# ANALYSIS VOLATILE COMPOUND OF GAHARU OIL COMPOSITION VIA SOLID PHASE MICRO EXTRACTION (SPME)

**NORSUZIEANA BT AB LATIF**

**UNIVERSITI MALAYSIA PAHANG**

# UNIVERSITI MALAYSIA PAHANG

## BORANG PENGESAHAN STATUS TESIS

JUDUL : **ANALYSIS VOLATILE COMPOUND OF GAHARU OIL COMPOSITION VIA SOLID PHASE MICRO EXTRACTION (SPME)**

SESI PENGAJIAN: **2008/2009**

Saya **NORSUZIEANA BT AB LATIF**
**(HURUF BESAR)**

mengaku membenarkan tesis Projek Sarjana Muda (PSM) ini disimpan di Perpustakaan Universiti Malaysia Pahang dengan syarat-syarat kegunaan seperti berikut:

1. Hakmilik kertas projek adalah di bawah nama penulis melainkan penulisan sebagai projek bersama dan dibiayai oleh UMP, hakmiliknya adalah kepunyaan UMP.
2. Naskah salinan di dalam bentuk kertas atau mikro hanya boleh dibuat dengan kebenaran bertulis daripada penulis.
3. Perpustakaan Universiti Malaysia Pahang dibenarkan membuat salinan untuk tujuan pengajian mereka.
4. Kertas projek hanya boleh diterbitkan dengan kebenaran penulis. Bayaran royalti adalah mengikut kadar yang dipersetujui kelak.
5. *Saya membenarkan/tidak membenarkan Perpustakaan membuat salinan kertas projek ini sebagai bahan pertukaran di antara institusi pengajian tinggi.
6. **Sila tandakan (✓)

| | | |
|---|---|---|
| ☐ | SULIT | (Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972) |
| ☐ | TERHAD | (Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan) |
| ☑ | TIDAK TERHAD | |

Disahkan oleh

_____ _____

(TANDATANGAN PENULIS) (TANDATANGAN PENYELIA)

Alamat Tetap: Lot 375 Kampung Pulau Kapas,
15200 Kota Bharu,
Kelantan Darul Naim.

**EN SAIFUL NIZAM BIN TAJUDDIN**
Nama Penyelia

Tarikh: 30 April 2009 Tarikh: 30 April 2009

CATATAN:
* Potong yang tidak berkenaan.
** Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT atau TERHAD.
◆ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertai bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (PSM).

"I hereby declare that I have read this thesis and in
my opinion this thesis is sufficient in terms of scope and
quality for the award of the degree of
Bachelor of Chemical Engineering"

Signature : .....

Supervisor : En Saiful Nizam bin Tajuddin

Date : .....

# ANALYSIS VOLATILE COMPOUND OF GAHARU OIL COMPOSITION VIA SOLID PHASE MICRO EXTRACTION (SPME)

**NORSUZIEANA BT AB LATIF**

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

**Faculty of Chemical & Natural Resources Engineering**
**Universiti Malaysia Pahang**

**April 2009**

I declare that this thesis entitled "**Analysis Volatile Compound of Gaharu Oil via Solid Phase Micro Extraction (SPME)**" 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."

Signature :……

Name : Norsuzieana bt Ab Latif

Date : 30 April 2009

*Special Dedication to my family members,*
*my friends, my fellow colleague*
*and all faculty members*

*For all your care, support and believe in me.*

## ACKNOWLEDGEMENT

I would like to express my humble thanks to ALLAH S.W.T. for the strength, inspiration and encouragement given to me through out the completion of this thesis without any obstacles. A lot of experiences and knowledge were gained along the way.

I wished to express my sincere appreciation to my supervisors, Mr. Saiful Nizam Bin Tajuddin and my panels for their critics, advices, motivation, friendship and input of ideas, relentless support, guidance and endless encouragement. I would also very thankful to my academic advisor, Mrs Jun Haslinda Bt Haji Shariffuddin, for her support and believe in me during my studies.

I'm very thankful to Universiti Malaysia Pahang (UMP) for providing good facilities in the campus. To all the staff in Faculty of Chemical & Natural Resources Engineering, a very big thanks you to all.

My sincere appreciation also extends to all my fellow colleagues and others who have provided assistance at various occasions. Their views and tips are useful indeed. Thank you for the time sacrificed to accompany me. And last but not least, I am grateful to all my family members.

## ABSTRACT

Gaharu (agarwood) is a fragrant wood that is usually derived from the diseased timber of the genus Aquilaria Thymelaeceae and often occurs as dark coloured patches or streaks in the tree. Due to its strong, unique scent and medicinal properties, gaharu oil is greatly valued as perfumery ingredient and incense. Gaharu may be classified into various grades; Grade A, B, C and D and they are often graded according to the physical properties, gaharu formation and its unique scent. The lower grades such as Grade C are often distilled to obtain gaharu oils. As part of an on-going research on the chemical profiling of some Malaysian gaharu oils and evaluation of their potential beneficial properties; gaharu oils obtained from different sources were analysed and compared by SPME and GCMS. Identification of the chemical components was based on comparison of the types of SPME fibers and chromatographic columns. The SPME device included a fused silica fiber coating partially cross-linked with 100μm Polydimethylsiloxane (PDMS), 75μm Carboxen/Polydimethylsiloxane (CAR/PDMS) and 65μm Polydimethylsiloxane /divinylbenzene (PDMS/DVB). The chromatographic column used were HP-5MS 5% Phenyl Methyl Siloxane and DB-WAX , 30 m x 250 μm i.d, film thickness 0.25 μm. Examination of the oils showed some variations and differences in terms of GCMS profiles, concentration and chemical components. Majority of the essential oil profiles were complex and made up of sesquiterpenoids and their oxygenated derivatives. However, common occurrences of chemical compounds such as benzaldehyde, 3-phenyl-butanone, alpha-guaiene and gamma- guaiene were detected.

## ABSTRAK

Gaharu (agarwood) adalah sejenis kayu wangian yang biasanya didapati daripada spesis *Aquilaria Thymelaeceae* dan mempunyai corak serta jalur berwana hitam. Berdasarkan sifat-sifat kekuatan, keunikan dan perubatan, minyak gaharu biasanya digunakan sebagai bahan pewangi dan kemenyan. Gaharu boleh dikelaskan kepada beberapa gred iaitu gred A,B,C dan D yang mana boleh dibeza berdasarkan sifat-sifat fizikal, pembentukan dan keunikan gaharu. Gred C iaitu gred yg rendah biasanya digunakan untuk penyulingan minyak gaharu. Penyelidikan berdasarkan bahan profil kimia dan kepentingan sifat-sifat dalam penghasilan minyak gaharu di Malaysia telah dibuat dengan menggunakan *SPME* dan *GCMS*. Identiti setiap komponen kimia diperolehi daripada perbezaan jenis fiber *SPME* dan kolum kromatografik *GCMS*. Jenis-jenis fiber *SPME* adalah *100µm Polydimethylsiloxane (PDMS), 75µm Carboxen/Polydimethylsiloxane (CAR/PDMS) dan 65µm Polydimethylsiloxane /divinylbenzene (PDMS/DVB).* Manakala kolum kromatografik adalah seperti *HP-5MS 5% Phenyl Methyl Siloxane dan DB-WAX , 30 m x 250 µm i.d,* ketebalan filem *0.25 µm.* Penyelidikan terhadap minyak gaharu menunjukkan perbezaan dalam bentuk profil GCMS, kepekatan minyak dan komponen-komponen kimia. Kebanyakan komponen seperti *sesquiterpenoids* dan kompaun sampingan hasil pengoksidaan sangat susah didapati dalam penghasilan profil minyak wangi. Walau bagaimanapun, di dalam analisa ini komponen-komponen seperti *benzaldehyde, 3-phenyl-butanone, alpha-guaiene and gamma- guaiene* diperolehi.

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLE

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of Study

Gaharu or agarwood is the resinous heartwood from aguilaria tree that is the occasional product of two to four general in the family Thymelaeaceae, with scientific name are Aquilaria agallocha, Aquilaria crassna and Aquilaria malaccensis Lam. This is the best unknown species. This resin is commonly named Aloeswood, Jinko, Jin Koh, Eagle wood, Oud or Ood ud. It's an evergreen tree native to Asia like northem India, Laos, Cambodia, Malaysia, Indonesia and Vietnam.

Gaharu has been used as medicine, perfume, and insence. Insence is traditionally used for rituals and religious ceremonies in the Far East. Gaharu is also believed to have tonic and therapeutic properties (Burkill, 1966; Okugawa et al., 1993). Its essential oil is in heavy demand in the perfume industry as evidenced by the recent expansion of the range of uses for Gaharu to include new products such as Gaharu essence, soap and shampoo (chakrabarty et al., 1994).

There are several techniques that allow the extraction of compounds responsible for the aroma of plants. The composition of the aromatic material obtained is strongly dependent on the method of isolation. The main techniques used at industrial scale are cold pressing, hydro distillation, extraction with organic solvents and extraction with compressed $CO_2$. In Malaysia, the techniques currently practiced in the industry for the extraction of oils are by hydro-distillation and solvent extraction (Nor Azah Mohd. Ali- 2002).Upon hydro distillation of Malaysian

Gaharu oil is obtained in 0.8% yield. These methods were chosen since it is much more suitable for a developing country like Malaysia. The advantages of these techniques are that they are economically viable and safe to operate. The odors of the oil can be described as complex mixture compound.

This essential oil will analyze with different type of fiber via Solid Phase Micro Extraction (SPME) and then injected to Gas Chromatography Mass Spectrometry (GCMS) to identify the chemical compound in Gaharu essential oils. SPME is currently a new and popular technique. It requires minimal accessory equipment low cost and is easy to use. Its major shortcomings are the lack of sensitivity and the limited range of volatiles which can be analyzed. Several chemical compounds such as agarospirol, guaiene, jinkohol and jinkohol 11 have been detected in Malaysian Gaharu oils.

Since Gaharu in the Malaysian market is categorized into several graded, the low quality graded are often distilled to produce essential oils. The essential oil from Gaharu brings high prices due to it rarity and high demand, usually the low grade is around RM30 000 and the superior grades priced up to RM60 000. The higher quality Gaharu wood can be recognized by its darker colour and strong aroma released upon burning its chips or quality incenses.

## 1.2 Objective

The main objectives of this preliminary study are:

1. To extract the Gaharu oils using hydro distill method.

2. To analyze Gaharu oil with different type of fiber in Solid Phase Microextraction (SPME).

3. To identify the compound of Gaharu oil with different type of column phase via Gas Chromatography Mass Spectrometry (GCMS).

4. To compare chemical compound present in each result from different column phase.

## 1.3 Scope of Study

The scope of this study is to extract and identified the important chemical constituent in Gaharu oils. We prepare the sample by using the hydro distillation extraction method to extract the essential oil from Gaharu.

The sample will analyze with different type of fiber via Solid Phase Micro Extraction (SPME). Headspace-SPME is identified as a solvent free sample preparation technique in which a fused silica fiber coated with polymeric organic liquid is introduced into the headspace above the sample. The adsorption of the analytes is followed by a thermal desorption process by introducing the SPME fiber into the injection port of a gas chromatography.

In this context, the headspace was considered to be an alternative method to clarify the question about the fragrance of Agarwood oil. The SPME device included a fused silica fiber coating partially cross-linked with 100μm Polydimethylsiloxane (PDMS), 75μm Carboxen/Polydimethylsiloxane (CAR/PDMS) and 65μm Polydimethylsiloxane/divinylbenzene (PDMS/DVB).

The last scope is to understand and know how Gas Chromatography Mass Spectrometry (GCMS) works to identify the compound of Gaharu oil with different type of column phase in GCMS. The column phases that we use are HP-5MS and DB-WAX. The chemical compound that present in the essential oil from Gaharu can be defined.

## 1.4 Problems Statement

Hydro-distillation is the oldest and most common method of extracting essential oil since it is economically viable and safe. Local institutions like the Forest Research Institute of Malaysia (FRIM) and The Malaysian Timber Industry Board (MTIB) play a major role in the essential oil technology transfer. Even though research were carried out at these institution, lack of documentation and research publication on their part, contributed to this study. Thus, this study want to see whether this method is proven can produce oil and give information on what important step during extraction of Gaharu oils.

Traditionally essential oils have been assessed on the basis of individual requirements. Thus, there is no standard to represent the chemical compounds in Gaharu essential oils in determining their quality. Physically characteristics of essential oils such as density, optical rotation, solubility in solvent, boiling temperature, odor, color and others are easily measured.

Today, the demand for meaningful essential oil standards has increased, because reproducibility of intended effects is essentially determined by a parity of concentration of essential oil component. Once the standard for active compounds in Gaharu essential oil is developed, then the quality of Gaharu oil can be identified. When analyzed their chemical compound via GCMS, that do not provide the full identification of the components and consequently do not give a guarantee of authenticity.

Several of the compounds such as agarospirol, jinkohol-eremol and kusenol have been reported to possibly contribute to the characteristic aroma of Gaharu (Nakanishi et al. 1984; Ishihara et al., 1993). The marker compound isolated will be used for standardizing the Gaharu essential oil contributing to value added products from Malaysian Gaharu and establishing a universal standard for Gaharu in the global market which is presently lacking.

## 1.5 Rationale and Significant

Hydro distillation method is the best for lab laboratory since its simple, cheap and easy to handle.

A novel method for profiling of essential oils from Aqualaria malaccensis (Gaharu) using newly identified marker compounds will be developed.

Essential oils from Aqualaria malaccensis (Gaharu) that have been identified will acquire added value in the global market.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Background of Aquilaria Malaccensis (Gaharu)

Aquilaria malaccensis is one of 15 tree species in the Indomalesian genus Aguilaria, family Thymelaeaceae, (Mabberley, 1997). It is 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). A. malaccensis and other species in the genus Aguilaria sometimes produce resin impregnated heartwood that is fragrant and highly valuable. There are many names for this resinous wood, including agar, agarwood, aloeswood, eaglewood, gaharu and kalamabak, this wood being in high demand for medicine, incense, and perfumes across Asia and the Middle East.

Aquilaria malaccensis is widely distributed in south and south-east Asia. There are differing accounts of the countries in which it occurs. According to Oldfield et al. (1998), A. malaccensis is found in 10 countries such as Bangladesh, Bhutan, India, Indonesia, Iran, Malaysia, Myanmar, Philippines, Singapore and Thailand.

Aquilaria species have adapted to live in various habitats, including those that are rocky, sandy or calcareous, well drained slopes and ridges and land near swamps. They typically grow between altitudes of 0-850 m, in locations with average daily temperatures of 20-22$^{o}$C (Afifi, 1995; Keller and sidiyasa, 1994; Wiriadinata, 1995).

Formation of Aquilaria occurs in the trunk and roots of trees that have been infected by a parasite ascomycetous mould, Phaeoacremonium parasitica, a dematiaceous (dark-walled) fungus. As a response, the tree produces a resin high in volatile organic compounds that aids in suppressing or retarding the fungal growth. While the unaffected wood of the tree is relatively light in colour, the resin dramatically increases the mass and density of the affected wood, changing its colour from pale beige to dark brown or black. In natural forest only about 7% of the trees are infected by the fungus. A common method in artificial forestry is to inoculate all the trees with the fungus. (http://www.organicessentialoils.in/AgarwoodOil.html).

High quality resin comes from a tree's natural immune response to a fungal attack. It is commonly known as agarwood. An inferior resin is created using forced methods where aquilaria trees are deliberately wounded, leaving them more susceptible to a fungal attack.

Some low cost agarwood oil is also developed through producers names Oud and high used in cosmetic preparations and other places. This quality is quite infamous and the oils are developed in particular carrier oil. Agar is the pathological product of a fungal disease contracted by the tree chiefly through wounds on the trunk. Since agar is located deep within the trunk, its detection is not easy. Generally such trees are distinguished by poor crown development, the presence of swellings or depressions on the bole. Depending upon the extent of the resin accumulation the heartwood is graded into four categories:

- Grade 1 Black/True Agar: mainly exported to Arabia as incense
- Grade 2 Bantang: mainly exported to Arabia as incense
- Grade 3 Bhuta or Phuta: sometimes extracted for a superior oil
- Grade 4 Dhum: used for oil

In true of black agar, the impregnation of the resin is intense and the wood resembles black stone. It is heavy to the extent that it sinks in water, and bears the

highest content but it is difficult to distill. True agar is mainly exported to the Middle East countries where it is used as incense. Bantang is brown in color without any black tone. Bhuta is also brown in color but interspersed with 50 per cent or more of yellow-colored wood. These two grades are also usually used in incense. Dhum is the lowest grade which is mostly yellow with scattered streaks of brown or black resin. It is chiefly distilled for the oil. Sometimes oil is also extracted from Bhuta and this oil is reported to be superior to that from Dhum.
(http://members.aol.com/ratrani/agarwood.html).

Figure 2.1: Pieces of *Aquilaria* wood lacking the dense and dark resinous agarwood caused by infection

Figure 2.2: Colour is one criterion in assessing the value of a gaharu. The darker the gaharu is, the higher value it fetches. The black and shiny resin on the top right (circled) is of Grade A.

(Source: http://www.forestry.gov.my/pdf/NST220308.pdf)

## 2.2 Aquilaria Malaccensis in Malaysia

A.malaccensis is distributed throughout Peninsular Malaysia, except for the States of Kedah and Perlis (Barden et al., 2000), but although the species has good geographical coverage, its occurrence in rather rare, with trees often locally scattered. La Frankie (1994) studied the population dynamics of A.malaccensis in Pasoh Forest Reserve and suggested a typical lowland Malaysia forest density of 2.5/ha and found that the growth rate varied between 0-1.95 cm/year.

Malaysia has a long history in the trade in A.malaccensis, 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 A.malaccensis products in domestic trade are woodchips and powder or sawdust (Chua, 2003). Some use 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).

## 2.3 Uses of Gaharu

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.

### 2.3.1 Incense

One of the traditional uses of Gaharu is for the production of incense, and is by far the most prized of all incenses. Incense made from high quality Gaharu is very expensive. Prices vary with the graded used. It is used in important religious ceremonies, rituals and meditation.

In the Arabian Country's People are used Agarwoods as an incense and burn it on coal. Both agarwood oil and incense are used for their fragrant properties, notably in the Middle East. Buddhist, Hindus and Muslims use Agarwood incense in religious ceremonies, while a revival of the "Koh doh" incense ceremony in Japan has rekindled interest in agarwood in that country.(http://essentialoilscompany.com).

### 2.3.2 Medicinal Uses

In Malaysia, Gaharu is used in various folk remedies for the treatment of weakness, stomach pains, after pregnancy, fever, chest pains, body pains, rheumatism, women diseases and dropsy (Chang, et al. 2002). Gaharu also used in curry flavor. A decoction of the wood used for abdominal pain, asthma, cancer, colic chest, congestion, diarrhea, hiccups, nausea, nerves and also regurgitation (Kim et al. 1997).

Gaharu have been recorded in traditional medical systems including Chinese (TCM), Tibetan, Ayurvedic (Indian) and Unani (Greek derived Islamic). External and Internal preparations have been used citing a variety of *Aquilaria* species. (http://www.equitech.biz/equitech_Silviculture)

- **TCM**

  Formulations in general seem to relieve spasms and other forms of stagnant or stuck energy particularly in the digestive (stomach, kidneys, liver, and bowel) and respiratory systems.

- **Ayurveda**

  In the Indian Ayurvedic healing system, the burning of agarwoods has a warming and centering effect on the chakras and promotes a deep meditational state. Agarwood heartwood is used in various Ayurvedic formulas including Chyavanprasha, Arimedadi Taila and Mahanarin Taila. Its uses have been described as a cardiac tonic, carminative & refrigerant.

- **Unani**

  It is used as a stimulant, stomachic, laxative (purgative in large doses) and as an aphrodisiac. It is also used in the Ayurvedic system against skin diseases and powdered heartwood is given for treatment of diahorrea, dysentery, vomiting and anorexia. Agarwood oil, mixed with essential oil from Piper betel is used against bronchial asthma. It is also reported as being used by the traditional vaidyas as a contraceptive and the leaves boiled in oil used to remove fish bones stuck in the throat.

- **Tibetan Medicine & Ethinic Psychiatry.**

  Oleoresin, wood and oil are used in Tibetan medicine and incense, especially prized is “black aloeswood”, (*Aquilaria agallocha*) which Clifford (1984) describes as being relied on by contemporary Tibetan doctors for treatment of a whole range of nervous and emotional disorders. Clifford further describes black aloeswood as the most commonly used minor tranquilliser.

### 2.3.3 Aromatherapy

Gaharu perfumes comprise Gaharu oil mixed with a carrier such as sandalwood oil. 'Attar oil,' for example, is water-based perfume containing Gaharu oil that is normally used by Muslims to lace prayer clothes. Gaharu essences have also recently been used to fragrance soaps and shampoos.

Miller and Miller (1995) in their book Ayerveda Aromatherapy *is* the energetic warming, balancing effects of oud (oil of *A. agallocha),* and its’ energy purifying and balancing, relaxant, rejeuvinative, transformative, clairvoyant and transcending actions. (http://www.equitech.biz/equitech_Silviculture)

## 2.4 Structure cell of Gaharu

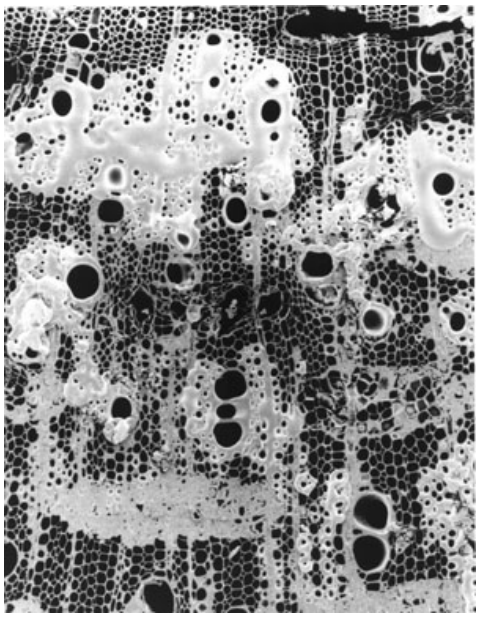


Figure 2.3: Cross section of Gaharu Cell

(Source: http://forestpathology.cfans.umn.edu/agarwoodadd.htm)

A cross section of an experimental tree with Gaharu observed by scanning electron microscopy showing copious amounts of resin formed in the wood cells. *Aquilaria* has an unusual anatomy and specialized cells within the xylem produce the resin. It is in species which have included phloem in the secondary cambium.

## 2.5 Essential Oil

Essential oils can be any products from herbal plans & tree who find via Hydro distill plant, $CO_2$ Plant (Supercritical Carbon Dioxide), Solvent Extraction, Cold-Pressed etc. Its can be any Oil, Concretes, Natural Aroma Compounds, Perfumes from the Leaves, Flowers, Bark, Roots, Wood, Seeds or Peel of Herbal Plans & Tree.

Essential oils are pure, natural and extremely concentrated substances derived from the flowers, leaves, stems, seeds, peels or barks of difference plants. They are

liquids obtained from the secretors structures in plant that evaporate at room temperature and have characteristic aromas. They are also called volatiles oils because of this ability to evaporate readily. The substances contain hormone-influencing substances, antibiotics and antiseptics. These oils are far more “alive” and potent than dried herbs. (http://www.edenbotanicals.com/agarwood).

Most flowers contain too little volatile oil to undergo expression and their chemical components are too delicate and easily denatured by the high heat used in steam distillation. Instead, a solvent such as hexane or supercritical carbon dioxide is used to extract the oils. Extracts from hexane and other hydrophobic solvent are called *concretes*, which is a mixture of essential oil, waxes, resins, and other lipophilic (oil soluble) plant material.

### 2.5.1 Physical Properties of Essential Oil

Usually essential oils is colorless, particularly when it fresh. But for gaharu oil, it can be distinguished by their colors; ‘reddish brown’ and ‘greenish brown’ (Fatmawati Adam *et al*., 2005). Essential oils also known as volatile oils because are easily to evaporate. Unlike vegetable oils expressed from nuts and seeds, essential oils are not actually oily. Some essential oils are viscous; others are fairly solid and most are somewhat watery (http://www.essentialwholesale.com/aromatherapy.html).

Essential oils are highly concentrated and should always be treated with care and respect. Like the herbal material from which the oils are obtained, essential oil should be strong properly. Whenever possible they should be kept in tightly sealed amber colored glass bottles. If amber glass is unavailable they should be stored in tight containers and kept from exposure to sunlight, fluorescent lights and excessive heat.

### 2.5.2 Chemical Properties of Essential Oil

Most people either use essential oils for their therapeutic effect or for the fragrance alone but it is also interesting to take note of the chemistry, of which the oils are made up from. It is interesting to know the make up the oils, but it is also humbling to take note of the fact that even with the best human efforts. Essential oils, like all organic compounds, are made up of hydrocarbon molecules and can further be classified as terpenes, alcohols, esters, aldehydes, ketones and phenols etc.

The oil is composed by one or more terpenes and some oxygenated derivatives. The oxygenated compounds usually have better organoleptic properties, so terpene separation is of interest. Nowadays preparation of essential oils with a high content on oxygenated terpenoids presents some difficulties, due to their delicate characteristics (Arce, Pobudkowska, Rodr´ıguez, & SotoEvery; 2007). Each single essential oil normally has more than a hundred components. When analyze essential oils with a chromatograph various organic components are found and the primary ones are as follows:

- Terpene hydrocarbons
    - Monoterpene hydrocarbons
    - Sesquiterpenes
- Oxygenated compounds
    - Phenols
    - Alcohols
        - Monoterpene alcohols
        - Sesquiterpene alcohols
    - Aldehydes
    - Ketones
    - Esters
    - Lactones
    - Coumarins
    - Ethers
    - Oxides

## 2.6 Chemical Components in Gaharu Oils

Generally, gaharu oils are mixtures of sesquiterpenes, sesquiterpenes alcohol, oxygenated compounds, chromone derivatives and resins. Some of the more important compounds are agarospirol, jinkohol-eromol, jinkohol and kusenol that may contribute to the characteristic aroma of gaharu. (Nakashini et.al 1984, Ishihara et.al 1993). Other compounds such as 2-(2-4'-methoxyphenylethyl) chromone produce a long lasting fragrance upon burning.

Based on the study by FRIM on several grades of gaharu obtained from the wild, samples from companies and through inoculation trials, chemical profiles of each grade such as grade A, B and C were different. Further comparison of Gas Chromatograms Mass Spectrometry of gaharu oils obtained from different sources in Peninsular Malaysia demonstrated that they were mostly of grade C quality. Several chemical compounds such as agarospirol, guaiene, jinkohol and jinkohol II have been detected in Malaysia gaharu oils.

### 2.6.1 Sesquiterpenes

Sesquiterpenes is the formation from biosynthesis reaction of isoprene. Molecule structure is $C_{15}H_{24}$ and exists in several derivatives such as alcohols, ketones, aldehydes, ethers and phenols. This sesquiterpenes is important to industry for example constituents of most odorant, natural and synthetic, employed in perfumery, aromatherapy and pharmaceutical.

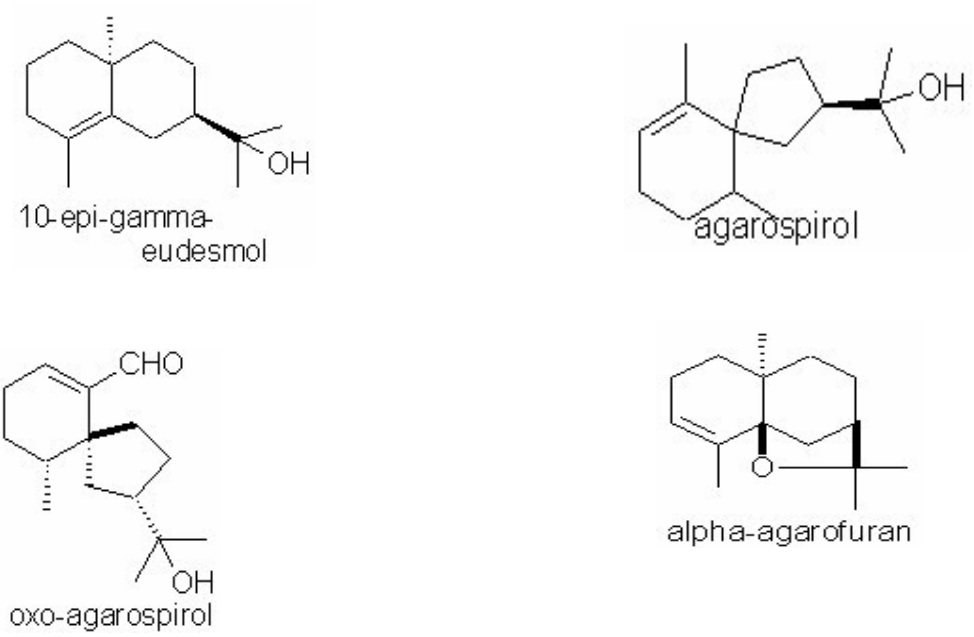



Figure 2.4**:** Gaharu chemical components

## 2.7 Extraction

Extraction is a separation process to separate the desired solute or removed an undesirable solute component from the solid phase where the solid is contacted with a liquid phase. The two phases are in intimate contact and the solutes can diffuse from the solid to the liquid phase, which causes a separation of the components originally in the solid. In particular, during extraction, the content of some components is significantly modified, depending on the extraction technique employed (Chiacchierini, Restuccia, & Vinci, 2007).

A process of extraction involves selectively removing one or more components of solid, liquid or gaseous mixtures into a separate phase. The substances being extracted will partition between the two immiscible phases that are in contact, depend on the relative solubility of the solute in each phases. (Gilbert *et al*, .2002).

There are various types of extractions. They range from food, drug and cosmetic extractions, which separate several compounds at one time, to the more sophisticated analytical extractions, which are designed to cleanly separate a selected compound or compounds from a complex mixture.

### 2.7.1 Extraction of Essential Oil

The essential oils from aromatic plants are for the most part volatile and thus, lend themselves to several methods of extraction such as hydro distillation, water and steam distillation, direct steam distillation, and solvent extraction (ASTA 1968, Guenther 1972, Heath 1981, Sievers 1928). The specific extraction method employed is dependent upon the plant material to be distilled and the desired end-product. The essential oils which impart the distinctive aromas are complex mixtures of organic constituents, some of which being less stable, may undergo chemical alterations when subjected to high temperatures. In this case, hydro distillation extraction is required to ensure no decomposition or changes have occurred which would alter the aroma and fragrance of the end product.
(http://www.hort.purdue.edu/newcrop/proceedings1990).

The most popular method for extraction in old times is hydro distillation, but as technological advances is made more efficient and economical methods being developed.

Although the extraction of essential oils may sound only to be of technical interest, it is one of the key points which determine the quality of the oil that is used, since a wrong or wrongly executed extraction, can damage the oil and alter the chemical signature of the essential oil.

Since Gaharu in Malaysia market is categorized into several grades, the low quality graded is often distilled to produce essential oils. The higher quality wood

that can be verified by the darker color and strong aroma released on burning is chipped or made into quality incense.

#### 2.7.1.1 Hydro Distillation Extraction

Hydro distillation is used in the manufacture and extraction of essential oil. This is the simplest and usually the cheapest process of distillation. Hydro distillation seems to work best for powders and very tough materials like roots, wood, or nuts. The main advantages of this method are that less steam is used, shorter processing time and a higher oil yield.

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 100$^{o}$C. Care needs to be taken in prevent 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.

## 2.8 Solid Phase Micro Extraction (SPME)

Solid Phase Micro Extraction (SPME) is currently a new and popular technique. It requires minimal accessory equipment (low cost) and is easy to use. Its major shortcomings are the lack of sensitivity (ppt) and the limited range of volatiles which can be analyzed. It works very well with the less volatile organics such as PCB's and can be automated.

SPME is a unique sample preparation technique that eliminates most of the drawbacks associated with extracting organics. SPME requires no solvents or complicated apparatus.

SPME has gained widespread acceptance as the technique of preference for many applications including: flavours, fragrances and contaminants in food; forensic and toxicology applications; environmental and biological matrices; organic volatiles in pharmaceutical compounds.

The extraction is performed by exposing the fibre into the sample vial. Samples can be agitated by orbital rotation and heated during extraction. Both the shaking speed and oven temperature are programmable. The oven door is kept closed during extraction to maintain constant temperature.

After the compounds have been thermally desorbed in the GC injector, the fibre may be fully cleaned again in the optional heated fibre cleaning station positioned at the back of the unit.

Advantages using SPME:

- Single step extraction (reduces sample preparation time by up to 70%)
- Minimal use of solvents
- Programmable extraction depth to perform both headspace and liquid extraction
- Oven door kept closed during extraction to keep temperature constant

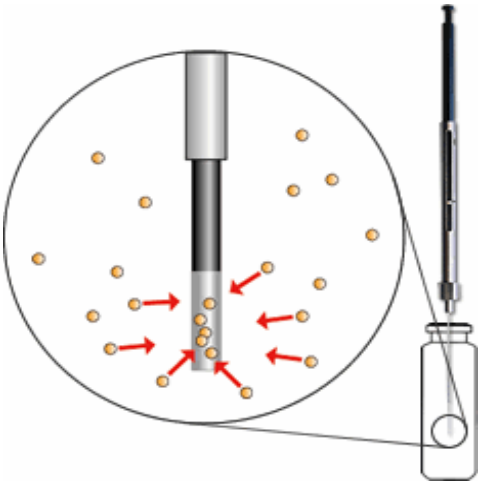


Figure 2.5: SPME: When placed into the headspace gas or a liquid, the compounds adsorb onto the fibre

### 2.8.1 SPME Process

Researchers at the Johns Hopkins University, Applied Physics Laboratory (JHU/APL) have further developed the Solid Phase Micro-Extraction (SPME) process. This a process whereby chemicals of interest are selectively and competitively adsorbed onto a silica fiber coated by a stationary chemical phase.

The adsorption process starts with the exposure of the fiber to the sample of interest and continues for the length of the exposure time. The silica fiber is a commercial off-the-shelf device available from SUPELCO (products for analysis and purification). In order to quantify the chemicals that are adsorbed onto the fiber, the fiber is inserted into the injector port of a gas chromatograph/mass spectrometer (GCMS). The chemicals are volatilized in the GCMS and analyzed using standard laboratory methods. Under commercial operations, the fibers are mounted into syringe-like holders that are used to extend the fibers and handle the fibers. Exposure to both liquid and gas environments is appropriate.

The present technology provides only for manual sampling and analysis methods in human-accessible locations in order to acquire a sample in fluid. The inventors at JHU/APL have improved the present technology by:

1. Providing for a self-contained sample collection and SPME fiber exposure means for locations that are not accessible to humans (i.e. onboard an unmanned undersea vehicle).

2. Using a sealed vessel that prevents degradation of the fluid sample prior to analysis while allowing the SPME fiber to remain in equilibrium with the sample.

This IP is applicable to any field or business linked to chemistry, intelligence, surveillance, reconnaissance, environmental monitoring.

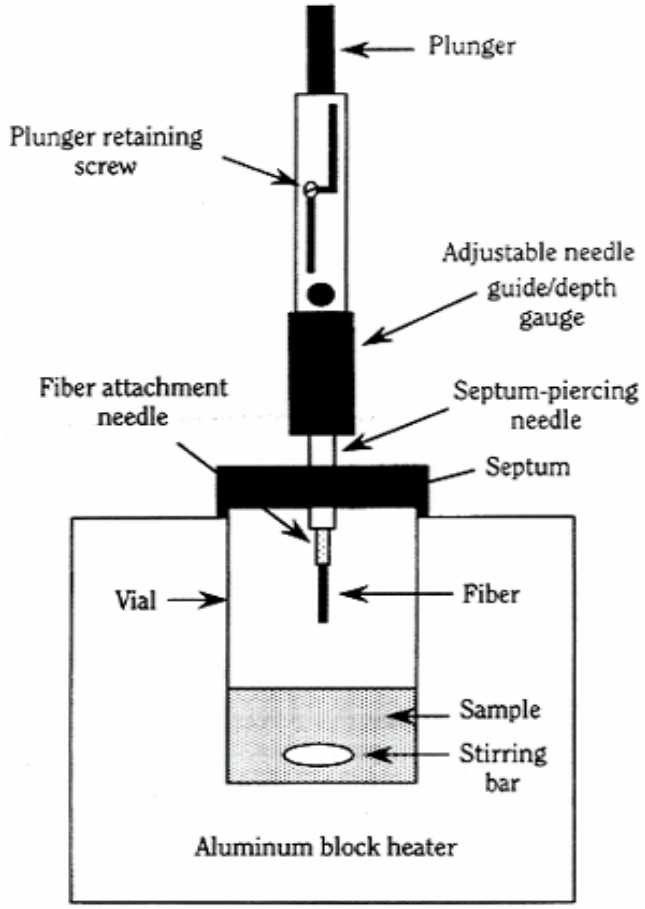



Figure 2.6: The SPME Process

### 2.8.2 Type of Fiber in SPME

#### 2.8.2.1 Polydimethylsiloxane (PDMS) fibers, *non-polar*

100 µm coating thickness is recommended for low molecular weight or volatile compounds, while non-polar semi volatiles or large molecular weight compounds are more effectively extracted with a 30 µm or a 7 µm PDMS fiber, respectively.

#### 2.8.2.2 Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) fibers

More volatile polar analytes, such as amines and alcohols, are adsorbed more efficiently and released faster with a 65 mm (PDMS/DVB)-coated fiber.

#### 2.8.2.3 Carboxen/Polydimethylsiloxane (CAR/PDMS) fibers

For trace level volatiles analysis, use a 75 µm PDMS/Carboxen fiber.

## 2.9 Gas Chromatography Mass Spectrometry (GCMS)

Gas Chromatography Mass Spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. GCMS can also be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification.

### 2.9.1 GCMS work

The GCMS instrument is made up of two parts. The gas chromatography (GC) portion separates the chemical mixture into pulses of pure chemicals and the mass spectrometer (MS) identifies and quantifies the chemicals.

The GC separates chemicals based on their volatility, or ease with which they evaporate into a gas. It is similar to a running race where a group of people begin at the starting line, but as the race proceeds, the runners separate based on their speed. The chemicals in the mixture separate based on their volatility. In general, small molecules travel more quickly than larger molecules. The MS is used to identify chemicals based on their structure. Some parts of the puzzle remain attached together and some individual pieces break off completely. By looking at these various pieces, you are still able to get an idea of what the original puzzle looked like. This is very similar to the way that the mass spectrometer works.

**1. Gas chromatography (GC)**

a. **Injection port -** One microliter (0.000001 L) of solvent containing the mixture of molecules is injected into the GC and the sample is carried by inert (non-reactive) gas through the instrument, usually helium. The inject port is heated to 300° C to cause the chemicals to become gases.
b. **Oven -** The outer part of the GC is a very specialized oven. The column is heated to move the molecules through the column. Typical oven temperatures range from 40° C to 320° C.
c. **Column -** Inside the oven is the column which is a 30 meter thin tube with a special polymer coating on the inside. Chemical mixtures are separated based on their volatile and are carried through the column by helium. Chemicals with high volatility travel through the column more quickly than chemicals with low volatile.

**2. Mass Spectrometer (MS)**

a. **Ion Source :** After passing through the GC, the chemical pulses continue to the MS. The molecules are blasted with electrons, which cause them to break into pieces and turn into positively charged particles called ions. This is important because the particles must be charged to pass through the filter.

b. **Filter :** As the ions continue through the MS, they travel through an electromagnetic field that filters the ions based on mass. The scientist using the instrument chooses what range of masses should be allowed through the filter. The filter continuously scans through the range of masses as the stream of ions come from the ion source.

c. **Detector :** A detector counts the number of ions with a specific mass. This information is sent to a computer and a mass spectrum is created. The mass spectrum is a graph of the number of ions with different masses that traveled through the filter.

**3. Computer**

a. The data from the mass spectrometer is sent to a computer and plotted on a graph called a mass spectrum.

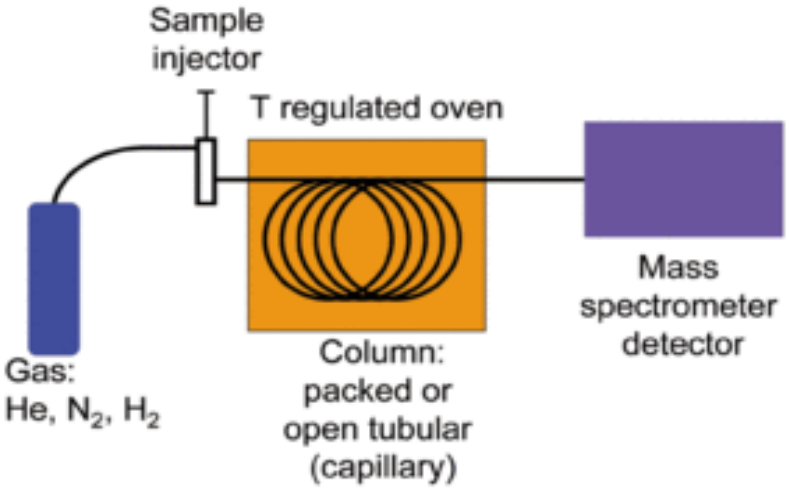



Figure 2.7:GCMS schematic

### 2.9.2 Analysis of Output

Each substance has a characteristic mass spectrum under particular controlled conditions. A technician can identify a specimen by comparing the specimen's mass spectrum with known compounds. Quantitative analysis is possible by measuring the relative intensities of the mass spectra.

Usually a mass spectrum will display a peak for the unfragmented molecule of the specimen. This is commonly the greatest mass detected, called the "parent mass." The parent mass is used to fit the pieces together from the other peaks in the mass spectrum. The parent mass reveals the mass of the molecule while the other peaks indicate the molecule's structure.

Determining the parent peak and consequently the molecular mass of the specimen is the most difficult part of MS analysis. Identifying the parent mass is outside the scope of this article. Assuming that a technician can correctly determine the molecular mass, the technician makes an educated guess of the specimen's identity and compares the mass spectrum to reference spectra for confirmation. The mass spectra for larger molecules containing carbon are complicated and require tedious calculations that are subject to error. Computers are commonly used for spectral analysis.

### 2.9.3 Stationary Phases in Gas Chromatographic Retention Data

The lists below contain names of stationary phases found in the gas chromatography retention database. More commonly encountered phases are listed at the top of each list.

Equivalent phases are listed on the same line (separated by semi-colons). Phases shown as equivalents are very similar to each other.

**1. Non-polar phases**

- DB-5; HP-5; SE-54; SE-52; CP Sil 8 CB; RTX-5; BPX-5; Ultra-2; 5 % Phenyl methyl siloxane; SPB-5; OV-3; PTE-5; NB-54; XTI-5; Mega 5MS; DB-5.625; RSL-200; MDN-5; OV-73; ZB-5; OV-5
- SE-30; DB-1; OV-101; OV-1; HP-1; Methyl Silicone; BP-1; SPB-1; Ultra-1; SP-2100; SF-96; RTX-1; E-301; DC-200; HP-101; RSL-150; Optima-1; ZB-1; 007-1; NB-30; LM-1; SPD-1
- Squalane
- Apiezon L
- CP Sil 5 CB
- DB-5MS; HP-5MS; RTX-5Sil MS
- Apolane
- Apiezon M
- Porapack Q
- CBP-1
- UCW-98
- PoraPLOT Q
- BP-5
- HP-PONA; Petrocol DH
- Cross-Linked Methylsilicone
- Apiezon
- PONA
- CP Sil 2
- Nonpolar
- LM-5
- JXR
- Vacuum Grease Oil (VM-4)
- MFE-73
- HT-5
- Silicon High Vacuum Grease (obsolete)
- RTX-5Sil
- PB-1

- SF96+Igepal
- C103H208
- PS-255
- Tridecane
- Silicone oil
- Apiezon LH + KF
- PE-1HT
- DB5-30W
- DB-1MS
- SE-30+Igepal
- Apiezon N
- PMS-1000
- CBP-5
- GP SP 2100 DB
- CP select for PCBs
- PoraPLOT
- OV101 (1%Carbowax 20M)
- RTX-1 PONA
- Triacontane
- HG-5
- Apiezon L + KF
- DB5-30N
- Permaphase DMS
- Sim Dist CB
- Optima 5
- Ultra-5
- Paraffin wax
- n-Dotriacontane
- PS-264
- Petrocol DH-100
- SE-30/SE-52
- C78, Branched paraffin

- SSP-1
- SE-33
- SE-52/54
- SPB-Sulfur
- MS5
- Polymethylsiloxane, (PMS-20000)
- OV-101 + Igepal

**2. Polar phases**

- Carbowax 20M; DB-Wax; PEG-20M; BP-20; Innowax; CP-Wax 52CB; AT-Wax; HP-Wax; RTX-Wax; Carbowax; Supelcowax; HP-20M; Stabilwax
- FFAP; SP-1000; OV-351
- Supelcowax-10
- HP-Innowax
- DB-FFAP
- PEG-40M
- PEG 4000
- Thermon 600T
- TC-WAX FFS
- CP-WAX 57CB
- Carbowax 20M-TPA
- Carbowax 20M + Igepal (20:1)
- PEGA
- Polyethylene Glycol
- CBP-20
- ZB-Wax
- Carbowax 4000
- Carbowax 40M
- Supelcowax-20M
- PEG-2000
- Carbowax 6000 + Hyprose SP 80 (40 : 60)

- Carbowax 400
- EPON 1001
- CP-Wax
- Stabilwax DA
- HP-FFAP
- Megawax
- Polyethylene Glycol 4000
- Emulphor-Q (polyethylene glycol, ca. 40)
- EC-WAX

# CHAPTER 3

# METHODOLOGY

## 3.1 Introduction

In analyzing and identified the Gaharu essential oil chemical composition, the available technique are using Solid Phase Micro Extraction and Gas Chromatography Mass Spectrometry. The methods that apply in this experiment are hydro distillation extraction process and Solid Phase Micro Extraction (SPME). Hydro distillation is use because water act as barrier to prevent the oil from overheating and in the same time protect it originality. By using the develop appliances, the extracted oil can be easily move from the tube by adding some hexane, collect, and let hexane vaporized by itself and we get the oil. The detail method is shows in flow chart below. After that it followed by the analysis the essential oil by SPME and GCMS.

## 3.2 Drying

In this process, the woods have to be completely dry from any moisture. Before drying process, cut the wood into small pieces to increase the surface area of chips and take a short time to complete the process. It is also to get free of any substance that can influence the impurities of oil when it has been extracted (Norazlina 2005). The process becomes completed when there were no changes of humidity inside tray dryer or in other word, humidity value become constant. Humidity can be measure by using humidity meter.

### 3.3 Grinding Process

Before this process, the gaharu wood is cut into pieces called chips but to maximize the surface area of wood, we carry out the second process which called grinding process. By this process, assume that every chips which have turn into sawdust with the size of 1 mm. Through this process, rate of extraction is increase when the contact between solvent and solid increasing because of increasing of gaharu sawdust area. More gaharu essential oil can be extracted when gaharu sawdust have higher surface area.

### 3.4 Soaking Process

Now move on into the next process which called soaking. After grinded into sawdust, gaharu must be soaked in water. The ratio of gaharu to water is 1:7 (Dong-ping *et al*, 1999) in range of three to seven days but in this experiment, 3 day soaking is enough for maximize effect. So, if 1 kilogram of gaharu sawdust was used, it needs to soak it into 700-800 mL of water. The purpose of soaking is to break down parenchymatous and oil glands (Chang *et al*, 2002).

### 3.5 Extraction Process

#### 3.5.1 Hydro distillation

To start the hydro distillation extraction process, 100 gram of sawdust of gaharu is weighing and drying it for 1 day in oven. After that, the drying of sawdust is soaking into water for 3 days in the flask. This process is in order to break down the parenchymatous cell and oil glands.

The soaking sawdust was then distilled in a Clevenger type apparatus for 3 days. To avoid the bumping process occur when water is boil which can affect the distillation process, the boiling chips also need to be insert in the flask. After the preparation is complete, start the method by heating process. Firstly, switch on the heating mantle and re-circulating cooler.

Along this experiment, set the temperature according to water boiling point which is 100 $^{O}$C. To make sure no heat loss while carry out the experiment, aluminum foil need to wrap over the appliances. This step is helping the system to reduce the error and give the maximum production of gaharu essential oil. For this type of extraction process, it will run for three days by using temperature at 100 $^{O}$C.

First flow for the process is steam and essential oil vapors are passed through the condenser. After condensation process, the vapors mixture will turn into liquid and it will be collected in at the end of receiving tube.

### 3.5.2 Collecting Process

Let the mixture for several days to make sure the layer of hydrosol and the gaharu essential oil is form. Then, collect the oil by suck it through the hole which made at the receiving tube. If the oil produce is very little, mix it with hexane, suck it, place it at the end of soxhlet extraction, let the hexane vaporized in room temperature and the rest is gaharu essential oil.

### 3.5.3 Water Removal

To make sure all gaharu essential oil is free from water or to get the pure gaharu oil, the sample must be punch with nitrogen and then place it in sample bottle for to analyze with SPME.

**THE FLOW DIAGRAM FOR HYDRO DISTILLATION PROCESS**

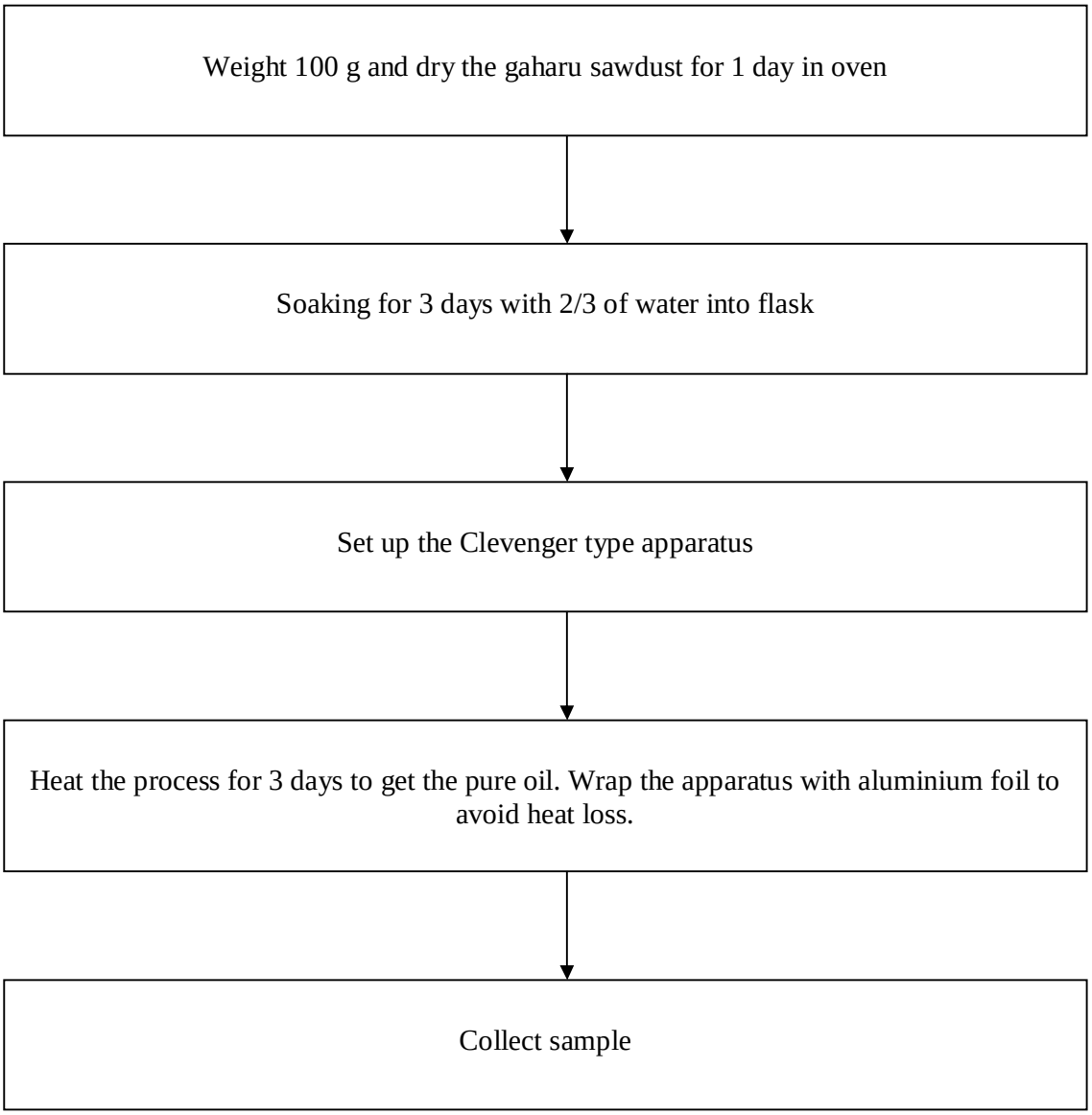



Figure 3.1: The flow diagram for hydro distillation process

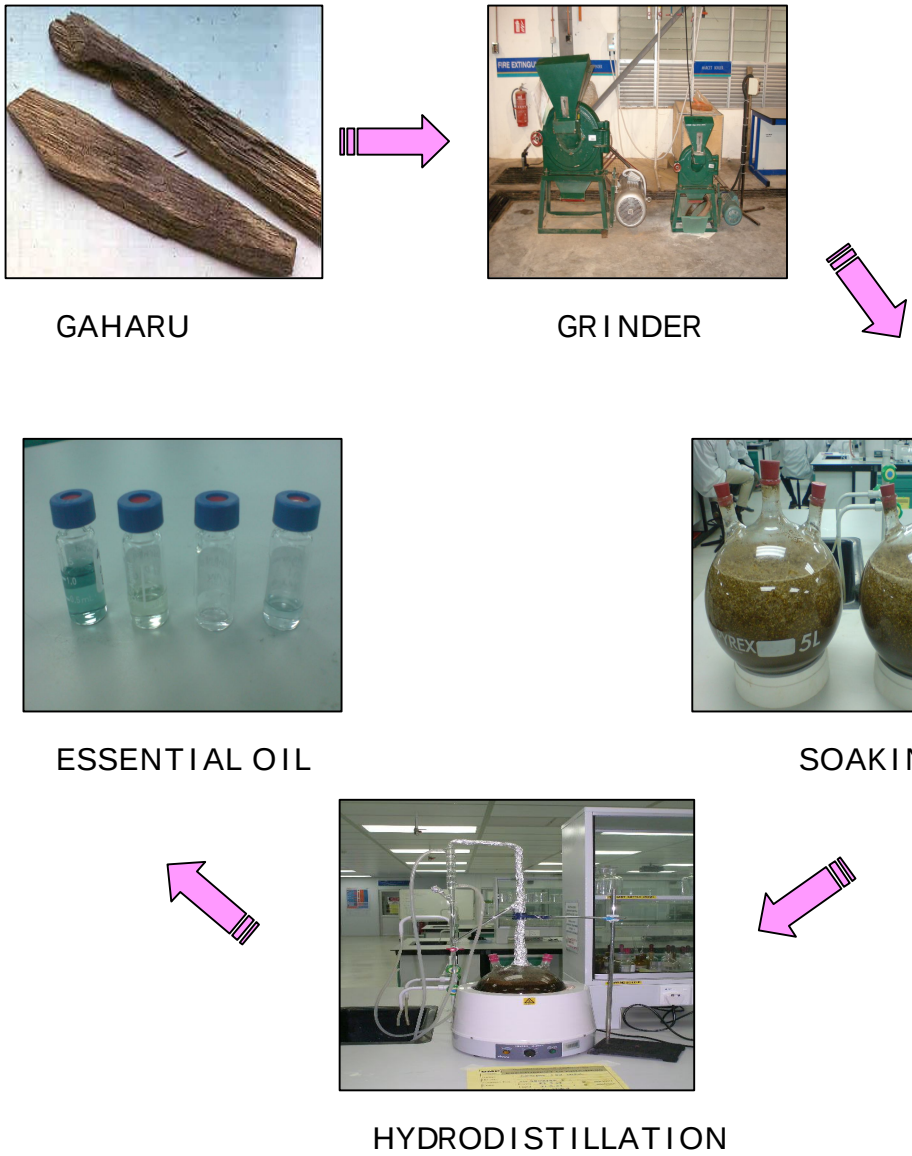



Figure 3.2 : The Schematic Diagram for Hydro distillation Process

### 3.6 Solid Phase Micro Extraction (SPME) Analyzed

The sample of gaharu oil is analyzing with SPME. The SPME device included a fused silica fiber coating partially cross-linked with 100µm Polydimethylsiloxane (PDMS), 75µm Carboxen/Polydimethylsiloxane (CAR/PDMS) and 65µm Polydimethylsiloxane/divinylbenzene (PDMS/DVB) were obtained from Supelco (Bellefonte, PA). The SPME fiber was conditioned at 200 ºC for 3 min in the GC injector.

1 µL of sampling oil was performed by inserting the syringe needle of the SPME assembly through the septum cap into the headspace above the sample. Volatiles were sorbed by extending the fiber into the headspace. After adequate sorption time, the fiber was withdrawn into the outer septum piercing needle, removed from the vial, and subsequently desorbed in the heated injection port of the GC. The sample was adsorbed about 5 to 7 minutes. Sample was desorbed for 3 min at 200 °C. Fibers can be cleaned to avoid analyte carryover by placing them in the injection port of the GC for a few minutes while maintaining the oven temperature at 250 °C. Any remaining analytes are in this way removed from the stationary phase of the fiber.

For comparison purposes, the different type of fiber are used and conventional headspace sampling was performed by inserting an air-tight syringe needle through the septum cap into the headspace above the sample and withdrawing 1 µL of the headspace. The syringe was then inserted into the injection port of a GCMS.

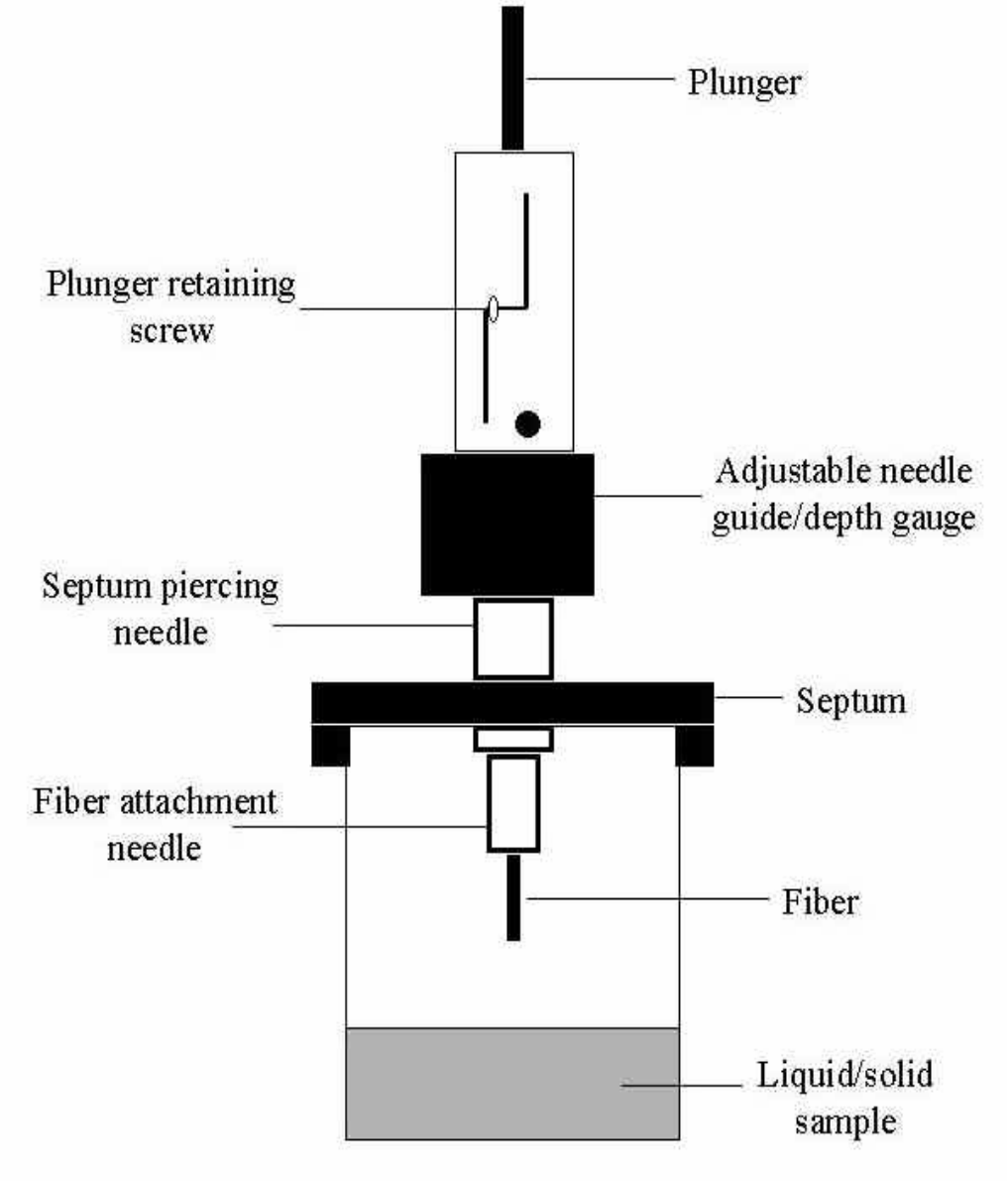



Figure 3.3: Schematic of the headspace SPME apparatus.

### 3.7 Gas Chromatography Mass Spectrometry Detector

All extracts were analyzed with a HP-5MS 5% Phenyl Methyl Siloxane and DB-WAX chromatographic column of GCMS. A 30 m x 250 μm i.d, film thickness 0.25 μm (95 % dimethyl-5 % diphenyl polysiloxane) capillary column (EQUITY 1) was used for all separations. SPME fibers were desorbed under splitless conditions. For all samples, the GC oven temperature was held at 40 °C during desorption, increased to 200 °C at 10 °C/min, and then held for 2 min (run time varied depending on desorption time). Injection port and transfer line temperatures were set at 230 °C and 250 °C, respectively. The GC carrier gas was helium at a column head pressure of 5 psi. The mass spectrometer was operated in electron impact mode and tuned to perfluorotributylamine (PFTBA). The mass spectrometer was scanned from *m*/*z* 50-350.

After a short, yet simple demonstration of the MS ChemStation software by the course instructor, we should be able to select peaks for identification. In our experience, students are increasingly computer-literate and experience no difficulty with operation of the Microsoft Windows-based HP ChemStation software. The software allows ready identification of mass spectra by reference comparison to spectra in a standard library database system (NIST).

**FLOW DIAGRAM FOR GCMS ANALYSIS**

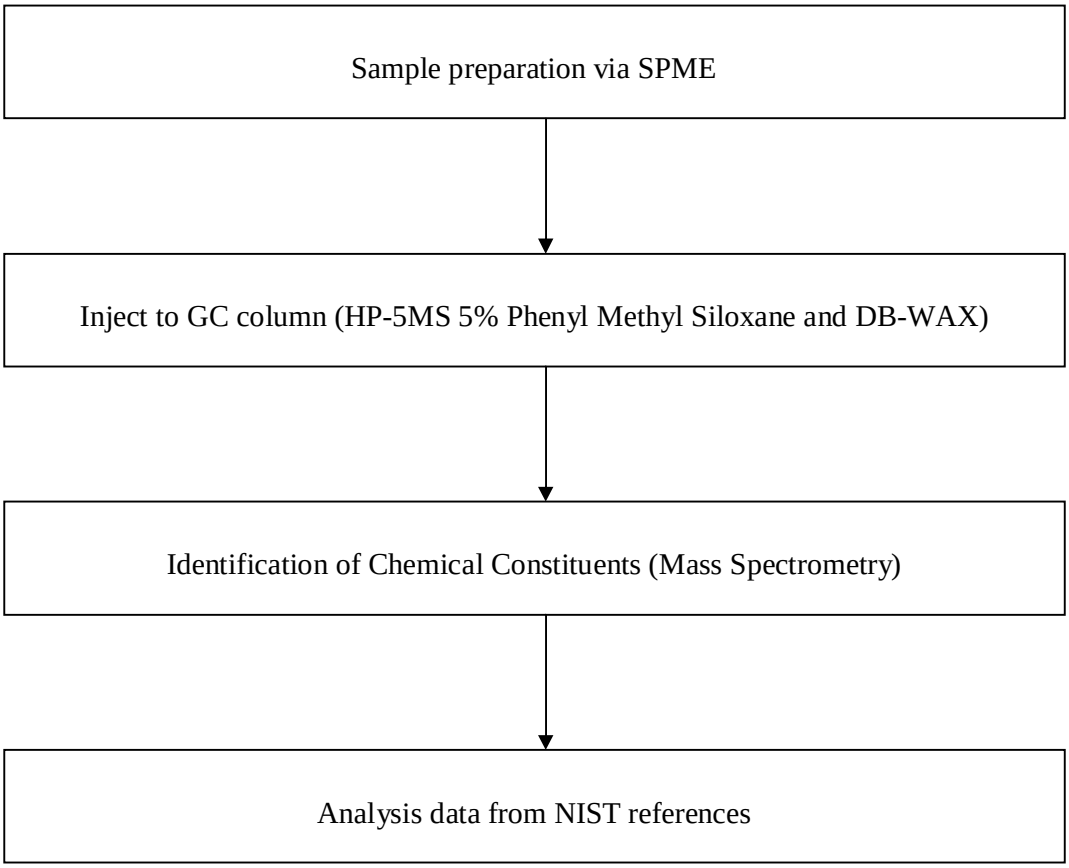



Figure 3.4: The flow diagram for GCMS analysis

### 3.7.1 Condition GCMS

Table 3.1: Gas Chromatograph Mass spectrometry Condition

| | |
|---|---|
| **Apparatus for GCMS** | Gas Chromatograph Mass Spectrometry HP5890 (Hewlett Packard Co.) |
| **Column** | (30m × 0.25mm, 0.25 µm film thickness) EQUITY 1 |
| **Stationary Phases** | HP-5MS 5% Phenyl Methyl Siloxane and DB-WAX |
| **Detector** | FID (Flame Ionization Detector) 250$^{o}$C |
| **Carrier Gas** | Helium |
| **Temperature Oven Programmed** | 200 $^{o}$C at 3 minutes |


Figure 3.5: Gas Chromatograph Mass Spectrometry HP5890

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Headspace-SPME Studies on Gaharu oil

Although SPME (Solid Phase Microextraction) has been originally developed for the rapid analysis pollutants in water it has found wide variety of use in the analysis of volatile organic compounds in foods, beverages, flavors, fragrances and essential oils. The components of the vapour phase are considered to be responsible for the odour of these products. Therefore headspace sampling has become the most preferred method in such analysis for the last decade.

Headspace-SPME is identified as a solvent free sample preparation technique in which a fused silica fiber coated with polymeric organic liquid is introduced into the headspace above the sample. The adsorption of the analytes is followed by a thermal desorption process by introducing the SPME fiber into the injection port of a gas chromatography.

In this context, the headspace was considered to be an alternative method to clarify the question about the fragrance of Agarwood oil. The SPME device included a fused silica fiber coating partially cross-linked with 100μm Polydimethylsiloxane (PDMS), 75μm Carboxen/Polydimethylsiloxane (CAR/PDMS) and 65μm Polydimethylsiloxane/divinylbenzene (PDMS/DVB). The SPME fibre was conditioned at 200 ºC for 3 min in the GC injector, according to the manufacturer's recommendations. For headspace sampling, 1 μL of gaharu essential oil was run with hydrodistillation method and introduced into a 4 ml glass vial.

The lists of components in gaharu essential oil that analyze by the GCMS are shown in table. Each table shows the result from different column of GCMS with the different type of fiber of headspace-SPME.

## 4.2 Spectrum and the constituents identified in the headspace-SPME experiments of Gaharu Essential Oil

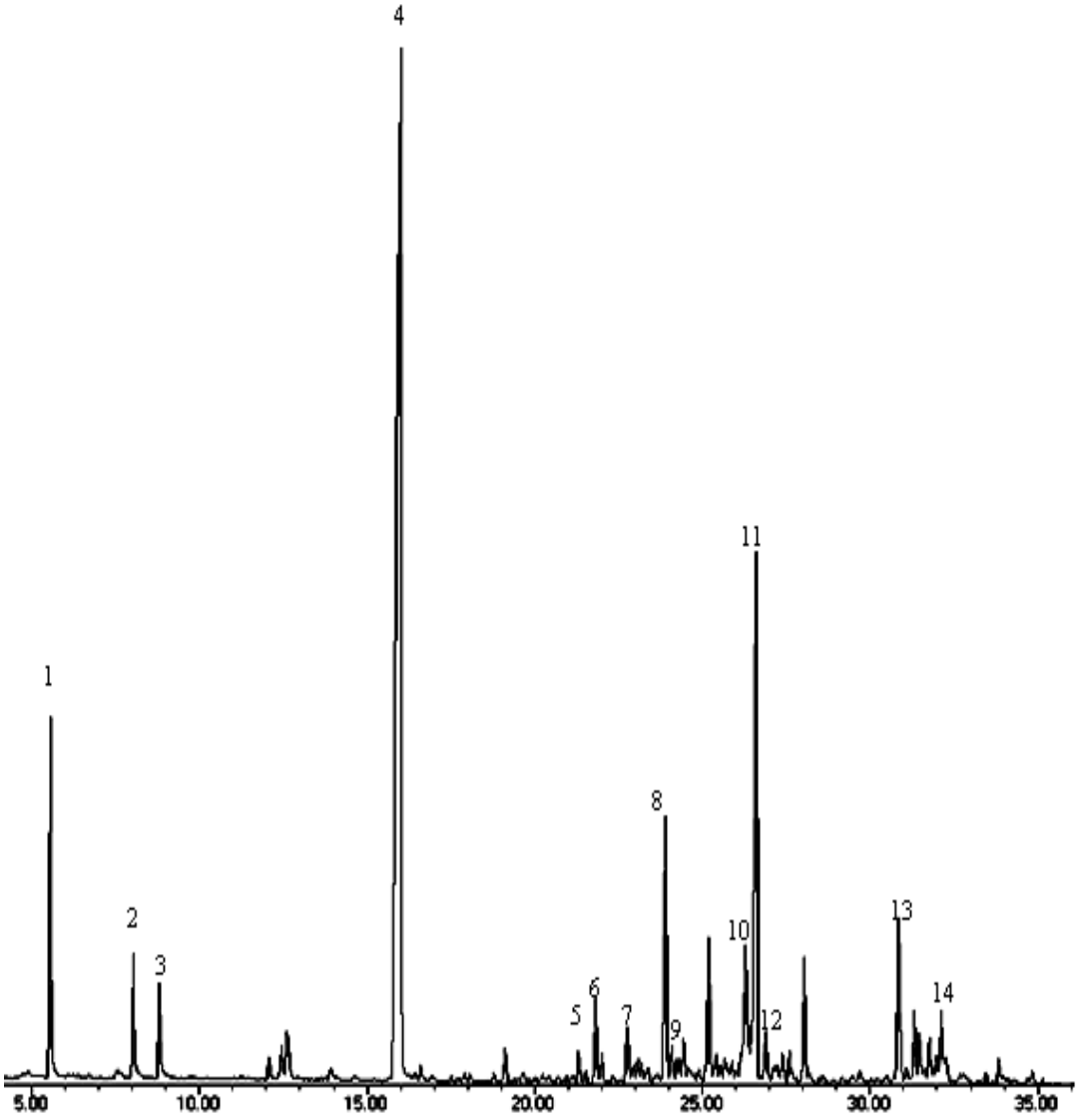



**Figure 4.1.** GCMS analysis of the headspace-SPME experiment of Agarwood Essential Oil (The chromatographic column used for analysis was HP-5MS 5% Phenyl Methyl Siloxane , 30 m x 250 μm i.d, film thickness 0.25 μm and 100μm Polydimethylsiloxane (PDMS) fiber).

**Table 4.1**. The constituents identified in the headspace-SPME (100μm PDMS fiber) experiments of Agarwood Essential Oil

| No | Compound | Quality | % Area | CAS No |
|---|---|---|---|---|
| 1. | Benzaldehyde | 97 | 5.33 | 000100-52-7 |
| 2. | Benzaldehyde, 2-hydroxy- | 94 | 2.29 | 000090-02-8 |
| 3. | Acetophenone | 95 | 1.51 | 000098-86-2 |
| 4. | 2-Butanone, 3-phenyl- | 96 | 40.27 | 000769-59-5 |
| 5. | Copaene | 97 | 0.62 | 003856-25-5 |
| 6. | 4-Ethylphenyl acetate | 83 | 1.70 | 003245-23-6 |
| 7. | α-Cedrene | 98 | 1.22 | 000469-61-4 |
| 8. | α-Guaiene | 99 | 5.16 | 003691-12-1 |
| 9. | α-Caryophyllene | 97 | 0.93 | 006753-98-6 |
| 10. | Eudesma-4(14),11-diene | 80 | 4.17 | 1000152-04-3 |
| 11. | δ-Guaiene | 99 | 11.69 | 003961-11-0 |
| 12. | Butyric acid,3-methyl-3-[2-isopropylphenyl]- | 83 | 1.20 | 092300-81-7 |
| 13. | τ-eudesmol | 94 | 3.44 | 001209-71-8 |
| 14. | β-Selinene | 91 | 1.03 | 017066-67-0 |

2-Butanone, 3-phenyl (40.27%),δ-Guaiene (11.69%), Benzaldehyde (5.33%), α-guaiene (5.16%), Eudesma-4(14),11-diene(4.17%), and τ-Eudesmol (3.44%) were the main constituents of the essential oil of Agarwood with the chromatographic column used for analysis was HP-5MS 5% Phenyl Methyl Siloxane , 30 m x 250 μm i.d, film thickness 0.25 μm and 100μm Polydimethylsiloxane (PDMS) fiber).

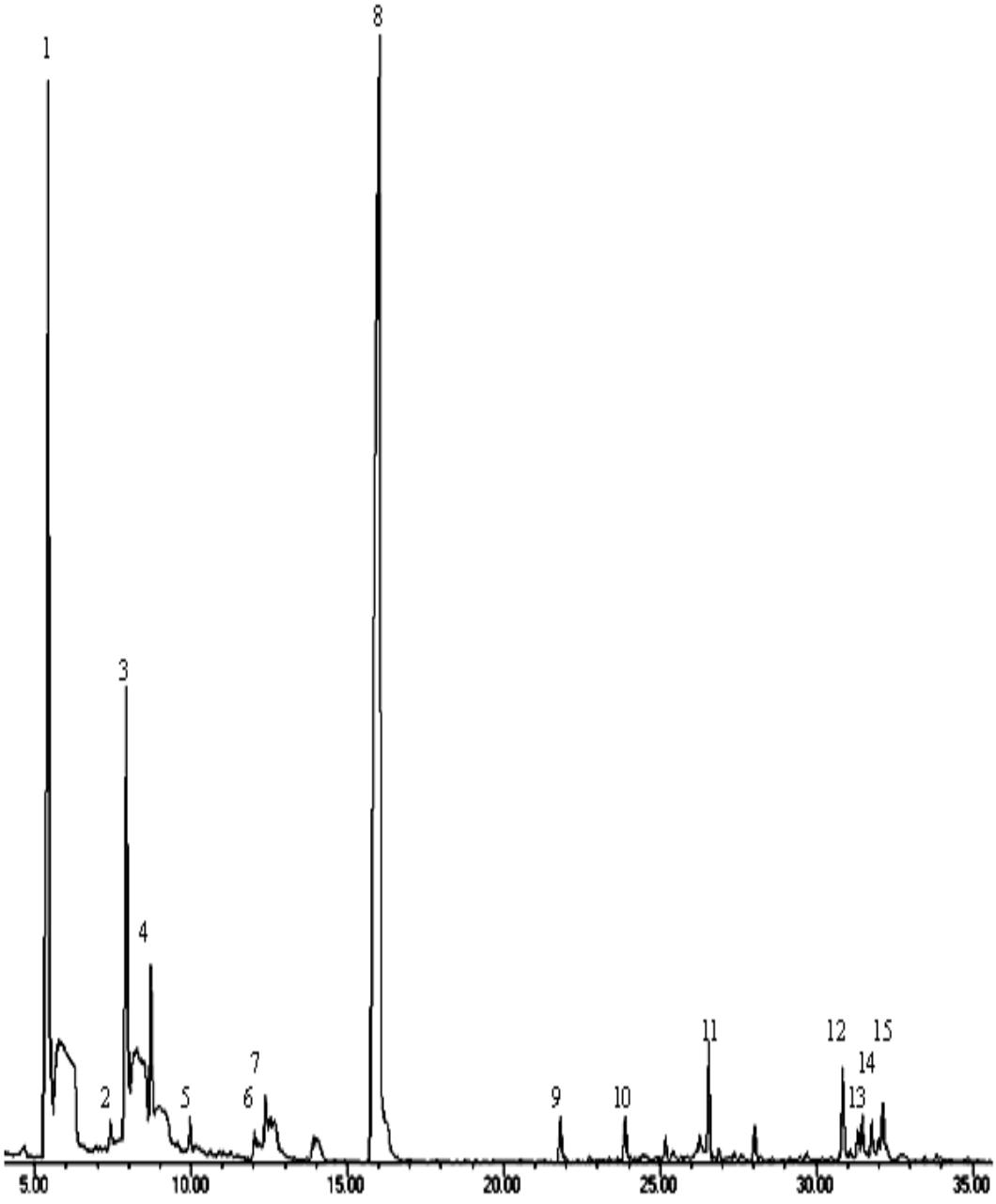



**Figure 4.2** GCMS analysis of the headspace-SPME experiment of Agarwood Essential Oil (The chromatographic column used for analysis was HP-5MS 5% Phenyl Methyl Siloxane , 30 m x 250 μm i.d, film thickness 0.25 μm and 75μm Carboxen/Polydimethylsiloxane (CAR/PDMS) fiber).

**Table 4.2.** The constituents identified in the headspace-SPME (75μm CAR/PDMS fiber) experiments of Agarwood Essential Oil

| No | Compounds | Quality | Area | CAS |
|---|---|---|---|---|
| 1 | Benzaldehyde | 97 | 18.25 | 100-52-7 |
| 2 | D-limonene | 93 | 0.38 | 5989-27-5 |
| 3 | Benzaldehyde, 2-hydroxy- | 94 | 7.38 | 90-02-8 |
| 4 | Acetophenone | 94 | 1.72 | 98-86-2 |
| 5 | Undecane | 91 | 0.43 | 1120-21-4 |
| 6 | Benzene,1-ethenyl-4-methoxy- | 96 | 0.37 | 637-69-4 |
| 7 | Ethanone, 1-(2-hydroxyphenyl)- | 95 | 1.16 | 118-93-4 |
| 8 | 2-butanone,3-phenyl- | 96 | 42.12 | 769-59-5 |
| 9 | 3,3-dimethyl-6-methylenecyclohexen | 81 | 0.62 | 20185-16-4 |
| 10 | A-Guaiene | 96 | 0.68 | 3691-12-1 |
| 11 | δ-Guaiene | 99 | 1.59 | 3691-11-0 |
| 12 | τ-Eudesmol | 94 | 1.32 | 1209-71-8 |
| 13 | Aromadendrene, (+)- | 70 | 0.63 | 489-39-4 |
| 14 | Aristolene | 78 | 0.45 | 1000150-14-9 |
| 15 | A-Bulnesene / δ-Guaiene | 89 | 0.62 | 3691-11-0 |

2-Butanone, 3-phenyl (42.12%), Benzaldehyde (18.25%), Benzaldehyde, 4-hydroxy- (7.38%), Acetophenone (1.72%) and δ-Guaiene (1.59%) were the main constituents of the essential oil of Agarwood with The chromatographic column used for analysis was HP-5MS 5% Phenyl Methyl Siloxane , 30 m x 250 μm i.d, film thickness 0.25 μm and 75μm Carboxen/Polydimethylsiloxane (CAR/PDMS) fiber).

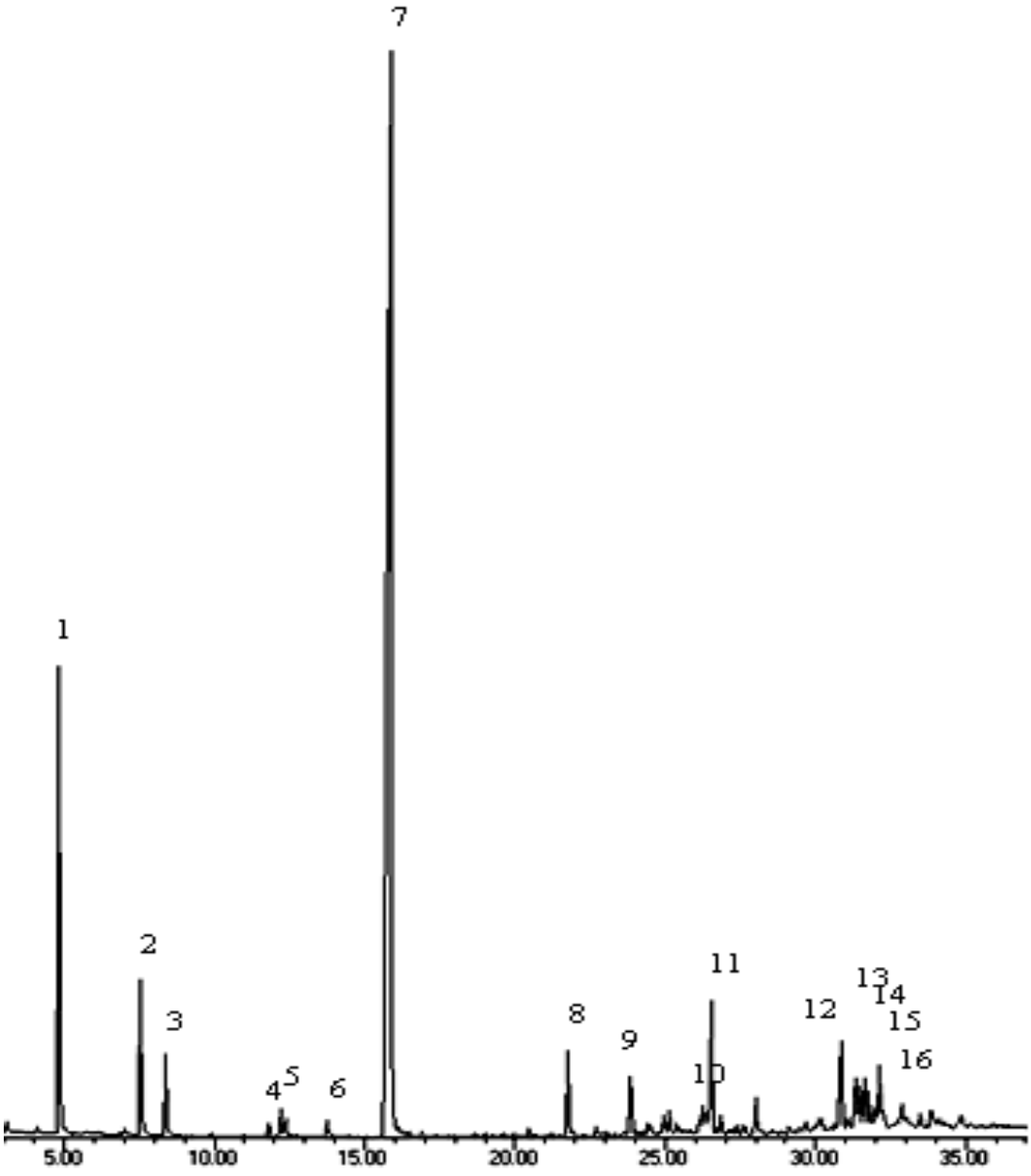



**Figure 4.3.** GCMS analysis of the headspace-SPME experiment of Agarwood Essential Oil (The chromatographic column used for analysis was HP-5MS 5% Phenyl Methyl Siloxane , 30 m x 250 µm i.d, film thickness 0.25 µm and 65µm Polydimethylsiloxane/divinylbenzene (PDMS/DVB)fiber).

**Table 4.3:** The constituents identified in the headspace-SPME (65μm PDMS/DVB fiber) experiments of Agarwood Essential Oil

| No | Compounds | Quality | % Area | CAS No. |
|---|---|---|---|---|
| 1. | Benzaldehyde | 97 | 6.79 | 000100-52-7 |
| 2. | Benzaldehyde, 4-hydroxy- | 89 | 3.45 | 000123-08-0 |
| 3. | Acetophenone | 95 | 1.34 | 000098-86-2 |
| 4. | Benzene, 1-ethenyl-4-methoxy- | 95 | 0.25 | 000637-69-4 |
| 5. | Ethanone, 1-(2-hydroxyphenyl)- | 94 | 0.52 | 000118-93-4 |
| 6. | Benzaldehyde, 3-methoxy- | 97 | 0.43 | 000591-31-1 |
| 7. | 2-Butanone, 3-phenyl- | 95 | 44.62 | 000769-59-5 |
| 8. | 1,3-Cyclopentadiene,5,5-dimethyl-1-ethyl- | 74 | 1.87 | 1000162-25-7 |
| 9. | α-Guaiene | 95 | 1.32 | 003691-12-1 |
| 10. | (-)-Isoaromadendrene-(V) | 83 | 1.13 | 1000156-14-3 |
| 11. | δ-Guaiene | 99 | 2.75 | 003961-11-0 |
| 12. | τ-eudesmol | 94 | 2.23 | 001209-71-8 |
| 13. | Agarospirol | 94 | 0.89 | 1460-73-7 |
| 14. | 2,6-bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol | 87 | 1.06 | 014035-34-8 |
| 15. | (-)-Aristolene | 83 | 0.94 | 6831-16-9 |
| 16. | (R)-Aromadendrene | 86 | 2.14 | 000489-39-4 |

2-Butanone, 3-phenyl (44.62%), Benzaldehyde (6.79%), Benzaldehyde, 4-hydroxy- (3.45%), δ-Guaiene (2.75%) and τ-eudesmol (2.23%) were the main constituents of the essential oil of Agarwood with the chromatographic column used for analysis was HP-5MS 5% Phenyl Methyl Siloxane , 30 m x 250 μm i.d, film thickness 0.25 μm and 65μm Polydimethylsiloxane/divinylbenzene (PDMS/DVB)fiber).

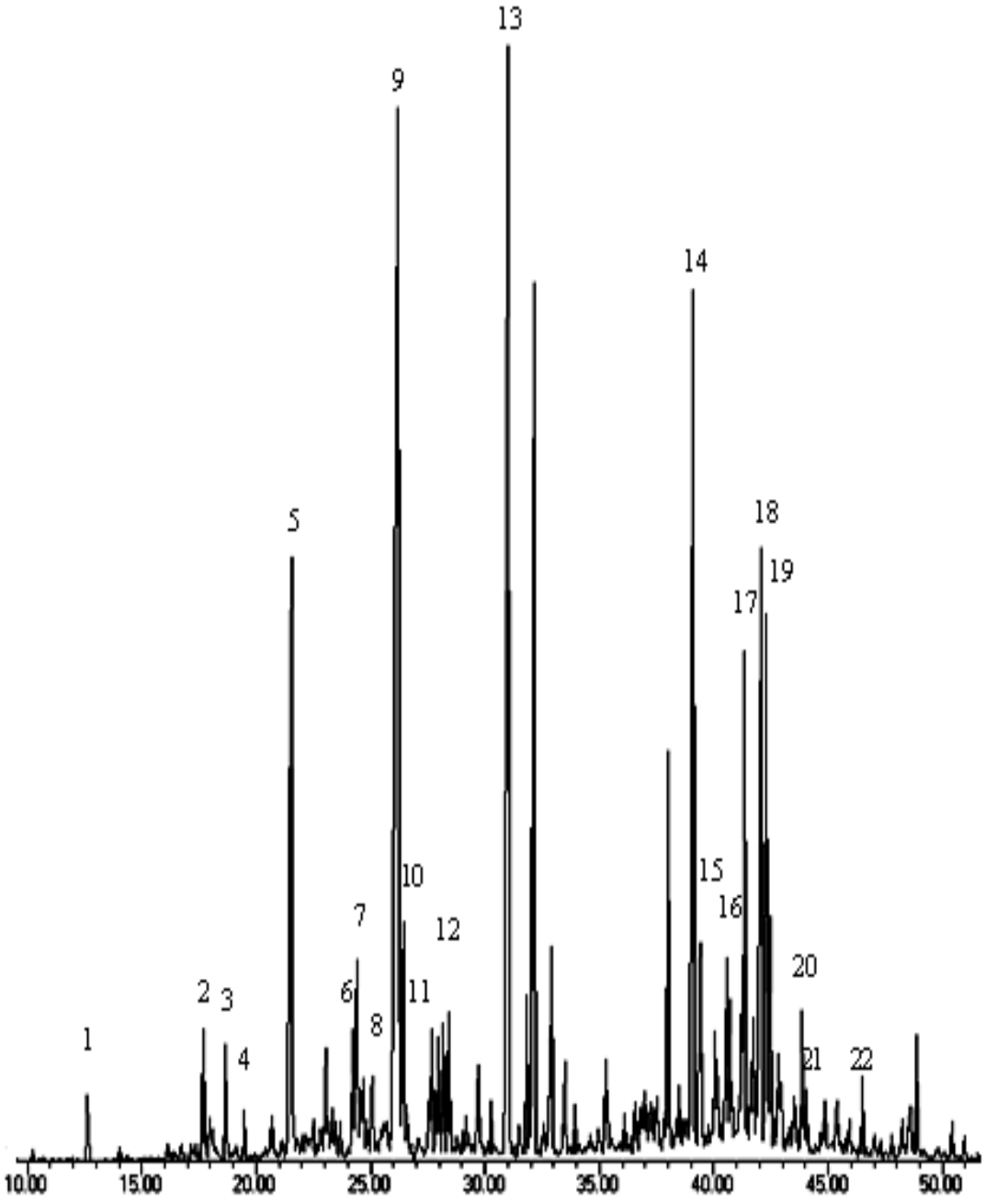



**Figure 4.4.** GCMS analysis of the headspace-SPME experiment of Agarwood Essential Oil (The chromatographic column used for analysis was DB-WAX , 30 m x 250 μm i.d, film thickness 0.25 μm and 100μm Polydimethylsiloxane (PDMS) fiber).

**Table 4.4.** The constituents identified in the headspace-SPME (100μm PDMS fiber) experiments of Agarwood Essential Oil

| No | Compounds | Quality | % Area | CAS No |
|---|---|---|---|---|
| 1 | Cyclohexasiloxane, dodecamethyl- | 91 | 0.46 | 000540-97-6 |
| 2 | α-Cubebene | 97 | 1.13 | 017699-14-8 |
| 3 | Benzaldehyde | 96 | 0.73 | 000100-52-7 |
| 4 | Cycloheptasiloxane,tetradecamethyl- | 92 | 0.27 | 000107-50-6 |
| 5 | α-Guaiene | 99 | 6.54 | 003691-12-1 |
| 6 | 1,4,7-Cycloundecatriene,1,5,9,9-tetramethyl-,Z,Z,Z- | 98 | 1.00 | 1000062-61-9 |
| 7 | Phenol,4,6-di(1,1-dimethylethyl)-2-methyl- | 70 | 1.55 | 000616-55-7 |
| 8 | α-Gurjunene | 97 | 0.6 | 000489-40-7 |
| 9 | δ-Guaiene | 99 | 17.13 | 003691-11-0 |
| 10 | Bi-1,3,5,7-cyclooctatetraene-1-yl | 90 | 2.02 | 006715-22-6 |
| 11 | Aromadendrene, dehydro- | 83 | 0.82 | 10000156-12-5 |
| 12 | Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl- | 98 | 0.87 | 000644-30-4 |
| 13 | 2-Butanone,3-phenyl- | 95 | 10.78 | 000769-59-5 |
| 14 | τ-Eudesmol | 93 | 7.88 | 001209-71-8 |
| 15 | β-Neoclovene | 84 | 2.19 | 056684-96-9 |
| 16 | τ-Gurjunene | 96 | 1.66 | 022567-17-5 |
| 17 | Agarospirol | 91 | 3.52 | 001460-73-7 |
| 18 | Tricycle[4.1.0.0(2,4)]heptanes,3,3,7,7-tetramethyl-5-(2-methyl-1-propenyl)- | 93 | 5.56 | 056348-21-1 |
| 19 | α-Selinene | 78 | 3.95 | 000473-13-2 |
| 20 | Bicycle[4.4.0]dec-1-ene,2-isopropyl-5-methyl-9-methylene | 70 | 1.56 | 150320-52-8 |
| 21 | β-Eudesmol | 98 | 0.87 | 000473-15-4 |
| 22 | Alloaromadendrene | 95 | 1.01 | 025246-27-9 |

δ-Guaiene (17.13%), 2-Butanone, 3-phenyl (10.78%), τ-eudesmol (7.88%), α-guaiene (6.54%) Tricycle[4.1.0.0(2,4)]heptanes,3,3,7,7-tetramethyl-5-(2-methyl-1-propenyl)- (5.56%) and Agarospirol (3.52%) were the main constituents of the essential oil of Agarwood with the chromatographic column used for analysis was DB-WAX, 30 m x 250 μm i.d, film thickness 0.25 μm and 100μm Polydimethylsiloxane (PDMS) fiber).

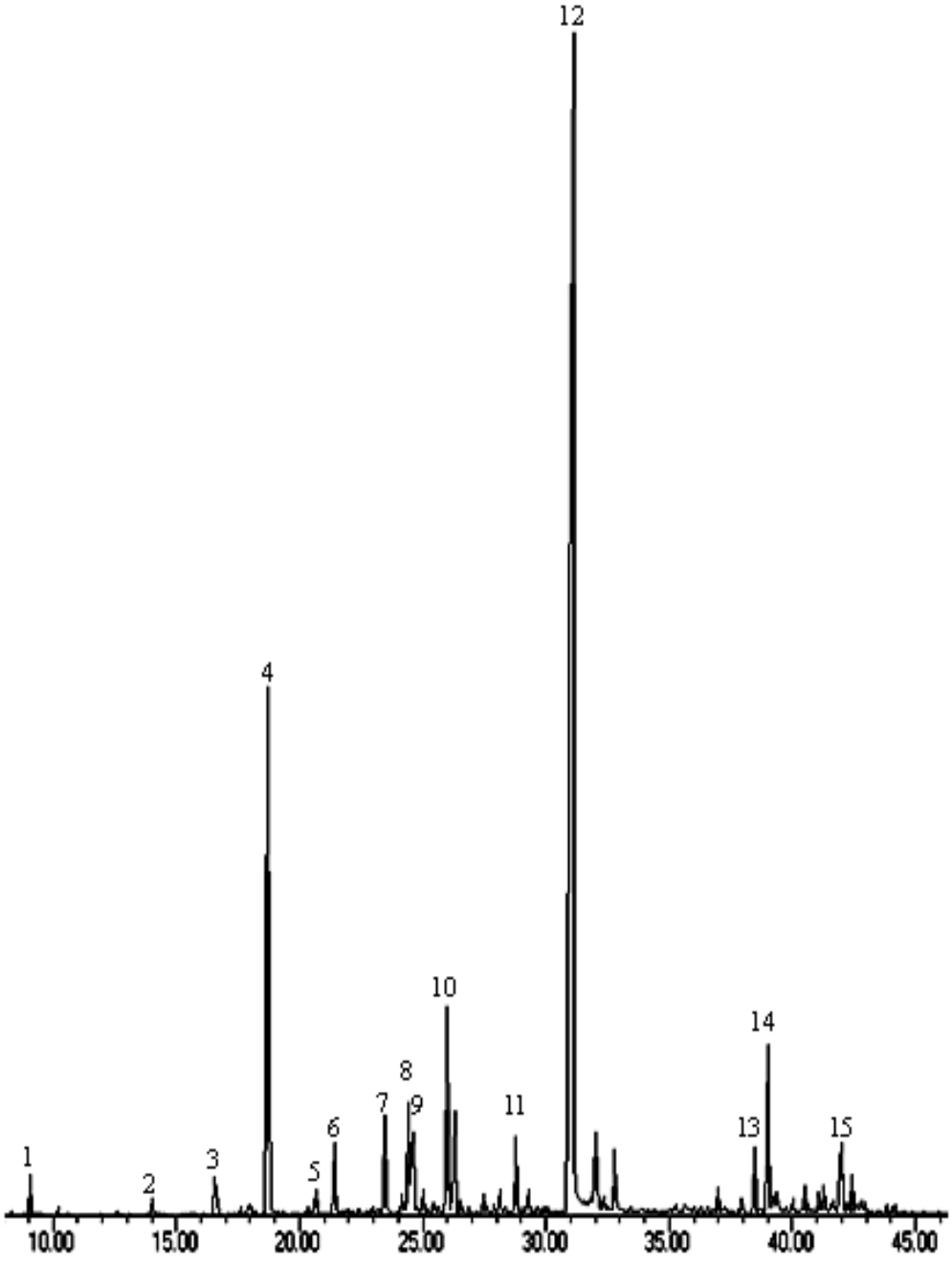



**Figure 4.5.** GCMS analysis of the headspace-SPME experiment of Agarwood Essential Oil (The chromatographic column used for analysis was DB-WAX, 30 m x 250 μm i.d, film thickness 0.25 μm and 75μm Carboxen/Polydimethylsiloxane (CAR/PDMS) fiber).

**Table 4.5.** The constituents identified in the headspace-SPME (75μm CAR/PDMS fiber) experiments of Agarwood Essential Oil

| No | Compounds | Quality | % Area | CAS |
|---|---|---|---|---|
| 1. | Styrene | 97 | 0.45 | 100-42-5 |
| 2. | Nonanal | 91 | 0.36 | 124-19-6 |
| 3. | Furfural | 91 | 1.19 | 98-01-1 |
| 4. | Benzaldehyde | 95 | 13.66 | 100-52-7 |
| 5. | 2-Furancarboxaldehyde,5-methyl- | 93 | 0.65 | 620-02-0 |
| 6. | A-Guaiene | 99 | 1.42 | 3691-12-1 |
| 7. | Acetophenone | 91 | 1.90 | 98-86-2 |
| 8. | Benzaldehyde,2-hydroxy- | 94 | 2.47 | 90-02-8 |
| 9. | Longifolene-(V4) | 76 | 1.44 | 61262-67-7 |
| 10. | δ-Guaiene | 99 | 4.34 | 3691-11-0 |
| 11. | Ethanone,1-(2-hydroxyphenyl)- | 94 | 1.53 | 118-93-4 |
| 12. | 2-Butanone,3-phenyl- | 95 | 49.80 | 769-59-5 |
| 13. | Phenol, 4-methyl- | 97 | 1.17 | 106-44-5 |
| 14. | τ-Eudesmol | 89 | 3.02 | 1209-71-8 |
| 15. | Neoisolongifolene | 92 | 2.02 | 1000156-12-4 |

2-Butanone, 3-phenyl (49.80%), Benzaldehyde (13.66%), δ-guaiene (4.34%), τ-Eudesmol (3.02%) and Benzaldehyde,2-hydroxy- (2.47%) were the main constituents of the essential oil of Agarwood with the chromatographic column used for analysis was DB-WAX, 30 m x 250 μm i.d, film thickness 0.25 μm and 75μm Carboxen/Polydimethylsiloxane (CAR/PDMS) fiber).

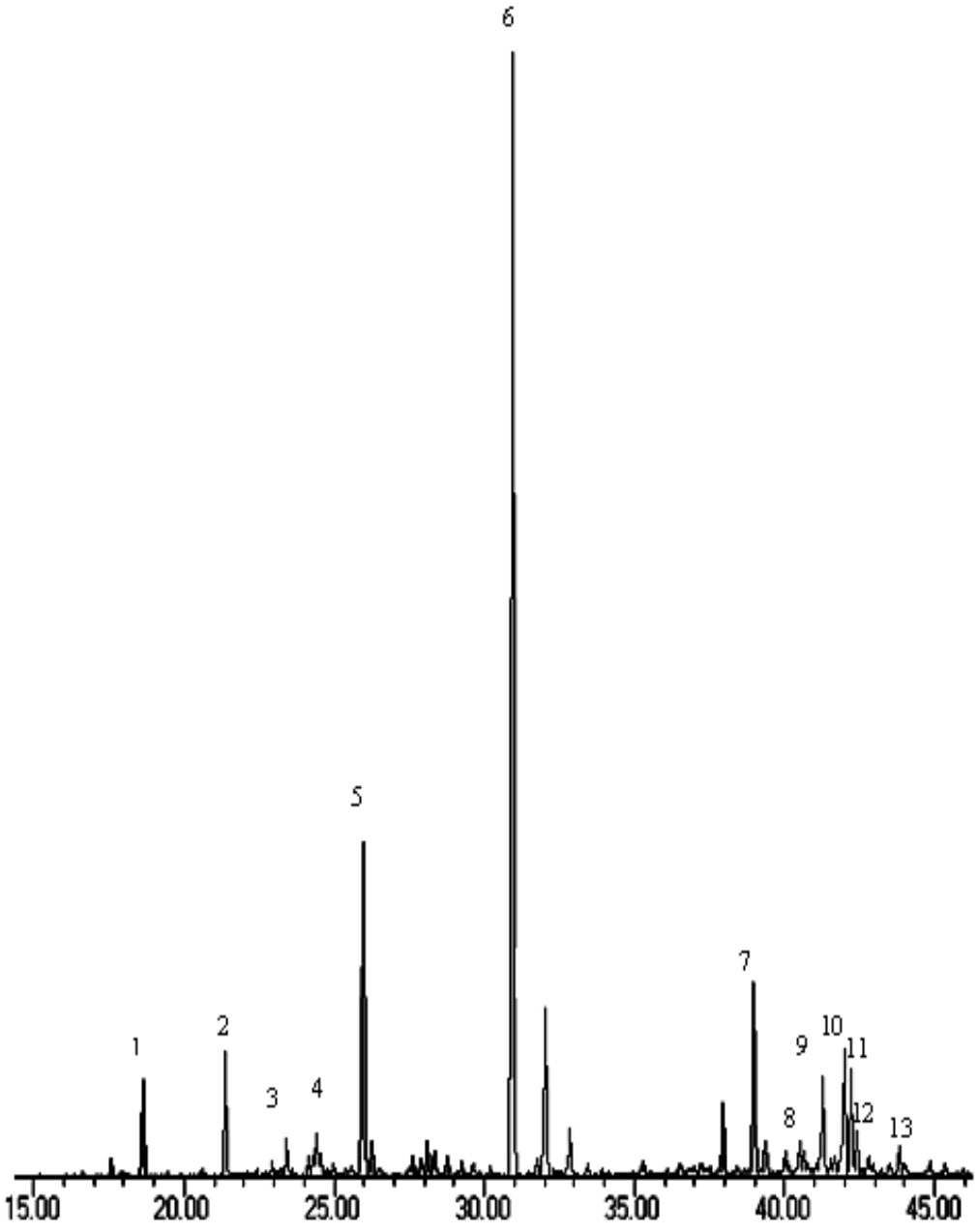



**Figure 4.6.** GCMS analysis of the headspace-SPME experiment of Agarwood Essential Oil (The chromatographic column used for analysis was DB-WAX, 30 m x 250 μm i.d, film thickness 0.25 μm and 65μm Polydimethylsiloxane/divinylbenzene (PDMS/DVB)fiber).

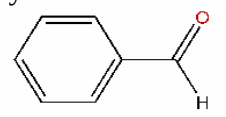
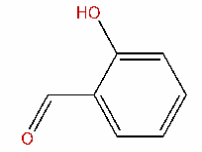
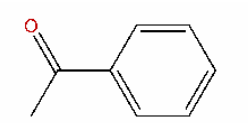
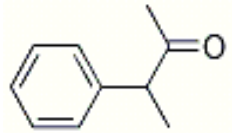
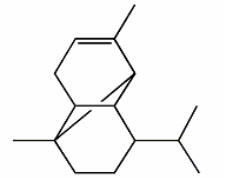
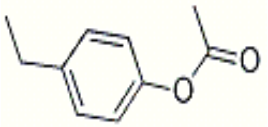
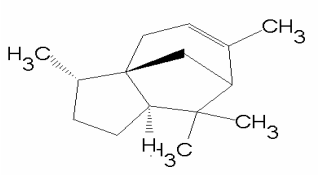
**Table 4.6.** The constituents identified in the headspace-SPME (65μm PDMS/DVB fiber) experiments of Agarwood Essential Oil

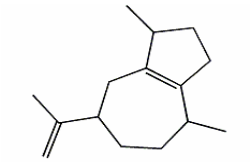
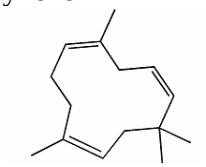
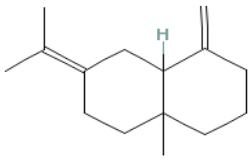
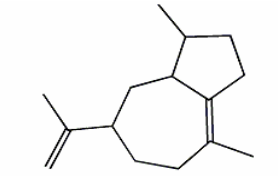
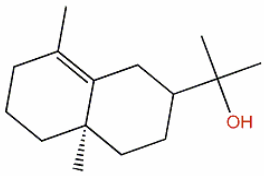
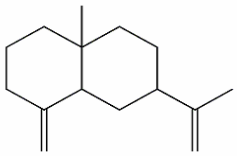
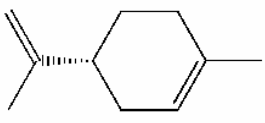
| No | Compound | Quality | % Area | CAS No |
|---|---|---|---|---|
| 1. | Benzaldehyde | 96 | 11.43 | 100-52-7 |
| 2. | A-Guaiene | 99 | 3.20 | 3691-12-1 |
| 3. | Acetophenone | 94 | 2.64 | 98-86-2 |
| 4. | α-Caryophyllene | 97 | 0.41 | 6753-98-6 |
| 5. | δ-Guaiene | 99 | 8.31 | 3691-11-0 |
| 6. | 2-Butanone,3-phenyl- | 95 | 49.06 | 000769-59-5 |
| 7. | δ-Selinene | 89 | 5.36 | 28624-23-9 |
| 8. | τ-Gurjunene | 95 | 0.95 | 22567-17-5 |
| 9. | Agarospirol | 90 | 1.93 | 1460-73-7 |
| 10. | Aristolene | 90 | 4.77 | 1000150-14-9 |
| 11. | Eudesma-3,7(11)-diene / Selina-3,7(11)-diene | 81 | 2.83 | 6813-21-4 |
| 12. | 1,6-Cyclodecadiene,1-methyl-5-methylene-8-(1-methylethyl)-,[s-(E,E)]- | 70 | 1.04 | 23986-74-5 |
| 13. | 1R,3Z,9S-2,6,10,10-tetramethylbicyclo[7.2.0]undeca-2,6-diene | 95 | 0.85 | 1000140-07-4 |

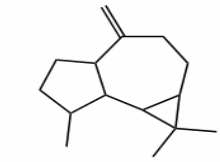
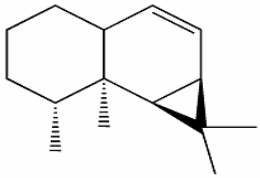
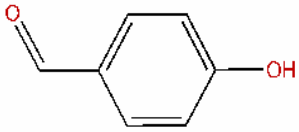
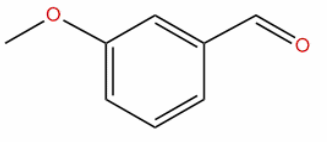
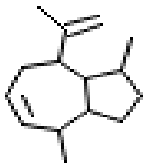
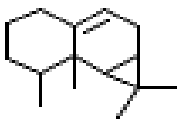
2-Butanone, 3-phenyl (49.06%), Benzaldehyde (11.43%), δ-guaiene (8.31%), δ-selinene (5.36%) and Aristolene (4.77%) were the main constituents of the essential oil of Agarwood with the chromatographic column used for analysis was DB-WAX, 30 m x 250 μm i.d, film thickness 0.25 μm and 75μm Carboxen/Polydimethylsiloxane (CAR/PDMS) fiber).

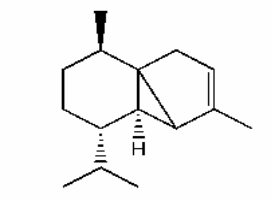
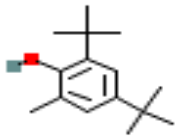
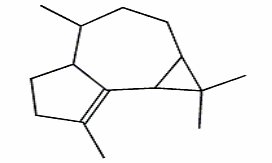
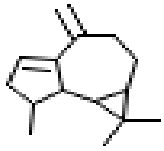
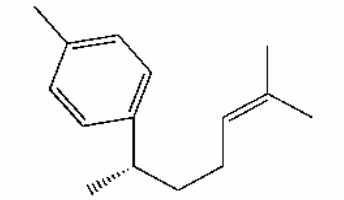
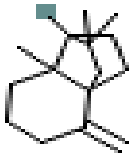
## 4.3 Analysis for each Chemical Compound in Gaharu Oil

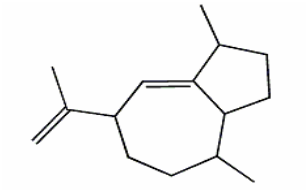
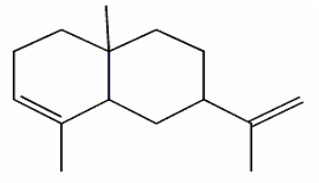
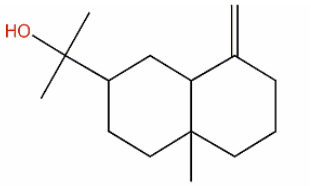
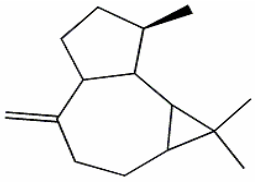
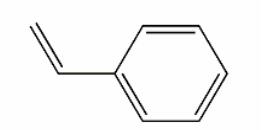
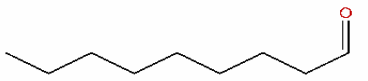
**Table 4.7**: Analysis Chemical Compound

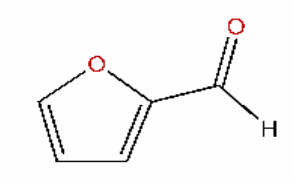
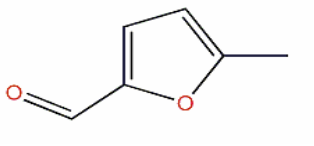
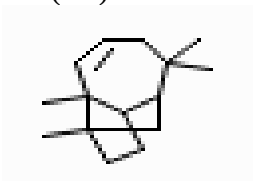
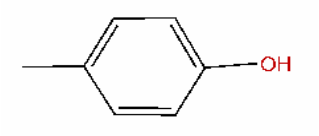
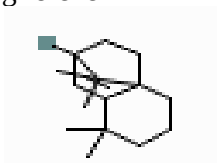
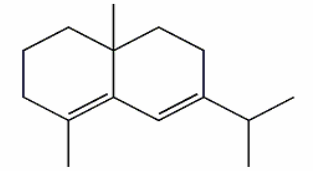
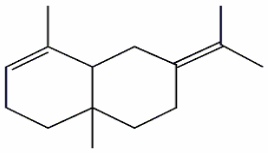
| No | Compound | Chemical Compound |
|---|---|---|
| 1. | Benzaldehyde  | **Formula**: $C_7H_6O$<br>**CAS:** 100-52-7<br>**Odor Characteristic**: Burnt sugar, Almond, Woody |
| 2. | Benzaldehyde, 2-hydroxy-  | **Formula:**$C_7H_6O_2$<br>**CAS:**90-02-8<br>Flora compound |
| 3. | Acetophenone  | **Formula**: $C_8H_8O$<br>**CAS:** 98-86-2 |
| 4. | 2-Butanone, 3-phenyl-  | **Formula**: $C_{10}H_{12}O$<br>**CAS:** 769-59-5 |
| 5. | Copaene  | **Formula:** $C_{15}H_{24}$<br>**CAS:** 3856-25-5<br>**Odor Characteristic**: Woody, Earthy |
| 6. | 4-Ethylphenyl acetate  | **Formula**: $C_{10}H_{12}O_2$<br>**CAS:** 3245-23-6 |
| 7. | α-Cedrene  | **Formula:** $C_{15}H_{24}$<br>**CAS:** 000469-61-4 |

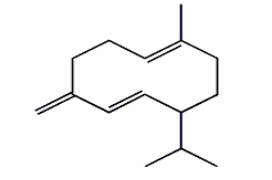
| | | |
|---|---|---|
| 8. | α-Guaiene  | **Formula**: $C_{15}H_{24}$<br>**CAS**:3691-12-1<br>**Odor Characteristic:**<br>Sweet-woody, Balsamic, Peppery |
| 9. | α-Caryophyllene  | **Formula:** $C_{15}H_{24}$<br>**CAS:**6753-98-6<br>**Odour Characteristic:**<br>Oily, Fruity, Woody |
| 10. | Eudesma-4(14),11-diene   | **Formula:** $C_{15}H_{24}$<br>**CAS:** 1000152-04-3 |
| 11. | δ-Guaiene / A-Bulnesene  | **Formula:** $C_{15}H_{24}$<br>**CAS:**3691-11-0 |
| 12. | τ-eudesmol   | **Formula**: $C_{15}H_{26}O$<br>**CAS**:1209-71-8<br>**Odour Characteristic:**<br>Sweet-woody |
| 13. | β-Selinene  | **Formula:**$C_{15}H_{24}$<br>**CAS:**17066-67-0<br>**Odor Characteristic:** Herbaceous |
| 14. | D-limonene  | **Formula:**$C_{10}H_{16}$<br>**CAS:**5989-27-5<br>**Odor Characteristic:**<br>Citrus-like, Orange, Fruity, Peely |

| | | |
|---|---|---|
| 15. | Aromadendrene, (+)-<br>  | **Formula:**$C_{15}H_{24}$<br>**CAS:**489-39-4 |
| 16. | Aristolene<br>  | **Formula:** $C_{15}H_{24}$<br>**CAS:** 1000150-14-9 |
| 17. | Benzaldehyde, 4-hydroxy-<br>  | **Formula:** $C_7H_6O_2$<br>**CAS:**123-08-0 |
| 18. | Benzaldehyde, 3-methoxy-<br>  | **Formula:** $C_8H_8O_2$<br>**CAS:**591-31-1 |
| 19. | (-)-Isoaromadendrene-(V)<br>  | **Formula:** $C_{15}H_{24}$<br>**CAS:** 1000156-14-3 |
| 20. | Agarospirol<br>  | **Formula:** $C_{15}H_{26}O$<br>**CAS:** 001460-73-7 |
| 21. | (-)-Aristolene<br>  | **Formula:** $C_{15}H_{24}$<br>**CAS:** 6831-16-9 |

| | | |
|---|---|---|
| 22. | α-Cubebene  | **Formula:**$C_{15}H_{24}$<br>**CAS:**17699-14-8<br>**Odor Characteristic:**<br>Mild waxy, Woody |
| 23. | Phenol,4,6-di(1,1-dimethylethyl)-2-methyl-  | **Formula:** $C_{15}H_{24}O$<br>**CAS:** 000616-55-7 |
| 24. | α-Gurjunene  | **Formula:** $C_{15}H_{24}$<br>**CAS:** 489-40-7<br>**Odour Characteristic:**<br>Earthy, Mango-like |
| 25. | Aromadendrene, dehydro-  | **Formula:** $C_{15}H_{22}$<br>**CAS:** 10000156-12-5 |
| 26. | Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl-<br>alpha-curcumene  | **Formula:** $C_{15}H_{22}$<br>**CAS:**644-30-4 |
| 27. | β-Neoclovene  | **Formula:** $C_{15}H_{24}$<br>**CAS:** 056684-96-9 |

| | | |
|---|---|---|
| 28. | τ-Gurjunene  | **Formula:** $C_{15}H_{24}$<br>**CAS**:22567-17-5 |
| 29. | α-Selinene  | **Formula:**$C_{15}H_{24}$<br>**CAS:**473-13-2<br>**Odor Characteristic:**<br>Pepper-like, Orange |
| 30. | β-Eudesmol   | **Formula:**$C_{15}H_{26}O$<br>**CAS**:473-15-4<br>**Odour Characteristic:**<br>Green, Woody, Yuzu-like, Sweet |
| 31. | Alloaromadendrene  | **Formula:** $C_{15}H_{24}$<br>**CAS:**25246-27-9 |
| 32. | Styrene  | **Formula:** $C_8H_8$<br>**CAS:**100-42-5<br>**Odour Characteristic:**<br>Pungent, Aromatic, Fragrant, Roasty |
| 33. | Nonanal   | **Formula**:$C_9H_{18}O$<br>**CAS:**124-19-6<br>**Odour Characteristic:**<br>Gravy, Green, Tallowy, Fruity, Gas, Chlorine, Floral, Waxy, Sweet, Melon, Soapy, Fatty, Lavender, Citrus fruit |

| | | |
|---|---|---|
| 34. | Furfural  | **Formula:**$C_5H_4O_2$<br>**CAS:**98-01-1<br>**Odor Characteristic:**<br>Woody, Almond, Sweet, Fruity, Flowery |
| 35. | 2-Furancarboxaldehyde,5-methyl-  | **Formula:**$C_6H_6O_2$<br>**CAS**:620-02-0<br>**Odor Characteristic:**<br>Caramel, Burnt sugar, Spicy, Acid, Coffee |
| 36. | Longifolene-(V4)  | **Formula:** $C_{15}H_{24}$<br>**CAS:** 61262-67-7 |
| 37. | Phenol, 4-methyl-  | **Formula:**$C_7H_8O$<br>**CAS:**106-44-5<br>**Odour Characteristic:**<br>Smokey, Phenolic |
| 38. | Neoisolongifolene  | **Formula:** $C_{15}H_{24}$<br>**CAS:** 1000156-12-4 |
| 39. | δ-Selinene  | **Formula:**$C_{15}H_{24}$<br>**CAS:**28624-23-9 |
| 40. | Eudesma-3,7(11)-diene / Selina-3,7(11)-diene  | **Formula**:$C_{15}H_{24}$<br>**CAS:**6813-21-4<br>**Odour Characteristic:**Woody |

| | | |
|---|---|---|
| 41. | 1,6-Cyclodecadiene,1-methyl-5-methylene-8-(1-methylethyl)-,[s-(E,E)]-<br>  | **Formula:** $C_{15}H_{24}$<br>**CAS:**23986-74-5<br>**Odor Characteristic:**<br>Oily, Green, Woody |
| 42. | 1R,3Z,9S-2,6,10,10-tetramethylbicyclo[7.2.0]undeca-2,6-diene<br>  | **Formula:** $C_{15}H_{24}$<br>**CAS:** 1000140-07-4 |

## 4.4 Analysis Comparison of Chemical Components between Columns of GCMS

**Table 4.8** Comparison between Chemical Component with difference Type of Fiber SPME and Column GCMS

| No | Compound | HP-5MS | | | DB-WAX | | |
|---|---|---|---|---|---|---|---|
| | | PDMS /DVB | CAR /PDMS | PDMS | PDMS /DVB | CAR /PDMS | PDMS |
| 1. | Benzaldehyde | √ | √ | √ | √ | √ | √ |
| 2. | Benzaldehyde, 2-hydroxy- | | √ | √ | | √ | |
| 3. | Acetophenone | √ | √ | √ | √ | √ | |
| 4. | 2-Butanone, 3-phenyl- | √ | √ | √ | √ | √ | √ |
| 5. | Copaene | | | √ | | | |
| 6. | 4-Ethylphenyl acetate | | | √ | | | |
| 7. | α-Cedrene | | | √ | | | |
| 8. | α-Guaiene | √ | √ | √ | √ | √ | √ |
| 9. | α-Caryophyllene | | | √ | √ | | |
| 10. | Eudesma-4(14),11-diene | | | √ | | | |
| 11. | δ-Guaiene / A-Bulnesene | √ | √ | √ | √ | √ | √ |
| 12. | Butyric acid,3-methyl-3-[2-isopropylphenyl]- | | | √ | | | |
| 13. | τ-eudesmol | √ | √ | √ | | √ | √ |
| 14. | β-Selinene | | | √ | | | |
| 15. | D-limonene | | √ | | | | |
| 16. | Undecane | | √ | | | | |
| 17. | Benzene,1-ethenyl-4-methoxy- | √ | √ | | | | |
| 18. | Ethanone, 1-(2-hydroxyphenyl)- | √ | √ | | | √ | |
| 19. | 3,3-dimethyl-6-methylenecyclohexen | | √ | | | | |
| 20. | Aromadendrene, (+)- | | √ | | | | |
| 21. | Aristolene | | √ | | √ | | |
| 22. | Benzaldehyde, 4-hydroxy- | √ | | | | | |
| 23. | Benzaldehyde, 3-methoxy- | √ | | | | | |
| 24. | 1,3-Cyclopentadiene,5,5-dimethyl-1-ethyl- | √ | | | | | |
| 25. | (-)-Isoaromadendrene-(V) | √ | | | | | |
| 26. | Agarospirol | √ | | | √ | | √ |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 27. | 2,6-bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol | √ | | | | | |
| 28. | (-)-Aristolene | √ | | | | | |
| 29. | (R)-Aromadendrene | √ | | | | | |
| 30. | Cyclohexasiloxane, dodecamethyl- | | | | | | √ |
| 31. | α-Cubebene | | | | | | √ |
| 32. | Cycloheptasiloxane,tetradecamethyl- | | | | | | √ |
| 33. | 1,4,7-Cycloundecatriene,1,5,9,9-tetramethyl-,Z,Z,Z- | | | | | | √ |
| 34. | Phenol,4,6-di(1,1-dimethylethyl)-2-methyl- | | | | | | √ |
| 35. | α-Gurjunene | | | | | | √ |
| 36. | Bi-1,3,5,7-cyclooctatetraene-1-yl | | | | | | √ |
| 37. | Aromadendrene, dehydro- | | | | | | √ |
| 38. | Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl- | | | | | | √ |
| 39. | β-Neoclovene | | | | | | √ |
| 40. | τ-Gurjunene | | | | √ | | √ |
| 41. | Tricycle[4.1.0.0(2,4)]heptanes,3,3,7,7-tetramethyl-5-(2-methyl-1-propenyl)- | | | | | | √ |
| 42. | α-Selinene | | | | | | √ |
| 43. | Bicycle[4.4.0]dec-1-ene,2-isopropyl-5-methyl-9-methylene | | | | | | √ |
| 44. | β-Eudesmol | | | | | | √ |
| 45. | Alloaromadendrene | | | | | | √ |
| 46. | Styrene | | | | | √ | |
| 47. | Nonanal | | | | | √ | |
| 48. | Furfural | | | | | √ | |
| 49. | 2-Furancarboxaldehyde,5-methyl- | | | | | √ | |
| 50. | Longifolene-(V4) | | | | | √ | |
| 51. | Phenol, 4-methyl- | | | | | √ | |
| 52. | Neoisolongifolene | | | | | √ | |
| 53. | δ-Selinene | | | | √ | | |
| 54. | Eudesma-3,7(11)-diene / Selina-3,7(11)-diene | | | | √ | | |
| 55. | 1,6-Cyclodecadiene,1-methyl-5-methylene-8-(1-methylethyl)-,[s-(E,E)]- | | | | √ | | |
| 56. | 1R,3Z,9S-2,6,10,10-tetramethylbicyclo[7.2.0]undeca-2,6-diene | | | | √ | | |

All of the gaharu oils gave out the distinctive gaharu aroma, however, the colour of the oils may vary from greenish brown to dark reddish brown. In general, all of the gaharu oils were complex mixtures of sesquiterpene hydrocarbons, sesquiterpene alcohols and aliphatic hydrocarbons and difficult to be identified based on MS alone. Some of the chemical constituents of the gaharu oils were identified by comparison of their mass spectral data with the existing Wiley library and reference library spectral data and comparison of their calculated retention indices with literature values.

The list of common chemical compounds detected in selected gaharu oils from differences of SPME fiber and chromatographic column are shown in Table 4.7. The results of this work indicate that there are some similarities and variations in the chemical composition of several of Grade C gaharu oil samples tested. Benzaldehyde, 2-Butanone, 3-phenyl-, alpha-Guaiene and gamma-Guaiene were some major of the chemical compounds found occurring in all the oils studied.

An aroma compound, also known as odorant, aroma, fragrance, flavor, is a chemical compound that has a smell or odor. A chemical compound has a smell or odor when two conditions are met is the compounds needs to be volatile, so it can be transported to the olfactory system in the upper part of the nose, and it needs to be in a sufficiently high concentration to be able to interact with one or more of the olfactory receptors. Benzaldehyde is the major aroma compound of aldehyde in essential oil. The odor characteristics are burnt sugar, almond, and woody.

The primary component found is the 2-Butanone 3-Phenyl. It is the monoterpene compounds that are found in nearly all essential oils and have a structure of 10 carbon atoms and at least one double bond. Alpha-Guaiaene and gamma-Guaiaene are the major sesquiterpenes consists of 15 carbon atoms and have complex pharmacological actions.

The others components are the oxygenated compound consist of phenols and alcohols. Phenols found normally have a carbon side chain. Due to the nature of

phenols, essential oils that are high in them should be used in low concentrations and for short periods of time, since they can lead to toxicity if used over long periods of time, as the liver will be required to work harder to excrete them. It can also cause skin and mucus membrane irritants and although they have great antiseptic qualities, like cinnamon and clove oil, they can cause severe skin reactions.

Alcohols found in the essential oil like monoterpene alcohol on the other hand have good antiseptic, anti-viral and anti-fungal properties with very few side effects such as skin irritation or toxicity and have an uplifting energizing effect. As for sesquiterpene alcohols, they are not commonly found in essential oils but when found they have great properties, which include liver and glandular stimulant, anti-allergen and anti-inflammatory. For the rest of the other components, aldehydes, ketones, esters, ethers, and oxides are found in a small quantity.

Gaharu oil is the interesting samples analyzed and popular choice of extraction of essential oil. These samples were easily extracted by SPME fibers due to their volatility. There are a few points of interest to note from our experiences with SPME. First of all, the fiber assembly is fragile, thus making it possible to bend the outer syringe needle or damage the fiber. Although the fiber assembly is easy to use, great care must be demonstrated to avoid damaging the fiber. Second, we liked the 7 μm bonded polydimethylsiloxane fiber because of its robustness. Bonded phase fibers can be submersed directly into a liquid matrix during sampling and can be rinsed with organic solvents. Another point of concern involves injection seals.

We chose to use a Merlin microseal instead of a septum for several reasons. The diameter of the outer syringe needle cores standard septa easily, requiring them to be replaced frequently. Pre-drilled septa work better and last longer, but the septum retainer nut has to be tightened beyond normal tightening to keep them from leaking. Due to the nature of the septum retainer nut, the fiber assembly will not rest firmly unless it is held in place. We found that a ring stand with a clamp works well for holding the fiber assemblies in place during desorption. A Merlin microseal not only lasts longer than standard septa, but also allows the base of the fiber assembly to rest freely on the microseal's housing.

GCMS profiles are currently being used to assist the detection and authentication of gaharu. Generating GC/MS profiles of gaharu samples of various grades and from different sources will be helpful towards developing suitable chemical marker for grading gaharu.

# CHAPTER 5

# CONCLUSION AND RECOMMENDATION

## 5.1 Conclusion

This study of analysis chemical compound in gaharu essential oil via solid phase micro extraction and gas chromatography mass spectrometry is successfully complete. The analysis is to compare the chemical compound that present in gaharu oil based on differentiate type of SPME fiber and column in GCMS.

This laboratory experiment presents an opportunity for students to use a relatively new, simple, yet rapid, solventless technique to extract volatile fragrance and flavor components from the headspace region of samples. A typical experiment involving SPME requires minimal sample preparation and analysis times are less than 30 minutes. Multiple analyses can be performed within the time span allotted for a typical laboratory class period. SPME offers a relatively inexpensive way to analyze fairly complex samples that might otherwise be difficult to analyze by conventional headspace analysis.

From this result, we can conclude that gaharu essential oils consists of mixtures of hydrocarbons such as terpenes, sesquiterpenes, oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols, phenol ethers), and a small percentage of viscid or solid nonvolatile residue (paraffin, waxes). The oxygenated compounds are the principal odor carriers, although the terpenes and sesquiterpenes, too, contribute in some degree to the total odor and flavor value of the oil.

## 5.2 Recommendations

From this analysis, the several important recommendations should be carried for the future research to know the chemical compound that obtains in gaharu essential oil. There are included:

1) Using gas chromatography with a flame ionization detector (FID) if a GCMS instrument is not available. Quantitative SPME experiments can also be performed.

2) Conduct the variety of process extraction to get the best quality of chemical compound and reduced the costing and timing.

3) Considered about new technologies for analytical equipment that can give the best and suitable research to analyze the compound.

4) The new scope and scale for analyzing should be recommended.

## REFERENCES

Anonymous. 1994. Aromatic offering to Arabia. Trade Digest Malaysia 1(2): 32 – 33

Burkill, I.H., 1966. A Dictionary of the economics products of Malaysia Peninsula, Vol.1. Ministry of Agriculture, Kuala Lumpur, pp.198-206

B. Norazlina. "Analysis of gaharu essential oil composition using gas chromatography/mass spectrometry for solvent extraction and hydrodistillation process". Degree thesis, University College Of Engineering And Technology Malaysia; 2005.

Chang Y. S., M. A. Nor Azah, A. Abu Said, E. H. Lok, S. Reader & A. Spiers (2002). "Gaharu" FRIM Technical Information. No. 69

Chakrabarty, K., A., Menon, V., 1994. Trade in Agarwood. WWF-TRAFFIC, India CIFOR, 1996. Manual of Forest Fruits, Seeds and Seedlings, Version 1.0. CD-Rom Publication No. 1, CIFOR, Bogor, Indonesia

Chakrabarty, K., Kumar, A. and Menon, V. (1994). *Trade in Agarwood*. Traffiq India and WWF-India, New Delhi. Pages 51

Compton J. & Ishihara A. (2004). *The Use & Trade of Agarwood in Japan*. Traffic East Asia-Japan.

Ding Hou, 1960. Thymelaeaceae. In: Van Steenis, C.G.G.J. (Ed), Flora Malesiana, Series 1, Vol. 6. Wolters-Noordhoff Publishing, Groningen, Thee Netherlands, pp. 1-15.

Dong-ping Q., Zhu-mei X., Shi-feng Y., Zhen-zhen G. (1999). "The Study on Seperating Thick Liquid and Residue in Processing Quick-dissolve Soya-

milk with Cold-pressing Soya-bean Cake". Proceedings of 99 International Conference on Agricultural Engineering.

Dr. Brian M. Lawrence, R. J. Reynolds, Tobacco Company (1985). *Progress in Essential Oil*. Supplied by The British Library-"The world's knowledge" volume 10. Pages 27-31.

Eurlings M.C.M & Gravendeel B. (2005). *TrnL-trnF sequence data imply paraphyly of Aquilaria & Gyrinops (Thymelaeaceae) and provide new perspectives for agarwood identification*. Plant Systematics & Evolution 254(1-2), 1-12.

F. Zich, J. Compton (2001). "Towards Sustainable Management of Papua New Guinea's Agarwood Resource". *A TRAFFIC Oceania Report In Conjunction With WWF South Pacific Programme*

Fatmawati Adam (2005). *Preliminary Study on Chemical Profiles of Gaharu Essential Oils (Aquilaria Malaccensis)*. Produced by Conventinal Hydrodistillation Process.

LaFrankie, J.V. 1994. Population dynamics of some tropical trees that yield non-timber forest products. Economic Botany 48(3): 301 – 309

Kazuo I. and Yajiro S. (1968). *Isolation and Structure Elucidation of Theaspirone, a component of tea essential oil*. Kyoto University; 11 March 1968

K.R.Varma, M.L. Maheshwari. "*The Constitution of Agarospirol, A Sesqiuterpenoid with new Skeleton"*. National Chem Laboratory, Poona India. 1964

L.T. Ng, Y.S. Chang & Azizol Abdul Kadir (1997). *A Review on Agar (Gaharu) Producing Aguilaria Species*. Journal of Tropical forest Products 2(2). Pages 272-285

Manfred Meier, Birgit Kohlenberg and Norbert A. Braun (2003). *Isolation Of Anisyl Acetone from Agarwood oil*. J.essent. oil Res., 15,54-56 . Jan/Feb 2003.

M. Ishihara, T. Tsuneya and K. Uneyama, (1993). *Fragrant sesquiterpene from Agarwood*. Phytochemistry.33 (5). Pages 1147-1155.

M. Ishihara and T. Tsuneya (1992). *Components of the Volatile Concentrate of Agarwood*. Journal Essential Oil Research.5. Pages 283-289.

M. Ishihara, T. Tsuneya, M. Shiga, K. Unemaya (1990). *Three sesquiterpene from Agarwood*. Phytochemistry.30. Pages 563-566.

Nakanishi, T., Yamagata, E, Yoneda, K, Nagashima, T, Kawasaki, I, Yoshida, T, Morill, H, and Miurall, I, (1983). *Three Fragrant sesquiterpene of Agarwood*. Faculty of Pharmaceutical Sciences, Osaka University, Japan. Pages 2066-2067

Nakanishi, T., Yamagata, E., Yoneda, K. and Miura, I. and Mori, H. (1983). *journal Chemical Society Perkin Trans. 1*, 601

Ng, L.T., Chang Y.S., Kadir, A.A., 1997. A review of Agar (Gaharu) producing Aquilaria spp. J. Trop. For. Prod. 2 (2), 272-285

Nobuchi, T. & Siripanatanadilok, S. 1991. Preliminary observation of Aquilaria crassna wood associated with formation of aloeswood. Bulletin of the Kyoto University Forests 63: 226 - 232

O. Normahwati. “The effect of temperature on the extraction of Gaharu Essential Oil using 500mL Hydro-Distillation Unit”. Degree thesis, University College Of Engineering And Technology Malaysia; 2005.

Okugawa, H.,Ueda, R., Matumoto, K., Kawanishi, K. & Kato, A. 1993. Effect of agarwood extracts on the central nervous system. Planta Medica 59(1): 32-36

Qi, S.Y. 1990. Accumulation of secondary metabolites in cell suspension culture of Aquilaria sinensis (Lour.) Gilg. (Thymelaeaceae). Vol. 3. pp. 1- 4 in Bhattacharyya, S.C., Sen, N. & Sethi, K.L., (Eds.). Proceeding on the 11$^{th}$ international vongress of essential oil, fragrance and flavors. November 12-16, 1989. New Delhi, India

Qi Shu-Yuan (1988). *Aquilaria Species in Vitro Culture and the Production of Eaglewood*. Biotetechnology in Agriculture & Forestry 33. Springer, pp 46-56.

Sadgopal., 1960. Exploratory studies in the development of essential oiland their constituent in aromatics plant. Part 1. Oil of agarwood. Soap, Perfumery and Cosmetics, London 33(1): 41-46

Sidiyasa. K., 1986. Jenis-jenis Gaharu in Indonesia (Gaharu in Indonesia). Jurnal Penelitian dan Perkembangan Kehutanan 2 (1), 7-16

Shimada, Y., Tominaga, T., Konishi, T., & Kiyosawa, S. 1982. Sudies on the agarwood (Jinko). I. Structures of 2- (2-phenylethyl) chromone derivatives. Chemical and pharmaceutical bulletin 30 (10): 3791 - 3795

Whitemore., T.C., 1972. Tree flora of Malaysia: A manual for foresers. Vol. 2. Longman TC, Kuala Lumpur

Yamada, I., 1995. Aloeswood forest and the maritime world. Southeast Asian Studies 33 (3), 181-186

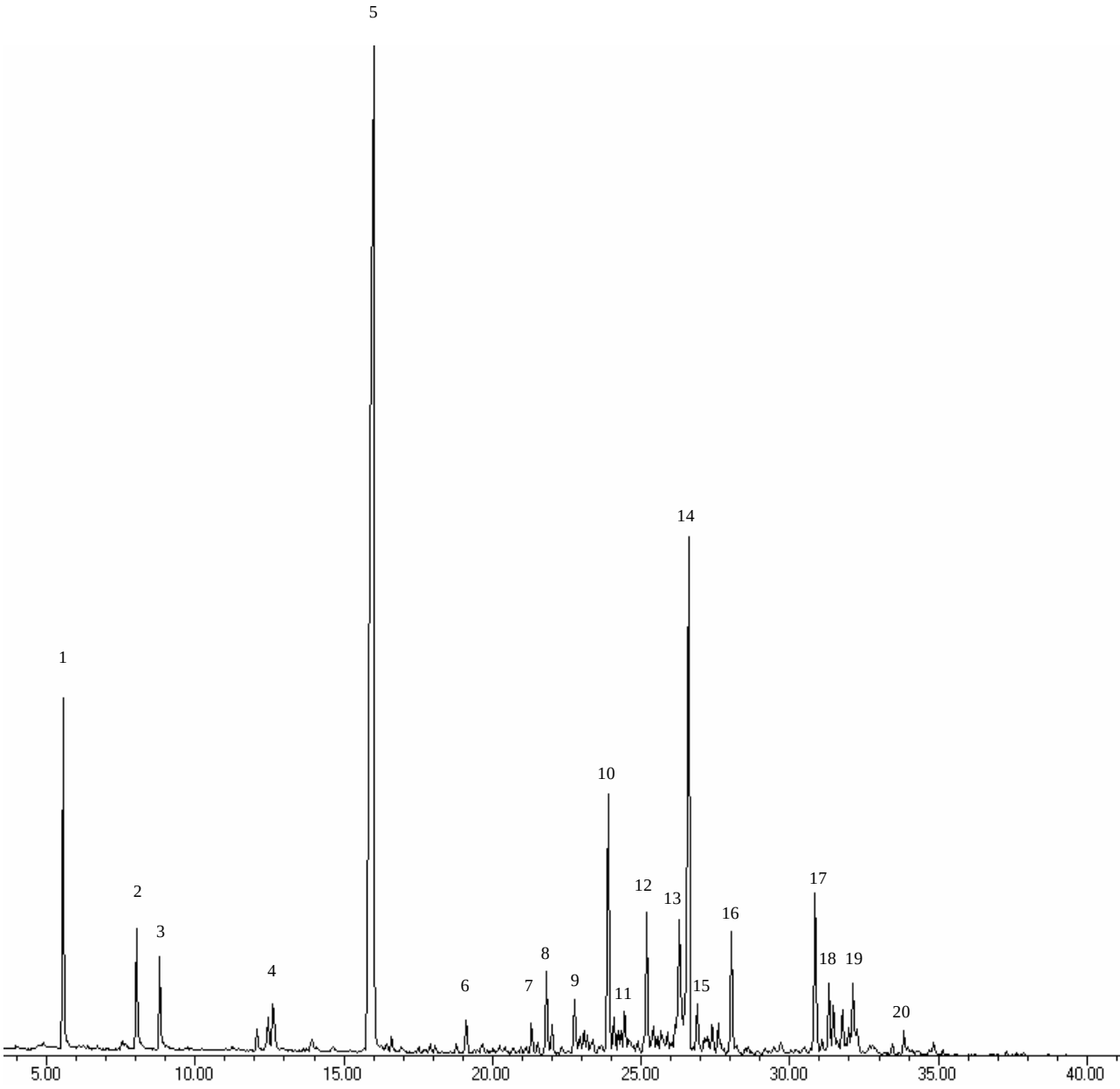
Y. S. Chang, M. A. Nor Azah, A. Abu Said, E. H. Lok, S. Reader & A. Spiers (2002). *Gaharu*. FRIM Technology Information. No 69.

Retrieved August 18, 2008, from *http://www.unimas.my/research/rimc/index.htm*

Retrieved August 18, 2008, from *http://www.cropwatch.org/za.garchem.htm*

# APPENDIXES

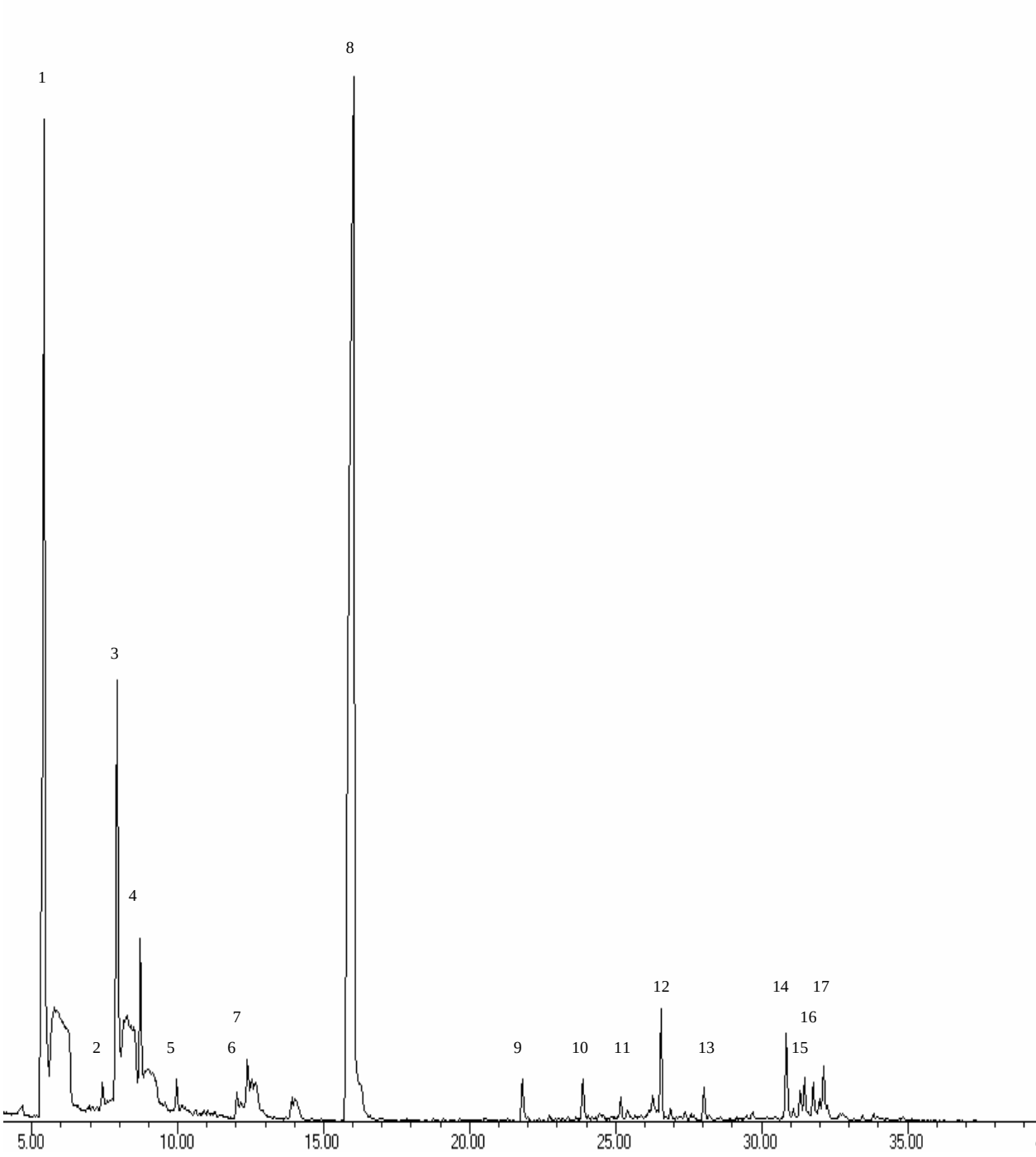
**Spectrum and the constituents identified in the headspace-SPME experiments of Agarwood Essential Oil**




GCMS analysis of the headspace-SPME experiment of Agarwood Essential Oil (The chromatographic column used for analysis was HP-5MS 5% Phenyl Methyl Siloxane , 30 m x 250 μm i.d, film thickness 0.25 μm and 100μm Polydimethylsiloxane (PDMS) fiber).

The constituents identified in the headspace-SPME (100μm PDMS fiber) experiments of Agarwood Essential Oil

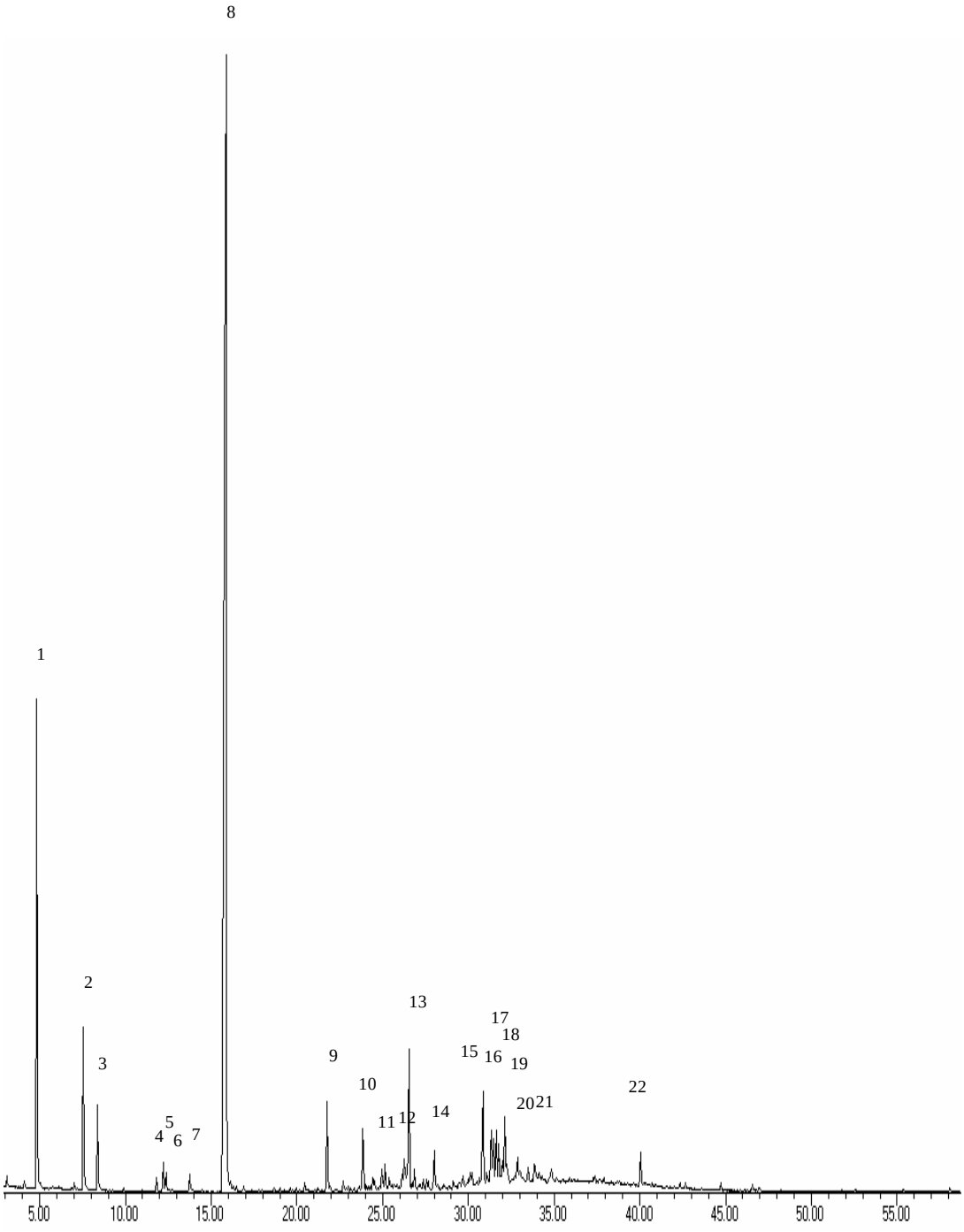
| No | Compound | Quality | % Area | CAS No |
|---|---|---|---|---|
| 1. | Benzaldehyde | 97 | 5.33 | 000100-52-7 |
| 2. | Benzaldehyde, 2-hydroxy- | 94 | 2.29 | 000090-02-8 |
| 3. | Acetophenone | 95 | 1.51 | 000098-86-2 |
| 4. | 1H-purin-6-amine,N-methyl- | 38 | 1.30 | 000443-72-1 |
| 5. | 2-Butanone, 3-phenyl- | 96 | 40.27 | 000769-59-5 |
| 6. | 1-phenyl-1-butene | 35 | 0.68 | 000824-90-8 |
| 7. | Copaene | 97 | 0.62 | 003856-25-5 |
| 8. | 4-Ethylphenyl acetate | 83 | 1.70 | 003245-23-6 |
| 9. | α-Cedrene | 98 | 1.22 | 000469-61-4 |
| 10. | α-Guaiene | 99 | 5.16 | 003691-12-1 |
| 11. | α-Caryophyllene | 97 | 0.93 | 006753-98-6 |
| 12. | Longipinane, (E)- | 44 | 3.06 | 1000156-14-5 |
| 13. | Eudesma-4(14),11-diene | 80 | 4.17 | 1000152-04-3 |
| 14. | δ-Guaiene | 99 | 11.69 | 003961-11-0 |
| 15. | Butyric acid,3-methyl-3-[2-isopropylphenyl]- | 83 | 1.20 | 092300-81-7 |
| 16. | Carvone | 6 | 2.30 | 000099-49-0 |
| 17. | τ-eudesmol | 94 | 3.44 | 001209-71-8 |
| 18. | (R)-aromadendrene | 55 | 1.23 | 000489-39-4 |
| 19. | β-Selinene | 91 | 1.03 | 017066-67-0 |
| 20. | 4,6,6-Trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)]octane | 55 | 0.23 | 1000190-22-2 |




GCMS analysis of the headspace-SPME experiment of Agarwood Essential Oil (The chromatographic column used for analysis was HP-5MS 5% Phenyl Methyl Siloxane , 30 m x 250 µm i.d, film thickness 0.25 µm and 75µm Carboxen/Polydimethylsiloxane (CAR/PDMS) fiber).

The constituents identified in the headspace-SPME (75μm CAR/PDMS fiber) experiments of Agarwood Essential Oil

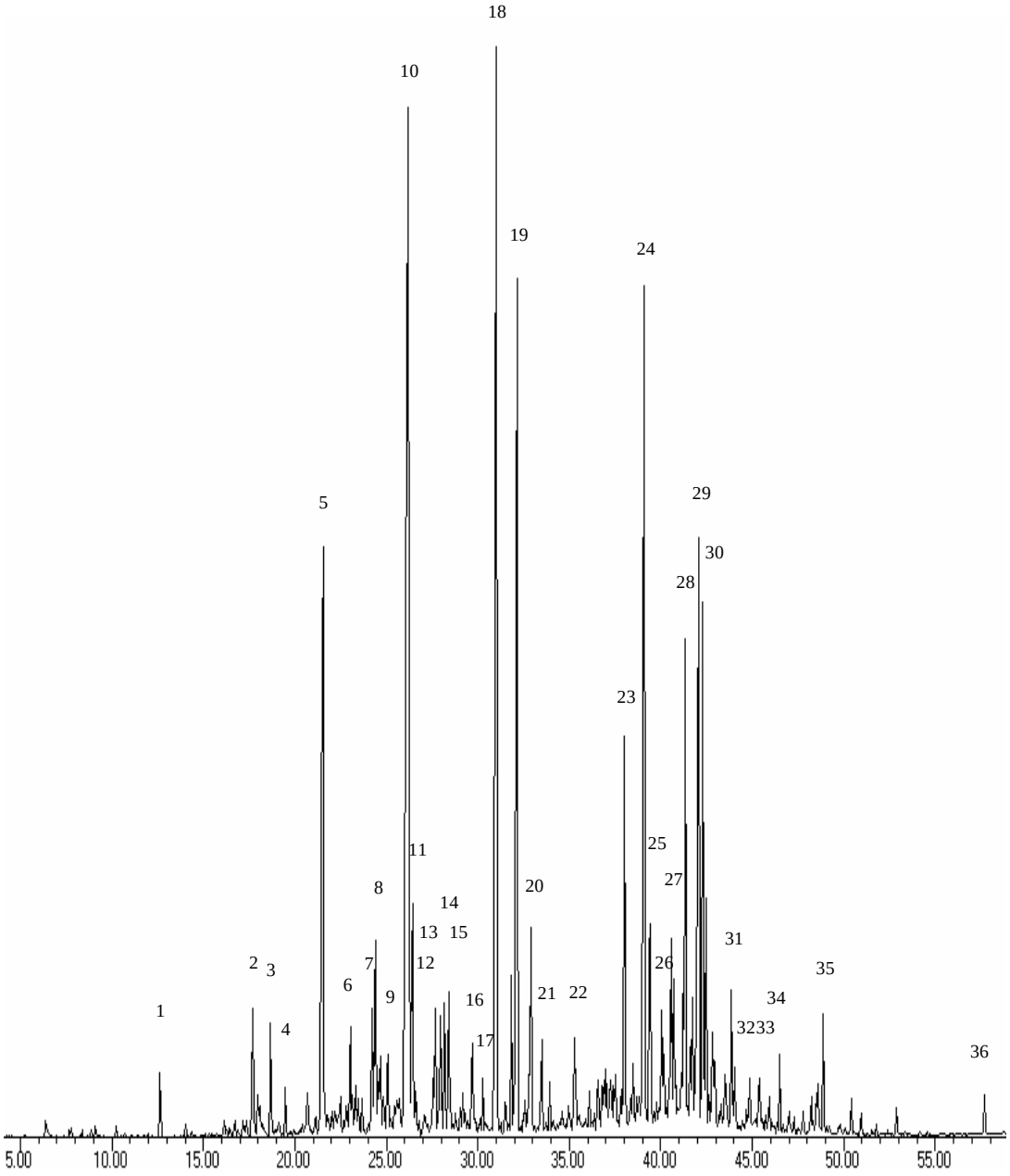
| No | Compounds | Quality | Area | CAS |
|---|---|---|---|---|
| 1 | Benzaldehyde | 97 | 18.25 | 100-52-7 |
| 2 | D-limonene | 93 | 0.38 | 5989-27-5 |
| 3 | Benzaldehyde, 2-hydroxy- | 94 | 7.38 | 90-02-8 |
| 4 | Acetophenone | 94 | 1.72 | 98-86-2 |
| 5 | Undecane | 91 | 0.43 | 1120-21-4 |
| 6 | Benzene,1-ethenyl-4-methoxy- | 96 | 0.37 | 637-69-4 |
| 7 | Ethanone, 1-(2-hydroxyphenyl)- | 95 | 1.16 | 118-93-4 |
| 8 | 2-butanone,3-phenyl- | 96 | 42.12 | 769-59-5 |
| 9 | 3,3-dimethyl-6-methylenecyclohexen | 81 | 0.62 | 20185-16-4 |
| 10 | A-Guaiene | 96 | 0.68 | 3691-12-1 |
| 11 | Tricyclo[5.2.2.0(1,6)]undecan-3-ol,2-methylene-6,8,8-trimethyl- | 52 | 0.39 | 1000159-37-6 |
| 12 | δ-Guaiene | 99 | 1.59 | 3691-11-0 |
| 13 | Cis-3-hexenyl heptine carbonate | 22 | 0.44 | 68698-58-8 |
| 14 | τ-Eudesmol | 94 | 1.32 | 1209-71-8 |
| 15 | Aromadendrene, (+)- | 70 | 0.63 | 489-39-4 |
| 16 | Aristolene | 78 | 0.45 | 1000150-14-9 |
| 17 | A-Bulnesene / δ-Guaiene | 89 | 0.62 | 3691-11-0 |




GCMS analysis of the headspace-SPME experiment of Agarwood Essential Oil (The chromatographic column used for analysis was HP-5MS 5% Phenyl Methyl Siloxane , 30 m x 250 µm i.d, film thickness 0.25 µm and 65µm Polydimethylsiloxane/divinylbenzene (PDMS/DVB)fiber).

The constituents identified in the headspace-SPME (65μm PDMS/DVB fiber) experiments of Agarwood Essential Oil

| No | Compounds | Quality | % Area | CAS No. |
|---|---|---|---|---|
| 17. | Benzaldehyde | 97 | 6.79 | 000100-52-7 |
| 18. | Benzaldehyde, 4-hydroxy- | 89 | 3.45 | 000123-08-0 |
| 19. | Acetophenone | 95 | 1.34 | 000098-86-2 |
| 20. | Benzene, 1-ethenyl-4-methoxy- | 95 | 0.25 | 000637-69-4 |
| 21. | Ethanone, 1-(2-hydroxyphenyl)- | 94 | 0.52 | 000118-93-4 |
| 22. | Benzenemethanol, .alpha.,4-dimethyl- | 56 | 0.42 | 000536-50-5 |
| 23. | Benzaldehyde, 3-methoxy- | 97 | 0.43 | 000591-31-1 |
| 24. | 2-Butanone, 3-phenyl- | 95 | 44.62 | 000769-59-5 |
| 25. | 1,3-Cyclopentadiene,5,5-dimethyl-1-ethyl- | 74 | 1.87 | 1000162-25-7 |
| 26. | α-Guaiene | 95 | 1.32 | 003691-12-1 |
| 27. | 3H-cyclopenta[1,3]cyclopropa[1,2]benzene-3,6(7H)-dione,1,2,3a,3b,4,5-hexahydro-3b-methyl-7-(1-methylethyl)- | 60 | 0.47 | 066708-18-7 |
| 28. | (-)-Isoaromadendrene-(V) | 83 | 1.13 | 1000156-14-3 |
| 29. | δ-Guaiene | 99 | 2.75 | 003961-11-0 |
| 30. | Bicycle[3.2.0]hept-2-ene,4-ethoxy-,endo- | 10 | 0.79 | 1000156-86-7 |
| 31. | τ-eudesmol | 94 | 2.23 | 001209-71-8 |
| 32. | Acetic acid,(4-oxo-2-thiazolidinylidene)-, methyl ester | 38 | 1.44 | 056196-66-8 |
| 33. | Agarospirol | 94 | 0.89 | 1460-73-7 |
| 34. | 2,6-bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol | 87 | 1.06 | 014035-34-8 |
| 35. | (-)-Aristolene | 83 | 0.94 | 6831-16-9 |
| 36. | (R)-Aromadendrene | 86 | 2.14 | 000489-39-4 |
| 37. | Hexahydro-5.lambda.(6)-thieno[3,4-b]pyrrol-2-one,1-(2-morpholin-4-ylethyl)-5,5-dioxo- | 64 | 0.31 | 1000303-88-9 |
| 38. | 2-(E)-Hexenoic acid, (4s)-amino-5-methyl- | 64 | 0.64 | 267008-79-7 |




GCMS analysis of the headspace-SPME experiment of Agarwood Essential Oil (The chromatographic column used for analysis was DB-WAX , 30 m x 250 μm i.d, film thickness 0.25 μm and 100μm Polydimethylsiloxane (PDMS) fiber).

The constituents identified in the headspace-SPME (100μm PDMS fiber) experiments of Agarwood Essential Oil

| No | Compounds | Quality | % Area | CAS No |
|---|---|---|---|---|
| 23 | Cyclohexasiloxane, dodecamethyl- | 91 | 0.46 | 000540-97-6 |
| 24 | α-Cubebene | 97 | 1.13 | 017699-14-8 |
| 25 | Benzaldehyde | 96 | 0.73 | 000100-52-7 |
| 26 | Cycloheptasiloxane,tetradecamethyl- | 92 | 0.27 | 000107-50-6 |
| 27 | α-Guaiene | 99 | 6.54 | 003691-12-1 |
| 28 | 1-(3-Methylbutyl)-2,3,4-trimethylbenzene | 45 | 0.55 | 107997-59-1 |
| 29 | 1,4,7-Cycloundecatriene,1,5,9,9-tetramethyl-,Z,Z,Z- | 98 | 1.00 | 1000062-61-9 |
| 30 | Phenol,4,6-di(1,1-dimethylethyl)-2-methyl- | 70 | 1.55 | 000616-55-7 |
| 31 | α-Gurjunene | 97 | 0.6 | 000489-40-7 |
| 32 | δ-Guaiene | 99 | 17.13 | 003691-11-0 |
| 33 | Bi-1,3,5,7-cyclooctatetraene-1-yl | 90 | 2.02 | 006715-22-6 |
| 34 | Aromadendrene, dehydro- | 83 | 0.82 | 10000156-12-5 |
| 35 | 7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol,4,4,11,11-tetramethyl- | 43 | 1.11 | 074842-43-6 |
| 36 | Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl- | 98 | 0.87 | 000644-30-4 |
| 37 | Benzene,1-methyl-2-(1-methylethyl)- | 30 | 1.13 | 000527-84-4 |
| 38 | 2,4-quinolinediol | 47 | 0.8 | 000086-95-3 |
| 39 | p-Propargyloxytoluene | 42 | 0.31 | 005651-90-1 |
| 40 | 2-Butanone,3-phenyl- | 95 | 10.78 | 000769-59-5 |
| 41 | 4-(p-acetoxyphenyl)-2-butanone | 38 | 7.12 | 003572-06-3 |
| 42 | Phenol,3,5-dimethyl- | 46 | 1.46 | 000108-68-9 |
| 43 | Ledol | 38 | 0.73 | 000577-27-5 |
| 44 | Bicycle[2.2.2]octa-2,5-diene,1,2,3,6-tetramethyl- | 38 | 0.34 | 062338-43-6 |
| 45 | Benzene,1,2,4-triethyl- | 55 | 2.79 | 000877-44-1 |
| 46 | τ-Eudesmol | 93 | 7.88 | 001209-71-8 |
| 47 | β-Neoclovene | 84 | 2.19 | 056684-96-9 |
| 48 | Bisabolol oxide A | 66 | 1.12 | 022567-36-8 |
| 49 | τ-Gurjunene | 96 | 1.66 | 022567-17-5 |
| 50 | Agarospirol | 91 | 3.52 | 001460-73-7 |
| 51 | Tricycle[4.1.0.0(2,4)]heptanes,3,3,7,7-tetramethyl-5-(2-methyl-1-propenyl)- | 93 | 5.56 | 056348-21-1 |
| 52 | α-Selinene | 78 | 3.95 | 000473-13-2 |
| 53 | Bicycle[4.4.0]dec-1-ene,2-isopropyl-5-methyl-9-methylene | 70 | 1.56 | 150320-52-8 |
| 54 | β-Eudesmol | 98 | 0.87 | 000473-15-4 |
| 55 | Alloaromadendrene | 95 | 1.01 | 025246-27-9 |
| 56 | 3,5,8-Trimethyl-3a,7,7a,8,9,9a-hexahydroazuleno[6,5-b]furan-2,6(3H,4H)-dione | 47 | 0.48 | 005655-56-1 |
| 57 | 2-(4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol | 43 | 0.7 | 1000190-51-8 |
| 58 | (-)-α-Panasinsen | 60 | 0.25 | 056633-28-4 |

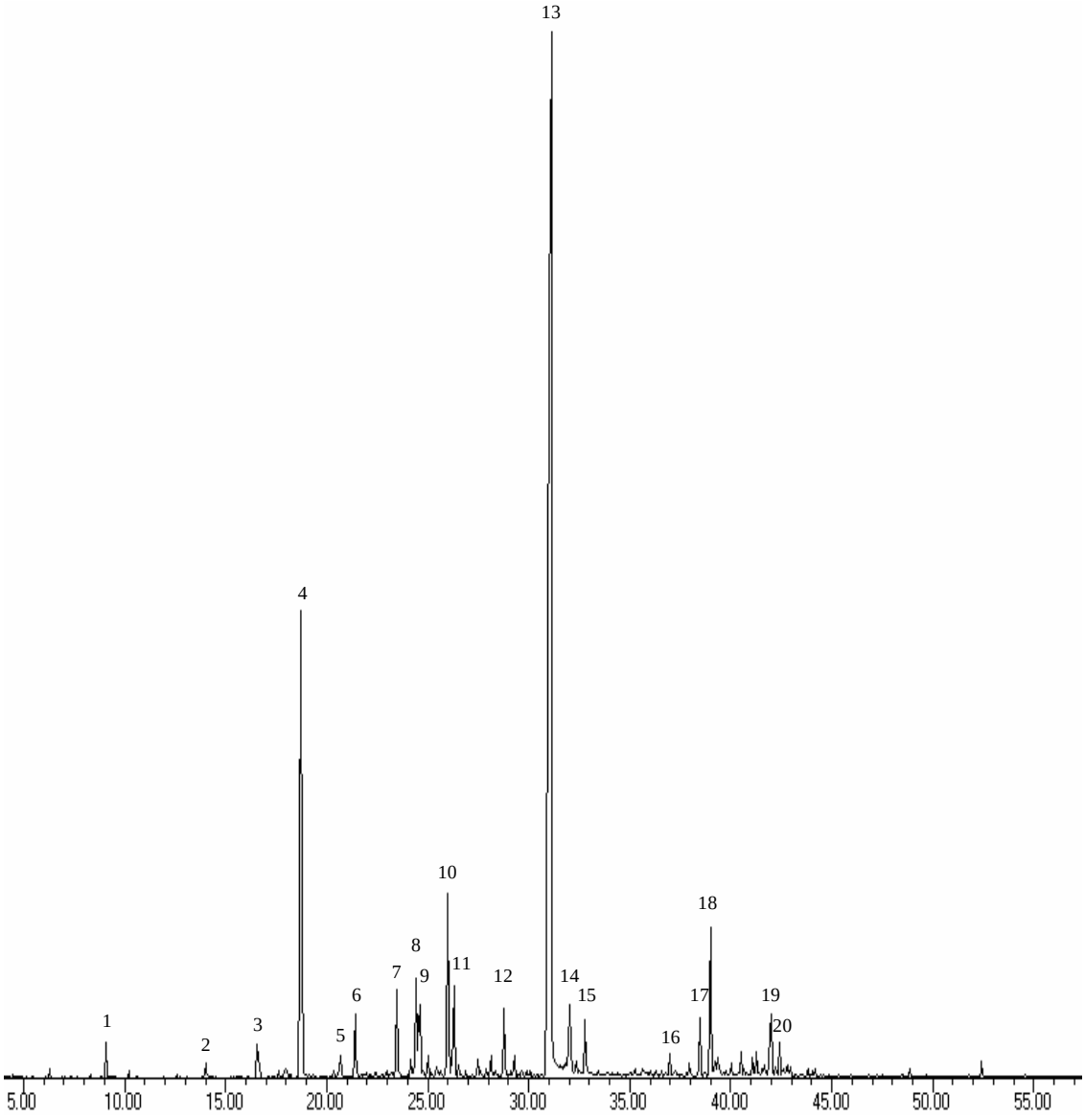
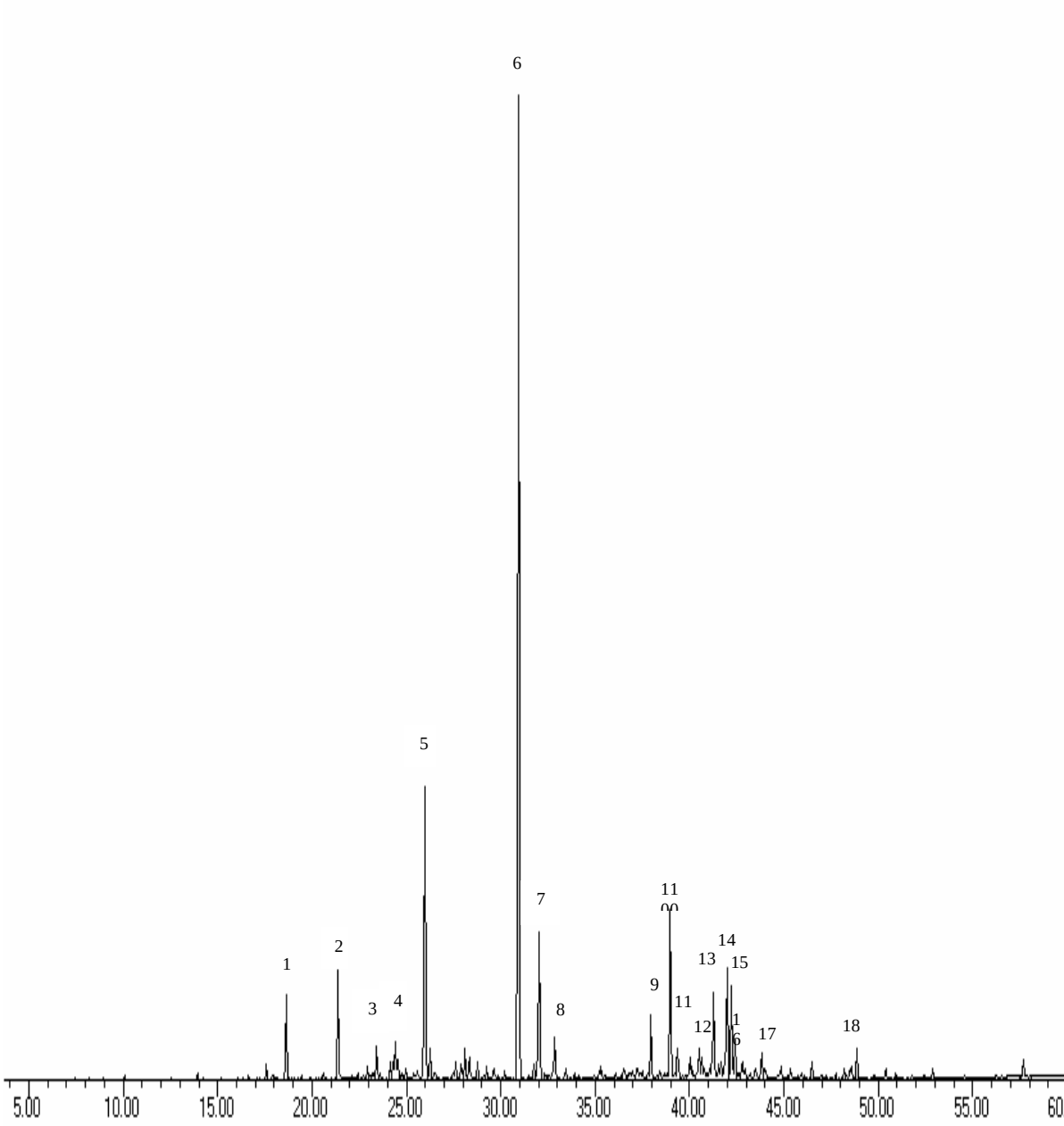



**Figure 4.7.** GCMS analysis of the headspace-SPME experiment of Agarwood Essential Oil (The chromatographic column used for analysis was DB-WAX, 30 m x 250 μm i.d, film thickness 0.25 μm and 75μm Carboxen/Polydimethylsiloxane (CAR/PDMS) fiber).

The constituents identified in the headspace-SPME (75μm CAR/PDMS fiber) experiments of Agarwood Essential Oil

| No | Compounds | Quality | Area | CAS |
|---|---|---|---|---|
| 16. | Styrene | 97 | 0.45 | 100-42-5 |
| 17. | Nonanal | 91 | 0.36 | 124-19-6 |
| 18. | Furfural | 91 | 1.19 | 98-01-1 |
| 19. | Benzaldehyde | 95 | 13.66 | 100-52-7 |
| 20. | 2-Furancarboxaldehyde,5-methyl- | 93 | 0.65 | 620-02-0 |
| 21. | α-Guaiene | 99 | 1.42 | 3691-12-1 |
| 22. | Acetophenone | 91 | 1.90 | 98-86-2 |
| 23. | Benzaldehyde,2-hydroxy- | 94 | 2.47 | 90-02-8 |
| 24. | Longifolene-(V4) | 76 | 1.44 | 61262-67-7 |
| 25. | δ-Guaiene | 99 | 4.34 | 3691-11-0 |
| 26. | Ethanone,1-(5,6,7,8-tetrahydro-2,8,8-trimethyl-4H-cyclohepta[b]furan-5-yl)- | 46 | 2.26 | 071596-88-8 |
| 27. | Ethanone,1-(2-hydroxyphenyl)- | 94 | 1.53 | 118-93-4 |
| 28. | 2-Butanone,3-phenyl- | 95 | 49.80 | 769-59-5 |
| 29. | 1H-Pyrazole,1,3-diphenyl- | 25 | 2.32 | 4492-01-7 |
| 30. | N-(Chroman-5-yl)acetamide | 42 | 1.14 | 1000306-69-8 |
| 31. | 3,7-cyclodecadien-1-one,3,7-dimethyl-10-(1-methylethylidene)-,(E,E)- | 55 | 0.40 | 6902-91-6 |
| 32. | Phenol, 4-methyl- | 97 | 1.17 | 106-44-5 |
| 33. | τ-Eudesmol | 89 | 3.02 | 1209-71-8 |
| 34. | Neoisolongifolene | 92 | 2.02 | 1000156-12-4 |
| 35. | τ-Cadinene | 55 | 0.65 | 39029-41-9 |




GCMS analysis of the headspace-SPME experiment of Agarwood Essential Oil (The chromatographic column used for analysis was DB-WAX, 30 m x 250 µm i.d, film thickness 0.25 µm and 65µm Polydimethylsiloxane/divinylbenzene (PDMS/DVB)fiber).

The constituents identified in the headspace-SPME (65μm PDMS/DVB fiber) experiments of Agarwood Essential Oil

| No | Compound | Quality | % Area | CAS No |
|---|---|---|---|---|
| 14. | Benzaldehyde | 96 | 11.43 | 100-52-7 |
| 15. | A-Guaiene | 99 | 3.20 | 3691-12-1 |
| 16. | Acetophenone | 94 | 2.64 | 98-86-2 |
| 17. | α-Caryophyllene | 97 | 0.41 | 6753-98-6 |
| 18. | δ-Guaiene | 99 | 8.31 | 3691-11-0 |
| 19. | 2-Butanone,3-phenyl- | 95 | 49.06 | 000769-59-5 |
| 20. | 1,3-Cyclopentadiene,5,5-dimethyl-1-ethyl- | 68 | 5.78 | 1000162-25-7 |
| 21. | Phenol,3,5-dimethyl- | 43 | 1.19 | 108-68-9 |
| 22. | 9-Oxabicyclo[4.3.0]non-6-en-8-one,7-(1-cyclopenten-3-one-1-yl)- | 47 | 1.93 | 1000160-28-8 |
| 23. | δ-Selinene | 89 | 5.36 | 28624-23-9 |
| 24. | 7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one | 64 | 1.44 | 473-08-5 |
| 25. | τ-Gurjunene | 95 | 0.95 | 22567-17-5 |
| 26. | Agarospirol | 90 | 1.93 | 1460-73-7 |
| 27. | Aristolene | 90 | 4.77 | 1000150-14-9 |
| 28. | Eudesma-3,7(11)-diene / Selina-3,7(11)-diene | 81 | 2.83 | 6813-21-4 |
| 29. | 1,6-Cyclodecadiene,1-methyl-5-methylene-8-(1-methylethyl)-,[s-(E,E)]- | 70 | 1.04 | 23986-74-5 |
| 30. | 1R,3Z,9S-2,6,10,10-tetramethylbicyclo[7.2.0]undeca-2,6-diene | 95 | 0.85 | 1000140-07-4 |
| 31. | 1,4-Methanoazulen-7(1H)-one,octahydro-4,8,8,9-tetramethyl-,(+)- | 49 | 0.91 | 018319-28-3 |