MONITORING OF QUALITY OF ESSENTIAL OIL FROM *ETLINGERA* SP. 5 (ZINGEBERACEAE) BY GC/MS & GC - FID

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

Faculty of Chemical Engineering and Natural Resources
Universiti Malaysia Pahang

APRIL 2008

"I declare that this thesis is the result of my own research except as cited references. The thesis has not been accepted for any degree and is concurrently submitted in candidature of any degree."

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Dedicated to my beloved mother Salmah Hj. Dahlan, brothers, sister, Siti Nurnadzmiah Bt. Mohd Nor and the most Allah S.W.T.…..

ACKNOWLEDGEMENT

First of all, thanks to Allah S.W.T for all His guidance and blessing through all the hardship encountered whilst completing this thesis. To beloved mother Salmah Hj. Dahlan, that rise up me from I'm little child until now where I'm a step closer to finish up my degree when this thesis is done for binding. To my big brother Mohd Khairilazwan Hj. Mat Ali who helping me buying my laptop where's it is really helping me in finished this thesis.

I would like to thank my supervisor Prof. Dr. Mashitah Bt. Mohd Yusoff who always given me the advise to this research and also Miss Nurul Husna Ab. Hamid, ones of the Research Officer under Prof. Dr. Mashitah Bt. Mohd Yusoff at Institute Medical Research (IMR) who always help me regarding to the research.

Not to forget my panel evaluation Associates Prof. Nordin B. Endut and En. Syaiful Nizam B. Hassan who dare to criticize, correct and advice to me regarding to the research. My fellow friends who also doing the research under Prof. Dr. Mashitah Bt. Mohd Yusoff where they always helping me in contributing the idea.

Furthermore, my Undergraduate Final Project Coordinator, Miss Sumaiya that keeps reminding me about the date of the submission and presentation and last but no least my future wife, Siti Nurnadzmiah Bt. Mohd Nor that keep supporting me from behind, giving me the courage and guts to finish up this research and also guiding me on how to do the thesis.

ABSTRACT

The aim of the present study is to monitor the quality of the essential oil from *Etlingera* sp. 5 (ZINGIBERACEAE) using GC/MS & GC – FID which this research is has been never reported before. So by provided the effect of storage on the quality of the essential oil, which can be obtained by GC/MS & GC – FID, will also yield useful information on the product. This research objective are to extract essential oil from the rhizome material of *Etlingera* sp. 5 by hydro-distillation, to develop a method of analysis of essential oil from the rhizome material of *Etlingera* sp. 5 by using GC/MS & GC-FID, and to monitor the effect of storage on the quality of the essential oil. The essential oil was extract by using hydro distillation with Clevenger type. To measure the quality of the essential oil, two tests had been come out there were light test and temperature test where the fresh essential oil will be separated into two according to the two tests. Clear & colorless vial was use to see the effect of light while the dark brown vial was use to see the effect of temperature to the quality of the essential oil. This two test of vials were put into two different places in the same room whereas for the light test vial was put in the glass cabinet while the temperature test vial was put near to the window where expose to direct sunlight. This monitoring was done in 3 weeks duration to see the changes when we test to the light and the temperature at normal room condition. This monitoring takes one week interval to analyze using GC – FID and to determine the major compounds, GC/MS will be use to detect the compounds. From the results, three major compounds had been detected and been analyzed which are Nerolidol, Farnesol and 1 – Tricosene. These three major compounds seen to have a slight change to the light and temperature test within three weeks of monitoring of the quality of the essential oil from *Etlingera* sp. 5. As for the conclusion, due to expose to light and temperature test, quality of essential oil from the Etlingera sp. 5 is affected to light and temperature. To continued research, there are suggested that this research is done for

a longer period of time to see the significant changes of the compounds and to determine the minimum quality of the essential oil base on the duration exposed.

ABSTRAK

Sasaran utama kajian ini adalah untuk meninjau kualiti pati minyak daripada *Etlingera* sp. 5 (ZINGIBERACEAE) menggunakan GC/MS dan GC – FID yang mana kajian ini tidak pernah dilaporkan sebelum ini. Maka sehubungan itu, dengan menyediakan kesan kualiti terhadap simpanan minyak pati yang mana boleh didapati dengan menggunakan GC/MS & GC – FID turut akan memberi manafaat pengetahuan terhadap produk. Objektif kajian ini adalah untuk mengestrak pati minyak daripada rizom *Etlingera* sp. 5 menggunakan penyulingan hidro, untuk membangunkan cara analisis minyak pati daripada rizom *Etlingera* sp. 5 menggunakan GC/MS & GC – FID dan untuk mengkaji kesan kualiti simpanan keatas minyak pati tersebut. Minyak pati tersebut diestrak menggunakan penyulingan hidro jenis Clevenger. Untuk mengukur kualiti pati minyak ini, dua ujian telah dilakukan iaitu ujian cahaya dan juga ujian suhu dimana pati minyak yang baru disuling dibahagikan kepada dua mengikut ujian. Vial yang terang dan tidak berwarna digunakan untuk melihat kesan cahaya manakala vial coklat gelap digunakan untuk melihat kesan suhu terhadap kualiti pati minyak. Dua vial ini diasingkan kepada dua tempat yang berasingan di dalam satu bilik yang sama dimana vial untuk ujian cahaya diletakkan di dalam cabinet gelas manakala vial untuk ujian suhu diletakkan di sebelah tingkap yang terdedah kepada cahaya matahari. Tinjauan ini berlangsung selama tiga minggu untuk melihat kesan perubahan apabila ujian cahaya dan ujian suhu dijalankan pada keadaan bilik yang normal. Tinjauan ini juga mengambil sela masa seminggu untuk menganalisa menggunakan GC – FID dan untuk menentukan komponen major, GC/MS digunakan untuk mengesan komponen pati minyak tersebut. Daripada keputusan tinjauan ini, tiga komponen major telah berjaya dikesan dan dianalisa iaitu Nerolidol, Farnesol dan 1 – Tricosene. Tiga komponen major ini dilihat mengalami sedikit perubahan terhadap ujian cahaya dan juga suhu yang berlangsung selama tiga minggu tinjauan kesan kualiti pati minyak

daripada *Etlingera* sp. 5. Sebagai konklusi, tinjauan terhadap ujian cahaya dan juga suhu dilihat member kesan kepada kualiti pati minyak daripada *Etlingera* sp. 5. Untuk meneruskan kajian ini, dicadangkan agar tinjauan ini dilakukan dalam masa yang lebih panjang untuk melihat perubahan komponen yang ketara dan untuk menentukan kualiti pati minyak yang minimum berdasarkan jangka masa pendedahan.

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CHAPTER 1

INTRODUCTION

1.0 Introduction

Zingiberaceae comprises about 1,200 species of which 1,000 occur in tropical Asia. The family is conventionally classified into distinct genera, each genus consists of usually several species, examples are *Curcuma* (e.g. “kunyit”), *Kaempferia* (e.g. “cekur”), *Alpinia* (e.g. “lengkuas”), *Zingiber* (e.g. “halia”) and *Etlingera* (e.g. “kantan”). In the Malesian region, a floristically distinct region which includes Malaysia, Indonesia, Brunei, Singapore, the Philippines and Papua New Guinea, there are 600 species encompassing 24 genera.

The commercial value of the family arises from its utility as ingredients of flavoring, colors and fragrance. The high content of essential oil in commercial gingers contributes to demands in the flavoring and fragrance industries. Essential oil exists in all ginger plants in the genus *Etlingera.* The highest content is usually in the rhizomes of this plant. Essential oils gradually loose freshness upon standing. The quality of the essential oil can be monitored by several methods. Gas chromatography-mass spectrometry (GC/MS) is the most common tool used in quality control today.

In the present study, the essential oil from the rhizomes of *Etlingera* sp. 5 is to be investigated.

1.1 Objective

The objectives of the study are as follows;

(i) To extract essential oil from the rhizome material of *Etlingera* sp. 5 by hydro-distillation,

(ii) To develop a method of analysis of essential oil from the rhizome material of *Etlingera* sp. 5 by using GC-MS and GC-FID, and

(iii) To monitor the effect of storage on the quality of the essential oil.

1.2 Scope of Study

The scope of study will involve collecting rhizome samples of *Etlingera* sp. 5 from a nearby forest, preparation of the sample for extraction, extraction of essential oil from the rhizome material of *Etlingera* sp. 5 by hydro-distillation, developing a method of analysis of the essential oil using GC-MS & GC-FID, and monitoring the effect of storage, i.e., effect of exposure to light and air, and standing, on the quality of the essential oil.

1.3 Problem Statement

GC-MS & GC-FID analysis of the essential oil from the rhizome material of *Etlingera* sp. 5 has never been reported before. The effect of storage on the quality of the essential oil, which can be obtained by GC-MS, will also yield useful information on the product.

CHAPTER 2

LITERATURE REVIEW

2.1 Gingers and Zingiberaceae

"Gingers" refers to edible ginger of commerce known in Malay as "halia" (scientific name: *Zingiber officinale*). Ginger is often used by Asians not only as a spice, but also as a medicinal plant with indications against several problems, such as stomachache, cardiovascular and motor diseases, also possessing anti-inflammatory activity. Botanically, *Zingiber* gives its name to the whole ginger family, Zingiberaceae. Zingiberaceae comprises about 1200 species of which 1000 occur in tropical Asia. The family is conventionally classified into distinct genera, each genera consist of usually several species, examples are *Curcuma* (e.g. "kunyit"), *Kaempferia* (e.g. "cekur"), *Alpinia* (e.g. "lengkuas"), *Zingiber* (e.g. "halia") and *Etlingera* (e.g. "kantan"). In the Malesian region, a floristically distinct region which includes Malaysia, Indonesia, Brunei, Singapore, the Phillipines and Papua New Guinea, there are 600 species encompassing 24 genera.

The commercial value of this family of plants arise from its utility as ingredients of flavoring, colours and fragrance. The high content of essential oil in commercial gingers contribute to demands in the flavouring and fragrance industries.

2.2 The Genus *Etlingera*

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 and, 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 very attractive plants. A total of 15 *Etlingera* species has been recorded in Peninsular Malaysia (Lim, 2001).

Plants of *Etlingera* have various traditional and commercial uses. Although trough out this research the species is unknown but we can expect that the other species that are from the same genus would have the same similarities of the physical and chemical content. There are no reports on the use of rhizomes of *Etlingera* species.

Inflorescences of *E. elatior* are widely cultivated throughout the tropics as spices for food flavouring and as ornamentals (Larsen, Ibrahim, Khaw, & Saw, 1999). Farms in Australia and Costa Rica are cultivating the species and selling its inflorescences as cut flowers (Larsen et al., 1999).

Flavonoids in the leaves of *E. elatior* have been identified as kaempferol 3-glucuronide, quercetin 3-glucuronide, quercetin 3-glucoside, and quercetin 3-rhamnoside (Williams & Harborne, 1977). Flavonoid content of inflorescences of *E. elatior* has been estimated to be 286 and 21 mg of kaempferol and quercetin (per kg dry weight), respectively (Miean & Mohamed, 2001). The taxonomic classification of *Etlingera* sp. 5 is outlined below.

Kingdom	*Plantae*
Division	*Magnoliphyta*
Class	*Liliopsida*
Order	*Zingiberales*
Family	*Zingiberaceceae*
Genus	*Etlingera*
Species	sp. 5

Since *Etlingera elatior* or “kantan” has been extensively studied, the present study will concentrate on a wild species of *Etlingera* collected near Bentong by a botanist and my supervisor. While comprehensive morphological examination is being carried out by the botanist at Universiti Malaya, the specimen is tentatively labeled as *Etlingera* sp. 5.

2.3 Uses of genus *Etlingera*

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, eaten raw or cooked (Noweg, Abdullah, & Nidang, 2003). While in Malaysia, fruits of *E. elatior* are used traditionally to treat earache, while leaves are applied for cleaning wounds (Ibrahim & Setyowati, 1999).

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

Leaves of *E. elatior*, mixed with other aromatic herbs in water, are used by post-partum women for bathing to remove body odour.

Inflorescences of *E. elatior* commonly used as ingredients of dishes such as laksa asam, nasi kerabu, and nasi ulam in Peninsular Malaysia (Larsen, Ibrahim, Khaw, & Saw, 1999)

2.4 Separation process principles

Separation processes is defined as any set of operations that separate solutions of two or more components into two or more products that differ in composition. Separation is done by exploiting the different of substance properties

base on their chemical and physical properties trough the use of separating agent (energy and mass). Separation process have three primary functions, there are:

i. Purification

In purification, undesired components in a feed mixture are removed from the desired species.

ii. Concentration

In concentration, a higher proportion of desired components that are initially dilute in a feed stream can be obtained.

iii. Fractionation

In fractionation, a feed stream of two or more components is segregated into product streams of different components, typically pure streams of each component.

2.4.1 Classification of separation process

Separation process mainly deal with the transfer and change of the energy, transfer and of materials, primarily by physical way and also by physical – chemicals way. Below is the classification of separation process:-

i. Evaporation

In evaporation, the vapor from a boiling liquid solution is removed are a more concentrated solution remains.

ii. Drying

Drying generally means removal of relatively small amounts of water from material. In drying, the water usually removed as a vapor by air.

iii. Distillation

Distillation is a method for separating the various components of a liquid solution which depends upon the distribution of these components between a vapor phase and a liquid phase.

iv. Absorption

Absorption is a mass – transfer process in which a vapor solute A in a gas mixture is absorbed by means of a liquid in which the solute is more or less soluble.

v. Adsorption

In adsorption processes one or more components of a gas or liquid stream are adsorbed on the surface of a solid adsorbent.

vi. Membrane separation

The membrane acts as a semipermeable barrier and separation occurs by the membrane controlling the rate of movement of various molecules between two liquid phases, two gas phases, or a liquid and a gas phase.

vii. Liquid – liquid extraction

In liquid – liquid extraction, the two phases are chemically quite different, which leads to a separation of the component according to physical and chemical properties.

viii. Ion exchange

Basically chemical reactions between ions in solution and ions in an soluble solid phase.

ix. Liquid – solid leaching

In leaching, the solid is contacted with a liquid phase to separate the desired solute constituent or remove an undesirable solute component from the solid phase.

x. Crystallization

Crystallization is another solid – liquid separation process, in which mass transfer of a solute from the liquid solution to a pure solid crystalline phase occurs.

xi. Mechanical – Physical separation processes

Mechanical – Physical separation processes are considered in several classifications that is Filtration, Settling and sedimentation, Centrifugal settling and sedimentation, Centrifugal filtration, and Mechanical size reduction and separation.

2.4.1.1 Evaporation

Objective of evaporation is to concentrate a solution consisting of a nonvolatile solute and a volatile solvent. In the vast majority of evaporations the solvent is water. Evaporation conducted by vaporizing the solvent to produce a concentrated solution of thick liquor. Evaporation differs from drying where the residue is a liquid, sometimes a highly viscous one, rather than a solid; it differs from distillation in that the vapor usually is a single component, and even when the vapor is a mixture, no attempt is made in the evaporation step to separate the vapor into fraction; it differ from crystallization in that emphasis is placed on concentrating a solution rather than forming and building crystals. In certain situation, for example, in the evaporation of brine produce a common salt, the line between evaporation and crystallization is far from sharp. Evaporation sometimes produces slurry of crystal in saturated mother liquor.

Normally, in evaporation the thick liquor is the valuable product, and the vapor is condensed and discarded. In one specific situation, however the reverse is true. Mineral bearing water often is evaporated to give a solid-free product for the boiler feed, for special process requirements, or for human consumption. This technique is often called *water distillation*, but technically it is evaporation. Large-scale evaporation processes have been developed and used for recovering potable water from seawater. Here the condensed water is the desired product. Only a fraction of the total water in the feed is recovered, the remainder is returned to the sea.

2.4.1.1.1 Processing Factors

The physical and chemical properties of the solution being concentrated and of the vapor being removed bear greatly on the type of evaporator used and the pressure and temperature of the process. Some of the properties which affect the processing methods are:

i. Liquid characteristic

The practical solution of an evaporation problem is profoundly affected by the character of the liquor to be concentrated. It is the wide variation in liquor characteristics (which demands judgment and experience in designing and operating evaporators) that broadens this operation from simple heat transfer to a separate art. Some of the most important properties of evaporating liquids are as follows.

ii. Concentration in the liquid

Usually, the liquid feed to an evaporator is relatively dilute, so its viscosity is low, similar to that of water, and relatively high heat-transfer coefficients are obtained. As evaporation proceeds, the solution may become very concentrated and quite viscous, causing the heat-transfer coefficient to drop markedly. Adequate circulation and/or turbulence must be present to keep the coefficient from becoming too low.

iii. Solubility

As solutions are heated and the concentration of the solute or salt increases, the solubility limit of the material in solution may be exceeded and crystal form. This may limit the maximum concentration in solution which can be obtained by evaporation. In most cases the solubility of the salt increases with temperature. This means that when a hot, concentrated solution from an evaporator is cooled to room temperature, crystallization may occur.

iv. Temperature sensitivity of material

Many products, especially food and other biological materials, may be temperature sensitive and degrade at higher temperature or after prolonged heating. Such

products include pharmaceutical products; food products such as milk, orange juice, and vegetable extract; and fine organic chemicals. The amount of degradation is a function of the temperature and the length of time.

v. Foaming or frothing

In some cases materials composed of caustic solutions, food solutions such as skim milk, and some fatty-acid solutions form a foam or froth during boiling. This foam accompanies the vapor coming out the evaporator and entrainment losses occur.

vi. Pressure and temperature

The boiling point of the solution is related to the pressure of the system. The higher the operating pressures of the evaporator, the higher the temperature at boiling. Also, as the concentration of the dissolved material in solution increases by evaporation, the temperature of boiling point may rise. This phenomenon is called *boiling-point rise* or *elevation*. To keep the temperature low in heat-sensitive materials, it is often necessary to operate under 1 atm pressure, that is, under vacuum.

vii. Scale deposition and materials of construction

Some solutions deposit solid materials called *scale* on the heating surfaces. These could be formed by decomposition products or by decreases in solubility. The result is that the overall heat-transfer coefficient decreases and the evaporator must eventually be cleaned. The materials used in construction of the evaporator should be chosen to minimize corrosion.

2.4.1.2 Boiling and Condensation

2.4.1.2.1 Boiling

Heat transfer to a boiling liquid is very important in evaporation and distillation as well as in other kind of chemical and biological processing, such as

petroleum processing, control of the temperature of chemical reactions, evaporation of liquid foods, and so on. The boiling point is usually contained in a vessel with a heating surface of tubes or vertical or horizontal plates which supply the heat for boiling. The heating surfaces can be heated by electrically or by a hot or condensing fluid on the other side of the heated surface. In boiling, the temperature of the liquid is the boiling point of this liquid at the pressure in the equipment. The heated surface is, of course, at a temperature above the boiling point. Bubbles of vapor are generated at the heated surface and rise through the mass of liquid. The vapor accumulates in a vapor above the liquid level and is withdrawn.

2.4.1.2.2 Condensation

Condensation of a vapor to a liquid and vaporization liquid to a vapor both involve a change of phase of a fluid with large heat-transfer-coefficients. Condensation occurs when a saturated vapor such as steam comes in contact with a solid whose surface temperature is below the saturation temperature, to form a liquid such as water. Normally, when a vapor condenses on a surface such as vertical or horizontal tube or other surface, a film of condensate is formed on the surface and flows over the surface by the action of gravity. It is this film of liquid between the surface and the vapor that forms the main resistance to heat transfer. This is called film-type condensation.

Another type of condensation, dropwise condensation, can occur, where small drops are formed on the surface. These drops grow and coalesce, and the liquid flows from the surface. During this condensation, large areas of tube are devoid of any liquid and are exposed directly to the vapor. Very high rates of heat transfer occur on these bare areas.

Dropwise condensation occurs on contaminated surfaces and when impurities are present. Film-type condensation is more dependable and more common. Hence, for normal design purpose, film-type condensation is assumed.

2.4.1.3 Distillation

The separation process known as distillation is method for separating the various components of a liquid solution which depends upon the distribution of these components between a vapor phase and a liquid phase. All components are presents in both phases. The vapor phase is created from the liquid phase by vaporization at the boiling point. The basic requirement for the separation of components by distillation is that the composition of the vapor be different from the composition of the liquid with which it is in equilibrium at the boiling point of the liquid.

Distillation is concerned with solutions where all components are appreciably volatile, such as ammonia-water or ethanol-water solution, where both components will be in the vapor phase. In evaporation, by contrast, of solution of salt and water, for example the water is vaporized but the salt is not. The process of absorption differs from distillation in that one of the components in absorption is essentially insoluble in the liquid phase. An example is absorption of ammonia from air by water, where air is insoluble in the water-ammonia solution.

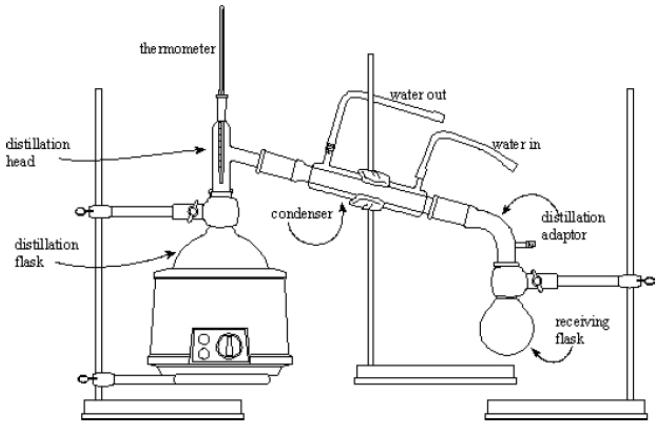


Figure 1.1: Simple Distillation

2.4.1.3.1 Oils Distillation

Essential oils are the volatile organic constituents of fragrant plant matter. They are generally composed of a number of compounds, including some that are solids at normal temperatures, processing different chemical and physical properties.

The aroma profile of the oil is a cumulative contribution from the individual compounds. The boiling points of most of these compounds range from 150 to 300°C at atmospheric pressure. If heated to this temperature, labile substances would be destroyed and strong resinification would occur. Hydro distillation permits the safe recovery of these heat sensitive compounds from the plant matter.

Hydro distillation involves the use of water or steam to recover volatile principles from plant materials. The fundamental feature of hydro distillation is that it enables a compound or mixture of compounds to be distilled and subsequently recovered at a temperature substantially below of the boiling point of the individual constituents.

2.4.1.3.2 Hydro-distillation

The high content of essential oil in commercial gingers contributes to demands in the flavouring and fragrance industries. Essential oil exists in all *Etlingera* species. Because of its high content of essential oil, *Etlingera elatior* for example, is highly demanded by the industry. The highest content is usually in the rhizomes of this plant. In the present study, the essential oil from the rhizomes of *Etlingera* sp. 5 is to be investigated.

Hydro-distillation is a method where the botanic material is completely immersed in water and then boiled. Through this procedure the oil will be extracted to a certain degree since the surrounding water will acts as a barrier in preventing the material from overheating.

A Clevenger-type hydro-distillation apparatus will be used. Upon cooling, the water and the essential oil will separate in the collector. This hydro-distillation process can be done at a reduced pressure (under vacuum) to decrease the temperature to less than 100^0C. This can be beneficial in protecting heat sensitive chemical compounds from rearrangement or complete decomposition which will affect the essential oil quality.

The difference between hydro-distillation and steam-distillation is that in the hydro-distillation the materials will be directly in contact with the water while in steam-distillation, the materials are not in direct contact with the water. Essential oil is extracted by passing steam through the raw material.

2.5 Essential Oil

The high content of essential oil in commercial gingers contribute to demands in the flavouring and fragrance industries. Essential oil exist in all ginger plants in the genus *Etlingera*. The highest content is usually in the rhizomes of this plant. In the present study, the essential oil from the rhizomes of *Etlingera* sp. 5 is to be investigated.

An essential oil is any concentrated, hydrophobic liquid containing volatile aroma compounds from plants. They are also known as volatile or ethereal oils, or simply as the "oil of" the plant material from which they were extracted, for example, oil of clove. The term essential indicates that the oil carries distinctive scent (essence) of the plant, not that it is an especially important or fundamental substance. Essential oils do not as a group needs to have any specific chemical properties in common, beyond conveying characteristic fragrances. They are not to be confused with essential fatty acids.

From the hydro-distillation process, we will obtain an essential oil of wild *Etlingera* sp. 5. One half of the essential oil will be put into an amber vial and stored in a refrigerator, while the other half is poured into a clear, colorless vial which is left on a laboratory shelf with a slightly loosened cap. The latter is done in order to investigate and determine the effect of exposure to heat and light and long standing on the quality of the essential oil. The essential oils will be analyzed using gas chromatography-mass spectrometer.

2.5.1 Physical Properties of Essential Oils

Essential oils actually are not oily, unlike the other essential oil extracted from vegetables and nuts. Some essential oils are viscous; others are fairly solid and most are somewhat watery (http://www.essentialwholesale.com/aromatherapy.html). Essential oils have a lipid-soluble molecular structure that allows them to pass through skin easily which is the basis for using them in massage and aromatherapy. There are about 3000 essential oils available throughout the whole world yet only 300 essential oils are used generally.

Essential oils are the most concentrated form from any botanical. It is commonly used in pharmacological because of its nature as an effective remedy for numerous of deceases. They are very volatile and should be kept in a very tight bottle so that they cannot evaporate so easily into the air. Essential oils should also be kept in a very small bottle if they are in small amount so that it does not get exposed to the air inside the bottle. Exposure to heat and light also can damage the quality of the essential oils so it must be stored in a dark or wrapped bottles and place with appropriate temperature.

2.5.2 Chemical Properties of Essential Oil

Essential oils have a very unique chemical property. Every single oil is normally has more than a hundred components (http://www.essentialoils.co.zalcomponents.htm). These components can be determined using analytical apparatus such as the gas chromatography and high performance liquid chromatography. Some of the chemical compounds that can be regularly found in the essential oils are sesquiterpenes, monoterpenes and phenols.

Sesquiterpenes consists of 15 carbon atoms and has a complex pharmacological action. It has anti-inflammatory and anti-allergy properties. Due to its nature essential oils that are high in phenols should be used in low concentration and in a short period of time. This is because they can lead to toxicity to the body as the liver will be required to work harder to excrete them if the body has accumulated

it over a long period of time. Although phenols have a very great antiseptic quality, they also can cause severe skin reactions and was then classified as skin and mucus membrane irritants.

Another chemical compounds regularly seen in essential oils are monoterpenes. It can be found nearly in all essential oils produced from the plant extraction process and have 10 carbons with at least 1 double bond structure. The 10 carbons are derived from 2 isoprene units and they can react readily to air and heat sources. Due to this, the higher the amounts of this compounds in the essential oils, the lesser the time it will last with high quality. The usage of it can be seen as a large broad generalization as these groups of chemicals vary greatly from the others. Some maybe used as anti-inflammatory, antiseptic, antiviral antibacterial therapeutic properties while some can be analgesic or stimulating with a tonic effect. Since some also have a stimulating effect on the mucus membrane they are also usually used as decongestants.

2.6 Gas Chromatography-Mass Spectrometer (GC-MS)

Gas-liquid chromatography is based on partitioning of the analyte between a gaseous mobile phase and a liquid phase immobilized on the surface of an inert solid packing or on the walls of capillary tubing. This concept was first enunciated in 1941 by A. J. P. Martin and R. L. M. Synge, who where responsible for the development of liquid-liquid partition chromatography (Skoog and West, 1996).

In gas chromatography, the components of a vaporized sample are fractioned as a consequence of being partitioned between a mobile gaseous phase and a liquid or a solid stationary phase held in a column. In performing this method, the sample is vaporized and injected onto the head of the chromatography column. Elution is brought about by the flow of an inert gaseous mobile phase. The efficiency of the GC is dependant on the compounds traveling through the column at different rates. The rate is depending on the factors as listed below:

i) Volatility of compound: low boiling (volatile) components will travel faster through the column than high boiling components.

ii) Polarity of compounds: polar compounds will move more slowly, especially if the column is polar.

iii) Column temperature: higher column temperature will rise up the compounds speed.

iv) Column packing polarity: usually all compounds move slower on polar column, but polar compounds will show a larger effect.

v) Flow rate of the gas: higher speed of the carrier gas flow will increase the speed of all compounds in the column.

vi) Length of the column: the longer the column, the longer time it takes. Longer column are used to obtain better separation result.

GC-MS are a combined technique in which a mass spectrometer is used as a detector for gas chromatography. The effluent from the gas chromatograph is passed into the inlet of a mass spectrometer, where the molecules of the gas are fragmented, ionized and analyzed using one of a variety of different types of mass analyzers.

GC-MS are different from the High Performance Liquid Chromatography (HPLC) because it uses gas as its mobile phase while the HPLC uses liquid as its mobile phase.

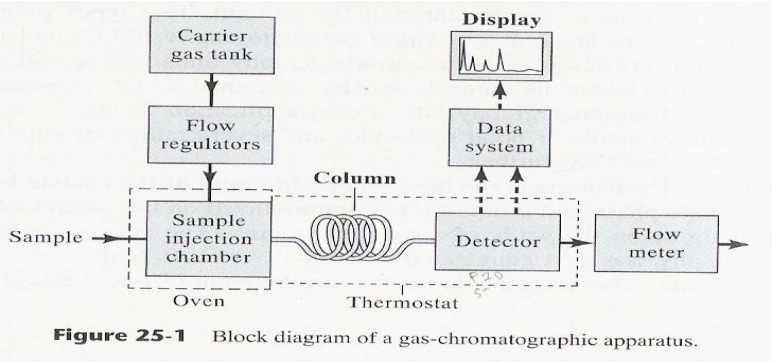
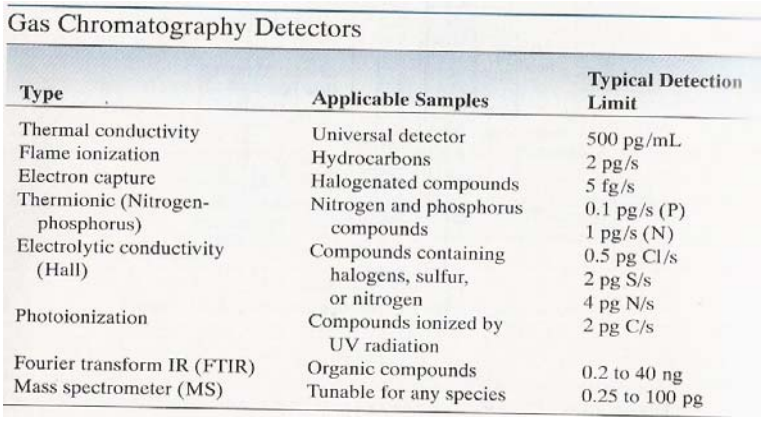


Figure 2.1: Block diagram of a gas – chromatographic apparatus



Gas Chromatography Detectors

Type	Applicable Samples	Typical Detection Limit
Thermal conductivity	Universal detector	500 pg/mL
Flame ionization	Hydrocarbons	2 pg/s
Electron capture	Halogenated compounds	5 fg/s
Thermionic (Nitrogen-phosphorus)	Nitrogen and phosphorus compounds	0.1 pg/s (P) 1 pg/s (N)
Electrolytic conductivity (Hall)	Compounds containing halogens, sulfur, or nitrogen	0.5 pg Cl/s 2 pg S/s 4 pg N/s
Photoionization	Compounds ionized by UV radiation	2 pg C/s
Fourier transform IR (FTIR)	Organic compounds	0.2 to 40 ng
Mass spectrometer (MS)	Tunable for any species	0.25 to 100 pg

Figure 2.2: Type of Gas Chromatography detectors

CHAPTER 3

METHODOLOGY

3.1 Material collection

The sample was collected near Bentong, Pahang by Professor Halijah Ibrahim, a botanist, for a separate taxonomic study. *Etlingera* sp. Voucher specimen was prepared, labeled as *Etlingera* sp. *5* and deposited at the Universiti Malaya herbarium.

3.2 Material preparation

First, rhizomes of *Etlingera* sp.5 rhizomes were washed to remove dirt and other debris. This is to ensure that foreign substances will not interfere with the distillation process. Then the rhizomes were milled, in order to increase its surface area so that it can be more efficiently extracted. Extraction efficiency is dependant on the surface area, the greater the surface area the greater the efficiency because contact between solvent and the materials is high. Then the milled sample is left in a drying oven at 39^{o}C overnight and the dried rhizome is shown as figure 3.1.

Figure 3.1: Dried rhizomes of *Etlingera* sp. 5

The dried sample (Figure 3.1) was carefully weighed and then left soaking in distilled water for six hours prior to hydrodistillation.

3.3 Hydro-distillation

In order to obtain essential oil from the rhizomes, an extraction process called the hydrodistillation process was used. Prior to it, the sample was soaked in water in order to break down the parenchymatous cells and oil glands. This would help in expediting the extraction process. Some of the parenchymatous cells and oil glands are also believed to break down during the washing and milling process but these are negligible.

The Clevenger-type apparatus (Figure 3.2) for distillation was set up and mixture of 50.0 gram of milled plant sample and 500 mL of water was put into a 1000 mL round bottomed flask. After that the heating mantle and water supply were switched on. The temperature was set at 80^0C for the extraction process which was for 6 hours.

In order to avoid heat loss, the apparatus was wrapped with aluminum foil. A couple of boiling chips were put into the flask to ensure bumping did not occur during distillation.

The essential oil then vaporized with the steam. Condensation occurred as the essential oil vapor and steam mixture passed through a condenser. The condensate which was a mixture of water and essential oil was then released into a separatory funnel. The essential oil and water were then separated and the essential oil was dried over anhydrous sodium sulfate. It was then filtered by gravity into a pre-weighed amber vial. The weight of the essential oil was determined by difference.

One half of the essential oil was poured into a clear, colorless vial and left on a laboratory shelf with a slightly loosened cap. The latter was done in order to investigate and determine the effect of exposure to heat and light and long standing on the quality of the essential oil.

Figure 3.2: A Hydrodistillation Set-up

Hydrodistillation was conducted for six hours until the liquid droplets collected were colorless. The percent of essential oil collected was 0.732% (or 0.366g based on dry weight).

3.4 Sample Preparation

The essential oil collected was stored in an amber vial and a small quantity subjected to instrumental analysis immediately to record the GC-FID chromatogram and identify compounds present by GC/MS. The remaining essential oil was put into

3 vials (Figure 3.2a and b), where one served as reference, one for the light exposure test and the other for temperature exposure test. Samples were prepared in 2ml a size, which is 1% sample.

In Light test show in figure 3.6, the temperature room is 31°C-33°C where the room humidity is around 42% - 45%, all of the three vials are placed in a glass cabinet. This light test is exposing to the sunlight and florescence light during working hours and only sunlight when not in working hours (Figure 3.3b). For the Temperature test, the vials are placed near a window, with direct sunlight every morning which the temperature is around 43°C- 49°C and the place humidity is around 33%-37% (Figure 3.3a). This room also are equip with air conditioner where the air conditioner are operate during working hours.

For the two sets of test, the essential oil is prepared using the fresh essential oil. 1 % of the essential are used to produce two samples, one for the light test and one for the temperature test. This 1 % of essential oil is prepared in 2 mL of Hexane, and then it is divided into two, with 1 mL for each test. The fresh essential oil are then put into the refrigerator to maintain its freshness and preserve it from contamination or exposed to heat or light. It is also will be used as the control parameter.

Each 1 mL of the sample prepared is put in vials. There are two types of vials used in this procedure, the clear & colourless vials for the effect of light test and the dark brown vials for the effect of temperature test. Three vials are used (Figure 3.4a and 3.4b) for each test, with the 1 mL of the sample prepared are divided into three to fill each vials for their test.

Figure 3.3a: Placement of vial for temperature test.

Figure 3.3b: Placement of vial for light test.

The monitoring will be held in 1 week interval, after each interval finish the essential oil will be analyze using GC-FID to identify the constituent that consist in the essential oil.

Figure 3.4a: Light Test Vials that use for GC-FID analysis

Figure 3.4b: Temperature Test Vials that use for GC-FID analysis

3.5 GC-FID Analysis

A gas chromatography- Flame Ionized Detector (GC-FID) instrument (Figure 3.5) was used to analyze the essential oil. The GC-FID can also be used to separate small amounts of materials and determine whether a desired component was present. The GC-FID consists of 3 essential parts. They are an injection block, a column and a detector.

For GC-FID analysis, the sample to be analyzed is introduced in the sample port, at about 50^{o}C above the boiling point of the least volatile component of the sample. A capillary column requires samples that are smaller by a factor of 10 or more. The sample splitter is often needed to deliver only a small known fraction (1:100 to 1:500) of the injected sample, with the remainder going to waste. After the sample of the essential oil is vaporized, it will be injected onto the head of the chromatography column. This sample will be transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid. The oven temperature is set between 50°C – 290°C at 3°C per minute increment; temperature injection is set at 220°C and inlet temperature at 250°C using the splitless mode. The sample will finally enter the detector after going through the column. Then the reading from the analyzed substance can be monitored using the computer.

Figure 3.5: A GC-FID instrument

3.6 Gas Chromatography Mass Spectrometer (GC/MS)

GC/MS (Figure 3.6) are a combined technique in which a mass spectrometer is used as a detector for gas chromatography. The effluent from the gas chromatograph is passed into the inlet of a mass spectrometer, where the molecules of the gas are fragmented, ionized and analyzed using one of a variety of different types of mass analyzers. This type of detector is commonly use and are tunable for any species with the typical detection limit from 0.25 to 100 pg. Identification of oils constituents, for example, is by comparison of mass spectra with the MS library in the Gas Chromatography – Mass Spectrometer (GC/MS).

Method that were use is the same when doing the monitoring using the GC – FID.

Figure 3.6: GC/MS instrument

The essential oil that been analyze is divided into one dark brown vial and one clear & colourless vial where the brown vial is put in a temperatured place, close seal while the colourless vial is let at a room temperature and expose to the light.

3.7 Essential Oil Quality

Essential oil samples quality will be monitored by GC-FID. The freshly hydrodistilled essential oil that had been analyzed was divided into one amber vial and one colourless vial. The amber vial was subjected to temperature, while the colourless vial was exposed to light. Monitoring was carried out every week by GC-FID.

3.8 Flow Diagram of Methodology

Figure 3.7 below showed the overall methodology from the beginning of the research until write up of the report.

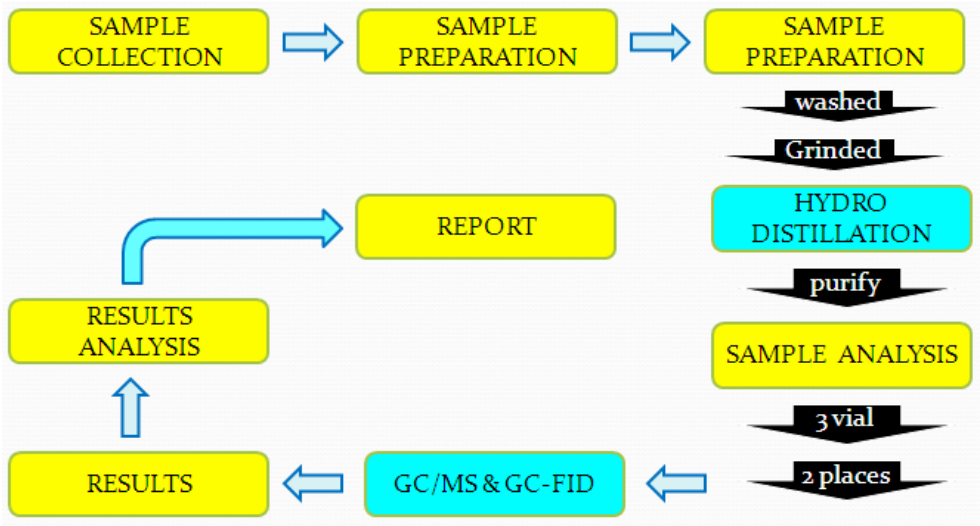


Figure 3.7: Flow Diagram of the methodology

CHAPTER 4

RESULTS AND DISCUSSION

4.1 GC-FID of essential oil of *Etlingera* sp. 5

Figure 4.1 illustrates the chromatographic profile of the freshly hydrodistilled essential oil of *Etlingera* sp. 5 which had been analyzed by GC-FID.

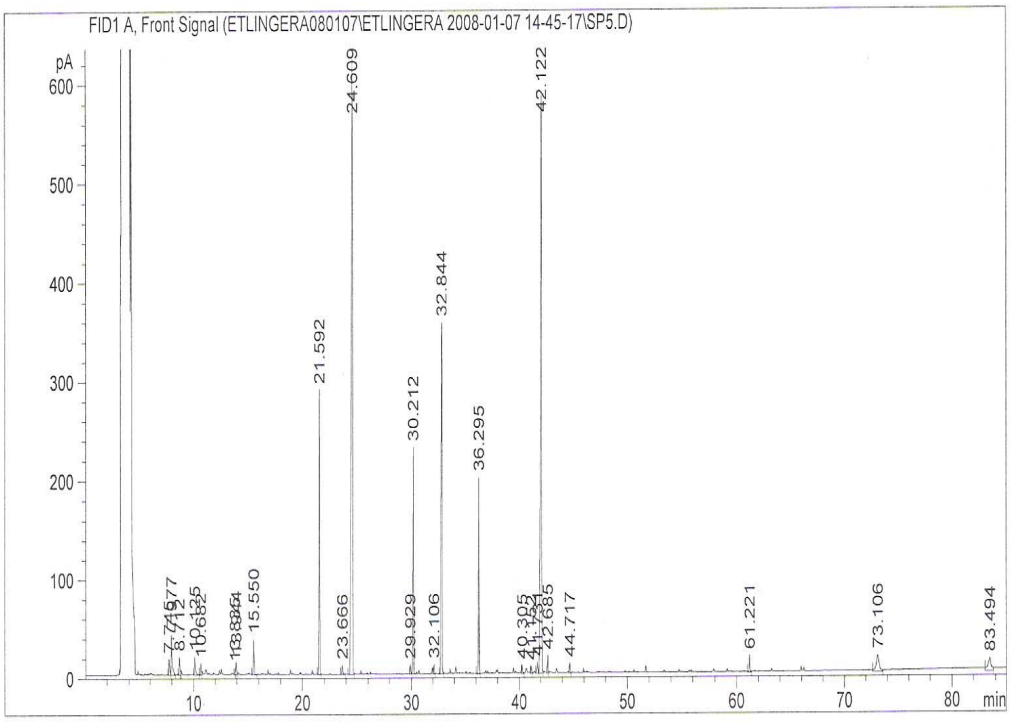


Figure 4.1: GC-FID of Freshly Hydrodistilled Essential Oil of *Etlingera* sp. 5

4.1.1 GC-FID of essential oil of *Etlingera* sp. 5 exposed to temperature

Figures 4.2a, b and c, illustrate the GC-FID generated chromatographic profile of the freshly hydrodistilled essential oil of *Etlingera* sp.5 which had been exposed to temperature (^{o}C) after one (1), two(2) and three(3) weeks, respectively.

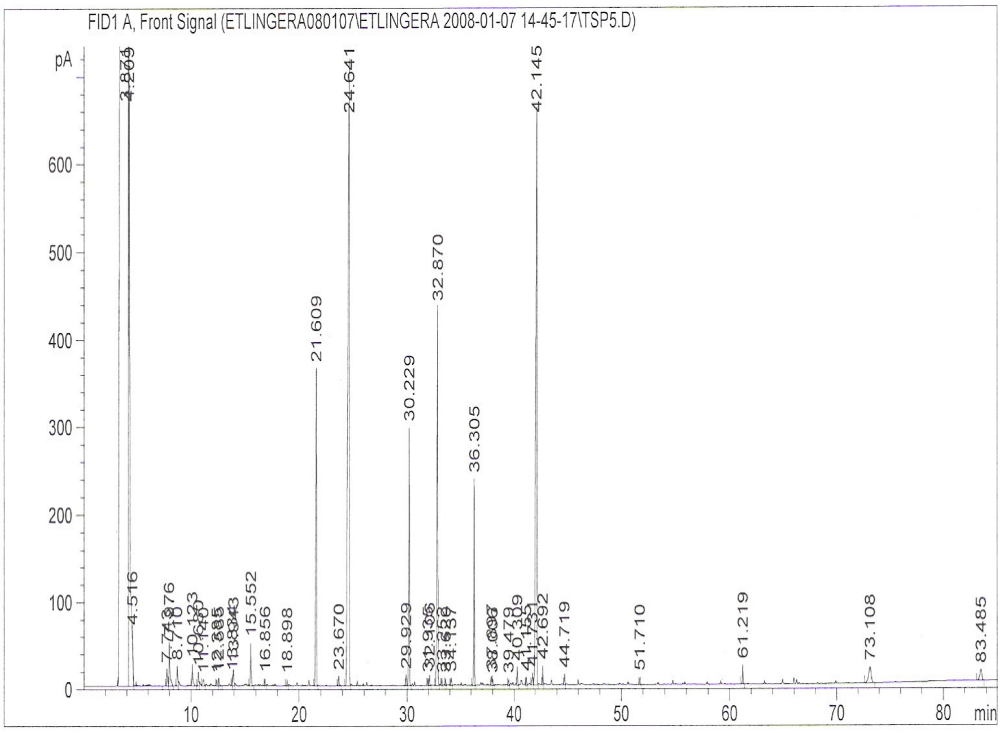


Figure 4.2a: GC-FID of Freshly Hydrodistilled Essential Oil of *Etlingera* sp. 5 after exposure to temperature at 33^{o}C - 37 ^{o}C for one (1) week

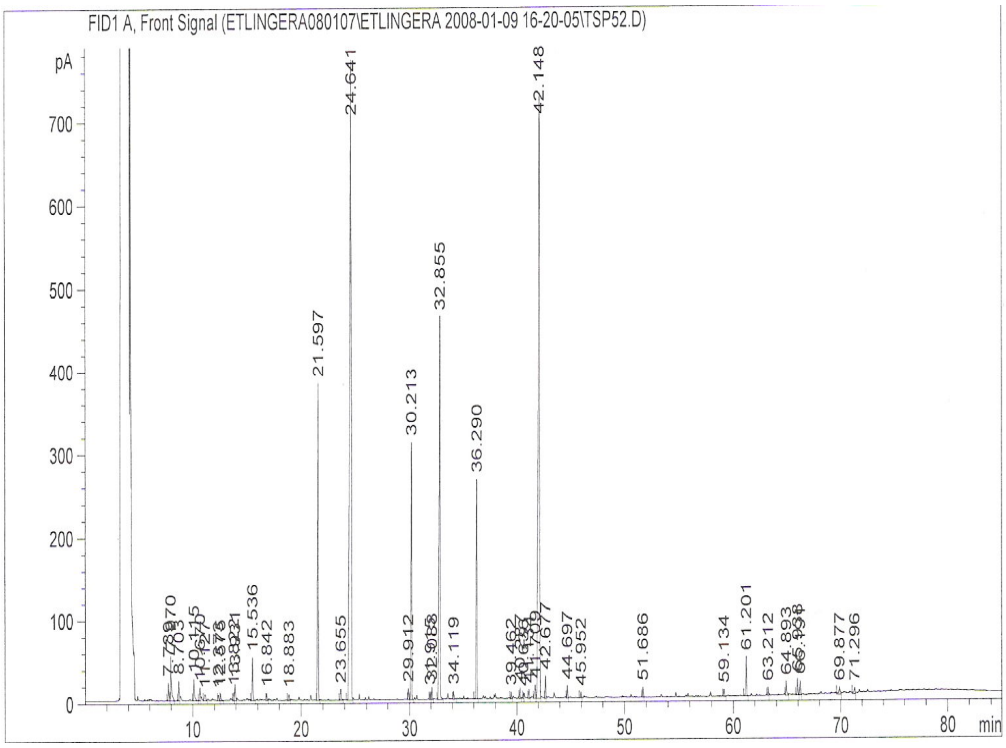


Figure 4.2b: GC-FID of Freshly Hydrodistilled Essential Oil of *Etlingera* sp. 5 after exposure to temperature at 33^{o}C - 37 ^{o}C for two (2) weeks

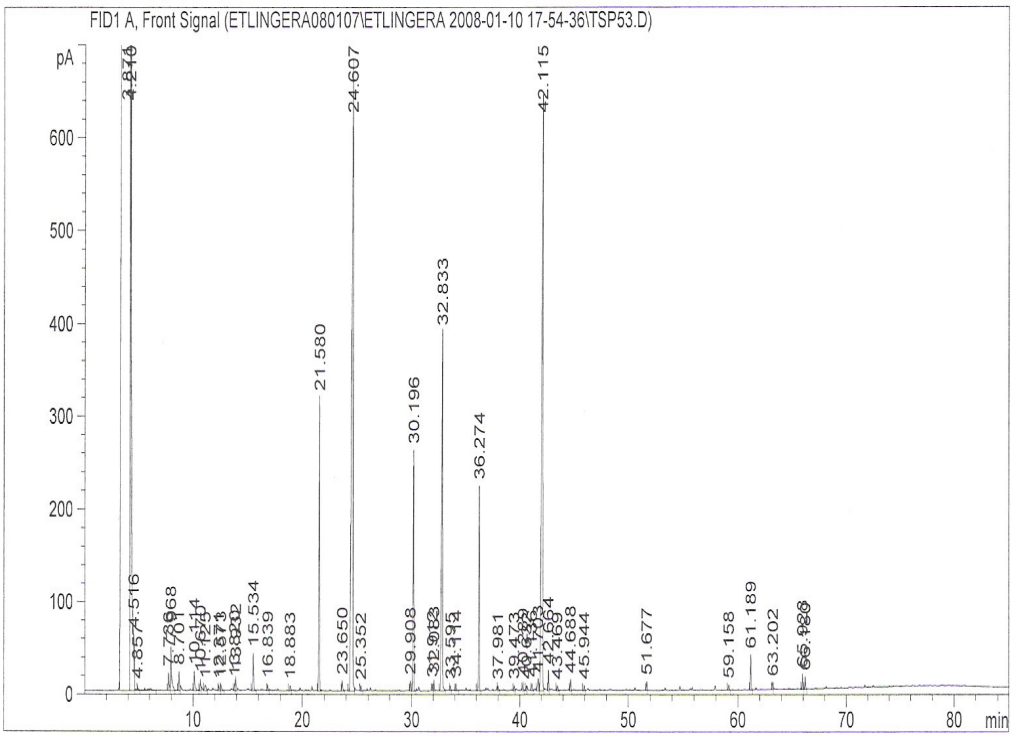


Figure 4.2c: GC-FID of Freshly Hydrodistilled Essential Oil of *Etlingera* sp. 5 after exposure to temperature at 33^{o}C - 37 ^{o}C for three (3) weeks

4.1.2 GC-FID of essential oil of *Etlingera* sp. 5 exposed to light

Figures 4.3a, b and c, illustrate the GC-FID generated chromatographic profile of the freshly hydrodistilled essential oil of *Etlingera* sp.5 which had been exposed to light after one (1), two(2) and three(3) weeks, respectively.

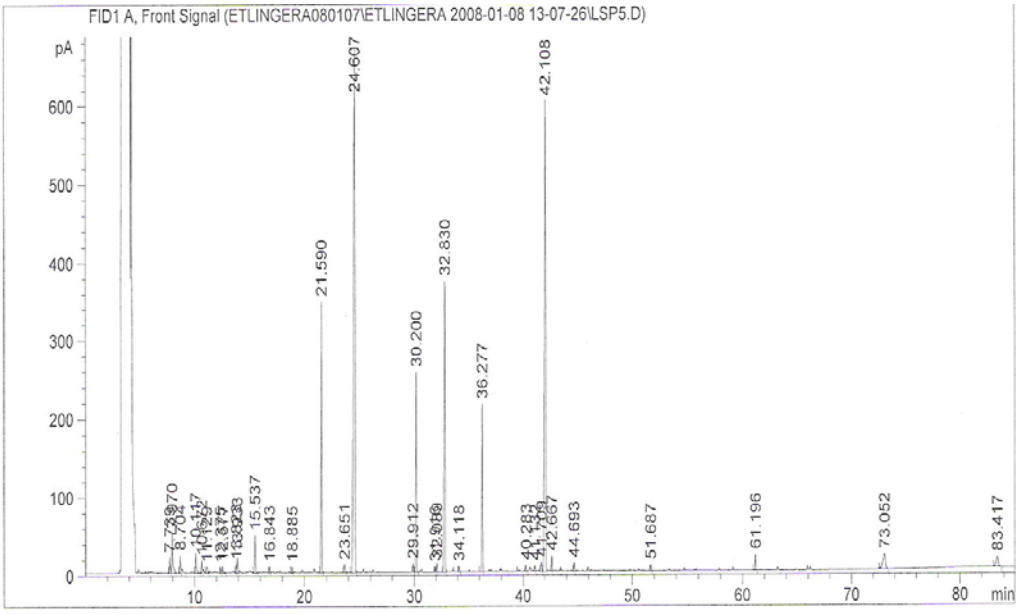


Figure 4.3a: GC-FID of Freshly Hydrodistilled Essential Oil of *Etlingera* sp. 5 after exposure to light for one (1) week

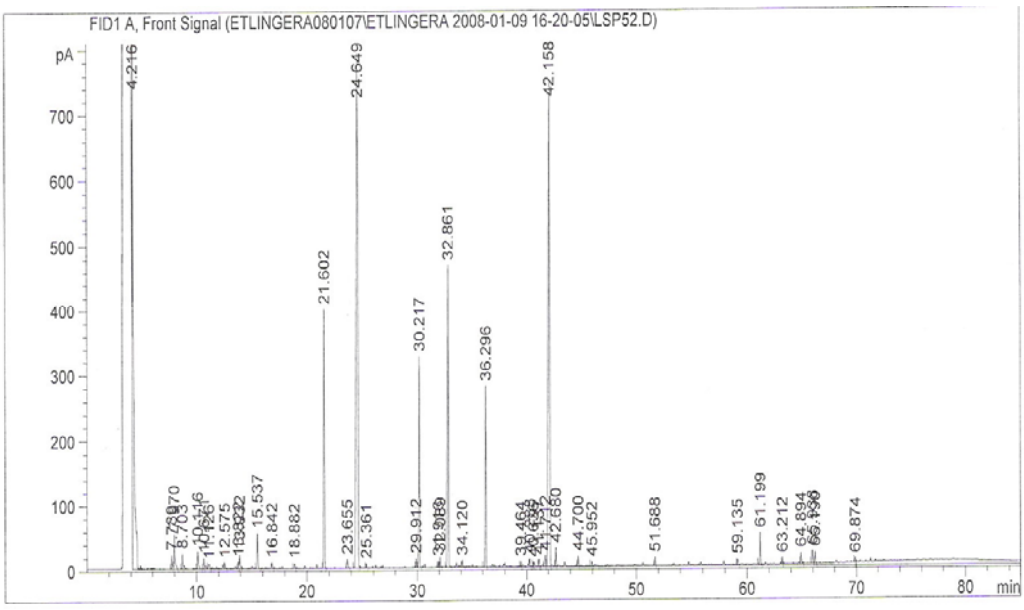


Figure 4.3b: GC-FID of Freshly Hydrodistilled Essential Oil of *Etlingera* sp. 5 after exposure to light for two (2) week

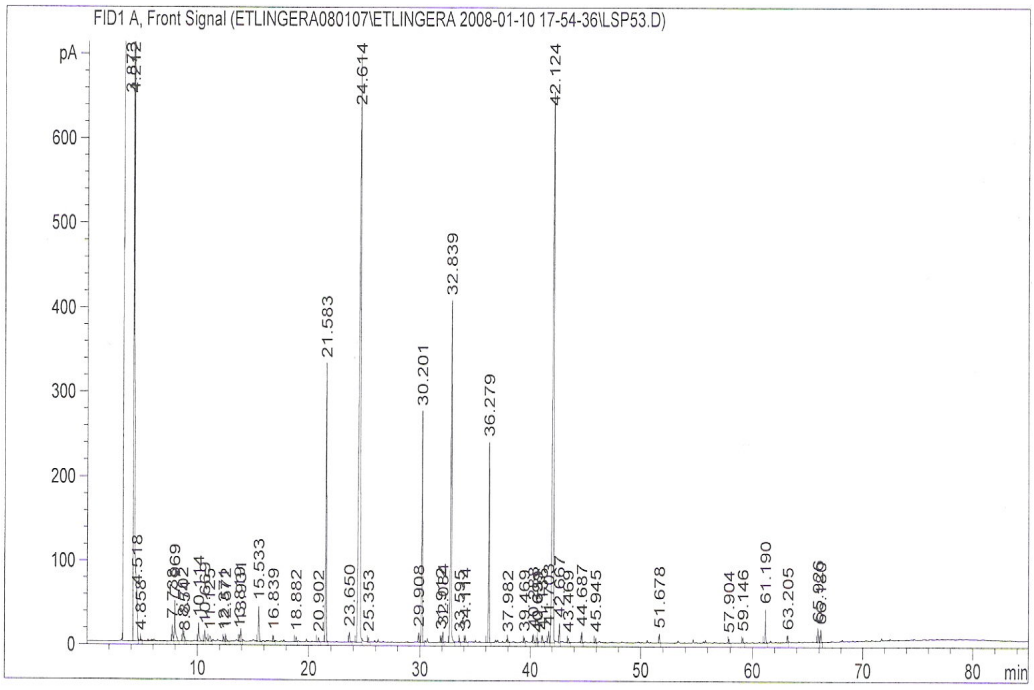


Figure 4.3c: GC-FID of Freshly Hydrodistilled Essential Oil of *Etlingera* sp.5 after exposure to light for three (3) week

4.1.3 GC/MS of essential oil of *Etlingera* sp. 5

Samples of *Etlingera* sp. 5 were subjected to GC/MS analyses using the established instrumental parameters optimized during GC-FID acquisitions of chromatograms. TIC's at 24.609, 32.106 and 42.122 minutes were selected as these peaks were found to be the major compounds in the freshly hydrodistilled essential oil. Mass spectra of the three TIC's were cross referenced with the GC/MS Library and the closest match were established.

Figure 4.4a illustrates the TIC selected at retention time 24.069 minutes while Figure 4.4b shows the mass spectrum arising from the MS analysis as Nerolidol (IUPAC: 3,7,11-trimethyl-1,6,10-dodecatriene-3-ol).

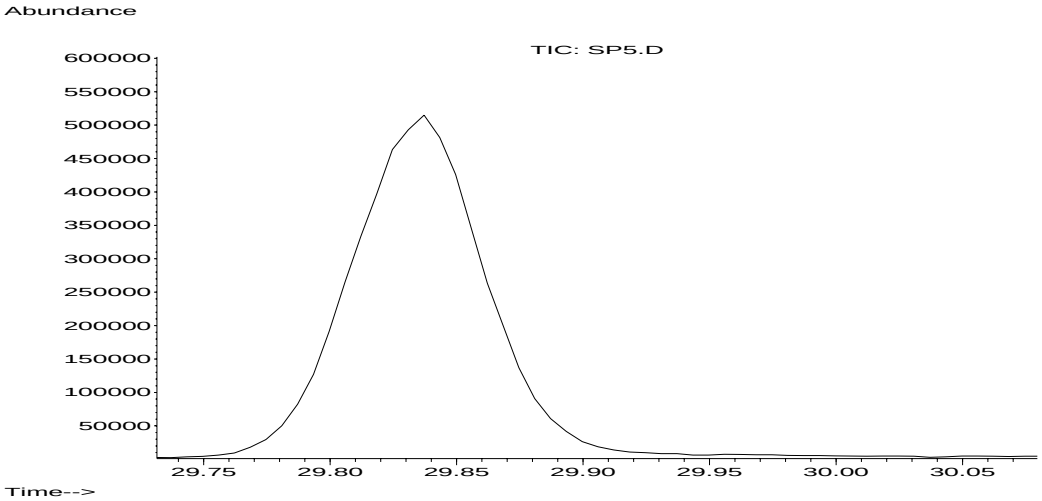


Figure 4.4a: TIC at retention time 24.069 minutes

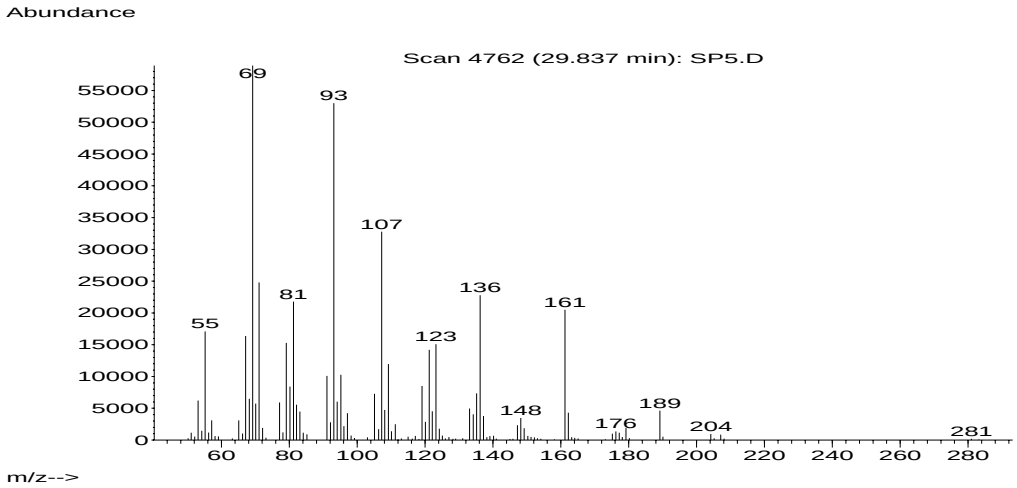


Figure 4.4b: Mass spectrum of Nerolidol

Figure 4.5a illustrates the second TIC selected at retention time 32.106 minutes while Figure 4.5b shows the mass spectrum arising from the MS analysis as Farnesol (IUPAC: 3,7,11-trimethyl-2,6,10-dodecatriene-1-ol).

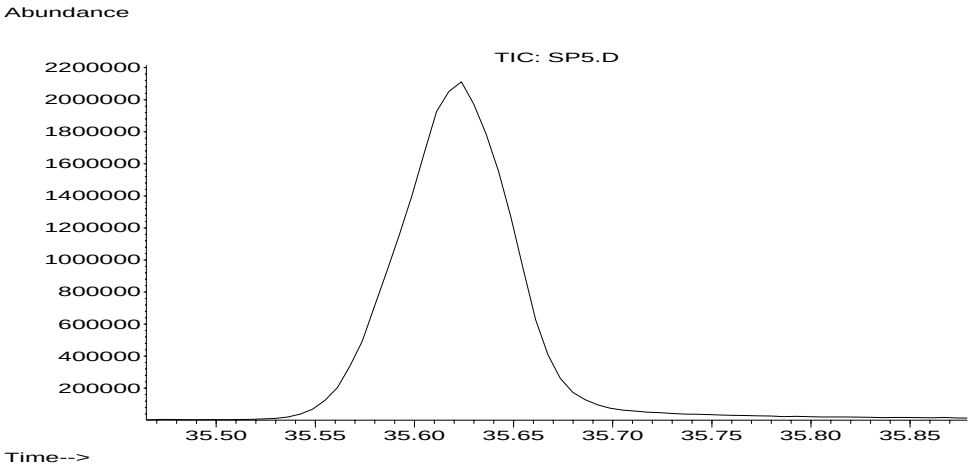


Figure 4.5a: TIC at retention time 32.106 minutes

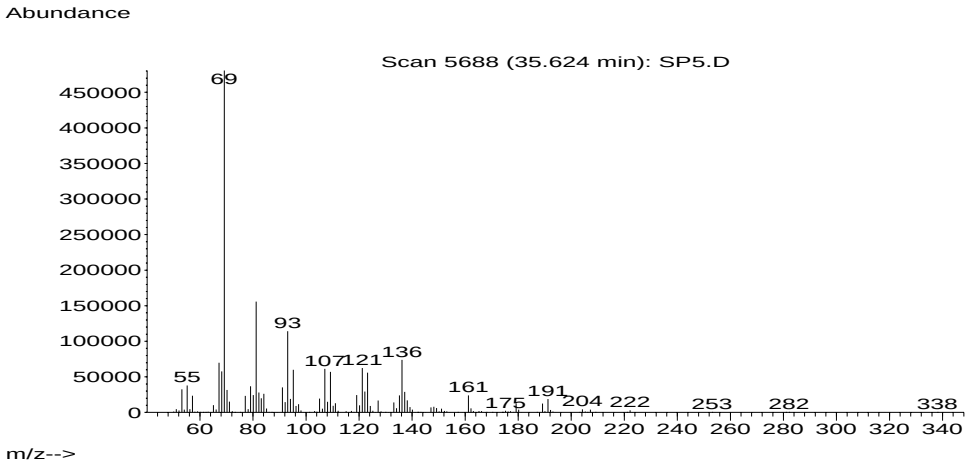


Figure 4.5b: Mass spectrum of Farnesol

Figure 4.6a illustrates the second TIC selected at retention time 42.122 minutes while Figure 4.6b shows the mass spectrum arising from the MS analysis as 1 – Tricosene.

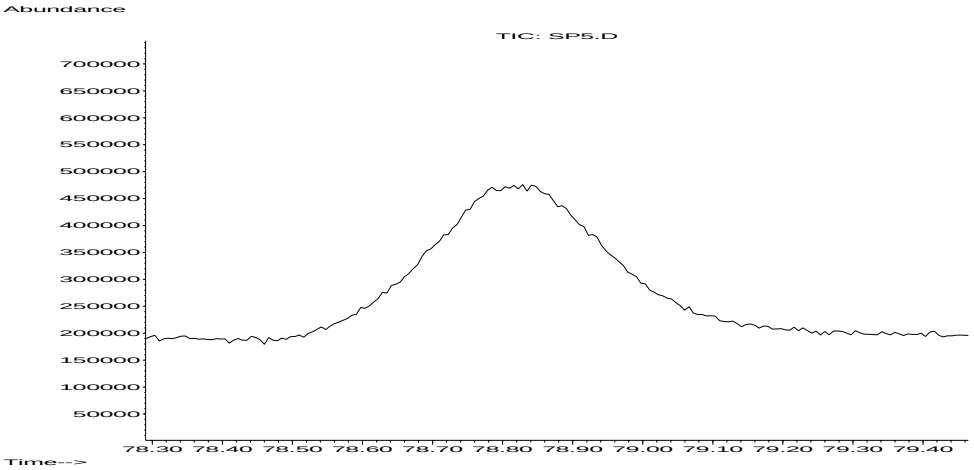


Figure 4.6a: TIC at retention time 42.122 minutes

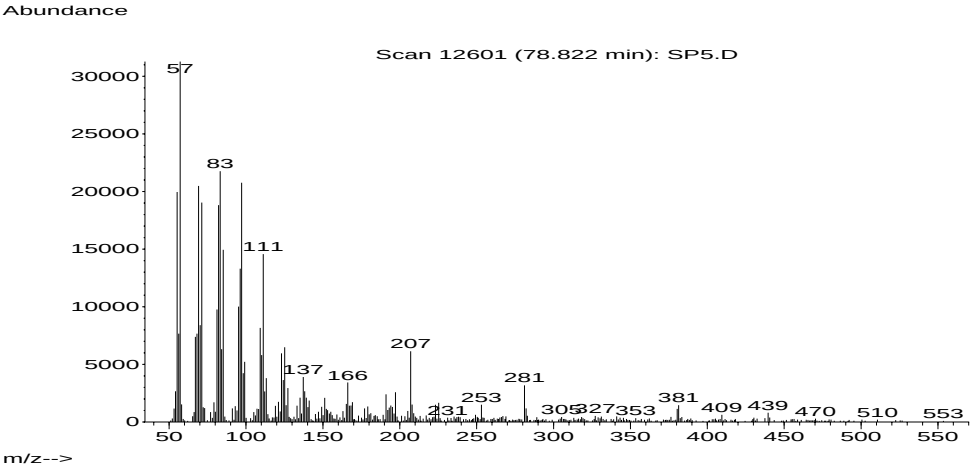


Figure 4.6b: Mass spectrum of 1 – Tricosene

4.1.4 Summary of Results

Table 4.1 summarizes the findings of the present study. The amount of compounds present in the oils are quantified by % area.

Table 4.1: Summary of Findings Based on GC-FID and GC/MS

No.	rt (min)	% Area							Compound
		Fresh oil	LSP51	LSP52	LSP53	TSP51	TSP52	TSP53	
1	24.609	31.038	30.8959	30.8494	30.6106	30.3680	30.6135	31.1406	Nerolidol
2	32.106	12.091	11.4732	12.0933	12.0702	12.4825	12.0544	12.0878	Farnesol
3	42.122	26.070	24.3894	26.3341	25.9703	24.3006	26.0855	25.7846	1- Tricosene

Legend –

- rt – retention time;
- LSP51 – Light Test Species 5 week 1
- LSP52 – Light Test Species 5 week 2
- LSP53 – Light Test Species 5 week 3
- TSP51 – Temperature Test Species 5 week 1
- TSP52 – Temperature Test Species 5 week 2
- TSP53 – Temperature Test Species 5 week 3
- % Area – Percentage Area of peak in Chromatogram

4.2 Discussion

Based on detection and identification by GC-FID and GC/MS, three compunds, namely, nerolidol, farnesol and 1 – Tricosene, were confirmed to be present in the freshly hydrodistilled essential oil sample of *Etlingera* sp. 5 in substantial quantities. The three compunds were then carefully monitored for changes in their quantity when samples of the oil were exposed to temperature and light over periods of time.

From the table 4.1 above, the three compounds are slightly change when the two test were use to see the changes of the compounds inside the *Etlingera* sp. 5 essential oil. The changes are not have significant changes to this two test but some how the changes are seen change near to the % area of the fresh essential oil.

These compounds were monitor within 3 weeks duration where as we can see the changes are having a slight change to the % area of the compounds this happen because of the unstable condition of the room that effect the results of the compounds because the room was only have a florescence light during working hours and the room also expose to the air conditioner during the working hours.

4.2.1 Nerolidol

Nerolidol (Figure 4.7), also known as peruviol, is a naturally occurring sesquiterpene found in the essential oils of many types of plants and flowers. There are two isomers of nerolidol, *cis* and *trans*, which differ in the geometry about the central double bond. Nerolidol is present in neroli, ginger, jasmine, lavender, tea tree and lemon grass. The aroma of nerolidol is woody and reminiscent of fresh bark. It is used as a flavoring agent and in perfumery. It is also currently under testing as a skin penetration enhancer for the transdermal delivery of therapeutic drugs (www.wikipedia.org). As expected, nerolidol is also present in *Etlingera* sp. 5 as this plant is a member of the ginger family. Exposure of the oil to light and temperature did not diminish the quantity of this compound in the oil.

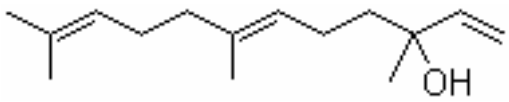


Figure 4.7: Nerolidol

4.2.1.1 Light Test

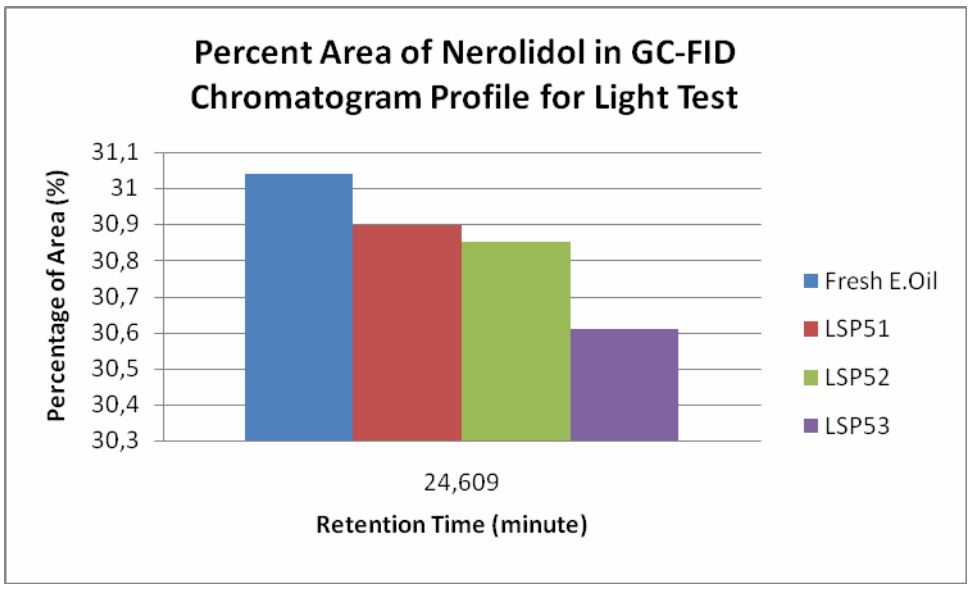


Figure 4.8: Percent Area of Nerolidol in GC-FID Chromatogram Profile for Light Test

From figure 4.8 as illustrated shows that the decreasing of the percent area of the Nerolidol when exposing to the Light for 3 weeks of monitoring. The changes that occur are having a small change and the percent of the changes is below than 10% which can be consider as none.

4.2.1.2 Temperature Test

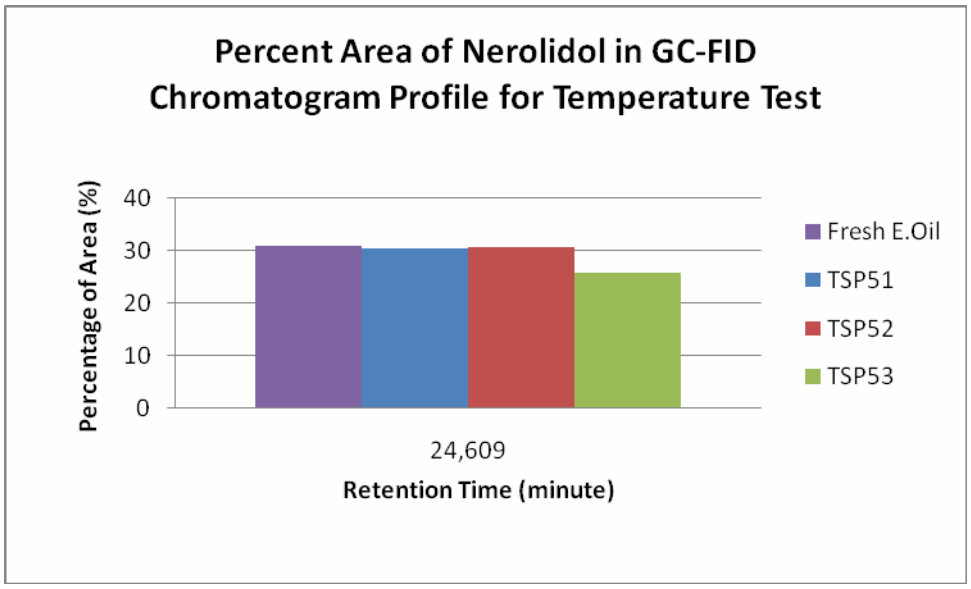


Figure 4.9: Percent Area of Nerolidol in GC-FID Chromatogram Profile for Temperature Test

Figure 4.9 illustrated the changes occurs along three weeks of monitoring where the changes seen to have a slightly change and the change are small which is below than 10% where can be consider as none.

4.2.2 Farnesol

Farnesol (Figure 4.10) is a naturally occurring sesquiterpene alcohol. A colorless liquid, it is insoluble in water, but miscible with oils. It is present in many essential oils such as neroli, cyclamen, ginger, lemon grass, tuberose, rose, balsam and tolu. It is used in perfumery to emphasize the odors of sweet floral perfumes. Farnesol is also a natural pesticide for mites and is a pheromone for several other

insects (www.wikipedia.org). As expected, farnesol is detected in *Etlingera* sp. 5 as this plant is a member of the ginger family. Exposure of the oil to light and temperature did not diminish the quantity of this compound in the oil.

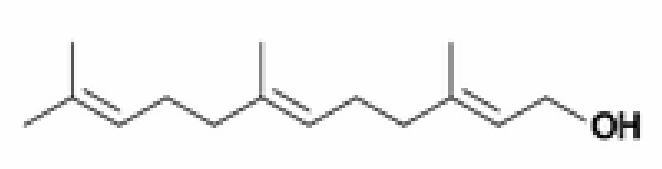


Figure 4.10: Farnesol

4.2.2.1 Light Test

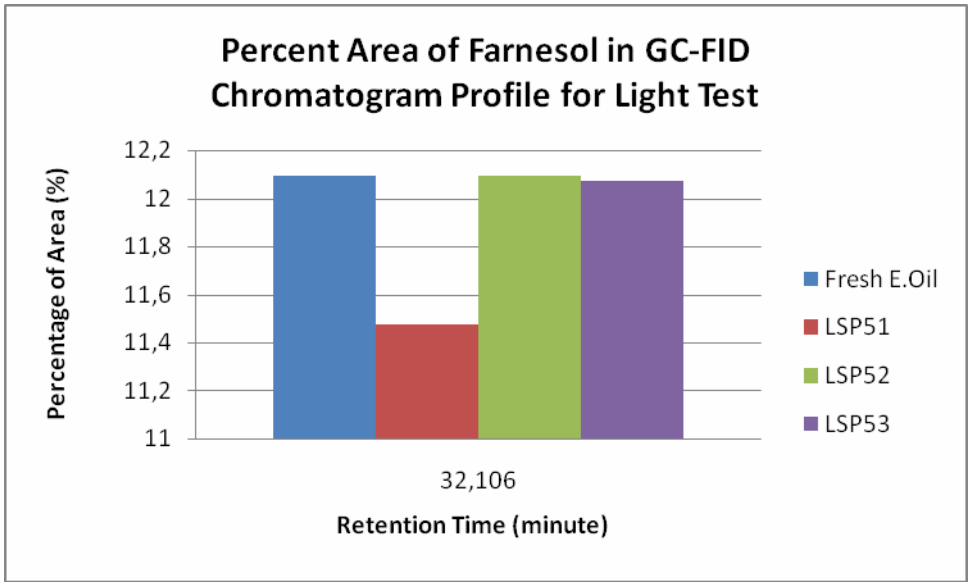


Figure 4.11: Percent Area of Farnesol in GC-FID Chromatogram Profile for Light Test

Figure 4.11 illustrated the changes of compound Farnesol in essential oil from *Etlingera* sp. 5 to the light test which is the changes are having fluctuated along three weeks of monitoring. The changes occur are small where it is less than 10% and this can be consider as none.

4.2.2.2 Temperature Test

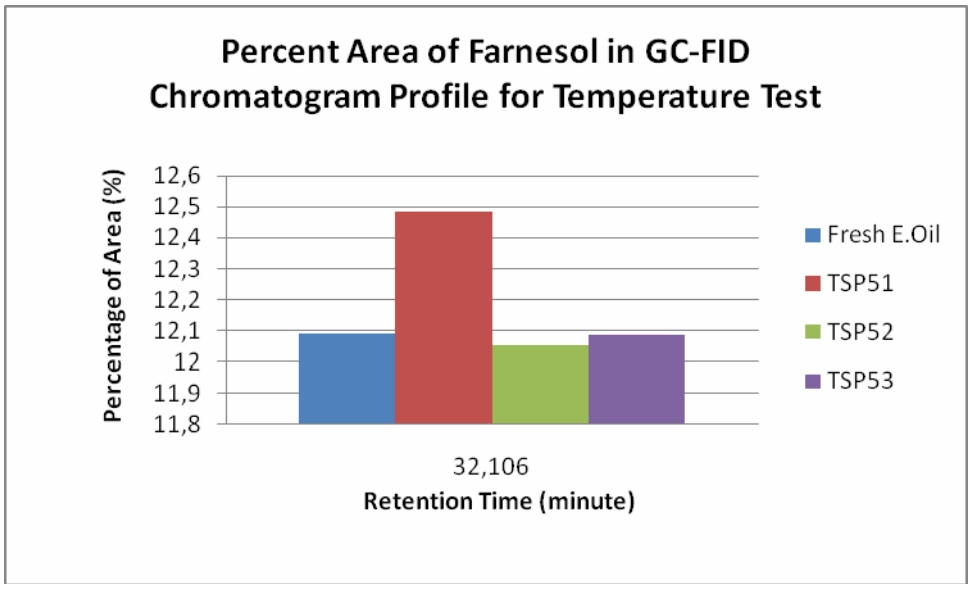


Figure 4.12: Percent Area of Farnesol in GC-FID Chromatogram Profile for Temperature Test

Figure 4.12 illustrated the changes of compound Farnesol in essential oil from *Etlingera* sp. 5 to the temperature test which is the changes are having a fluctuated along the three weeks of monitoring. The changes occur are small where it is less than 10% and this can be consider as none.

4.2.3 1 – Tricosene

1 – Tricosene (Figure 4.13) is a colorless liquid with a molecular weight of 322.62. It has the structural formula $CH_3(CH_2)_{12}CH=CH(CH_2)_7CH_3$. The CAS Number is 27519-02- 04. Trade names for this active ingredient include “muscalure” and “muscamone”. 1 – Tricosene is classified as a biochemical pesticide because, even though chemically synthesized, it is identical to the sex pheromone of the female house fly, *Musca domestica*. It is also used as an insect attractant as it has a “non-toxic” mode of action. 1 – Tricosene has been detected in *Etlingera* sp. 5. Exposure of the oil to light and temperature did not diminish the quantity of this compound in the oil.

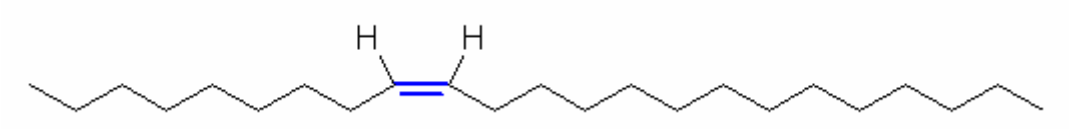


Figure 4.13: 1 – Tricosene

4.2.3.1 Light Test

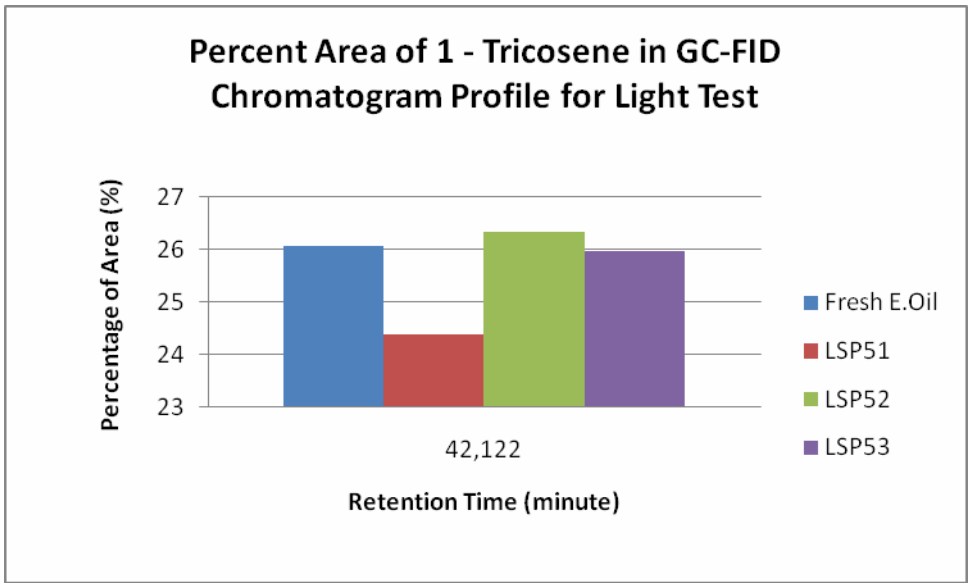


Figure 4.14: Percent Area of 1 – Tricosene in GC-FID Chromatogram Profile for Light Test

Figure 4.14 illustrated the changes of compound 1 – Tricosene in essential oil from *Etlingera* sp. 5 to the light test which is the changes are having a fluctuated along the three weeks of monitoring. The changes occur are small where it is less than 10% and this can be consider as none.

4.2.3.2 Temperature Test

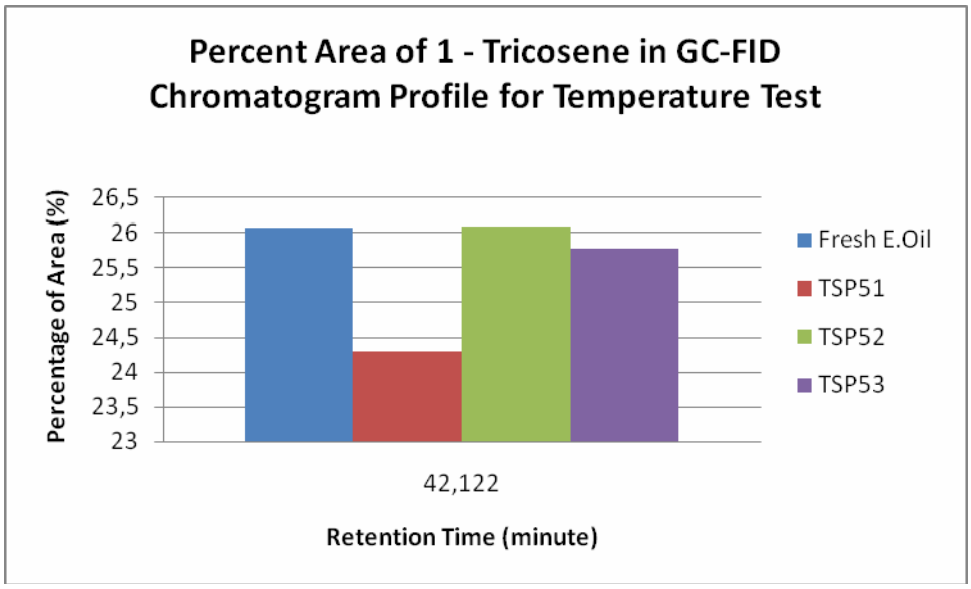


Figure 4.15: Percent Area of 1 – Tricosene in GC-FID Chromatogram Profile for Temperature Test

Figure 4.15 illustrated the changes of compound 1 – Tricosene in essential oil from *Etlingera* sp. 5 to the temperature test which is the changes are having a fluctuated along the three weeks of monitoring. The changes occur are small where it is less than 10% and this can be consider as none.

4.2.4 Summary of Discussion

The three compounds detected in *Etlingera* sp. 5 were found to be stable during storage. This monitoring only takes three weeks and the changes may have a significant change if the period of monitoring become longer. The other factor that may influence the slight change of the % Area is the place is not constantly in condition. Such as, inside the room the light are exposed to florescence light and sun light but after working hours there only sun light.

The conditions chosen were average household parameters of light and temperature. These compounds contribute in a major way to the fragrance industry as

well related industries. The presence of these compounds in a plant like *Etlingera* sp. 5 must be noted as this plant can be potentially exploited to manufacture these types of compounds. Plant manufacturing chemicals is now very popular in the biotechnology arena where genes of plants can be modified to allow the plant to generate specific types of chemical compound as industrial feedstock, especially for pharmaceutical and cosmeceutical uses.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

From the result, we may conclude that the *Ethligera sp. 5* was having a changers base on the compound inside the essential oil and the quality of the essential oil also changes due to the exposed to the light and temperature.

So, in order to preserve and maintain the high quality of essential oils; the essential oils should be kept in a dark place or dark container to avoid contact with light. It also should be kept in a closed container and at a cold place to avoid quality reduced because of the heat.

5.2 RECOMMENDATION

To get a significant change of the compounds in the essential oil, a longer period of monitoring was suggested. 3 weeks of monitoring was not enough to examine the minimum quality of the essential oil of *Etlingera* sp. 5.

Beside that, there was suggested that this research was using only the GC/MS or GC-FID only so that the effect of equipment was not interrupted the results.

The condition of the monitoring also was suggested that to have an maintained and constant condition to avoid any possible matter that affect the results

REFERENCE

C.A. Williams and J.B. Harborne, (1977) The leaf flavonoids of Zingiberales, *Biochemical Systematics and Ecology* **5**: 221–229.

Geankoplis, C.J. (2003). *Transport Processes and Separation Process Principles.* 4 ed. United States of America: Prentice – Hall.

Graham, R.C. (1993). *Data Analysis for the Chemical Sciences-A Guide to Statistical Techniques*. New York: VCH Publisher Inc.

Ibrahim and Setyowati, 1999 Ibrahim, H., & Setyowati, F.M., (1999). Etlingera. In: de Guzman, C.C., & Siemonsma, J.S. (Eds.), Plant resources of south-east Asia, Vol. 13, Pudoc, Wageningen, pp. 123–126.

Khaw, 2001 S.H. Khaw, The genus *Etlingera* (Zingiberaceae) in Peninsular Malaysia including a new species, *Gardens' Bulletin Singapore* 53**:**191–239.

Larsen, K., Ibrahim, H., Khaw S.H. and Saw, L.G. (1999). In *Gingers of Peninsular Malaysia and Singapore*, Wong, K.M. (ed.). Natural History Publications (Borneo): Kota Kinabalu, Sabah. Pp 57 – 66, 135

Lim, C.K. (2000) Taxonomic notes on *Etlingera* Giseke (Zingiberaceae) in Peninsular Malaysia: the "*Nicolaia*" taxa, *Folia Malaysiana* . 1: 1–12.

Lim, C.K. (2001) Taxonomic notes on *Etlingera* Giseke (Zingiberaceae) in Peninsular Malaysia: the "*Achasma*" taxa and supplementary notes on the "*Nicolaia*" taxa, *Folia Malaysiana* 2: 141–178.

Miean K.H. and Mohamed S (2001), Flavonoid (myricetin, quercetin, kaempferol, luteolin and apigenin) content of edible tropical plants, *Journal of Agricultural and Food Chemistry* 49**:** 3106–3112.

Noweg T, Abdullah A.R. and Nidang D (2003), Forest plants as vegetables for communities bordering the Crocker Range National Park, *ASEAN Review of Biodiversity and Environmental Conservation (ARBEC)* pp. 1–18.

Sirirugsa, 1999 Sirirugsa, P., (1999). Thai Zingiberaceae: species diversity and their uses. <http://www.iupac.org/symposia/proceedings/phuket97/sirirugsa.html> Accessed 11.09.07.

Skoog, D.A. and West, D.M. (1996). *Fundamental of Analytical Chemistry*. 7ed. Philadelphia: Saunders College Publishing Co.

Skoog, D.A., West, D.M., Holler, F.J. and Crouch, S.R. (2000). *Analytical Chemistry An Introduction*, 7 ed. Singapore: Thomson Learning.

Retrieved August 25, 2007 From World Wide Web:
http://www.essentialoils.co.zalcomponents.htm

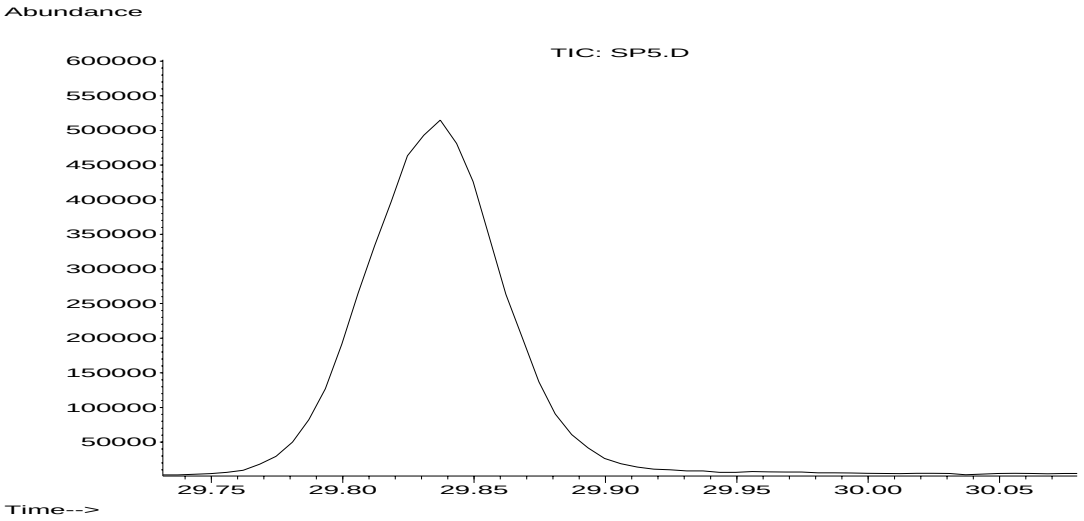
Retrieved August 25, 2007 From World Wide Web:
http://www.essentialwholesale.com/aromatherapy.html

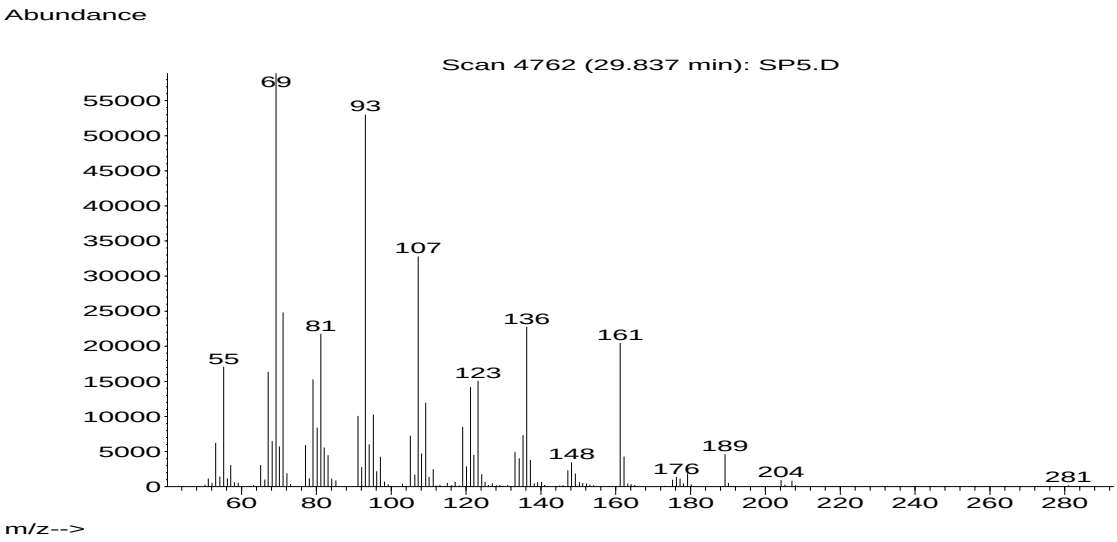
APPENDIX A

CHROMATOGRAM PROFILE OF MAJOR COMPOUNDS DATA FROM GC – FID

APPENDIX B

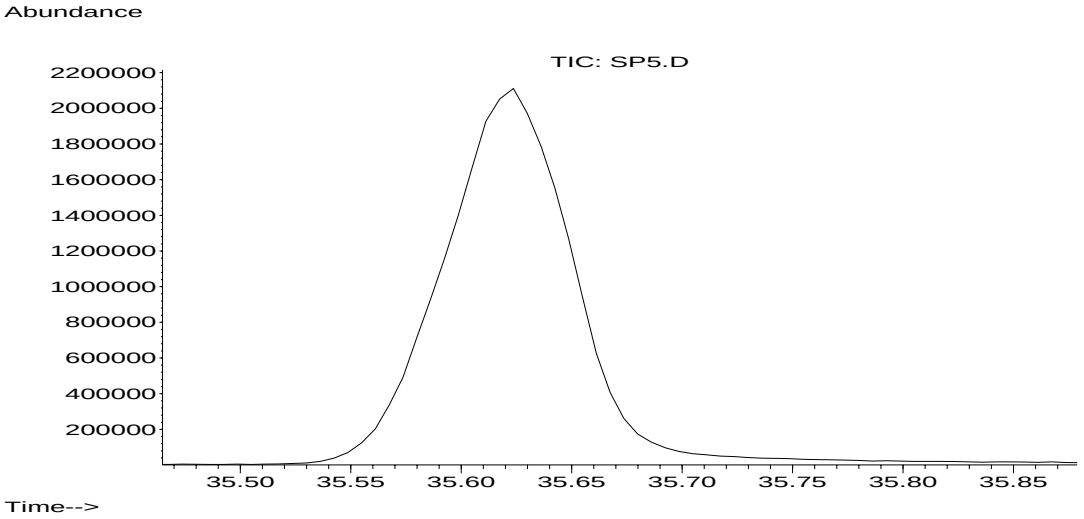
MAJOR COMPOUNDS DATA FROM GC/MS

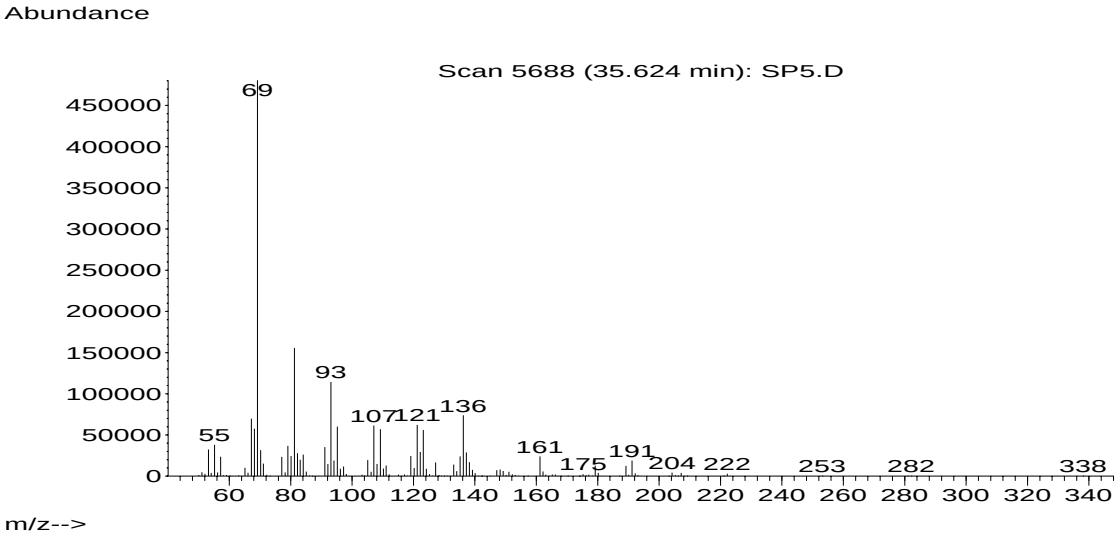




Scan 4762 (29.837 min): SP5.D
SP5

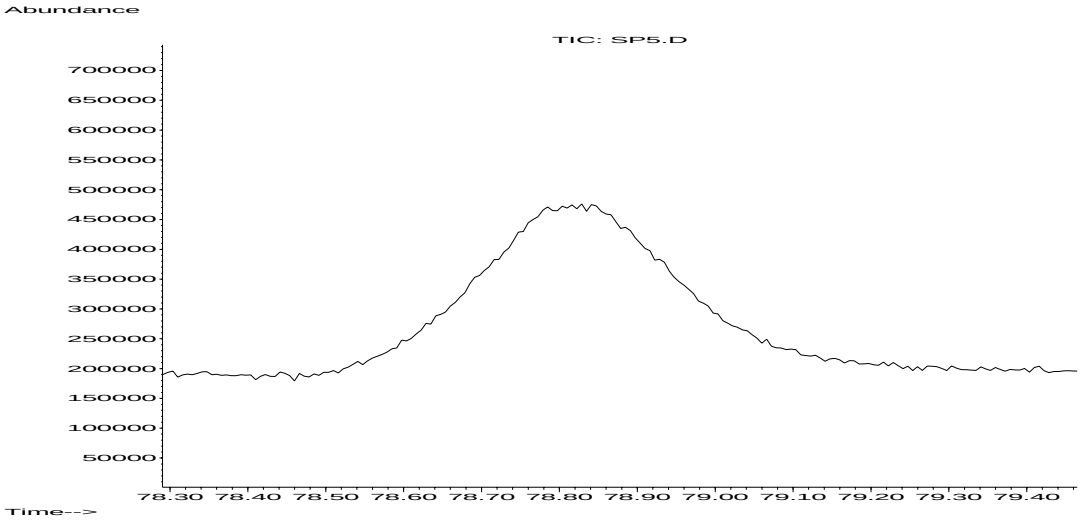
1	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)- $$.+/-.-trans-Nerolidol							222		
	C15H26O	91		040716-66-3	148727	144	8	0	77	
	0	62	27	74	9580					
2	Nerolidol $$ 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl- (CAS) $$ 3,7,11-Trimethyldodeca-1,6,10-trien-3-ol $$ 3,7,11-Trimethyl-1							222	C15H26O	91
	007212-44-4	148268	152	0	0	82	0	62	1	81
	9968									
3	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl- $$ Nerolidol $$ 3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol $$ 3,7,11-Trimethyldodeca-1							222	C15H26O	87
	007212-44-4	148267	125	27	1	66	8	54	24	56
	9206									
4	Farnesol $$ 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- (CAS) $$ Farnesyl alcohol $$ 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol $$							222	C15H26O	87
	004602-84-0	148294	128	21	0	79	8	54	33	62
	9008									
5	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-							222	C15H26O	68
	040716-66-3	148728	137	8	1	74	22	40	16	59
	7669									

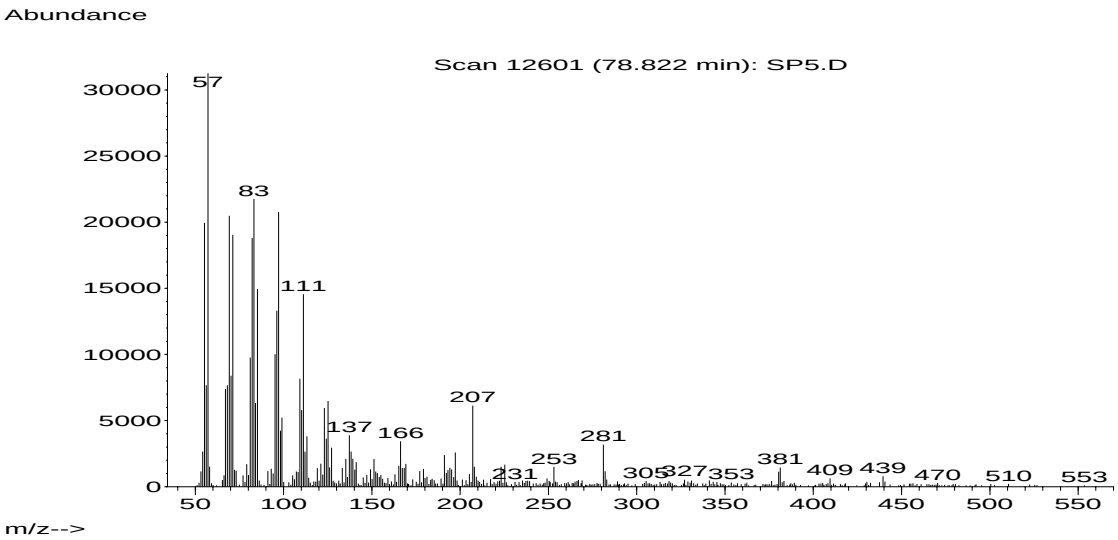




Scan 5688 (35.624 min): SP5.D
SP5

1	FARNESOL ISOMER B	222	C15H26O	95	*	000000-00-0				
	148700	90	24	0	68	17	74	0	95	9916
2	Farnesol isomer a	222	C15H26O	93	*	000000-00-0				
	148279	80	36	0	69	23	66	0	93	9871
3	FARNESOL ISOMER A	222	C15H26O	93	*	000000-00-0				
	148699	80	36	0	69	23	66	0	93	9871
4	Farnesol $$ 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- (CAS) $$ Farnesyl alcohol $$ 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol $$			222	C15H26O	90				
	004602-84-0	148299	92	37	1	89	2	57	0	47
	9985									
5	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-			222	C15H26O	87	*			
	004602-84-0	148286	74	43	1	86	14	54	0	81
	9865									





Scan 12601 (78.822 min): SP5.D
SP5

1	1-Tricosene	322	C23H46	72	*	018835-32-0	273786	96		
	70	0	54	62	42	55	93	8872		
2	1-Pentatriacontanol	509	C35H72O	70		055517-90-3				
	370258	135	70	0	61	29	41	36	68	9518
3	14-.BETA.-H-PREGNA $$ 14-.BETA.-PREGNA $$ 14B-PREGNANE								288	
	C21H36		64		000000-00-0		236429	111	58	1
	33	37	0	64	9606					
4	erythro-9,10-Dibromopentacosane			508	C25H50Br2		50	*		
	000000-00-0	370089	73	127	3	85	49	25	0	74
	8715									
5	Z-10-Methyl-11-tetradecen-1-ol propionate				282	C18H34O2		50	*	
	000000-00-0	228742	79	85	3	87	58	24	0	84
	8441									