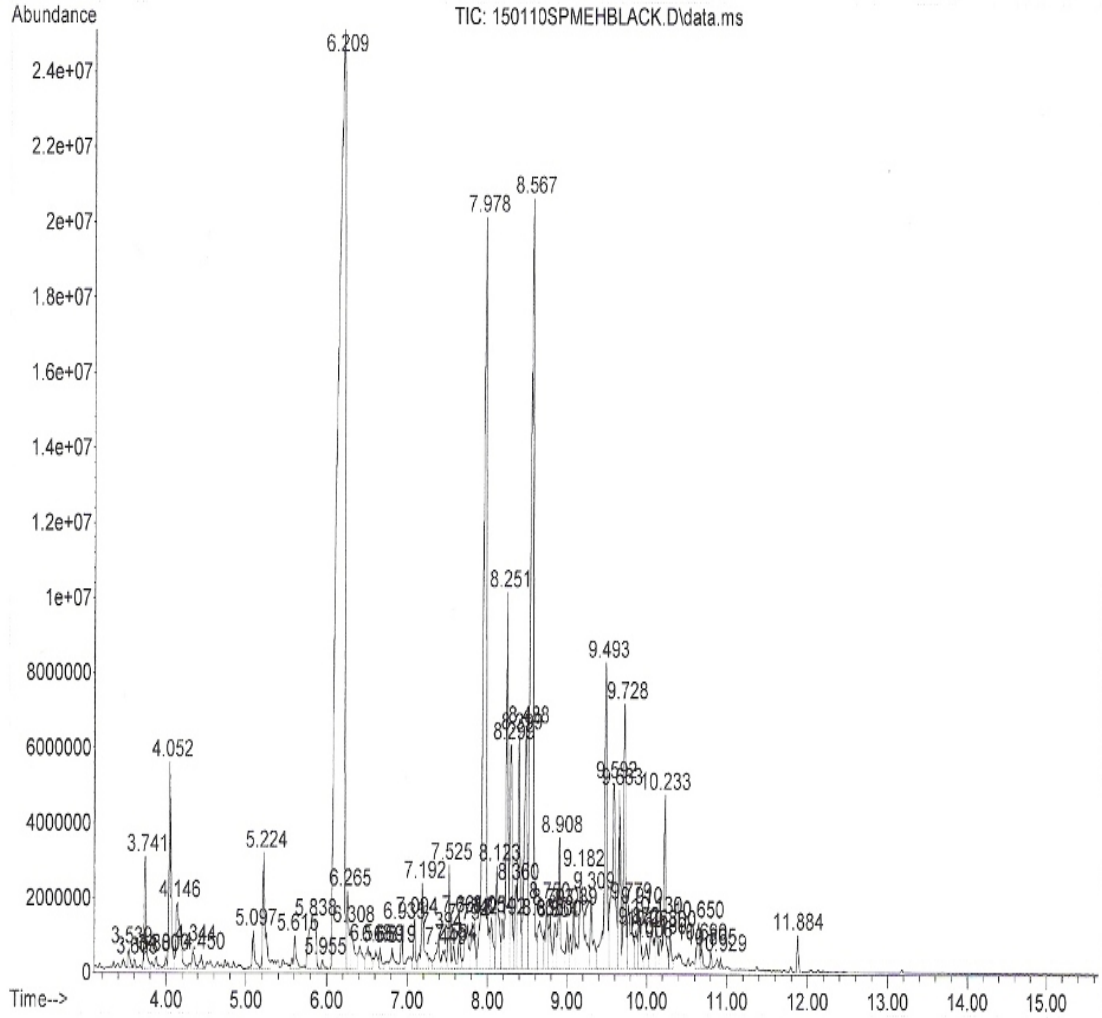


Figure 4.1: Chromotogram of gaharu essential oil with CAR/PDMS

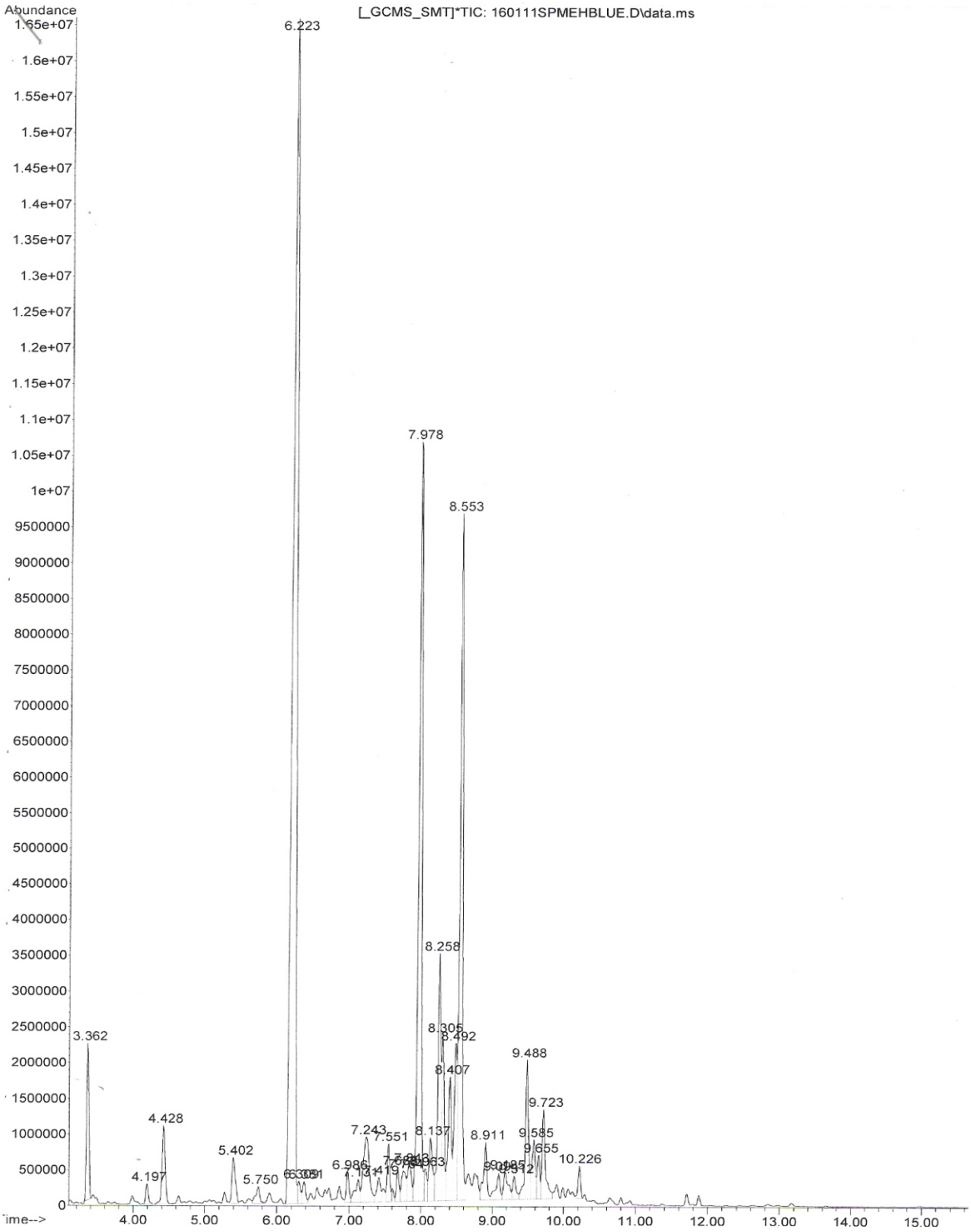


Figure 4.2: Chromotogram of gaharu essential oil with PDMS/DVB

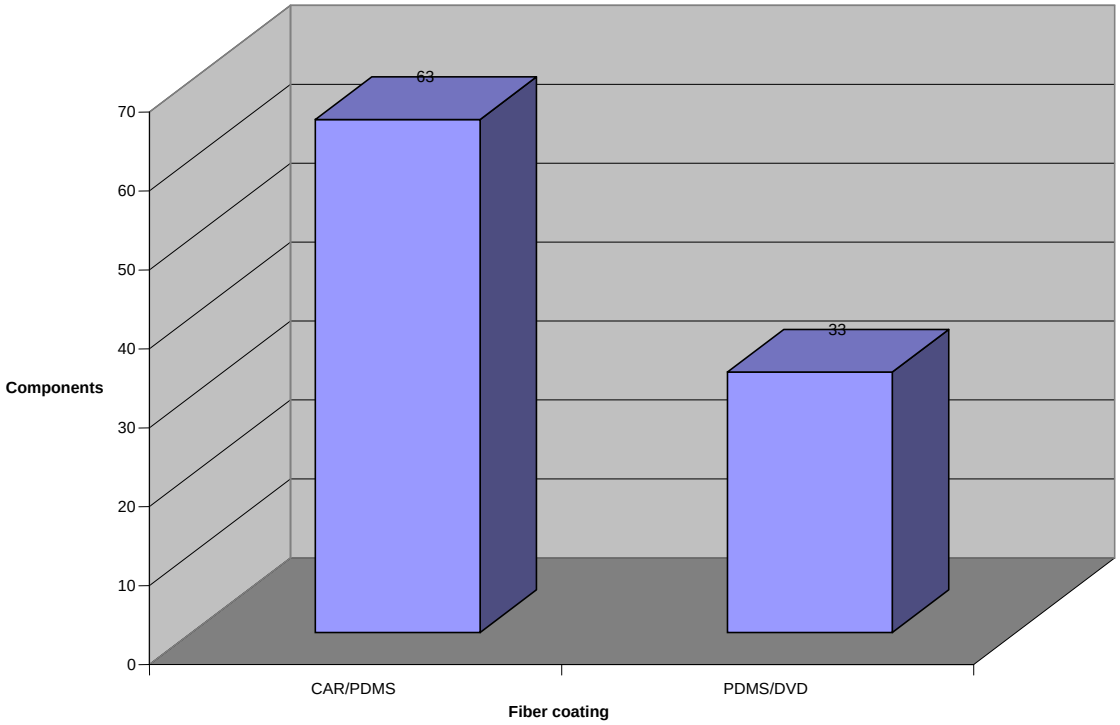


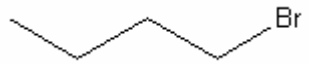
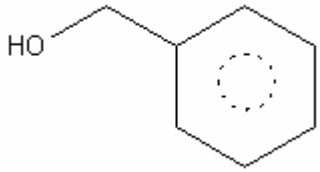
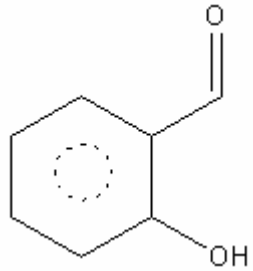
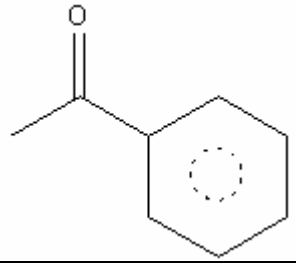
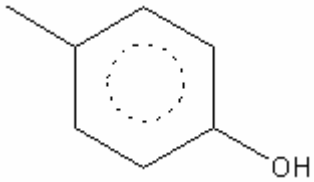
Figure 4.3: The amount of gaharu essential oil components detected by each fiber

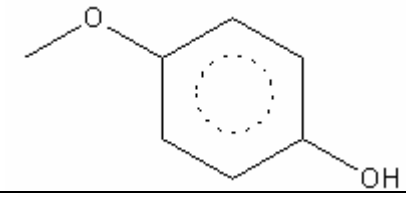
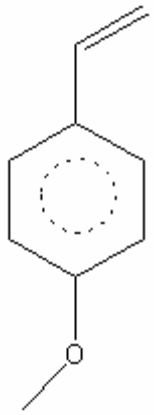
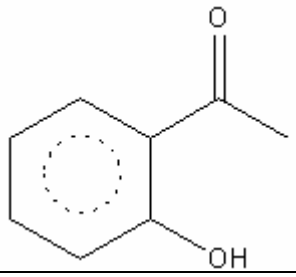
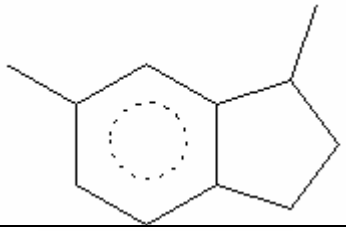
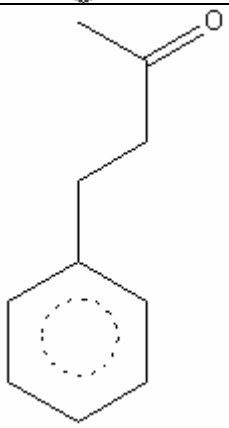
Analysis of the chromatogram from GC-MS showed the amount of components extracted by each fiber (Figure 4.3). As seen from figure 4.3, among the two fibers, the CAR/PDMS fiber has the best extraction efficiency. Although both the fibers are partially crosslinked, they differ by their thickness of the fiber. The thickness of PDMS/DVB fiber is 65µm which is slightly lower than the CAR/PDMS fiber. Followed by that, it shows that the thicker fiber gives higher sensitivity in extracting large amount of components as stated by H. Kataoka (2000). The immobilization of the fiber by partially crosslinked does not differentiate the fibers in extraction of analytes because the gaharu essential oil which was used as a sample is a water-miscible organic solvent.

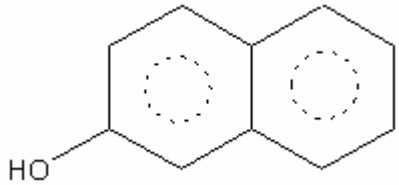
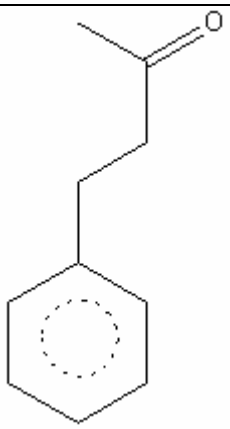
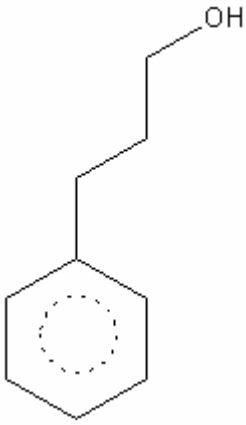
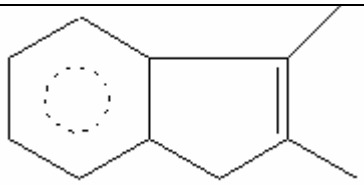
4.2 Identification of Components of Gaharu Essential Oil via SPME GC-MS

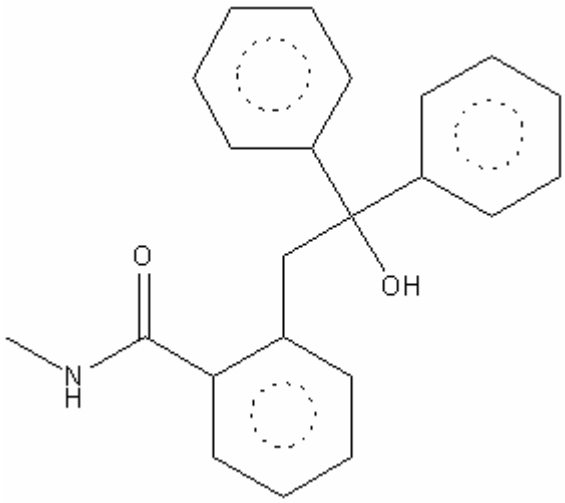
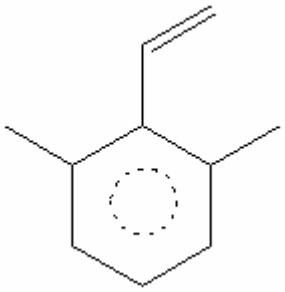
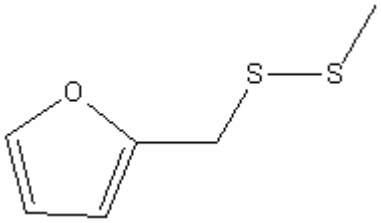
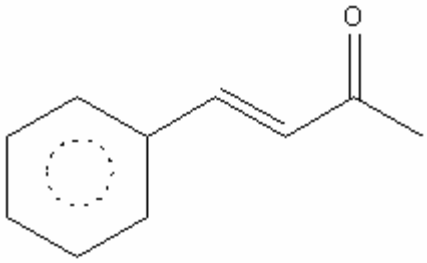
A total of 96 compounds were tentatively identified using GC-MS. A nominal total of 63 compounds for CAR/PDMS fiber as shown in Table 4.1 and 33 compounds for PDMS/DVB were detected for gaharu essential oil using GC-MS as shown in Table 4.2.

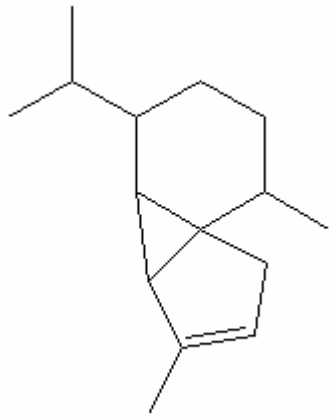
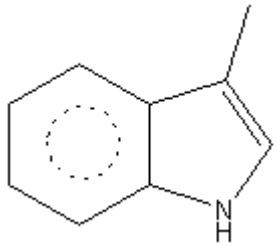
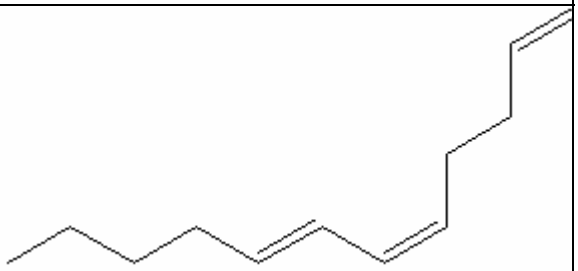
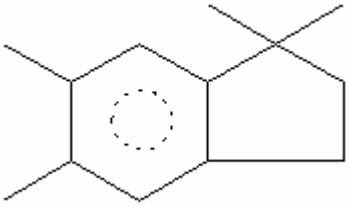
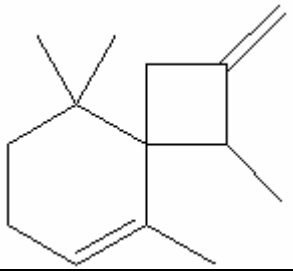
Table 4.1: Components of the gaharu essential oil for CAR/PDMS

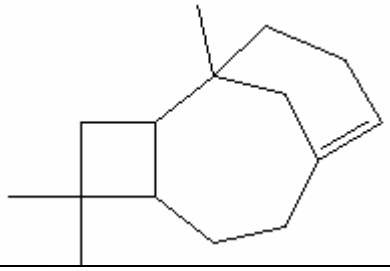
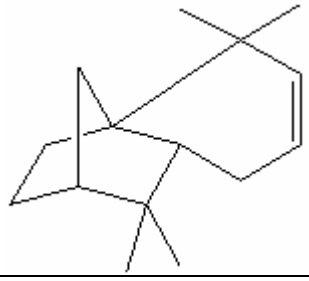
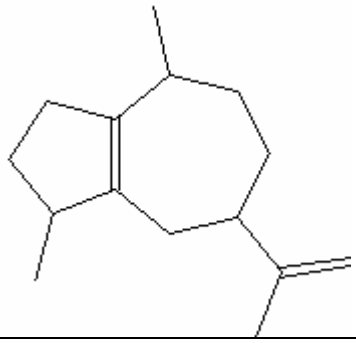
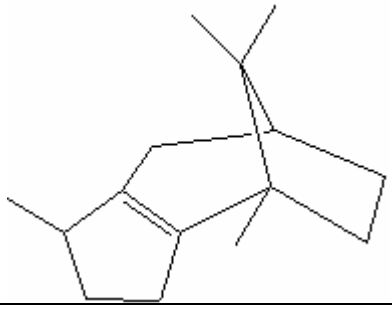
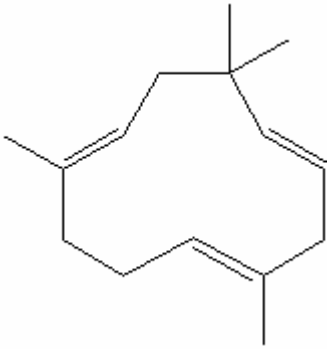
Peak No.	**Compound**	**Peak Area (%)**	**Structure**
1	Butane, 1-bromo-	3.539	
2	Benzyl Alcohol	3.608	
3	Benzaldehyde-2-hydroxy	3.741	
4	Acetophenone	4.052	
5	Phenol, 4-methyl-	4.146	

6	Mequinol	4.344	
7	Hexadecane	4.450	
8	Benzene, 1-ethenyl-4-methoxy	5.097	
9	Ethanone, 1-(2-hydroxyphenyl)-	5.224	
10	1H-Indene, 2, 3-dihydro-1,6-dimethyl-	5.615	
11	2-Butanone, 4-phenyl-	5.838	

12	2-Naphtalenol	5.955	
13	2-Butanone, 4-phenyl	6.209	
14	Benzene propanol	6.265	
15	1H-Indene,2,3-dimethyl-	6.308	

16	Benzomide, 0-(2-hydroxy-2, 2-diphenylethyl-N-methyl-	6.819	
17	Benzene, 2-ethenyl-1, 3-dimethyl-	6.935	
18	Furan, 2-[(methyldithio)methyl]-	7.094	
19	3-Buten-2-one, 4-phenyl-, (E)-	7.192	

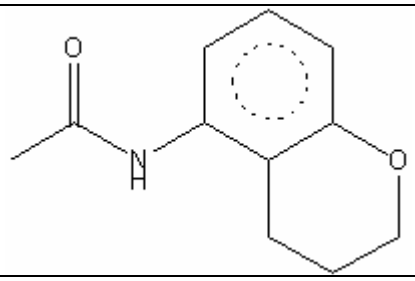
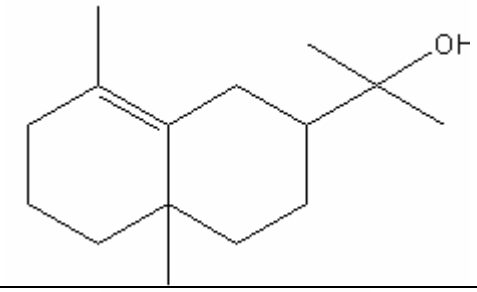
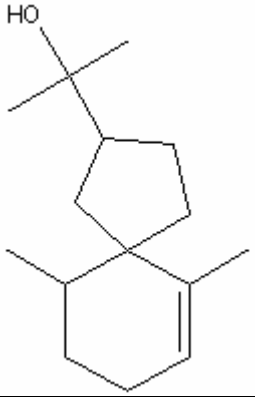
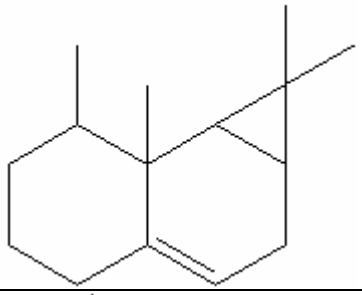
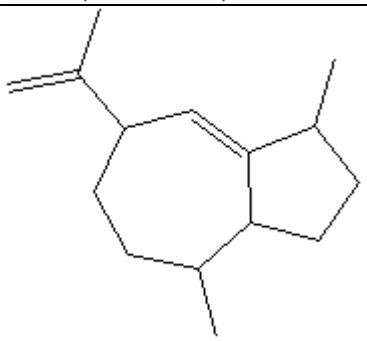
20	Alpha-Cubebene	7.394	
21	1H-Indole, 3-methyl	7.450	
22	1Z-5, E-7-Dodecatriene	7.525	
23	1H-Indene, 2, 3-dihydro-1, 1, 5, 6-tetramethyl-	7.584	
24	1, 5, 9, 9-tetramethyl-2-methylene-spiro[3, 5]non-5-ene	7.661	

25	1S, 2S, 5R-1, 4, 4-trimethyltricyclo [6. 3. 1. 0(2, 5)] dodec-8(9)-ene	7.734	
26	Isolongifolene, 9, 10-dehydro-	7.842	
27	Azulene, 1, 2, 3, 4, 5, 6, 7, 8-octahydro-1, 4-dimethyl-7-1(1-methylethenyl)-[1S-(1.alpha, 4.alpha, 7alpha)]-	7.978	
28	4, 7-Methanoazulene, 1, 2, 3, 4, 5, 6, 7, 8-pctahydro-1, 4, 9, 9-tetramethyl-[1S-(1.alpha, 4.alpha, 7.alpha)]-	8.054	
29	Alpha-caryophyllene	8.123	

30	Azulene, 1, 2, 3, 4, 5, 6, 7, 8-octahydro- 1, 4-dimethyl-7-(1-methylethenyl)-[1S-(1.alpha, 4.alpha, 7.alpha)]-	8.192	
31	Naphthalene, 1, 2, 4a, 5, 6, 8a-hexahydro-4,7-dimethyl-1-(1-methyl-ethyl)-1(1R-(1.alpha, 4.alpha, 8a.alpha)]-	8.251	
32	1H-cycloprop[e]azulen-1-01, decahydro-1, 1, 7-trymethyl-4-methylene-,[1aR-(1a.alpha, 4.alpha, 7.beta, 7a.beta, 7b.alpha)]-	8.299	OH
33	2-Isopropenyl-4a, 8-dimethyl-1, 2, 3, 4, 4a, 5, 6, 7-octahydronaphthalene	8.360	
34	Naphthalene, 1 ,2 ,3 ,4 , 4a, 5, 6, 8a-octahydro-4a, 8-dimethyl-2-(1-methylethenyl)-, [2R(2.alpha, 4a.alpha, 8a.beta)]-	8.399	

35	Caryophyleine-(13)	8.488	
36	Azulene, 1, 2, 3, 5, 6, 7, 8, 8a-octahydro-1, 4, dimethyl-7-(1-methylethenyl)-[1S-(1.alpha, 7.alpha, 8a.beta)]-	8.567	
37	Azulene, 1, 2, 3, 3a, 4, 5, 6, 7-octahydro-1, 4-dimethyl-7-(1-methylethenyl)-,[1R-(1.alpha, 3a.beta, 4.alpha, 7.beta)]-	8.665	
38	2,4-Quinolinediol	8.750	OH N H O
39	1H-Indene, 2, 3, 3a, 4-tetrahydro-3, 3a, 6-trimethyl-1-(1-methylethyl)-	8.793	

40	Alpha-calacorene	8.854	
41	2, 6-dimethyl-5-heptanal	8.908	
42	Longifolene-(V4)	9.007	
43	4, 6, 6-trimethyl-2-(3-methylbuta-1,3-dienyl-3-oxatricyclo[5.1.0.0(2, 4)]octane	9.089	
44	Diethyl phthalate	9.198	

45	N-(chroman-5-yl)acetamide	9.309	
46	2-Naphthalenemethanol, 1, 2, 3, 4, 4a, 5, 6, 7-octahydro-.alpha., .alpha., 4a, 8-tetramethyl-, (2R-cis)-	9.493	
47	Agarospirol	9.592	
48	(-)-Aristolene	9.663	
49	Azulene, 1, 2, 3, 3a, 4, 5, 6, 7-octahydro-1, 2-dimethyl-7-(1-methylethenyl)-,[1R-(1.alpha., 3a.beta., 4.alpha., 7.beta.)]-	9.779	

50	Tricyclo[3.2.1.02, 7]oct-3-ene, 2, 3, 4, 5-tetramethyl-	9.872	
51	Trans-Z-.alpha.-Bisabolene epoxide-	9.910	
52	1, 6-Dimethylhepta-1, 3, 5-triene	10.006	
53	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	10.068	
54	Cycloheptene, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1vinyl-	10.130	
55	7-oxabicyclo[4.1.0]heptane, 2, 2, 6-trimethyl-1-(3-methyl-1, 3-butadienyl)-5-methylene-	10.373	

56	Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-,[1S-(1.alpha., 2.beta., 4.beta.)]-	10.406	
57	1H-cycloprop[e]azulene, decahydro-1, 1, 7-trimethyl-4-methylene-,[1aR(1a.alpha., 4a.beta., 7.alpha., 7a.beta., 7b.alpha)]-	10.580	
58	1, 3, 6-Trimethyladamantane	10.650	
59	1-Cyclohexene-1-acrylic acid, 2, 6, 6-trimethyl-3-oxo-, methyl ester	10.690	
60	Humulen-(IV)-	10.805	

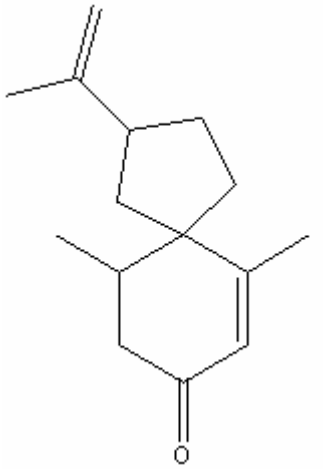
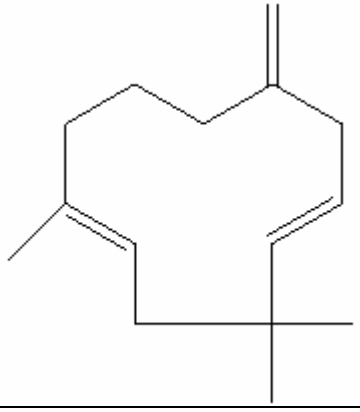
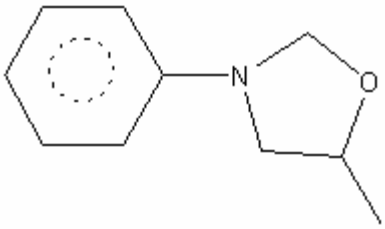
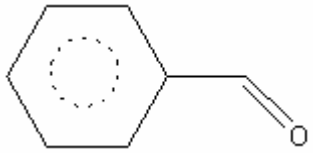
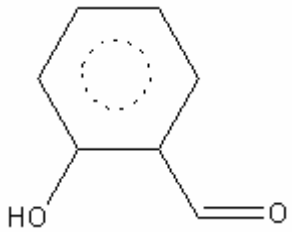
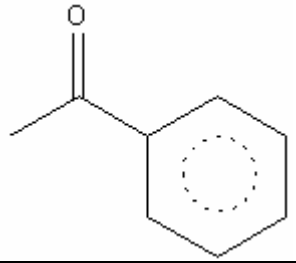
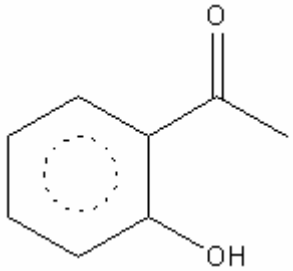
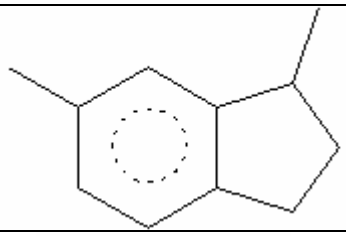
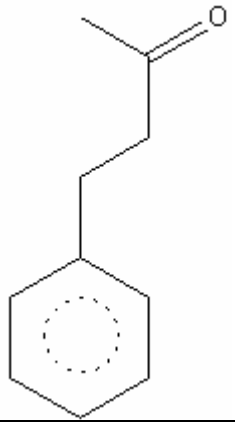
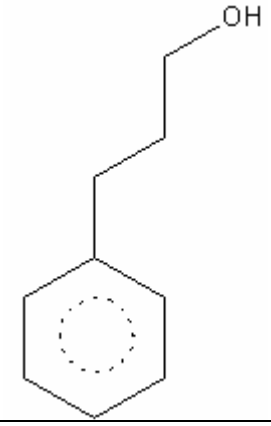
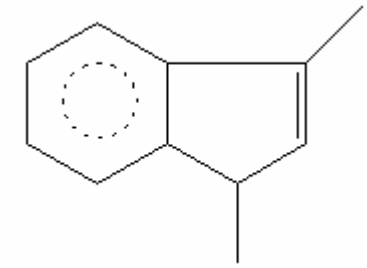
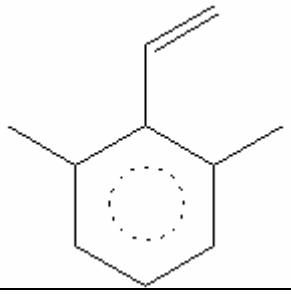
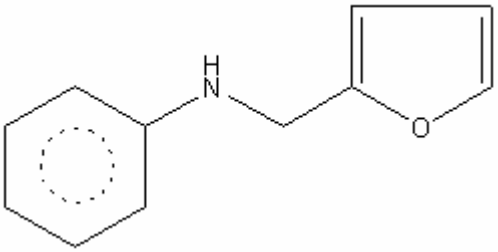
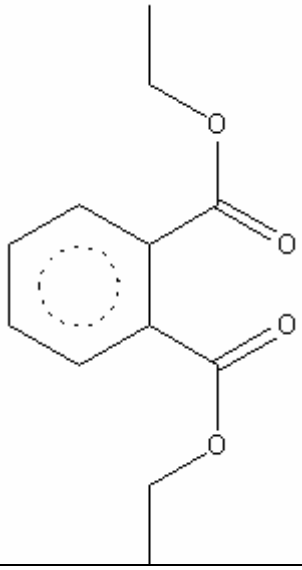
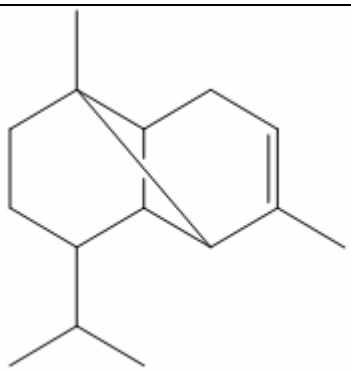
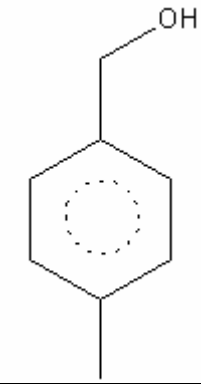
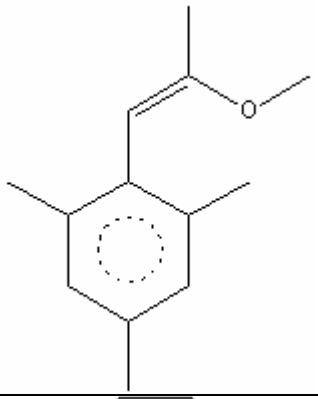
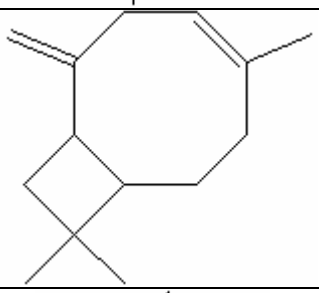
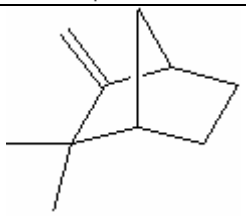
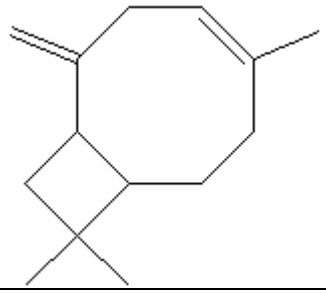
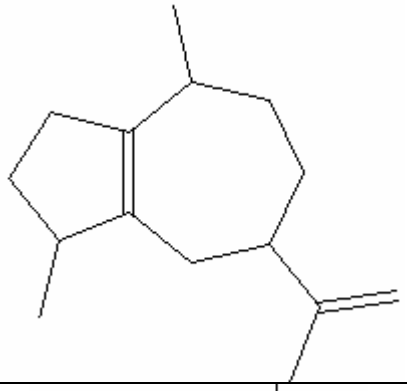
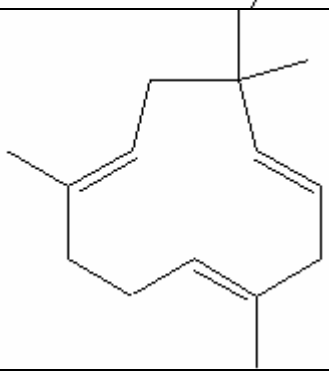
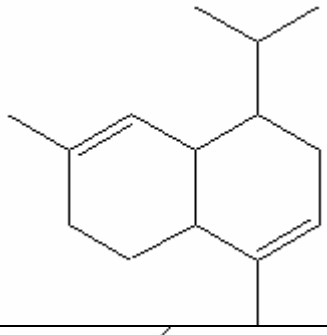
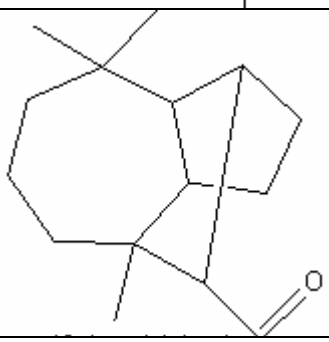
61	Solavetivone	10.886	
62	.beta.-Humulene	10.929	
63	5-methyl-3-phenyl-1, 2-oxazolidine	10.180	

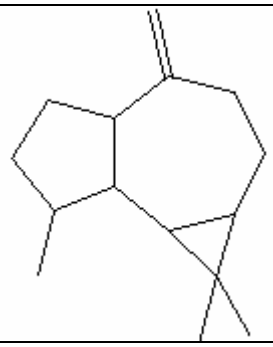
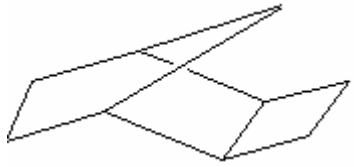
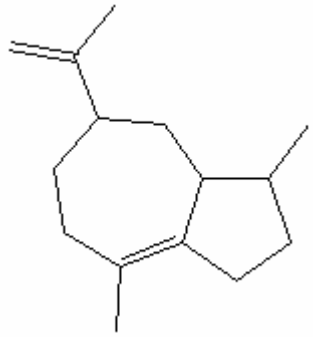
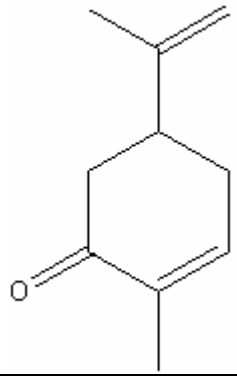
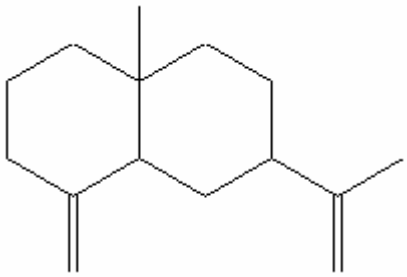
Table 4.2: Components of gaharu essential oil for PDMS/DVB

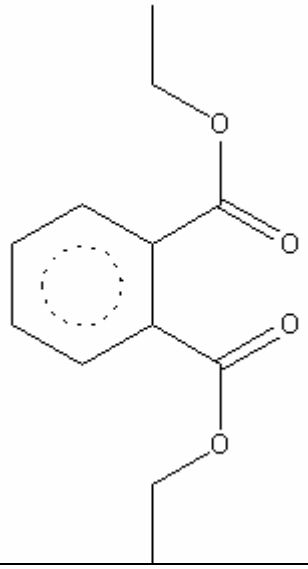
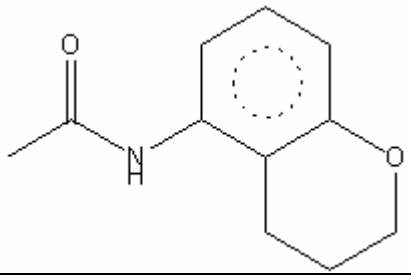
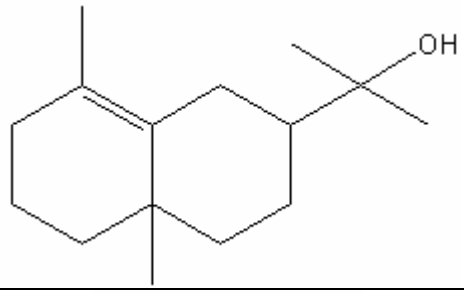
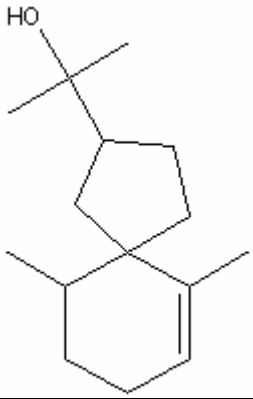
Peak No.	**Compound**	**Peak Area (%)**	**Structure**
1	Benzaldehyde	3.362	
2	Benzaldehyde, 2-hydroxy	4.197	
3	Acetophenone	4.428	
4	Ethanone, 1-(2-hudroxyphenyl)-	5.402	
5	1H-Indene, 2, 3-dihydro-1, 6-dimethyl	5.750	
6	2-Butanone, 4-phenyl	6.223	

7	Benzenepropanol	6.309	
8	1H-Indene, 1, 3-dimethyl-	6.381	
9	Benzene,2-ethenyl-1, 3-dimethyl-	6.986	
10	N-Furfurylaniline	7.131	
11	Diethyl Phthalate	7.243	

12	Copaene	7.419	
13	Benzenemethanol, 4-methyl	7.551	
14	Benzene, 2-(2-methoxy-1-propenyl)-1, 3, 5-trimethyl	7.607	
15	Bicyclo[7.2.0]undec-4-ene, 4, 11, 11-trimethyl-8-methylene	7.683	
16	Bicyclo[2.2.1]heptane, 2, 2-dimethyl-3-methylene,-(IR)-	7.757	

17	Caryophyllene	7.834	
18	Azulene, 1, 2, 3, 4, 5, 6, 7, 8-octahydro-1, 4, dimethyl-7-(1-methylethenyl)-[1S-(1.alpha., 4.alpha., 7.alpha)]-	7.978	
19	.alpha.caryophllene	8.137	
20	Naphthalene, 1, 2, 4a, 5, 6, 8a-hexahydro-4, 7-dimethyl-1-(1-methylethyl)-,[1R-(1.alpha., 4a.alpha., 8a.alpha.)]-	8.258	
21	Longifolenaldehyde	8.305	

22	1H-Cycloprop[e]azulene, decahydro-1, 1, 7-trimethyl-4-methylene-,[1aR-(1a.alpha., 4a.beta., 7.alpha., 7a.beta., 7b.alpha.)]-	8.407	
23	Tricyclo[4.2.1.0(2, 5)]nonane	8.492	
24	Azulene, 1, 2, 3, 5, 6, 7, 8, 8a,-octahydro-1, 4-dimethyl-7-(1-methylethenyl-[1S-(1.alpha., 7.alpha., 8a.beta.)-	8.553	
25	Carvone	8.911	
26	Eudosma-4(14), II-diene	9.011	

27	Diethyl Phthalate	9.185	
28	N-(Chroman-5-yl)acetamide	9.312	
29	2-Napthalene methanol, 1, 2, 3, 4, 4a, 5, 6, 7-octahydro-.alpha., .alpha., 4a, 8-tetramethyl-, (2R-cis)-	9.488	
30	Agarospirol	9.585	

31	.beta.-Neoclovene	9.655	
32	1H-Cycloprop[e]azulene, decahydro-1, 1, 7-trimethyl-4-methylene-,[1aR-(1a.alpha., 4a.alpha., 7.alpha., 7a.beta., 7b.alpha.)]-	9.723	
33	.alpha.-Farnesene	10.226	

These component numbers are rather arbitary given that the composition of overlapped peaks is not readily quantified. However, only half or even less of the compounds was tentatively identified by matching peak apex mass spectra with mass spectra in the library. Identified compounds had matching quality of 9% to 99% between the mass spectra of the compounds obtained from the samples to the mass spectra from the NIST library; compounds with matching quality less than 50 % could not be considered as qualitative result since it does not match more than half a percentage. The inability to identify compounds is largely ascribed to their overlapping with other compounds, leading to poor quality spectra for those compounds which reduce the match quality with the MS library.

A wide range of different compound classes were identified in gaharu essential oil, including essential oil-type components, aldehydes, alcohols, pyrazines, ketones, pyrans, acids and mono aromatics. Gaharu essential oil which was extracted from a wood grade C by two different fibers contains sesquiterpene alcohol which produces its characteristic aroma. The gaharu is traditionally used to produce incense

in the Far East because of the strong aroma of it. As quoted by Ishihara (1991), there is an absence of sesquiterpenes such as (-)-guaia-1(10), 11-dien-15-al, (-)-selina-3, 11-dien-9-one and (+)-selina-3, 11-dien-9-ol in this gaharu grade C which proves that the wood used in this experiment is a lesser grade gaharu. There is a presence of agarospirol in the gaharu essential oil which only present in lesser grade gaharu. Agarospirol determines the grade of the wood that was used in this experiment. Other compounds such 2-[(1S,8S)-1,8-dimethyl-1,2,3,4,6,7,8,8a-octahydronaphthalen-2 yl]propan-2-ol or known as Karanone, (4aR,5S)-4a,5-dimethyl-3-propan-2-ylidene-5,6,7,8-tetrahydro-4H-naphthalen-2-one or dihydrokaranone and Oxo-agarospirol which characterize the wood as a lesser grade
wood were not present in the gaharu essential oil. This might be due to characteristics of the fiber used.

Benzyl alcohol which was found with the CAR/PDMS fiber had a 96% of matching quality with the mass spectra of NIST library. It gives a mild aromatic smell to the oil and a useful solvent due to its polarity, low toxicity, and low vapor pressure. Gaharu is used in production of soap and perfume because of the present of Benzyl alcohol. Followed by that, there are also other compounds found in the analysis of gaharu essential oil that can be used in perfume industry. Benzaldehyde and acetophenone are also used in the perfume industry. Benzaldehyde is an aromatic aldehyde which has almond like odor, whereas Acetophenone is a crystalline ketone which smells as cherry or almond and used in chewing gums.

There are four types of Indene compounds shown in Table 4.1 and 4.2. These indene compounds polycyclic hydrocarbon that consists of fused aromatic rings and do not contain heteroatoms. Some of them are known or suspected carcinogens, and are linked to other health problems. They are primarily formed by incomplete combustion of carbon-containing fuels such as wood. However, in gaharu essential oil production, there were no combustion occurred, so the indene contain might be due to over heating of gaharu in the oven.

Diethyl phthalate was found in analysis of both types of fibers. Diethyl phthalate gives a bitter taste and it does not have harmful effect to human. According

to internet resources, it is used in toys and food packaging production. Although it is quite surprising to find it in gaharu essential oil, this might be not true that it is a compound found in gaharu because it might be the content of the injection port since it is a same type material used in food packaging.

There are around seven types of Azulene found in gaharu essential oil analyzed by both the fibers. It is a monoterpene that consist of two isoprene units and isomer of napthalene. Basically they are used in cosmetics. Other compounds such as caryophyllene are bicyclic sesquiterpenes that consist of two fused rings and Copaene are tricyclic sesquiterpene that consist of oily liquid hydrocarbon. Moreover, there is quite a number of Naphthalene present in gaharu essential oil. It is an aromatic hydrocarbon that has characteristic of odor. It is detectable at concentration as low as 0.08ppm by mass. It is used in production of mothballs and over dosage of it can damage the red blood cells.

The compounds extracted by both the fiber from the gaharu essential oil can be concluded as semi-polar or non-polar because CAR/PDMS and PDMS/DVB fibers have low polarity and strong retention. Moreover, the compounds found in gaharu essential oil are mostly hydrocarbon and hydrocarbons are non-polar compounds. There is similarity in compounds extracted by both the fibers. There are eleven compounds found for extraction with both the fibers which are benzaldehyde, 2-hydroxy, acetophenone, ethanone, 1-(2-hydroxyphenyl)-, 1H-Indene, 2, 3-dihydro-1, 6-dimethyl, 2-butanone, 4-phenyl, Azulene, 1, 2, 3, 4, 5, 6, 7, 8-octahydro-1, 4, dimethyl-7-(1-methylethenyl)-[1S-(1.alpha., 4.alpha., 7.alpha)]-, .α-caryophllene, Naphthalene, 1, 2, 4a, 5, 6, 8a-hexahydro-4, 7-dimethyl-1-(1-methylethyl)-,[1R-(1.alpha., 4a.alpha., 8a.alpha.)]-, 1H-Cycloprop[e]azulene, decahydro-1, 1, 7-trimethyl-4-methylene-,[1aR-(1a.alpha., 4a.beta., 7.alpha., 7a.beta., 7b.alpha.)]-, Azulene, 1, 2, 3, 5, 6, 7, 8, 8a,-octahydro-1, 4-dimethyl-7-(1-methylethenyl-[1S-(1.alpha., 7.alpha., 8a.beta.)-, 2-Napthalene methanol, 1, 2, 3, 4, 4a, 5, 6, 7-octahydro-.alpha., .alpha., 4a, 8-tetramethyl-, (2R-cis)- and agarospirol. Although the oil used in this analysis with two types of fibers are produced by the same method and extracted at same conditions, there are still differences in the compounds that have been

extracted by each fiber. This is due to the characteristics of the fiber such as thickness, polarity and retention.

CHAPTER 5

CONCLUSIONS

5.1 Conclusions

In this study and investigation, a simple procedure using solid phase microextraction (SPME) as an extractant has been described to recover the components from the gaharu essential oil by hydrodistillation. Employing this method, a total of 96 compounds have been recovered by SPME with two types of fiber, CAR/PDMS and PDMS/DVB.

Gaharu essential oil analysis is very important for the quality control of the gaharu products and the monitoring of harmful contaminants. The choice of analytical method depends on the presence of the target compounds in the oil and the variety and complexity of the sample. Therefore, sample preparation for a complex matrix greatly influences the reliable and accurate analysis of essential oil sample. The application of SPME in sample preparation for gaharu essential oil is very little yet increasingly important. The SPME technique described in this research is very effective as a sample preparation technique for qualitative and quantitative analyses. The headspace (HS-SPME) that was used in this research has effectively been useful by increasing the lifetime of the fiber and extracting a large amount of compounds. The main advantages of SPME are simplicity, rapidity, solvent elimination, high sensitivity, small sample volume, lower cost and simple automation. It is concluded that the CAR/PDMS and PDMS/DVB SPME fibers can be successfully applied for non-polar and semi-polar compounds in oil samples and can be easily couples with GC-MS.

Followed by that, it is proven by the research that the gaharu grade C has agarospirol which is only present in lesser grade gaharu. In conclusion, the odor of gaharu essential oil is composed of the sesquiterpenes such as benzaldehyde, benzel alcohol, acetophenone and agarospirol, all of which have strong oriental woody note. The compounds found in the analysis are concluded as non-polar or semi-polar since the fiber that we have used is possible to extract low polarity compounds.

The affinity of the fiber coating for an analyte is the most important factor in SPME. Fiber coating of different polarity and thickness were selected for each compound. Most of the compounds from the gaharu essential oil were extracted with 75µm CAR/PDMS by HS sampling and analyzed in combination with GC-MS. So this proves that, the thicker the fiber, the more the compounds can be extracted.

5.2 Recommendations for Future Study

The first and foremost improvement that needs to be paid attention is the optimization of the extraction with solid phase microextraction (SPME). In fiber SPME, the amount of analyte extracted onto the fiber depends not only on the polarity and thickness of the stationary phase but also the extraction time and the concentration of analyte in sample. Extraction of analyte is also typically improved by agitation, addition of salt to sample, changing the pH and temperature. Agitation accelerated the transfer of analytes from the sample matrix to the coating fiber. Extraction efficiency is also improved by adding soluble salts to the sample, such as sodium chloride or potassium carbonated. In general, the sample is acidified for the extraction of acidic analytes and is made alkaline for extraction of basic analytes. Furthermore, a volatile acid or base is used for DI-SPME and a non volatile acid or base is used for HS-SPME. An increase in extraction temperature causes an increase in extraction rate. It is recommended to analyze the extraction time and other SPME parameters for an accurate and precise analysis.

Additionally, more types of fiber should be used for this analysis. Since the CAR/PDMS and PDMS/DVB are partially crosslinked and more or less the same type of retention and polarity, it does not differ much in their analysis. At least more than three types of fibers should be used at various temperature, thickness, agitation speed, pH and also SPME parameters. This could possibly increase the number of compounds extracted by SPME and more polar compounds could be found in gaharu essential oil.

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APPENDIX A

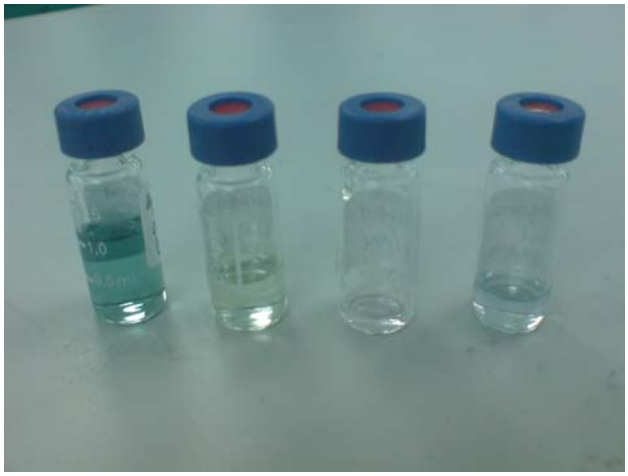

Figure A-1 : 4mL vial used for sample preparation

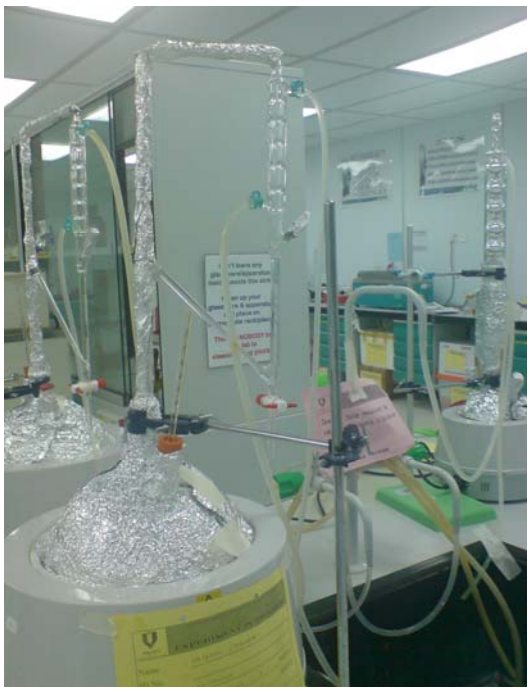

Figure A-2 : Hydrodistillation apparatus

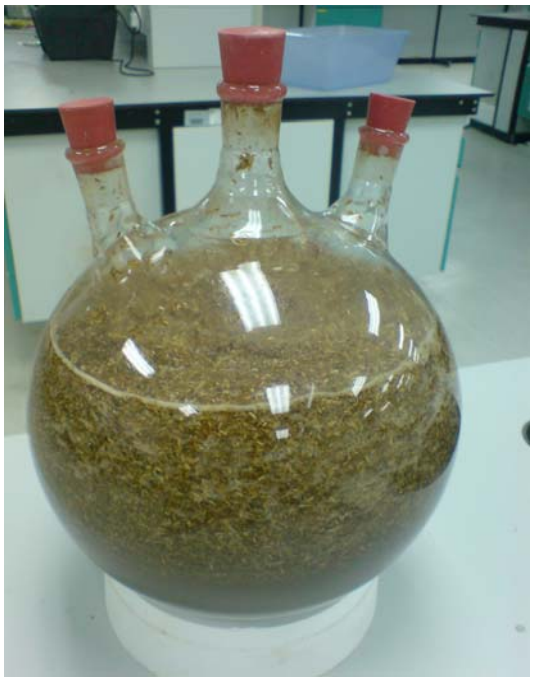

Figure A-3: Soaking the raw material

Figure A-4: Gas Chromotography-Mass Spectroscopy