MONITORING THE QUALITY OF ESSENTIAL OIL FROM *ETLINGERA* SP.4 (ZINGIBERACEAE) BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

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BORANG PENGESAHAN STATUS TESIS[υ]

JUDUL : **MONITORING THE QUALITY OF ESSENTIAL OIL FROM *ETLINGERA* SP.4 (ZINGIBERACEAE) BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)**

SESI PENGAJIAN: **2007/2008**

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CHUA SHARE KUNG

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

Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

APRIL 2008

I declare that this thesis entitled "*Monitoring the Quality of Essential Oil from Etlingera sp.4 (Zingiberaceae) by Gas Chromatography-Mass Spectrometry (GC-MS)*" 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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Special dedication to my family members, my supervisor, my beloved friends and all faculty members

For all your love, care support, and believe in me. Thank you so much.

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ABSTRACT

Essential oils are volatile, fragrant oils that occur in plants and in general contribute to their characteristic odors, flavors, or other properties. Studies have revealed that there is a spectrum of essential oils present in Zingibereaceae species, which are used widely as spice, flavouring and medicinal sources. The focus of current research was given to a wild species named as sp.4 from the genus *Etlingera* whose most species are still in undeveloped stage. The extraction of essential oil from the rhizome material of *Etlingera* sp.4 was conducted by hydro-distillation process using an all glass Clevenger-type apparatus. The compositions of essential oil isolated were further analyzed by gas chromatography-mass spectrometry (GC-MS) analysis. In addition, the effects of storage conditions including exposure to heat and light on the quality of essential oil were observed for 3 weeks. The changes in the compositions of essential oil were monitored by comparing the area of peaks. Based on the quantitative analysis of the GC-MS results, it was discovered that extreme storage conditions will result in the loss of certain volatile oil components in essential oils. Thus, optimum storage condition must be applied on natural essential oil to preserve its freshness and potency.

ABSTRAK

Minyak pati merupakan minyak yang mudah meruap dan berbau wangi yang menyumbangkan bau, rasa dan ciri-ciri penting lain kepada tumbuhan yang berkenaan. Kajian telah mendapati bahawa terdapat pelbagai jenis minyak pati yang wujud dalam spesies keluarga Zingiberaceae. Minyak pati halia digunakan secara meluas sebagai rempah, bahan perasa dan sumber perubatan. Kajian ini memberi tumpuan terhadap sejenis spesies dari genus *Etlingera* yang dinamakan sebagai sp.4. Minyak pati daripada bahagian rizom *Etlingera* sp.4 akan diekstrak menggunakan kaedah penyulingan hidro lalu dianalisa menggunakan GC-MS. Kesan pendedahan cahaya dan haba terhadap kualiti minyak pati tersebut juga diperhatikan selama 3 minggu. Perubahan komposisi minyak pati diperhatikan dengan membandingkan keputusan GC-MS. Berdasarkan analisis GC-MS secara kuantitatif, didapati bahawa kaedah penyimpanan yang kurang sesuai akan menyebabkan kehilangan sebahagian kandungan minyak pati dan mengurangkan kualitinya. Oleh itu, minyak pati semula jadi wajarlah disimpan dalam keadaan yang optimum untuk mengekalkan kesegaran dan kualitinya.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Herbs have been extensively used throughout human history as sources of food, medicine, beauty enhancers, and fragrances. The herbal–related market includes herbs used as food or food additives, cosmetic ingredients, and herbal medicines. The current estimates for this market ranges between USD 40 to100 billion with an average growth rate of 15 to 20 percent annually. (Ramlan, 2006)

In view of the potential size of market, it is important for Malaysia to build up herbal industry with its rich biological heritage, cultural background, and trade links. In fact, Malaysia is listed as the 12th most bio-diverse nation in the world and ranks 4th in Asia with over 15,000 flowering plants and over 3000 species medicinal plants. However, only about 50 out of the 3000 species are used commercially and even less are researched scientifically for their medicinal properties.

Among the valuable species of medicinal plants, local scientists and researchers have shown increasing interests in plants of Ginger family due to its wide potentials. Studies have revealed that there is a spectrum of essential oils present in Zingiberaceae species (Ibrahim & Zakaria, 1987; Zakaria *et al.*, 1989). A large number of species, particularly the strongly aromatic ones, could be screened to production of medicinal essential oils. Many active components, such as Zingiberene, have been found in the essential oil of ginger.

Ginger (*Zingiber officinale*) has a long history of being used as a spice and medicinal source. Chinese use ginger as common traditional medicine for the treatment of many diseases, such as common cold (Yu *et al*., 2006). In recent years, more and more pharmaceutical effects have been found about ginger. It can act as an aphrodisiac, a carminative, a rubifacient, an anti-asthmatic and as a stimulant to the gastrointestinal tract (U. Bhandari, 1998). Ginger is often used for the treatment of stomachache, and cardiovascular and motor diseases. It also possesses anti-inflammatory activity and regulates bacterial growth, as well as providing protection for immune-depressed patients who are HIV positive (Penna, 2003).

The family of Zingiberaceae is conventionally classified into distinct genera, each genus consists of usually several species. Some examples of distinct genera are *Curcuma, Kaempferia, Alpinia, Zingiber* and *Etlingera*. The focus of current research will be given to the genus *Etlingera* whose most species are still in undeveloped stage. Until now there are still no comprehensive reports on the use of rhizomes of *Etlingera* species. As a matter of fact, all ginger plants of genus *Etlingera* contain essential oil, where the highest content usually exists in rhizomes part.

Since *Etlingera elatior* or "kantan" has been extensively studied, the present study will thus concentrate on a wild species of *Etlingera* collected near Bentong. While comprehensive morphological examination is being carried out by the botanist at Universiti Malaya, the specimen will be named *Etlingera* sp. 4. The properties of essential oil extracted from *Etlingera* sp. 4 will be investigated in detail.

In general, an analytical procedure for essential oil from natural plants comprises two steps which are extraction and analysis. The Clevenger-type apparatus based on hydro-distillation will be used to extract essential oil from specimen in this case. Hydro-distillation is recommended for small scale operation as it protects the quality of essential oil from heat destruction. On the other hand, gas chromatography-mass spectrometry (GC-MS) is applied to determine the composition of essential oil for their reliable accuracy.

1.2 Objective

The main objective of the present study is to monitor the quality of essential oil from *Etlingera* sp.4 (Zingiberaceae) by gas chromatography-mass spectrometry (GC-MS).

1.3 Scope of Study

In order to achieve the objectives of this research, the scopes need to be identified. At initial stage, the study involves collecting rhizome samples of *Etlingera* sp.4 (Zingiberaceae) from a nearby forest and preparation of samples before the extraction process. The pre-treatment process of samples is immediately followed by the isolation of essential oils by hydro-distillation process using an all glass Clevenger-type apparatus.

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After purification procedure, the profile of pure essential oil will be further monitored by gas chromatography-mass spectrometry (GC-MS) analysis. A most suitable analysis method will be developed by trial and errors.

In addition, the effects of storage conditions including exposure to heat and light on the quality of essential oil will be observed. The storage condition is important to preserve the quality and freshness of natural essential oil.

1.4 Problem Statement

GC/MS analysis of the essential oil from the rhizome material of *Etlingera* sp. 4 has never been reported before. Research work is essential to develop the potentials from rhizome part of genus *Etlingera.*

In addition, the quality of natural essential oils is easily affected by its storage

conditions such as exposure to light and air as well as the changes in surroundings temperature. Appropriate handling and processing methods are crucial to minimize the loss of volatile oil components in essential oil.

Therefore, it is desirable to develop potential methods where the qualities of essential oil can be monitored efficiently. In the present study, the properties of essential oils under various conditions are interpreted from the result of gas chromatography-mass spectrometry (GC-MS) analysis. The results yield useful information on the effects of storage conditions to quality of essential oil.

CHAPTER 2

LITERATURE REVIEW

2.1 Ginger

2.1.1 Overview of Ginger

The word “Ginger” comes from the ancient Sanskrit word “singabera”, meaning shaped like a horn. Ginger is a tropical herb extensively grown for its pungently aromatic underground stem or rhizome which is an important export crop valued for its powder, oil and oleoresin (NEPC, 1999). It is a very valuable herbaceous perennial, said to be originated from central Asia and was among the first vegetatively cultivated plants. Today, ginger is cultivated all over tropic and sub-tropic Asia, where 50% of the world's harvest is produced in India. On the other hand, approximately 1000 species occur in tropical Asia while about 600 species are present in South East Asia.

The Zingiberaceae, or Ginger family, is a moderately-sized family of relatively advanced monocotyledonous plants of the order Zingiberales. The family is conventionally classified into at least 51 genera and 1500 species (Chen & Chen, 1998).Some distinct genera include *Curcuma, Kaempferia, Alpinia, Zingiber* and *Etlingera*.

Zingiberaceous species provide potential resources for a variety of uses ranging from medicine to food. The plants are basically rhizomatous, aromatic herbs

ranging in size from as small as 15cm in *Camptandra parvula* to as tall as 5m as observed in a number of genera from the tribe *Alpineae*. A comprehensive introduction to Zingiberaceae can be found in earlier reports and papers (Ibrahim, 1989; 1990).

2.1.2 Physical Properties

Zingiberaceous plants are rhizomatous, perennial and aromatic herbs often of large size, bearing white or yellowish-green flowers either terminally on aerial leaf shoots or from ground level.

Ginger is a knotted, thick, beige underground stem (rhizome). The stem extends roughly from one to three feet above ground, surrounded by the long, narrow, spear-shaped and green leaves. The rhizome produces a refreshing and lemon-like smell, as well as pungent taste.

2.1.3 Chemical Composition

Ginger contains ginger oil and oleoresin that account for the characteristic aroma of ginger. On the other hand, the oleoresin fraction of ginger rhizomes consists of both volatile oils and nonvolatile pungent compounds which can be extracted with solvents such as acetone or alcohol.

The volatile oil components in ginger consist mainly of sesquiterpene hydrocarbons, predominantly zingiberene (35%), curcumene (18%) and farnesene (10%). An insignificant percentage of at least 40 different monoterpene hydrocarbons are present with 1, 8-cineole, linalool, borneol, neral, and geraniol being the most abundant (Combest, 2003). Many of these volatile oil constituents contribute to the distinctive aroma and taste of ginger, however most of them are not unique to ginger.

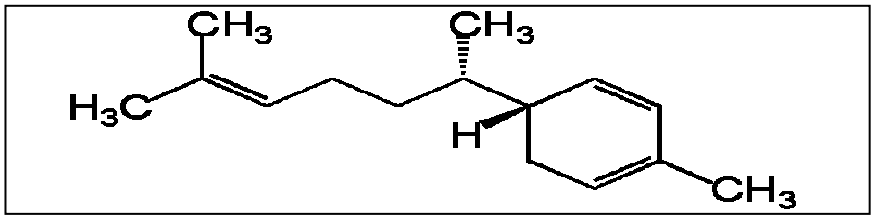

Figure 2.1 Structure of Zingiberene (A. Heinrich, 2004)

Several nonvolatile constituents of ginger are being responsible for its characteristic pungent flavor as well as pharmacological actions. The principle components of this fraction are the gingerols and shogoal. Shogoal is dehydroxylated derivatives of gingerols whose concentrations increase in dried ginger after prolonged storage.

In addition to the extractable oleoresins, ginger contains many fats, waxes, carbohydrates, vitamins and minerals. Ginger rhizomes also contain a potent proteolytic enzyme called zingibain.

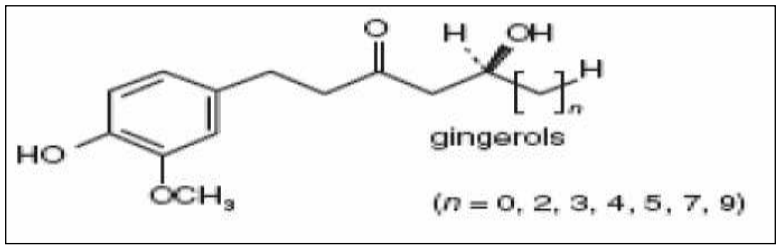

Figure 2.2 Structure of Gingerol (A. Heinrich, 2004)

2.2 Genus *Etlingera*

2.2.1 Overview

The genus *Etlingera* from the family of Zingiberaceae is distributed from India to the Pacific Islands with centres of species richness are assumed in Borneo and New Guinea. Presently at least 70 species are known from the Malesian region, which refers to a floristically distinct region including Malaysia, Indonesia, Brunei, Singapore, Phillipines and Papua New Guinea (Poulson, 2002).Works by Lim (2001) shown that a total of 15 *Etlingera* species has been recorded in Peninsular Malaysia.

2.2.2 Physical Properties

Species of *Etlingera* can be more than 5 m tall and become dominant in gaps. *Etlingera Giseke* of the family Zingiberaceae are tall forest plants, with larger species reaching 6 m in height (Khaw, 2001). In the *Phaeomeria* group, inflorescences are borne on erect stalks protruding from the ground; whereas in the *Achasma* group, inflorescences are subterranean with flowers appearing at soil level (Lim, 2000, 2001). The varying shades of pink and red colours of bracts and flowers make *Etlingera* species well known for their attractiveness.

2.2.3 Usage

Etlingera is among the most diverse and attractive genera found in Ginger family. Plants of *Etlingera* display high commercial values for their wide applications and usages. In Sabah, Malaysia, the hearts of young shoots, flower buds and fruits of *E. elatior*, *E. rubrolutea*, and *E. littoralis* are consumed by indigenous communities as condiment, either eaten raw or cooked (Noweg, Abdullah, & Nidang, 2003). In

Thailand, fruits and cores of young stems of *E. littoralis* and flowers of *E. maingayi* are eaten as vegetables (Sirirugsa, 1999).

Inflorescences of *E. elatior* or locally known as "bunga kantan" are widely cultivated throughout the tropics as spices for food flavouring and as ornamentals. They are commonly used as ingredients of some local dishes such as *laksa asam*, nasi kerabu, and nasi ulam in Peninsular Malaysia (Larsen *et al.*, 1999). In addition, farms in Australia and Costa Rica are cultivating the species and selling its inflorescences as cut flowers (Larsenet al., 1999).

For medicinal uses, fruits of *E. elatior* are used traditionally to treat earache, while leaves are applied for cleaning wounds in Malaysia (Ibrahim & Setyowati, 1999). Leaves of *E. elatior*, mixed with other aromatic herbs in water, are used by post-partum women for bathing to remove body odour.

Figure 2.3 *Etlingera elatior* (P. Ròebére, 2003)

2.3 Essential Oils

Essential oils are volatile, fragrant oils that occur in plants and in general contribute to their characteristic odors, flavors, or other such properties (Heravi, 2006). They are found in various parts of the plant body such as seeds, flower petals, bark, rhizomes, roots or leaves. They are also concentrated in certain special groups of cells.

Essential oils are extremely concentrated form of any botanical. For instance, essential oils made from rose plants require 4,000 pounds of rose petals to make one pound of essential oil. This explains the high commercial value of essential oils.

Essential oils have been used for thousands of years. Essential oils were the primary source of perfumes for the ancient civilizations of Egypt, India, Greece, and Rome. Nowadays, owe to their special properties, essential oils are widely used as perfumes, food flavorings, medicines and as fragrant and antiseptic additives in many common products. Essential oils are also applied in the healing practice of aromatherapy, one of the fastest-growing alternative health treatments in the 1990s.

Natural essential oils can be isolated from plants by various techniques, depending upon the nature of the plant body. Distillation process is widely applied where water or steam is used to remove the essential oils from dried or fresh plants. Mechanical expression method is sometimes useful to squeeze the oil out of plants by machines. Furthermore, other techniques may use alcohol or solvents to extract essential oils from plant materials with delicate chemical components.

Unlike vegetable oils expressed from nuts and seeds, essential oils are not oily. Essential oils are not fat-based, but they have a lipid-soluble molecular structure which allows them to pass easily to human skin. Essential oils are highly volatile, they evaporate into air easily. In addition, essential oils are sensitive to heat and light. Thus, they should be stored in dark or wrapped bottles and places with appropriate temperature to preserve their quality.

2.4 Separation Processes

Separation Processes is defined as any set of operations that separate two or more components into two or more products that differ in composition (Noble & Terry, 2004). The aim of separation is attained by exploiting the differences between chemical and physical properties of the substances through mass or energy.

Most chemical materials and biological substances occur in mixture form of different components in the gas, liquid, or solid phase. To separate one or more of components from the original chemical mixture, it must be contacted with another phase. The two phases are brought into more or less intimate contact with each other so that a solute or solutes can diffuse from one to the other. (Geankoplis, 2003)

The two bulk phases are usually miscible in each other to certain extent only. The two-phase pair can be gas-liquid, gas-solid, liquid-liquid, or liquid-solid. During the contact of the two phases, the components of the original mixture redistribute themselves between the two phases. The phases are then separated by simple physical methods. By choosing the proper conditions and phases, one phase is enriched while the other is depleted in one or more components.

There are a few examples of common separation process:

i. Absorption

When the two contacting phases are a gas and liquid, this operation is called absorption. A solute or several solutes are absorbed from the gas into the liquid phase in absorption.

ii. Distillation

In the distillation process, a volatile vapor phase and a liquid phase that vaporizes are involved.

iii. Liquid-liquid extraction

When the two phases are liquids, where a solute or solutes are removed from one liquid phase to another liquid phase, the process is called liquid-liquid extraction.

iv. Leaching

If a fluid is being used to extract a solute from a solid, the process is called leaching. Sometimes this process is also called extraction.

v. Membrane processing

Separation of molecules by the use of membranes is a relatively new separation process and is becoming more important. The relatively thin, solid membrane controls the rate of movement of molecules between two phases.

vi. Crystallization

Solute components soluble in a solution can be removed from a solution by adjusting the conditions, such as temperature or concentration, so that the solubility of one or more of the components is exceeded and they crystallize out a solid phase.

vii. Adsorption

In an adsorption process, one or more components of a liquid or gas stream are adsorbed on the surface or in the pores of a solid adsorbent and a separation are obtained.

viii. Ion exchange

In an ion exchange process, certain ions are removed by an ion-exchange solid. This separation process closely resembles adsorption.

2.4.1 Distillation Process

Distillation is one of the separation methods that used for separating homogeneous mixtures based upon equilibration of liquid and vapor phases. Substances that differ in volatility appear in different proportions in vapor and liquid phases at equilibrium with one another. Thus, vaporizing part of a volatile liquid produces vapor and liquid products that differ in composition. This outcome constitutes a separation among the components in the original liquid. Through appropriate configurations of repeated vapor-liquid contacting, the degree of separation among components differing in volatility can be increased significantly.

The choice of distillation methods gives effects on the percentages of essential oil components (Kiran, Babu, & Kaul, 2005). In simple terms, distillation of aromatic herbs implies vaporizing the oils from the trichomes and plant cell membranes of the herb in presence of high temperature and moisture. The vapor mixture is then cooled to separate out the oil from water. It is the most widely used and cost effective method in use today for producing majority of the essential oils throughout the world.

Distillation process is sometimes coupled with extraction process to isolate certain compound from mixtures. However, in this process, the solvent must be recovered for reuse (usually by distillation), and the combined operation is more complicated and often more expensive than ordinary distillation without extraction (McCabe, Smith & Harriott, 2001).

On the other hand, distillation process is commonly applied to purify a liquid, or to concentrate the alcohol in a fermented liquid. It is also by far the most common method of separation in the petroleum, natural gas, and petrochemical industries. Its many applications in other industries include air fractionation, solvent recovery and recycling, separation of light isotopes such as hydrogen and deuterium, and production of alcoholic beverages, flavors, fatty acids, and food oils.

There are several different types of distillation that have been developed, such as flash distillation, fractional distillation, steam distillation, azeotropic distillation and hydro-distillation.

2.4.1.1 Steam Distillation

Steam distillation is a special type of distillation process for temperature sensitive materials like natural aromatic compounds. It is the oldest and most popular form of essential oil extraction method used by commercial scale producers.

Many organic compounds tend to decompose at high sustained temperatures. Separation by normal distillation would then not be an ideal option, so water or steam is introduced into the distillation apparatus. By adding water or steam, the boiling points of the compounds are depressed, allowing them to evaporate below the temperatures at which the deterioration of the quality of material becomes appreciable. If the substances to be distilled are very sensitive to heat, steam distillation can also be combined with vacuum distillation. After distillation, the vapours are condensed as usual, usually yielding a two-phase system of water and the organic compounds, allowing for simple separation.

In the production of natural essential oil, the desired plant material is placed onto a still. A still is a specialized piece of equipment that is used in the distillation process. It consists of a vessel into which heat is added and a device that is used for cooling.

The plant is first placed into the vessel. Next steam is added and passed through the plant. The heat from the steam helps to open the pockets of the plant that contain the plant's aromatic molecules or oils. The molecules of these volatile oils then escape from the plant material and evaporate into the steam.

The vapors carrying these molecules travel within a closed system towards

the cooling device. Cold water is normally used to cool the vapors. As they cool, they condense and transform into a liquid state. The liquid is collected in a container and same with any type of oil-water mixture, it separates. The oils float towards the top while the water settles below. From there, simple method is used to extract the oils that have been separated. These are the highly condensed, aromatic oils used in aromatherapy.

The water is not discarded, however. The water, which also contains the plant's aroma along with the other parts of the plant that are water soluble, are the hydrosols - a milder form of the essential oils. Rose hydrosol, for example, is commonly used for its mild antiseptic and soothing properties, as well as its pleasing floral aroma.

The steam is created at a pressure higher than that of the atmosphere. Therefore, its boiling point is above 100 degrees Celsius and this results in an extraction process that is safe and fast. If the temperature is allowed to become too hot, however, the plant material as well as its essential oils can easily become damaged.

2.4.1.2 Hydro-distillation

Although several methods of extracting essential oil have been developed, most essential oils in market are still produced by hydro-distillation (Reverchon et al., 1992). Hydro-distillation can extract up to 97% of the essential oil component from plant while the remaining 3% is extracted by other method. (Masango P., 2001)

The hydro-distillation or water distillation process is similar to steam distillation. The only difference is that instead of introducing the heat from the bottom and up through the still, as happens in steam distillation, the heat passes into the still from the top. It is cooled from below, which makes collection of the essential oils easier.

The hydro-distillation process involves placing the desired plant material in a still and then submerging it in water. The water is then brought to a boil. The heat helps open the pockets containing the plant's aromatic molecules so they can be extracted. When the condensed material cools down, the water and essential oil is separated and oil decanted, to be used as essential oil.

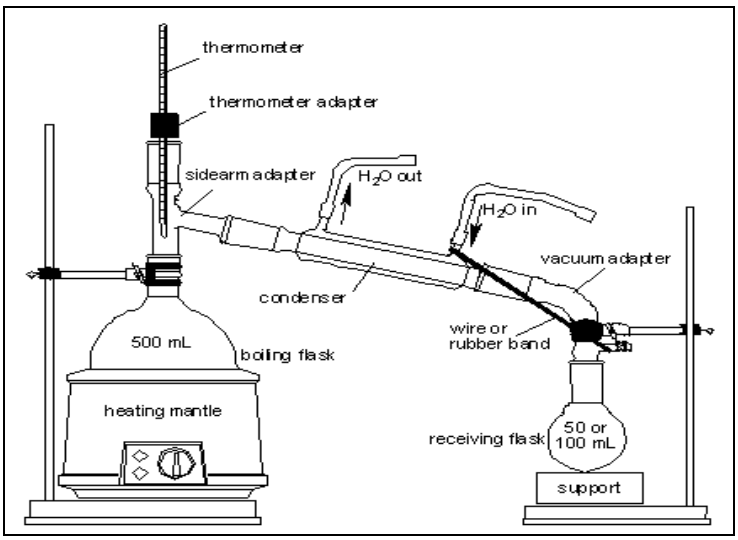

Figure 2.4 Hydro-distillation Apparatus (University of Wisconsin, 2002)

The water in this case provides protection for the plant because it acts as a barrier to prevent it from overheating. Less pressure is used as well as a lower temperature than that which is used in the steam distillation method. Water distillation can be done at reduced pressure (under vacuum) to reduce the temperature to less than 100 degrees Celsius, which is beneficial in protecting the botanical material, as well as the essential oils. This works well with plants that cannot tolerate high heat. In fact, hydro-distillation results in a higher yield of essential oils because less steam and consequently less processing time are involved.

In a water/steam combination distillation method, plant material is submerged into heated water and steam is forced through the water, opening the pockets containing the aroma molecules. When cooled, the essential oils condense and are collected as described above.

2.5 Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample.

The combination of gas chromatography and mass spectrometry allows a much finer degree of substance identification than either unit used separately. In fact, the mass spectrometry process normally requires a very pure sample while gas chromatography is easily confused by different molecular types that both possess similar retention time and similar pattern of mass spectrum. Combining the two processes makes it extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer.

Furthermore, the complementary relationship enables the retention times and mass spectral data to be accessed easily. GC-MS display a high degree of accuracy in detecting the existence of specific substance in a mixture. Many scientists consider GC-MS analysis as a tool for conclusive proof of identity.

2.5.1 History

The use of a mass spectrometer as the detector in gas chromatography was developed during the 1960s. These sensitive devices were bulky, fragile, and originally limited to laboratory settings. The development of affordable and miniaturized computers has simplified the operation of this instrument, as well as allowed a greater efficiency for sample analysis.

The invention of gas chromatography- mass spectrometry (GC-MS) allowed, for the first time, the analysis of mixtures without laborious separation by hand. The development of GC-MS was the trigger for the development of modern mass spectrometry.

In year 1996, the top-of-the-line high-speed GC-MS units completed analysis of fire accelerants in less than 90 seconds, whereas first-generation GC-MS would have required at least 16 minutes. This has led to their widespread adoption in a number of fields.

2.5.2 Instrumentation

The GC-MS is composed of two major building blocks which are the gas chromatograph and the mass spectrometer. The equipments are arranged where the effluent from GC instrument is the feed to the MS instrument

2.5.2.1 Gas Chromatograph

A chromatograph consists of a flowing mobile phase, an injection port, a separation column containing the stationary phase, a detector, and a data recording system.

Mobile phases of gas chromatography are carrier gases that must be chemically inert, such as nitrogen, helium, argon, and carbon dioxide. The flow rate of the gas influences how fast a compound will travel through the column; the faster the flow rate, the lower the retention time.

The injection port consists of a rubber septum through which a syringe needle is inserted into a flash vaporiser port to inject the sample. The sample has to be vaporized prior to GC analysis, thus the sample injection port is usually maintained at a temperature of 50°C higher than the boiling point of the least volatile component in the sample mixture.

There are two general designs of column for gas chromatography, which are packed columns and capillary columns, where the latter provides much higher

separation efficiency. The columns are contained in an oven, the temperature of which is precisely controlled electronically. The optimum temperature for column is dependant upon the boiling point of the sample. Following the rule of thumb, a temperature slightly above the average boiling point of sample will result in an elution time of 2 - 30 minutes. Minimal temperatures give good resolution, but increase elution times.

Table 2.1 Types of detectors (Sheffield Hallam University, 2006)

Detector	Type	Support gases	Selectivity	Detectability	Dynamic range
Flame ionization (FID)	Mass flow	Hydrogen and air	Most organic compounds	100 pg	10^7
Thermal conductivity (TCD)	Concentration	Reference	Universal	1 ng	10^7
Electron capture (ECD)	Concentration	Make-up	Halides, nitrates, nitriles, peroxide, anhydrides, organometallics	50 fg	10^5
Nitrogen-phosphorus	Mass flow	Hydrogen and air	Nitrogen, phosphorus	10 pg	10^6
Flame photometric (FPD)	Mass flow	Hydrogen and air possibly oxygen	Sulphur, phosphorus, tin, boron, arsenic, germanium, selenium, chromium	100 pg	10^3
Photo-ionization (PID)	Concentration	Make-up	Aliphatics, aromatics, ketones, esters, aldehydes, amines, heterocyclics, organosulphurs, some organometallics	2 pg	10^7
Hall electrolytic conductivity	Mass flow	Hydrogen, oxygen	Halide, nitrogen, nitrosamine, sulphur		

Various types of detectors are used in gas chromatography, where each of them tends to give different types of selectivity. As shown by Table 2.1, detectors can be grouped into concentration dependant detectors and mass flow dependant detectors. The signal from a concentration dependant detector is related to the concentration of solute in the detector, and does not usually destroy the sample. Dilution with make-up gas will lower the detectors response. On the contrary, mass flow dependant detectors usually destroy the sample, and the signal is related to the rate of solute molecules entering the detector. The response of a mass flow dependant detector is unaffected by make-up gas.

2.5.2.2 Mass Spectrometer

All mass spectrometers consist of three basic parts: an ion source, a mass analyzer, and a detector system.

The ion source ionizes the analyte, the ions resulted are then transported by magnetic or electric fields to the mass analyzer. Techniques for ionization play the role to determining what types of samples can be analyzed by mass spectrometry. Electron ionization and chemical ionization are used for gases and vapors.

Mass analyzers separate the ions according to their mass-to-charge ratio. All mass spectrometers are based on dynamics of charged particles in electric and magnetic fields in vacuum. There are many types of mass analyzers, using either static or dynamic fields, and magnetic or electric fields, but all operate according to this same principle.

The detector records the charge induced or current produced when an ion passes by or hits a surface. In a scanning instrument, the signal produced in the detector versus where the instrument is in the scan will produce a mass spectrum, a record of ions as a function of m/q. Typically, some type of electron multiplier is used, other detectors including Faraday cups and ion-to-photon detectors. As the

number of ions leaving the mass analyzer at a particular instant is typically quite small, significant amplification is often necessary to generate a signal.

2.5.3 Analysis

The gas chromatograph separates different molecules in a mixture by utilizing their different partitioning behaviour between the mobile gas phase and the stationary phase. Each type of molecule takes different retention time to come out of the gas chromatograph, and this allows the mass spectrometer downstream to evaluate and identify the molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio. Each molecule has a specific fragment spectrum which allows for its detection.

The primary goal of chemical analysis is to determine the identity of certain substance. Two types of analysis are possible, comparative and original. Comparative analysis is done by comparing the relative concentrations among the atomic masses in the generated spectrum to a spectrum library. The analysis is best performed by a computer as a myriad of visual distortions might take place due to variations in scale. In addition, computers can correlate more data simultaneously, leading to a higher efficiency of analysis.

Another analysis measures the peaks in relation to one another, with the tallest peak receiving 100% of the value, and the others receiving proportionate values, with all values above 3% being accounted for. The parent peak represents the total mass of the unknown compound which can then be used to fit to a chemical formula assumed to be present in the compound. Once a chemical formula has been matched to the spectrum, the molecular structure and bonding can be further identified, provided it must be consistent with the characteristics interpreted by GC/MS. The fitting is normally done automatically by programmes which come with the machine, given a list of possible elements in the sample.

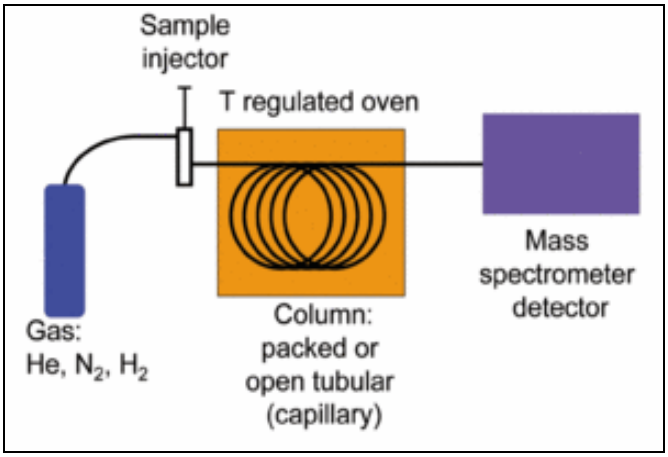

Figure 2.5 GC-MS Schematic Diagram (Sheffield Hallam University, 2006)

2.5.4 Applications

The GC-MS has been widely heralded as a "gold standard" for substance identification as it is used to perform a specific test. A specific test positively identifies the actual presence of a particular substance in sample, but not just merely indicates that the substance falls into which category.

The cost of GC-MS equipment has fallen significantly, however its reliability has increased at the same time. This has contributed to the wide application of GC-MS in environmental studies where cost is always a major consideration. Nowadays, GC-MS is becoming the tool of choice for tracking organic pollutants in the environment. It displays high sensitivity and efficiency in identifying most organic environmental samples such as pesticides.

The contribution of GC-MS in law enforcement is proven vividly as it is widely used for detection of illegal narcotic and explosive materials. The technology also enables the analysis of particles from human body and even fire debris in order to help link a criminal to a crime.

CHAPTER 3

METHODOLOGY

3.1 Overview

The isolation of essential oil from plant body must be conducted in the right path to optimize the yield and quality of essential oils. There are three main stages to fulfill this study, which are pre-treatment of sample, extraction of essential oil and the analysis of essential oil.

3.1.1 Pre-treatment of Sample

Pre-treatment of plant before the extraction of essential oil is important to minimize the loss of volatile oil components (Ebewele and Jimoh, 1981).

The sample for current research, fresh rhizomes of *Etlingera* sp.4 will be collected by a botanist and my supervisor near Bentong. A voucher specimen is prepared, labelled as *Etlingera* sp. 4 and deposited at Universiti Malaya herbarium. The harvest of ginger for dried spices and essential oil is best at full maturity, when the leaves turn yellow. (Plotto, 2004)

After harvest, the washing and drying processes should be carried out as quickly as possible to gain optimum yield and to prevent the growth of bacteria or mould. In addition, unpeeled or coated rhizomes are preferably used for essential oil

or oleoresin extraction to improve the yield. The roots and leaves are removed and the rhizomes are washed thoroughly to eliminate any impurities such as debris and shoots.

Next, the sample is grinded into smaller pieces to increase the drying rate as well as the efficiency of essential oil extraction. Samples with larger surface area will result in higher extraction rate because the contact between solvents and samples is enhanced.

The ginger rhizomes are further dried in oven at a temperature of 40°C for about 8 to12 hours, until a constant weight is attained.

Figure 3.1 Dried ginger rhizomes

3.1.2 Extraction of Essential Oil

Prior to the extraction process, the sample is soaked in water in order to break down the parenchymatous cells and oil glands. This will help in the extraction process and expedite the process. Some of the parenchymatous cells and oil glands are also believed to break down during the washing and milling process but it is negligible.

The samples will be hydro-distilled using an all glass Clevenger-type apparatus, according to the method recommended by the European Pharmacopoeia

(European Pharmacopoeia, 1983) to produce essential oil. The Clevenger apparatus based on distillation is the best method to determine the essential oil content of plant. (Ferhat et al., 2005) In addition, hydro-distillation is applied in this study as it is beneficial in protecting the botanical material, as well as the essential oils.

The mixture of 50 gram of mashed samples and 500 mL of distilled water is put into the flask of distillation unit. Next, the heating mantle and water supply is switched on. The samples are subjected to hydro-distillation process at 80 ^{o}C for 6 hours.

The apparatus is wrapped with aluminum foil to minimize heat loss. A couple of boiling chips were put into the flask to ensure bumping do not occur during distillation.

Figure 3.2 Clevenger-type hydro-distillation apparatus

During the extraction process, the essential oil will vaporize with the steam and condense as it passes through a condenser. The condensate which was a mixture of water and essential oil was then separated using a separating funnel. Anhydrous sodium sulphate will be added to the essential oil to eliminate the remaining water. It is then filtered by gravity into a pre-weighed amber vial and the weight of the

essential oil is then determined.

2ml of sample solution was made which it consisted of 1% of essential oil with hexane as the solvent. The remaining essential oil was kept in an amber vial and stored in temperature condition of -4°C. To determine the effects of light to the quality of the essential oil, 1ml of sample solution was poured into a clear and colourless vial which is left on a laboratory shelf. Whereas, to identify the effects of heat to the quality of the essential oil, another 1ml of sample solution was kept in an amber vial which was placed near to windows with direct sunlight.

Figure 3.3 Samples exposed to light

Figure 3.4 Samples exposed to heat

3.1.3 Analysis of Essential Oil

The essential oils were analyzed using an Agilent 5975C Series GC/MSD equipped with a HP5-MS capillary fused silica column (30m, 0.320mm I.D.; 0.25µm film thickness). The oven temperature program was initiated at 70°C then raised at a rate of 10°C/min to 300°C, held for 8 min. Other operating conditions were as follows: carrier gas, He (99.999%); with a flow rate of 1 ml/min; injector temperature, 250°C; splitless; detector temperature, 310°C.

The total ion chromatogram obtained was auto-integrated by ChemStation and the constituents were identified by comparison with published mass spectra database or with authentic compounds and confirmed by comparison of their retention indices (Wiley, 1990).

Figure 3.5 Gas chromatography-mass spectrometry (GC-MS)

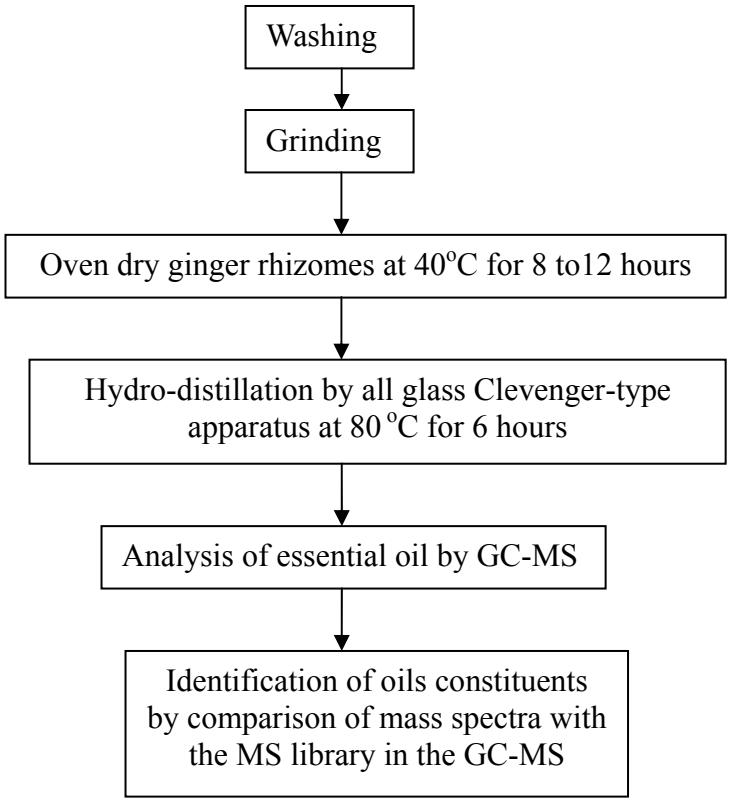

Figure 3.6 Flow diagram of ginger essential oil extraction process

CHAPTER 4

RESULT AND DISCUSSION

4.1 Yield of Essential Oil

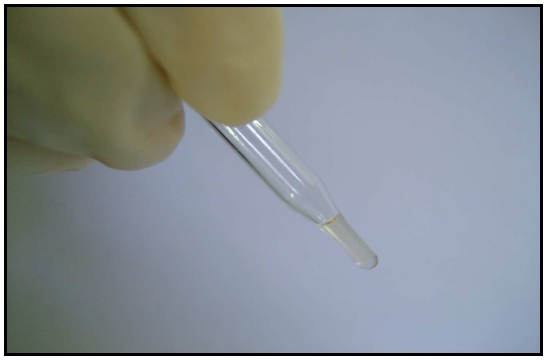

Figure 4.1 The essential oil extracted by hydro-distillation

The hydro-distillation process has proven its capability in extracting the essential oil from the rhizomes of *Etlingera* sp.4. The percentage of yield is computed based on the constant dry weight of specimen. For 120g of dried ginger rhizome, 0.05g of essential oil is extracted. In other words, the percentage of yield is around 0.04167%.

Table 4.1 Yield of Essential Oil

Weight of dried specimen (g)	120
Weight of essential oil (g)	0.05
Percentage of yield (%)	0.04167

4.2 Physical Characteristics of Essential Oil

Table 4.2 Physical Characteristics of Essential Oil

Types of Essential Oil	Appearance	Odour
(i) Freshly distilled	Pale yellow liquid	Pungent and strong
(ii) Stored in optimum condition	Pale yellow liquid	Pungent and strong
(iii) Exposed to light, heat and long-standing conditions	The original colour gradually grow faint	Pungency decreases

The physical appearance of essential oil under various conditions was observed, with the freshly distilled sample as reference. The freshly distilled essential oil was pale yellow in colour, with a pungent and fresh spicy-woody smell.

The optimum storage condition implies that the essential oil was kept in amber vial and stored at a temperature of -4°C. Observation of the essential oil revealed that the physical characteristics of fresh sample were maintained well. Thus, it is advisable that natural essential oil should be kept away from light and heat to preserve their freshness and potency.

Upon exposure to light and heat under long-standing condition, the original colour of essential oil gradually grows faint. In addition, the significant smell and pungency of essential oil also decreased. Therefore, it is proven that extreme storage conditions can leave detrimental effects on the quality of essential oils.

4.3 GC-MS Analysis of Essential Oil

The quality of essential oil isolated from sample is determined by gas chromatography-mass spectrometry (GC-MS). The freshly distilled essential oil was

analyzed by GC-MS, and the result was obtained as the reference (Figure 4.2). On the other hand, both of the samples that were exposed continuously to heat and light for 3 weeks were analyzed by GC-MS at an interval of one week. The results were compared to the reference to monitor any changes in the composition of the essential oils upon exposure to extreme storage conditions.

4.3.1 Freshly Distilled Essential Oil

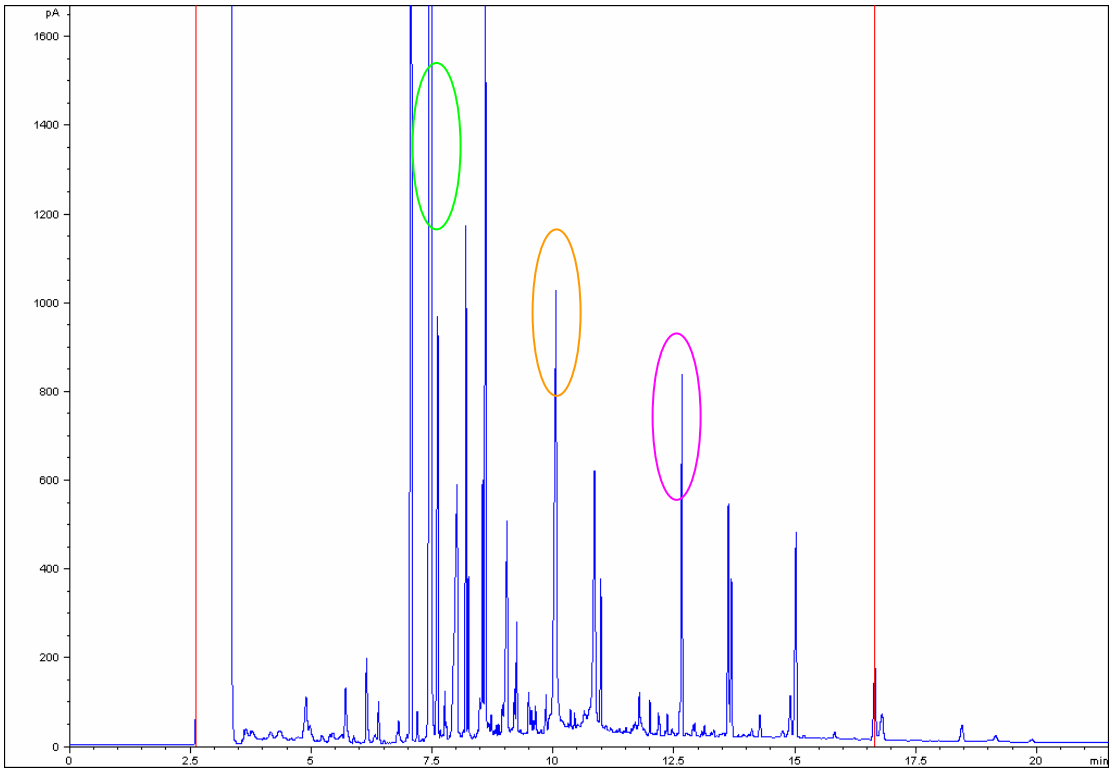

Figure 4.2 Freshly Distilled Sample

The GC-MS analysis of freshly distilled essential oil would provide the reference data for further comparison of oil constituents. A total of 28 major peaks were computed from the chromatogram. However, only 3 peaks would be analyzed and compared in detail in the present study.

The highest peak was determined at a retention time of 7.477 minutes, with a height of 4665.54834 pA and percentage area of 30.04%. Comparison with published

mass spectra database had identified this major compound as 1-dodecanol (CAS No.112-53-8). 1-Dodecanol also known by its trivial name dodecyl alcohol and lauryl alcohol is clear oily liquid at room temperature. Its molecular structure is shown in Figure 4.3. It is a fatty alcohol with characteristic odour which is unpleasant at high concentrations but delicate and floral on dilution. 1-Dodecanol is commonly used to make surfactants, lubricating oils, pharmaceuticals and as an emollient in cosmetics.

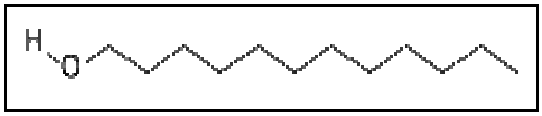

Figure 4.3 Molecular Structure of 1-Dodecanol

Next, the peak at a retention time of 10.054 minutes, with a height of 1012.36346 pA and percentage area of 7.87% was selected. This compound was determined as palmitic acid, or hexadecanoic acid in IUPAC nomenclature (CAS No.57-10-3). It is one of the most common fatty acids found in animals and plants. Palmitic acid presents in the form of colourless or white crystals. It is widely used in the manufacture of pharmaceuticals, soaps, cosmetics, and food packaging

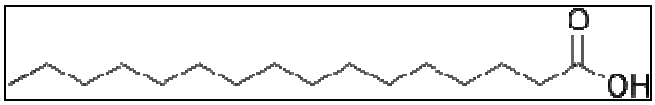

Figure 4.4 Molecular Structure of Hexadecanoic acid

The last peak was chosen at a retention time of 12.658 minutes, with a height of 828.15918 pA and percentage area of 3.16%. This compound was identified as cyclododecane (CAS No. 294-62-2), normally found in the form of white crystalline powder. Cyclododecane is mainly used as intermediate for the production of polyamides, polyesters, synthetic lubricating oils and nylon.

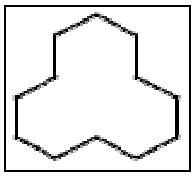

Figure 4.4 Molecular Structure of Cyclododecane

4.3.2 Light Test

To determine the effects of light to the quality of the essential oil, 1ml of sample solution was poured into a clear and colourless vial which is left on a laboratory shelf. The storage condition is determined as followed:

(i) Temperature = 31-33°C
(ii) Relative humidity = 42-45%

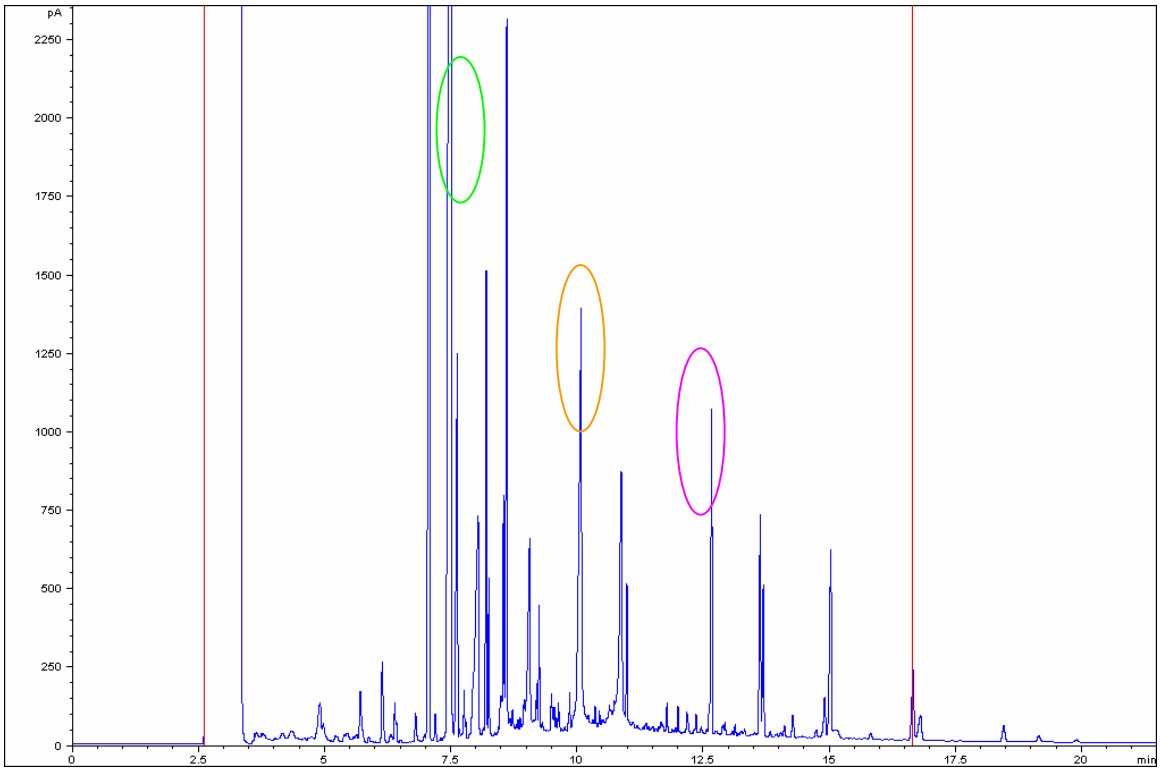

Figure 4.6 Sample of light test (1st week)

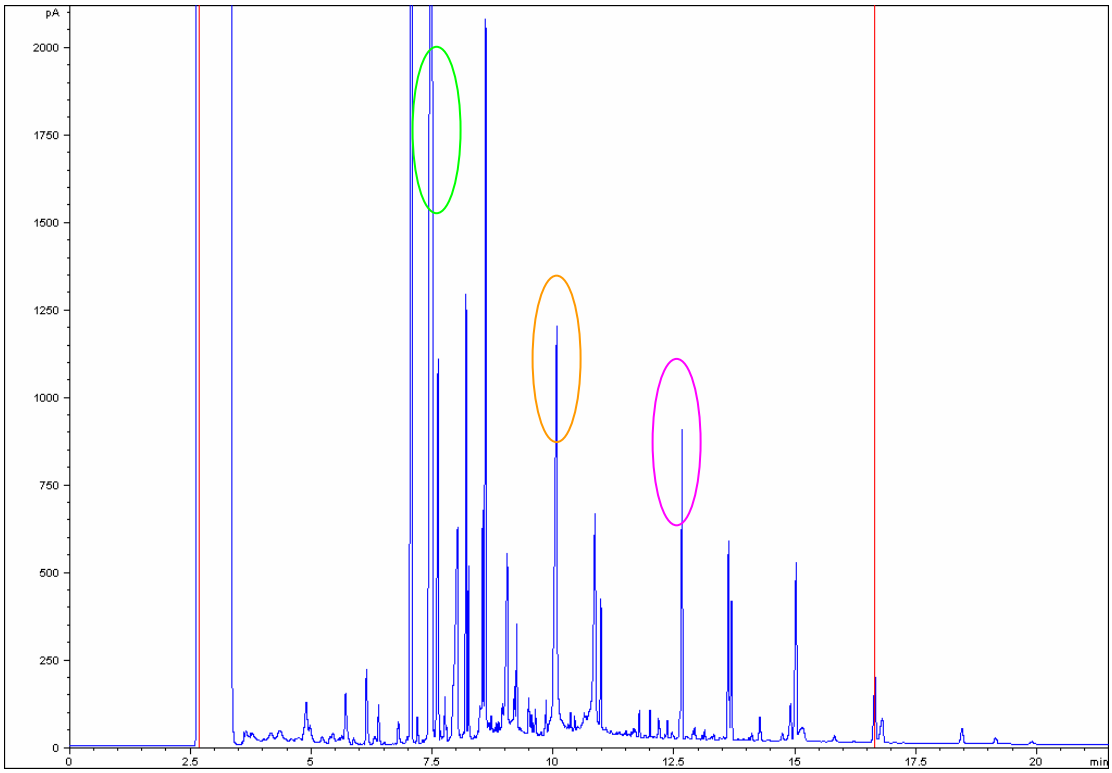

Figure 4.7 Sample of light test (2nd week)

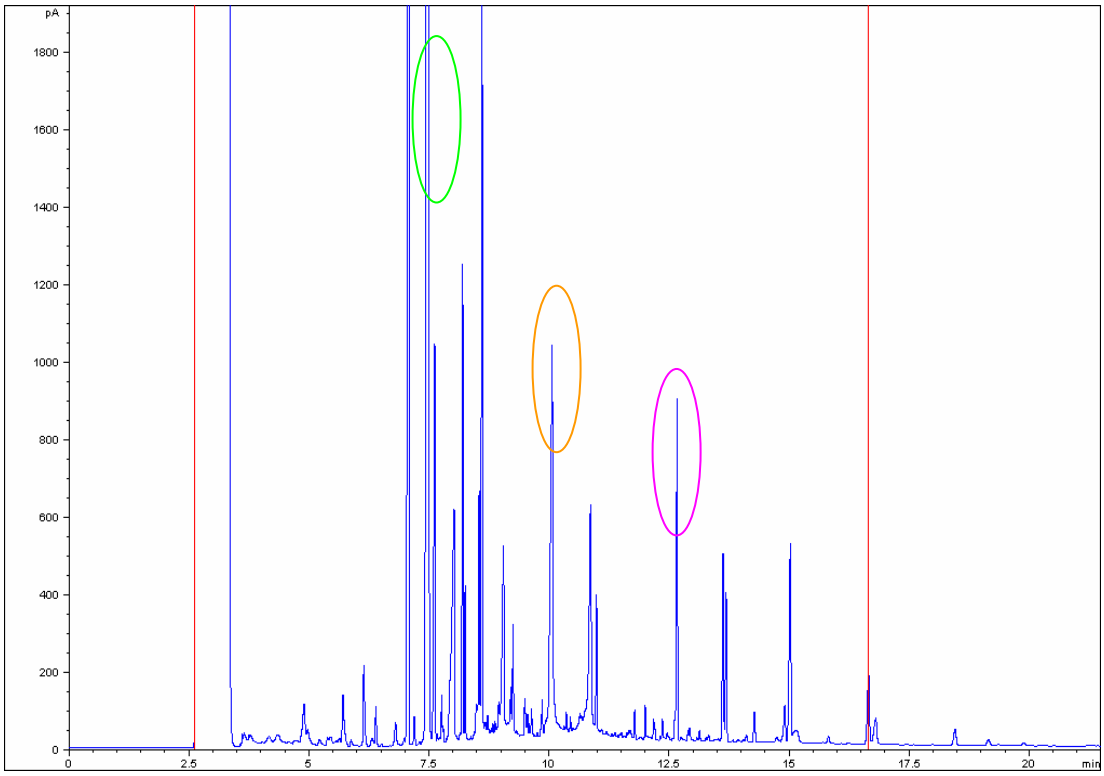

Figure 4.8 Sample of light test (3rd week)

The changes in the compositions of 1-dodecanol, hexadecanoic acid and cyclododecane upon exposure to light were monitored continuously for 3 weeks. The characteristics of each peak were summarized as followed:

Table 4.3 Result Summary of Light Test

Compound	Week No.	Width (min)	Area (pA*s)	Height (pA)	Area (%)
1-Dodecanol	1st week	0.0468	19907.2	5756.3584	30.10
	2nd week	0.0431	17446.3	5414.33887	30.03
	3rd week	0.0428	15732.4	4981.02344	30.73
Hexadecanoic acid	1st week	0.0557	6041.21045	1376.09619	9.13
	2nd week	0.0566	5321.2915	1192.24463	9.16
	3rd week	0.0573	4626.09131	1032.70862	9.04
Cyclododecane	1st week	0.03	2085.4978	1057.16553	3.15
	2nd week	0.0286	1658.58911	893.94965	2.85
	3rd week	0.0286	1656.20325	895.35291	3.24

Quantitative analysis of chromatogram can be obtained from peak height or peak area measurements. The actual peak area was recalculated by omitting the solvent peak. The area of the peak is proportional to the number of molecules generating the signal.

According to the result summary above, a general trend of decrease was observed for the peak area as well as the peak height of the compounds. Thus, it was evident that the essential oil of *Etlingera* sp.4 was suffered from loss of certain volatile components upon exposure to light. The components might be denaturalized, disappeared or changed into another form of structure.

4.3.3 Heat Test

To identify the effects of heat to the quality of the essential oil, 1ml of sample solution was kept in an amber vial which was placed near to windows with direct sunlight. The storage condition is determined as followed:

(i) Temperature =33-37°C

(ii) Relative humidity = 43-49%

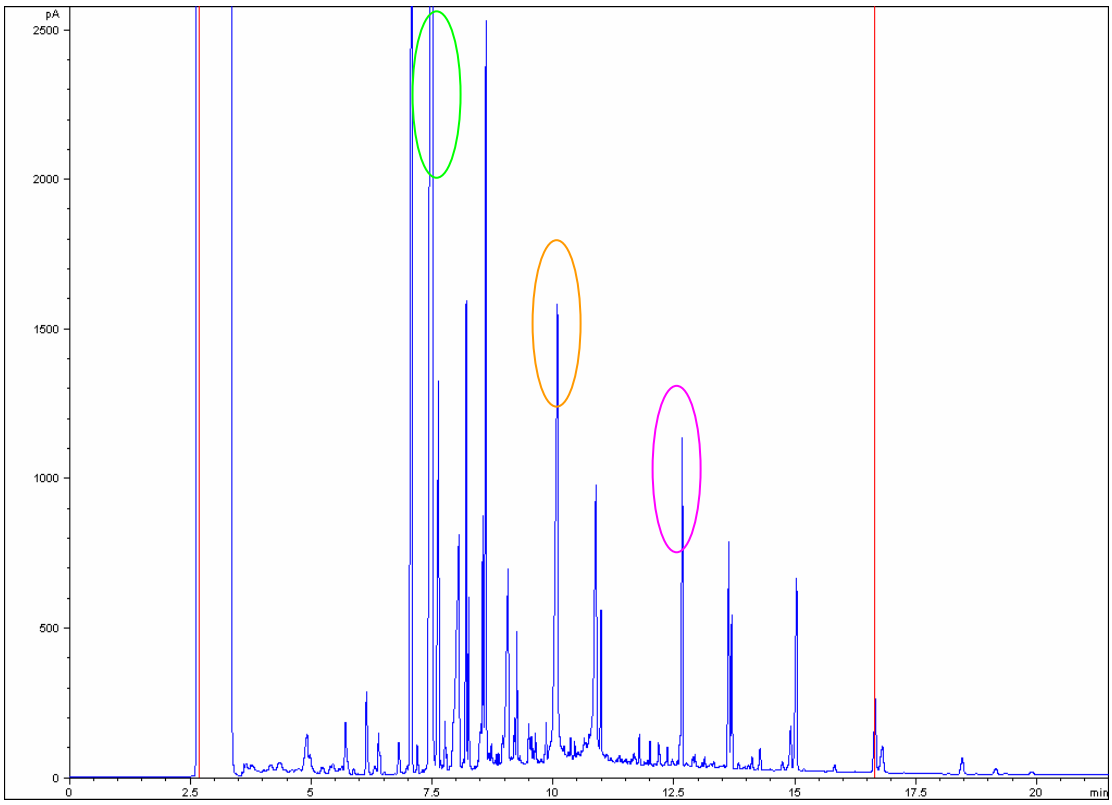

Figure 4.9 Sample of heat test (1st week)

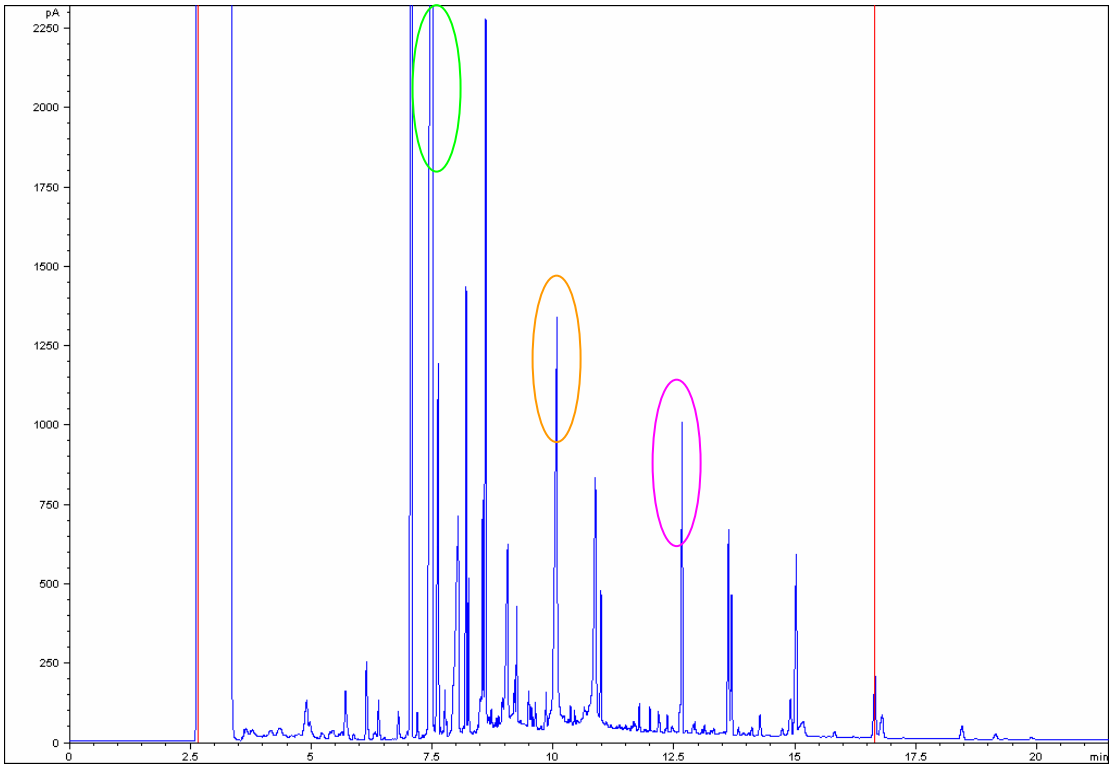

Figure 4.10 Sample of heat test (2nd week)

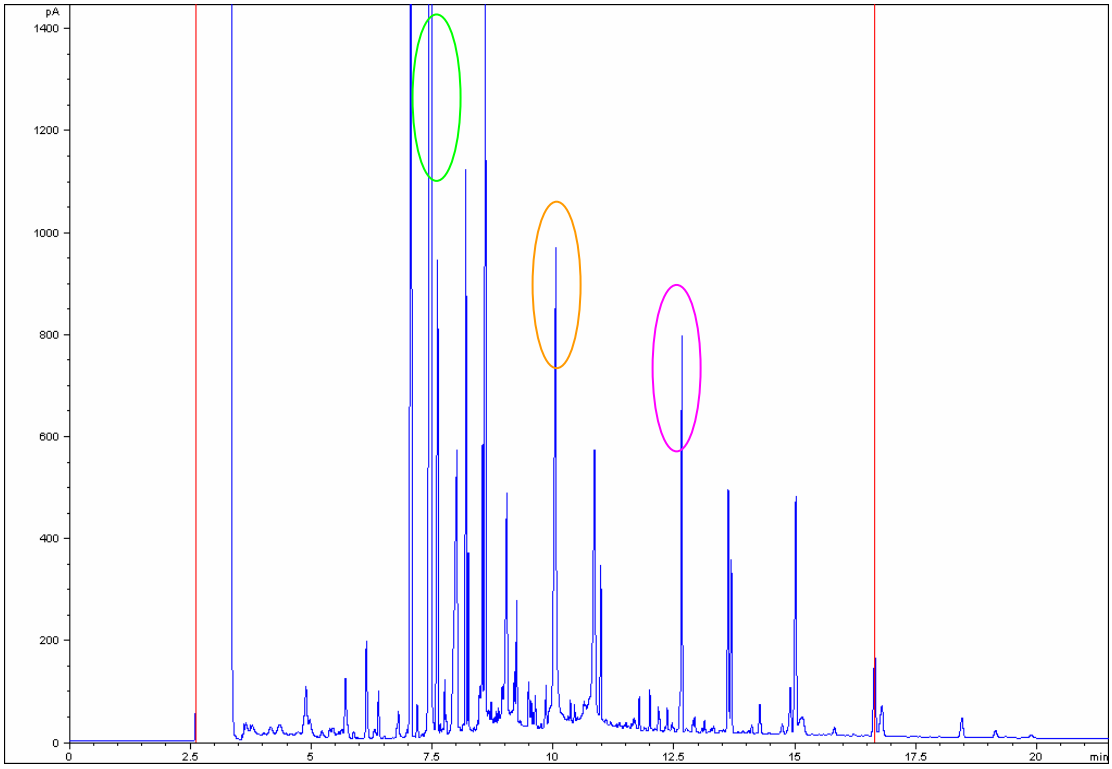

Figure 4.11 Sample of heat test (3rd week)

The changes in the compositions of 1-dodecanol, hexadecanoic acid and cyclododecane upon exposure to prolonged heat were monitored continuously for 3 weeks. The characteristics of each peak were summarized as followed:

Table 4.4 Result Summary of Heat Test

Compound	Week No.	Width (min)	Area (pA*s)	Height (pA)	Area (%)
1-Dodecanol	1st week	0.0480	22273.4	6113.40918	30.12
	2nd week	0.0457	19042.4	5597.22559	30.69
	3rd week	0.0439	13615.5	4611.48633	32.59
Hexadecanoic acid	1st week	0.0588	7055.77979	1572.87366	9.54
	2nd week	0.0559	5772.05615	1322.97229	9.30
	3rd week	0.0420	2886.39014	910.29456	6.91
Cyclododecane	1st week	0.0314	2299.4187	1121.70325	3.11
	2nd week	0.0298	1952.4104	999.35284	3.15
	3rd week	0.0275	1365.03516	776.83099	3.27

Quantitative analysis of chromatogram was done on peak area and peak height measurements. The actual peak area was recalculated by omitting the solvent peak. The area of the peak is proportional to the number of molecules generating the signal.

The result summary above had displayed a general trend of decrease for the peak area as well as the peak height of the enlisted compounds. Apparently, the essential oil of *Etlingera* sp.4 was suffered from loss of certain volatile components upon exposure to heat. The components might be denaturalized, disappeared or changed into another form of structure.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The hydro-distillation process displayed high efficiency in isolating essential oil from the rhizomes of *Etlingera* sp.4, giving a yield of 0.04167%. This method of obtaining essential oil is economical and environmental friendly as water is used as the major medium.

Gas chromatography-mass spectrometry (GC-MS) provided an accurate method of substance identification. By utilizing the differences in retention time, the constituents of essential oils were determined. In addition, the changes in the compositions can be monitored by comparing the peak areas and peak heights. The area of peak is proportional to the amount of compound present.

The storage conditions play an important role in determining the quality and compositions of natural essential oils. Current study had revealed that exposure to heat and light tends to leave detrimental effects on the quality of essential oil. Long-standing essential oils under inappropriate storage conditions tend to lose their characteristic colour and odours gradually.

Based on the quantitative analysis of the GC-MS results, it was discovered that extreme storage conditions will result in the loss of certain volatile oil components. Volatile oil components are usually responsible for the distinctive properties and qualities of essential oils. In other words, poor storage conditions tend to decrease the quality and commercial value of natural essential oil.

In fact, studies shown that terpene hydrocarbons which are the main constituents of most aromatic essential oils have a tendency to get oxidized under the influence of light and air. Thus, to preserve the freshness and potency of essential oil, optimum storage condition must be applied. It is highly recommended that natural essential oils should be kept in amber vials and stored at a temperature of -4°C.

5.2 Recommendations

The present study is important in providing useful information related to the storage and preservation of natural ginger essential oils. As essential oil content defines the taste and smell of the product and colour is the most important parameter for visual appraisal by consumers, colour and content of essential oil have been in focus for quality research. Therefore, further efforts shall be developed in the relevant fields to maintain and further increase the quality of essential oils.

The effects of temperature variations, light and air exposure as well as other factors to the quality of essential oils shall be investigated in detail. Preservation of some crucial components of essential oils is highly important to determine the value and quality of that essential oil itself.

Some problems were faced during the method development stage of GC-MS analysis. As the constituents of the essential oil of *Etlingera* sp.4 is too complex, longer time was needed to get an ideal condition for analysis. Thus, it is recommended to replace the GC column of 30m length with a longer column which provides higher resolving power.

The essential oil from the rhizomes of *Etlingera* sp.4 is currently still in undeveloped stage. Thus, more researches shall be continued in future to discover the benefits and values of the plant. Essential oils from the other parts of plant, such as leaves, petals and stems can be extracted for further analysis too.

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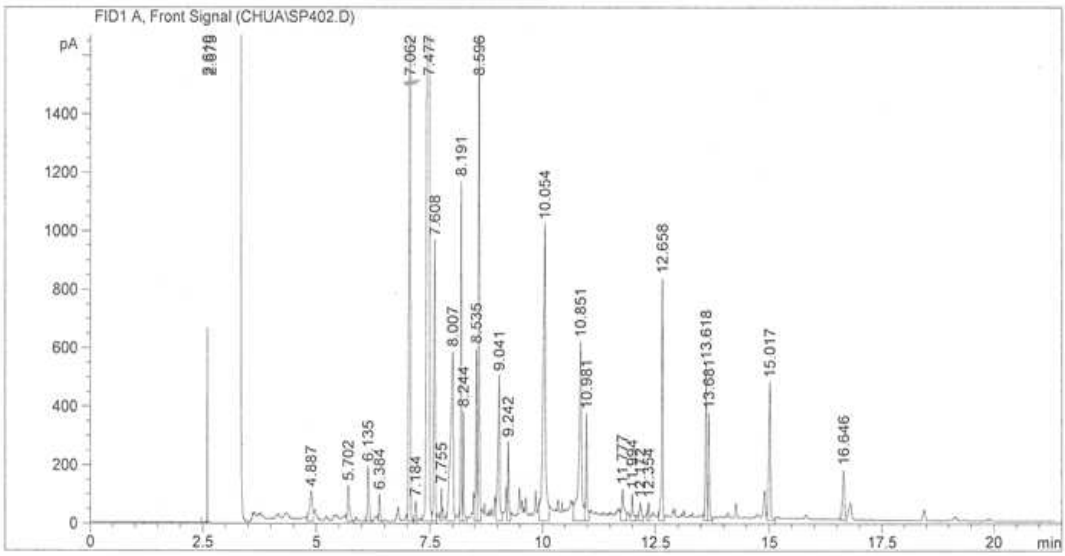
LIST OF APPENDICES

APPENDIX A (Analysis of Freshly Distilled Samples)

Appendix A1: Results of Analysis

Data File C:\CHEM32\1\DATA\CHUA\SP402.D

```
==================================================================
Acq. Operator    : husna
Acq. Instrument  : Instrument 1                    Location : Vial 101
Injection Date   : 1/11/2008 8:50:00 AM
                                                 Inj Volume : External
Acq. Method      : C:\CHEM32\1\METHODS\ETLINGERA.M
Last changed     : 1/11/2008 8:43:27 AM by husna
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\METHODS\AEGYPTI.M
Last changed     : 1/11/2008 2:27:20 PM by husna
                   (modified after loading)
Method Info      : Aedes aegypti pheromone
```




```
==================================================================
                        Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A, Front Signal
```

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	2.610	BV S	0.0343	1.51615e6	5.65460e5	14.71311
2	2.675	VB S	0.2614	8.74090e6	5.57162e5	84.82409
3	4.887	VV T	0.0627	464.73465	104.93169	0.00451
4	5.702	VV T	0.0441	371.18958	123.29853	0.00360
5	6.135	VV T	0.0329	422.73419	190.05682	0.00410
6	6.384	VV T	0.0274	168.85049	94.06725	0.00164
7	7.062	VV T	0.0295	6037.12402	3064.73462	0.05859
8	7.184	VV T	0.0286	130.46727	68.82692	0.00127

Data File C:\CHEM32\1\DATA\CHUA\SP402.D

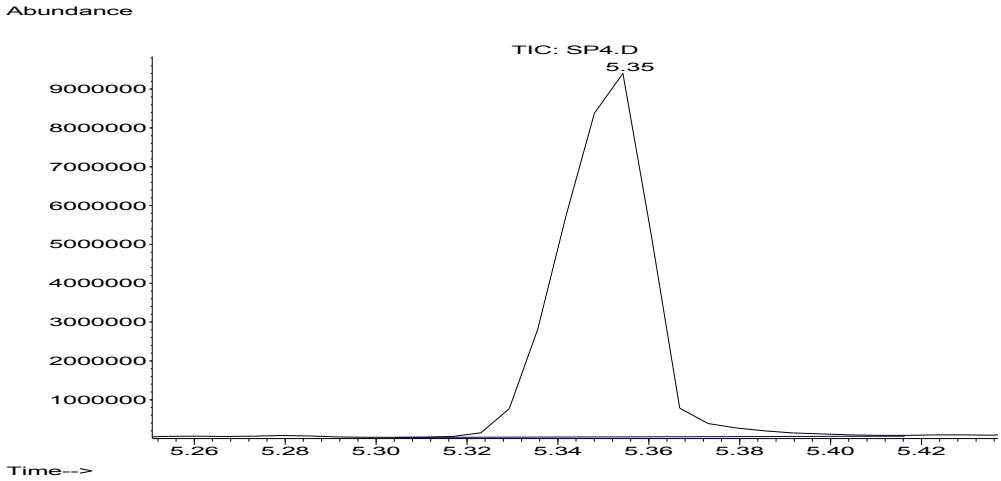
	Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
(a)	9	7.477	VV T	0.0463	1.43248e4	4665.54834	0.13901
	10	7.608	VV T	0.0301	1984.54700	959.84442	0.01926
	11	7.755	VV T	0.0224	170.36433	114.50321	0.00165
	12	8.007	VV T	0.0566	2466.98853	580.70557	0.02394
	13	8.191	VV T	0.0238	1723.53552	1163.50916	0.01673
	14	8.244	VV T	0.0223	538.46130	373.60422	0.00523
	15	8.535	VV T	0.0247	974.14246	591.13171	0.00945
	16	8.596	VV T	0.0251	2953.85303	1803.16931	0.02867
	17	9.041	VV T	0.0427	1479.25256	497.11481	0.01436
	18	9.242	VV T	0.0272	494.56836	271.78476	0.00480
(b)	19	10.054	VV T	0.0477	3751.83472	1012.36346	0.03641
	20	10.851	VV T	0.0579	2533.94043	611.27344	0.02459
	21	10.981	VV T	0.0265	676.92542	366.79834	0.00657
	22	11.777	VV T	0.0467	386.86591	109.46995	0.00375
	23	11.994	VV T	0.0338	225.21906	92.87114	0.00219
	24	12.172	VV T	0.0415	194.80106	63.17365	0.00189
	25	12.354	VV T	0.0291	127.10221	62.78181	0.00123
(c)	26	12.658	VV T	0.0272	1508.09778	828.15918	0.01463
	27	13.618	VV T	0.0308	1066.84534	534.20764	0.01035
	28	13.681	VV T	0.0310	735.31323	365.52768	0.00714
	29	15.017	VV T	0.0407	1238.73315	470.42346	0.01202
	30	16.646	VV T	0.0468	539.43274	170.86760	0.00523
	Totals :				1.03047e7	1.14198e6	

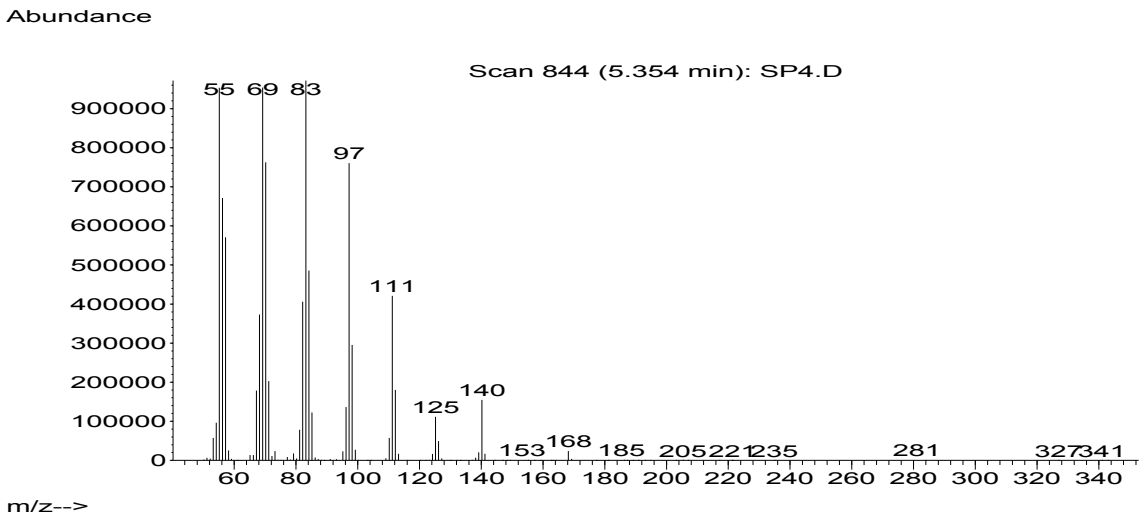
*** End of Report ***

Appendix A2: Identification of Selected Compounds

Scan 844 (5.354 min): SP4.D

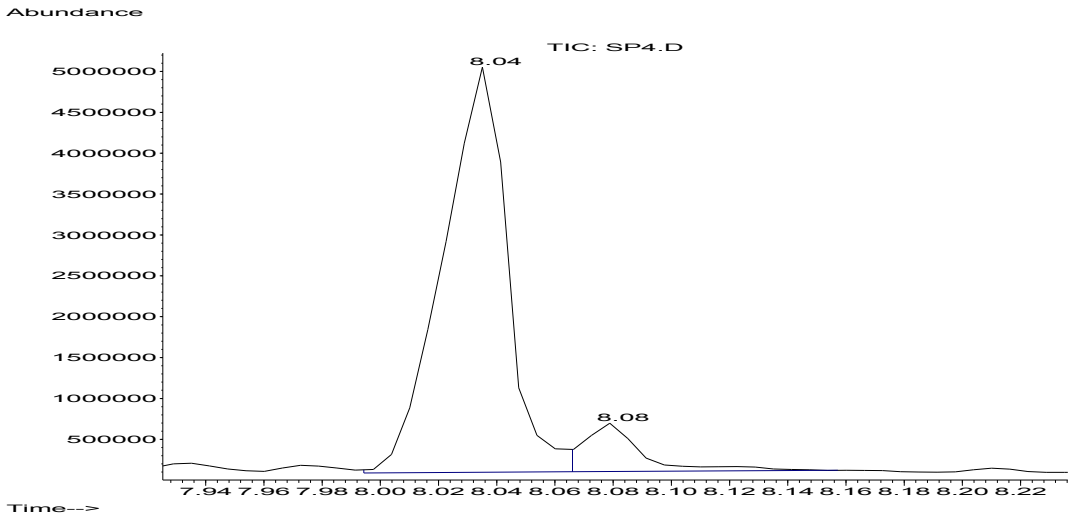
1 1-Dodecanol (CAS) $$ n-Dodecanol $$ CO 12 $$ S 1298 $$ Dodecanol $$ Pisol $$ Alfol 12 $$ Sipol L 12 $$ Lauryl 24 $$ Siponol 25 186 C12H26O 91
000112-53-8 95940 138 10 0 75 3 62 34 72 9906
2 Cyclododecane 168 C12H24 91 * 000294-62-2 71567
101 25 1 87 14 62 48 83 8707
3 1-Dodecanol (CAS) $$ n-Dodecanol $$ CO 12 $$ S 1298 $$ Dodecanol $$ Pisol $$ Alfol 12 $$ Sipol L 12 $$ Lauryl 24 $$ Siponol 25 186 C12H26O 91
000112-53-8 95944 150 0 0 72 0 62 40 78 9685
4 1-Dodecanol (CAS) $$ n-Dodecanol $$ CO 12 $$ S 1298 $$ Dodecanol $$ Pisol $$ Alfol 12 $$ Sipol L 12 $$ Lauryl 24 $$ Siponol 25 186 C12H26O 91
000112-53-8 95945 140 7 0 91 0 62 28 64 9698
5 1-Undecanol (CAS) $$ n-Undecanol $$ 1-Hendecanol $$ n-Undecan-1-ol $$ Undecyl alcohol $$ Hendecyl alcohol $$ n-Undecyl alcohol 172 C11H24O 91
000112-42-5 76845 122 18 1 82 3 60 26 53 9484

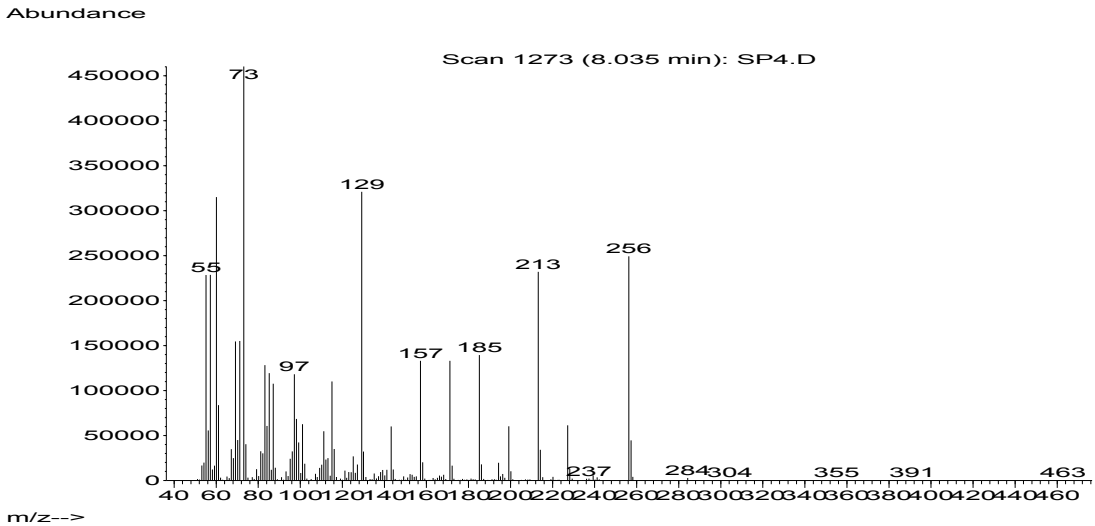




Scan 1273 (8.035 min): SP4.D

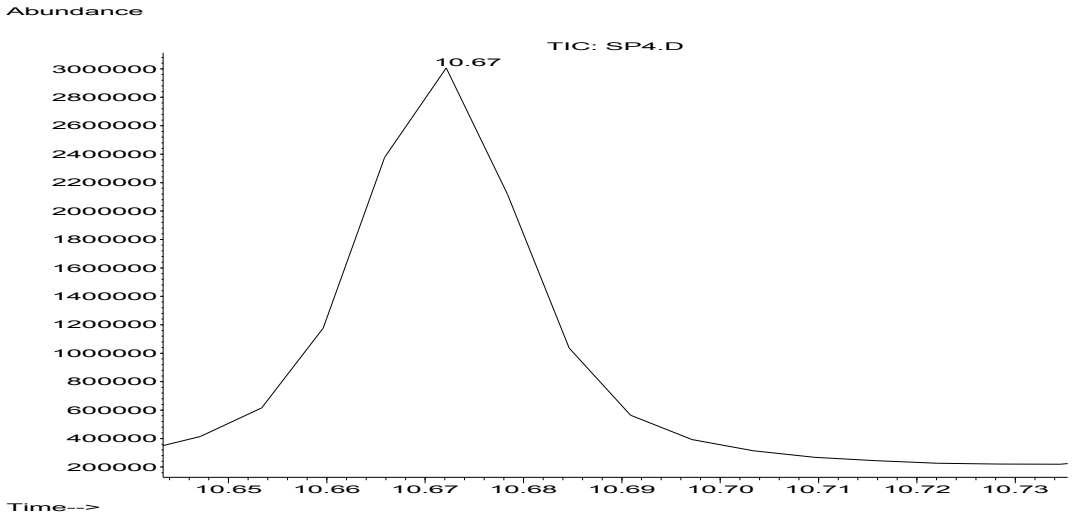
1 n-Hexadecanoic acid 256 C16H32O2 99 * 000057-10-3
195432 150 19 0 68 10 81 0 99 9908
2 Hexadecanoic acid (CAS) $$ Palmitic acid $$ Palmitinic acid $$ n-Hexadecoic acid $$ n-Hexadecanoic acid $$ Pentadecanecarboxyli 256 C16H32O2 99 *
000057-10-3 195440 164 4 0 81 0 80 64 99
8586
3 Hexadecanoic acid (CAS) $$ Palmitic acid $$ Palmitinic acid $$ n-Hexadecoic acid $$ n-Hexadecanoic acid $$ Pentadecanecarboxyli 256 C16H32O2 99 *
000057-10-3 195439 142 21 0 87 17 80 60 99
7033
4 Hexadecanoic acid (CAS) $$ Palmitic acid $$ Palmitinic acid $$ n-Hexadecoic acid $$ n-Hexadecanoic acid $$ Pentadecanecarboxyli 256 C16H32O2 97 *
000057-10-3 195436 117 41 0 90 21 78 75 97
7176
5 Hexadecanoic acid (CAS) $$ Palmitic acid $$ Palmitinic acid $$ n-Hexadecoic acid $$ n-Hexadecanoic acid $$ Pentadecanecarboxyli 256 C16H32O2 95 *
000057-10-3 195435 114 39 0 76 21 74 54 95
8906

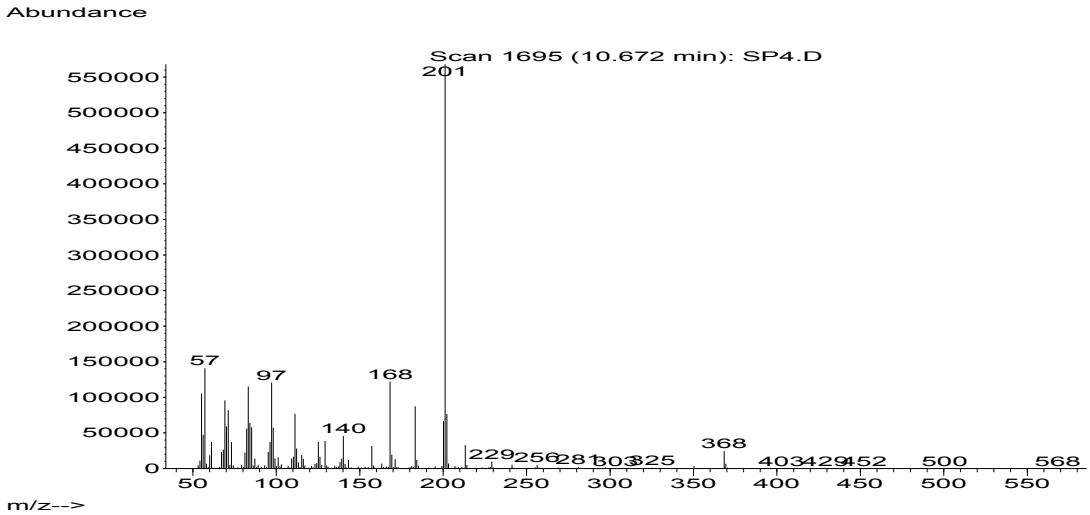




Scan 1695 (10.672 min): SP4.D

1 tris(exo-2-bicyclo[2.2.1]heptyl)borane $$ Borane, tris(bicyclo[2.2.1]hept-2-yl)-, stereoisomer (CAS) $$ Borane, tri-2-norbornyl 296 C21H33B 46 *
022801-27-0 245730 46 124 3 74 45 20 0 44
9195
2 Dodecanoic acid, undecyl ester 354 C23H46O2 38
003658-44-4 302043 98 61 1 29 56 14 0 56
9484
3 Methyl 4,6-decadienyl ether 168 C11H20O 35 *
000000-00-0 71277 84 12 0 16 66 11 58 80
3109
4 1-Dodecanethiol $$ n-Dodecanethiol $$ n-Dodecyl mercaptan $$ n-Lauryl mercaptan $$ Dodecane-1-thiol $$ Dodecyl mercaptan $$ Lau 202 C12H26S 35 *
000112-55-0 118005 80 28 1 15 68 11 40 66
3171
5 Cyclododecane (CAS) $$ CYCLODODECAN 168 C12H24 15 *
000294-62-2 71561 71 18 0 15 72 2 41 59
3042



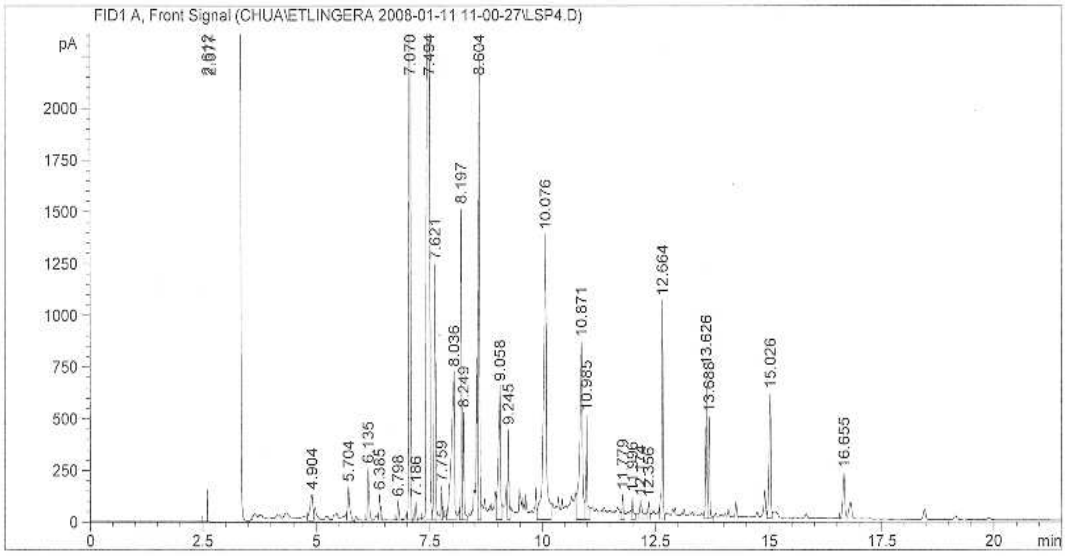


APPENDIX B (Results of Analysis-Light Test)

Appendix B1: Sample of 1st Week

```
Data File C:\CHEM32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\LSP4.D
Sample Name: LSP4

    ==================================================================
    Acq. Operator   : husna                          Seq. Line :   4
    Acq. Instrument : Instrument 1                    Location : Vial 104
    Injection Date  : 1/11/2008 12:31:10 PM                Inj :   1
                                                    Inj Volume : External
    Acq. Method     : C:\Chem32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\ETLINGERACHUA.M
    Last changed    : 1/11/2008 11:00:27 AM by husna
    Analysis Method : C:\CHEM32\1\METHODS\AEGYPTI.M
    Last changed    : 1/11/2008 2:23:08 PM by husna
                      (modified after loading)
    Method Info     : Aedes aegypti pheromone
```




```
    ==================================================================
                              Area Percent Report
    ==================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]    [pA*s]      [pA]       %
    ----|-------|----|-------|----------|----------|--------|
       1   2.612 BV S  0.0334 1.48031e6  5.68217e5  14.47488
       2   2.677 VBAS  0.2609 8.68032e6  5.53926e5  84.87843
       3   4.904 VV T  0.0724  621.72400  128.60913  0.00608
       4   5.704 VV T  0.0444  498.80295  164.25713  0.00488
       5   6.135 VV T  0.0340  582.08936  255.88544  0.00569
       6   6.385 VV T  0.0260  215.74695  126.04054  0.00211
       7   6.798 VV T  0.0340  217.11205   93.73896  0.00212
       8   7.070 VV T  0.0345 8418.98340 3784.52173  0.08232
       9   7.186 VV T  0.0300  178.15945   90.18437  0.00174

Instrument 1 1/11/2008 2:23:50 PM husna                          Page   1 of 2
```

Data File C:\CHEM32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\LSP4.D
Sample Name: LSP4

	Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
(a)	10	7.494	VV T	0.0468	1.99072e4	5756.35840	0.19466
	11	7.621	VV T	0.0314	2744.69458	1236.40112	0.02684
	12	7.759	VV T	0.0225	244.65370	163.51173	0.00239
	13	8.036	VV T	0.0661	3517.46875	718.62262	0.03439
	14	8.197	VV T	0.0252	2406.54858	1504.49097	0.02353
	15	8.249	VV T	0.0223	773.57800	520.56183	0.00756
	16	8.604	VV T	0.0281	4129.54346	2282.97656	0.04038
	17	9.058	VV T	0.0472	2283.41968	646.35974	0.02233
	18	9.245	VV T	0.0244	705.07416	434.22162	0.00689
(b)	19	10.076	VV T	0.0557	6041.21045	1376.09619	0.05907
	20	10.871	VV T	0.0595	3675.54907	858.35406	0.03594
	21	10.985	VV T	0.0303	1065.56958	501.35965	0.01042
	22	11.779	VV T	0.0315	269.41940	120.75421	0.00263
	23	11.996	VV T	0.0319	247.89581	109.42854	0.00242
	24	12.174	VV T	0.0456	312.93213	91.04543	0.00306
	25	12.356	VV T	0.0317	184.59174	82.04881	0.00180
(c)	26	12.664	VV T	0.0300	2085.49780	1057.16553	0.02039
	27	13.626	VV T	0.0302	1459.67505	718.12561	0.01427
	28	13.688	VV T	0.0308	984.02985	492.05533	0.00962
	29	15.026	VV T	0.0422	1637.46094	603.33923	0.01601
	30	16.655	VV X	0.0514	727.00708	223.76976	0.00711
	Totals :				1.02268e7	1.14628e6	

==========

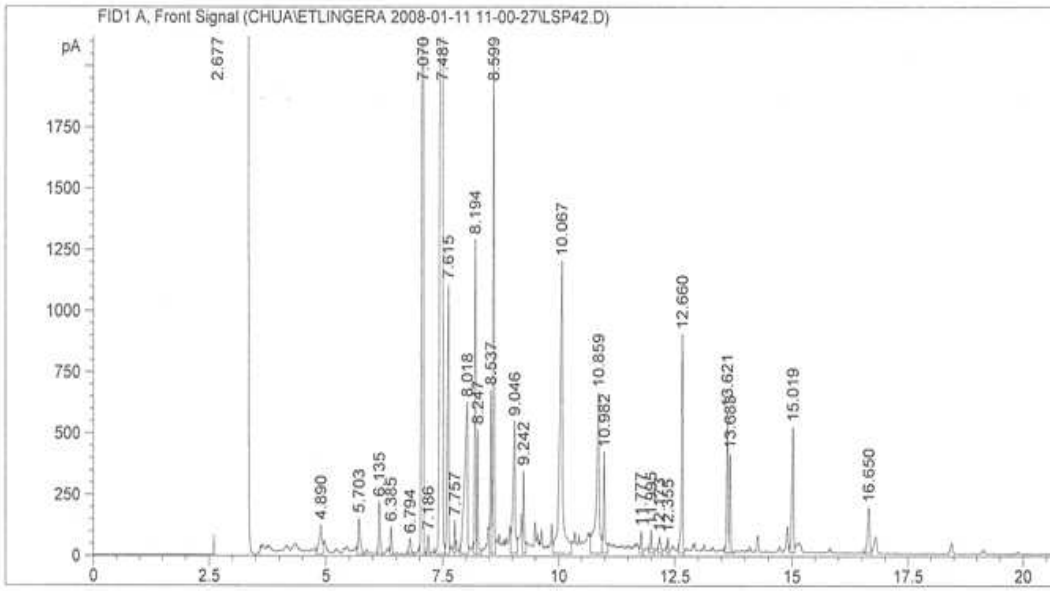
*** End of Report ***

Appendix B2: Sample of 2nd Week

Data File C:\CHEM32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\LSP42.D
Sample Name: LSP42

==========

Acq. Operator : husna
Seq. Line : 5
Acq. Instrument : Instrument 1
Location : Vial 105
Injection Date : 1/11/2008 12:59:27 PM
Inj : 1
Inj Volume : External
Acq. Method : C:\Chem32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\ETLINGERACHUA.M
Last changed : 1/11/2008 11:00:27 AM by husna
Analysis Method : C:\CHEM32\1\METHODS\AEGYPTI.M
Last changed : 1/11/2008 2:23:52 PM by husna
(modified after loading)
Method Info : Aedes aegypti pheromone



==========

Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A, Front Signal

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	2.677	VB S	0.2607	8.59843e6	5.49450e5	99.32887
2	4.890	VV T	0.0652	547.43854	120.15925	0.00632
3	5.703	VV T	0.0448	447.38632	145.96956	0.00517
4	6.135	VV T	0.0340	487.31665	214.45145	0.00563
5	6.385	VV T	0.0283	212.36058	113.77782	0.00245
6	6.794	VV T	0.0424	176.32204	64.47900	0.00204
7	7.070	VV T	0.0334	8326.74316	3829.70288	0.09619
8	7.186	VV T	0.0289	148.58633	77.38318	0.00172
(a) 9	7.487	VV T	0.0431	1.74463e4	5414.33887	0.20154

Data File C:\CHEM32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\LSP42.D
Sample Name: LSP42

	Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
	10	7.615	VV T	0.0306	2317.50879	1100.45996	0.02677
	11	7.757	VV T	0.0221	197.66428	134.96754	0.00228
	12	8.018	VV T	0.0570	2728.85718	617.88977	0.03152
	13	8.194	VV T	0.0246	1991.67297	1283.06531	0.02301
	14	8.247	VV T	0.0225	738.16895	506.30127	0.00853
	15	8.537	VV T	0.0235	1028.07361	665.51514	0.01188
	16	8.599	VV T	0.0255	3528.58594	2058.87354	0.04076
	17	9.046	VV T	0.0451	1788.02710	540.93738	0.02066
	18	9.242	VV T	0.0274	636.74957	338.93118	0.00736
(b)	19	10.067	VV T	0.0566	5321.29150	1192.24463	0.06147
	20	10.859	VV T	0.0612	2938.70630	656.27869	0.03395
	21	10.982	VV T	0.0288	823.07227	411.85376	0.00951
	22	11.777	VV T	0.0290	184.46214	91.54604	0.00213
	23	11.995	VV T	0.0316	209.51198	93.57165	0.00242
	24	12.173	VV T	0.0383	192.26659	68.39465	0.00222
	25	12.355	VV T	0.0290	126.16960	63.98858	0.00146
(c)	26	12.660	VV T	0.0286	1658.58911	893.94965	0.01916
	27	13.621	VV T	0.0295	1132.80090	573.38086	0.01309
	28	13.683	VV T	0.0304	790.81244	403.70123	0.00914
	29	15.019	VV T	0.0405	1365.91223	513.90204	0.01578
	30	16.650	VV X	0.0507	605.36353	187.41055	0.00699
	Totals :				8.65652e6	5.71827e5	

*** End of Report ***

Appendix B3: Sample of 3rd Week

```
Data File C:\CHEM32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\LSP43.D
Sample Name: LSP43

==================================================================
Acq. Operator   : husna                          Seq. Line :   6
Acq. Instrument : Instrument 1                    Location : Vial 106
Injection Date  : 1/11/2008 1:27:31 PM                 Inj :   1
                                                 Inj Volume : External
Acq. Method     : C:\Chem32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\ETLINGERACHUA.M
Last changed    : 1/11/2008 11:00:27 AM by husna
Analysis Method : C:\CHEM32\1\METHODS\AEGYPTI.M
Last changed    : 1/11/2008 2:25:31 PM by husna
                  (modified after loading)
Method Info     : Aedes aegypti pheromone
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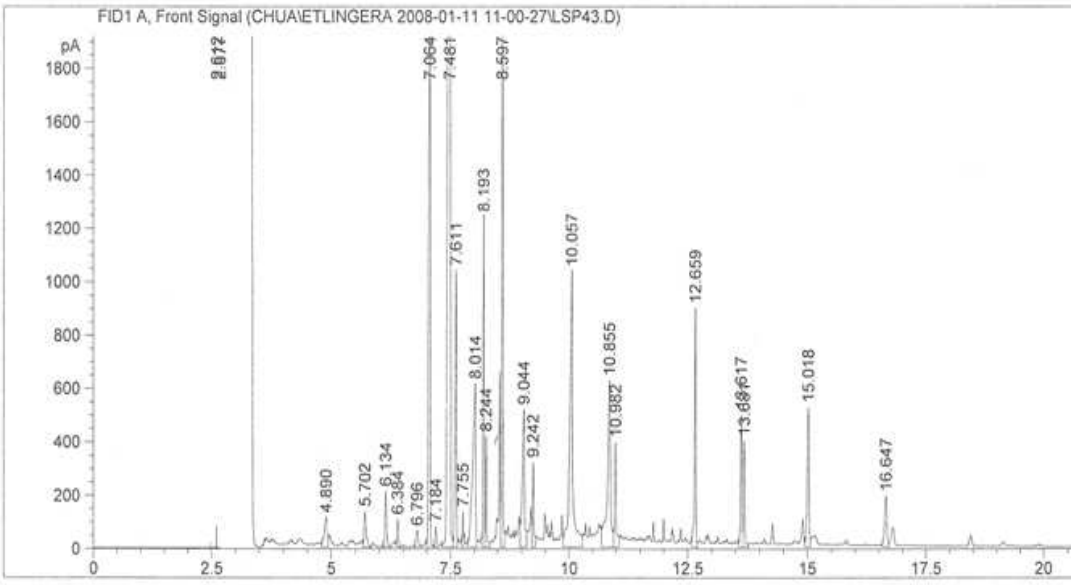



```
==================================================================
                        Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A, Front Signal

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [pA*s]      [pA]       %
----|-------|----|-------|----------|----------|--------|
   1   2.612 BV S  0.0342 1.50604e6  5.62914e5  14.79031
   2   2.677 VBAS  0.2606 8.62540e6  5.51209e5  84.70691
   3   4.890 VV T  0.0640  497.42035  110.55534   0.00488
   4   5.702 VV T  0.0447  407.59085  133.27484   0.00400
   5   6.134 VV T  0.0338  472.72818  209.71594   0.00464
   6   6.384 VV T  0.0277  190.22723  104.76461   0.00187
   7   6.796 VV T  0.0445  196.02371   62.60614   0.00193
   8   7.064 VV T  0.0298 6604.65430 3234.52197   0.06486
   9   7.184 VV T  0.0297  152.49162   76.80624   0.00150

Instrument 1 1/11/2008 2:27:18 PM husna                    Page   1 of 2
```

Data File C:\CHEM32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\LSP43.D
Sample Name: LSP43

	Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
(a)	10	7.481	VV T	0.0428	1.57324e4	4981.02344	0.15450
	11	7.611	VV T	0.0305	2176.97388	1036.68787	0.02138
	12	7.755	VV T	0.0223	198.09468	133.27519	0.00195
	13	8.014	VV T	0.0596	2679.58447	611.32465	0.02632
	14	8.193	VV T	0.0245	1910.79187	1238.40930	0.01877
	15	8.244	VV T	0.0225	622.02924	414.07516	0.00611
	16	8.597	VV T	0.0264	3281.63306	1921.90674	0.03223
	17	9.044	VV T	0.0495	1722.95667	515.33081	0.01692
	18	9.242	VV T	0.0274	595.01294	316.31732	0.00584
(b)	19	10.057	VV T	0.0573	4626.09131	1032.70862	0.04543
	20	10.855	VV T	0.0635	2832.56665	623.92346	0.02782
	21	10.982	VV T	0.0296	809.87823	392.43924	0.00795
(c)	22	12.659	VV T	0.0286	1656.20325	895.35291	0.01626
	23	13.617	VV T	0.0304	997.05432	496.92267	0.00979
	24	13.681	VV T	0.0315	818.95099	397.55255	0.00804
	25	15.018	VV T	0.0386	1374.09094	523.13873	0.01349
	26	16.647	VV T	0.0498	640.16602	189.99165	0.00629
Totals :					1.01826e7	1.13378e6	

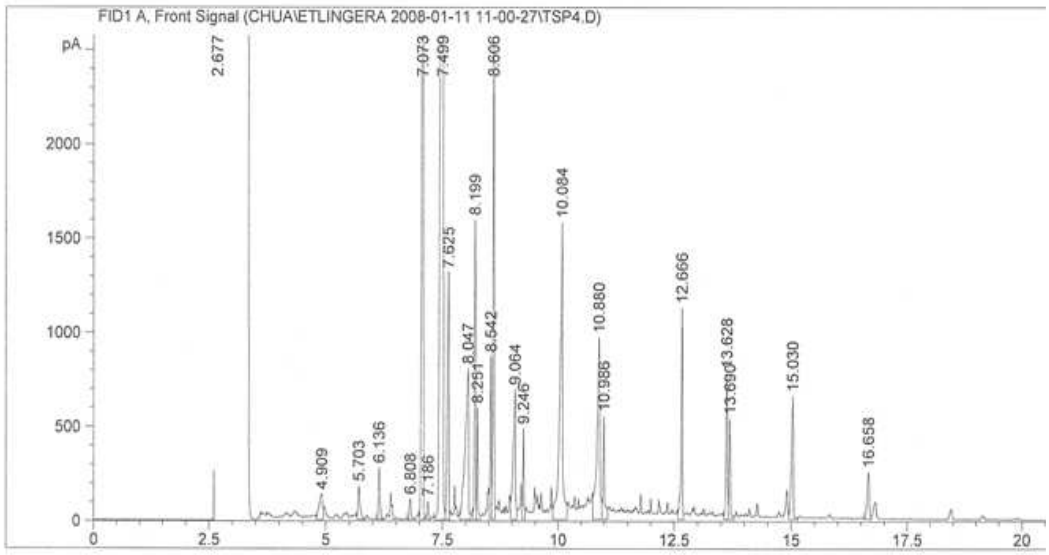
*** End of Report ***

APPENDIX C (Results of Analysis-Heat Test)

Appendix C1: Sample of 1st Week

```
Data File C:\CHEM32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\TSP4.D
Sample Name: TSP4

    ==================================================================
    Acq. Operator   : husna                          Seq. Line :   1
    Acq. Instrument : Instrument 1                    Location : Vial 101
    Injection Date  : 1/11/2008 11:06:49 AM                Inj :   1
                                                    Inj Volume : External
    Acq. Method     : C:\Chem32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\ETLINGERACHUA.M
    Last changed    : 1/11/2008 11:00:27 AM by husna
    Analysis Method : C:\CHEM32\1\METHODS\AEGYPTI.M
    Last changed    : 1/11/2008 2:16:57 PM by husna
                      (modified after loading)
    Method Info     : Aedes aegypti pheromone
```




```
    ==================================================================
                              Area Percent Report
    ==================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]    [pA*s]      [pA]       %
    ----|-------|----|-------|----------|----------|--------|
       1   2.677 VBAS  0.2600 8.61437e6  5.51440e5  99.14878
       2   4.909 VV T  0.0673  695.58948  135.76620  0.00801
       3   5.703 VV T  0.0446  548.38141  177.43515  0.00631
       4   6.136 VV T  0.0356  652.70117  276.05545  0.00751
       5   6.808 VV T  0.0427  297.67545  109.66187  0.00343
       6   7.073 VV T  0.0348 9506.09375 4135.34082  0.10941
       7   7.186 VV T  0.0314  204.39038   97.77908  0.00235
    (b) 8  7.499 VV T  0.0480 2.22734e4  6113.40918  0.25636
       9   7.625 VV T  0.0326 2980.11011 1309.37024  0.03430

Instrument 1 1/11/2008 2:18:09 PM husna                          Page   1 of 2
```

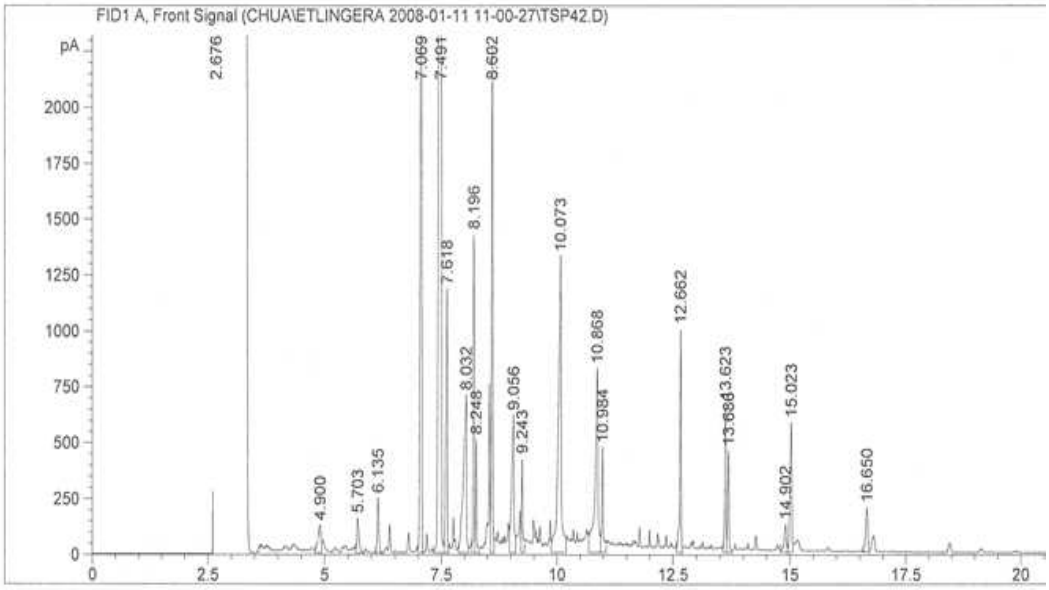
Data File C:\CHEM32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\TSP4.D
Sample Name: TSP4

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
10	8.047	VV T	0.0683	4067.72144	799.79901	0.04682
11	8.199	VV T	0.0263	2628.61792	1588.21313	0.03025
12	8.251	VV T	0.0221	865.14801	591.07892	0.00996
13	8.542	VV T	0.0243	1393.54578	863.97821	0.01604
14	8.606	VV T	0.0272	4560.27002	2510.69824	0.05249
15	9.064	VV T	0.0489	2469.06665	687.24030	0.02842
16	9.246	VV T	0.0232	747.22400	478.21088	0.00860
(b) 17	10.084	VV T	0.0588	7055.77979	1572.87366	0.08121
18	10.880	VV T	0.0590	4131.10937	964.30811	0.04755
19	10.986	VV T	0.0305	1174.53809	548.49866	0.01352
(c) 20	12.666	VV T	0.0314	2299.41870	1121.70325	0.02647
21	13.628	VV T	0.0321	1670.31262	776.83679	0.01922
22	13.690	VV T	0.0319	1109.63684	531.13904	0.01277
23	15.030	VV T	0.0409	1809.54407	650.75513	0.02083
24	16.658	VV T	0.0490	816.37823	247.55814	0.00940
Totals :				8.68833e6	5.77728e5	

*** End of Report ***

Appendix C2: Sample of 2nd Week

```
Data File C:\CHEM32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\TSP42.D
Sample Name: TSP42
  ûé
  ==================================================================
  Acq. Operator   : husna                          Seq. Line :   2
  Acq. Instrument : Instrument 1                    Location : Vial 102
  Injection Date  : 1/11/2008 11:35:05 AM                Inj :   1
                                                  Inj Volume : External
  Acq. Method     : C:\Chem32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\ETLINGERACHUA.M
  Last changed    : 1/11/2008 11:00:27 AM by husna
  Analysis Method : C:\CHEM32\1\METHODS\AEGYPTI.M
  Last changed    : 1/11/2008 2:18:11 PM by husna
                    (modified after loading)
  Method Info     : Aedes aegypti pheromone
```




```
  ==================================================================
                          Area Percent Report
  ==================================================================

  Sorted By             :      Signal
  Multiplier            :      1.0000
  Dilution              :      1.0000
  Use Multiplier & Dilution Factor with ISTDs


  Signal 1: FID1 A, Front Signal
```

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	2.676	VBAS	0.2586	8.68945e6	5.59350e5	99.29096
2	4.900	VV T	0.0707	598.00244	125.14565	0.00683
3	5.703	VV T	0.0450	479.77066	155.30911	0.00548
4	6.135	VV T	0.0339	556.65253	246.11871	0.00636
5	7.069	VV T	0.0321	8168.88770	3792.23730	0.09334
(a) 6	7.491	VV T	0.0457	1.90422e4	5597.22559	0.21759
7	7.618	VV T	0.0316	2586.75977	1180.19604	0.02956
8	8.032	VV T	0.0621	3355.09106	704.10956	0.03834
9	8.196	VV T	0.0256	2275.59473	1424.25293	0.02600

Data File C:\CHEM32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\TSP42.D
Sample Name: TSP42

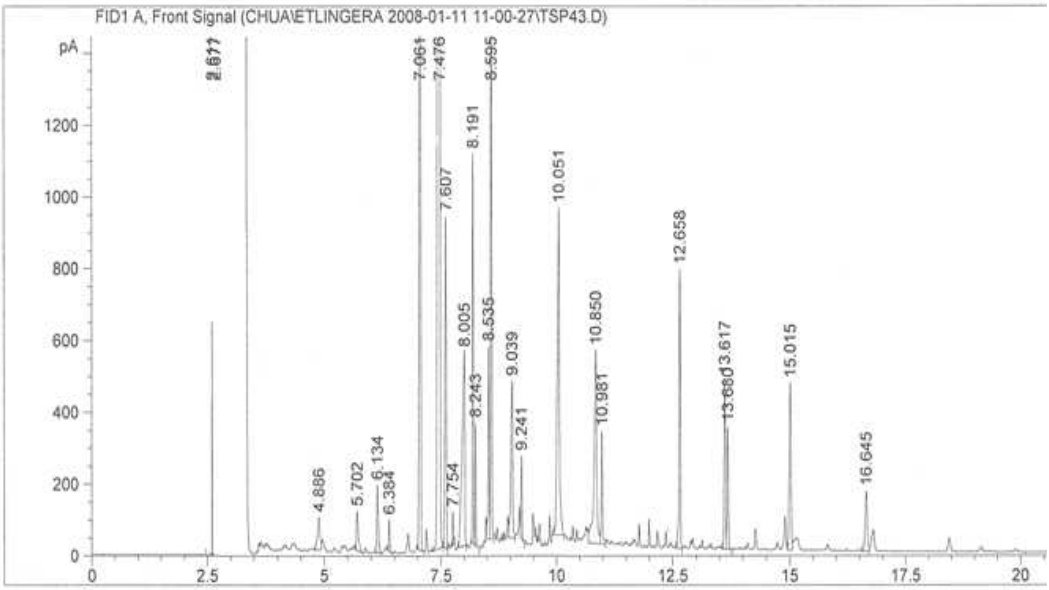
	Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
	10	8.248	VV T	0.0230	757.90814	506.41345	0.00866
	11	8.602	VV T	0.0268	3961.94360	2273.39624	0.04527
	12	9.056	VV T	0.0477	2193.57178	613.62982	0.02507
	13	9.243	VV T	0.0268	764.91663	418.30896	0.00874
(b)	14	10.073	VV T	0.0559	5772.05615	1322.97229	0.06596
	15	10.868	VV T	0.0622	3715.81274	823.38080	0.04246
	16	10.984	VV T	0.0302	993.25903	468.73843	0.01135
(c)	17	12.662	VV T	0.0298	1952.41040	999.35284	0.02231
	18	13.623	VV T	0.0323	1370.24231	657.20709	0.01566
	19	13.686	VV T	0.0315	931.81183	453.00269	0.01065
	20	14.902	VV T	0.0482	413.02063	124.40459	0.00472
	21	15.023	VV T	0.0381	1515.60974	578.61212	0.01732
	22	16.650	VV T	0.0495	646.27026	198.44928	0.00738
	Totals :				8.75150e6	5.82012e5	

*** End of Report ***

Appendix C3: Sample of 3rd Week

Data File C:\CHEM32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\TSP43.D
Sample Name: TSP43

```
==================================================================
Acq. Operator   : husna                          Seq. Line :   3
Acq. Instrument : Instrument 1                    Location : Vial 103
Injection Date  : 1/11/2008 12:03:08 PM                Inj :   1
                                                 Inj Volume : External
Acq. Method     : C:\Chem32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\ETLINGERACHUA.M
Last changed    : 1/11/2008 11:00:27 AM by husna
Analysis Method : C:\CHEM32\1\METHODS\AEGYPTI.M
Last changed    : 1/11/2008 2:21:48 PM by husna
                  (modified after loading)
Method Info     : Aedes aegypti pheromone
```




```
==================================================================
                        Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: FID1 A, Front Signal

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	2.611	BV S	0.0345	1.52099e6	5.64170e5	14.84877
2	2.677	VB S	0.2617	8.68043e6	5.52712e5	84.74333
3	4.886	VV T	0.0544	353.15106	91.68201	0.00345
4	5.702	VV T	0.0410	302.01889	110.21680	0.00295
5	6.134	VV T	0.0316	400.42728	190.14830	0.00391
6	6.384	VV T	0.0250	145.69742	89.50266	0.00142
7	7.061	VV T	0.0276	5718.60791	3017.14136	0.05583
(s) 8	7.476	VV T	0.0439	1.36155e4	4611.48633	0.13292
9	7.607	VV T	0.0289	1820.15784	925.99829	0.01777

Data File C:\CHEM32\1\DATA\CHUA\ETLINGERA 2008-01-11 11-00-27\TSP43.D
Sample Name: TSP43

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
10	7.754	VV T	0.0182	101.22992	89.65070	0.00099
11	8.005	VV T	0.0529	2176.45117	541.22424	0.02125
12	8.191	VV T	0.0228	1565.03821	1085.60583	0.01528
13	8.243	VV T	0.0196	407.72760	338.23212	0.00398
14	8.535	VV T	0.0232	817.04407	538.16144	0.00798
15	8.595	VV T	0.0244	2703.12012	1709.74072	0.02639
16	9.039	VV T	0.0390	1181.32715	437.68204	0.01153
17	9.241	VV T	0.0221	339.25885	231.98485	0.00331
(b) 18	10.051	VB T	0.0420	2886.39014	910.29456	0.02818
19	10.850	VV X	0.0552	2089.91772	539.20593	0.02040
20	10.981	VV X	0.0251	529.20898	315.38486	0.00517
(c) 21	12.658	VV X	0.0275	1365.03516	776.83099	0.01333
22	13.617	VV X	0.0293	901.85986	472.85846	0.00880
23	13.680	VV X	0.0298	651.02106	340.87674	0.00636
24	15.015	VV X	0.0386	1180.68945	466.10922	0.01153
25	16.645	VV X	0.0485	531.13757	167.51782	0.00519
Totals :				1.02432e7	1.13488e6	

==========

*** End of Report ***

APPENDIX D (Research Gantt Charts)

Appendix D1: Gantt Chart for Undergraduate Research Project I

RESEARCH ACTIVITIES	WEEK														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1.Research and selection of title															
2.Information gathering															
3.Determination of objective and scope of research															
4.Indentification of problem statement															
5.Preparation of report															
-Introduction															
-Literature Review															
-Methodology															
-Expected result															
6.Submission of report (first draft)															
7.Correction of report (first draft)															
8.Preparation of seminar 1															
9.Seminar 1 presentation															
10.Correction and preparation of final draft															
11.Submission of report (final draft)															
12.Preparation of Undergraduate Research Project II															

Appendix D2: Gantt Chart for Undergraduate Research Project II

RESEARCH ACTIVITIES	WEEK														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
EXPERIMENT															
1.Specimen collection															
2.Pre-treatment of sample															
3.Extraction of essential oil															
3.GC-MS analysis															
4.Identification of oil qualities															
REPORT & SEMINAR															
5.Preparation of report															
-Result															
-Discussion															
-Conclusion															
6.Submission of report															
7.Correction of report															
8.Preparation of seminar 2															
9.Seminar 2 presentation															
10.Correction and preparation of final report															
11.Submission of final report															