ANALYSIS OF AGARWOOD OIL COMPOSITION VIA PREPARATIVE THIN LAYER CHROMATOGRAPHY

SITI FARIDAH BT AB RAHMAN

UNIVERSITI MALAYSIA PAHANG

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ANALYSIS OF AGARWOOD OIL COMPOSITION VIA PREPARATIVE THIN LAYER CHROMATOGRAPHY

SITI FARIDAH BT AB RAHMAN

Thesis submitted to the Faculty of Chemical and Natural Resources Engineering in
Partial Fulfillment of the Requirement for the
Degree of Bachelor Engineering in Chemical Engineering

Faculty of Chemical Engineering & Natural Resources
Universiti Malaysia Pahang

APRIL, 2009

I declare that this thesis entitled "*Analysis of Agarwood Oil Composition Via Preparative Thin Layer Chromatography"* is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Date : 30 APRIL 2009

Special Dedication of This Grateful Feeling to My…

Beloved father and mother;
Mr. Ab Rahman B Hamad & Mrs. Rohani Bt Mamat

Loving Friends and Lecturers

For Their Love, Support and Best Wishes.

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ABSTRACT

Agarwood which is also known as Aquilaria is the most valuable wood in the world with higher prices and demands nowadays. The widely uses of agarwood in meditation field, essential oil production and etc makes agarwood one of the precious things on earth. The study was carried out to analysis compounds present in agarwood oil by using Preparative Thin Layer chromatography. In this study, used of *Aquilaria Maleccencis* from Malaysia as the sample and it can be classified in grade C. After extraction, isolation was carrying out to isolate it complex component present and detected by UV irradiation to afford 4 spots. Each spots, i.e spots 1, 2, 3 and 4 numbered in order of increasing polarity and each separated spot was confirmed by GC-MS. Results from GC-MS was analyzed to confirm presented of sesquiterpenes as a mojor active compound in agarwood oil and comparison between sample was made between commercial sample, i.e. Maha and Kelantan samples. This study showed a marked similar compound presented in the oil compositions among the sample and commercial samples.

ABSTRAK

Kayu Agar atau lebih dikenali sebagai Aquilira adalah antara kayu yang paling berharga dan mempunyai permintaan dan harga yang tinggi. Penggunaan kayu Agar secara meluas dalam bidang perubatan, pengahasilan minyak wangi dan sebagainya menjadikan kayu Agar sebagai sesuatu yang berharga di dunia. Kajian ini dijalankan untuk menganalisa kumpulan yang wujud dalam minyak kayu Agar dengan menggunakan kaedah *preparative Thin Layer Chromatography*. Dalam kajian ini, Gred C dari spesis *Aquilaria Malaccensis* dari Malaysia telah digunakan sebagai sampel. Selepas proses pengekstrakan, kumpulan komplek yang terdapat di dalam minyak tersebut, diklaskan dengan menggunakan kaedah *Preparative Thin Layer Chromatography* dan menghasilkan 4 tanda selepas diimbas di bawah pengcahayaan UV. Setiap tanda tersebut, dinomborkan dengan 1, 2, 3 & 4 mengikut kepolaran dan analisis oleh GC-MS dilakukan untuk mengesahkan kompaun yang wujud dalam setiap tanda tersebut. Keputusan dari GC-MS membuktikan kumpulan *sesquiterpenes* adalah kompaun yang banyak hadir dalam minyak kayu Agar. Seterusnya, perbandingan dilakukan ke atas sampel dari makmal dan komersial (MAHA&Kelantan). Dalam kajian ini, menunjukkan wujudnya persamaan kompaun dalam setiap sampel yang dianalisa.

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CHAPTER 1

INTRODUCTION

1.1 Research Background

The fragrant wood of *Aquilaria* species is known as agarwood, aloe wood, eaglewood or gaharu depending on the country. In Malaysia, the tree of *Aquilaria* is called karas and its fragrant wood is known as gaharu. The wealth of names for this dark and heavy wood reflects its widespread and varied use over thousand of years. It is also used highly regarded for use during Buddhist and Islamic cultural activities as well as an important ingredient in many traditional medicines and cosmetic. agarwood is considered to be a pathological product produced by fungal invasion of the host. The oil obtained from agarwood is described as a stimulant, car diatonic and carminative (Qi Shu-Yuan-1992). Each kilograms of high-quality agarwood can fetch up to RM30000 in the global market and prices are expected to surge as demand continues to rise (Raduan Md Taib-2008).

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). The odors of the oil can be described as complex mixture compound. The technique most widely used for purify assessment is liquid chromatography (LC) with mass spectrometric (MS), ultraviolet (UV) and preparative thin layer chromatography (PTLC). PTLC is a new isolation device and it's has proven to purified synthesis product successfully (Piia K.Salo- 2006). The present of active component was analysis by (GC-MS) Gas Chromatograph- Mass Spectrometer (Tamuli-2007)

1.2 Objective

The objective of this project is:

I. To extract agarwood oil by using hydro-distillation method.
II. To purify agarwood oil produced by using Preparative Thin –Layer Chromatography (PTLC)
III. To analysis of component in agarwood oil via Gas Chromatography-mass spectrometer (GCMS)
IV. To compare chemical compound in agarwood oil from lab scale (hydro-distillation method) production with commercial scale.

1.3 Scope of study

There are some important tasks to be carried out in order to achieve the objective of this study. The important scopes have been identified for this research in achieving the objective:

I. In this study, one type of samples is being used that is Gred C, *Aqualaria Malaccensis*. The agarwood oil would be extracted by the hydro-distillation method
II. In this research, purification and analysis would be done to determine all components present in the oil from agarwood. The fraction component from sample obtained from extraction would be isolated by Preparative Thin –Layer Chromatography (PTLC). The present of component was confirmed by using Gas Chromatography- mass spectrometer (GCMS). By doing so, some important component in essential oil from Agarwood would be determined.
III. Two types of samples from commercial scale are being used that is from MAHA and Kelantan industrial.

1.4 Problem statement

In Malaysia, agarwood oil extractions are mostly done by hydro-distillation. 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 agarwood.

Today, the demand for significant oil of agarwood standards has increased. Nevertheless, the complex chemical compound in its oil has not been well researched and there is no full identification of the component and no standardizing for quality of its oil. Sometime, adulteration occur during manufacturing standardize. Thus, the challenge is to come out with standard quality control and characteristic for active compound in agarwood oil. Once the standard of active component in agarwood oil is developed, then the quality of agarwood oil can be identified. So this study will purify and analysis compound in oil by using PTLC and GCMS due to identify active component that make agarwood essential oil became “black gold of the forest”.

1.5 Benefit and significant of research

I. Good laboratory practice in performing laboratory testing of hydro-distillation method of oil from agarwood will be developed.
II. Active component in essential oil of agarwood will recognized and acquire to compare with global market standards.
III. Comparison active component in oil of agarwood between lab and industrial scale will be developed.

CHAPTER 2

REVIEW OF RELATED RESEARCH

2.1 Agarwood (*Aquilaria malaccensis*)

Aquilaria malaccensis is one of 15 tree species in the Indomalesian genus *Aquilaria*, family Thymelaeaceae (Mabberley-1997). It is a large evergreen tree growing over 15-30 m tall and 1.5 – 2.5 m in diameter, and has white flowers (Chakrabarty- 1994). *Aquilaria malaccensis* and other species in the genus *Aquilaria* sometimes produce resin-impregnated heartwood that is fragrant and highly valuable. It widely distributed in south and south – East Asia. There are differing accounts of the countries in which it occurs. According to Oldfield (1998), *Aquilaria malaccensis* is found in 10 countries which are Malaysia, Indonesia, Iran, Myanmar, Philippines, Singapore, Bangladesh, Bhutan, India and Thailand.

Aquilaria species have adapted to live in various habitats, including those that are rocky, sandy, well drained slopes and ridges and land near swamps. Agarwood is traded in several raw forms, ranging from large sections of trunk to finished products such as incense and perfumes. Agarwood powder is generally much less expensive that chips or flakes, with prices varying from around USD20-60/kg. Grading agarwood is a subjective and complicated process based on size, color, shape, weight, density and flammability. The Forest Research Institute Malaysia (FRIM) categorizes agarwood as grades A, B, and C based on chemical analysis.

2.2 Extraction by hydro distillation

There are a few conventional and modern methods of extracting essential oils. It can be extracted by hydro-distillation, cold pressing, effleurage, hydro-diffusion, supercritical fluid extraction, vapor-cracking, turbo-extractor and microwave extraction. In Malaysia, the techniques currently practiced in the industry for the extraction of essential oils are by hydro-distillation; steam, water and water / steam distillation and solvent extraction (Nor Azah Mohd. Ali- 2002).

According to Guenther (1972), the equipment required for carrying on distillation of plant materials depends upon the size of the operation and the type of distillation to be used. There are, however three main parts, which in varying size, form the base for all three types of hydro-distillation. The three universally employed parts are:

(1) The retort or still proper
(2) The condenser
(3) The receiver for the condensate or oil separator

According to Guenther (1972), the ratio between quantity of condensed water and time may be designated as rate of distillation (kg/hr). If the velocity of the rising steam is too low, the steam will stagnate in the condenser of the charge and complete exhaustion is impossible. Hence, if the velocity is too high, the steam may break through the charge, form steam channel, hurl plant particles into the condenser and partly clogging it.

Hydro-distillation is commonly used for the extraction of essential oil. The essential oils of plants such as caraway, clove and sandalwood are examples of plants extracted by this process. When extracted they have an oil yield of 0.10 to 15.0 percent of essential oil (Derksen-1990).

2.3 Chemical of compound in agarwood oil

2.3.1 Chemical compounds

Agar is considered to be a pathological product produced by fungal invasion of the host (Qi Shu-Yuan-1992). Since 1938, few workers have been studying about agar formation and reported the agar zones to be associated with mold and decay fungi (Bose-1938; Bhattacharyya-1952; Jalaluddin-1977; Venkataramanan- 1985; Beniwal-1989; Tamuli -2000; Mitra and Gogoi-2001). Among different fungal species reported to be associated with agar zones, few could exhibit pathogenesis with the development of disease symptoms while others seem to be of saprophytic nature in different eco-geographical condition.

Maheshwari-1963 isolated three new sesquiterpenic furanoids of the selinane group from agarwood oil, obtained from the fungus infected plant and their structures and absolute configurations determined by degradative studies and physical measurements. Varma-1965 examined that degradative studies and physical measurements supported by an unambiguous synthesis of the derived ketone have led to the assignment of a novel spiroskeleton to agarospirol, a sesquiterpene alcohol isolated from the essential oil of infected agarwood. Paknikar and Dhavlikar-1975 and Paknikar and Naik-1975 reported that on hydrogenation of α- agarofuran and β-agarofuran the same dihydroagarofuran was obtained.

Thomas and Ozainne-1976 reported some naturally occurring dihydroagarofuran and isodihydroagarofuran to unequivocally show that the dihydroagarofuran found was indeed dihydro-β-agarofuran and isodihydroagarofuran was isodihydro- β-agarofuran; two separate compounds. Pant and Rastogi-1980 and Bhandari-1982 isolated a new sesquiterpene, agarol and a couinarinolignan, aquillochin, respectively, from the oil of agarwood. Nagashima-1983 further

characterized the presence of two more sesquiterpene alcohols, jinkohol II and jinkoh- eremol, from the Indonesia agarwood oil.

Nakanishi-1984 again reported that a benzene extract of an Indonesian sample of 'Jinkoh' agarwood was found to contain α-agarofuran, 10-epi- γ- eudesmol and oxo-agarospirol. Ishihara-1991 characterized seven new sesquiterpenes based on the guaiane skeleton in a sample of agarwood oil. Five new eudesmane sesquiterpenes and three other compounds further characterized by Ishihara-1993 in a sample of agarwood extract produced in the laboratory from *A. agallocha* of Vietnamese origin.

The list of common chemical compounds detected in selected agarwood oils from various locations is shown in Table 1. The results of this work indicate that there are some similarities and variations in the chemical composition of several of Grade C agarwood oil samples tested reported by Nor Azah-2008.

Table 1: Common chemical compounds in agarwood oils

Chemical compounds	RI	Selangor (%)	Kelantan (%)	Pahang (%)	Terengganu (%)
3-phenyl-2-butanone	1249	1.50	5.77	7.80	0.79
α-guaiene	1448	-	0.67	-	-
β-agarofuran	1477	1.69	1.98	0.69	0.50
α-agarofuran	1553	4.83	2.96	1.48	1.57
Nor-ketoagarofuran	1557	2.09	-	-	-
10-epi-γ-eudesmol	1618	11.54	9.03	8.10	3.32
Agarospirol	1631	14.86	5.49	7.11	18.86
β-eudesmol	1649	-	-	-	5.74
Jinkoh-eremol	1650	10.62	7.70	6.31	-
kusunol	1659	18.94	-	-	-
Jinkohol II	1751	4.71	-	-	-

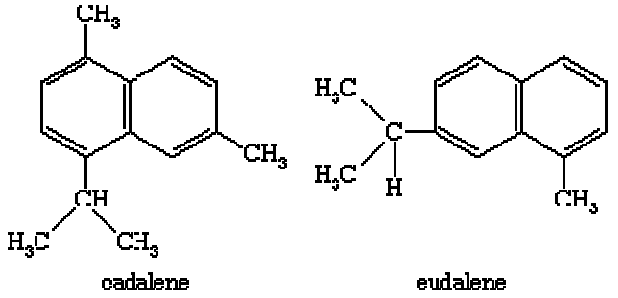
2.3.2 Sesquiterpenes as major component in agarwood oil

Generally, agarwood oils are mitures of sesquiterpenes, sesquiterpene alcohols,oxgyenated compounds, chromone derivatives and resins. Some importants compounds are agarospirol, jinkohol-eremol, jinkohol and kesenol that may contribute to characteristic aroma of agarwood (Nakanishi-1984,Ishihara-1993).

The name *terpene* specifically refers to naturally occurring compounds that are derivatives of a single isoprene unit. The smallest terpene molecules, those containing 10 carbon atoms are called monoterpenes. The larger molecules, increased by one isoprene unit at a time, are called sesquiterpenes ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$), triterpenes ($C_{30}H_{48}$), and tetraterpenes ($C_{40}H_{64}$). The monoterpenes are mostly volatile, which accounts for their fragrances.

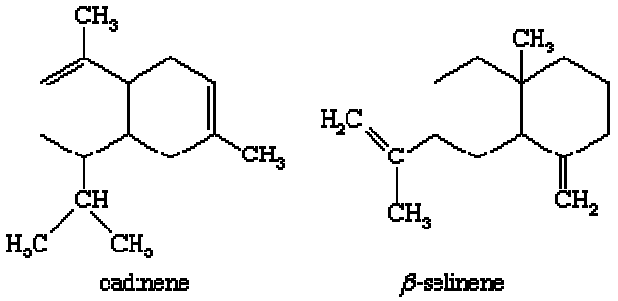
Terpenes of higher molecular weight are less volatile, although sesquiterpenes contribute to the flavours of some foods. Refer to research report by Masakazu- 1993, agarwood oil contained large amount of oxygenates sesquiterpene and chromone derivatives. Because sesquiterpenes are of lower volatility than the monoterpenes, sesquiterpenes ($C_{15}H_{24}$), are isolated from their natural sources by distillation with steam or by extraction. They are purified by vacuum fractional distillation or by chromatography.

The sesquiterpenes demonstrate an even greater complexity of structure than the monoterpenes, and oxygenated sesquiterpenes are commonly encountered. Two arrangements of isoprene units are found in bicyclic sesquiterpenes, the cadalene and the eudalene types, and the carbon skeleton of a sesquiterpene may frequently be determined by heating it with sulfur or selenium to effect dehydrogenation to the corresponding naphthalenic hydrocarbons: cadalene, 4-isopropyl-1,6-dimethylnaphthalene; or eudalene, 7-isopropyl-1-methylnaphthalene. In those cases in which sulfur dehydrogenation fails to yield information about the carbon skeleton of a sesquiterpene, a systematic degradation by oxidation to compounds of known structure is necessary.



cadalene eudalene

Cadinene, the principal component of oils of cubeb and cade, is a typical sesquiterpene of the cadalene type. It is optically active oil with a boiling point of 274 °C (525 °F). β-Selinene, present in celery oil, is typical of the eudalene type.



cadinene β-selinene

2.4 Isolated of Agarwood oil

Agarwood oil is the complex compound, so we need to purify and isolate this complex compound in order to identify a major constituent in agarwood oil. There are many technique most widely used for purify such as Liquid chromatography (LC) with mass spectrometric (MS), ultraviolet (UV), diode- array (DAD), evaporative light scattering (ELSD), capillary electrophoresis (CE) and Thin Layer chromatography (TLC). Although is the main technique for quality control of synthesis and purification, TLC and Preparative layer chromatography (PLC) are important. They enable simultaneous analysis of many samples on one plate, solvent consumption is low, plate are disposable, so there are no memory effect, and it is possible to use several detection method in sequence. TLC is furthermore, an easy, inexpensive method that can be used in any laboratory (Piia K. Salo- 2006).

2.4.1 Theory of Thin Layer Chromatography (TLC)

To thoroughly understand the process of TLC, as well as all types of chromatography, we must travel to the molecular level. All forms of chromatography involve a dynamic and rapid equilibrium of molecules between the two phases. As shown in **Figure 2.1**, there are:

1. **free** - completely dissolved in the liquid or gaseous mobile phase and
2. **absorbed** - stuck on the surface of the solid stationary phase.

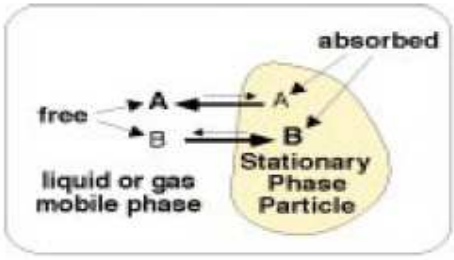


Figure 2.1: Mixture of A & B frees in mobile phase and absorbed on the stationary phase.

Molecules are continuously moving back and forth between the free and absorbed states with millions of molecules absorbing and millions of other molecules desorbing each second. The equilibrium between the free and absorbed states depends on three factors:

I. Polarity and size of the molecule
II. Polarity of the stationary phase
III. Polarity of the solvent

Thus, one has three different variables to change in chromatography. The polarity of the molecules is determined by their structures. By selecting different stationary and mobile phases, one can change the equilibrium between the free and

absorbed states. It is important to understand chromatography at this molecular level because this allows one to choose mobile and stationary phases that will separate just about any mixture of molecules.

Since the A molecules spend more time in the mobile phase, they will be carried through the stationary phase faster and move farther in a given amount of time. Since B is absorbed to the stationary phase more than A, B molecules spend less time in the mobile phase and therefore move through the stationary phase particles more slowly. The B molecules don't move as far in the same amount of time. The consequences of this flowing mobile phase are that A is gradually separated from B by moving ahead in the flow. This separation process is depicted in **Figure 2.2**.

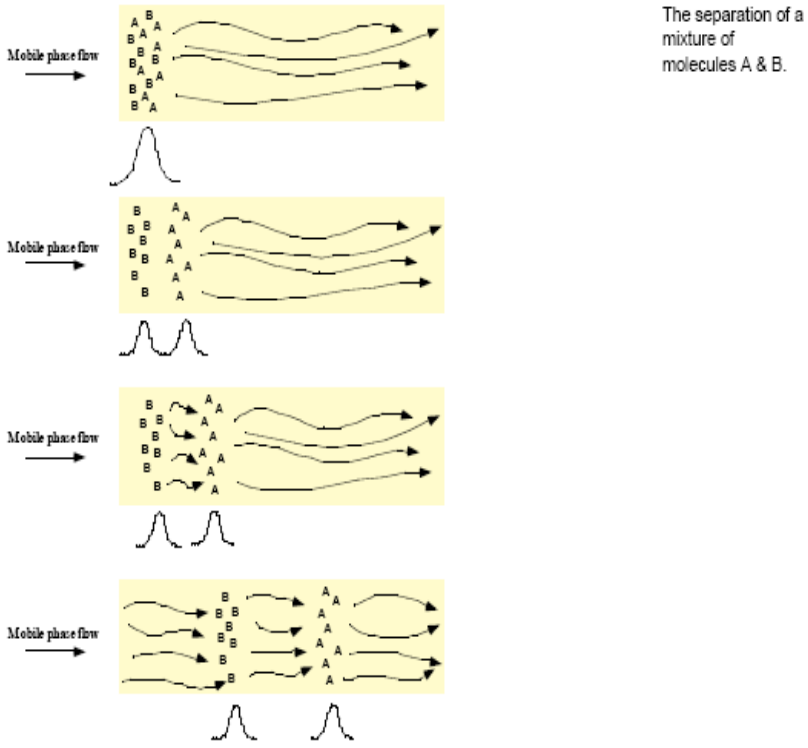


Figure 2.2: Mixture of A & B separated by a moving mobile phase while being absorbed on the stationary phase.

In TLC, the stationary phase is typically alumina (Al2O3xH2O)n or silica gel (SiO2**.**xH2O)n. The covalent network of these absorbents creates very polar materials. The structure of silica is shown below:

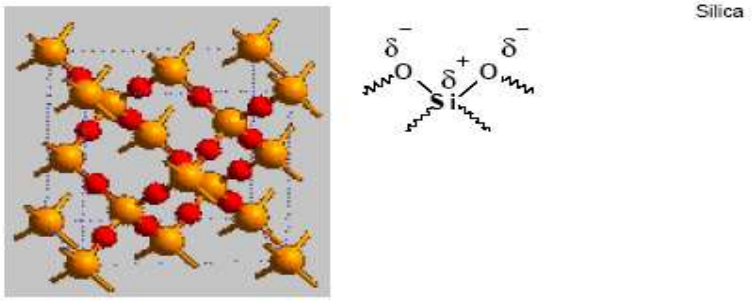


Figure 2.3: Structure of Silica (SiO2**.**xH2O)n.

The electropositive character of the aluminum or silicon and the electronegative oxygen create a very polar stationary phase. Therefore, the more polar the molecule to be separated, the stronger the attractive force to the stationary phase.

2.4.2 Spotting the TLC Plate

One advantage TLC has over other separation methods is that it is truly a micro scale technique. Only a few micrograms of material in solution are necessary to observe the solute on a TLC plate. Dissolve a few milligrams of material in a volatile solvent creating a dilute solution. Choose a volatile solvent that completely dissolves the sample. However, if it is partially soluble, since such only low concentrations are needed, normally we will be able to observe the compound. Once the sample is prepared, a spotting capillary must be used to add the sample to the plate. The spotting capillaries must be extremely small. In fact, the opening at the end of a regular Pasteur pipet is too big for spotting a TLC plate. The solution can be drawn up the tube by capillary action (hence the name) and spotted on the plate at the hash mark labeled in pencil.

This is known as the origin and is shown in **Figure 2.4**. Since a TLC plate can run three, if not four mixtures at one time, it is very important to properly label the plate. Notice that pencil is always used to mark a TLC plate since the graphite carbon is inert. If organic ink is used to mark the plate, it will chromatograph just as any other organic compound and give incorrect results.

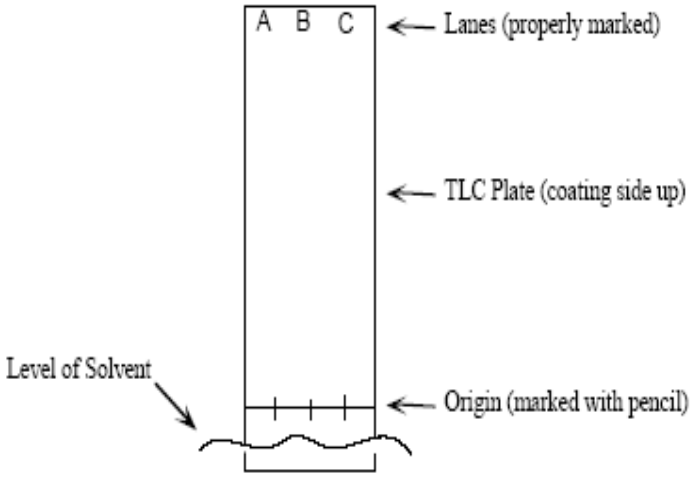


Figure 2.4: TLC Plate ready to be spotted.

The solvent should evaporate quickly leaving your mixture behind on the plate. Spot the plate a couple of times to ensure the material is present, but do not spot too much sample. If too much solute is added to the plate, a poor separation will result. Smearing, smudging and spots that overlap will result making identification of separated components difficult.

2.4.3 Development

Once the dilute solution of the mixture has been spotted on the plate, the next step is the development. Just like paper chromatography, the solvent must be in

contact with the stationary phase. **Figure 2.5** shows a wide-mouth bottle commonly used to develop TLC plates.

Figure 2.5: Development Chamber.

The bottle is filled with a small amount of the mobile phase and capped with a cork. In addition, a piece of filter paper is put in the bottle to help create an atmosphere saturated with solvent. If the spots are submerged in the solvent, they are washed off the plate and lost. Once the solvent has run within a centimeter of the top of the plate, remove it with tweezers. Using a pencil, immediately draw a line across the plate where the solvent front can be seen. The proper location of this solvent front line will be important for later calculations.

2.4.4 Visualization

Some organic compounds are colored. If you are fortunate enough to be separating organic molecules that are colored such as dyes, inks or indicators, then visualizing the separated spots is easy. However, since most organic compounds are colorless, this first method does not always work. In most cases observed the

separated spots by UV light works well. TLC plates normally contain a fluorescent indicator which makes the TLC plate glow green under UV light of wavelength 254 nm. Compounds that absorb UV light will quench the green fluorescence yielding dark purple or bluish spots on the plate. Simply put the plate under a UV lamp, and the compounds become visible to the naked eye. Lightly circle the spots, so that you will have a permanent record of their location for later calculations.

2.4.5 R*f* Values

In addition to qualitative results, TLC can also provide a chromatographic measurement known as an R*f* value. The R*f* value is the "retardation factor" or the "ratio-to-front" value expressed as a decimal fraction. The R*f* value can be calculated as:

$$R_f = \frac{\text{distance spot travels}}{\text{distance solvent travels}}$$

This number can be calculated for each spot observed on a TLC plate. Essentially it describes the distance traveled by the individual components. If two spots travel the same distance or have the same R*f* value then it might be concluded that the two components are the same molecule. For R*f* value comparisons to be valid; however, TLC plates must be run under the same exact conditions. These conditions include the stationary phase, mobile phase, and temperature.

Just as many organic molecules have the same melting point and color, many can have the same R*f* value, so identical R*f* values doesn't necessarily mean identical compounds. Additional information must be obtained before this conclusion can be made. It is important to restate that this number is only significant when the same

chromatographic conditions are used. **Figure 2.6** shows a diagram of a typical TLC plate and how the distances are measured to calculate the R*f* value.

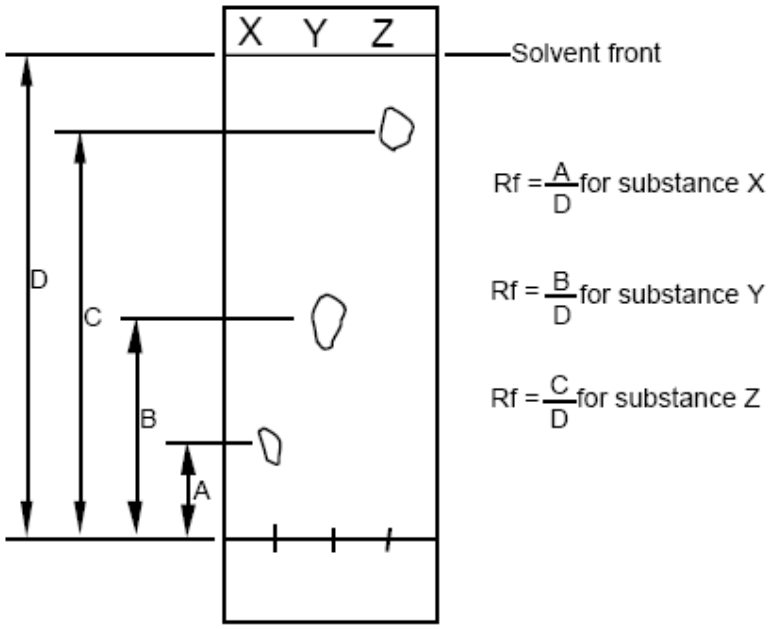


Figure 2.6: Calculation of R*f* value.

2.4.6 Preparative Plates

TLC can be used on a micro scale to monitor a reaction and determine if the product or products were successfully produced using only microgram quantities of materials. It is difficult to separate gram quantities using TLC and therefore column chromatography is used at this scale. However, larger TLC plates, called a Preparative Plates, can be used for separations of milligram quantities of materials because they are coated with thick layers (1-3mm) of stationary phase. Once the plate is developed, the spots are scraped off the plate along with the absorbent such as in **figure 2.7**. Each separate component is then extracted from the stationary phase with a polar solvent.

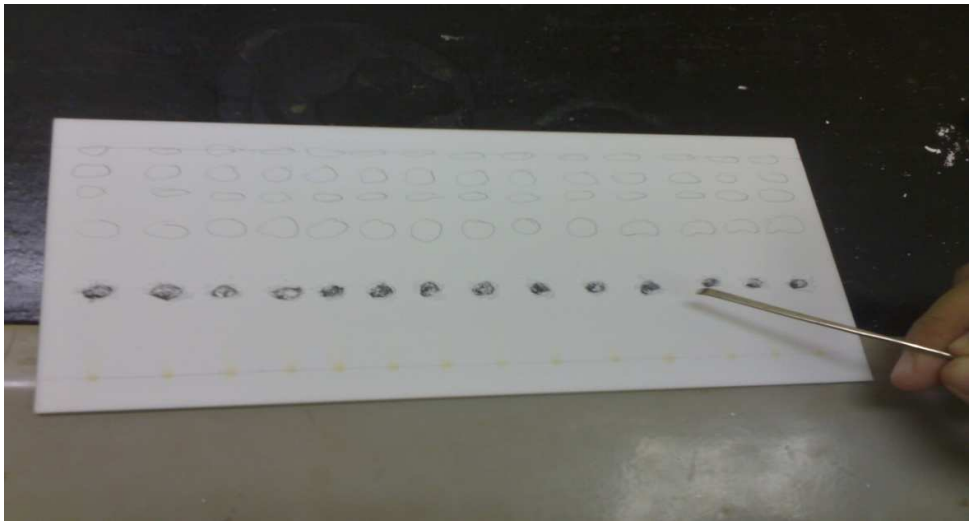


Figure 2.7: The spots are scraped off the plate

2.5 Analysis agarwood oil.

There were many methods which we can use to analysis and detect the component of agarwood oil occurs in a sample. In this research, we use gas chromatography-mass spectrometer or mostly known as GCMS to search for every component in the oil produce from Aquilaria malaccensis.

2.5.1 Gas chromatography-mass spectrometer (GCMS)

Gas chromatography mass spectrometry (GC/MS) is an instrumental technique, comprising a gas chromatograph (GC) coupled to a mass spectrometer (MS), by which complex mixtures of chemicals may be separated, identified and quantified. This makes it ideal for the analysis of the hundreds of relatively low molecular weight compounds found in environmental materials. In order for a compound to be analyzed by GC/MS it must be sufficiently volatile and thermally stable. In addition, functionalized compounds may require chemical modification (derivatization), prior to analysis, to eliminate undesirable adsorption effects that would otherwise affect the quality of the data obtained.

Samples are usually analyzed as organic solutions consequently materials of interest (e.g. soils, sediments, tissues etc.) need to be solvent extracted and the extract subjected to various 'wet chemical' techniques before GC/MS analysis is possible. The sample solution is injected into the GC inlet where it is vaporized and swept onto a chromatographic column by the carrier gas (usually helium). The sample flows through the column and the compounds comprising the mixture of interest are separated by virtue of their relative interaction with the coating of the column (stationary phase) and the carrier gas (mobile phase). The latter part of the column passes through a heated transfer line and ends at the entrance to ion source (Fig. 2.8) where compounds eluting from the column are converted to ions.

Two potential methods exist for ion production. The most frequently used method is electron ionization (EI) and the occasionally used alternative is chemical ionization (CI). For EI a beam of electrons ionize the sample molecules resulting in the loss of one electron. A molecule with one electron missing is called the molecular ion and is represented by $M^{+\cdot}$ (radical cat ion). When the resulting peak from this ion is seen in a mass spectrum, it gives the molecular weight of the compound. Due to the large amount of energy imparted to the molecular ion it usually fragments producing further smaller ions with characteristic relative abundances that provide a 'fingerprint' for that molecular structure.

This information may be then used to identify compounds of interest and help elucidate the structure of unknown components of mixtures. CI begins with the ionization of methane (or another suitable gas), creating a radical which in turn will ionize the sample molecule to produce $[M+H]^+$ molecular ions. CI is a less energetic way of ionizing a molecule hence less fragmentation occurs with CI than with EI, hence CI yields less information about the detailed structure of the molecule, but does yield the molecular ion; sometimes the molecular ion cannot be detected using EI, hence the two methods complement one another. Once ionized a small positive is used to repel the ions out of the ionization chamber.

The next component is a mass analyzer (filter), which separates the positively charged ions according to various mass related properties depending upon the analyzer used. Several types of analyzer exist: quadrupoles (Fig 2.9), ion traps, magnetic sector, time-of-flight, radio frequency, cyclotron resonance and focusing to name a few. The most common are quadrupoles and ion traps. After the ions are separated they enter a detector the output from which is amplified to boost the signal. The detector sends information to a computer that records all of the data produced, converts the electrical impulses into visual displays and hard copy displays. In addition, the computer also controls the operation of the mass spectrometer.

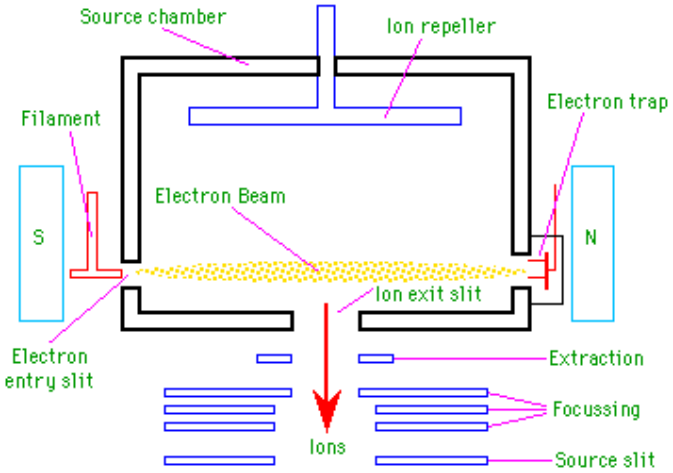


Figure 2.8 A schematic of an ion source

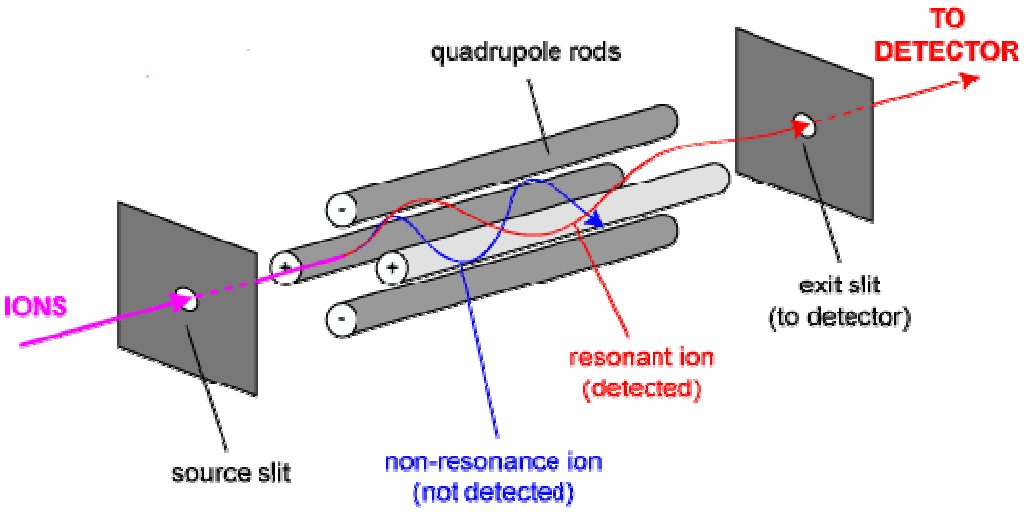


Figure 2.9 A schematic of a quadrupole analyzer

CHAPTER 3

METHODOLOGY

3.1 Introduction

There are several processes and steps that need to be done and followed in order to archive the objective of this project. Generally, major process is extraction process which is sample of agarwood oil is collected from hydro distillation process and than isolated by preparative TLC before through the analysis process to confirm its compound by using GC-MS.

3.2 Drying Process

The raw material (sawdust of agarwood) need go through the drying process before started extraction process which is it should to be completely dry from any moisture and to get free of any substance that can influence the impurities of oil when it has been extracted. The using of small pieces of agarwood (sawdust) is to increased rate of extraction because in sawdust shape it will increase surface area between solvent and solid. The process becomes completed when there were no changes of humidity inside tray dryer or in other word, humidity value become constant. This process are set up at 50-60^{o}C for 1 days drying period.

3.3 Soaking process

The purpose of soaking is to break down parenchymatous and oil glands (Chang *et al*, 2002). The ratio distilled water to sawdust of agarwood is 7:1 and in range period 3 to 7 days (Dong-ping *et al*, 1999). During this research, 100 g of sawdust of agarwood are used, so 700 ml of water are used for 3 days soaking.

Soaking of sawdust: water (1:7)

Figure 3.1: Soaking process

3.4 Hydro Distillation process (extraction)

The agarwood oil extraction was done using sets of Hydro distillation equipments unit. Figure 3.2 showed the equipment used in this research. The equipment set up consists of a condenser and mantle heater.

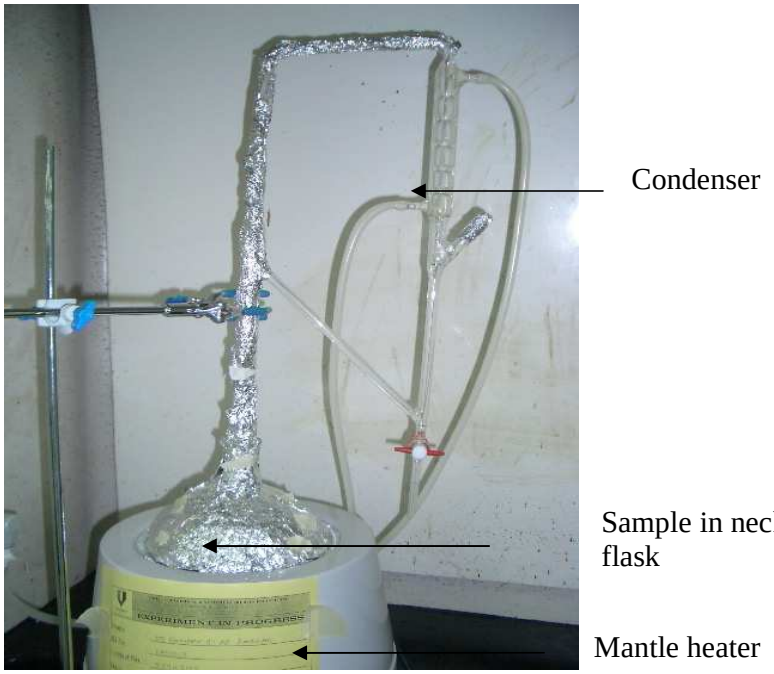


Figure 3.2: Equipment set up

The extraction was carried out at atmospheric pressure. First, 100 g of ground agarwood were put into the neck flash and later added with 700 ml of distilled water and connected to the rest of the apparatus. Then wrap over the equipment by aluminum foil in order to maintain the temperature. Add up small amount of hexane at Dean stark. After that, the mantle heater was switched on. The experiment was carried out for 8 hours for 3 days. During the experiment, the temperatures are maintained at boiling water (100^{o}C) and connecting the condenser with distilled water to re-circulating the heat. This will allow continuous condensation of the vapor and to maintain the pressure of the extraction to be at atmospheric pressure.

First flow for the process is steam and oil vapors are passed through the condenser. After condensation process, the vapors mixture will turn back into liquid and it will be collected in at Dean stark. At the end of the experiment, the agarwood oil was collected from the Dean stark. The oil collected was then dried over anhydrous sodium sulfate to make sure the oil is free from water. The experiments were repeated three times.

3.5 Preparative Thin Layer Chromatography and Isolation Procedure

Preparative Thin Layer Chromatography was performed on 20cm x 10cm PLC plates (Merck) coated with 2mm layer of silica gel 60 F_{254} develop with ethyl acetate- n-hexane (1:5 v/v) as mobile phases. This preparation applied on 3 samples which are from LAB sample and two from industries scale (MAHA & KELANTAN) samples. Sample solutions were applied, manually to TLC adsorbent, by use spotting capillary as thin 1.5 cm rectangular bands 1.5 cm apart. Then, the plates were twice eluted vertically in a saturated chamber to a distance of 8 cm, and performed in fume hood. The elution time was approximately 30 minute and the zone of the synthesis product was isolated as figure 3.3. The isolation zones were detected visually under UV lamps and the spots occurs outlined with pencil while being viewed in the UV-light. The sample zone was scraping off from plate. Then, to purify sample zone was insert into reservoir unit and 1mL Chloroform was added. The sample zone was run in Ultrasonic for 15 minute to centrifuge. Then, used syringe to filter and final purify of the product isolated from PLC was confirmed by use GC-MS and R*f* value was calculated.

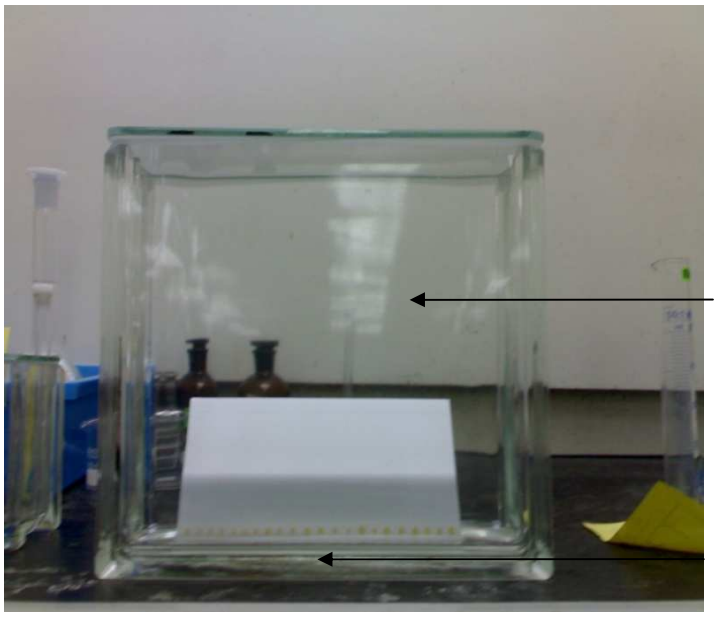


Figure 3.3: Preparative Thin Layer Chromatography

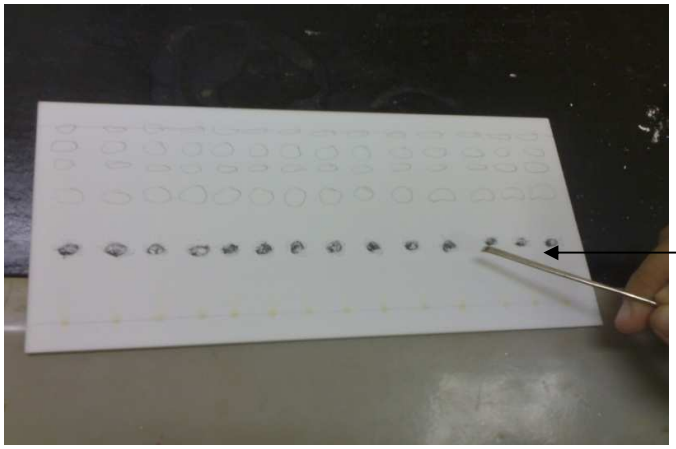


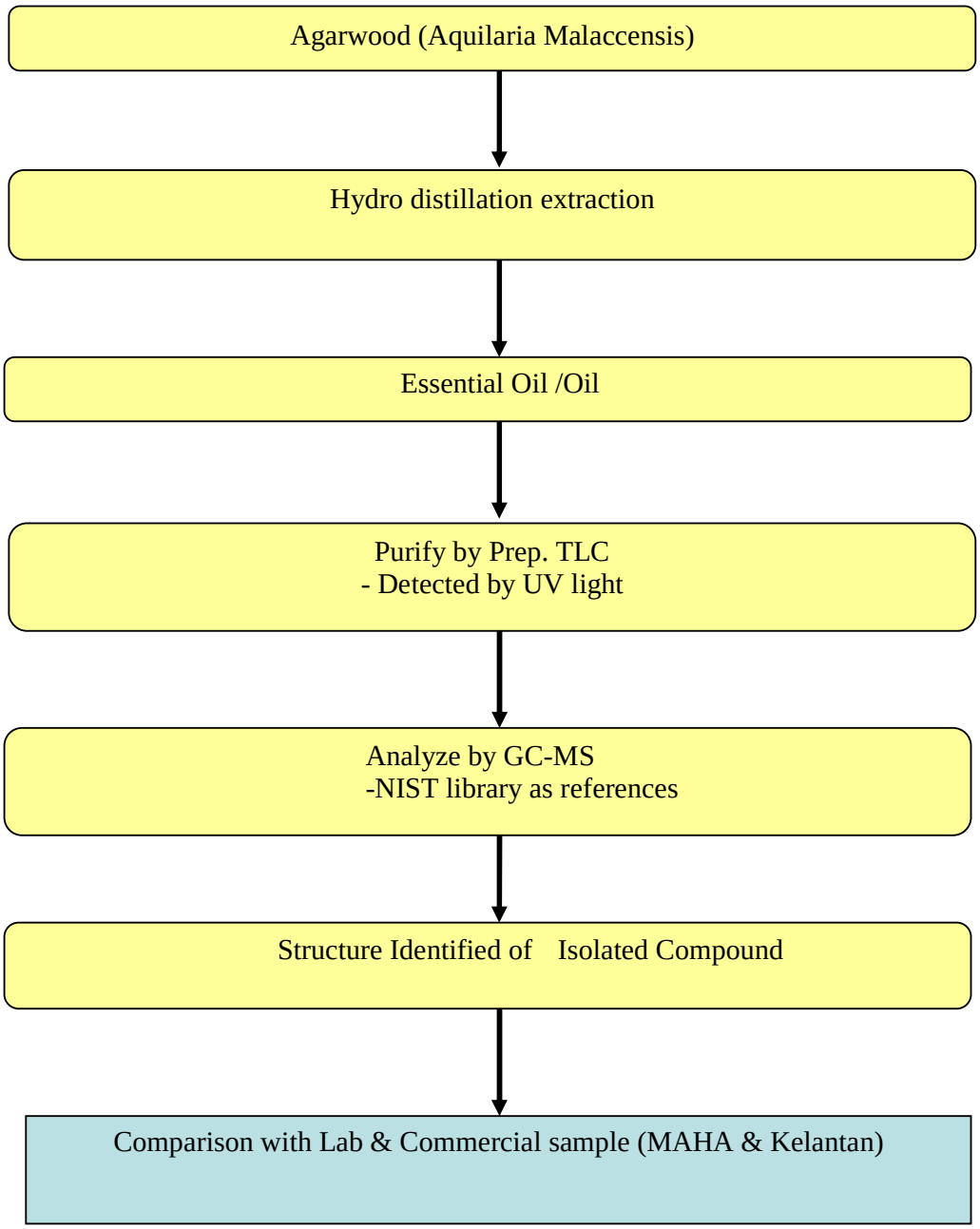
Figure 3.4: The spots are scraped off from the plate

3.6 Analysis by GC/MS

The extracts were analyzed and confirmed by GC-MS with Hewlett-Packard gas chromatograph and MS mass detector (7890 with 6590 mass spectrometry detector). The 14 spots (samples) dissolved in n-hexane was injected in split mode with using gas helium as a carrier gas. The GC separates chemicals based on their volatility, or ease with which they evaporate into a gas. The MS is used to identify chemicals based on their structure. Each sample was run for 70 minute using DB-wax capillary column.(30m x 0.25µm x 0.25) Identification of chemical component was based on the comparison of the spectral data with existing NIST library and literature references.

Figure 3.5: Component in gas chromatography mass spectrometer

SUMMARY OF RESEARCH FLOW



CHAPTER 4

RESULT & DISCUSSION

4.1 Introduction

The experiment of extracting agarwood essential oil by using hydro distillation method followed by purification and analysis is completed. The procedure that we used in this experiment is followed step by step by counting on the precaution steps and several assumptions to ensure we get the maximum result from the experiment. From the results obtained, fragrant compounds of agarwood are sesquiterpenoids and chromone derivatives, which are the main source of agarwood's peculiar odor. Agarwood oils vary in their composition; some oils contain a large amount of sesquiterpene compounds and others contain principally benzylacetone. Analyses of the volatile components of agarwood oils used in this study were performed on PTLC-GCMS.

4.2 R*f* value from Preparative TLC

All samples were subjected to prep. TLC (2mm thickness: developed with C_6H_6-EtOAc, 5:1, detected by UV irradiation) to afford spots. Once the spots are located, their Rf values can be calculated and the results obtained is presented in table 4.0.

Table 4.0: Summaries of relative of R*f* value

Samples / R_f	Spot 1	Spot 2	Spot 3	Spot 4	Spot 5
LAB	0.288	0.75	0.775	0.887	-
MAHA	0.175	0.413	0.775	0.887	0.913
KELANTAN	0.275	0.625	0.788	0.887	0.95

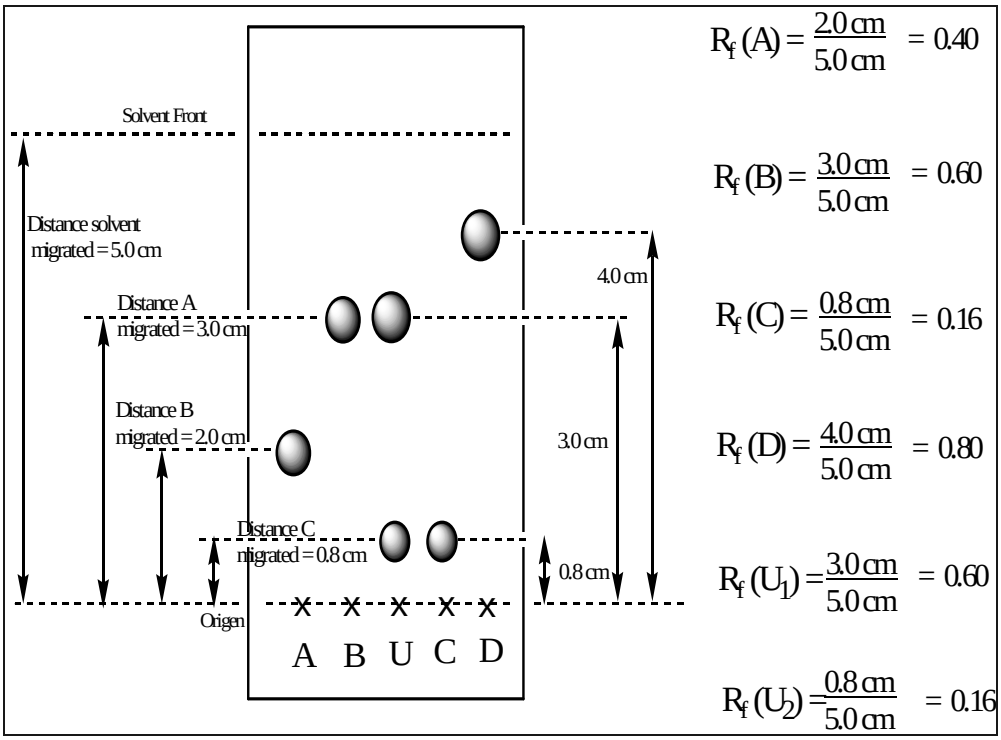
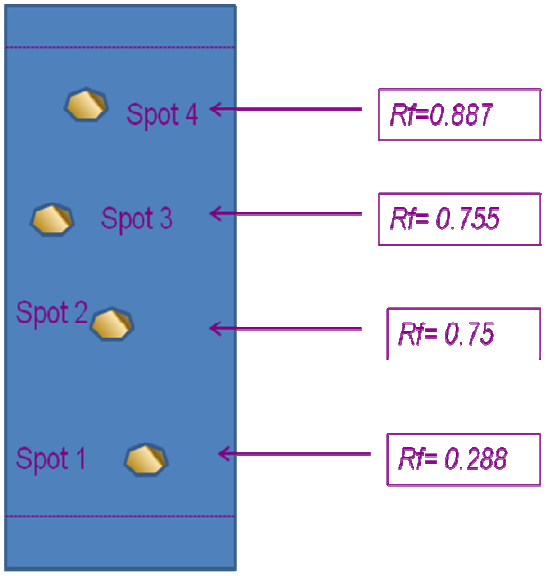


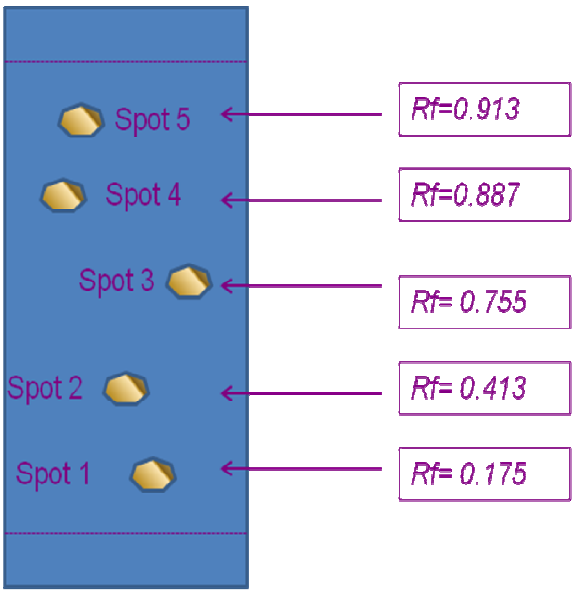
Figure 4.0: R*f* value calculation

Results of R_f from Lab sample



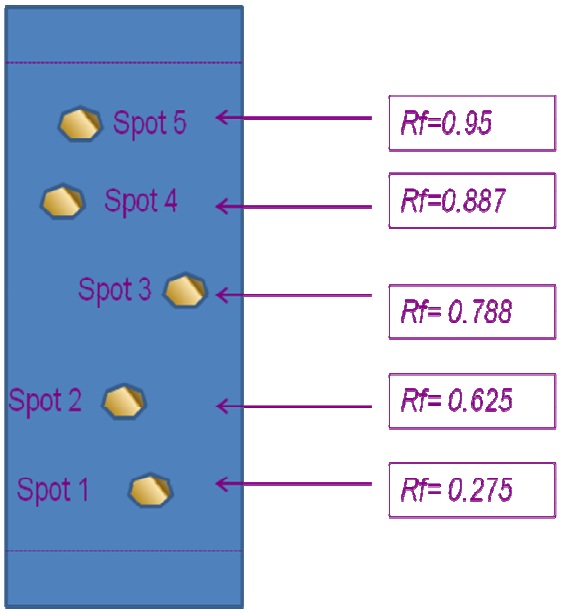
-Sample solutions from Lab sample was performed on 20cm x 10cm PLC plates (Merck) coated with 2mm layer of silica gel 60 F_{254} develop with ethyl acetate-n-hexane (1:5 v/v) as mobile phases.
-The present of spot was detected by UV irradiation to afford 4 spots. Each spots, i.e spots 1, 2, 3 and 4 numbered in order of increasing polarity.

Results of R_f from MAHA sample



-Sample solutions from MAHA -sample was performed on 20cm x 10cm PLC plates (Merck) coated with 2mm layer of silica gel 60 F_{254} develop with ethyl acetate- n-hexane (1:5 v/v) as mobile phases.
-The present of spot was detected by UV irradiation to afford 4 spots. Each spots, i.e spots 1, 2, 3,4 and 5 numbered in order of increasing polarity.

Results of R_f from KELANTAN sample



-Sample solutions from MAHA sample was performed on 20cm x 10cm PLC plates (Merck) coated with 2mm layer of silica gel 60 F_{254} develop with ethyl acetate- n-hexane (1:5 v/v) as mobile phases.
-The present of spot was detected by UV irradiation to afford 4 spots. Each spots, i.e spots 1, 2, 3,4 and 5 numbered in order of increasing polarity.

4.3 Discussion on the R*f* value

In preparative TLC, the solvent gradually moves up the plate via capillary action, and it carries the deposited substances along with it at different rates. The result showed is that each component of the deposited mixture is moved a different distance up the plate by the solvent. The components then appear as a series of spots at different locations up the plate. Substances can be identified from their so-called R_f values. The retention factor, or R_f, is defined as the distance traveled by the compound divided by the distance traveled by the solvent.

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent front}}$$

The R_f for a compound is a constant from one experiment to the next only (Lab, MAHA & KELANTAN samples) if the chromatography conditions below are also constant:

- solvent system
- adsorbent
- thickness of the adsorbent
- amount of material spotted
- temperature

But, since these factors are difficult to keep constant from experiment to experiment, relative Rf values are generally considered. “Relative R_f” means that the values are reported relative to a standard, or it means the R_f values of compounds run on the same plate at the same time. The larger R_f of a compound, the larger the distance it travels on the TLC plate. When comparing two different compounds run under identical chromatography conditions, the compound with the larger R_f is less polar because it interacts less strongly with the polar adsorbent on the TLC plate. Compound of low polarity will have a larger R_f value than a polar compound if run on the same plate.

Actually, the R_f can provide corroborative evidence as to the identity of a compound. If the identity of a compound is suspected but not yet proven, an authentic sample of the compound, or standard, is spotted and run on a TLC plate side by side (or on top of each other) with the compound in question. If two substances have the same R_f value, they are likely (but not necessarily) the same compound. From the results obtained, it showed spot 4 from all samples applied have a same R_f values which is 0.887. Its means at spot 4 should have same compounds when we compare within that samples. If they have different Rf values, they are definitely different compounds. Table 4 showed summaries of R_f values. This result was confirmed by GC-MS.

4.4 Result from GC-MS.

After carry out the extracting and preparative TLC, the spot through analyzing process by using GC-MS, then the result is founded and analyzed in table 4.1 to 4.14. All components in the sample can be search according to the retention time and percentage of quality. Because agarwood is the complex mixture, we choose just for 70% of quantity of chemical component present in the graph. The lists of components in agarwood oil that analyze by the GCMS are shown in table.

4.4.1 Spectrum and the constituents identified in the GC-MS experiments of agarwood oil from Lab samples- without PLTC.

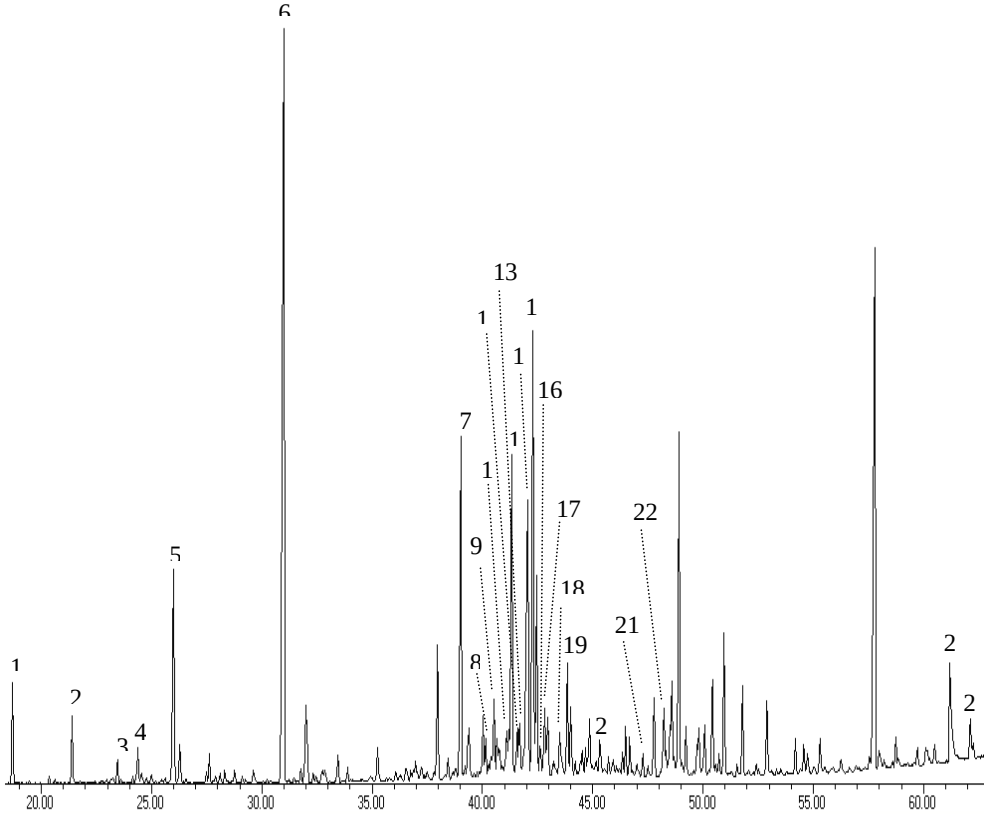


Figure 4.1: Analysis of agarwood oil using 30m x 0.25mm i.d x 0.25µm DB- Wax column, ,T= 60-246^{o}C, ΔT= 3^{o}C/min.

Table 4.1: Analysis of Lab Sample by GC-MS without through PTLC Main components of Agarwood oil using 30m x 0.25mm i.d x 0.25µm DB- Wax column, T= 60-246°C, ΔT= 3°C/min.

No.	Main components	Formula	Quality	% Area	CAS no.
1	Benzaldehyde	C_7H_6O	96	1.39	100-52-7
2	α-Guaiene	$C_{15}H_{24}$	99	0.99	12/1/3691
3	Acetophenone	C_8H_8O	94	0.33	98-86-2
4	Benzaldehyde, 2-hydroxy-	$C_7H_6O_2$	94	0.58	90-02-8
5	α-Bulnesene	$C_{15}H_{24}$	99	3.81	3691-11-0
6	2-Butanone, 3-phenyl-	$C_{10}H_{12}O$	95	16.24	769-59-5
7	τ-Eudesmol	$C_{15}H_{26}O$	93	5.71	1209-71-8
8	α-Bisabolol oxide B	$C_{15}H_{26}O_2$	87	0.33	26184-88-3
9	β-Panasinsene	$C_{15}H_{24}$	90	0.87	159-39-0
10	τ-Eudesmol	$C_{15}H_{26}O$	99	0.25	1209-71-8
11	Agarospirol	$C_{15}H_{26}O$	86	4.28	1460-73-7
12	.(+)-Aromadendrene	$C_{15}H_{24}$	89	0.42	489-39-4
13	β-Humulene	$C_{15}H_{24}$	91	0.51	116-04-1
14	Neoisolongifolene	$C_{15}H_{24}$	90	6.44	156-12-4
15	Eudesma-3,7(11)-diene	$C_{15}H_{24}$	84	7.24	6813-21-4
16	τ-eudesmol	$C_{15}H_{26}O$	98	0.46	1209-71-8
17	β-Eudesmol	$C_{15}H_{26}O$	91	0.8	473-15-4
18	D-longifolene	$C_{15}H_{24}$	91	0.49	475-20-7
19	Alloaromadendrene	$C_{15}H_{24}$	96	1.48	25246-27-9
20	Tricyclo[3.2.1.02,7]oct-3-ene, 2,3,4,5-tetramethyl-	$C_{12}H_{18}$	90	0.6	62338-44-7
21	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	$C_{15}H_{22}O$	86	0.27	188-66-5
22	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	$C_{15}H_{24}$	90	0.56	159-38-5
23	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	95	2.66	57-10-3
24	3-Pentanone, 1,5-diphenyl-	$C_{17}H_{18}O$	90	0.43	5396-91-8

4.4.2 Spectrum and the constituents identified in the PTLC experiments of agarwood oil from Lab samples

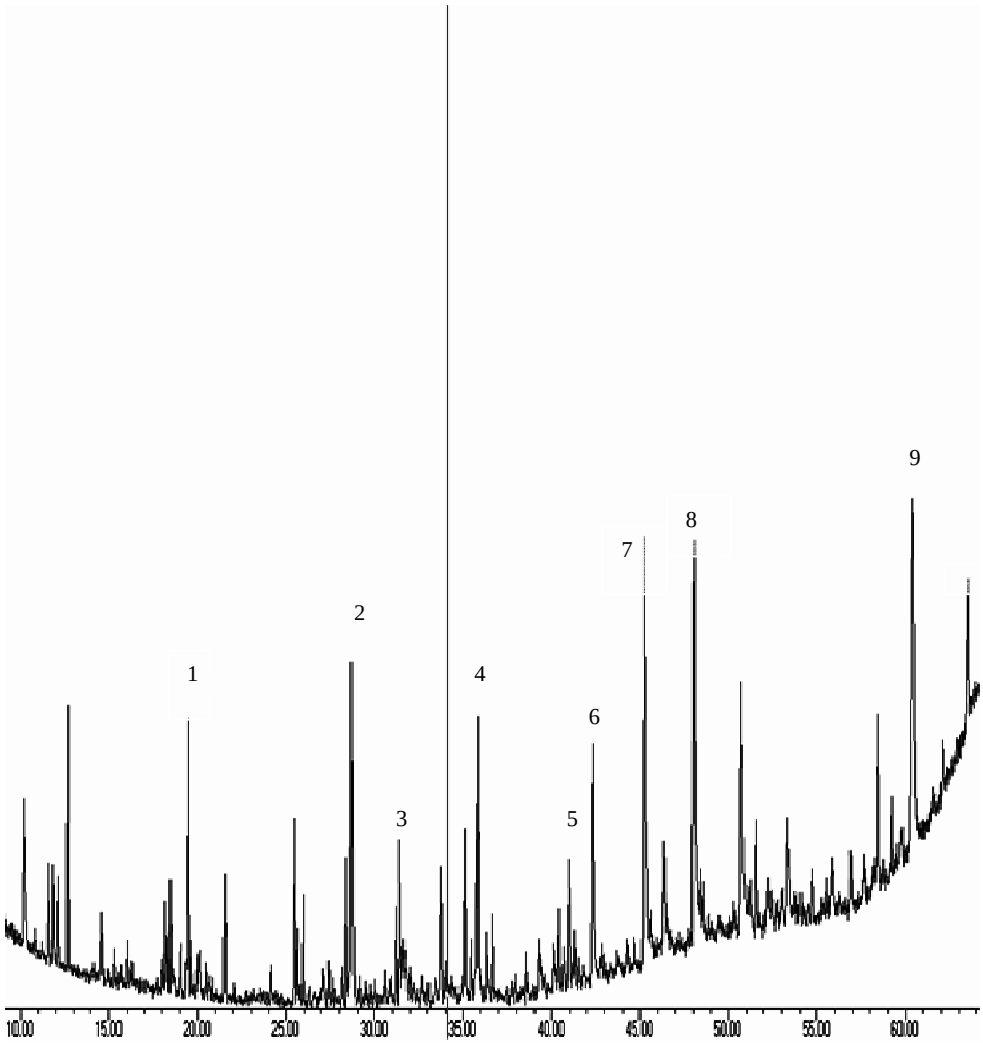


Figure 4.2: Analysis of Spot 1-LAB sample

GCMS analysis of the PTLC experiment (spot 1) of agarwood oil (The chromatographic column used for analysis was DB-wax, 30 m x 0.25 μm x 0.25 i.d. T= 60-246^{o}C, ΔT= 3^{o}C/min.

Table 4.2: GC-MS analysis of Spot 1-LAB sample

Peak no.	Area %	**ID**	Ref#	Cas#	Qual
1	0.02	Cyclohexasiloxane	$C_{12}H_{36}O_6Si_6$	000540-97-6	91
2	0.05	1,7-Octadiene, 2-methyl-6-methylene	$C_{10}H_{16}$	001686-30-2	70
3	0.02	2-Propenoic acid, tridecyl ester	$C_{16}H_{30}O_2$	003076-04-8	91
4	0.03	Diphenyl ether	$C_{12}H_{10}O$	000101-84-8	76
5	0.02	3-Allyl-6-methoxyphenol	$C_{10}H_{12}O_2$	000501-19-9	95
6	0.04	Octadecane, 2,6,10,14-tetramethyl-	$C_{22}H_{46}$	054964-82-8	86
7	0.07	Tricosane	$C_{23}H_{48}$	000638-67-5	91
8	0.06	Tetracosane	$C_{24}H_{50}$	000646-31-1	96
9	0.11	Antioxidant 425	$C_{25}H_{36}O_2$	000088-24-4	99

Antioxidant 425 (0.11%), Tricosane (0.07%),Tetracosane (0.06%) and 1,7-Octadiene, 2-methyl-6-methylene (0.05%) were the main constituents of the oil of agarwood in spot 1 with chromatographic column used for analysis was DB-wax, 30 m x0. 25 μm x 0.25 i.d.

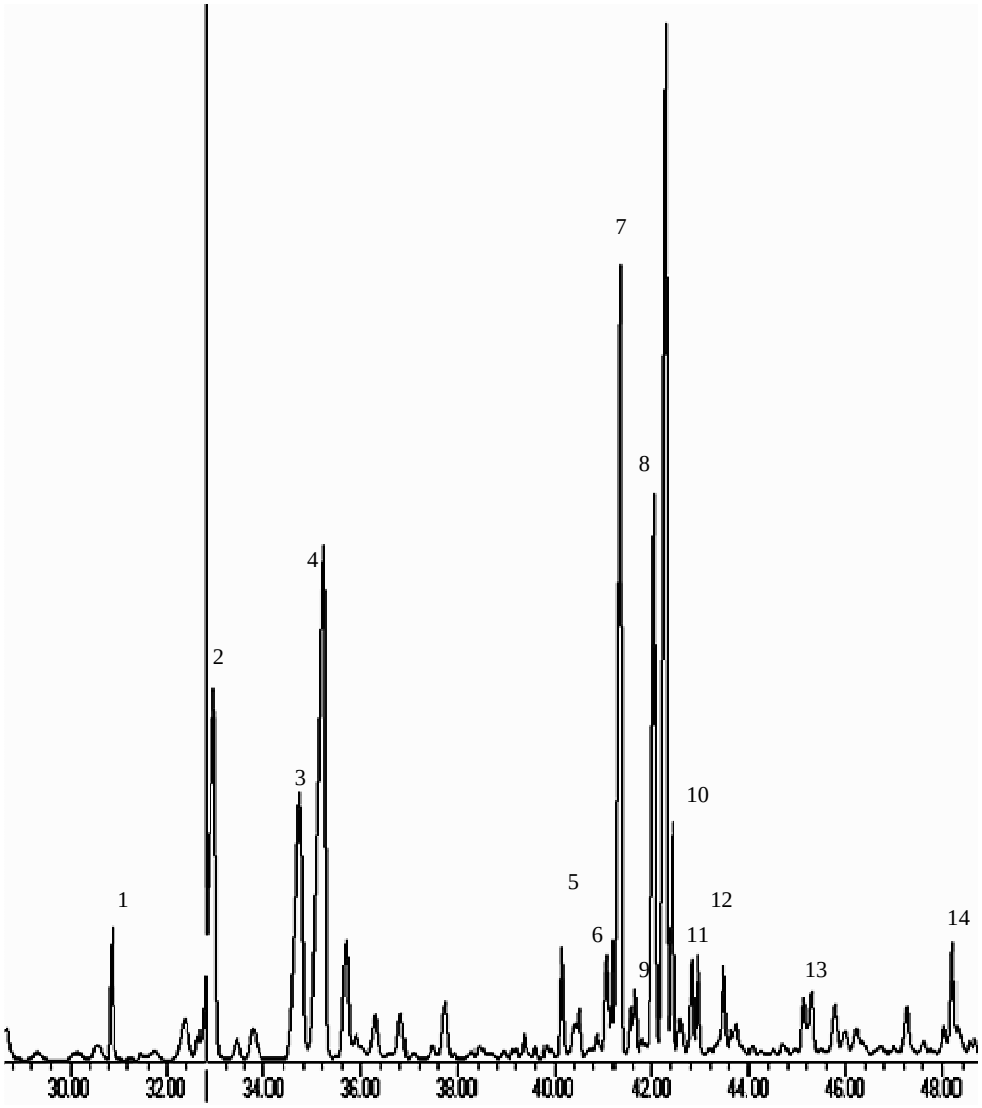


Figure 4.3: GC-MS analysis of Spot 2-LAB sample

GCMS analysis of the PTLC experiment (spot 2) of agarwood oil (The chromatographic column used for analysis was DB-wax, 30 m x 0.25 μm x 0.25 i.d. T= 60-246^{o}C, ΔT= 3^{o}C/min.

Table 4.3: Analysis of Spot 2-LAB sample

Peak no.	Area %	ID	Ref#	Cas#	Qual
1	0.46	2-Butanone, 4-phenyl	$C_{10}H_{12}O$	002550-26-7	97
2	3.2	Agarospirol	$C_{15}H_{26}O$	001460-73-7	91
3	2.46	Aristolene	$C_{15}H_{24}$	1000150-14-9	89
4	4.69	.+)-Aromadendrene	$C_{15}H_{24}$	000489-39-4	90
5	0.41	α-Bisabolol oxide B	$C_{15}H_{26}O_2$	026184-88-3	94
6	0.31	τ-Eudesmol	$C_{15}H_{26}O$	001209-71-8	99
7	3.29	Agarospirol	$C_{15}H_{26}O$	001460-73-7	91
8	2.50	Aristolene	$C_{15}H_{24}$	1000150-14-9	89
9	4.77	α-Guaiene	$C_{15}H_{24}$	003691-11-0	87
10	0.31	β-Selinenol	$C_{15}H_{26}O$	000473-15-4	99
11	0.28	Benzene	$C_{12}H_{18}$	000087-85-4	76
12	0.41	Eudesma-4(14),11-diene	$C_{15}H_{24}$	1000152-04-3	89
13	0.06	4áH,5à-Eremophila-1(10),11-diene 4à,10à-Dimethyl-6á-isopropyl-ë1,9-octalin	$C_{15}H_{24}$	004630-07-3	93
14	0.31	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	$C_{15}H_{24}$	1000159-38-5	86

2-Butanone, 4-phenyl (0.46%), Agarospirol (3.2%), Aristolene (2.46%), .+)-Aromadendrene (4.69 %), Aristolene (2.5%), ë-Guaiene (4.77%), β-Selinenol (0.31%), Benzene, hexamethyl-(0.28%), Eudesma-4(14),11-diene (0.41%), Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl- (0.31%) were the main constituents of the essential oil of Agarwood in spot 2 with chromatographic column used for analysis was DB-wax, 30 m x 0.25 μm x 0.25 i.d.

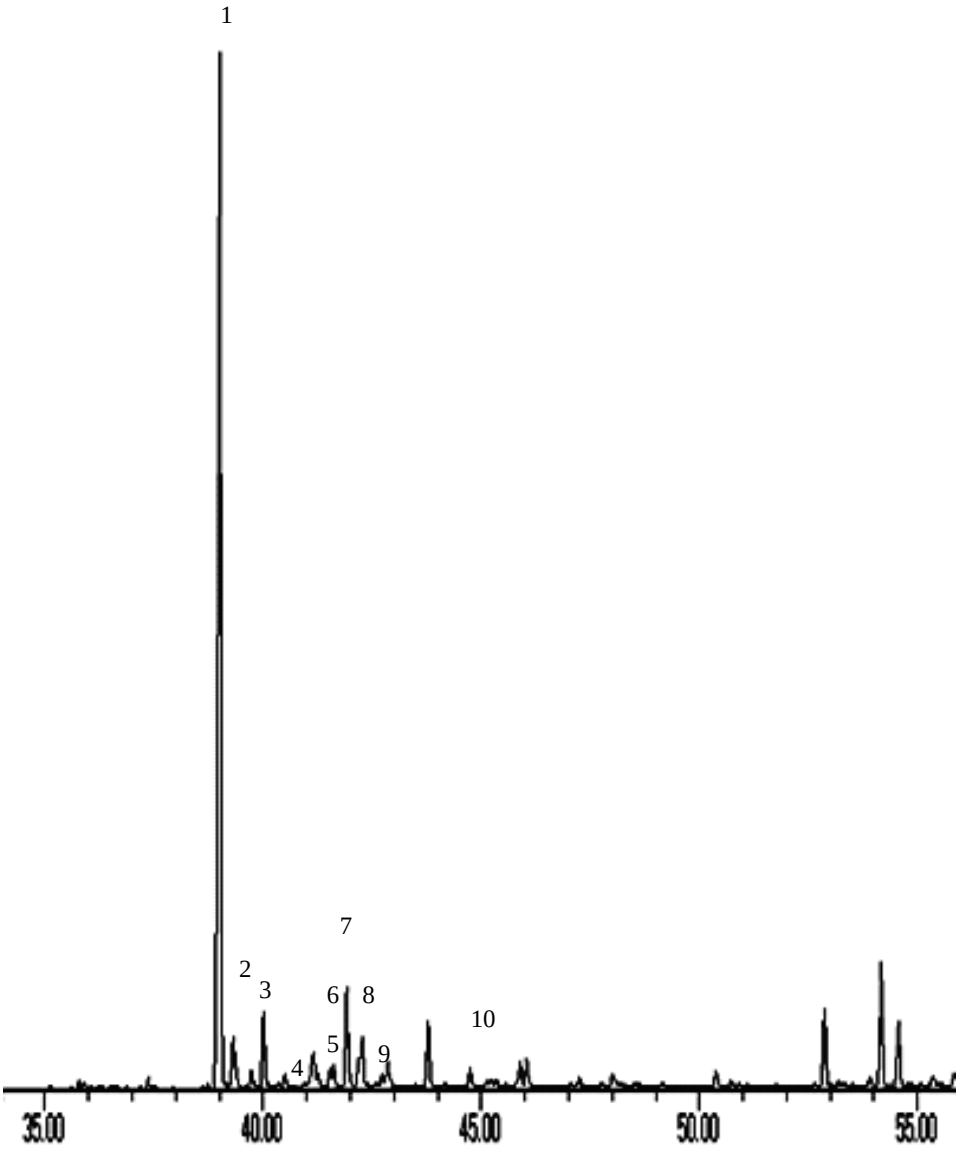


Figure 4.4: GC-MS analysis of Spot 3-LAB sample

GCMS analysis of the PTLC experiment (spot 3) of agarwood oil (The chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d. T= 60-246^{o}C, ΔT= 3^{o}C/min.

Table 4.4: Analysis of Spot 3-LAB sample

Peak no.	Area %	ID	Mol. formula	Cas#	Qual
1	3.02	τ-Eudesmol	$C_{15}H_{26}O$	001209-71-8	91
2	0.28	β-Selinene	$C_{15}H_{24}$	017066-67-0	90
3	0.18	Aristolone	$C_{15}H_{22}O$	006831-17-0	89
4	0.05	γ-Gurjunene	$C_{15}H_{24}$	022567-17-5	90
5	0.15	Napthhalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-.[1R-(1.alpha.,7.beta.,8a.alpha.)]-	$C_{15}H_{24}$	004630-07-3	96
6	0.05	Agarospirol	$C_{15}H_{26}O$	001460-73-7	91
7	0.30	β-Selinene	$C_{15}H_{24}$	017066-67-0	90
8	0.07	.beta.-Humulene 1H-Cycloprop[e]azulene,decahydro-1,1,7-trimethylene-,[1aR(1a.alpha.4a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-	$C_{15}H_{24}$	000116-04-1	91
9	0.16	β-Panasinsene	$C_{15}H_{24}$	1000159-39-0	91
10	0.18	Aristolone	$C_{15}H_{22}O$	006831-17-0	89

γ-Eudesmol(3.02%), β-Selinene (0.28%), Aristolone (0.18%), Napthhalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-.[1R-(1.alpha.,7.beta.,8a.alpha.)]- (0.15%), β-Selinene (0.30%), Aristolone (0.18%),β-Panasinsene (0.16%) were the main constituents of the essential oil of Agarwood in spot 3 with chromatographic column used for analysis was DB-wax, 30 m x 0.25 μm x 0.25 i.d.

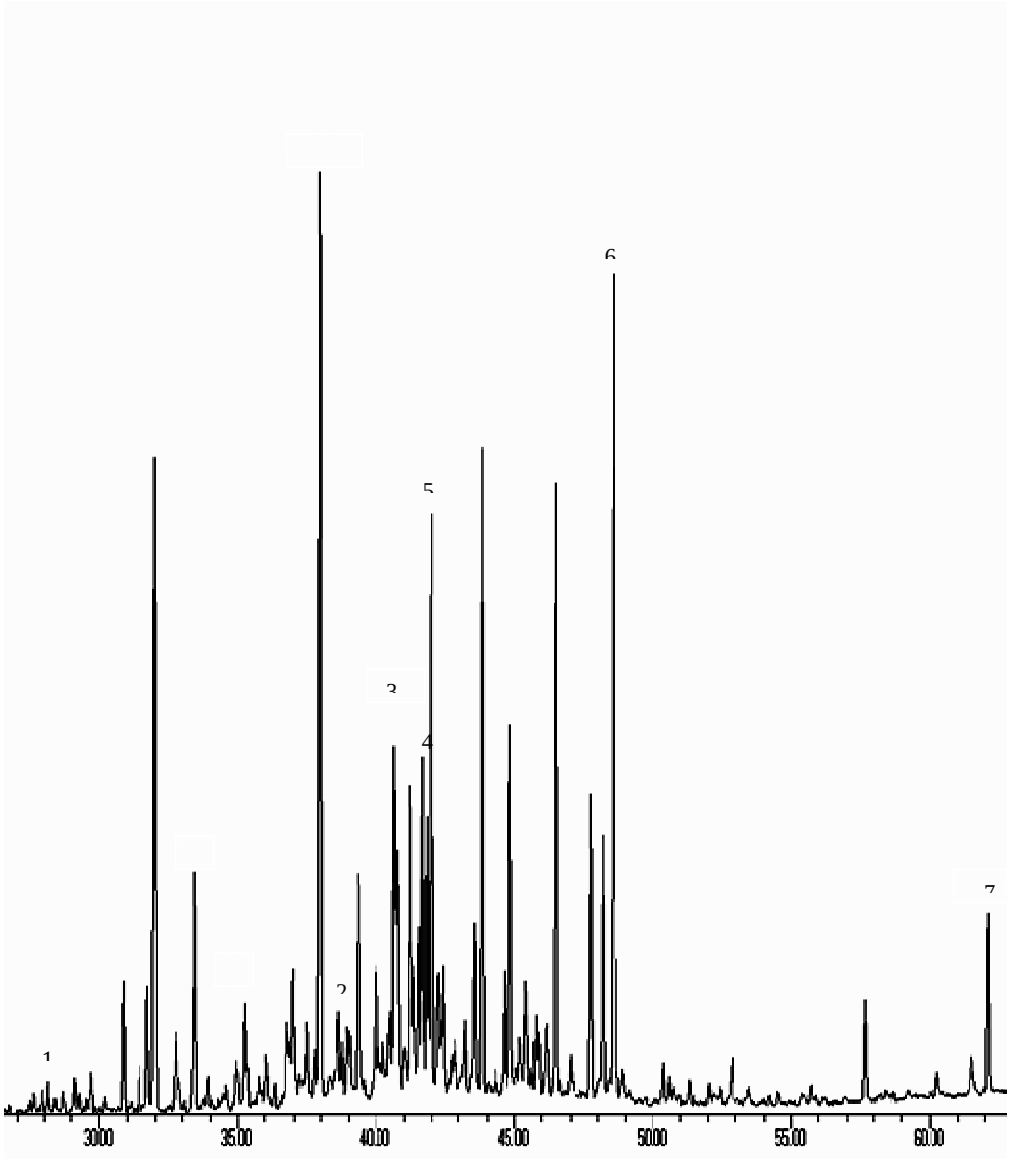


Figure 4.5 : GC-MS analysis of Spot 4-LAB Sample

GCMS analysis of the PTLC experiment (spot 4) of agarwood oil (The chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d, T= 60-246^{o}C, ΔT= 3^{o}C/min.

Table 4.5: Analysis of Soot 4-Lab Sample

No	Area %	ID	Molecule formula	Cas#	Qual
1	0.28	α-Guaiene	$C_{15}H_{24}$	003691-11-0	98
2	0.40	4,6,6-Trimethyl-2-(3-methylbuta-1, 3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)]octane	$C_{15}H_{22}O$	1000190-22-2	95
3	0.13	Spiro[4.5]dec-6-en-8-one, 1,7-dimethyl-4-(1-methylethyl)-	$C_{15}H_{24}O$	039510-36-6	87
4	0.65	6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	$C_{15}H_{22}O$	086917-79-5	83
5	0.88	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	$C_{15}H_{24}$	1000159-38-5	92
6	1.08	Cycloheptane, 4-methylene-1-methyl -2-(2-methyl-1-propen-1-yl)-1-viny l-	$C_{15}H_{24}$	1000159-38-5	70
7	0.24	3-Pentanone, 1,5-diphenyl-	$C_{17}H_{18}O$	005396-91-8	94

α-Guaiene(0.23%), 4,6,6-Trimethyl-2-(3-methylbuta-1, 3-dienyl)-3 oxatricyclo[5.1.0.0(2,4)]octane (0.4%), 6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one (0.65%), Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl- (0.88%), Cycloheptane, 4-methylene-1-methyl -2-(2-methyl-1-propen-1-yl)-1-viny l-(1.08%) were the main constituents of the essential oil of agarwood in spot 4 with chromatographic column used for analysis was DB-wax, 30 m x 0.25 μm x 0.25 i.d.

4.4.3 Spectrum and the constituents identified in the PTLC experiments of agarwood oil from MAHA samples.

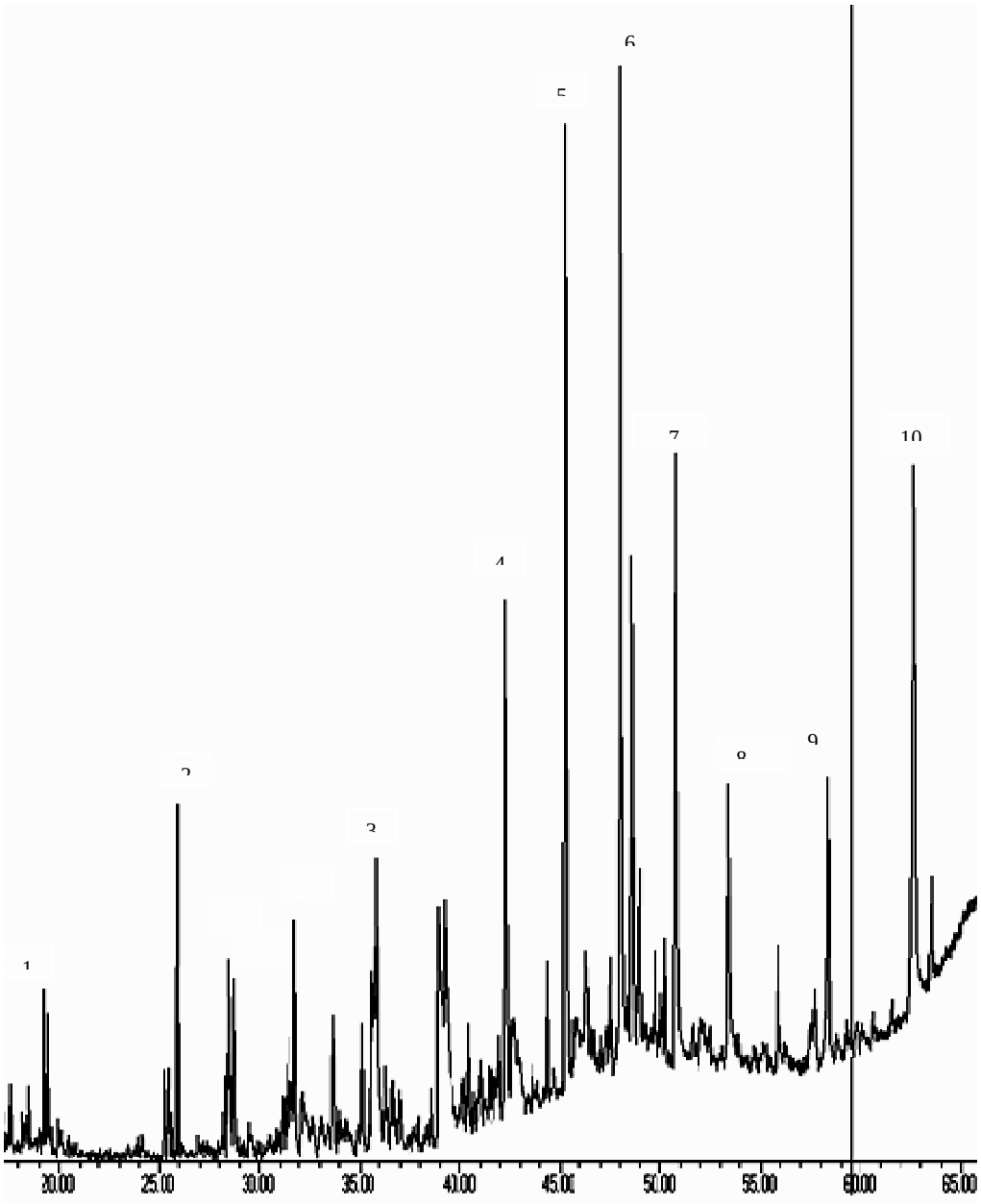


Figure 4.6: GC-MS analysis of Spot 1-MAHA sample

GCMS analysis of the PTLC experiment (spot 1) of agarwood oil (The chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d. T= 60-246^{o}C, ΔT= 3^{o}C/min.

Table 4.6: Analysis of Spot 1-MAHA sample

Peak no.	Area %	ID	Molecule formula	Cas#	Qual
1	0.02	Cycloheptasiloxane, tetradecamethy	$C_{14}H_{42}O_7Si_7$	000107-50-6	93
2	0.06	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	000556-68-3	91
3	0.03	Cyclononasiloxane	$C_{18}H_{54}O_9Si_9$	000556-71-8	90
4	0.13	Docosane	$C_{22}H_{46}$	000629-97-0	93
5	0.21	Hexadecane	$C_{16}H_{34}$	000544-76-3	96
6	0.23	Tetracosane	$C_{24}H_{50}$	000646-31-1	99
7	0.12	γ-Elemene	$C_{15}H_{24}$	030824-67-0	83
8	0.17	Pentacosane	$C_{25}H_{52}$	000629-99-2	93
9	0.09	3-Eicosene, (E)-	$C_{20}H_{40}$	074685-33-9	93
10	0.23	Phenol, 2,2'-methylenebis [6-(1,1-dimethylethyl)-4-ethyl-	$C_{25}H_{36}O_2$	000088-24-4	93

Docosane (0.13%), Hexadecane (0.21%), Tetracosane (0.23%), Pentacosane (0.17%), Phenol, 2,2'-methylenebis [6-(1,1-dimethylethyl)-4-ethyl- (0.23%) were the main constituents of the essential oil of agarwood in spot 1 with chromatographic column used for analysis was DB-wax, 30 m x 0.25 μm x 0.25 i.d.

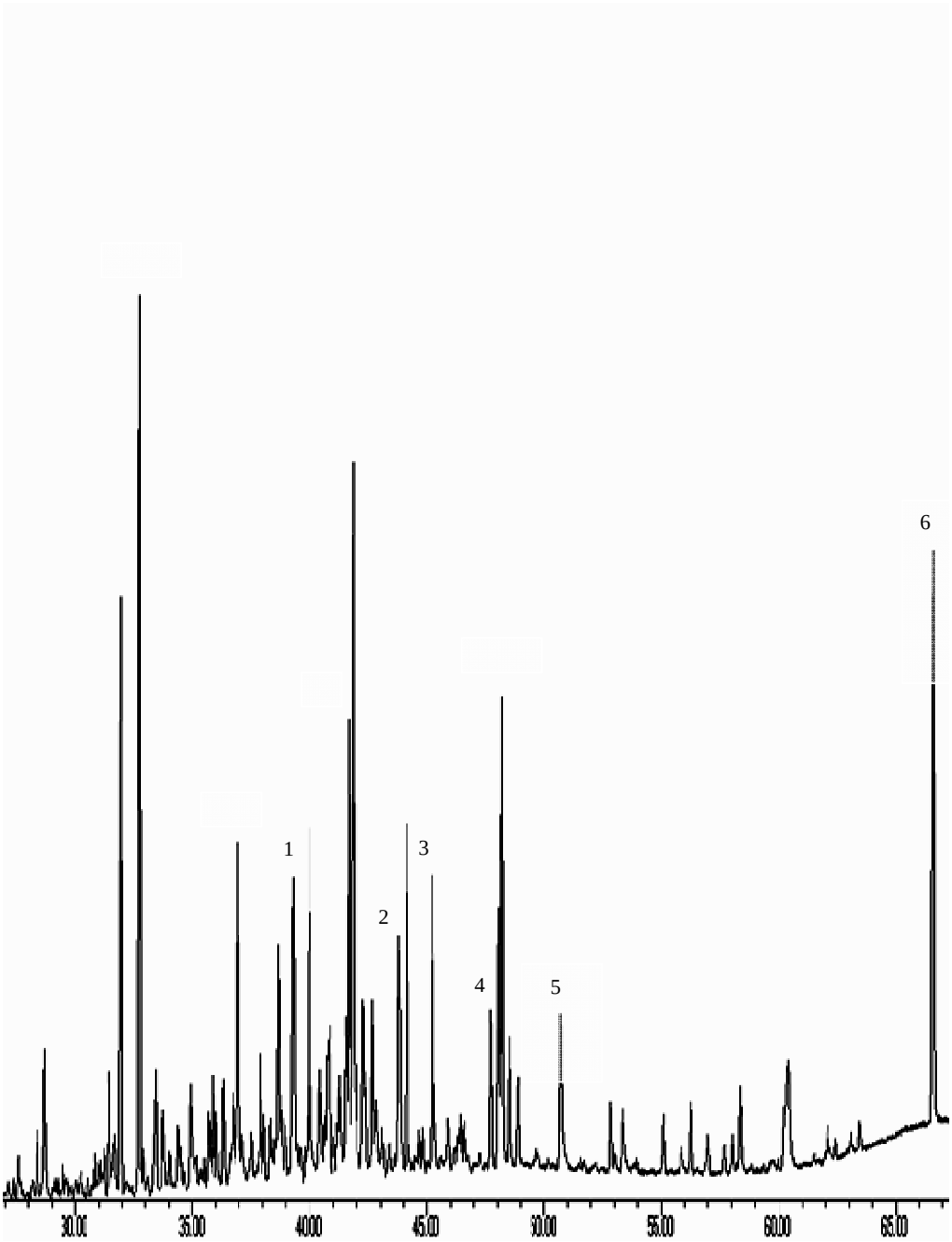


Figure 4.7: GC-MS analysis of Spot 2-MAHA sample

GCMS analysis of the PTLC experiment (spot 2) of agarwood oil (The chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d. T= 60-246^{o}C, ΔT= 3^{o}C/min.

Table 4.7: Analysis of Spot 2-MAHA sample

Pek no.	Area %	ID	Molecule comp	Cas#	Qual
1	0.22	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	$C_{15}H_{22}O$	1000188-66-5	93
2	0.25	Cycloheptane, 4-methylene-1-methyl -2-(2-methyl-1-propen-1-yl)-1-viny l-	$C_{15}H_{24}$	1000159-38-5	90
3	0.23	Heptacosane	$C_{27}H_{56}$	000593-49-7	91
4	0.09	Tetracosane	$C_{24}H_{50}$	000646-31-1	98
5	0.13	Tetratetracontane	$C_{44}H_{90}$	007098-22-8	87
6	0.41	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	004376-20-9	91

1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (0.41%), Heptacosane (0.23%), Cycloheptane, 4-methylene-1-methyl -2-(2-methyl-1-propen-1-yl)-1-viny l- (0.25%), 2(1H)Naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-(0.22%) were the main constituents of the essential oil of agarwood in spot 2 with chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d.

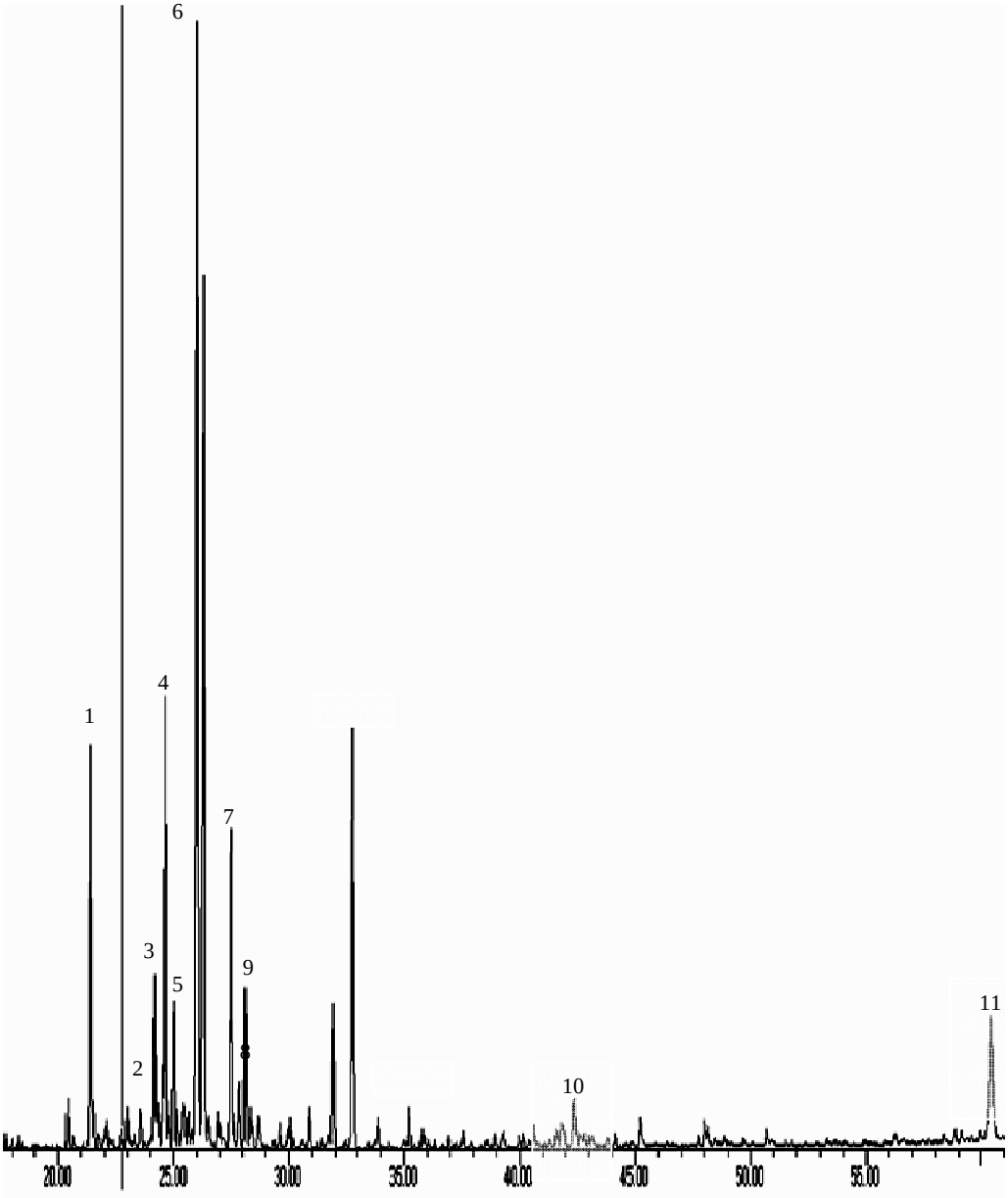


Figure 4.8: GC-MS analysis of Spot 3-MAHA sample

GCMS analysis of the PTLC experiment (spot 3) of agarwood oil (The chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d. T= 60-246^{o}C, ΔT= 3^{o}C/min

Table 4.8: Analysis of Spot 3-MAHA sample

Peak no.	Area %	ID	Molecule Formula	Cas#	Qual
1	0.55	α-Guaiene	$C_{15}H_{24}$	003691-12-1	99
2	0.21	1,4,7,-Cycloundecatriene, 1,5,9,9- tetramethyl-, Z,Z,Z-	$C_{15}H_{24}$	1000062-61-9	98
3	0.04	γ-Selinene	$C_{15}H_{24}$	000515-17-3	95
4	0.58	Aristolene	$C_{15}H_{24}$	1000150-14-9	90
5	0.25	α-Gurjunene	$C_{15}H_{24}$	000489-40-7	96
6	2.24	α-Guaiene	$C_{15}H_{24}$	003691-11-0	99
7	0.48	τ-Cadinene	$C_{15}H_{24}$	000483-76-1	98
8	0.12	α-Gurjunene	$C_{15}H_{24}$	000489-40-7	95
9	0.23	α-Curcumene	$C_{15}H_{22}$	000644-30-4	97
10	0.10	Cadalene	$C_{15}H_{18}$	000483-78-3	98
11	0.51	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl-	$C_{25}H_{36}O_2$	000088-24-4	99

α-Guaiene (0.55%), 1,4,7,-Cycloundecatriene, 1,5,9,9- tetramethyl-, Z,Z,Z-(0.21%) Aristolene (0.58%),α-Gurjunene (0.25%), α–Guaiene (2.24%), τ-Cadinene (0.48%), α-Gurjunene (0.12%), α-Curcumene (0.23%), Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl-(0.51%) were the main constituents of the essential oil of agarwood in spot 3 with chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d.

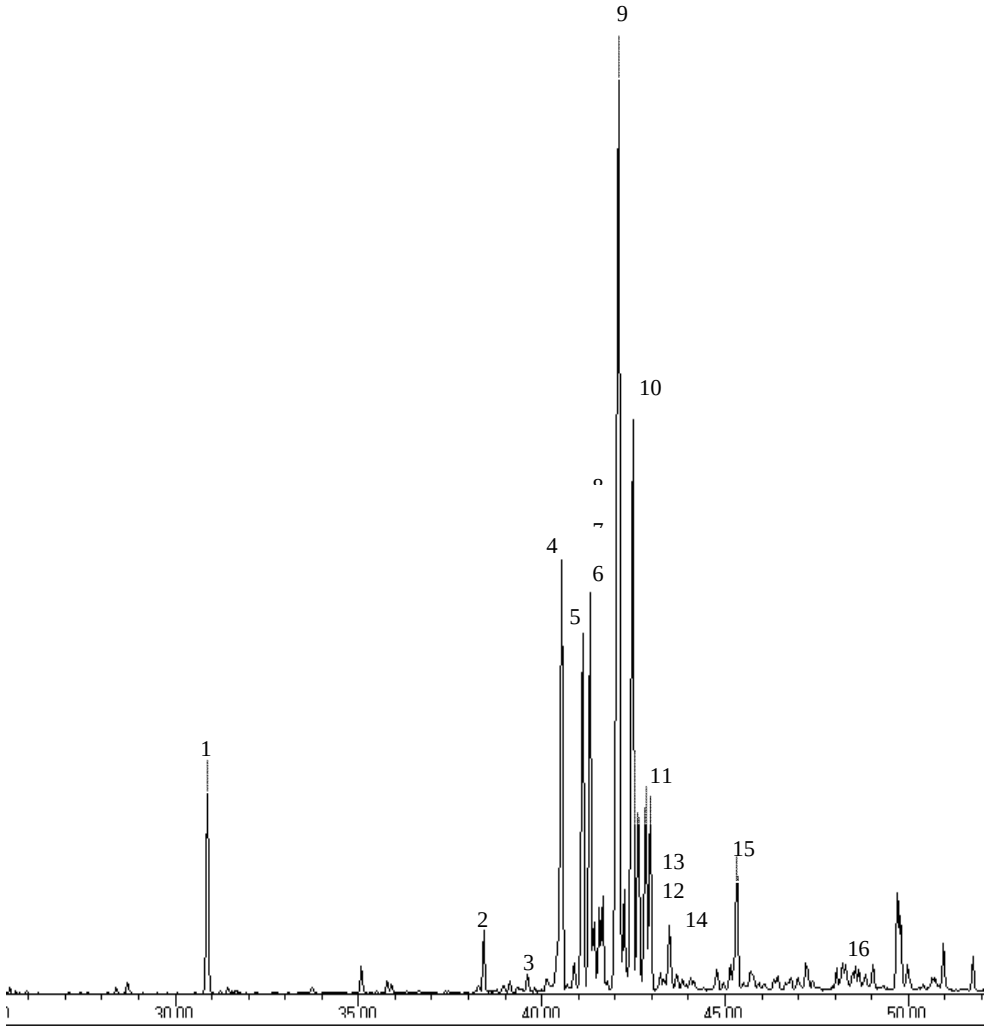


Figure 4.9: GC-MS analysis of Spot 4-MAHA sample

GCMS analysis of the PTLC experiment (spot 4) of agarwood oil (The chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d

Table 4.9: Analysis of Spot 4-MAHA sample

Peak no.	Area %	ID	Molecule comp	Cas#	Qual
1	0.97	2-Butanone, 3-phenyl-	$C_{10}H_{12}O$	000769-59-5	96
2	0.24	Elemol	$C_{15}H_{26}O$	000639-99-6	91
3	2.19	Guaiene	$C_{15}H_{24}$	000088-84-6	86
4	1.77	τ-Eudesmol	$C_{15}H_{26}O$	001209-71-8	99
5	1.55	Agarospirol	$C_{15}H_{26}O$	001460-73-7	91
6	0.13	4a,trans-8a-Perhydro-cis-2-(2-hydroxy-2-propyl)-4a,cis-8-dimethylnaphthalene	$C_{15}H_{28}O$	006770-16-7	83
7	0.14	α-Amorphene	$C_{15}H_{24}$	000483-75-0	70
8	0.21	Hinesol	$C_{15}H_{26}O$	023811-08-7	94
9	5.82	β-Panasinsene	$C_{15}H_{24}$	1000159-39-0	86
10	0.24	α-Guaiene	$C_{15}H_{24}$	003691-11-0	87
11	0.80	α-Eudesmol	$C_{15}H_{26}O$	000473-16-5	96
12	0.77	β-Selinenol	$C_{15}H_{26}O$	000473-15-4	99
13	0.56	Benzene	$C_{12}H_{18}$	000087-85-4	90
14	0.30	γ-Gurjunene	$C_{15}H_{24}$	022567-17-5	93
15	0.58	Benzene	$C_{12}H_{18}$	000087-85-4	78
16	0.30	Aristolone	$C_{15}H_{22}O$	006831-17-0	89

2-Butanone, 3-phenyl (0.97%), Elemol (0.24%), Guaiene (2.19%), τ-Eudesmol (1.77%), Agarospirol (1.55%), Hinesol (0.21%), β-Panasinsene (5.82%), τ-Guaiene (0.24%), α-Eudesmol (0.8%), β-Selinenol (0.77%), Benzene (0.56%), γ-Gurjunene (0.3%), Aristolone (0.3%) were the main constituents of the essential oil of agarwood in spot 4 with chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d.

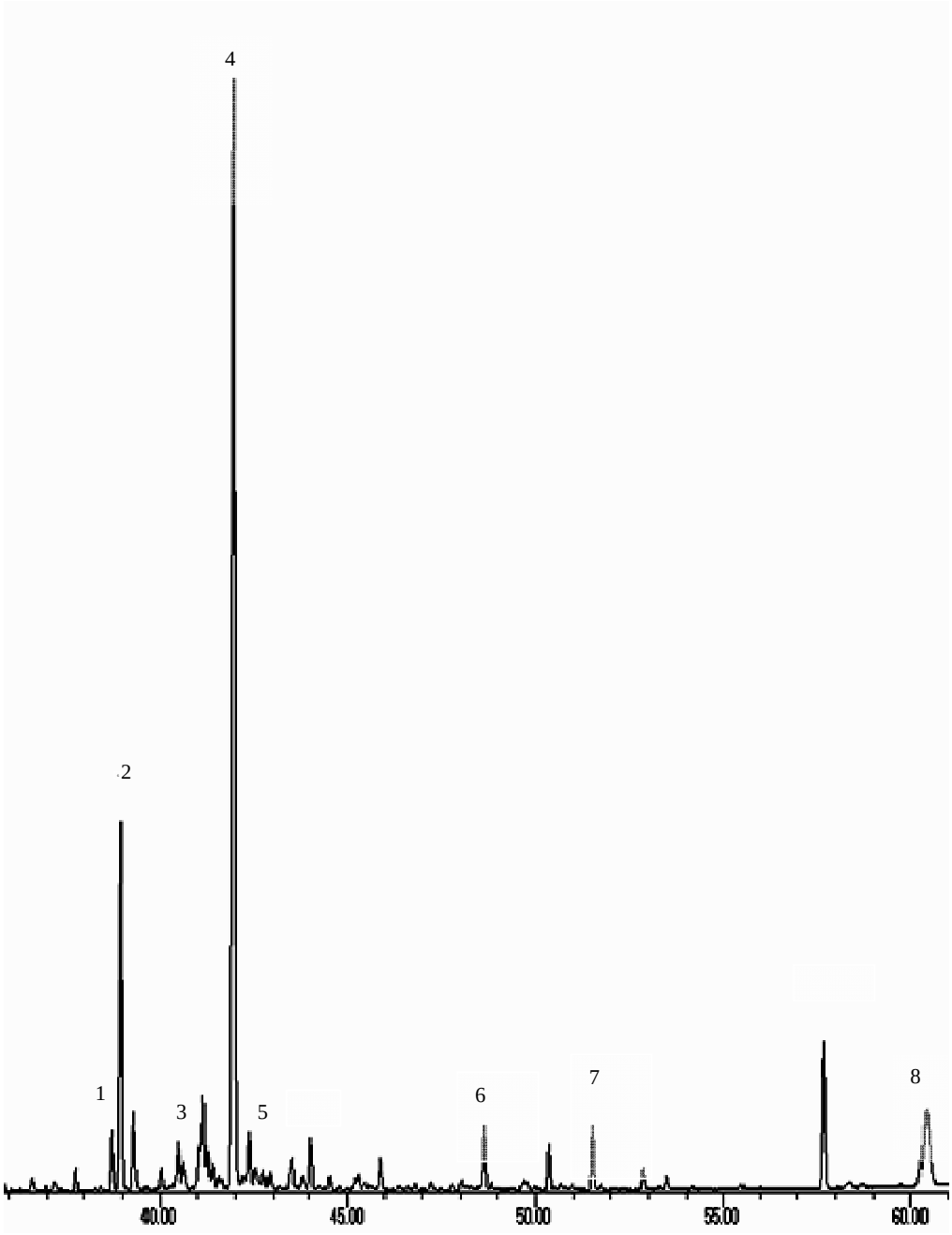


Figure 4.10: GC-MS analysis of Spot 5-MAHA sample

GCMS analysis of the PTLC experiment (spot 5) of agarwood oil (The chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d. T= 60-246^{o}C, ΔT= 3^{o}C/min.

Table 4.10: Analysis of Spot 5-MAHA sample

Peak no.	Area %	ID	Molecule Formula	Cas#	Qual
1	0.12	Isoledene	$C_{15}H_{24}$	1000156-10-	93
2	0.82	ñ)-Cadinene	$C_{15}H_{24}$	005951-61-1	95
3	0.09	(+)-Aromadendrene	$C_{15}H_{24}$	000489-39-4	90
4	2.91	β-Selinene	$C_{15}H_{24}$	017066-67-0	95
5	0.10	Germacrene D	$C_{15}H_{24}$	023986-74-5	78
6	0.15	2(3H)-Naphthalenone, 4,4a,5,6,7,8- hexahydro-4a,5-dimethyl-3-(1-methylethylidene)-, (4ar-cis)-	$C_{15}H_{22}O$	019598-45-9	99
7	0.14	Phthalic acid, isobutyl octyl este	$C_{23}H_{36}O_5$	1000309-04-5	90
8	0.48	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl-	$C_{25}H_{36}O_2$	000088-24-4	98

ñ)-Cadinene (0.82%), Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl- (0.48%), 2(3H)-Naphthalenone, 4,4a,5,6,7,8- hexahydro-4a,5-dimethyl-3-(1-methylethylidene)-, (4ar-cis)- (0.15%), β-Selinene (2.91%) were the main constituents of the essential oil of Agarwood in spot 5 with chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d.

4.4.4 Spectrum and the constituents identified in the PTLC experiments of agarwood oil from Kelantan samples.

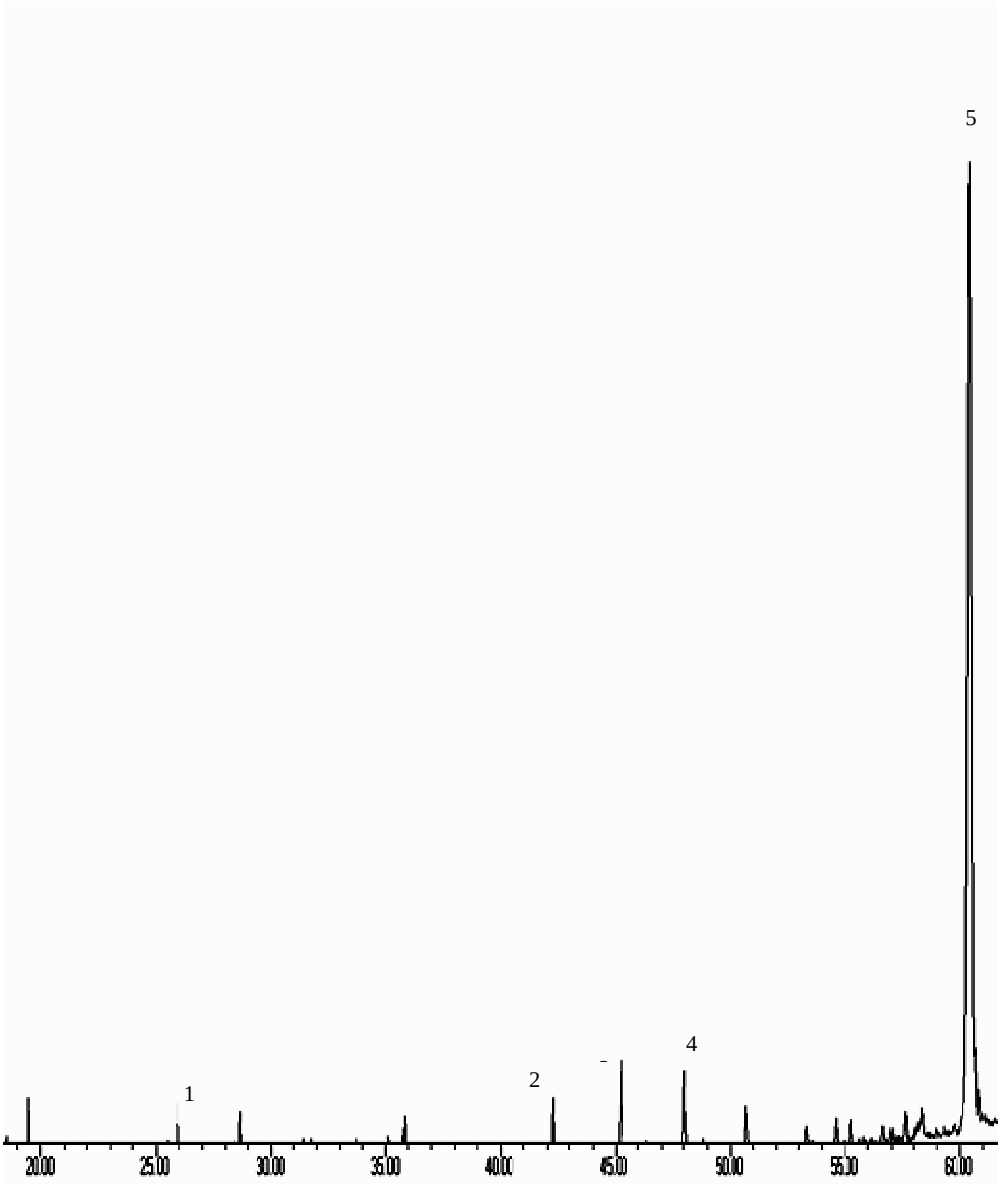


Figure 4.11: GC-MS analysis of Spot 1-KELANTAN sample

GCMS analysis of the PTLC experiment (spot 1) of agarwood oil (The chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d T= 60-246^{o}C, ΔT= 3^{o}C/min.

Table 4.11: Analysis of Spot 1-KELANTAN sample

Peak no.	Area %	ID	Mol.formula	Cas#	Qual
1	0.02	α- myrcene	$C_{10}H_{16}$	001686-30-2	81
2	0.04	Docosane	$C_{22}H_{46}$	000629-97-0	93
3	0.04	Tritetracontane	$C_{43}H_{88}$	007098-21-7	91
4	0.05	Heneicosane, 11-(1-ethylpropyl)-	$C_{26}H_{54}$	055282-11-6	91
5	1.20	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl-	$C_{25}H_{36}O_2$	000088-24-4	70

Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl- (1.2%) were the main constituents of the essential oil of agarwood in spot 1 with chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d.

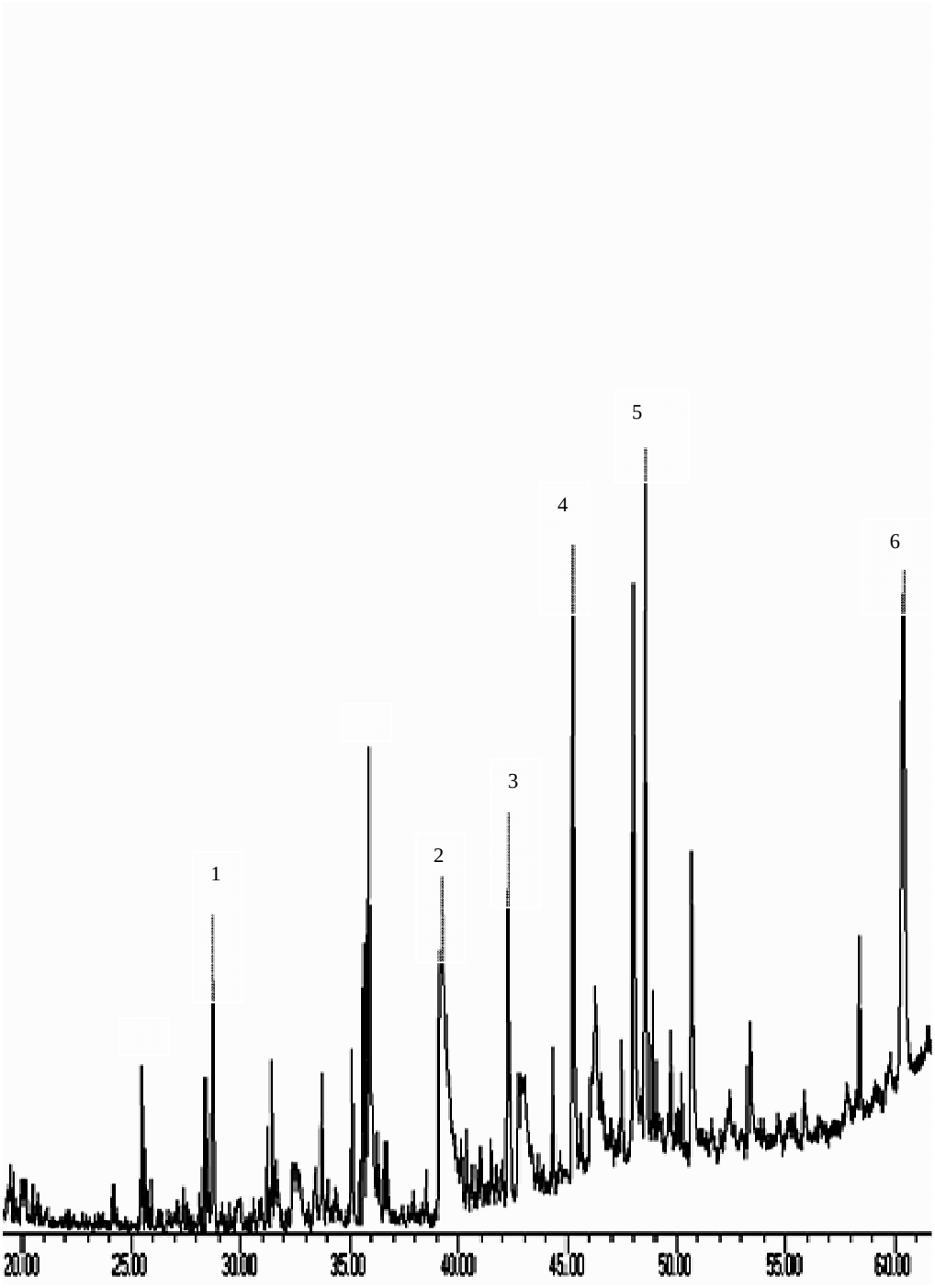


Figure 4.12: GC-MS analysis of Spot 2-KELANTAN sample

GCMS analysis of the PTLC experiment (spot 2) of agarwood oil (The chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d T= 60-246^{o}C, ΔT= 3^{o}C/min.

Table 4.12: Analysis of Spot 2-KELANTAN sample

Peak no.	Area %	ID	Molecule Formula	Cas#	Qual
1	0.03	α-Myrcene	$C_{10}H_{16}$	001686-30-2	70
2	0.03	Docosane	$C_{22}H_{46}$	000629-97-0	93
3	0.06	Eicosane	$C_{20}H_{42}$	000112-95-8	91
4	0.05	Tetracosane	$C_{24}H_{50}$	000646-31-1	93
5	0.03	Pentacosane	$C_{25}H_{52}$	000629-99-2	90
6	0.14	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl-	$C_{25}H_{36}O_2$	000088-24-4	99

Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl- (0.14%) and Eicosane (0.06%) were the main constituents of the essential oil of agarwood in spot 2 with chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d.

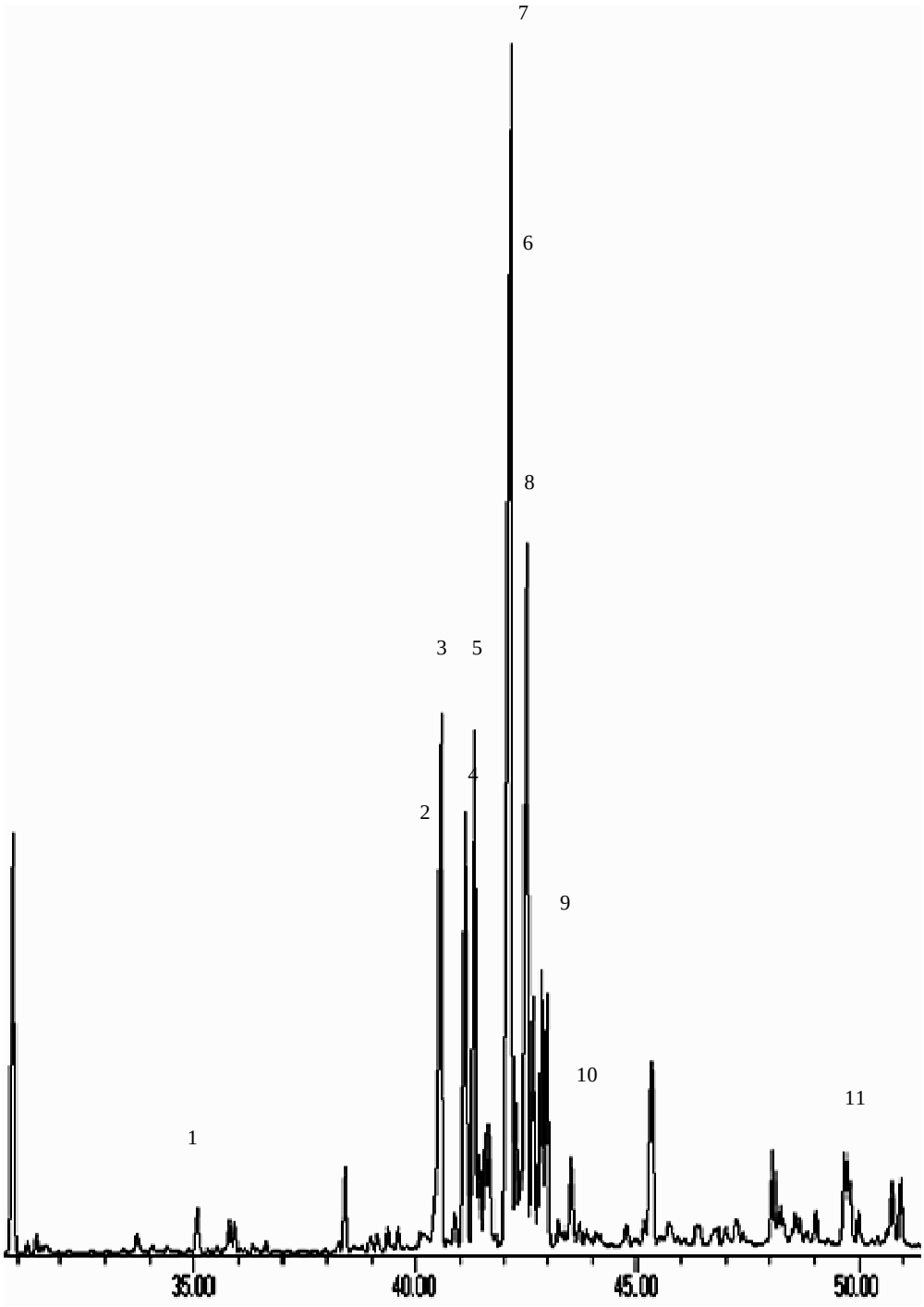


Figure 4.13: GC-MS analysis of Spot 3-KELANTAN sample

GCMS analysis of the PTLC experiment (spot 3) of agarwood oil (The chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d T= 60-246^{o}C, ΔT= 3^{o}C/min.

Table 4.13: Analysis of Spot 3- Kelantan Sample

Peak no.	Area %	ID	Mol. Formula	Cas#	Qual
1	0.32	Elemol	$C_{15}H_{26}O$	000639-99-6	91
2	2.84	β-Panasinsene	$C_{15}H_{24}$	1000159-39-0	86
3	2.23	τ-Eudesmol	$C_{15}H_{26}O$	001209-71-8	99
4	2.09	Agarospirol	$C_{15}H_{26}O$	001460-73-7	91
5	0.19	Selina-3,7(11)-diene	$C_{15}H_{24}$	006813-21-4	83
6	0.24	Hinesol	$C_{15}H_{26}O$	023811-08-7	94
7	7.78	Neoisolongifolene	$C_{15}H_{24}$	1000156-12-4	86
8	0.29	α-Guaiene	$C_{15}H_{24}$	003691-11-0	87
9	0.98	α-Eudesmol	$C_{15}H_{26}O$	000473-16-5	96
10	0.92	β-Selinenol	$C_{15}H_{26}O$	000473-15-4	99
11	0.39	γ-Gurjunene	$C_{15}H_{24}$	022567-17-5	93

γ-Gurjunene (0.39%), β-Selinenol (0.92%), α-Eudesmol (0.98%), α-Guaiene (0.29%), Neoisolongifolene (7.78%), Agarospirol (2.09%), γ-Eudesmol (2.23%), β-Panasinsene (2.84%), Elemol (0.32%) were the main constituents of the essential oil of Agarwood in spot 3 with chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d.

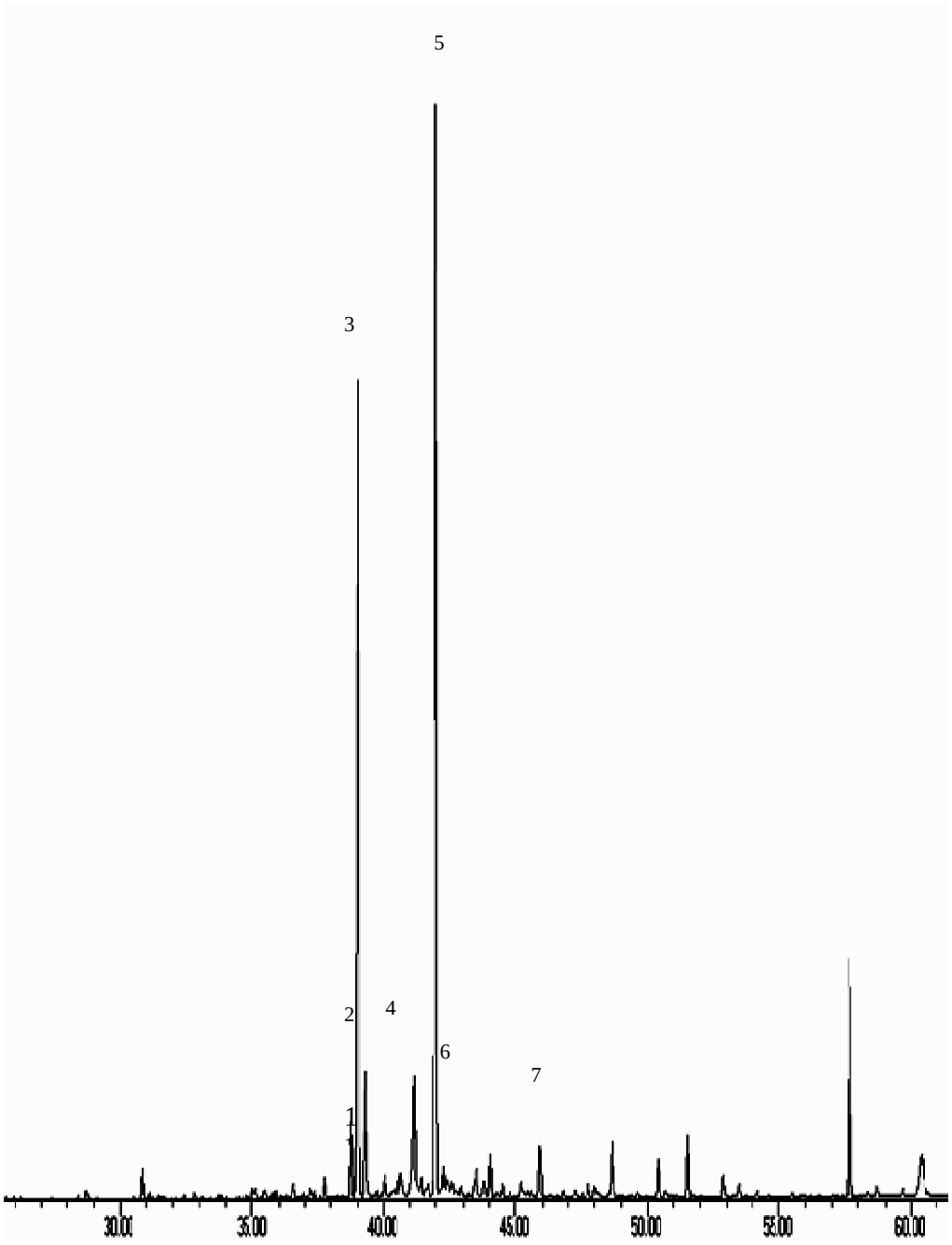


Figure 4.14: GC-MS analysis of Spot 4-KELANTAN sample

GCMS analysis of the PTLC experiment (spot 4) of agarwood oil (The chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d T= 60-246^{o}C, ΔT= 3^{o}C/min.

Table4.14: Analysis of Spot 4-KELANTAN sample

Peak no.	Area %	ID	Mol.formula	Cas#	Qual
1	0.07	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	$C_{15}H_{24}$	016728-99-7	95
2	0.24	Isoledene	$C_{15}H_{24}$	1000156-10-8	91
3	3.20	γ-Eudesmol	$C_{15}H_{26}O$	001209-71-8	91
4	0.49	4áH,5à-Eremophila-1(10),11-diene	$C_{15}H_{24}$	004630-07-3	74
5	4.56	β-Selinene	$C_{15}H_{24}$	017066-67-0	95
6	0.07	1,1,3a-Trimethyl-7-methylenedecahydro-1H-cyclopropa[a]naphthalene	$C_{15}H_{24}$	020071-49-2	96
7	0.17	2(3H)-Naphthalenone, 4,4a,5,6,7,8- hexahydro-4a,5-dimethyl-3-(1-methylethylidene)-, (4ar-cis)-	$C_{15}H_{22}O$	019598-45-9	99

α-Selinene (4.56%), γ-Eudesmol (3.2%) and Isoledene (0.24%), 4áH,5à-Eremophila-1(10),11-diene (0.49%), were the main constituents of the essential oil of agarwood in spot 4 with chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d.

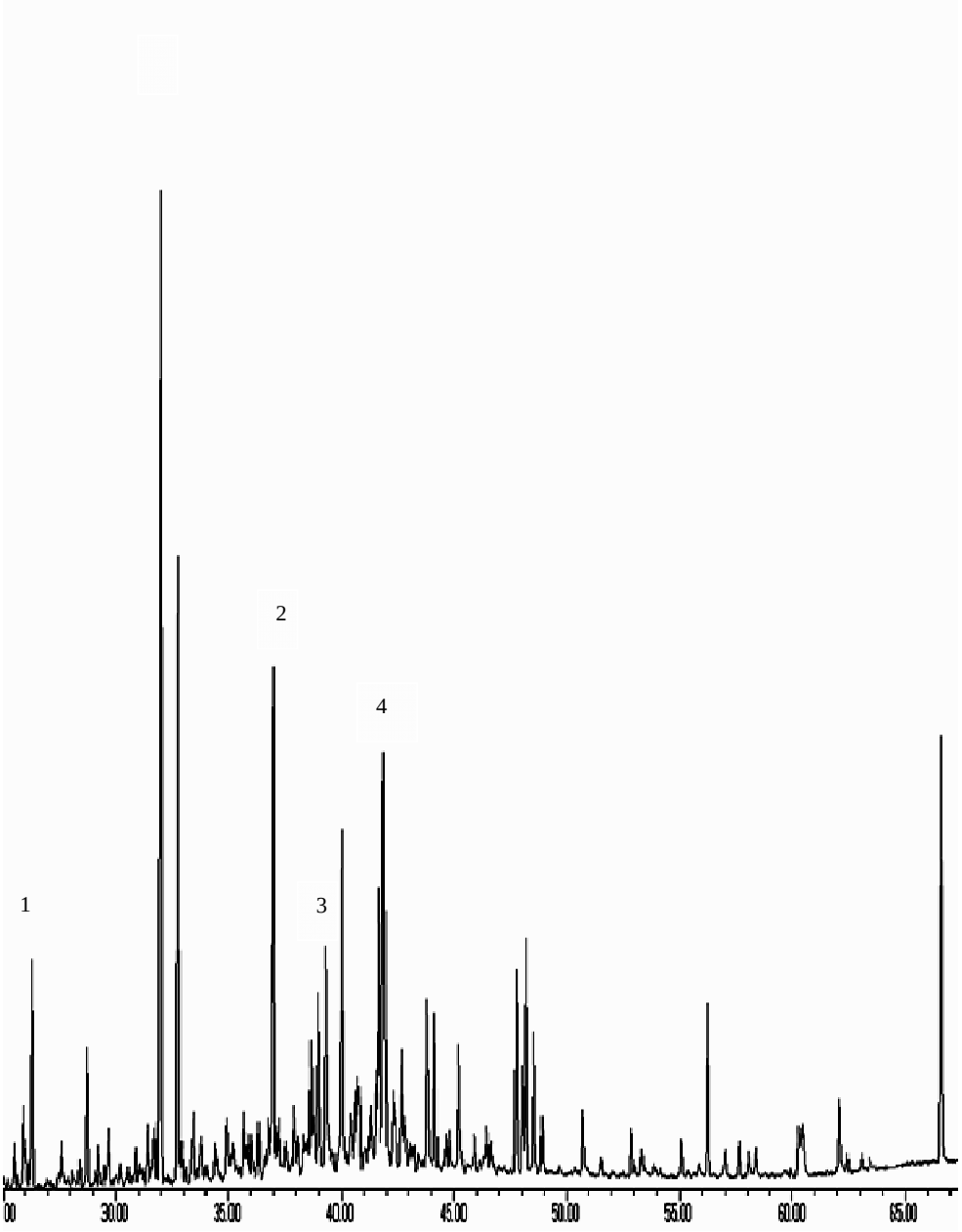


Figure 4.15: GC-MS analysis of Spot 5-KELANTAN sample

GCMS analysis of the PTLC experiment (spot 5) of agarwood oil (The chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d T= 60-246^{o}C, ΔT= 3^{o}C/min.

Table 4.15: Spot 5-KELANTAN sample

Peak no.	Area %	Component	Mol.formula	Cas#	Qual
1	0.07	τ-Guaiene	$C_{15}H_{24}$	003691-11-0	97
2	0.30	(ñ)-Cadinene	$C_{15}H_{24}$	005951-61-1	95
3	0.39	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	$C_{15}H_{22}O$	1000188-66-5	90
4	0.37	Cycloheptane, 4-methylene-1-methyl -2-(2-methyl-1-propen-1-yl)-1-vinyl-	$C_{15}H_{24}$	1000159-38-5	95

Cycloheptane, 4-methylene-1-methyl -2-(2-methyl-1-propen-1-yl)-1-vinyl- (0.37%), 2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)- (0.39%) and (ñ)-Cadinene (0.30%) were the main constituents of the essential oil of agarwood in spot 5 with chromatographic column used for analysis was DB-wax, 30 m x 0.25µm x 0.25) i.d.

4.5 Discussion on Analysis by GC-MS

All spots obtained from TLC procedure was analyzed and confirmed by GC-MS and we considered compound within 70% and above of quality are presented in table 4.1 to 4.14. The results showed, complex compound in agarwood oil is separated due TLC procedure depends on their polarity. Table 4.16 showed components identified as sesquiterpenes and chromones from agarwood presented in the samples.

The results showed 8 component exist as α-guaiene, Agarospirol, τ-Eudesmol, β-Selinenol, β-Selinene, β-Panasinsene, γ-Gurjunene, Cycloheptane 4-methylene-1-methyl -2-(2-methyl-1-propen-1-yl)-1-vinyl- are presented in 3 samples. While (+)-Aromadendrene, Aristolene are presented in lab and Maha samples and 4áH,5à-Eremophila-1(10),11-diene are presented in lab and Kelantan samples.

Table 4.16: Comparison of component presented in lab, Maha and Kelantan samples.

no	Compounds	Molecule formula	Lab Sample	Maha Sample	Kelantan Sample
1	α-guaiene	$C_{15}H_{24}$	✓	✓	✓
2	Agarospirol	$C_{15}H_{26}O$	✓	✓	✓
3	τ-Eudesmol	$C_{15}H_{26}O$	✓	✓	✓
4	β-Selinene	$C_{15}H_{24}$	✓	✓	✓
5	β-Panasinsene	$C_{15}H_{24}$	✓	✓	✓
6	β-Selinenol	$C_{15}H_{26}O$	✓	✓	✓
7	γ-Gurjunene	$C_{15}H_{24}$	✓	✓	✓
8	Cycloheptane, 4-methylene-1-methyl -2-(2-methyl-1-propen-1-yl)-1-vinyl-	$C_{15}H_{24}$	✓	✓	✓
9	(+)-Aromadendrene	$C_{15}H_{24}$	✓	✓	-
10	Aristolene	$C_{15}H_{22}O$	✓	✓	
11	4áH,5à-Eremophila-1(10),11-diene	$C_{15}H_{24}$	✓	-	✓

CHAPTER 5

CONCLUSION

5.1 Conclusion

Back to the objective of this research, this experiment was carry out to see whether hydro distillation methods can be used to extracted the Agarwood essential oil and then purify of active components present by preparative TLC and analysis its component by GC-MS. After all the research have been done, some conclusion we can make to prove the theory and new founded for those problem. The conclusions are

I. Hydro distillation method is one of the methods which can be used to extract agarwood essential oil. This was proven from the analysis when one of the main components of agarwood which is Agarospirol was detected.

II. From preparative TLC of 3 samples, the component was separated due their polarity. Preparative TLC was shown to be an easy and efficient method for purification of synthesis products.

III. Analysis by GC-MS was shown and confirmed component present in agarwood oil and proved that sesquiterpenes was the major active component present in agarwood oil.

IV. From this study, lab scale production of agarwood oil was archived standard when compare with MAHA and KELANTAN standards.

5.2 Recommendations

From this research, some recommendation can be made to improve the result of the analysis. The recommendations are:

I. Add more parameter to make sure the distillation process can produce more agarwood oil such as increase the pressure for the system where this method is applied in combination of hydro distillation method.

II. Research must be carry out in clean condition. This reason is to avoid other derivatives interrupt the experiment especially in analysis process which GCMS will detect other compound that didn't have any related with the agarwood oil. All appliances must be clean up perfectly before running another experiment.

III. During preparative TLC, some of the constituents in the mixture may not be very stable on the TLC plate. Thus, once the plates are dried, they must be examined immediately under short wavelength UV (254nm) and long wavelength UV (365nm) light.

IV. Sometimes a substance will move along a TLC plate as a long streak, rather than as a single discrete spot. This is the result of spotting the plate with too much substance, more than the moving solvent can handle. The solvent moves as much substance as it can, but a substantial amount of substance is left behind. The substance is dragged along by the solvent leaving a trail of substance that may sometimes span the entire distance between the starting line and the solvent front. Streaking can be eliminated by systematically diluting the spotting solution until development and visualization show the substances moving as single spots, rather than elongated streaks.

V. A common problem in TLC is uneven advance of solvent along the plate. Instead of a straight line, the solvent front may appear to bow either up or down in the center. Uneven advance of solvent leads to uneven advance of substance spots, and inaccurate R_f values result. A frequent cause of uneven solvent advance is the use of a developing chamber that does not have a flat bottom. Glass bottles usually have bottoms that curve upward from the edges to the center.

VI. Carefully scrape all the silica gel outlined for each sample component from the TLC plate. Take care not to contaminate a component with materials from other spots on the plate to avoid them mix together with other spots.

VII. Sample spots made using TLC capillaries should be no larger than 1-2 mm in diameter, because component spots in the developed plate will be no smaller than, and will usually be larger than, the size of the initial spot. If the initial spot is larger than 2 mm in diameter, then components with similar R_f values may not be resolved because their spots will be so large that they will overlap considerably and may appear to be one large spot. Small initial spots, on the other hand, maximize the potential of complete separation of components

VIII. For better comparison between all samples, the preparative TLC should be performed on a single plate because it is difficult to duplicate all the factors which influence R_f exactly from experiment to experiment.

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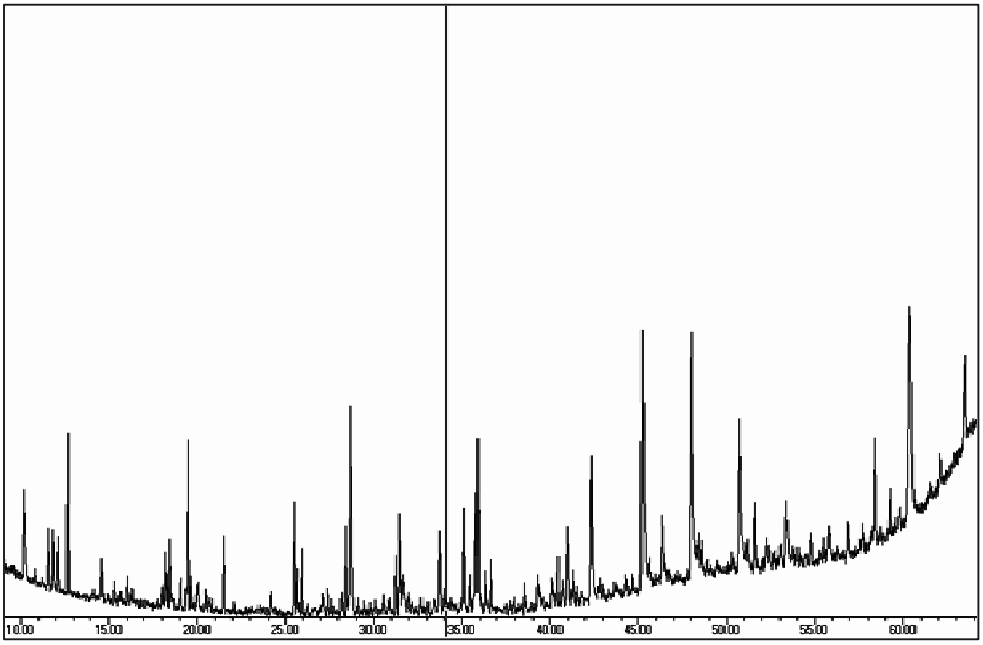
APPENDIX

Spot 1-Lab Sample



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		Benzenepropanenitrile, .beta.-oxo-	20712	000614-16-425
		Benzenecarbothioic acid	16683	000098-91-914
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		Cyclohexasiloxane, dodecamethyl-	179152	000540-97-691
		Cyclohexasiloxane, dodecamethyl-	179153	000540-97-680
		Acetic acid, [bis[(trimethylsilyl) oxy]phosphinyl]-, trimethylsilyl e ster	155042	053044-27-238
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		Ethane, 1,1,2,2-tetrachloro-	33584	000079-34-591
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		Cycloheptasiloxane, tetradecamethy l-	185541	000107-50-664
		2-Benzo[1,3]dioxol-5-yl-8-methoxy-3-nitro-2H-chromene	140587	1000275-63-137
		Tetrasiloxane, 3,5-diethoxy-1,1,1, 7,7,7-hexamethyl-3,5-bis(trimethyl siloxy)-	185549	072439-78-227
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		Hydrogen chloride	46007	647-01-09
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		2-Chloroethylamine	10120	00689-98-59
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		Cycloisolongifolene, 8,9-dehydro-	58528	1000151-28-053
		Naphthalene, 1,2,3,4-tetrahydro-1, 6-dimethyl-4-(1-methylethyl)-, (1Scis)-	58550	00483-77-230

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	Phenol, 4-(1,1-dimethylpropyl)-	32060	000080-46-638	
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	2-Propenoic acid, tridecyl ester	94777	003076-04-891	
	2-Propenoic acid, pentadecyl ester	113378	043080-23-590	
	Dodecyl acrylate	85325	002156-97-086	
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	Diphenyl ether	36402	000101-84-876	
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	3-Allyl-6-methoxyphenol	31757	000501-19-995	
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	Eugenol	31715	000097-53-091
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	Tricosane	139232	000638-67-591
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	Tetracosane	146923	000646-31-196
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	Sulfurous acid, 2-propyl tridecyl ester	128318	1000309-12-446
	Hexadecane	76093	000544-76-342
25	51.562 0.01 C:\Database\NIST05a.L		
	Hydrogen chloride	46	007647-01-09
	Hydrogen chloride	45	007647-01-09
	4-Nitropyridazine 1-oxide	18169	028147-45-74
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	Tridecanol, 2-ethyl-2-methyl-	86872	1000115-66-146
	Tritetracontane	188549	007098-21-746
	Tetratetracontane	188837	007098-22-843
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	2-t-Butyl-4-quinolinealdehyde	66578	1000255-75-135
	Furo[2,3-b]quinoline, 2,3-dihydro-2,4,8-trimethyl-	66602	1000304-15-730
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	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl-	160659	000088-24-499
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	15-Crown-5	70737	033100-27-538
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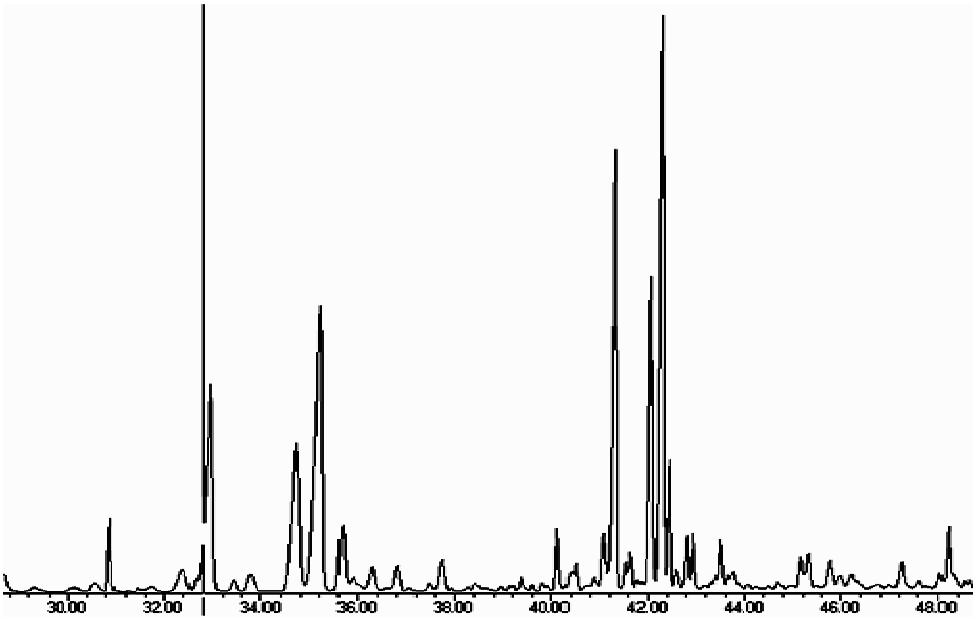
Spot 2- Lab Sample



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Acq On : 8 Oct 2008 14:54

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Search Libraries: C:\Database\NIST05a.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	3.407	0.51	C:\Database\NIST05a.L			
			Trichloromethane	8713	000067-66-3	74
			Methane, dichloronitro-	12675	007119-89-3	64
			Trichloromethane	8712	000067-66-3	64
2	4.198	48.97	C:\Database\NIST05a.L			
			Trichloromethane	8713	000067-66-3	91
			Trichloromethane	8712	000067-66-3	64
			Trichloromethane	8714	000067-66-3	53
3	4.262	17.41	C:\Database\NIST05a.L			
			Trichloromethane	8713	000067-66-3	90

	Trichloromethane	8714	000067-66-383
	Trichloromethane	8712	000067-66-383
4	4.433 6.45 C:\Database\NIST05a.L		
	Trichloromethane	8714	000067-66-397
	Trichloromethane	8712	000067-66-395
	Methane, oxybis[dichloro-	44703	020524-86-183
5	30.871 0.46 C:\Database\NIST05a.L		
	2-Butanone, 4-phenyl-	21740	002550-26-797
	2-Butanone, 3-phenyl-	21739	000769-59-597
	2-Butanone, 4-phenyl-	21741	002550-26-795
6	32.377 0.38 C:\Database\NIST05a.L		
	2-Naphthalenemethanol, 1,2,3,4,4a, 5,6,7-octahydro-.alpha.,.alpha.,4a ,8-tetramethyl-, (2R-cis)-	72997	001209-71-899
	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- , (1.alpha.,4a.beta.,8a.alpha.)-(. +/-.)-	60045	005951-61-195
	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a .beta.,7b.alpha.)]-	60090	000489-40-791
7	32.943 3.28 C:\Database\NIST05a.L		
	Agarospirol	72903	001460-73-791
	Hinesol	72894	023811-08-790
	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methyleth enyl)-, [1R-(1.alpha.,7.beta.,8a.a lpha.)]-	60048	004630-07-364
8	34.727 2.40 C:\Database\NIST05a.L		
	Aristolene	59784	1000150-14-989
	Neoisolongifolene	59831	1000156-12-483
	.beta.-Panasinsene	59841	1000159-39-083
9	35.240 4.69 C:\Database\NIST05a.L		
	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR -(1a.alpha.,4a.alpha.,7.alpha.,7 beta.,7b.alpha.)]-	60080	000489-39-490
	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl) -, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	60034	003691-11-086
	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl) -, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	60031	003691-11-084
10	35.678 0.69 C:\Database\NIST05a.L		
	Naphthalene, 1,2,3,4,4a,5,6,8a-oct	60062	039029-41-964

ahydro-7-methyl-4-methylene-1-(1-m
ethylethyl)-, (1.alpha.,4a.beta.,8 a.alpha.)-
1,6-Cyclodecadiene, 1-methyl-5-met 59960 023986-74-560
hylene-8-(1-methylethyl)-, [s-(E,E)]-
Guaiol 72885 000489-86-158

11 36.286 0.25 C:\Database\NIST05a.L
2-Naphthalenemethanol, decahydro-. 73010 000473-15-498
alpha.,.alpha.,4a-trimethyl-8-meth
ylene-, [2R-(2.alpha.,4a.alpha.,8a
.beta.)]-
2-Naphthalenemethanol, decahydro-. 73009 000473-15-486
alpha.,.alpha.,4a-trimethyl-8-meth
ylene-, [2R-(2.alpha.,4a.alpha.,8a
.beta.)]-
2-Naphthalenemethanol, 1,2,3,4,4a, 73024 000473-16-558
5,6,8a-octahydro-.alpha.,.alpha.,4
a,8-tetramethyl-, [2R-(2.alpha.,4a
.alpha.,8a.beta.)]-

12 36.820 0.29 C:\Database\NIST05a.L
Benzene, hexamethyl- 30800 000087-85-481
1,2,3,4-Tetrahydro-2,3-dimethylqui 30653 013311-77-880
noxaline
5-Hydroxy-3-methyl-1-indanone 30532 057878-30-576

13 37.728 0.30 C:\Database\NIST05a.L
Eudesma-4(14),11-diene 59851 1000152-04-395
1H-3a,7-Methanoazulene, 2,3,6,7,8, 60044 000560-32-7 91
8a-hexahydro-1,4,9,9-tetramethyl-,
(1.alpha.,3a.alpha.,7.alpha.,8a.b
eta.)-
Naphthalene, decahydro-4a-methyl-1 60015 017066-67-086
-methylene-7-(1-methylethenyl)-, [
4aR-(4a.alpha.,7.alpha.,8a.beta.)]

14 40.132 0.41 C:\Database\NIST05a.L
2-Furanmethanol, tetrahydro-.alpha 83897 026184-88-394
.,.alpha.,5-trimethyl-5-(4-methyl-
3-cyclohexen-1-yl)-, [2S-[2.alpha.
,5.beta.(R*)]]-
2H-Pyran-3-ol, tetrahydro-2,2,6-tr 83896 022567-36-816
imethyl-6-(4-methyl-3-cyclohexen-1
-yl)-, [3S-[3.alpha.,6.alpha.(R*)]]-
Copaene 59778 003856-25-510

15 41.093 0.31 C:\Database\NIST05a.L
2-Naphthalenemethanol, 1,2,3,4,4a, 72997 001209-71-899
5,6,7-octahydro-.alpha.,.alpha.,4a
,8-tetramethyl-, (2R-cis)-
1H-Cycloprop[e]azulene, 1a,2,3,4,4 60090 000489-40-790
a,5,6,7b-octahydro-1,1,4,7-tetrame

			thyl-, [1aR-(1a.alpha.,4.alpha.,4a .beta.,7b.alpha.)]-	
			Naphthalene, decahydro-4a-methyl-1 -methylene-7-(1-methylethylidene)- , (4aR-trans)-	59990 000515-17-383
16	41.328	3.29	C:\Database\NIST05a.L	
			Agarospirol	72903 001460-73-791
			Hinesol	72894 023811-08-787
			Naphthalene, 1,2,3,5,6,7,8,8a-octa hydro-1,8a-dimethyl-7-(1-methyleth enyl)-, [1S-(1.alpha.,7.alpha.,8a. alpha.)]-	60053 010219-75-764
17	41.574	0.06	C:\Database\NIST05a.L	
			Aristolene	59784 1000150-14-986
			(-)-Aristolene	59805 006831-16-983
			Naphthalene, 1,2,3,4,4a,5,6,8a-oct ahydro-4a,8-dimethyl-2-(1-methylet hylidene)-, (4aR-trans)-	60004 006813-21-478
18	42.055	2.50	C:\Database\NIST05a.L	
			Aristolene	59784 1000150-14-989
			.beta.-Panasinsene	59841 1000159-39-083
			Neoisolongifolene	59831 1000156-12-483
19	42.300	4.77	C:\Database\NIST05a.L	
			Azulene, 1,2,3,5,6,7,8,8a-octahydr o-1,4-dimethyl-7-(1-methylethenyl) -, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	60033 003691-11-087
			Azulene, 1,2,3,5,6,7,8,8a-octahydr o-1,4-dimethyl-7-(1-methylethenyl) -, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	60034 003691-11-081
			Naphthalene, 1,2,3,4,4a,5,6,8a-oct ahydro-4a,8-dimethyl-2-(1-methylet henyl)-, [2R-(2.alpha.,4a.alpha.,8 a.beta.)]-	60063 000473-13-270
20	42.439	0.72	C:\Database\NIST05a.L	
			(+)-Epi-bicyclosesquiphellandrene	59869 054324-03-753
			Naphthalene, 1,2,3,4,4a,5,6,8a-oct ahydro-4a,8-dimethyl-2-(1-methylet hylidene)-, (4aR-trans)-	60005 006813-21-453
			Naphthalene, 1,2,3,4,4a,5,6,8a-oct ahydro-7-methyl-4-methylene-1-(1-m ethylethyl)-, (1.alpha.,4a.beta.,8 a.alpha.)-	60057 039029-41-953
21	42.813	0.31	C:\Database\NIST05a.L	
			2-Naphthalenemethanol, decahydro-. alpha.,.alpha.,4a-trimethyl-8-meth ylene-, [2R-(2.alpha.,4a.alpha.,8 .beta.)]-	73010 000473-15-499

2-Naphthalenemethanol, decahydro-. 73009 000473-15-455
alpha.,.alpha.,4a-trimethyl-8-meth
ylene-, [2R-(2.alpha.,4a.alpha.,8.beta.)]-
p-Isopropylphenetole 32040 004132-79-025

22 42.941 0.28 C:\Database\NIST05a.L
Benzene, hexamethyl- 30800 000087-85-47
Tricyclo[3.2.1.02,7]oct-3-ene, 2,3 30912 062338-44-776
,4,5-tetramethyl-
Benzene, hexamethyl- 30802 000087-85-476

23 43.497 0.41 C:\Database\NIST05a.L
Eudesma-4(14),11-diene 59851 1000152-04-389
Naphthalene, decahydro-4a-methyl-1 60015 017066-67-087
-methylene-7-(1-methylethenyl)-, [
4aR-(4a.alpha.,7.alpha.,8a.beta.)]
1,4-Methanoazulene, decahydro-4,8, 60024 000475-20-764
8-trimethyl-9-methylene-, [1S-(1.a
lpha.,3a.beta.,4.alpha.,8a.beta.)]

24 45.152 0.06 C:\Database\NIST05a.L
Naphthalene, 1,2,3,5,6,7,8,8a-octa 60046 004630-07-393
hydro-1,8a-dimethyl-7-(1-methyleth
enyl)-, [1R-(1.alpha.,7.beta.,8a.a
lpha.)]-
Naphthalene, 1,2,3,5,6,7,8,8a-octa 60047 004630-07-386
hydro-1,8a-dimethyl-7-(1-methyleth
enyl)-, [1R-(1.alpha.,7.beta.,8a.a
lpha.)]-
Naphthalene, 1,2,3,5,6,7,8,8a-octa 60053 010219-75-783
hydro-1,8a-dimethyl-7-(1-methyleth
enyl)-, [1S-(1.alpha.,7.alpha.,8a.
alpha.)]-

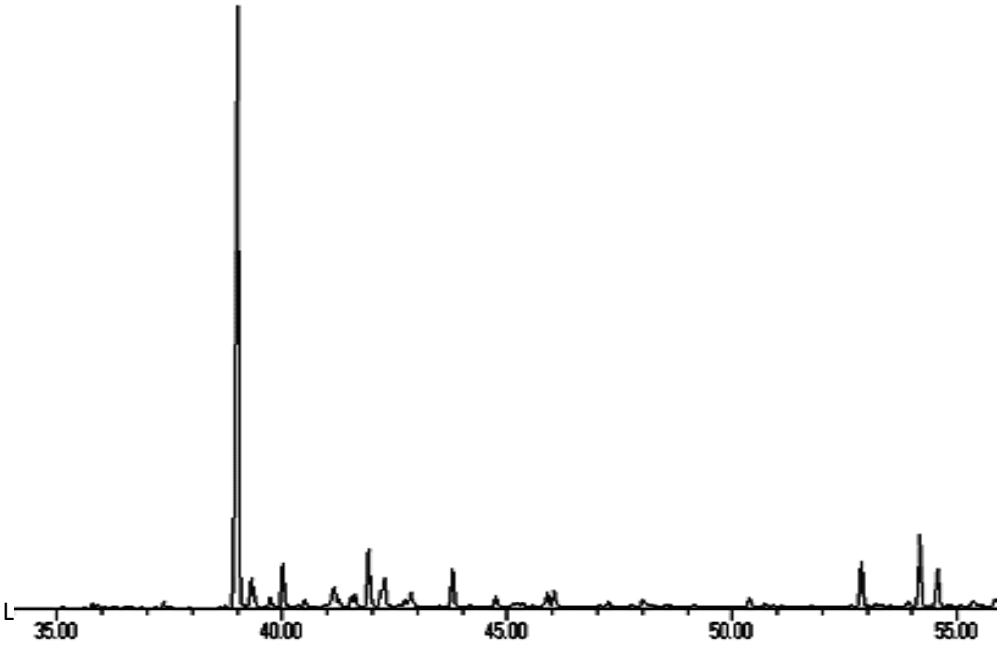
25 45.772 0.23 C:\Database\NIST05a.L
Glaucyl alcohol 71328 087745-32-276
Tricyclo[3.3.0.0(2,8)]octan-3-one, 49987 1000150-11-827
4-methyl-4-(2-methyl-2-propenyl)-
Bicyclo[4.3.0]nonane, 7-methylene- 59915 1000156-11-927
2,4,4-trimethyl-2-vinyl-

26 47.257 0.26 C:\Database\NIST05a.L
2(1H)Naphthalenone, 3,5,6,7,8,8a-h 69976 1000188-66-590
exahydro-4,8a-dimethyl-6-(1-methyl
ethenyl)-
Naphthalene, 1,2,3,5,6,7,8,8a-octa 60047 004630-07-383
hydro-1,8a-dimethyl-7-(1-methyleth
enyl)-, [1R-(1.alpha.,7.beta.,8a.a lpha.)]-
2,2,7,7-Tetramethyltricyclo[6.2.1. 69957 1000189-49-9 83
0(1,6)]undec-4-en-3-one

27 48.218 0.31 C:\Database\NIST05a.L

Cycloheptane, 4-methylene-1-methyl -2-(2-methyl-1-propen-1-yl)-1-viny l-	59957	1000159-38-5 86
2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-4a,5-dimethyl-3-(1-methy lethylidene)-, (4ar-cis)-	69988	019598-45-9 84
Glaucyl alcohol	71328	087745-32-2 50

Spot 3-Lab Sample



Data Path : D:\Data\PSM-GAHARU(2008)\
Data File : HPTLC3.LAB.D
Acq On : 8 Oct 2008 16:12
ALS Vial : 3 Sample Multiplier: 1
Search Libraries: C:\Database\NIST05a.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
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			Trichloromethane	8713	000067-66-374	
			Trichloromethane	8712	000067-66-364	
			Methane, dichloronitro-	12675	007119-89-364	
2	4.038	39.03	C:\Database\NIST05a.L			
			Trichloromethane	8714	000067-66-391	

	Ethane, 1,2,2-trichloro-1,	35125	000354-21-278
	1-difluo ro-		
	Trichloromethane	8712	000067-66-374
3	4.134 8.74 C:\Database\NIST05a.L		
	Trichloromethane	8714	000067-66-391
	Trichloromethane	8713	000067-66-391
	Trichloromethane	8712	000067-66-374
4	4.177 6.95 C:\Database\NIST05a.L		
	Trichloromethane	8713	000067-66-391
	Trichloromethane	8714	000067-66-353
	Trichloromethane	8712	000067-66-342
5	4.230 23.25 C:\Database\NIST05a.L		
	Trichloromethane	8713	000067-66-390
	Trichloromethane	8714	000067-66-383
	Trichloromethane	8712	000067-66-383
6	4.444 7.90 C:\Database\NIST05a.L		
	Trichloromethane	8714	000067-66-397
	Trichloromethane	8712	000067-66-395
	Methane, oxybis[dichloro-	44703	020524-86-183
7	30.924 3.64 C:\Database\NIST05a.L		
	2-Butanone, 3-phenyl-	21739	000769-59-596
	2-Butanone, 4-phenyl-	21740	002550-26-796
	2-Butanone, 4-phenyl-	21741	002550-26-795
8	39.010 3.02 C:\Database\NIST05a.L		
	2-Naphthalenemethanol, 1,2,3,4,4a, 5,6,7-octahydro-.alpha.,.alpha.,4a ,8-tetramethyl-, (2R-cis)-	72997	001209-71-891
	1H-Cycloprop[e]azulene, 1a,2,3,4,4 a,5,6,7b-octahydro-1,1,4,7-tetrame thyl-, [1aR-(1a.alpha.,4.alpha.,4a .beta.,7b.alpha.)]-	60090	000489-40-789
	2-Naphthalenemethanol, 1,2,3,4,4a, 5,6,7-octahydro-.alpha.,.alpha.,4a ,8-tetramethyl-, (2R-cis)-	72998	001209-71-889
9	40.015 0.18 C:\Database\NIST05a.L		
	2H-Pyran-3-ol, tetrahydro-2,2,6-tr imethyl-6-(4-methyl-3-cyclohexen-1 -yl)-, [3S-[3.alpha.,6.alpha.(R*)]-	83896	022567-36-852
	2-Furanmethanol, tetrahydro-.alpha .,.alpha.,5-trimethyl-5-(4-methyl- 3-cyclohexen-1-yl)-, [2S-[2.alpha,5.beta.(R*)]]-	83897	026184-88-349
	6-Aza-2-thiothymine	19414	000615-76-935
10	41.927 0.28 C:\Database\NIST05a.L		
	Naphthalene, decahydro-4a-methyl-1	60025	017066-67-090

-methylene-7-(1-methylethenyl)-, [
4aR-(4a.alpha.,7.alpha.,8a.beta.)]
Naphthalene, decahydro-4a-methyl-1 60015 017066-67-090
-methylene-7-(1-methylethenyl)-, [
4aR-(4a.alpha.,7.alpha.,8a.beta.)]
2,10,10-Trimethyltricyclo[7.1.1.0(59752 1000210-81-960
2,7)]undec-6-en-8-one

11 43.785 0.18 C:\Database\NIST05a.L
2H-Cyclopropa[a]naphthalen-2-one, 69993 006831-17-089
1,1a,4,5,6,7,7a,7b-octahydro-1,1,7
,7a-tetramethyl-, (1a.alpha.,7.alp
ha.,7a.alpha.,7b.alpha.)-
2(3H)-Naphthalenone, 4,4a,5,6,7,8- 69988 019598-45-955
hexahydro-4a,5-dimethyl-3-(1-methy
lethylidene)-, (4ar-cis)-
5(1H)-Azulenone, 2,4,6,7,8,8a-hexa 69984 006754-66-149
hydro-3,8-dimethyl-4-(1-methylethy
lidene)-, (8S-cis)-

12 52.876 0.22 C:\Database\NIST05a.L
Naphthalene, 5-butyl-1,2,3,4-tetra 48777 066325-42-647
hydro-
3-Pyridinecarbonitrile, 6-ethyl-5- 21387 110253-41-337
methyl-
Benzene, 1-(1-methyl-2-propenyl)-4 48792 057438-46-735
-(2-methylpropyl)-

13 54.179 0.33 C:\Database\NIST05a.L
1,6-Dimethylhepta-1,3,5-triene 9726 1000196-61-050
Benzenemethanol, 4-methyl- 9648 000589-18-445
Benzenemethanol, 4-methyl- 9653 000589-18-445

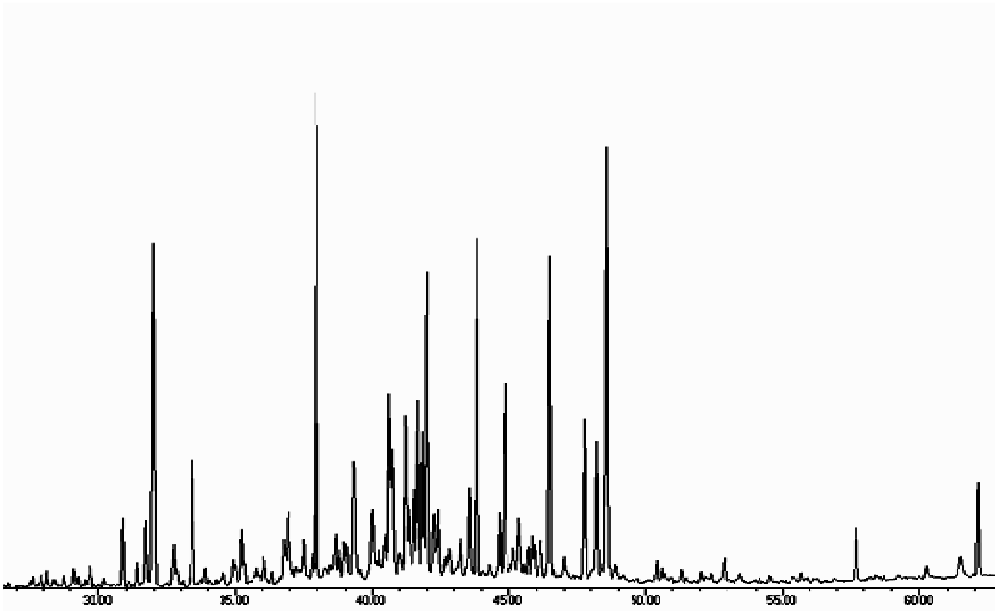
14 54.563 0.19 C:\Database\NIST05a.L
1H-Indene, 2,3-dihydro-1,1,3-trime 29565 002613-76-535
thyl-
1H-Indene, 2,3-dihydro-1,1,5-trime 29566 040650-41-735
thyl-
1H-Benzimidazole, 2-(1-methylethyl 29371 005851-43-430
)-

15 57.768 4.96 C:\Database\NIST05a.L
1,3-Cyclopentadiene, 5,5-dimethyl- 9746 1000162-25-738
1-ethyl-
Phenol, 3,4-dimethyl-, methylcarba 42272 002425-10-738
mate
Phenol, 2-ethyl- 9607 000090-00-638

16 58.729 0.30 C:\Database\NIST05a.L
1,4-Cyclohexadiene, 3,3,6,6-tetram 15305 002223-54-315
ethyl-

1-Hydroxy-6-(3-isopropenyl-cyclopr op-1-enyl)-6-methyl-heptan-2-one	72783	1000189-14-914
Tricyclo[4.1.0.0(2,4)]heptane, 3,3 ,7,7-tetramethyl-5-(2-methyl-1-pro penyl)-	59968	056348-21-114

Spot 4- Lab Sample



Library Search Report

Data Path : D:\Data\PSM-GAHARU(2008)\
Data File : HPTLC4.LAB.D
Acq On : 9 Oct 2008 9:17
ALS Vial : 2 Sample Multiplier: 1

Search Libraries: C:\Database\NIST05a.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	3.418	0.81	C:\Database\NIST05a.L			
			Trichloromethane	8713	000067-66-364	
			Methane, dichloronitro-	12675	007119-89-364	
			Ethane, 1,2,2-trichloro-1,1-difluo ro-	35125	000354-21-253	
2	4.048	41.19	C:\Database\NIST05a.L			
			Trichloromethane	8714	000067-66-391	
			Trichloromethane	8712	000067-66-383	

		Ethane, 1,2,2-trichloro-1,1-difluo ro-	35125	000354-21-278
3	4.198	38.90 C:\Database\NIST05a.L		
		Trichloromethane	8713	000067-66-391
		Trichloromethane	8712	000067-66-353
		Methane, oxybis[dichloro-	44703	020524-86-140
4	4.433	8.14 C:\Database\NIST05a.L		
		Trichloromethane	8714	000067-66-397
		Trichloromethane	8712	000067-66-390
		Methane, oxybis[dichloro-	44703	020524-86-183
5	25.957	0.28 C:\Database\NIST05a.L		
		Azulene, 1,2,3,5,6,7,8,8a-octahydr o-1,4-dimethyl-7-(1-methylethenyl) -, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	60035	003691-11-098
		Azulene, 1,2,3,5,6,7,8,8a-octahydr o-1,4-dimethyl-7-(1-methylethenyl) -, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	60033	003691-11-098
		Azulene, 1,2,3,5,6,7,8,8a-octahydr o-1,4-dimethyl-7-(1-methylethenyl) -, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	60034	003691-11-092
6	30.871	0.16 C:\Database\NIST05a.L		
		2-Butanone, 4-phenyl-	21740	002550-26-797
		2-Butanone, 3-phenyl-	21739	000769-59-596
		2-Butanone, 4-phenyl-	21741	002550-26-795
7	31.725	0.15 C:\Database\NIST05a.L		
		1-[1-Methyl-1-(4-methyl-cyclohex-3 -enyl)-ethyl]-1H-pyrrole	59088	1000192-41-827
		Phenol, 4-amino-	5407	000123-30-827
		Thujol	24014	1000152-08-227
8	31.982	1.06 C:\Database\NIST05a.L		
		Hydrazine, 2-[fluorobis(1-methylpr opyl)silyl]-1,1-dimethyl-	70772	066436-26-835
		5-Acetyl-2-ethylsulfanyl-6-methyl- nicotinonitrile	70862	303146-26-118
		Acetamide, N-(4-aminophenyl)-N-met hyl-	32618	000119-63-111
9	33.445	0.34 C:\Database\NIST05a.L		
		cis-Z-.alpha.-Bisabolene epoxide	71387	1000131-71-253
		Bicyclo[4.1.0]heptane, 3-methyl-	5859	041977-47-3 25
		3-Dodecyne	33484	006790-27-8 22
10	35.240	0.07 C:\Database\NIST05a.L		
		1-Naphthalenamine, 5,6,7,8-tetrahy dro-	21435	002217-41-6 30

		2-Naphthalenecarboxylic acid, 8-et henyl-3,4,4a,5,6,7,8,8a-octahydro- 5-methylene-	69905	001451-36-125
		1,3,5-Cycloheptatriene, 2,5-diethy l-7,7-dimethyl-	40393	1000156-99-522
11	37.974	1.21 C:\Database\NIST05a.L		
		Benzene, 1,2,4-triethyl-	30810	000877-44-155
		9-Oxabicyclo[4.3.0]non-6-en-8-one, 7-(1-cyclopenten-3-one-1-yl)-	69762	1000160-28-851
		4,6,6-Trimethyl-2-(3-methylbuta-1, 3-dienyl)-3-oxatricyclo[5.1.0.0(2, 4)]octane	69975	1000190-22-250
12	39.352	0.41 C:\Database\NIST05a.L		
		4,6,6-Trimethyl-2-(3-methylbuta-1, 3-dienyl)-3-oxatricyclo[5.1.0.0(2, 4)]octane	69975	1000190-22-295
		(-)-Caryophyllene-(I1)	59854	1000156-13-194
		Cyclolongifolene oxide, dehydro-	69941	1000156-11-483
13	39.993	0.18 C:\Database\NIST05a.L		
		2(3H)-Naphthalenone, 4,4a,5,6,7,8- hexahydro-4a,5-dimethyl-3-(1-methy lethylidene)-, (4ar-cis)-	69988	019598-45-986
		D-Norandrostan-16-one, (5.alpha.)-	98843	032319-06-558
		Disulfide, diphenyl	69621	000882-33-745
14	40.645	0.25 C:\Database\NIST05a.L		
		Cyclolongifolene oxide, dehydro-	69941	1000156-11-438
		Benzenamine, N-2-butenyl-N-methyl-	30201	030211-55-335
		1,3,5-Cycloheptatriene, 2,5-diethy l-7,7-dimethyl-	40393	1000156-99-535
15	41.200	0.28 C:\Database\NIST05a.L		
		Humulane-1,6-dien-3-ol	72919	1000140-23-160
		(+)-Camphor-10-sulfonyl chloride	91498	021286-54-435
		1H-Pyrrole, 2-ethyl-3,5-dimethyl-	9901	032990-59-335
16	41.563	0.13 C:\Database\NIST05a.L		
		Spiro[4.5]dec-6-en-8-one, 1,7-dime thyl-4-(1-methylethyl)-	71412	039510-36-687
		2-Butanol, 4-(2,2-dimethyl-6-methy lenecyclohexylidene)-	52862	068238-73-338
		1H-Cyclodecapyrazole, 4,5,6,7,8,9, 10,11-octahydro-	41599	034176-71-135
17	41.692	0.35 C:\Database\NIST05a.L		
		Benzene, 1-cyclohexyl-2-methoxy-	49942	002206-48-650
		Phenol, 2-cyclohexyl-4-methyl-	49938	001596-09-445
		Ethanone, 1-[5-(2-furanylmethyl)-2 -furanyl]-	49730	052805-84-243

18 41.852 0.28 C:\Database\NIST05a.L
9H-Cycloisolongifolene, 8-oxo- 69939 1000155-43-086
Isolongifolen-5-one 69932 1000159-37-183
Benzene, 1,2,4-triethyl- 30816 000877-44-174

19 42.001 0.65 C:\Database\NIST05a.L
6-Isopropenyl-4,8a-dimethyl-4a,5,6 69971 086917-79-583
,7,8,8a-hexahydro-1H-naphthalen-2-
one
1,6-Cyclodecadiene, 1-methyl-5-met 59960 023986-74-555
hylene-8-(1-methylethyl)-, [s-(E,E
)]-
Cyclohexane, 1-ethenyl-1-methyl-2, 60003 000515-13-942
4-bis(1-methylethenyl)-, [1S-(1.al
pha.,2.beta.,4.beta.)]-

20 42.226 0.14 C:\Database\NIST05a.L
4,6,6-Trimethyl-2-(3-methylbuta-1, 69975 1000190-22-286
3-dienyl)-3-oxatricyclo[5.1.0.0(2,
4)]octane
Cyclolongifolene oxide, dehydro- 69941 1000156-11-470
1H-Cycloprop[e]azulene, decahydro- 60076 025246-27-9 64
1,1,7-trimethyl-4-methylene-, [1aR
-(1a.alpha.,4a.beta.,7.alpha.,7a.b
eta.,7b.alpha.)]-

21 43.550 0.25 C:\Database\NIST05a.L
Cyclopentane-3'-spirotricyclo[3.1. 48802 078578-93-570
0.0(2,4)]hexane-6'-spirocyclopenta
ne
Bicylo[4.1.0]heptane, 7-bicyclo[4. 48793 1000152-39-955
1.0]hept-7-ylidene-
1,4-Dimethyl-2-cyclopentylbenzene 39045 062379-92-450

22 43.828 0.88 C:\Database\NIST05a.L
Cycloheptane, 4-methylene-1-methyl 59957 1000159-38-592
-2-(2-methyl-1-propen-1-yl)-1-vinyl
Humulen-(v1) 59795 1000159-39-466
4,6,6-Trimethyl-2-(3-methylbuta-1, 69975 1000190-22-246
3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)]octane

23 44.651 0.14 C:\Database\NIST05a.L
5,7-Indolinedicarboxaldehyde, 1-me 49257 092287-89-330
thyl-
4-(4-Chlorophenyl)pyridine 49318 005957-96-030
1,3,4-Oxadiazole, 2-(4-dimethylami 49171 1000308-79-327
nophenyl)-
24 44.843 0.46 C:\Database\NIST05a.L
2H-Cyclopropa[a]naphthalen-2-one, 69993 006831-17-049
1,1a,4,5,6,7,7a,7b-octahydro-1,1,7
,7a-tetramethyl-, (1a.alpha.,7.alp

		ha.,7a.alpha.,7b.alpha.)-		
		Cyclolongifolene oxide, dehydro-	69941	1000156-11-446
		1s,4R,7R,11R-1,3,4,7-Tetramethyltricyclo[5.3.1.0(4,11)]undec-2-en-8-one	69972	137235-42-846
25	45.366	0.17 C:\Database\NIST05a.L		
		Humulen-(v1)	59795	1000159-39-444
		Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	60003	000515-13-927
		N1-(4,4-Dimethyl-1,3-thiazolan-2-yliden)-2,6-dimethylaniline	80982	281211-67-418
26	46.488	0.78 C:\Database\NIST05a.L		
		Benzene, 1,2-diethyl-3,4-dimethyl-	30866	054410-75-230
		(3,7-Dimethyl-octa-2,4,6-trienylideneamino)-acetonitrile	48671	1000187-75-730
		Benzene, 1,4-diethyl-2-methyl-	21907	013632-94-518
27	47.770	0.47 C:\Database\NIST05a.L		
		Humulen-(v1)	59795	1000159-39-447
		1-Propyl-3-(propen-1-yl)adamantane	70021	057040-46-746
		Cyclolongifolene oxide, dehydro-	69941	1000156-11-430
28	48.197	0.39 C:\Database\NIST05a.L		
		Neoisolongifolene, 8,9-epoxy-	69938	1000155-47-453
		Benzene, 1,4-dimethyl-2,5-bis(1-methylethyl)-	50036	010375-96-950
		Quinoline, 3-(methylthio)-	39685	051934-46-449
29	48.592	1.08 C:\Database\NIST05a.L		
		Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	59957	1000159-38-570
		2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	69976	1000188-66-547
		Cyclolongifolene oxide, dehydro-	69941	1000156-11-438
30	57.672	0.15 C:\Database\NIST05a.L		
		Phenol, 3,4-dimethyl-, methylcarbamate	42272	002425-10-743
		Phenol, 2-ethyl-	9598	000090-00-643
		Phenol, 3,5-dimethyl-	9619	000108-68-943
31	62.105	0.24 C:\Database\NIST05a.L		
		3-Pentanone, 1,5-diphenyl-	84022	005396-91-894
		3-Pentanone, 1,5-diphenyl-	84020	005396-91-890
		2-Cyanophenyl .beta.-phenylpropion	92690	040123-48-658