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JUDUL: **A Study on Microwave-Assisted Extraction of Patchouli Essential Oil: Effect of Hexane as Solvent**

SESI PENGAJIAN: **2007/2008**

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A Study on Microwave-Assisted Extraction of Patchouli Essential Oil: Effect of Hexane as Solvent

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in partial fulfillment of the requirements for the degree of
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"I declare that this thesis 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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Dedicated, in thankful appreciation for support,
encouragement and understanding
to my beloved family and friends
especially Valice Wong

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ABSTRACT

The objective of this research was to extract patchouli essential oil with microwave assisted extraction (MAE) by using hexane as solvent extraction. This research focuses on effect of varying microwave power at 300 W, 400 W and 500 W at different time of extraction and influence of dielectric properties on extraction of patchouli oil. Hexane was used because it is a good extraction solvent Patchouli or *Pogostemon cablin* accumulates large amounts of essential oil. Patchouli oil contains *sesquiterpene* alcohols as its main active component or also known as *patchoulol.* In this research, the methods used were drying, grinding, extraction, separation and analysis. From the research, the optimum yield of *patchoulol* was 82.166% at microwave power 400 W and time extraction of 10 minutes and the major components of *Pogostemon Cablin* were *patchoulol, α-bulnesene, α-guaiene and β-patchoulene.*

ABSTRAK

Objektif kajian ini adalah mengekstrak minyak pati patchouli dengan menggunakan pengekstrakan kaedah mikro gelombang dan menggunakan heksana sebagai pelarut pengekstrakan. Kajian ini akan merangkumi kesan pengaruh kuasa mikro gelombang pada 300 W, 400 W dan 500 W pada perbezaaan masa pengekstrakan dan pengaruh pemalar dielektrik mikro gelombang dalam pengekstrakan minyak patchouli. Heksana digunakan sebagai pelarut kerana heksana merupakan pelarut pengekstrakan yang baik. Patchouli atau *Pogostemon cablin* mengandungi banyak kandungan minyak pati. Minyak patchouli mengandungi alkohol *sesquiterpene* sebagai komponen aktif utama atau lebih dikenali sebagai *patchoulol.* Kaedah yang digunakan dalam kajian ini ialah pengeringan, pengecilan, pengekstrakan, pemisahan dan analisis. Daripada kajian ini, penghasilan minyak *patchoulol* yang paling baik ialah 82.166% pada kuasa mikro gelombang 400 W dan masa pengekstrakan 10 minit dan komponen utama dalam *Pogostemon Cablin* ialah *patchoulol, α-bulnesene, α-guaiene and β-patchoulene.*

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LIST OF ABBREVIATIONS

MAE	-	Microwave Assisted Extraction
SME	-	Solvent Microwave Extraction
GC	-	Gas Chromatograph
MS	-	Mass Spectrometer
W	-	Watt

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CHAPTER 1

INTRODUCTION

1.1 Pogostemon Cablin

Patchouli oil is often obtained by steam distillation and solvent extraction of the dried leaves of *Pogostemon cablin*, a plant from the *Lamiaceae* family, and is widely appreciated for its characteristic pleasant and long lasting woody, earthy, camphoraceous odor. Patchouli oil serves as an important ingredient in many fine fragrance products like perfumes, soaps and cosmetic products [1]. Patchouli oil is essential oil that contains sesquiterpene alcohols as its main active component or also known as *patchoulol.*

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1.2 Microwave Assisted Extraction

Microwave assisted extraction (MAE) is a new separation technique that combines the uses of the energy of microwave radiation and traditional solvent extraction. Microwave extraction of biologically active compounds was first presented by Ganzler as a novel and effective sample preparation technique in 1986 [2]. By using a closed system, extraction can be performed at higher temperatures and extraction time can be reduced drastically. This technique can be applied to both liquid and gas phase extraction. Several studies showed that microwave assisted extraction has many advantages, such as shorter time extraction, less solvent needed, higher extraction rate and better products with lower cost [3,4]

1.3 Problem Statement

The concentration and purification of patchouli oil have been important processes for many years. The conventional methods used so far are mainly based on solvent extraction and steam distillation. The drawbacks linked to these techniques have led to the searching for new alternative extraction processes. The commercial methods currently used for folding or unfolding essential oils are fractional vacuum distillation, selective solvent extraction and chromatographic separation. All these methods have drawbacks such as low yields, formation of by-products owing to the time of exposure to high temperatures and the presence of toxic organic residues in the extracts.

1.4 Objective

The objective of this research was to extract patchouli essential oil with microwave assisted extraction (MAE) by using hexane as solvent extraction.

1.5 Scope of Research Work

This research focus on two main scopes:

(i) Investigate the influence of microwave power and extraction time

(ii) Determine the influence of dielectric properties on extraction of patchouli oil

CHAPTER 2

LITERATURE REVIEW

2.1 Essential Oil

Essential oil is defined as concentrated, hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are aromatic substances that are widely used in the perfume industries, in the pharmaceutical sector and in the food and human nutrition field. They are mixtures of more than 200 compounds that can be grouped into two fractions, a volatile fraction, that constitutes 90-95% of the whole oil and contains *monoterpenes* and *sesquiterpene* hydrocarbons and their oxygenated derivatives, along with aliphatic aldehydes, alcohols and esters, and a non-volatile residue, that constitutes from 5 to 10% of the whole oil and contains hydrocarbons, fatty acids, sterols, carotenoids, waxes, coumarins, psoralens and flavonoids [5]. The *terpene* fraction, ranging from approximately 99% of the volatile fraction in grapefruit oil to 60% in bergamot oil, makes little contribution to the favour or fragrance of the oil. Since *terpenes* are mostly unsaturated compounds, they are decomposed by heat, light and oxygen to produce undesirable compounds which can give off-flavours and off-aromas. The oxygenated fraction is highly odoriferous and is mainly responsible for the characteristic favors.

Essential oils are produced using several techniques. Distillation uses water and steam to remove the oils from dried or fresh plants, and the expression method uses machines to squeeze the oil out of plants. Other techniques may use alcohol or solvents to remove essential oils from plant materials. Essential oils generally have very complex chemical constituents, containing many different substances and compounds. Scientific research has isolated hundreds of chemicals in essential oils, and has shown many essential oils to have anti-bacterial, anti-fungal, and anti-parasitic.

2.2 *Pogostemon Cablin*

Pogostemon Cablin is both a plant and essential oil (patchouli oil) obtained from the leaves of a plant of the same name. The scent of patchouli is heavy and strong. It has been used for centuries in perfumes, and is grown in the East and West India. Figure 2.1 show the picture of *Pogostemon Cablin*.

Figure 2.1 *Pogostemon Cablin* [6]

Patchouli oil has a rich musky-sweet, strong spicy and herbaceous smell with a nearly hidden fruity note. It is light yellow to dark golden brown in color and is a viscous liquid. Patchouli oil has found widespread uses in modern industry. It is a component in about a third of modern, high-end perfumes, including more than half of perfumes for men. It is used as a scent in products like paper towels, laundry detergents, and air fresheners. The essential oil is obtained by steam distillation of the dried leaves of the plant, a process which provides a relatively high yield of the oil. Patchouli is mostly grown in Indonesia. The yield of oil from the dried leaves is about 2 %. An important component of the essential oil is *patchoulol*. *Patchoulol* is the volatile oil of *Pogostemon Cablin* and commonly used as an indicator for the quality assessment of dried *Pogostemon Cablin*. Figure 2.2 shows the chemical structure of *patchoulol*.

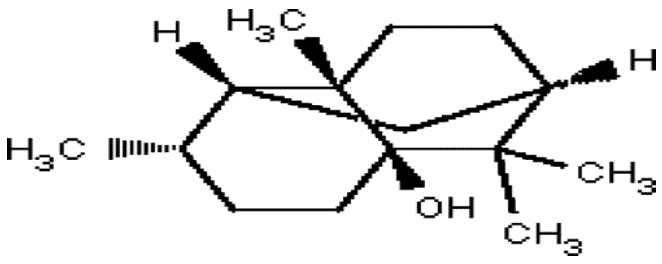

Figure 2.2 Structure of *patchoulol* (patchouli alcohol) [7]

Patchoulol or patchouli alcohol ($C_{15}H_{26}O$) is a *terpene* extracted from Patchouli. Other names for patchoulol are Patchouli camphor. The IUPAC name is *3,4,4αβ,5,6β,7,8,8α-Octahydro, 4α,8αβ,9,9-tetramethyl, 1,6-methanonaphthalen-1β(2H)-ol.* IUPAC nomenclature is a system of naming chemical compounds and of describing the science of chemistry in general.

Based on their components, *Pogostemon cablin* volatile oils from different regions can be classified into three types, they have a characteristic of high level of *pogostol* [8], high level of *patchoulol* [9,10], and high level of *patchoulol* and *pogostol* [11,12], respectively. *Patchoulol* has been known for well over a century. It is a major constitute of the pungent oil from the East Indian shrub *Pogostemon cablin* and widely used in fragrances. Patchouli alcohol was considered as Ca^{2+} antagonist and antibacterial component in *Pogostemon cablin*. In addition, *pogostol* was also considered as antibacterial agent. Patchouli oil contains many chemical compositions. Table 2.1 shows the composition of patchouli oil in patchouli leaves.

Table 2.1 Composition Patchouli Oil in Patchouli Leaves [13]

Compound	Patchouli Oil Weight Percentage
β-patchoulene	2.10%
α -bulnesene	16.60%
(+)-germacrene	1.00%
trans-β-caryophyllene	3.50%
α-humulene	0.79%
(-) pogostol	2.54%
Seychellene	7.40%
Patchoulol	34.80%
γ-patchoulene	7.90%
guai-4,11-diene	4.00%
α-patchoulene	7.90%
Norpatchoulenol	0.82%
α-guaiene	13.80%
Other sesquiterpenes	4.71%

2.2.1 Uses of Patchouli Oil

There are many uses of patchouli oil recorded. One of the major uses is in the aromatherapy treatment. The oil is used as a topical remedy for skin problems such as acne, eczema, inflamed, cracked, chapped and irritated skin. It is known as a cell rejuvenator and helpful in healing wounds and scars. As an antifungal, patchouli oil has been used to treat athlete's foot. For the hair, patchouli oil has been used for dandruff and to aid oily hair. For the nerve system, patchouli essential oil helps to reduce tension, insomnia and anxiety. It is also know as uplifting fragrance that helps to soothe away everyday cares, and to bring about a sense of nourishment. In this way, and due to its wine-like intoxicating aroma, patchouli oil is also known an aphrodisiac.

In the perfume industry, patchouli acts as a base note and fixative par excellence. A little patchouli oil, used as a fixative can be used in many natural perfume formulations. Patchouli oil mixes well with many essential oils including sandalwood, frankincense, bergamot, myrrh, jasmine, rose and the citrus oils. The traditional use of the patchouli is as incense in temples. It is said to assist in grounding and centering the mind prior to meditation. It is also produces a strong connection to the earth as it is an aid to connection with the natural beauty of the planet.

2.3. Microwave Assisted Extraction (MAE)

Microwave Assisted Extraction is a simple technique that provides a novel way of extracting soluble products into a fluid, from a wide range of materials, helped by microwave energy [14]. The technique can be applied to both liquid phase extraction and gas phase extraction. The extraction process in liquid phase extraction like isolation of essential oil is based on a basic physical principle, namely the different ability to absorb microwave energy depending on the chemical nature of the species being subjected to microwave irradiation. The parameter generally used as a measurement of this physical property is the dielectric constant. Thus, liquid phase extraction assisted by microwaves is based on the fact that it is possible to immerse the matrix to be extracted into a solvent that is characterized both by a low dielectric constant and a relative transparency to microwaves.

The first applications of the technique dealt with the extraction of essential oils from plant products [15]. The kinetics of the microwave extraction of rosemary leaves in hexane, ethanol or hexane-ethanol mixtures, as well as the influence of factors such as the source of the leaf, the microwave energy, duration of irradiation and sample load, on the rate of extraction of the compounds have been reported [16]. In more recent research, MAE has been coupled with liquid chromatography for the determination of herbicides in plant tissue [17].

Moreover, MAE offers other advantages over conventional techniques, such as reduced energy consumption, smaller volumes of chemical solvents, use of less toxic solvents and a smaller quantity of waste products. Compared with the traditional methods, MAE has many advantages, such as shorter time, less solvent, higher extraction rate, better products with lower cost because the microwaves heat the solvent or solvent mixture directly, and the direct interaction of microwaves with the free water molecules presents in the glands and vascular systems, which results in

the subsequent rupture of the plant tissue and the release of the active compounds into the organic solvent.

2.3.1 Microwave Theory

Microwaves are non-ionizing electromagnetic waves of frequency between 300 MHz to 300 GHz and positioned between the X-ray and infrared rays in the electromagnetic spectrum [18]. In modern day science, microwaves serve two major purposes: communication and as energy vectors. The latter application is the direct action of waves on materials that has the ability to convert a part of the absorbed electromagnetic energy to heat energy. Microwaves are made up of two oscillating perpendicular field's like electric field and magnetic field and the former is responsible for heating [18]. Unlike conventional heating which depends on conduction where convection loses the heat energy to the environment. Whereas in case of MAE, heating occurs in a targeted and selective manner with practically no heat being lost to the environment as the heating occurs in a closed system. This unique heating mechanism can significantly reduce the extraction time which normally less than 30 minutes as compared to Soxhlet [19].

The principle of heating using microwave is based upon its direct impact with polar materials/solvents and is governed by two phenomenon's: ionic conduction and dipole rotation, which in most cases occurs simultaneously [18,20]. Ionic conduction refers to the electrophoretic migration of ions under the influence of the changing electric field. The resistance offered by the solution to the migration of ions generates friction, which eventually heats up the solution. Dipole rotation means realignment of the dipoles of the molecule with the rapidly changing electric field. Figure 2.3 show the difference between conventional heating and microwave heating.

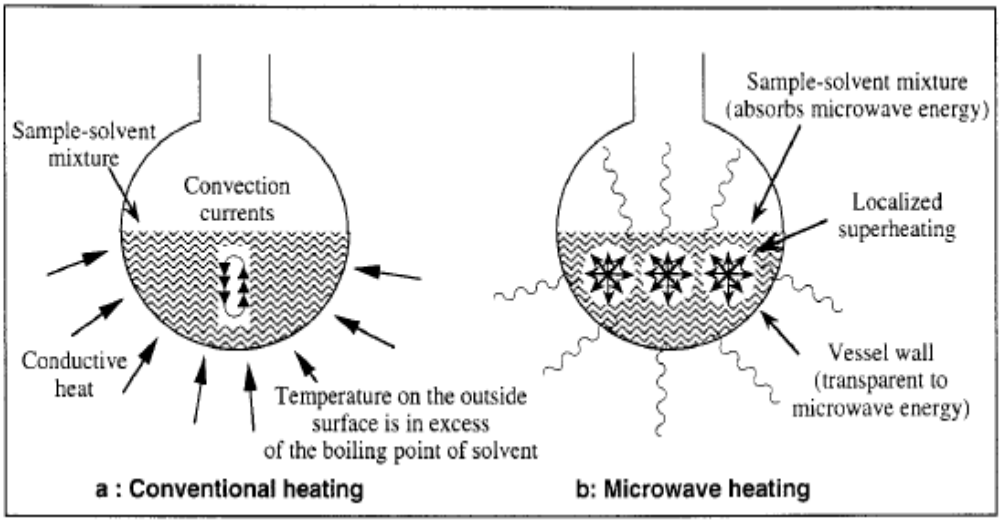

Figure 2.3 Representation of the two heating modes: convection (a) and microwave energy (b) (from Neas and Collins, 1988 [21]).

2.3.2 FACTORS AFFECTING MAE

2.3.2.1 Solvent Nature

A correct choice of solvent is fundamental for obtaining an optimal extraction process. Solvent choice for MAE is dictated by the solubility of the target analyst, by the interaction between solvent and plant matrix, and finally by the microwave absorbing properties of the solvent [20]. Preferably the solvent should have a high selectivity towards the analyst of interest excluding unwanted matrix components. This is important particularly in the extraction of pesticide and organic pollutants from soil sample. Another important aspect is the compatibility of the extracting solvent with further chromatographic analytical steps. MAE can also be performed with the same solvent as used for the conventional extraction methods.

However, the optimal extraction of solvents for MAE cannot always be deduced from those used in conventional procedures. MAE of ginger using hexane gave lesser yield than the Soxhlet extraction procedure [22]. On the other hand use of ethanol as the extracting solvent gave significantly higher yield than Soxhlet ethanol extraction. This can be accounted due to the difference in dielectric properties of the solvent. Hexane is transparent to microwave and so does not heats up under microwave, whereas ethanol has good microwave absorbing capacity and hence heats up faster and can enhance the extraction process. Thus, dielectric properties of the solvent towards microwave heating play an important role in microwave extraction. It was established that both the efficacy and selectivity of MAE depend significantly on the dielectric constant of the extracting solvent mixture. Table 2.2 shows dielectric constant for some solvents commonly used in MAE.

Table 2.2 Dielectric constant for some solvents commonly used in MAE [23]

Solvent	Dielectric constant (ε)
Acetone	20.7
Acetonitrile	37.5
Ethanol	24.3
Hexane	1.89
Methanol	32.6
2-propanol	19.9
Water	78.3

2.3.2.2 Extraction time

As in other extraction technique, time is another parameter whose influence needs to be taken into account. Generally, by increasing the extraction time, the quantity of analysts extracted is increased, although there is the risk that degradation may occur. Often 15 – 20 minute is sufficient, but even 5 minute have been demonstrated to have given excellent recovery [24,25]. MAE of polyphenols and caffeine was found to increase up to 4 min and later decreased with the increase of time [26]. In the extraction of artemisnin an overall high of 92% extraction was achieved with 12 min after which extraction yield dropped down [27], as over exposure may lead to thermal degradation of effective constituents. A proper study on optimization of extraction time is vital because extraction time may vary with different plant part used. Irradiation time is also influenced by the dielectric properties of the solvent. Solvents like water, ethanol, and methanol may heat up tremendously on longer exposure thus risking the future of thermolabile constituents.

2.3.2.3 Microwave Power

Microwave power and irradiation time are two such factors, which influences each other to a great extent. A combination of low or moderate power with longer exposure may be a wise approach. Amount of ginsenosides extracted by MAE method under different microwave conditions were studied [28]. In general, the extraction efficiency was improved by raising microwave power from 30 to 150 W. During short extraction time such as 1 and 2 minutes, recovery was enhanced with increased microwave power. The difference of the ginsenosides extracted between 30W and 150W appeared to be more significant with short extraction time compared to long extraction time. High power with prolonged exposure always involves the risk of thermal degradation.

Reports on the other hand also shows that varying of power from 400 W to 1200 W had no significant effects on the yield of flavonoids extraction but the extraction time was shortened by 45 min when using 1200 W [29]. At higher power level settings the extraction pattern was same whereas purity reduced substantially. Rapid rupture of cell wall takes place at higher temperature when kept at higher power, as a result together with the desired analytes impurities are also leached out into the solvent.

Whereas at low power levels the cell wall rupture might take place gradually this enables selective MAE. In closed vessel systems, the chosen power settings depends on the number of samples to be extracted during a single extraction run, as up to 12 vessels can be treated in a single run [20]. The power must be chosen correctly to avoid excessive temperature, which could lead to solute degradation and overpressure inside the vessel.

CHAPTER 3

METHODOLOGY

3.1 Introduction

In order to extract the patchouli essential oil, the technique used in this research was solvent microwave extraction (SME) where hexane will act as solvent.

3.2 Pre-treatment Methods

Two pre-treatment methods which were drying and grinding methods should apply before proceeds to solvent microwave extraction methods. Drying was a mass transfer process resulting in the removal of water moisture or moisture from another solvent, by evaporation. The patchouli leaves was dried by using Desiccators at room temperature for 48 hour where inside the desiccators contain silica gel that able to absorb water. After the drying process, the blender was used to ground the patchouli leaves into the small particle. The purpose of grinding methods was to reduce the size into relatively fine particles. The fine particle of patchouli leave was put in the beaker for weighing by using the high precision electronic balance.

3.3 Extraction Methods

Extractions were performed using an Ethos microwave lab station from Milestone Inc. operated via a compact terminal touch screen display with Milestone Easy-Control software as shown in Figure 3.1. The temperature was set at 55^{o}C and pressure at 1 bar. 150ml hexane solvent was used in this research. Solvent hexane and the patchouli were mixed at the large glassware.

Figure 3.1 Microwave extractor

Figure 3.2 shows that patchouli leave with solvent after extraction. After that, the patchouli oil was filtered by using the filter paper to remove the patchouli leave for separation process.

Figure 3.2 Patchouli leave with solvent after extraction

3.4 Separation Methods

After extraction process, the patchouli oil with the solvent hexane is separated by using the rotary evaporator. The purpose of separation is to remove large amount of traces hexane solvent in the patchouli oil. The rotary speed was set at 3 rpm and temperature at 60^{o}C.

3.5 Analysis Methods

The patchouli oil was analyzed by GC–MS Agilent 6890 gas chromatography instrument coupled to an Agilent 5973 mass spectrometer and an Agilent Chem Station software as shown in Figure 3.3 to determine qualitative analysis of the volatiles. Compounds were separated on a 30m×0.25mm capillary column coated with 0.25 µm film 5% phenyl methyl siloxane. The column temperature was at 80^{o}C for injection, temperature program began at 10^{o}C min^{-1} to 110^{o}C, then at 3^{o}C min^{-1} to 120^{o}C and held for 10 min. Furthermore, the temperature was increased at 2^{o}C min^{-1} to 134^{o}C, at 1^{o}C min^{-1} to 143^{o}C and held for 5 min, then at 5^{o}C min^{-1} to 240^{o}C, finally at 20^{o}C min^{-1} to 280^{o}C and held for 5 min. Split injection (2µl) was conducted with a split ratio of 1:10 and helium was used as carrier gas of 1.0 ml min^{-1} flow-rate. The spectrometers were operated in electron-impact (EI) mode, the scan range was 40–550 amu, the ionization energy was 70 eV and the scan rate was 0.34 s per scan. The inlet, ionization source temperature were 250^{o}C min and 280^{o}C, respectively.

Figure 3.3 GC-MS Agilent

Figure 3.4 show the summary of methodology that been conduct in this research

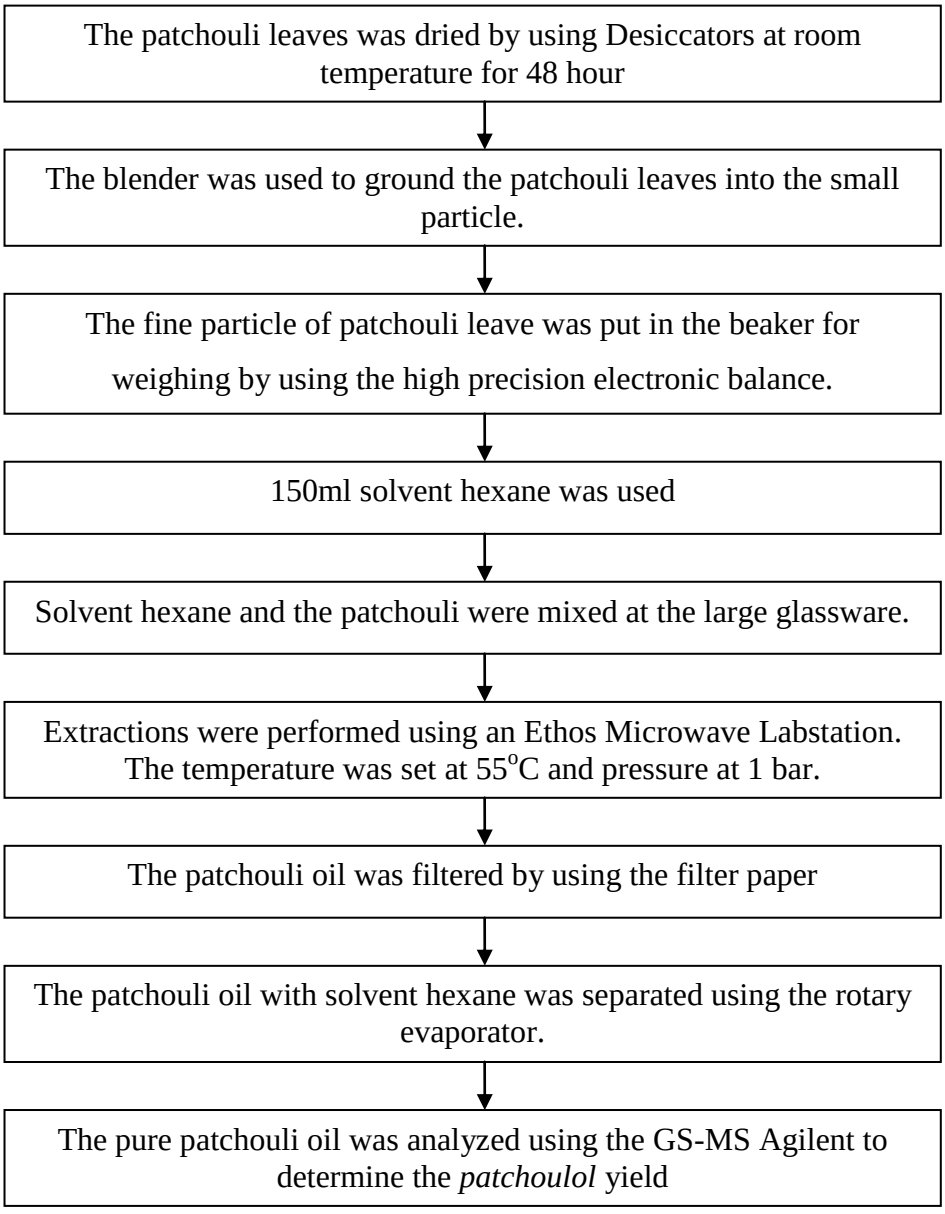

Figure 3.4 Summary of methodology

CHAPTER 4

RESULTS AND DISCUSSSION

4.1 Influence of Microwave Power and Extraction Time on *Patchoulol* Yield

In this research, pure patchouli oil with little traces of hexane solvent was analyzed with GC-MS Agilent to determine the component in the patchouli oil and the *patchoulol* yield. Table 4.1 shows the result of the research on the effect of influence of microwave power and extraction time on *patchoulol* yield.

Table 4.1 Results of influence of microwave power and extraction time on *patchoulol* yield

No Research	Power (W)	Time (min)	Patchouli leave (g)	Hexane + patchouli oil after extraction (ml)	Hexane + Patchouli oil after separation (ml)	*Patchoulol* Yield (%)
1	300	5	10	112	3.6	0
2	300	10	10	106	4	76.073
3	400	5	10	106	4.2	82.166
4	400	10	10	110	4	66.215
5	500	5	10	109	3.7	0
6	500	10	10	107	3.4	0

In general, the extraction efficiency was improved by raising microwave power from 300W to 500W because the microwave radiations would increase the mechanisms of interaction between the microwaves and the materials. Besides that, increasing the extraction time resulted in a higher percentage of *patchoulol* extracted from patchouli oil.

From the Table 4.1, it shows that power 300W at 5 minutes and 400W at 10 minutes and 5 minutes had yield *patchoulol*. The highest *patchoulol* yield was power 400W at 5 minutes. There is no *patchoulol* yield at 300W at 5 minutes because the time extraction time is short and low power extraction. Hexane has low dielectric constant which it heats up slow and unable to extract the *patchoulol* and other component.

On the other hand, there is no *patchoulol* yield at 500W at 5 minutes and 10 minutes because the high power of extraction. The high power of extraction causes the thermal degradation of *patchoulol*. Thermal degradation refers to molecular deterioration because of overheating. It occurs at a temperature at which some components of the material were separating or reacting with one another to modify the macrostructure or microstructure.

There was drop in *patchoulol* yield at power 400W and time 10 min because the solvents heat up tremendously on longer exposure thus risking the thermolabile constituents. Thermolabile refers to a substance which is subject to destruction or change in response to heat.

4.1.1 GC-MS Analysis Results

From the GC-MS analysis, research 2, 3 and 4 had yielded the two same components which are *patchoulol* and *pogostol*. The oil analyzed in this research belongs to the third type which is high level of *patchoulol* and *pogostol.* In research 2 and 4, there is other component of patchouli oil was detected which were *β-patchoulene*, *α-guaiene* and *α-bulnesene* [8]. There is no other component detected in research 3 because the extraction time was short. Therefore, longer time of extraction would increase the extraction efficiency in this research. Table 4.2 shows the comparison retention time main compounds of *Pogostemon cablin* essential oil.

Table 4.2 Comparison retention time main compounds of the *Pogostemon cablin* essential oil

Compounds	Research 2 Retention Time	Research 3 Retention Time	Research 4 Retention Time
Patchoulol	45.189	45.178	45.172
Pogostol	45.029	45.040	45.023
β-patchoulene	35.937	-	36.635
α-guaiene	35.937	-	35.942
α-bulnesene	45.029	-	45.023

From Table 4.2, the peak of retention time of *patchoulol* in research 2, 3 and 4 almost same with each other. The little difference occurs because different microwave power and time extraction was used. Research 2 and 4 had components *α-bulnesene* and *pogostol* at the same peak of retention time while experiment 3 did not contain the *α-bulnesene* because short extraction time. Therefore, the 4 major components of *Pogostemon Cablin* was *patchoulol*, *α-bulnesene, α-guaiene and β-patchoulene.*

4.2 Influence of Dielectric Properties on Extraction of *Patchoulol* Yield

In this research, the pure patchouli oil with little traces of hexane solvent and water was analyzed with GC-MS Agilent to determine the component in the patchouli oil and the *patchoulol* yield. Table 4.3 shows the result of the research on the effect of influence of dielectric properties on *patchoulol* yield.

Table 4.3 Result of Influence of dielectric properties on *patchoulol* yield

No Research	Patchouli leave (g)	Water (ml)	Hexane (ml)	Hexane + water + patchouli oil after extraction (ml)	Hexane + water + patchouli oil after separation (ml)	*Patchoulol* Yield (%)
7	10	15	135	102	3.5	61.739
8	10	30	120	95	3.1	0
9	10	45	105	78	2.8	0
10	10	60	90	65	2.3	0

Generally, absorption of the microwave energy increases with the dielectric constant of the molecule, resulting in power dissipated inside the solvent and material and generating more effective molecular movement and heating [12,13]. Water was a relatively good absorber (ε = 78.3) of microwave energy but not a good extraction solvent. However, hexane was a good extraction solvent but not a good absorber of microwave energy (ε = 1.89).

From Table 4.3, only research 7 had yielded *patchoulol* when the water was 10% weight percentage and hexane was 90% weight percentage. There was no yield of *patchoulol* at research 8, 9 and 10 when the water weight percentage increase. Research 4 with 100% weight percentage hexane at power 400 W and time 10

minutes had yielded 66.215% *patchoulol* while research 7 with 90% weight percentage hexane and 10% weight percentage water at same parameter had reduce the *patchoulol* yield to 61.739%.

In this research, the dielectric properties of the solvent towards microwave heating play an important role in microwave extraction. Water heat up tremendously than the hexane because the ability to absorb the microwave energy. Therefore, hexane will need longer time of extraction in order to increase the extraction efficiency as it takes more time to absorb the microwave energy.

4.2.1 GC-MS Analysis

From the GC-MS analysis, only research 7 had yielded *patchoulol*, the main component in patchouli oil. In research 7, there was other component of patchouli oil detected which was *β-patchoulene*, *seychellene, α-Selinene*, *α-patchoulene*, *norpatchoulenol*, *pogostol* and *α-bulnesene* [8]. Research 8, 9 and 10 did not extract any components of patchouli oil because water was not a good solvent extraction.

The peak of retention time of *patchoulol* research 7 almost same with research 2, 3 and 4. Research 7 able to extract more components more than research 4 at the same power and time extraction because some presence of water able to heat up the faster and increase the extraction efficiency. Therefore, the 5 major components of *Pogostemon Cablin* of research 7 was *patchoulol*, *α-bulnesene, α-patchoulene, α-Selinene and β-patchoulene.*

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The objective of this research was to extract patchouli essential oil with microwave assisted extraction (MAE) by using hexane as solvent. In this research, the patchouli leave that used was contains high level of *patchoulol* and *pogostol*. Power and time extraction play important role in microwave assisted extraction (MAE). The higher percentage extract of *patchoulol* was obtained at power 400W and time 10 minutes. Besides that, MAE able reduces the extraction time and obtained higher percentage extract of *patchoulol*.

In this research, the dielectric properties of the solvent towards microwave heating play an important role in microwave extraction. Water heat up faster than the hexane in the microwave because water had the higher ability to absorb the microwave energy. Although water had the higher dielectric properties, but it is not a good extraction solvent. Solvent hexane was a good extraction solvent but need longer time to absorb microwave energy as it was not good absorber of microwave energy. From GC-MS analysis, the major components of *Pogostemon Cablin* were *patchoulol, α-bulnesene, α-guaiene and β-patchoulene.*

5.2 Recommendations

It has been proven that MAE offers a huge potential in the area of essential oil extraction. There are some recommendations for further improvement in future research:

i. Use another solvent like ethanol and methanol because it is a good extraction solvent and absorber of microwave energy.
ii. Future study of microwave assisted extraction should be done in large scale as it will save production cost and time.
iii. Longer time of extraction for solvent hexane at the right extraction power to get the optimum yield of *patchoulol.*
iv. Use another species of patchouli leaves which posses high level of *pogostol* and high level of *patchoulol.*

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APPENDIX A

Grant Chart for Undergraduate Research Project I

Subject \ Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Tittle comfirmation	■														
Discussion with supervisor	■	■	■	■	■	■	■	■		■	■	■	■		■
Preparation of report															
a) Abstract							■								
b) Introduction		■	■												
c) Literature Review			■	■											
d) Methodology				■	■										
e) Expected Result and Discussion					■										
f) Conclusion					■										
g) Reference							■								
Submission 1st draft report								■							
Seminar 1 presentation									■						
Preparation final draft										■	■	■	■		
Submission final draft report														■	
Preparation for PSM II															■

APPENDIX B

Grant Chart for Undergraduate Research Project II

Subject \ Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Literature Research	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Experiment familiarization	■	■													
Experiment work															
a) Pre-treatment methods	■	■	■	■	■	■	■	■	■	■					
b) Extraction methods	■	■	■	■	■	■	■	■	■	■					
c) Separation methods	■	■	■	■	■	■	■	■	■	■					
d) Analysis methods	■	■	■	■	■	■	■	■	■	■					
Discussion with supervisor	■	■	■	■	■		■	■	■	■	■	■	■	■	■
Preparation of Full Thesis															
a) Abstract												■			
b) Introduction	■														
c) Literature Review		■	■												
d) Methodology		■	■	■	■										
e) Result and Discussion						■	■	■	■	■	■				
f) Conclusion												■			
g) Reference												■			
h) Appendix												■			
Preparation of technical paper												■			
Seminar II presentation													■		
Submission final draft thesis														■	
Submission of full thesis															■
Submission of technical paper															■

APPENDIX C

GC-MS analysis: Research 1

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                                                  Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\PATCHOULI.M
Last changed    : 13/03/2008 11:35:20  by fiza
Analysis Method : C:\CHEM32\1\METHODS\NUTMEG PSM.M
Last changed    : 14/03/2008 12:21:52  by fiza
                  (modified after loading)
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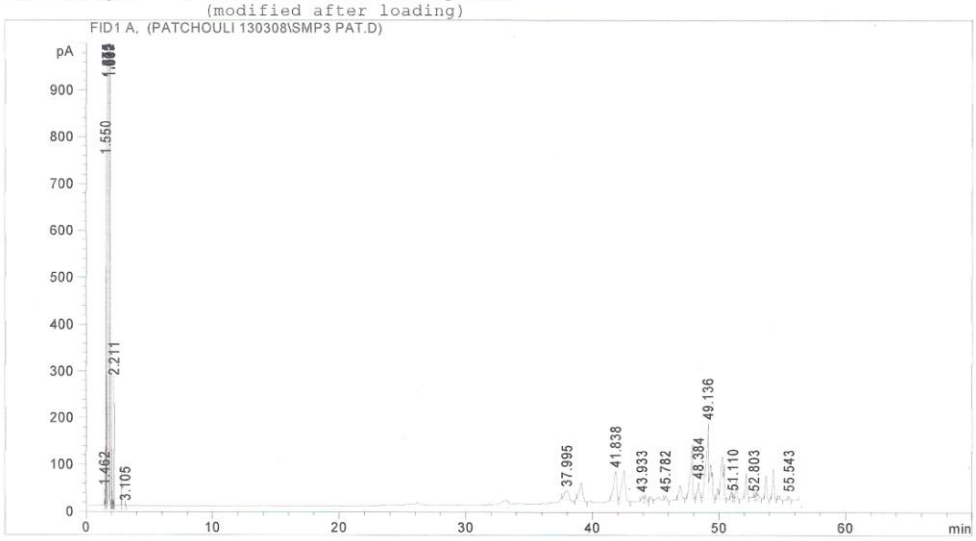


```
==========================================================================
                        External Standard Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      09/11/2005 15:02:23
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount         :      2.00000  [ppm]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          [pA*s]                  [ppm]
-------|------|----------|----------|----------|--|------------------
  2.169 VV         9.25360   13.45225  124.48185
  2.211 VB       211.82648    7.65873 1622.32140
  2.308            -           -          -
  2.359            -           -          -
  2.788 BB        83.07826    7.30717  607.06717
  4.814            -           -          -

Totals :                              2353.87042


2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
```

Data File C:\CHEM32\1\DATA\PATCHOULI 130308\SMP3 PAT.D
Sample Name: patchouli 3

Integration Results

Signal 1: FID1 A,

Peak #	Time [min]	Type	Area [pA*s]	Height [pA]	Width [min]	Start [min]	End [min]
1	1.462	BV	30.83645	33.44904	0.0138	1.438	1.501
2	1.520	VV	86.44424	113.93655	0.0119	1.501	1.534
3	1.550	VV	729.36328	739.61090	0.0155	1.534	1.590
4	1.607	VV	254.99718	261.36902	0.0154	1.590	1.645
5	1.675	VV S	1.59267e4	1.14854e4	0.0222	1.645	1.700
6	1.715	VV S	7559.36230	4255.52637	0.0296	1.700	1.736
7	1.802	VV S	8.01303e5	3.32106e5	0.0402	1.736	1.843
8	1.887	VB S	4545.17969	6635.11182	0.0110	1.843	1.945
9	1.982	BB	2.00734	2.93541	0.0110	1.967	2.005
10	2.034	BV	262.79727	286.17197	0.0147	2.014	2.060
11	2.075	VB	24.93998	29.47372	0.0129	2.060	2.105
12	2.136	BV	7.95143	10.15358	0.0121	2.116	2.146
13	2.169	VV	9.25360	6.56771	0.0194	2.146	2.187
14	2.211	VB	211.82648	269.81876	0.0132	2.187	2.242
15	2.788	BB	83.07826	82.28876	0.0168	2.768	2.822
16	3.105	BB	1.78063	1.45367	0.0193	3.080	3.142
17	37.995	BB	361.33148	18.50825	0.2318	37.585	38.615
18	39.122	BB	640.56470	37.03511	0.2148	38.735	39.575
19	41.838	BV	1070.42371	62.48431	0.2059	41.412	42.049
20	42.512	VB	1231.89197	65.33131	0.2240	42.049	42.995
21	43.933	BV	107.25565	10.72614	0.1197	43.772	44.056
22	44.198	VB	126.65145	13.28438	0.1172	44.056	44.408
23	44.562	BB	108.69543	9.53155	0.1360	44.425	44.792
24	45.782	BB	96.47518	8.50251	0.1364	45.642	46.068
25	46.918	BB	406.57547	28.46336	0.1706	46.675	47.308
26	47.894	BV	1410.57544	116.34180	0.1585	47.445	48.113
27	48.384	VV	356.08417	33.16934	0.1342	48.113	48.601
28	49.136	VV	2090.62256	160.17036	0.1663	48.601	49.212
29	49.340	VV	765.99371	71.29499	0.1293	49.212	49.434
30	49.477	VB	308.05280	54.72771	0.0797	49.434	49.675
31	49.923	BV	188.60239	23.17892	0.1019	49.749	50.011
32	50.258	VV	1135.70654	88.48461	0.1567	50.011	50.355
33	50.420	VB	369.42789	71.86764	0.0730	50.355	50.555
34	50.966	BV	109.49111	15.93982	0.0901	50.816	51.051
35	51.110	VV	54.61675	9.44913	0.0764	51.051	51.206
36	51.386	VB	321.34100	43.49831	0.0922	51.206	51.598
37	52.177	BB	433.89307	50.64148	0.1090	51.978	52.395
38	52.803	BV	21.76453	4.15597	0.0661	52.725	52.869
39	52.958	VV	69.89442	11.45034	0.0792	52.869	53.036
40	53.129	VB	75.75340	8.01970	0.1132	53.036	53.335
41	53.733	BB	406.43008	48.33926	0.1031	53.552	53.955
42	54.301	BB	514.19995	63.91293	0.1009	54.102	54.518
43	54.781	BB	60.76591	8.33130	0.0981	54.628	54.998
44	55.543	BB	58.12566	8.02109	0.0905	55.406	55.738

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Misc Info  :
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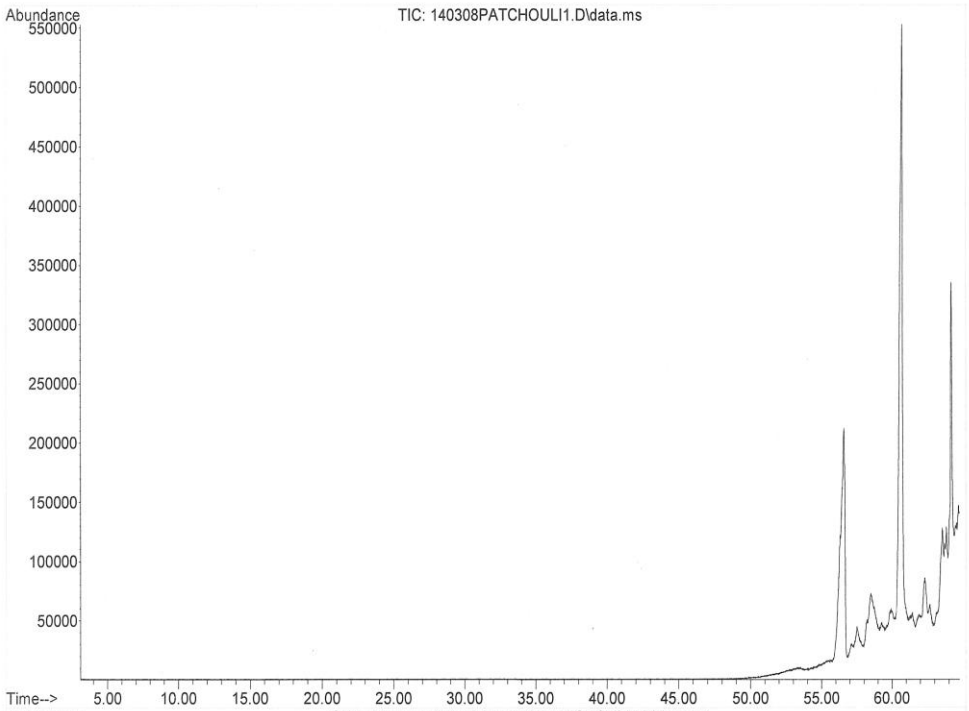
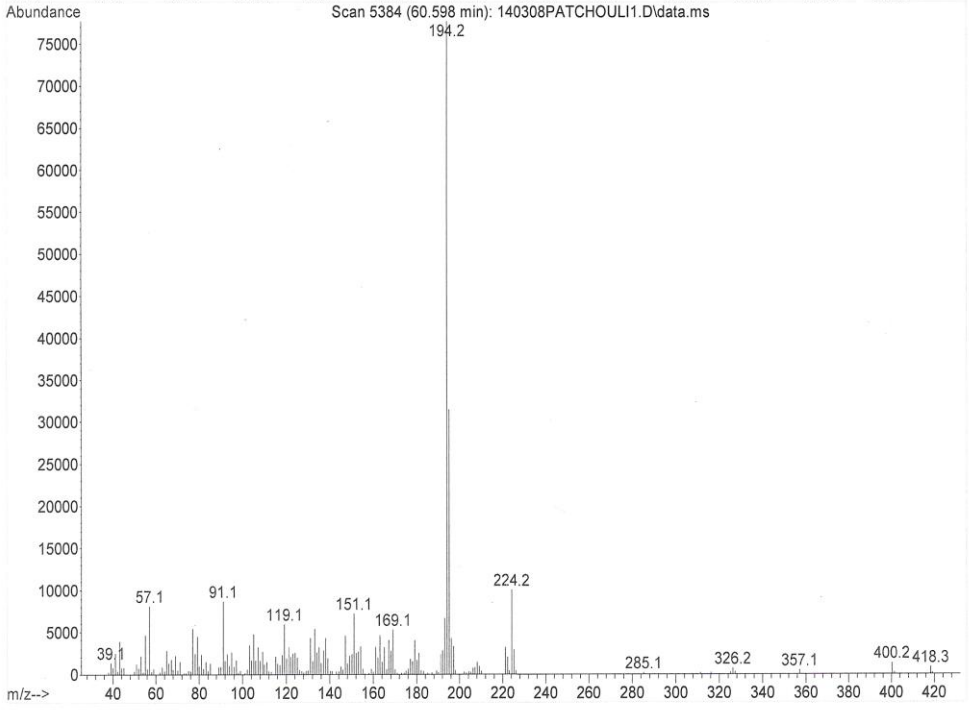
GC-MS





Library Search Report

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Misc :
ALS Vial : 1 Sample Multiplier: 1

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C:\Database\Flavor2.L Minimum Quality: 30

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

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
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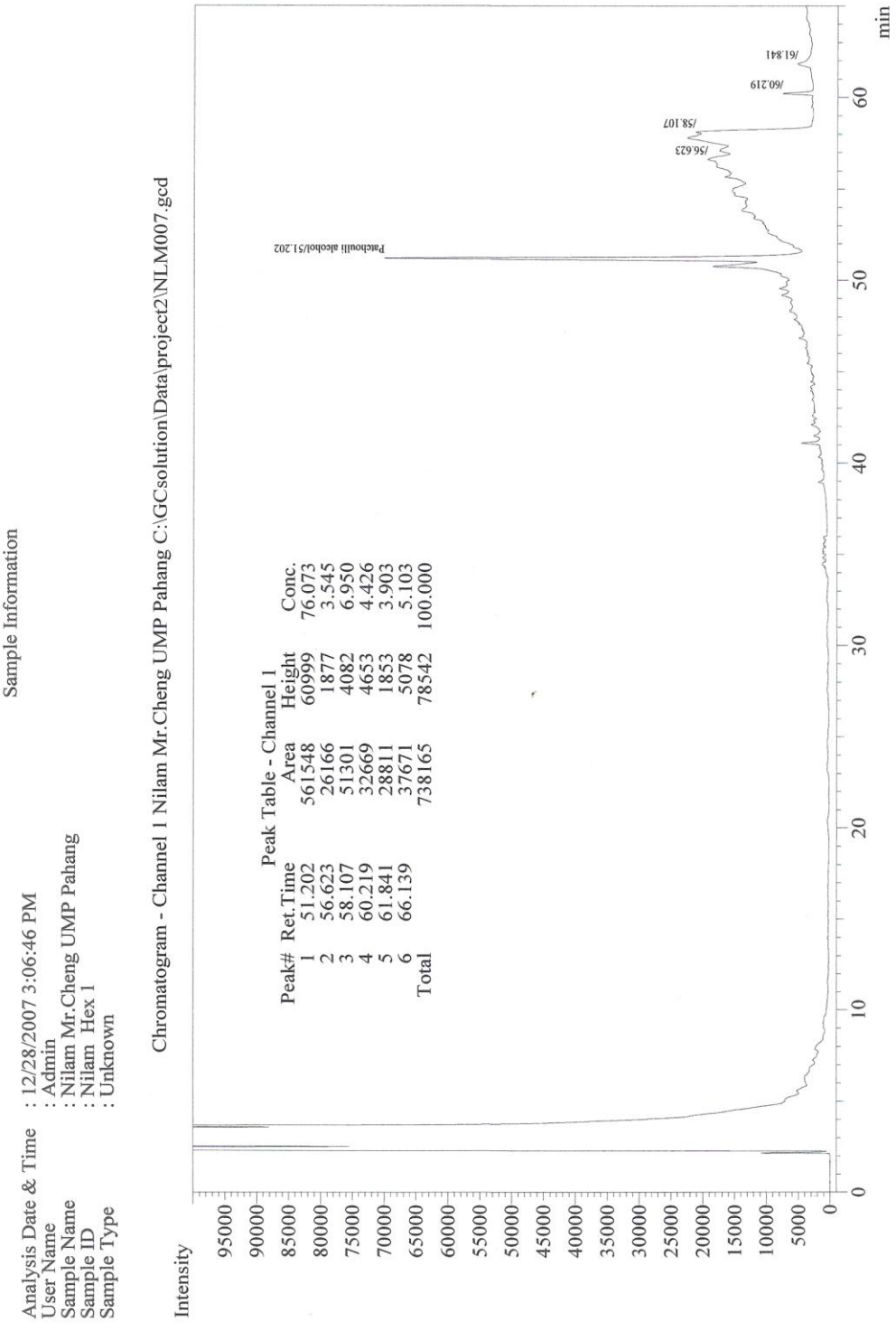
			7H-Furo[3',2':4,5]furo[2,3-c]xanthen-7-one, 3a,12c-dihydro-12c-hydroxy-6,8-dimethoxy-	154351	020797-80-2	87
			9,9'-Biphenanthrene	154697	020532-03-0	58
			1,3-Difluoro-5-pentafluorophenyldimethylsilyloxybenzene	154218	1000299-10-3	50
2	58.494	5.33	C:\Database\NIST05a.L			
			5H-Dibenz[b,e]azepine, 6,11-dihydro-	53660	000449-55-8	56
			Desmethylnomifensine	74439	073999-34-5	49
			5H-Dibenz[b,f]azepine-5-carboxamide, 10,11-dihydro-	83808	003564-73-6	46
3	60.716	46.94	C:\Database\NIST05a.L			
			N-(4-Methoxyphenyl)-2-hydroxyimino-acetamide	53297	1000143-61-3	95
			2-Hydroxy-4-isopropyl-7-methoxytropone	52474	089647-85-8	53
			Phenol, 2,6-dimethoxy-4-(2-propenyl)-	52464	006627-88-9	49
4	63.547	3.85	C:\Database\NIST05a.L			
			2-Cyclopenten-1-one, 3-methyl-2-(1,3-pentadienyl)-, (E,Z)-	30784	022610-80-6	30
			Tetrapentacontane, 1,54-dibromo-	190704	1000156-09-4	25
			2-Cyclopenten-1-one, 3-methyl-2-(2,4-pentadienyl)-, (Z)-	30782	022610-79-3	25
5	63.707	1.11	C:\Database\NIST05a.L			
			2-Hydroxy-4-isopropyl-7-methoxytropone	52474	089647-85-8	50
			2(1H)-Naphthalenone, octahydro-4a,7,7-trimethyl-, trans-	52865	054699-31-9	43
			2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-	52320	001135-24-6	41
6	63.835	1.78	C:\Database\NIST05a.L			
			Aciphyllene	59792	087745-31-1	55
			2,3-Dehydro-4-oxo-.beta.-ionone	59625	077503-87-8	27
			2-Cyclopenten-1-one, 3-methyl-2-(2,4-pentadienyl)-, (Z)-	30782	022610-79-3	25
7	64.177	11.63	C:\Database\NIST05a.L			
			4(1H)-Quinazolinone	21337	000491-36-1	38
			4-Hydroxy-1,7-naphthyridine	21349	053454-31-2	35
			4(1H)-Quinazolinone	21336	000491-36-1	35
8	64.551	0.44	C:\Database\NIST05a.L			
			Cyclohexaneethanol, 4-methyl-.beta.-methylene-, trans-	25803	015714-12-2	30
			Bicyclo[2.2.2]octan-2-one, 5-acetyl-8,8-dimethyl-	52682	1000277-97-9	30
			3-Hexen-2-one, 3-cyclohexyl-4-ethyl-	62952	1000150-19-1	18

APPENDIX D

GC-MS analysis: Research 2



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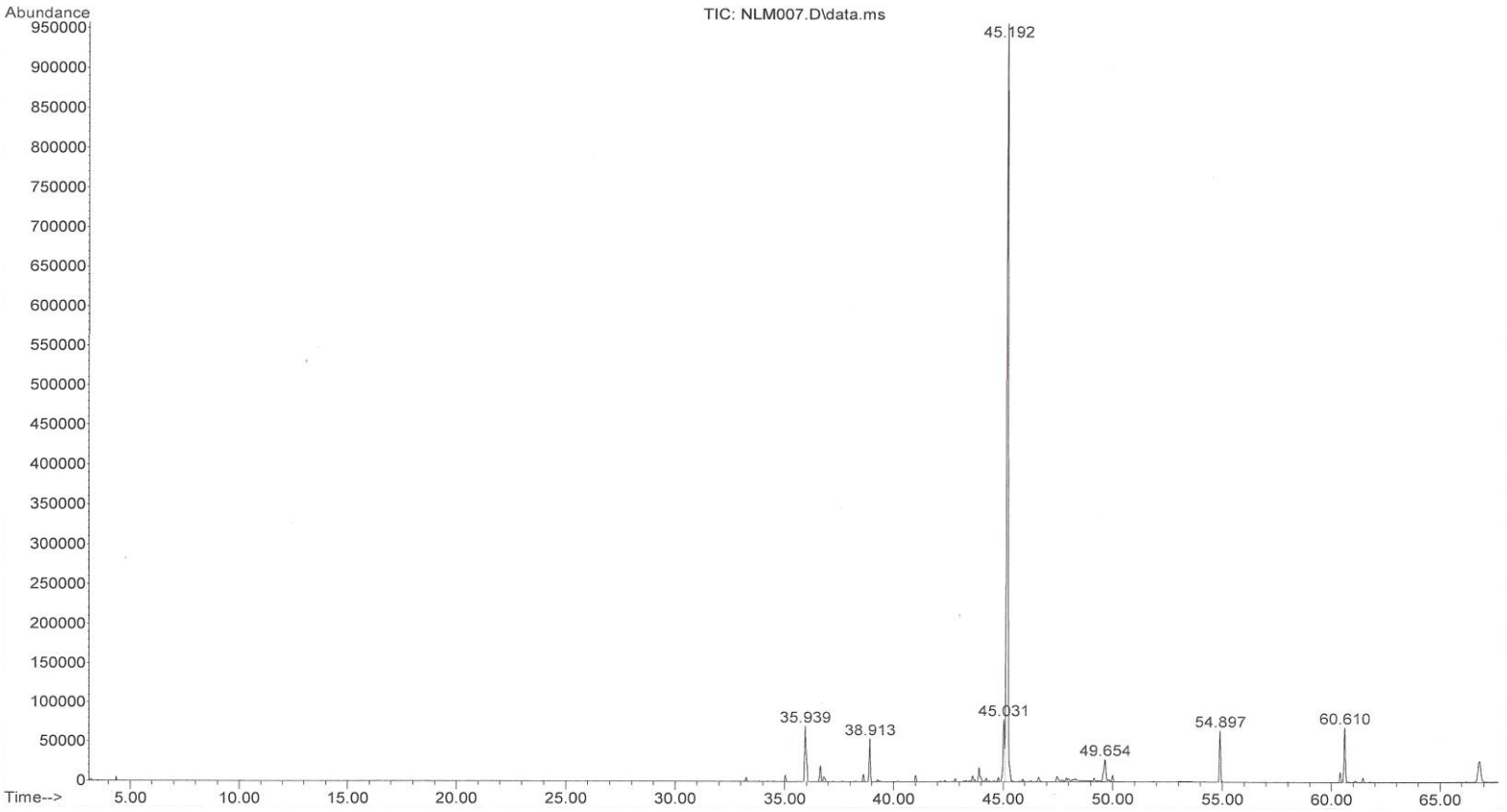
Instrument : GCMSD
Sample Name: Nilam Hex 1
Misc Info : UMP Pahang
Vial Number: 15



Library Search Report

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Sample : Nilam Hex 1
Misc : UMP Pahang
ALS Vial : 15 Sample Multiplier: 1

Search Libraries: C:\Database\HPCH2205.L Minimum Quality: 0
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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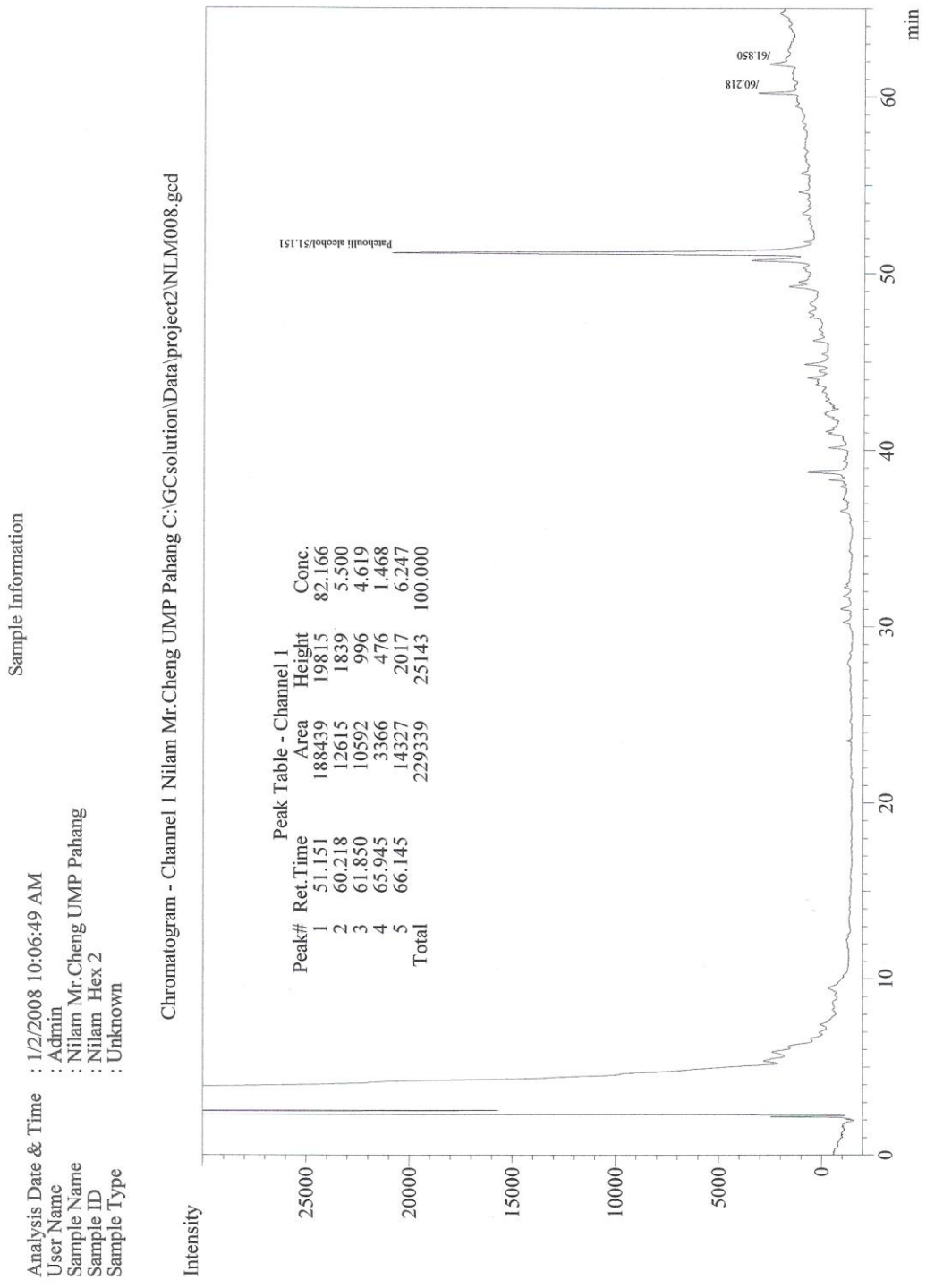
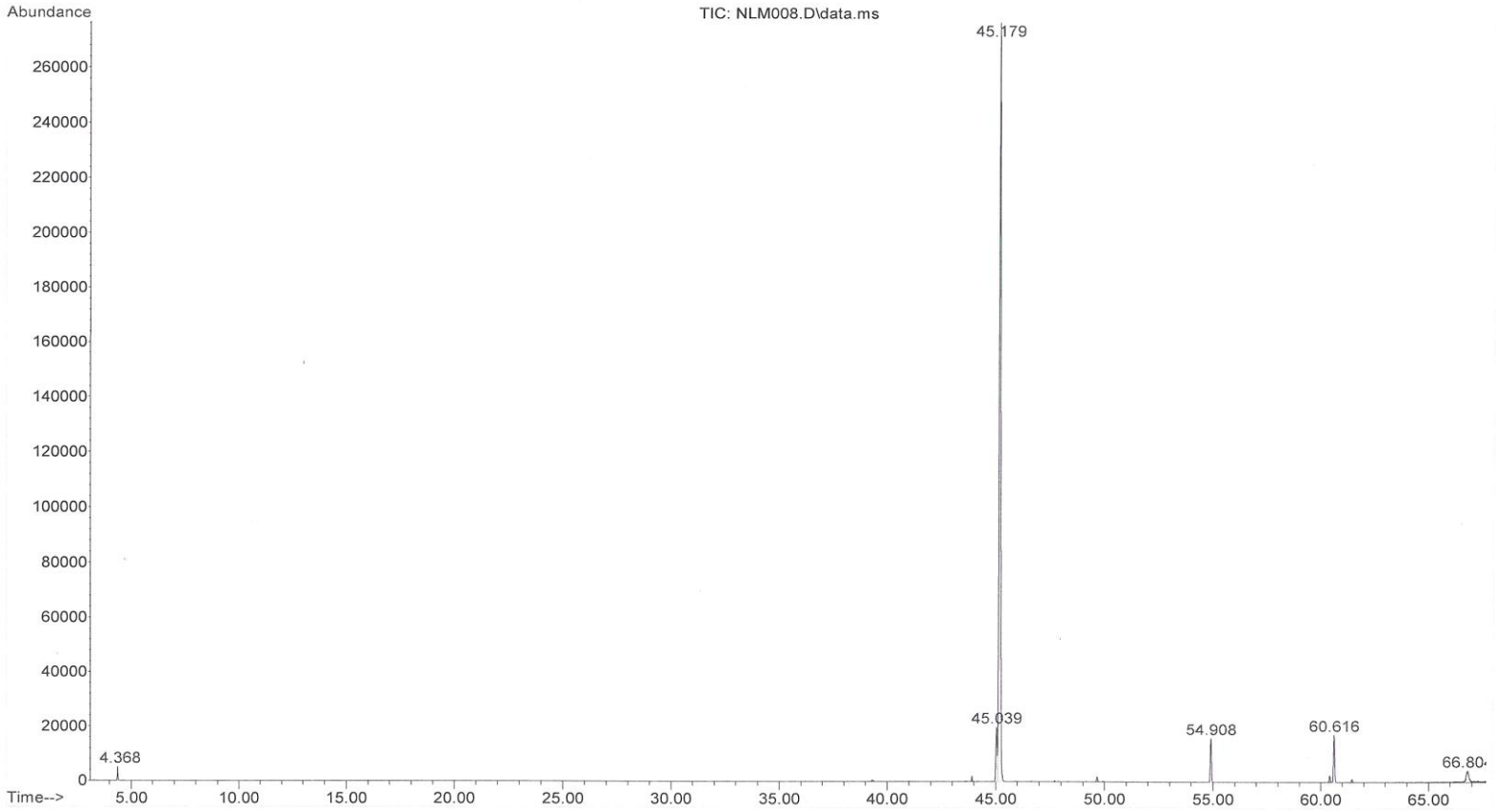
		26.20	Guaiene<alpha->	831	003691-12-1	95
		27.33	Caryophyllene<9-epi-(E)->	1360	068832-35-9	53
		28.91	Patchoulene<gamma->	1242	000508-55-4	49
2	38.912	3.35	C:\Database\HPCH2205.L			
		29.20	Bulnesene<alpha->	1244	003691-11-0	99
		24.21	Sativene	1314	006813-05-4	60
		28.68	Viridiflorene	6	021747-46-6	58
3	45.029	5.66	C:\Database\HPCH2205.L			
		34.89	Pogostol	1645	021698-41-9	55
		29.20	Bulnesene<alpha->	1244	003691-11-0	43
		23.78	Panasinsene<beta->	1232	056684-97-0	30
4	45.189	74.42	C:\Database\HPCH2205.L			
		35.08	Patchouli alcohol	1239	005986-55-0	98
		35.68	Cedranol<5-iso->	402	013567-45-8	52
		35.74	Valeranone	1530	055528-90-0	22
5	49.652	2.60	C:\Database\HPCH2205.L			
		29.35	Modhephen-8-beta-ol	634	210482-74-9	78
		34.42	Aromadendrene epoxide-<allo-	107	085760-81-2	35
		37.06	Thujopsenal	1887	000470-41-7	30
6	54.899	3.82	C:\Database\HPCH2205.L			
		44.64	Methyl hexadecanoate	955	000112-39-0	96
		21.23	Methyl decanoate	1091	000110-42-9	58
		29.82	Methyl dodecanoate	1094	000111-82-0	53
7	60.610	4.40	C:\Database\HPCH2205.L			
		29.77	Dodecadienol<2E,4E->	2107	018485-38-6	12
		46.92	Eicosene<1->	1217	003452-07-1	10
		45.07	Cyclohexadecanolide	797	000109-29-5	10

APPENDIX E

GC-MS analysis: Research 3



File :D:\DATA DIS. 2007\NLM008.D
Operator : Majid
Acquired : 28 Dec 2007 9:22 using AcqMethod MPATI CTC1.M
Instrument : GCMSD
Sample Name: Nilam Hex 2
Misc Info : UMP Pahang
Vial Number: 16



Operator : Majid
Sample : Nilam Hex 2
Misc : UMP Pahang
ALS Vial : 16 Sample Multiplier: 1

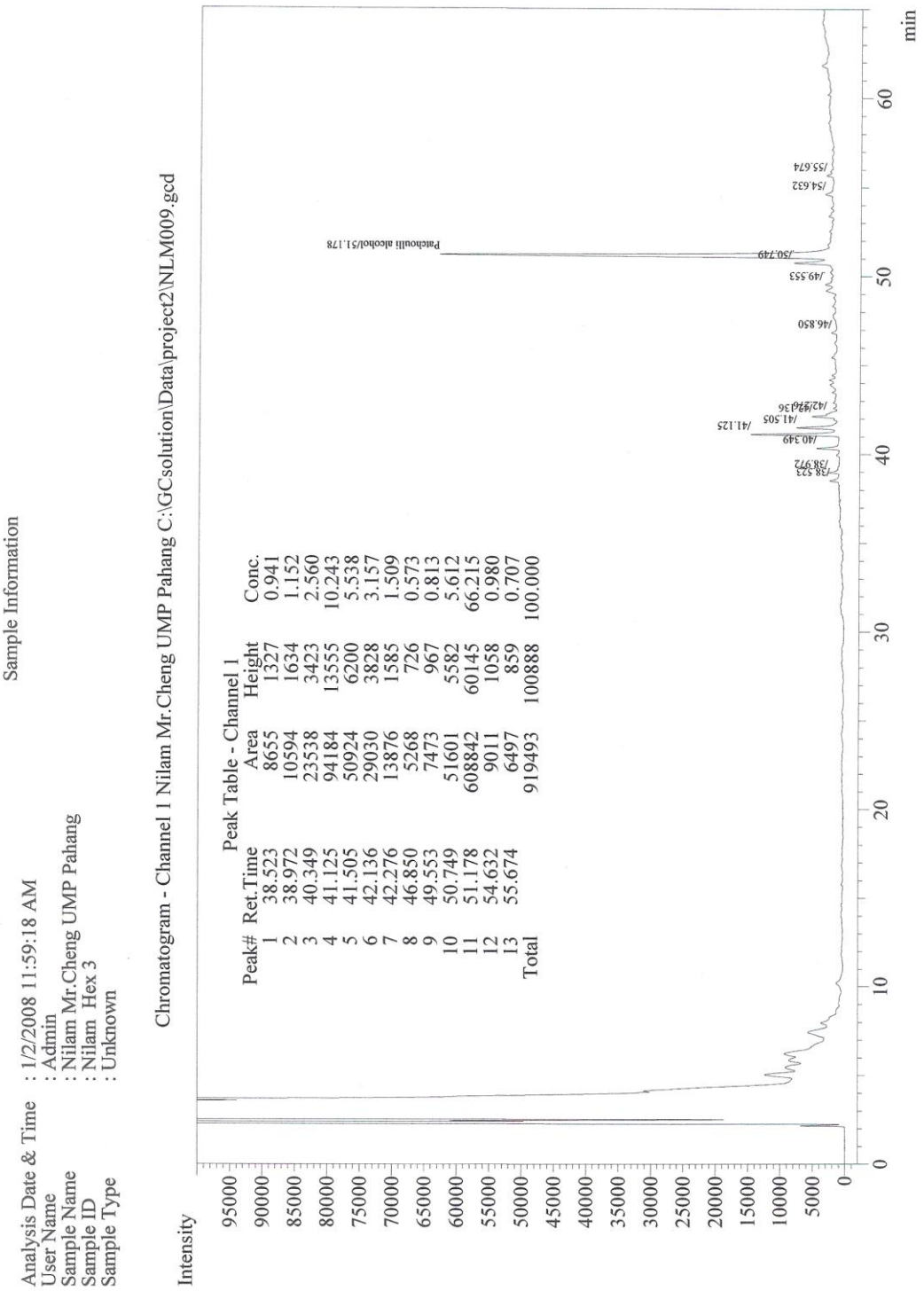
Search Libraries: C:\Database\HPCH2205.L Minimum Quality: 0
C:\Database\NIST05a.L Minimum Quality: 0

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

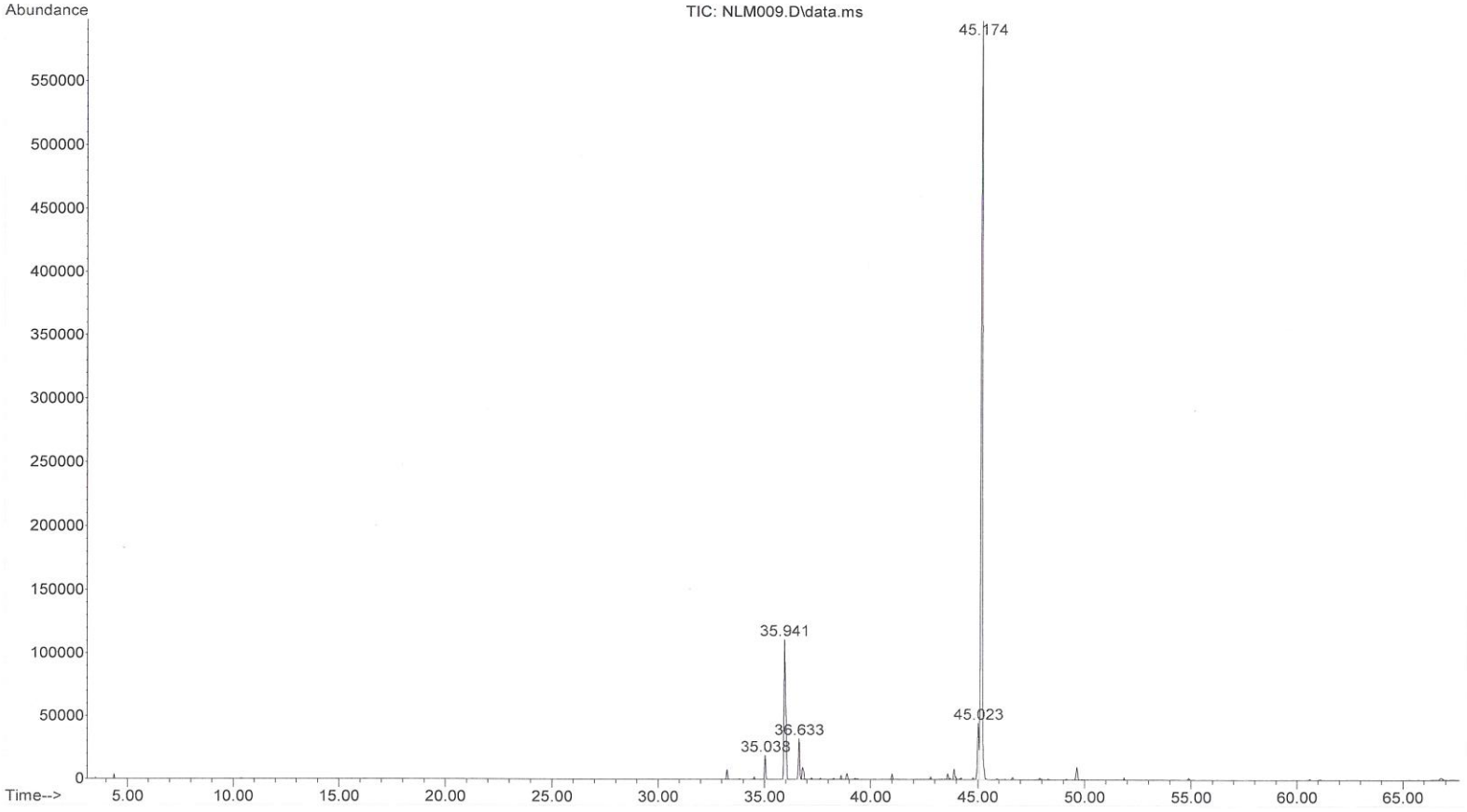
Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	4.369	0.59	C:\Database\HPCH2205.L			
		3.10	Hexenal<3E->	1975	069112-21-6	3
		3.72	Pentanone<4-hydroxy-4-methyl-2->	1750	000123-42-2	2
		22.83	Hydroxy citronellol	825	000107-74-4	2
2	45.040	5.14	C:\Database\HPCH2205.L			
		34.89	Pogostol	1645	021698-41-9	74
		26.08	Elemene<gamma->	742	029873-99-2	60
		32.50	Globulol	1867	051371-47-2	49
3	45.178	84.09	C:\Database\HPCH2205.L			
		35.08	Patchouli alcohol	1239	005986-55-0	98
		35.68	Cedranol<5-iso->	402	013567-45-8	83
		35.74	Valeranone	1530	055528-90-0	14
4	54.911	3.73	C:\Database\HPCH2205.L			
		44.64	Methyl hexadecanoate	955	000112-39-0	96
		37.57	Methyl tetradecanoate	1111	000124-10-7	53
		29.82	Methyl dodecanoate	1094	000111-82-0	42
5	60.616	4.16	C:\Database\HPCH2205.L			
		45.07	Cyclohexadecanolide	797	000109-29-5	38
		11.38	Nonenal<6Z->	2131	002277-19-2	23
		29.77	Dodecadienol<2E,4E->	2107	018485-38-6	12
6	66.807	2.28	C:\Database\HPCH2205.L			
		23.75	Thujic acid	1850	000499-89-8	2
		32.89	Rosifoliol	1595	063891-61-2	2
		17.19	Thymol, methyl ether	1506	001076-56-8	2

APPENDIX F

GC-MS analysis: Research 4



File :D:\DATA DIS. 2007\NLM009.D
Operator : Majid
Acquired : 28 Dec 2007 10:34 using AcqMethod MPATI CTC1.M
Instrument : GCMSD
Sample Name: Nilam Hex 3
Misc Info : UMP Pahang
Vial Number: 17



Operator : Majid
Sample : Nilam Hex 3
Misc : UMP Pahang
ALS Vial : 17 Sample Multiplier: 1

Search Libraries: C:\Database\HPCH2205.L Minimum Quality: 0
C:\Database\NIST05a.L Minimum Quality: 0

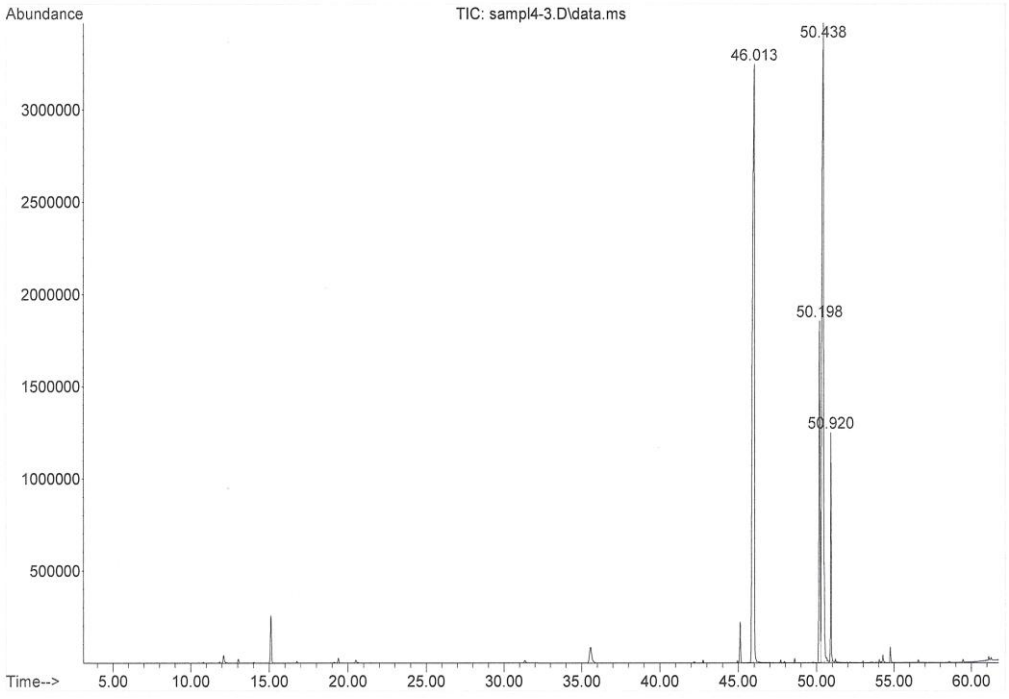
Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	35.038	1.73	C:\Database\HPCH2205.L			
		25.36	Caryophyllene(E-)	830	000087-44-5	99
		24.95	Caryophyllene<Z->	386	000118-65-0	58
		22.18	Silphinene	2167	074284-57-4	50
2	35.942	15.47	C:\Database\HPCH2205.L			
		26.20	Guaiene<alpha->	831	003691-12-1	95
		27.33	Caryophyllene<9-epi-(E)->	1360	068832-35-9	62
		28.37	Selinene<beta->	420	017066-67-0	43
3	36.635	3.41	C:\Database\HPCH2205.L			
		26.92	Patchoulene<alpha->	1241	000560-32-7	99
		26.44	Prenyl limonene<cis->	1347	135267-20-8	49
		30.03	Bisabolene<(E)-gamma->	357	053585-13-0	49
4	45.023	4.82	C:\Database\HPCH2205.L			
		34.89	Pogostol	1645	021698-41-9	93
		29.20	Bulnesene<alpha->	1244	003691-11-0	64
		27.80	Gurjunene<gamma->	762	022567-17-5	50
5	45.172	74.57	C:\Database\HPCH2205.L			
		35.08	Patchouli alcohol	1239	005986-55-0	98
		35.68	Cedranol<5-iso->	402	013567-45-8	50
		41.09	Longifolol acetate<iso->	1031	205697-15-0	14

APPENDIX G

GC-MS analysis: Research 5

```
File       :D:\Data\Patchouli\sampl4-3.D
Operator   : nora
Acquired   : 18 Mar 2008  15:00     using AcqMethod PATCHOULI PSM.M
Instrument :    GCMSD
Sample Name: Sample 4
Misc Info  :
Vial Number: 4
```



Library Search Report

Data Path : D:\Data\Patchouli\
Data File : sampl4-3.D
Acq On : 18 Mar 2008 15:00
Operator : nora
Sample : Sample 4
Misc :
ALS Vial : 4 Sample Multiplier: 1

Search Libraries: C:\Database\NIST05a.L Minimum Quality: 0
C:\Database\Flavor2.L Minimum Quality: 0

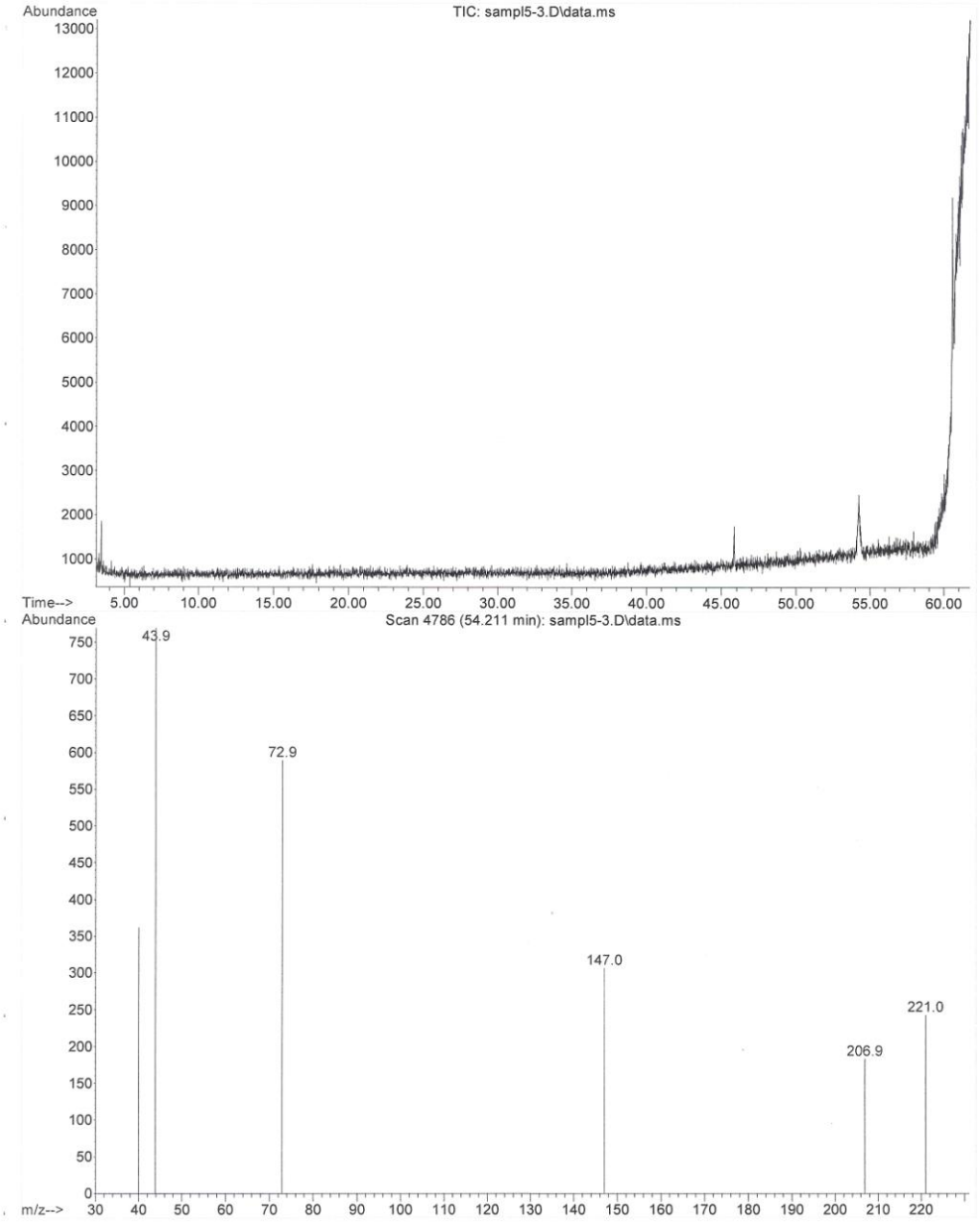
Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	46.018	41.04	C:\Database\NIST05a.L			
			Pentadecanoic acid, 14-methyl-, methyl ester	105662	005129-60-2	96
			Hexadecanoic acid, methyl ester	105644	000112-39-0	95
			Hexadecanoic acid, methyl ester	105639	000112-39-0	93
2	50.194	12.48	C:\Database\NIST05a.L			
			9,12-Octadecadienoic acid, methyl ester	121093	002462-85-3	99
			9,12-Octadecadienoic acid (Z,Z)-, methyl ester	121105	000112-63-0	99
			9,12-Octadecadienoic acid (Z,Z)-, methyl ester	121106	000112-63-0	99
3	50.440	40.42	C:\Database\NIST05a.L			
			9-Octadecenoic acid (Z)-, methyl ester	122323	000112-62-9	99
			9-Octadecenoic acid (Z)-, methyl ester	122321	000112-62-9	99
			8-Octadecenoic acid, methyl ester	122297	002345-29-1	99
4	50.921	6.05	C:\Database\NIST05a.L			
			Octadecanoic acid, methyl ester	123709	000112-61-8	98
			Octadecanoic acid, methyl ester	123700	000112-61-8	98
			Octadecanoic acid, methyl ester	123708	000112-61-8	97

APPENDIX H

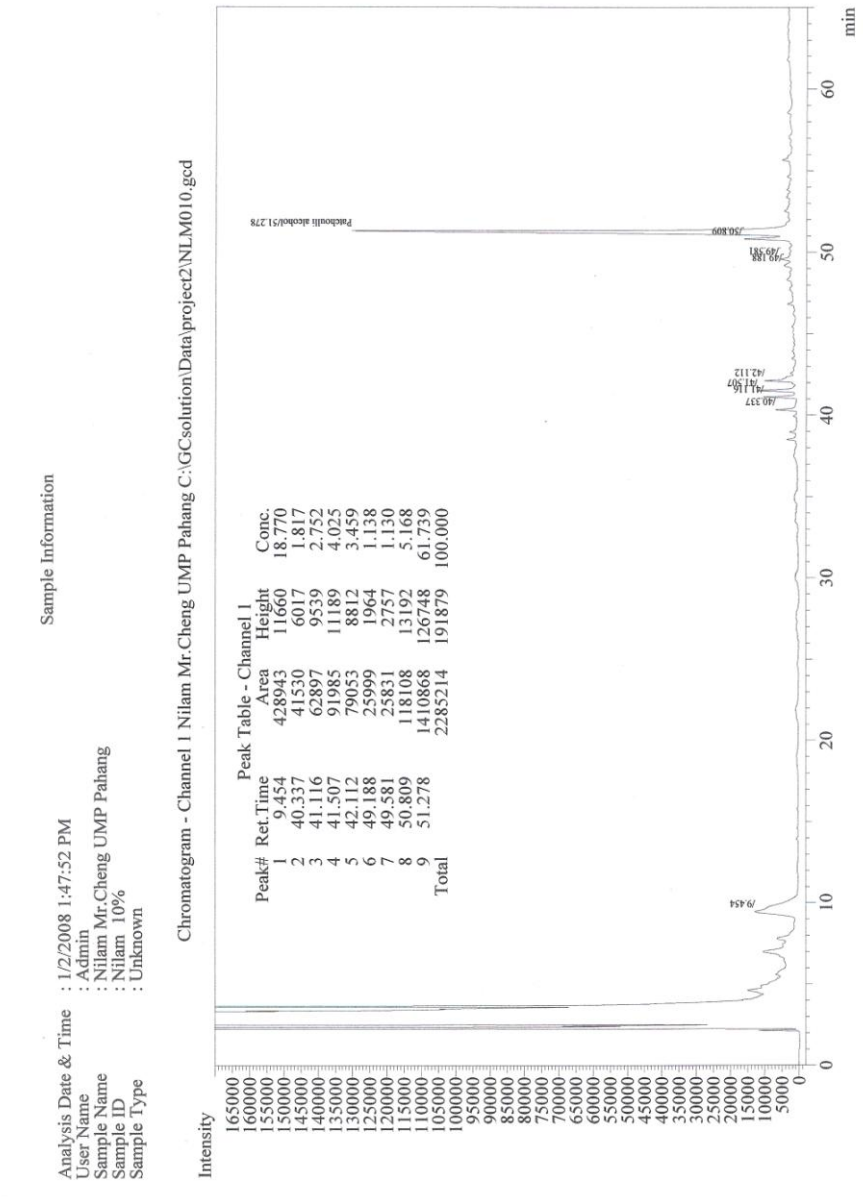
GC-MS analysis: Research 6

```
File       :D:\Data\Patchouli\sampl5-3.D
Operator   : nora
Acquired   : 18 Mar 2008  16:06     using AcqMethod PATCHOULI PSM.M
Instrument :    GCMSD
Sample Name: Sample 5
Misc Info  :
Vial Number: 5
```

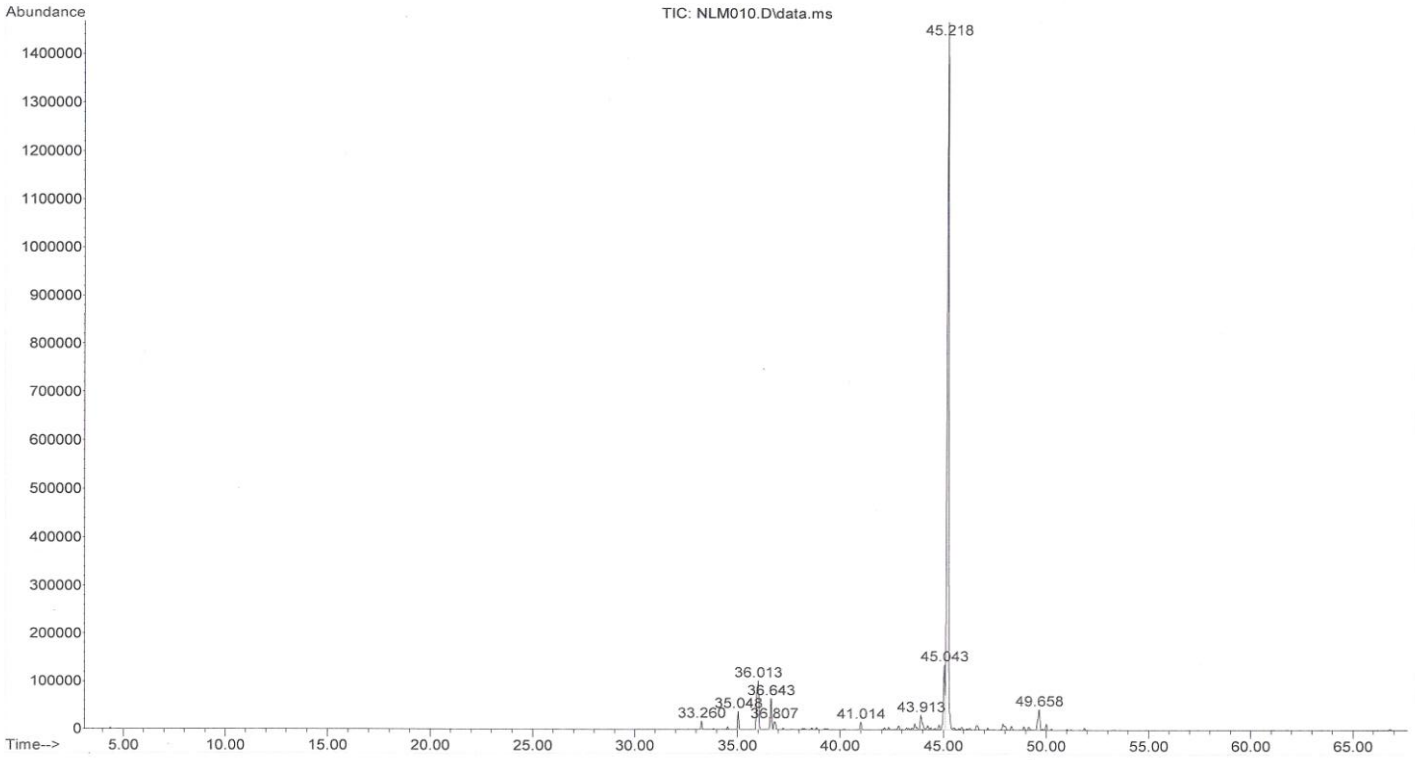


APPENDIX I

GC-MS analysis: Research 7



File :D:\DATA DIS. 2007\NLM010.D
Operator : Majid
Acquired : 28 Dec 2007 11:46 using AcqMethod MPATI CTC1.M
Instrument : GCMSD
Sample Name: Nilam Hex 10%
Misc Info : UMP Pahang
Vial Number: 18



Data Path : D:\DATA DIS. 2007\
Data File : NLM010.D
Acq On : 28 Dec 2007 11:46
Operator : Majid
Sample : Nilam Hex 10%
Misc : UMP Pahang
ALS Vial : 18 Sample Multiplier: 1

Search Libraries: C:\Database\HPCH2205.L Minimum Quality: 0
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	33.259	0.59	C:\Database\HPCH2205.L			
		23.70	Patchoulene<beta->	1240	000514-51-2	98
		29.97	Zonarene	1051	041929-05-9	70
		21.70	Presilphiperfol-7-ene	636	080931-09-5	68
2	35.050	1.31	C:\Database\HPCH2205.L			
		25.36	Caryophyllene(E-)	830	000087-44-5	98
		24.95	Caryophyllene<Z->	386	000118-65-0	93
		27.16	Aromadendrane<dehydro->	198	085048-01-7	59
3	36.011	6.86	C:\Database\HPCH2205.L			
		26.50	Seychellene	1243	020085-93-2	99
		40.11	Eudesmol acetate<beta->	693	055868-98-9	35
		22.20	Silphiperfol-5-ene<7-epi->	637	138752-23-5	32
4	36.641	2.80	C:\Database\HPCH2205.L			
		26.92	Patchoulene<alpha->	1241	000560-32-7	99
		26.44	Prenyl limonene<cis->	1347	135267-20-8	49
		30.03	Bisabolene<(E)-gamma->	357	053585-13-0	43
5	36.807	1.02	C:\Database\HPCH2205.L			
		27.07	Aromadendrene<allo->	467	025246-27-9	89
		27.80	Gurjunene<gamma->	762	022567-17-5	83
		26.39	Guaiadiene<6,9->	1795	037839-64-8	76
6	41.012	0.63	C:\Database\HPCH2205.L			
		31.00	Norpatchoulenol	1644	041429-52-1	98
		33.08	Sesquithuriferol	963	117468-55-0	35
		11.74	Dimethyl phenol<2,6->	750	000576-26-1	18
7	43.913	1.86	C:\Database\HPCH2205.L			
		28.89	Aciphyllene	1642	087745-31-1	62
		28.66	Valencene	1528	004630-07-3	56
		34.89	Pogostol	1645	021698-41-9	50

Library Search Report

Data Path : D:\DATA DIS. 2007\
Data File : NLM010.D
Acq On : 28 Dec 2007 11:46
Operator : Majid
Sample : Nilam Hex 10%
Misc : UMP Pahang
ALS Vial : 18 Sample Multiplier: 1

Search Libraries: C:\Database\HPCH2205.L Minimum Quality: 0
C:\Database\NIST05a.L Minimum Quality: 0

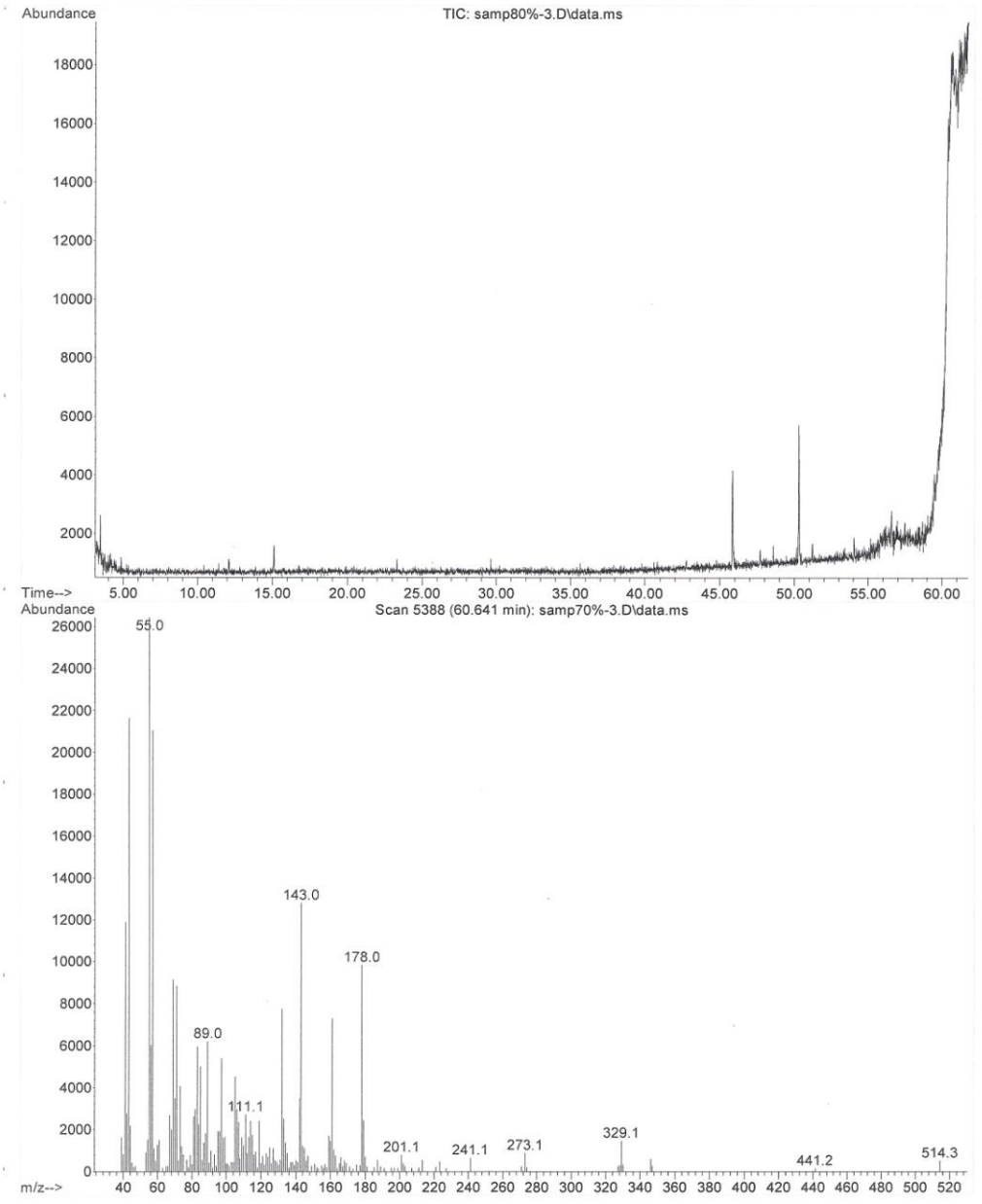
Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
8	45.040	6.18	C:\Database\HPCH2205.L			
		34.89	Pogostol	1645	021698-41-9	91
		28.74	Selinene<alpha->	421	000473-13-2	64
		29.20	Bulnesene<alpha->	1244	003691-11-0	45
9	45.218	76.10	C:\Database\HPCH2205.L			
		35.08	Patchouli alcohol	1239	005986-55-0	98
		35.68	Cedranol<5-iso->	402	013567-45-8	50
		35.74	Valeranone	1530	055528-90-0	22
10	49.658	2.66	C:\Database\HPCH2205.L			
		29.35	Modhephen-8-beta-ol	634	210482-74-9	46
		34.81	Vulgarone B	220	064180-68-3	20
		23.75	Thujic acid	1850	000499-89-8	16

APPENDIX J

GC-MS analysis: Research 8

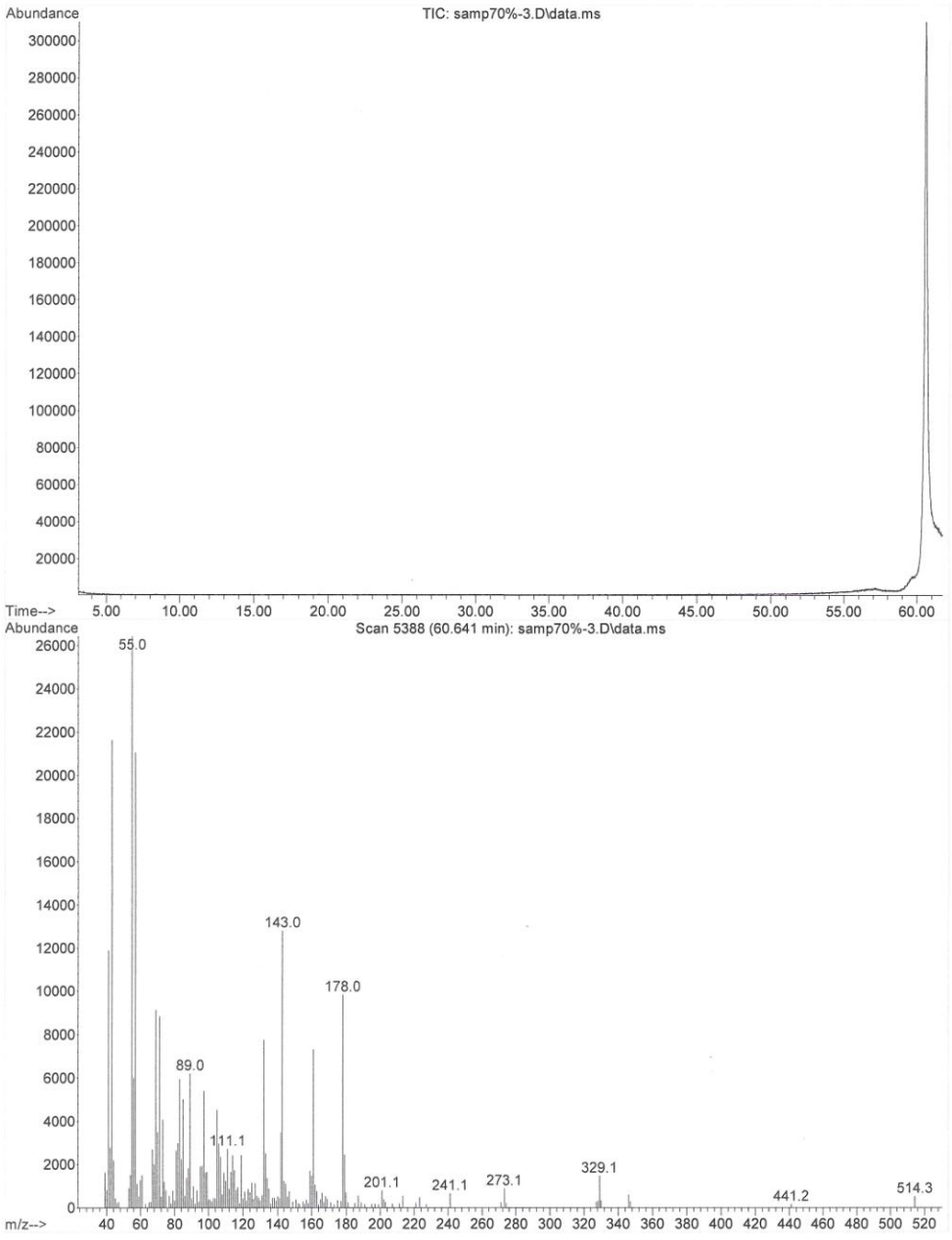
```
File       :D:\Data\Patchouli\samp80%-3.D
Operator   : nora
Acquired   : 18 Mar 2008  13:54      using AcqMethod PATCHOULI PSM.M
Instrument :     GCMSD
Sample Name: Sample 3
Misc Info  :
Vial Number: 3
```



APPENDIX K

GC-MS analysis: Research 9

```
File       :D:\Data\Patchouli\samp70%-3.D
Operator   : nora
Acquired   : 18 Mar 2008  12:48      using AcqMethod PATCHOULI PSM.M
Instrument :    GCMSD
Sample Name: Sample 2
Misc Info  :
Vial Number: 2
```



APPENDIX L

GC-MS analysis: Research 10

```
File       :D:\Data\Patchouli\samp60%-3.D
Operator   : nora
Acquired   : 18 Mar 2008  11:42     using AcqMethod PATCHOULI PSM.M
Instrument :    GCMSD
Sample Name: Sample 1
Misc Info  :
Vial Number: 1
```