MONITORING OF QUALITY OF ESSENTIAL OIL FROM *ETLINGERA* SP. 2 (ZINGIBERACEAE) BY GC AND GC-MS

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" 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."

Signature : ……

Name of Candidate : Hazrin bin Azemi

DEDICATION

Thanks to ALLAH SWT for His Blessing…

Specially dedicated to my beloved family, lecturers and friends…

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First of all, thanks to Allah s.w.t for all His guidance and blessing through all the hardship encountered whilst completing this thesis. In preparing this thesis, I was in contact with many people, researchers, academicians and practitioners. They have contributed towards my understanding and thoughts. In particular, I wish to express my sincere appreciation to my supervisor, Prof. Dr. Mashitah bt. Yusoff for her help, encouragement, guidance, critics and friendship.

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ABSTRACT

Ginger essential oil is highly regarded in the world and has a high commercial value in the world market. There are many types of ginger in the *Zingiber* family and the one that are studied is a wild species and due to comprehensive morphological examination being carried out by the botanist at Universiti Malaya, the specimen is named *Etlingera* sp. 2. The main objective of this test is to monitor the quality of its essential oil after being exposed to the heat and light within three weeks period. The specimen essential oil is collected using hydro-distillation process and the compounds of the essential oil are determined using GC-MS analysis. The samples are analyzed at the end of every week using the GC method. From the data analysis, the percentage area of three major compounds which is Trans-anethole, p-Anisaldehyde and Camphor are compared to the fresh sample analysis. The analysis due to both test show there are slight changes in the compound percentage area, which is less than 10% meaning that the changes are considered none. The samples from both tests maintain slightly the same properties as the fresh one. From this test, it shows that in the period of three weeks, the changes to the essential oil are minimal and the quality of the essential oil after three weeks is still the same like the freshly extracted.

ABSTRAK

Pati minyak halia adalah sangat berharga dan mempunyai nilai komersil yang tinggi di pasaran antarabangsa. Terdapat banyak jenis halia dari keluarga Zingiber dan halia yang dikaji adalah dari spesis liar dan sedang dikaji secara morfologikal di Universiti Malaya. Oleh yang demikian, spesimen dinamakan sebagai Etlingera sp. 2 objektif utama ujian ini dijalankan adalah untuk menilai kualiti pati minyaknya selepas dibiarkan terdedah kepada haba dan cahaya dalam jangka masa tiga minggu. Spesimen pati minyak ini didapatkan melalui kaedah penyulingan air dan kandungannya dikaji menggunakan analisis GC-MS. Sampel dianalisa setiap akhir minggu ujian menggunakan analisis GC. Peratus luas dari tiga komponen utama yang dikaji Trans-anethole, p-Anisaldehyde dan Camphor dan dibandingkan dengan analisis dari sampel yang segar. Analisis dari kedua-dua ujian menunjukkan sedikit perubahan kepada peratusan luas komponen, iaitu kurang dari 10%. Ini bermakna perubahan yang berlaku adalah kecil dan boleh dianggap tiada. Sampel dari kedua-dua ujian mengekalkan sifat yang sama seperti yang segar. Daripada ujian ini, ia menunjukkan bahawa dalam jangka masa tiga minggu perubahan kepada pati minyak halia adalah sangat kecil dan kualitinya tetap sama seperti yang baru disulingkan.

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Ginger (*Zingiber officinale*) has a long history of being used as a spice and as a medicinal plant. It is cultivated on a large scale worldwide, including India, China, Jamaica and Nigeria. Ginger is often used for the treatment of stomachache, and cardiovascular and motor diseases. It can act as an aphrodisiac, a carminative, a rubifacient, an anti-asthmatic and as a stimulant to the gastrointestinal tract (Bhandari, U., *et al*, 1998). It also possesses anti-inflammatory activity and regulates bacterial growth, as well as providing protection for immune-depressed patients, such as individuals who are HIV positive (Yusof, Y.A.M., *et al*, 2003).

Ginger products, such as essential oil and oleoresin, are internationally commercialized for use in food and pharmaceutical processing. Many active components, such as Zingiberene, have been found in the essential oil of ginger. In Malaysia, it is commonly known as "halia". Botanically, the Zingiber give the name to all of its family, zingeberaceae. This ginger family comprises of 1200 species of which 1000 of them can be found in the 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 Malesian region, the region that comprises Malaysia, Brunei, Singapore, Indonesia, Papua New Guinea and the Philippines, there are 600 species

encompassing 24 genera. As a result of its usage in flavoring, coloring and fragrance, the commercial value for each genus also arise.

1.2 Problem statement

The quality of wild *Etlingera* that are collected by a botanist and my supervisor are not yet to be determined for its genera and its physical and chemical properties. It is named as species 2 as it is still yet to be determined. This study also would like to know the effects that heat and light can cause to the essential oils.

1.3 Objective

The objectives of the study are as follows;

(i) To extract essential oil from the rhizome material of *Etlingera* sp. 2 by hydro-distillation,

(ii) To develop a method of analysis of essential oil from the rhizome material of *Etlingera* sp. 2 by using GC and GC-MS, and

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

1.4 Scope of Research

GC and GC-MS analysis of the essential oil from the rhizome material of *Etlingera* sp. 2 has never been reported before. The effect of storage on the quality of the essential oil, which can be obtained by using GC and GC-MS, will also yield useful information on the product.

CHAPTER 2

LITERATURE REVIEW

2.1 *Etlingera*

This study will be about the *Etlingera* genus, which has 10 species estimated by botanists spread and distributed around Peninsular Malaysia and Singapore (Larsen *et al.* 1999). 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 (Foster, 2000). 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 will be named *Etlingera* sp. 2.

In Malesian region, the region that comprises Malaysia, Brunei, Singapore, Indonesia, Papua New Guinea and the Philippines, there are 600 species encompassing 24 genera. As a result of its usage in flavoring, coloring and fragrance, the commercial value for each genus also arise.

Essential oil exists in all *Etlingera* species. Because of its high content of essential oil, *Etlingera* is frequently used in the industry and have high demand for its flavor and fragrance. The highest content is usually in the rhizomes of this plant. In the present study, the essential oil from the rhizomes of *Etlingera* sp. 2 is to be investigated.

(a)

(b)

Figure 2.0: (a) Red Torch *Etlingera* (b) White *Etlingera*
(Source: http://naturepark.freeservers.com/ginger/042Etlingera_torch.htm)

2.2 Type of Extraction Method

Extraction is a commonly used technique in order to separate soluble essences from natural products such as from wild *Etlingera* rhizomes to produce an essential oil. There are several methods can be used to extract essential oil. The most

common techniques used in extracting essential oil from plants are hydro-distillation, steam-distillation, supercritical fluid extraction and solvent extraction.

2.2.1 Hydro-distillation

The high content of essential oil in commercial gingers contributes to demands in the flavoring 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. 2 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.

2.2.2 Steam distillation

In steam distillation, live steam is used. Through this technique, both water and steam are used but the difference is that the materials are not in direct contact with the water. Essential oil is extracted by passing steam through the raw material. The heat of the steam will work as a force to pull out the tiny intercellular pockets that hold the essential oil to open and release them. The heat of the steam must be at an appropriate temperature so that it can produce the force enough for the process and not so high because it can destroy the material itself.

As they are released, it will travel with the steam through the tube to the still's condensation chamber. As the steam cools down, it will condense into water. The essential oil will forms a film on the surface of the water and to separate it, the film will be decanted or skimmed off the top.

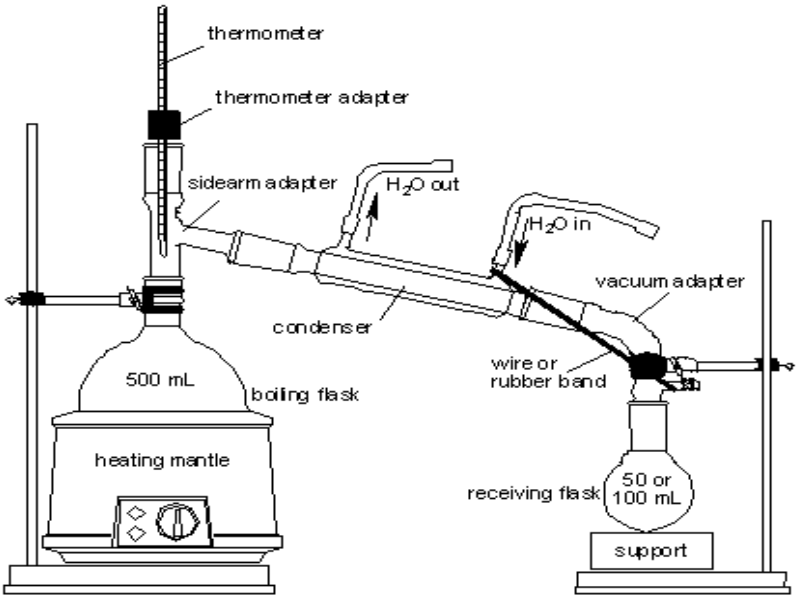


Figure 2.1: Steam Distillation Apparatus

2.2.3 Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) is the application of fluids in their supercritical state for extraction of components from solid materials. This process is new and gives a better quality of extraction but has a high capital cost. This non-toxic and non-combustible environmental friendly method use carbon dioxide as its solvent. Other advantages of this method are it give high efficiency, high extraction rate and also have more selectivity.

Supercritical fluid extractions were performed at certain pressures and temperatures for duration of several minutes, static, followed by extraction. In order to prevent sample plugging, the restrict point was warmed electrically. The essential oil was extracted from the plant using supercritical CO_2 under various conditions.

2.2.4 Solvent Extraction

This is another method of extraction, used on delicate plants. It yields a higher amount of essential oil at a lower cost. In this process, a chemical solvent such as hexane is used to saturate the material and pull out the aromatic compounds. This renders a substance called a concrete. The concrete can be dissolved in alcohol to remove the solvent. When the alcohol evaporates, the essential oil will be left behind as remains. The disadvantage of using this method is that the residue of the solvent may remain in the absolute and can cause side effect.

2.2.5 Super Critical Carbon Dioxide (CO_2) Extraction

The end result of super critical carbon dioxide (CO2) extraction - one of the newest extraction technologies - is a super-concentrated, high-quality version of essential oil. This rapid extraction method uses lower temperatures and higher

pressure to transform carbon dioxide, a gas, into a liquid. It's an inert solvent meaning that it's non-reactive and therefore cannot form another chemical compound.

When the extraction process is complete, the carbon dioxide is returned back to a gaseous state therefore, no residual remains. All that is left is pure essential oil. Although this technology produces one of the purest forms of essential oil, it is not yet widely used. The equipment needed for this extraction process is very expensive, which keeps production costs high. And because of production costs are high, so too are the costs of the essential oils that are produced via carbon dioxide extraction.

2.3 Type of Analysis Method

The essential oil that was obtained after the extraction process will be determined by using analysis method. There are several common analysis methods that can be carried out such as gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC).

2.3.1 Gas Chromatography (GC)

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.

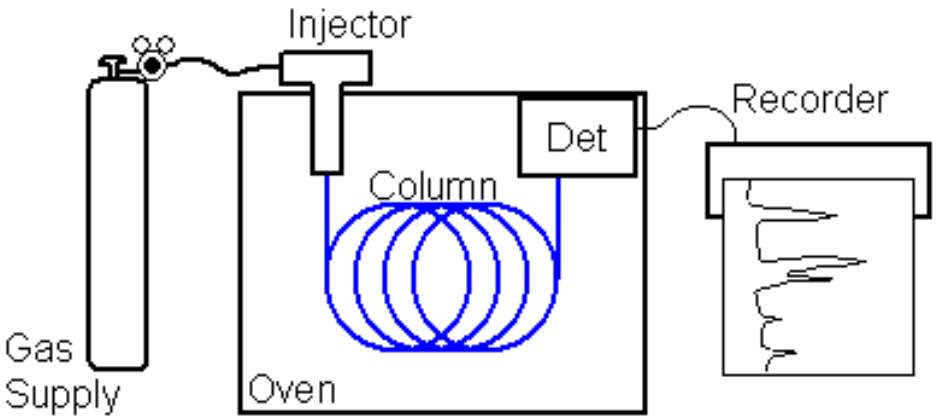


Figure 2.2: Schematic GC
(Source: http://faculty.kutztown.edu/betts/html/GC.htm)

2.3.2 Gas Chromatography-mass Spectrometry (GC-MS)

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.

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.

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.

2.3.3 High Performance Liquid Chromatography (HPLC)

This method is the most versatile and widely used type of elution chromatograph. It is used to separate and determine species in a variety of organic, inorganic and biological materials. It differs from the GC and GC-MS because it uses liquid as its mobile solvent. It has column chromatography in which the stationary

phase is made up of small particles and the mobile phase is forced through the particles by high pressure.

TABLE 31-1

Gas Chromatographic Detectors

Type	Applicable Samples	Typical Detection Limit
Flame ionization	Hydrocarbons	0.2 pg/s
Thermal conductivity	Universal detector	500 pg/mL
Electron capture	Halogenated compounds	5 fg/s
Mass spectrometer	Tunable for any species	0.25–100 pg
Thermionic	Nitrogen and phosphorous 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	Organic compounds	0.2 to 40 ng

Table 2.0: Gas Chromatographic Detectors

2.4 Essential Oils

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. In the present study, the essential oil from the rhizomes of *Etlingera* sp. 2 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, such as 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. 2. This essential oil will then be determined using the gas chromatography-mass spectrometer. After the extraction of the *Etlingera* rhizomes materials complete, the essential oil will be put into a thick brown bottle, a wrapped bottle and another one will be put in a refrigerator. This is done in order to investigate and determine the effect of exposure to heat and light at a certain amount of time.

2.5 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 (Anonymous, 2005). Essential oils have a lipid-soluble molecular structure that allows them to pass through skin easily. The essential oils can penetrate through fat-layer of the skin quickly; making methods such as massaging become an effective treatment. 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. An 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.

2.6 Chemical Properties of Essential Oils

Essential oils have a very unique chemical property. Although in small amounts, it contain very large amount of goodness. Every single oil is normally has more than a hundred components (Anonymous, 2006). These components can be determined using analytical apparatus such as the gas chromatogram and high performance liquid chromatogram. Some of the chemical that can be regularly found in the essential oils are sesquiterpenes, monoterpenes and phenols.

For sesquiterpenes, it consists of 15 carbon atoms and has a complex pharmacological action. It has anti-inflammatory and anti-allergy properties. As for phenols, due to its nature essential oils that are high with it 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 require working harder to excrete them if used for 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.

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Materials

Wild *Etlingera* were collected near Bentong, Pahang by a botanist and my supervisor. A voucher specimen was prepared, labeled as *Etlingera* sp. 2 and deposited at Universiti Malaya herbarium.

3.2 Methodology

3.2.1 Hydro-distillation Methods

To obtain the essential oil from the rhizomes, an extraction process called the hydro-distillation process will be used.

3.2.1.1 Materials Preparation

Firstly the wild *Etlingera* sp.2 rhizomes are cleaned and washed to remove dirt and other unneeded substances around it. This is to make sure that other

substances that are not from the wild *Etlingera* will not disturb the distillation process or get extracted also. Then the materials are leaved over a night under the temperature of 40^{o}C in lab oven. The final rhizome weight that is obtained from this process is 87.1 gram.

3.2.1.2 Grinding and Drying Process

Then the rhizomes will be cut and mash into smaller pieces, in order to increase its surface area so that it can be easily extracted during the distillation process. The rate of extraction depends on the surface area, the higher the surface area the higher extraction rate because contact between solvent and the materials are high. After that, the materials will be hair dried according to the certain level of heat. This is done so that the materials will not be dried completely to avoid less extracted essential oil from it.

Figure 3.1: Dried sample of *Etlingera* sp.2

3.2.1.3 Soaking Process

The materials are soaked in water in order to break down the parenchymatous cells and oil glands. This will help in the extraction process and make the process faster. Some of the parenchymatous cells and oil glands are also believed to break down in during the washing and cutting process but it will be neglected.

3.2.1.4 Extraction Process

The equipment of the distillation process is set up and then the mixture of 37.5 gram of mashed materials and 262.5 mL of water is put into the flask of distillation unit. After that the heating mantle and water supply are switched on. The temperature is set at 80^0 C for the extraction process and then the process was run for 6 hours.

In order to avoid heat loss, all the apparatus will be wrapped with aluminums foil. A boiling chip will be inserted in the flask to ensure that occurred when water is boiled, did not affect distillation process.

The essential oil present in the distillation flask will vaporize and the steam is passed through a condenser. The condensate which has the mixture of water and essential oil is collected in a receiving flask. At the receiving flask, the essential oil is decanted, cleaned, made moisture free by using anhydrous sodium sulfate and traded.

Figure 3.2: Hydro-distillation set-up.

3.2.1.5 Sample preparation

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 vials for the effect of light test and the brown vials for the effect of temperature test. Three vials are used for each test, with the 1 mL of the sample prepared are divided into three to fill each vials for their test.

In light test, all of the three vials are placed in a glass cabinet. The room temperature is about 31^{o}C to 33^{o}C and the room humidity is 42% to 45%. The same room temperature and room humidity apply to the temperature test, but in the temperature test the vials are placed near a window, with direct sunlight every morning.

Figure 3.3a: Placement of vial for temperature test.

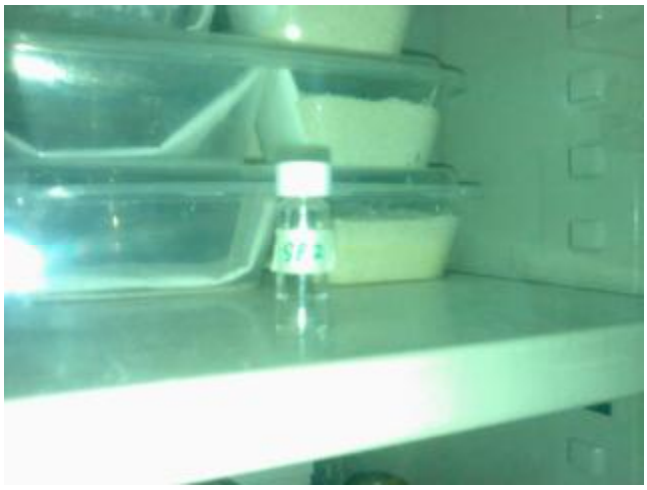


Figure 3.3b: Placement of vial for light test.

3.2.2 GC-MS Analysis

A gas chromatography-mass spectrometer (GC/MS) was used to analyze the essential oil. The GC/MS can also be used to separate small amounts of materials and determine whether a desired component was present. The GC consists of 3 essential parts which is an injection block, a column and a detector.

For GC analysis, the sample to be analyzed is put in the sample port, with about 50^{o}C above the boiling point of the least volatile component of the sample. Splitless injection was used for this GC-MS method, which means that the entire sample goes into the GC to be analyzed. The column oven temperature was programmed to rise to an initial temperature of 50 °C to 290 °C at 3 °C min−1 in 80 minutes, and are hold about 5 minute to make sure the entire sample are burned. Helium was used as the carrier gas with a flow rate of 1 mL min−1.

After the sample of the essential oil vaporized, it was injected onto the head of the chromatography column. This sample was 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. It is then transferred to the detector with the temperature of 300 °C. The sample was finally entered the detector after going through the column. There the sample will be analyzed one by one with a mass spectrometer detector. This type of detector is commonly use and are tunable for any species with the typical detection limit from 0.25 to 100pg. Then the reading from the analyzed species can be monitored using the computer.

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.

In this part, the GC that is being used is the Agilent 7890A type model G3440A, with the GC system of Agilent Technologies. For the injector, the type

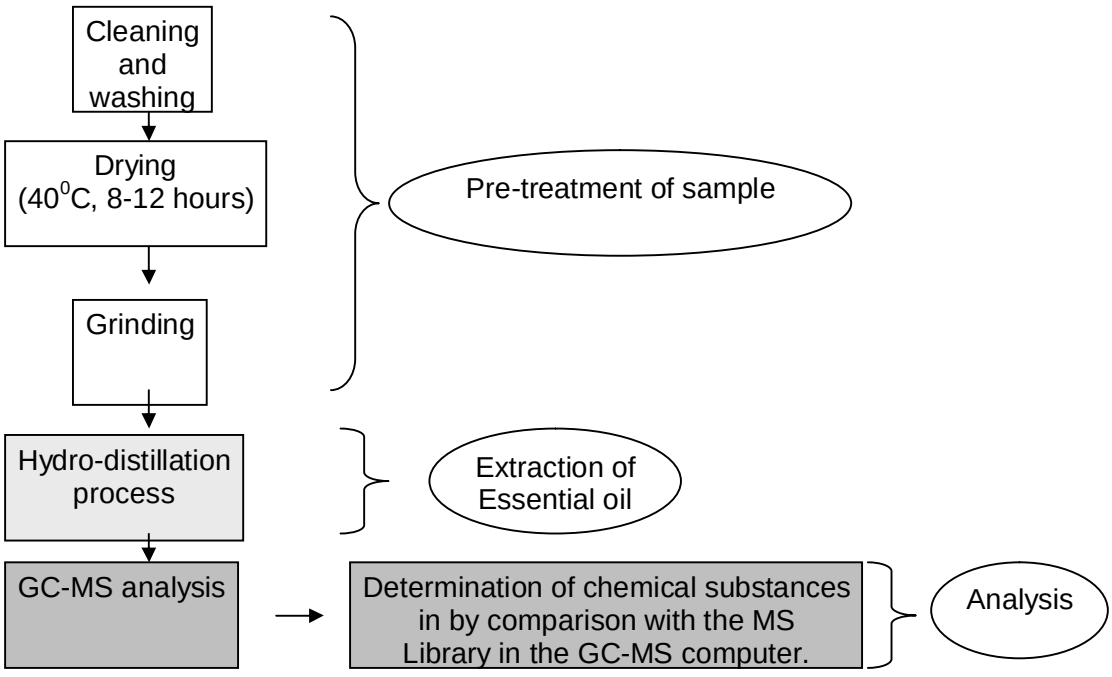
used is 7683 B Series, Agilent Technologies with split less inlet injection detector. The column used are of the J & W Scientific Columns, manufactured by Agilent Technologies, USA type HP-5, with length 30 meters, I.D 0.32 mm and film 0.25μm. The GC also used n-Hexane as the carrier gas.

Figure 3.4: GC-MS

Figure 3.5a: Sampling vials of heat test for GC analysis

Figure 3.5b: Sampling vials of light test for GC analysis

3.3 Flow of Methodology



CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Introduction

Because of the usage of the wild species of *Etlingera* that are yet to be known, there are no other indicators of how and what are the expected results will be. Assuming that the quality of the essential oils will reduce due to exposure to air, heat and light, the results are expected to be varying for each of the sample from the four sample bottles.

The controlled sample bottles are expected to have the finest quality of essential oil, while the sample in the exposed bottle will have the quality of its essential oil reduced. During the period given, the exposed sample will be determined for its quality and it is expected than the longer the sample are exposed to air, heat and light, the lower its quality will be.

4.2 Results

The expected results are to be tabulated as follows;

4.2.1 Yield Of Product

Weight of dry specimen (g)	Weight of essential oil (g)	% yield (based on dry weight)
87.1	0.641	0.736

4.2.2 Gas Chromatography

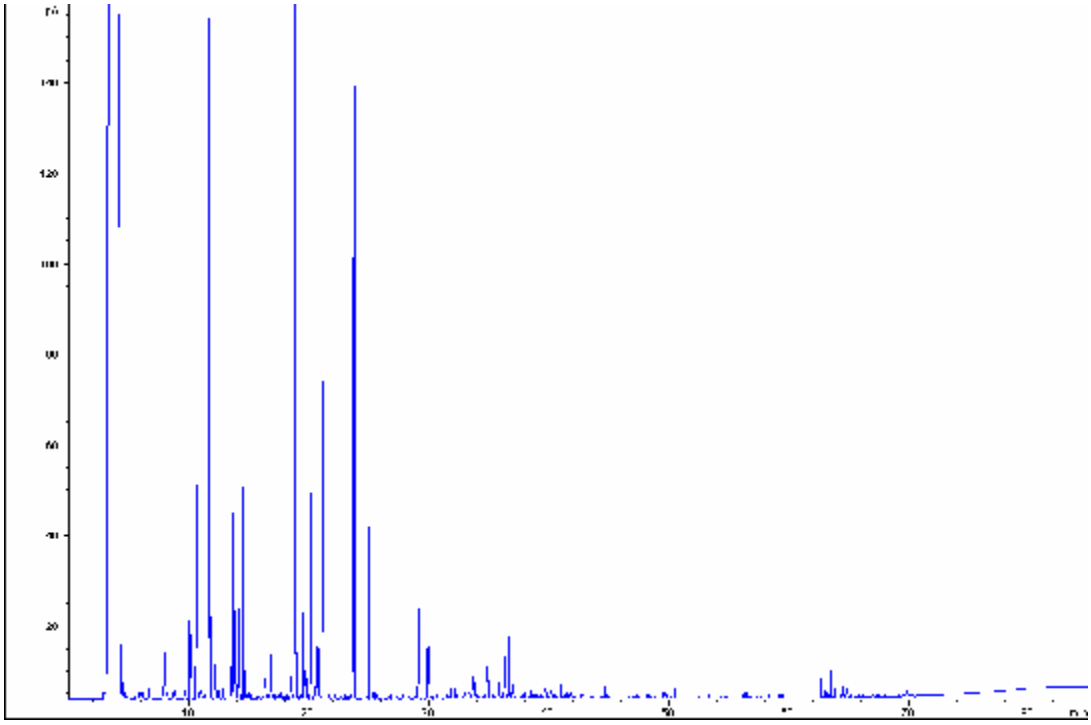


Figure 4.1: GC-MS analysis for fresh *Etlingera* sp.2 essential oil

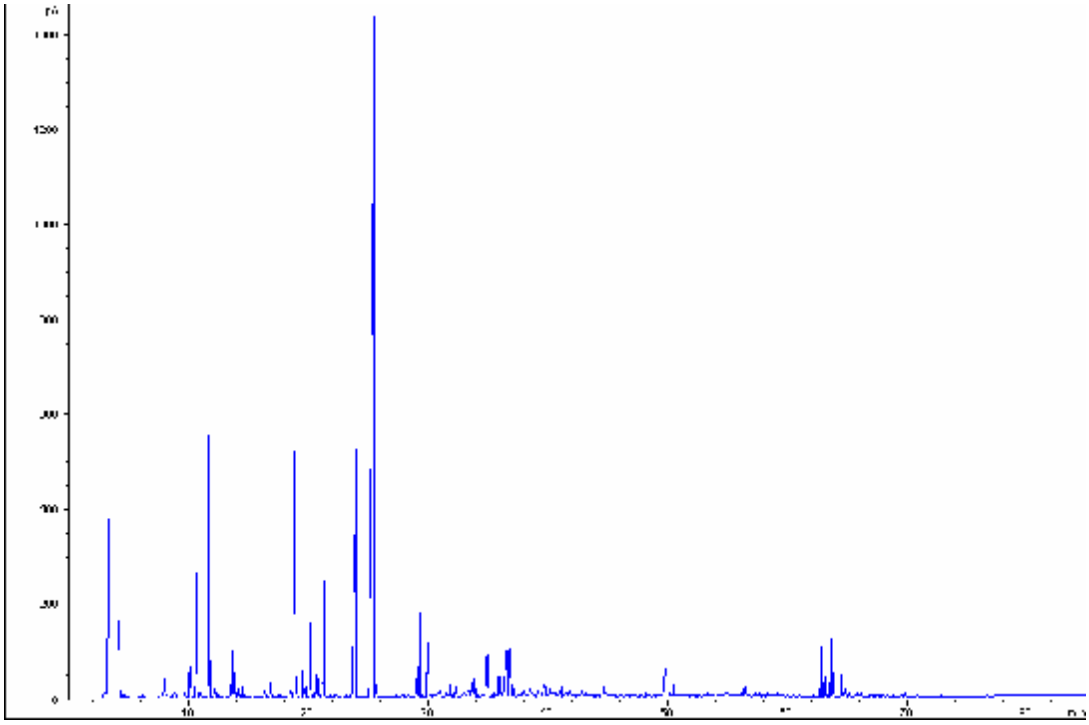


Figure 4.2: GC-MS analysis for test of heat week 1

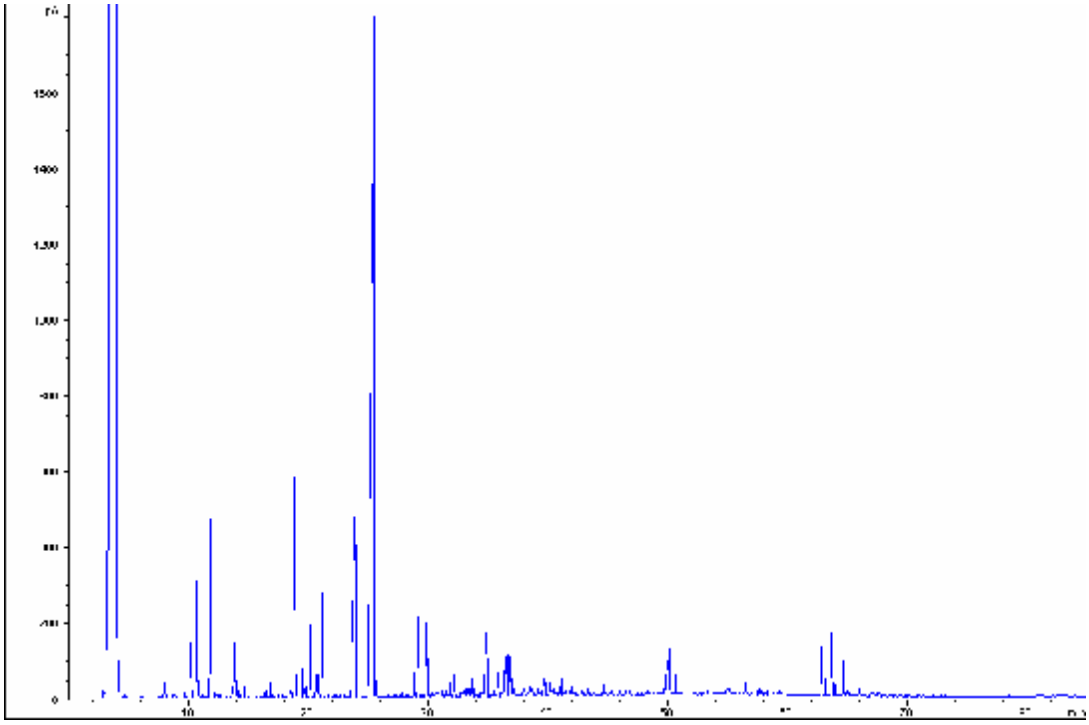


Figure 4.3: GC-MS analysis for test of heat week 2

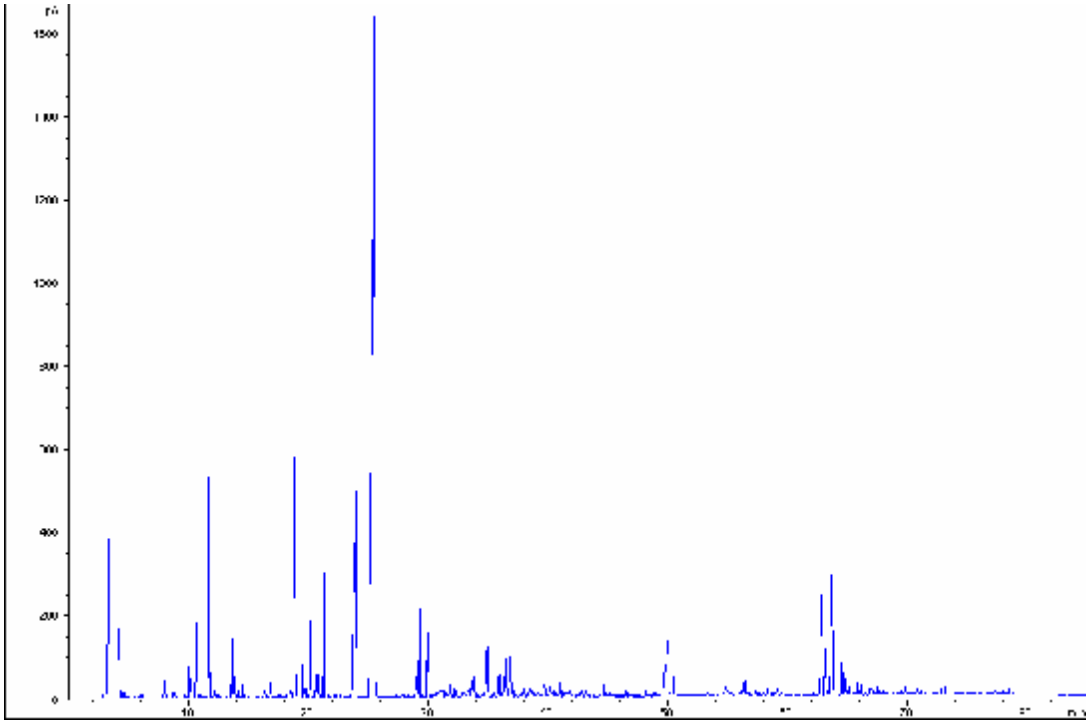


Figure 4.4: GC-MS analysis for test of heat week 3

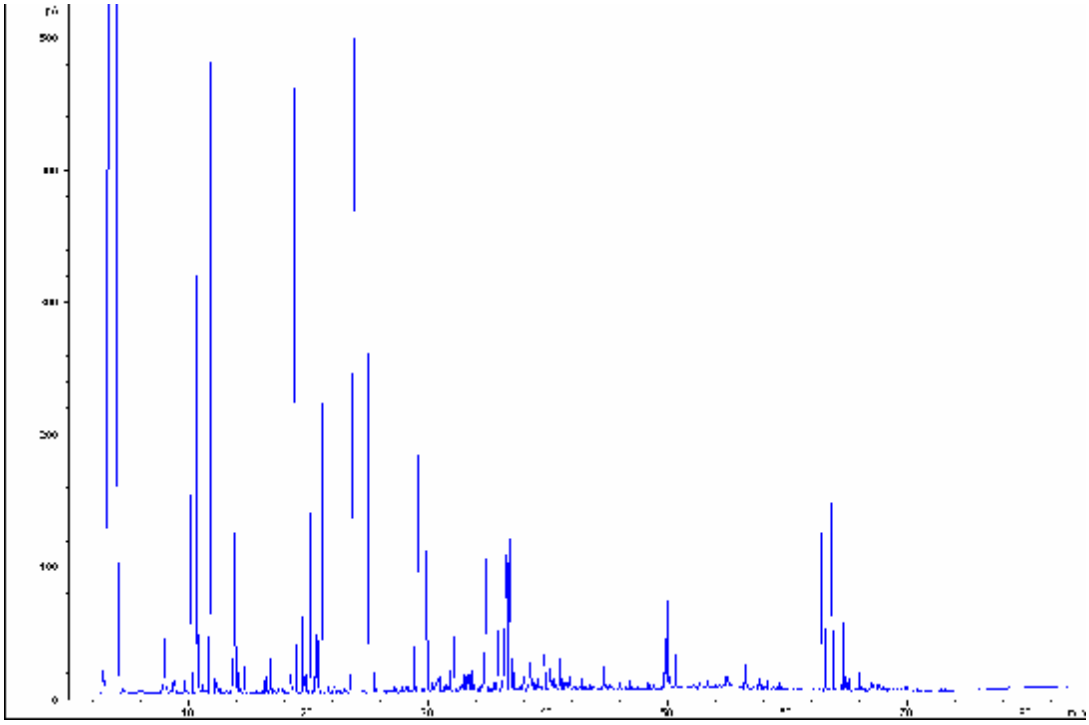


Figure 4.5: GC-MS analysis for test of light week 1