UNIVERSITI MALAYSIA PAHANG

BORANG PENGESAHAN STATUS TESIS

JUDUL: **MONITORING ESSENTIAL OIL QUALITY OF *ETLINGERA SP1* BY GC AND GC-MS**

SESI PENGAJIAN: **2007/2008**

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Nama Penyelia II	: .....
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Nama Penyelia III	: .....
Tarikh	: .....

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MONITORING ESSENTIAL OIL QUALITY OF *ETLINGERA SP1* BY GC AND GC-MS

SIVANESAN SHANMUGAM

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

Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

MAY 2008

I declare that this thesis entitled “Monitoring Essential Oil Quality of *Etlingera sp1* BY GC and GC-MS” is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : …….....

Name of Candidate : …….....

Date : …….....

DEDICATION

Specially dedicated to my late grandmother, family and friends for their love and care…

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Completion and submission of a thesis of this kind involves lots of hard work and sacrifices. This thesis is a result of almost a year of study whereby I have been accompanied and fostered by many. It is a pleasant moment that I have now the opportunity to express my gratitude to all of them.

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ABSTRACT

Etlingera sp.1 of the family *Zingiberaceae* is being studied for changes in its essential oil quality during storage. *Zingiberaceae* comprises about 1200 species of which 1000 is distributed throughout Tropical Asia. Essential oil exists in all species in the genus *Etlingera.* The highest content is usually in the rhizomes of this plant. Essential oil extracted from the rhizomes is primarily used in fragrance making and have high commercial value due to its antibacterial and therapeutic properties. Suitable extraction method needs to be chosen as essential oils are composed of heat-sensitive chemical constituents. In this study, the Clevenger-type hydrodistillation method was employed due to ease of use and milder extracting condition. Essential oil composition was analyzed using GC and GC-MS. Generally, gas chromatographic techniques are used to separate mixtures of chemical constituents into individual components Compounds present were identified using GC-MS while GC was used for weekly analysis. n-Hexane was chosen as solvent as previous studies using n-hexane reportedly produced optimum results. Comparative analysis conducted over a three weeks period indicated that the quality of essential oil exposed to light and temperature underwent minor changes.

ABSTRAK

Etlingera sp.1 dari famili *Zingiberaceae* telah dikaji perubahan ke atas kualiti minyak patinya semasa penyimpanan. *Zingiberaceae* terdiri daripada 1200 spesis dengan seribu darinya tertabur di keseluruhan Asia Tropika. Minyak pati wujud di semua spesis dalam genus *Etlingera.* Kandungan tertinggi minyak pati ialah di dalam rizom tumbuhan. Minyak pati yang diekstrak daripada rizom digunakan di dalam persediaan fragran dan mempunyai nilai kormesial tinggi sebagai antibakteria dan terapeutik. Kaedah pengekstrakan yang sesuai adalah perlu disebabkan komposisi minyak pati adalah sensitif haba. Di dalam kajian ini, metod penyulingan hidro jenis Clevenger digunakan kerana mudah dan kondisi pengekstrakannya yang sederhana. Komposisi minyak pati dianalisa menggunakan GC dan GC-MS. Amnya, teknik kromatografi gas diguna untuk memisahkan campuran kimia kepada kompaun individu. GC-MS diguna untuk mengenalpasti kompaun yang hadir dalam sampel manakala GC diguna untuk analisis mingguan. n-Heksana dipilih sebagai pelarut memandangkan kajian-kajian sebelumnya melaporkan hasil yang optimum dengan penggunaannya. Perbandingan analisis yang dibuat selama tiga minggu mendapati kualiti minyak pati telah mengalami perubahan kecil-kecilan.

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LIST OF ABBREVATIONS AND SYMBOLS

T	=	Temperature
t	=	Time
cm	=	Centimeter
m	=	Meter
g	=	Gram
°C	=	Degree Celcius
K	=	Kelvin
%	=	Percentage
g/mL	=	gram per milliliter
mg/L	=	Milligram per litre
rpm	=	rotation per minute

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CHAPTER 1

INTRODUCTION

1.1 Background

Zingiberaceae, or the family of ginger in layman term, is a family of flowering plants consisting of aromatic lasting herbs with rhizomes, comprising 52 genera and more than 1300 species, distributed throughout tropical Africa, Asia and the Americas (Wikipedia, 2007).The most popular species from this family is the edible ginger of commerce known in Malay as "halia" (scientific name: *Zingiber officinale*).Noticeably, from its total population, around 1000 species occur in tropical Asia.Many species are important ornamental plants, spices, or medicinal plants. Attractive genera include the shell gingers (*Alpinia*), Siam or summer tulip (*Curcuma alismatifolia*), *Globba*, ginger lily (*Hedychium*), *Kaempferia*, torch-ginger *Nicolaia*, *Renealmia*, and ginger (*Zingiber*). Spices include ginger (*Zingiber*), galangal or Thai ginger (*Alpinia galanga* and others), melegueta pepper (*Aframomum melegueta*), myoga (*Zingiber mioga*), turmeric (*Curcuma*), cardamom (*Amomum*, *Elettaria*).

1.2 Problem Statement

Quality of essential oils degrades easily upon exposure to light, air and changing climate. The study is to monitor changes in the essential oil of an *Etlingera* species using

GC-FID and GC-MS under different conditions, namely, temperature and light, over time.

1.3 Objectives

The objective of this study is to perform a comparison study between samples of essential oil obtained from *Etlingera sp.1*.The aim of this experiment is to monitor the changes in essential oil quality *Etlingera sp.1* using GC-FID and GC-MS upon certain length of time to heat and light and to identify the compounds present.

1.4 Research Scope

Variation in *Etlingera sp.1* essential oil sample is being studied for its property changes over 3 weeks period.

CHAPTER 2

LITERATURE REVIEW

2.1 The Genus *Etlingera* - An overview

Kingdom	*Plantae*
Subkingdom	*Viridaeplantae*
Phylum	*Tracheophyta*
Subphylum	*Spermatophytina*
Intraphylum	*Angiosperma*
Division	*Magnoliphyta*
Class	*Liliopsida*
Order	*Zingiberales*
Family	*Zingiberaceceae*
Genus	*Etlingera*
Species	*sp.1*
Scientific name	*?*
Common name	?

Figure 2.1: Taxonomy of Ginger (Farlex, 2004)

The genus *Etlingera* is distributed from India to the Pacific Islands with Borneo and New Guinea seem to be the most popular location of this species. To date, botanists estimate about 10 species of *Etlingera* distributed throughout Peninsular Malaysia and Singapore. The varying shades of pink and red colours of bracts and flowers make *Etlingera* species very attractive plants. Plants of *Etlingera* have various traditional and commercial uses. While comprehensive morphological examination is being carried out by the botanist at Universiti Malaya, the specimen that used is this study will be named *Etlingera* sp. 1

Even though we do not have specific information on this species, we can expect similarities from previous researches done on the other species which belong to the same genus, *Etlingera.* For example, 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). In Thailand, fruits and cores of young stems of E. littoralis are edible, and flowers of E. maingayi are eaten as vegetables (Sirirugsa, 1999). Inflorescences of *E. elatior* are widely cultivated throughout the tropics as spices for food flavoring and as ornamentals. They are commonly used as ingredients of dishes such as laksa asam, nasi kerabu, and nasi ulam in Peninsular Malaysia (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). In Malaysia, fruits of *E. elatior* are used traditionally to treat earache, while leaves are applied for cleaning wounds (Ibrahim & Setyowati, 1999). Leaves of *E.* elatior, mixed with other aromatic herbs in water, are used by post-partum (depression after pregnancy) women for bathing to remove body odor. Phytochemical studies on rhizomes of E. *elatior* led to the isolation of two new and six known compounds of diarylheptanoids, labdane diterpenoids, and steroids (Habsah et al., 2005). Ethanolic extracts from the flower shoots of *E. elatior* have antimicrobial activity and are cytotoxic to heal cells (Mackeen et al., 1997).Past studies on the antioxidant activity of ginger species were confined to rhizomes (Jitoe et al., 1992; Habsah et al., 2000; Zaeoung, Plubrukarn, & Keawpradub, 2005). Their rhizomes have been reported to contain antioxidants. (Chan et al., 2007)

2.1.1 Ecology

Species of *Etlingera* can be more than 5 m tall and become dominant in gaps (*E. megalocheilos, E. brevilabrum, E. coccinea, E. fimbriobracteata*).The reproductive

biology has been investigated by Ms. Louise Pedersen (Univ. Copenhagen) who found that spider hunters are important pollinators (Axel, 2007).

2.1.2 Medicine

The species sp.1 that is being studied is likely to have medicinal and therapeutic properties just like other members of the *Zingiberaceae* family. Many previous studies have proved this to be true. An organic extract of *Etlingera* aff. rosea B.L. Burtt & R.M. Sm. (*Zingiberaceae*) was found to exhibit significant cytotoxic activity when evaluated against a panel of human cancer cell lines. Leaves of *Etlingera* species inhibited Gram-positive bacteria. With promising antioxidant and antibacterial properties, leaves of *Etlingera* species have great potential to be developed into natural preservatives and herbal products, applicable to the food and pharmaceutical industries. Unlike the commercial use of rhizomes, the harvesting of leaves does not result in destructive sampling of plants (Chan et al, 2007).

2.1.3 Selected plants from the genera *Etlingera*

Figure 2.2: *Etlingera Elatior* “Yamamoto” (http://www.alohatropicals.com)

Figure 2.3: *Etlingera Elatior* "Thai Queen" (http://www.alohatropicals.com)

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Figure 2.11: *Etlingera velutina*, Sabah (Axel, 2007)

Figure 2.12: *Etlingera venusta* "malay rose" (Axel, 2007)

2.1.4 Essential oil

The essential oil of a material is the name given to the mixtures of substances extracted from a biological system and contains the essential components that provide the characteristic smell or flavor of that material. They are also known as volatile or refined oils or simply as the oil of the plant material from which they were extracted. The term essential indicates that the oil carries distinctive fragrance (essence) of the plant, importantly or core substance.For example, peppermint oil, patchouli oil, jasmine oil and etc. Essential oils are usually a highly complex mixture of a wide variety of substances.

The oil may often be simulated by a fairly simple mixture of artificial compounds blended in the same percentage as the original oil but the aroma or taste often lacks the validity of the original essential oil (Yoshiro, 1976). Essential oils are extracted by different methods. The material is sometimes leached with water and the oil steam distilled from the aqueous mixture. The natural material may also be solvent extracted and the oil recovered by distillation. Extraction must be done with care as many of the components of essential oils are temperature sensitive. Essential oils analysis without the use of gas chromatography would be extremely difficult. Prior to the technique being developed, only the major components of the oils could be separated, achieved by distillation with high efficiency columns (Holttum, 1965).

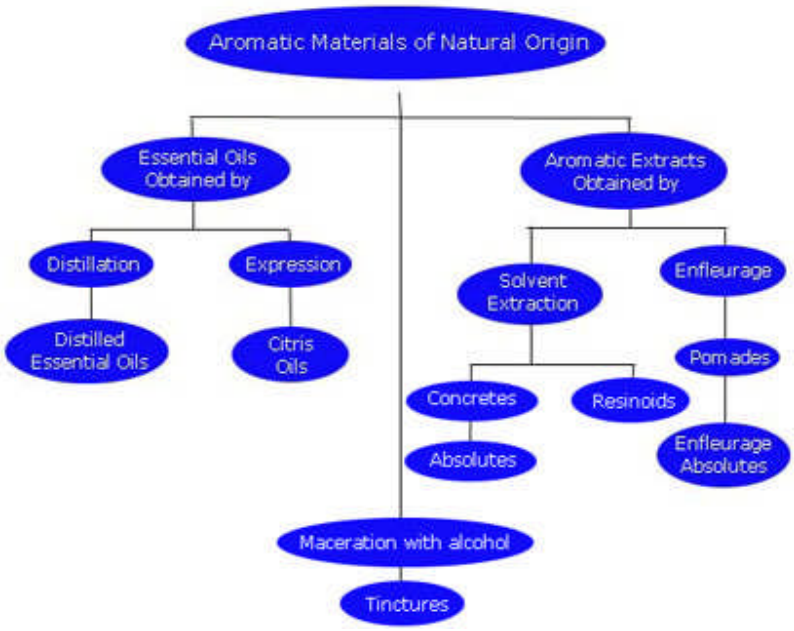


Figure 2.13: Essential Oil extraction chart (www.healing.about.com)

The extraction method depends on the plant material as well as the type of end product that is desired. Essential oils been used medicinally at different periods in the past. Medical applications proposed by those who sell medicinal oils vary from skin treatments to remedies for cancer, and are often based on historical use of these oils for these purposes.

Interest in essential oils has revived in recent times, with the popularity of aromatherapy, a branch of alternative medicine which claims that the specific aromas carried by essential oils have curative effects.

Berries	Leaves	Flowers
• Allspice • Juniper **Seeds** • Almond • Anise • Nutmeg oil **Wood** • Camphor • Cedar • Rosewood • Sandalwood **Rhizome** • Ginger	• Basil • Bay leaf • Cinnamon • Common sage • Eucalyptus • Lemon grass • Oregano • Patchouli • Peppermint • Pine • Rosemary • Spearmint **Resin** • Frankincense • Myrrh	• Chamomile • Clary sage • Clove • Geranium • Hyssop • Jasmine • Lavender • Marjoram • Orange • Rose • Ylang-ylang **Peel** • Bergamot • Lime • Orange • Tangerine **Root** • Valerian

Figure 2.14: Plant bases of various Essential Oils (www.healing.about.com)

2.1.5 Gas Chromatography

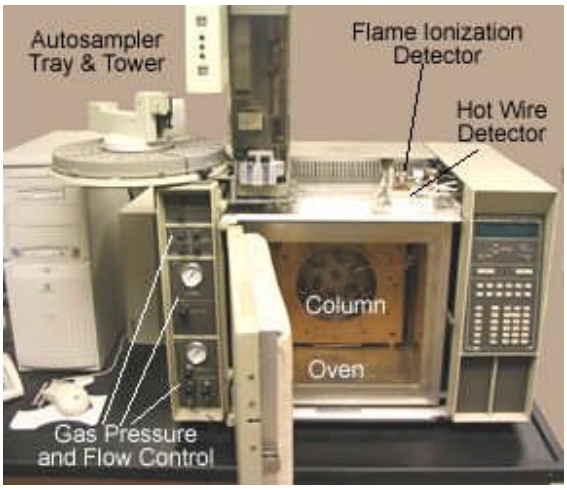


Figure 2.15: Gas Chromatography (http://www.j-chrom-sci.com, 2007)

Gas chromatography is a chromatographic technique that can be used to separate organic compounds that are volatile. A gas chromatograph consists of a flowing mobile phase, an injection port, a separation column containing the stationary phase, a detector, and a data recording system. The organic compounds are separated due to differences in their partitioning behavior between the mobile gas phase and the stationary phase in the column.

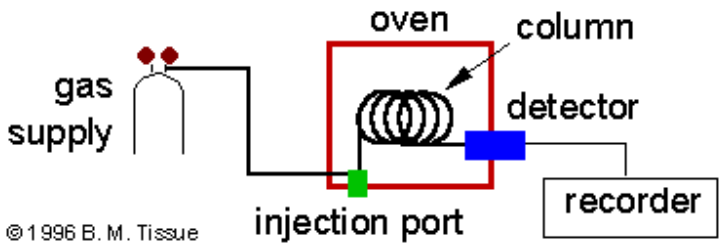


Figure 2.16: Simplified Gas Chromatograpy Parts
(http://www.j-chrom-sci.com, 2007)

The process of gas chromatography is carried out in a specially designed instrument. A very small amount of liquid mixture is injected into the instrument and is volatilized in a hot injection chamber. Then, it is swept by a stream of inert carrier gas

Through a heated column which contains the stationary, high-boiling liquid. As the mixture travels through this column, its components go back and forth at different rates between the gas phase and dissolution in the high-boiling liquid, and thus separate into pure components. In gas chromatography (GC) the gaseous mobile phase is forced through the stationary phase using pressure. Just before each compound exits the instrument, it passes through a detector. When the detector detects a compound, it sends an electronic impulse to the recorder, which responds by printing a peak on a piece of paper.

The GC consists of an injection block, a column, and a detector. An inert gas flows through the system. The injection chamber is a heated cavity which serves to volatilize the compounds. The sample is injected by syringe into this chamber through a port which is covered by a rubber septum. Once inside, the sample becomes vaporized and is carried out of the chamber and onto the column by the carrier gas.

inside of the injector port

the septum

the column

the detector inside the housing

Figure 2.17: Parts in Gas Chromatography

Each instrument will have a different setting for:

- column temperature
- injection port temperature
- detector temperature

2.1.5.1 Recorders

Two devices are used to record the area under peaks. Those are integrating recorders and computer programs. Each type of device records the messages sent to them by the detector as peaks, calculates the retention time, and calculates the area under each peak; all of this information is included in the printout. For similar compounds, the area under a GC peak is roughly proportional to the amount of compound injected. If a two-component mixture gives relative areas of 75:25, we may conclude that the mixture contains approximately 75% of one component and 25% of the other.

2.1.5.2 Retention Time (RT)

The retention time, RT, is the time it takes for a compound to travel from the injection port to the detector; it is reported in minutes. The retention time is measured by the recorder as the time between the moment we press start and the time the detector sees a peak. If we do not press start at the same time we inject your sample, the RT values will not be consistent from run to run.

2.1.5.3 Factors which affect GC separations

Efficient separation of compounds in GC is dependent on the compounds traveling through the column at different rates. The rate at which a compound travels through a particular GC system depends on the factors listed below:

- **Volatility of compound**: Low boiling (volatile) components will travel faster through the column than will high boiling components

- **Polarity of compounds**: Polar compounds will move more slowly, especially if the column is polar.
- **Column temperature**: Raising the column temperature speeds up all the compounds in a mixture.
- C**olumn packing polarity**: Usually, all compounds will move slower on polar columns, but polar compounds will show a larger effect.
- **Flow rate of the gas** through the column: Speeding up the carrier gas flow increases the speed with which all compounds move through the column.
- **Length of the column**: The longer the column, the longer it will take all compounds to elute. Longer columns are employed to obtain better separation.

2.1.5.4 Mass Spectroscopy

Gas chromatography separates the components of a mixture while mass spectroscopy characterizes each of the components individually. By combining the two techniques, we can both qualitatively and quantitatively evaluate a sample containing a number of chemicals.

As the individual compounds elute from the GC column, they enter the electron ionization detector. There, they are bombarded with a stream of electrons causing them to break apart into fragments. These fragments can be large or small pieces of the original molecules. The fragments are actually charged ions with a certain mass. The mass of the fragment divided by the charge is called the mass to charge ratio (M/Z). Since most fragments have a charge of +1, the M/Z usually represents the molecular weight of the fragment.

2.1.6 Applications of GC-MS

When GC is combined with MS, a powerful analytical tool is assembled. A researcher can take an organic solution, inject it into the instrument, separate the individual components, and identify each of them. Below are some examples of GC separations.

Petroleum

Gasoline

Hydrocarbon gas analysis

Fuel and fuel oil analysis

Oxygenated additives in gasoline

Environmental

Determination of pesticides

Detection of disinfection by-products in drinking water

Underground storage tank leakage

Air pollution analysis

Pharmaceutical

Residual solvents in pharmaceutical formulations

Determination of drugs in race horse urine

Forensics

Blood alcohol analysis

Determination of illicit drugs

Determination of drug impurities to track sources

Food and Flavor

Perfume analysis

Quality control of alcoholic beverages

Fatty acid analysis

Detection of 145 components in rose oil
Volatile compounds in food packaging

2.1.7 Distillation methods

The vast majority of true essential oils are produced by distillation. There are different processes used, however. In all of them, water is heated to produce steam, which carries the most volatile chemicals of the aromatic material with it. The steam is then chilled (in a condenser) and the resulting distillate is collected. The Essential Oil will normally float on top of the Hydrosol (the distilled water component) and be separated off.

2.1.7.1 Steam Distillation

True Steam distillation uses an outside source of steam which pipes the steam into the distillation unit, sometimes at high pressure. The steam passes through the aromatic material, and exits into the condenser.

2.1.7.2 Hydrodistillation

Figure 2.18: Clavenger Type Hydrodistillation apparatus
(http://heartmagic.com.eooptions)

The botanicals are fully submerged in water, producing a "soup", the steam of which contains the aromatic plant molecules. This is the most ancient method of distillation and the most versatile. The oil and water are then separated; the water, referred to as a 'hydrosol,' can be retained as it will have some of the plant essence. Rose hydrosol, for example, is commonly used for its mild antiseptic and soothing properties, as well as its pleasing floral aroma. A number of factors determine the final quality of a steam-distilled essential oil. Aside from the plant material itself, most important are time, temperature, and pressure, and the quality of the distillation equipment. Essential oils are very complex products; each is made up of many, sometimes hundreds, of distinct molecules which come together to form the oil's aroma and therapeutic properties. Some of these molecules are fairly delicate structures that can be altered or destroyed by adverse environmental conditions. So, much like a fine meal is more flavorful when made with patience, most oils benefit from a long, slow 'cooking' process. The temperature of the extraction chamber cannot be too high. The same goes for pressure. Higher temperatures and pressures result in a 'harsh' aroma – more chemical than floral –

and reduce the oil's therapeutic effects. Also, the essential oil extraction period must be allowed to continue for a certain period of time in order to flush all the oil's components from the plant, as some are released more quickly than others. The risk, of course, is that the still can run dry, or be overheated. Hydrodistillation seems to work best for powders (i.e., spice powders, ground wood, etc.) and very tough materials like roots.

Table 2.1 Boiling points of some common essential oil components, °C

Constituents	BP	(at atm pressure)	BP
alpha-Pinene	154.75	Citral	228.00
Camphene	159.50	Geraniol	229.65
Myrcene	171.50	Carvone	230.84
alpa-Phellandrene	175.79	Thymol	231.32
Cineole	176.40	Safrole	234.50
p-Cymene	176.80	Cuminic aldehyde	235.50
Dipentene	177.60	Carvacrol	237.70
Fenchone	193.53	Anethole	239.50
Linalool	198.30	Cinnamic aldehyde	251.00
beta-Tujone	201.00	Eugenol	252.66
Citronellal	206.93	Caryophyllene	260.50
Borneol	212.00	Isoeuganol	266.52
l-Menthol	216.00	Zinguberene	269.50
alpha-terpinoel	217.50	Dillapiole	285.00
Dihydrocarvone	222.40	Coumarin	301.72
Bornyl acetate	223.00	*alpha*-Santalol	301.99
Citronellol	223.42	*beta*-Santalol	309.00

2.1.7.3 Water & Steam distillation

A water and steam distillation arrangement can be compared to a kitchen steamer basket, with the botanicals supported in a "basket" over boiling water, thus exposing the plant material only to the rising steam vapors. This is the best method for distilling leafy materials, but doesn't work well for woods, roots, seeds, etc.

2.1.7.4 Solvent Extraction

Very delicate aromatics can't survive the process of distillation. To capture their magical aromas, a process of solvent extraction is used. An extracting unit is loaded with trays of blossoms. The blossoms are washed repeatedly with a solvent (usually hexane.) The solvent dissolves all extractable matter from the plant which includes non-aromatic waxes, pigments and highly volatile aromatic molecules. The solution containing both solvent and dissolvable plant material is filtered and the filtrate subjected to low pressure distillation to recover the solvent for further use. The remaining waxy mass is what is called the concrete and can contain as much as 55% volatile oil. The concentrated concretes are processed further to remove the waxy materials which dilute the pure essential oil. To prepare the absolute from the concrete, the waxy concrete is warmed and stirred with alcohol (usually ethanol). During the heating and stirring process the concrete breaks up into minute globules. Since the aromatic molecules are more soluble in alcohol than is the wax an efficient separation of the two takes place. But along with the aromatic molecules a certain amount of wax also becomes dissolved and this can only be removed by agitating and freezing the solution at very low temperatures.

2.1.7.5 Carbon Dioxide Extraction

When CO_2 (carbon dioxide) is subjected to high pressure, the gas turns into liquid. This liquid CO_2 can be used as a very inert, safe, "liquid solvent." which will extract the aromatic molecules in a process similar to that used to extract absolutes. The advantage is that no solvent residue remains since at normal pressure and temperature, the CO_2 simply reverts to a gas and evaporates. CO_2 extraction has given us essences of some aromatics that don't yield essential oils, Rose Hip Seed, and Calendula, for examples. If the same essential oil is available both as a steam distilled essential oil and a CO_2 extracted essence, the CO_2 seems to have a richer, more intense scent, since more of the aromatic chemicals are released through this process.

2.1.7.6 Cold Pressing

It is like the spray of orange essential oil that can be released by scoring skin of the fruit. The cold pressed citrus oils are commercial produced just this way, by machines which score the rind and capture the resulting oil. Although many citrus oils are also produced by steam distillation, they seem to lack the vibrancy of the cold pressed oils.

2.1.7.7 Florasols/ Phytols

This extraction method uses a new type of gaseous solvents. In the late 1980s Dr. Peter Wilde first recognized the unique properties of these solvents for the extraction of aromatic oils and biologically active components from plant materials, for use in the food, pharmaceutical, aromatherapy and perfume industries. Refrigerant, (R134a) is the solvent upon which the process is based .Extraction occurs at or below ambient temperatures; hence there is no thermal degradation of the products. The extraction process utilizes the selectivity of the solvent and produces free flowing clear oil free of waxes (http://naturesgift.com).

Choosing the right method is a work of dedication at every step, from observation to fine-tuning the distillation process, produces truly fine oil. The making of a fine essential oil relies far more on technical expertise than it does on the particular extraction method. There are, however, legitimate reasons to select one method of extracting essential oil over another – some plants simply require a particular process to produce fine oil, and the oil needed for a particular application may only be made by one process.

CHAPTER 3

METHODOLOGY

3.1 Introduction

The experiment was conducted focusing on the achievement of the research objective. The detailed procedure of the experimental work is discussed throughout this chapter. This include the six phases; washing of the rhizomes, grinding process, drying, hydrodistillation, exposure of the samples to light and temperature, and finally the GC analysis . From the GC and GC-MS analysis conducted all the necessary result has been recognized.

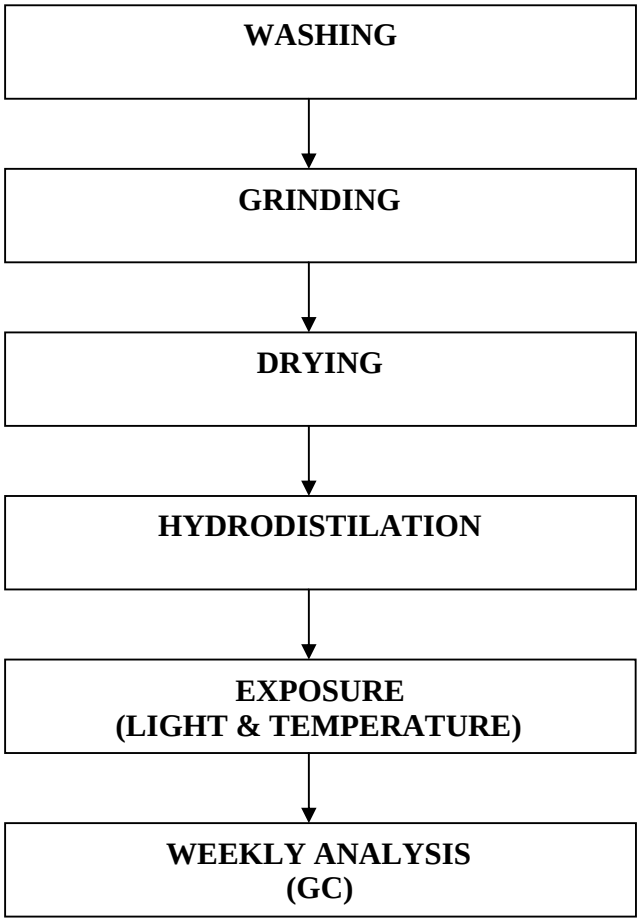


Figure 3.1: Summary of Methodology

3.2 Sample Preparation

Wild species of *Etlingera* species collected near Bentong by a botanist and my supervisor. Samples prepared from the *Etlingera* sp.1 rhizomes. The rhizomes cleaned and sliced into small pieces. Than the samples weighed before dried in the oven at a moderate temperature around or < 40°C. Temperature is a main concern here as the constituents in the rhizomes can be destroyed by excessive heat. The dried samples were weighed.

3.3 Isolation of the Essential Oil

First, the entire dried sample was immersed in water in a round bottom flask. The mixture was allowed to stand for few hours. Aluminum foil will be used to wrap the exposed glass to minimize heat loss. Cooling water circulated through the condenser before conducting the experiment. During the extraction, the temperature of the heating mantle to be controlled to ensure that the vapor pressure temperature does not exceed certain temperature. Water and essential oil mixture evaporated and separated. The essential oil was on the top of the water. Then essential oil mixed with sodium sulfate anhydrous to absorb the water that mix with essential oil to ensure there is no more moisture content, before analyzed using GC and GC-MS

$$\text{Yield of essential Oil (\%)} = \frac{\text{Weight of essential oil (g) obtained}}{\text{Weight of ground, dried sample (g) used}} \times 100\% \quad \text{(eq.3.1)}$$

3.4 Sample Analysis

The separation performed in a GC and GC-MS. The carrier gas used was helium with a constant flow rate. The temperature of the injector and detector fixed. Essential oil samples were diluted in n-hexane. Raw data obtained electronically in the form of retention times and percent area.

3.4.1 GC-MS Analysis (operating conditions)

The role of GC-MS here was mainly to identify the compounds. Injector volume set to be at 1 µL with an inlet temperature of 250˚C (splitless).Column flow fixed at 1 mL/min. Owen temperature was in the range of 70˚C -300˚C with 10˚C/ min hold time for 8 minutes. Detector temperature preset at 310˚C.

3.4.2 GC Analysis (operating conditions)

GC or commonly known as GC-FID used for weekly analysis of samples. Injector volume set to be at 1 μL with an inlet temperature of 250˚C (splitless).The column flow fixed at 1 mL/min. Owen temperature is in the range of 70˚C -300˚C with 15˚C/ min hold time for 10 minutes. Detector temperature preset at 310˚C

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Results

The data were obtained from the sample for each experiment conducted with similar parameters. While the detailed morphological study is still being done and the handling of the wild species of *Etlingera* are yet to be known, there are no other indicators on how and what are the predictable results will be. At the same time it is likely to exhibit same pattern as other members of the *Zingiberacae* family essential oil. Presumptuous that the quality of the essential oil will deteriorate due to exposure to heat and light, the results are predictable to vary for each of the sample vials.

The tightly controlled sample vial displayed the finest quality of essential oil, while the exposed vials had the quality of its essential oil reduced. Throughout three experimental weeks, the exposed sample analyzed in periodic to monitor the quality.

4.1.1 Yield Of Product

Table 4.1 Yield of Essential oil

Weight of dry specimen (g)	Weight of essential oil (g)	% yield (based on dry weight)
87.4	0.79	0.903

4.1.2 Physical Characteristics Of Product

Table 4.2 Product physical characteristics

Essential oil	General appearance	Color	Odor
Freshly distilled	Dark Ointment-like look	Dark yellow	Strong pine-like smell
Storage in refrigerator	Mild	Yellow	Moderate pine-like smell
Exposed to light and temperature	Diluted	Clearer	Reduced smell

4.1.3 Gas Chromatography

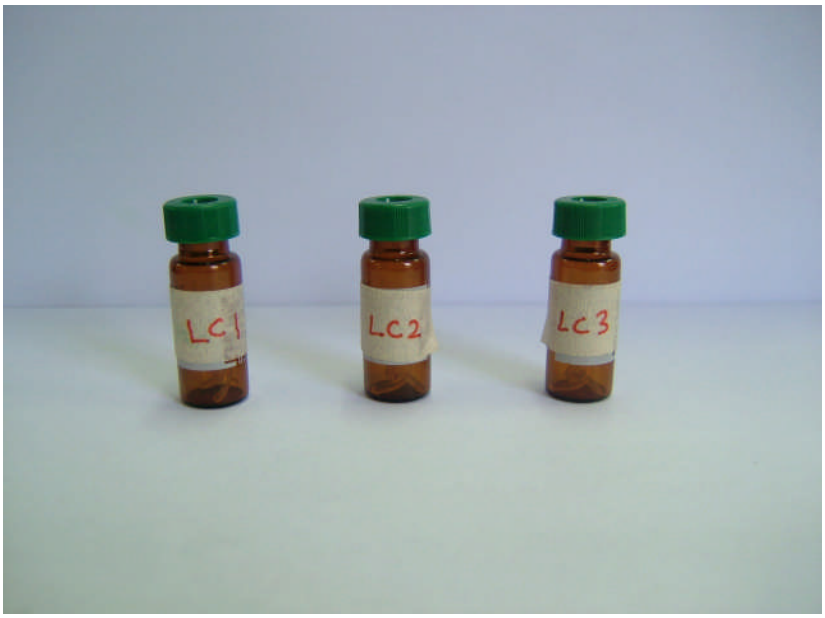


Figure 4.1: Light test samples

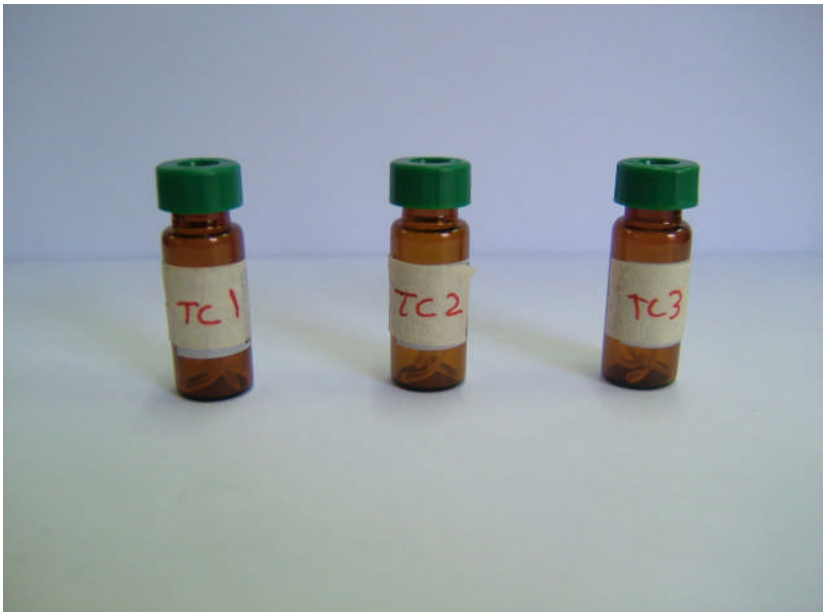
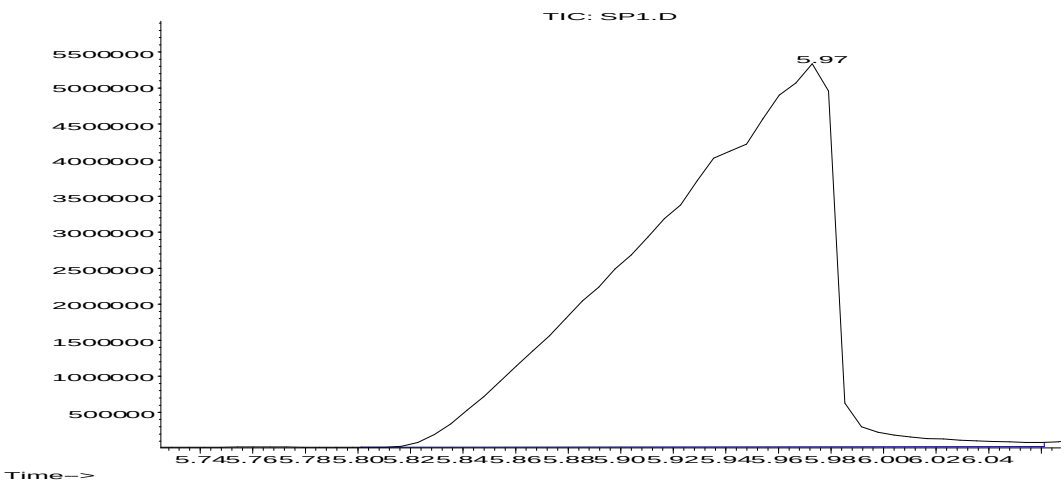


Figure 4.2: Temperature test samples

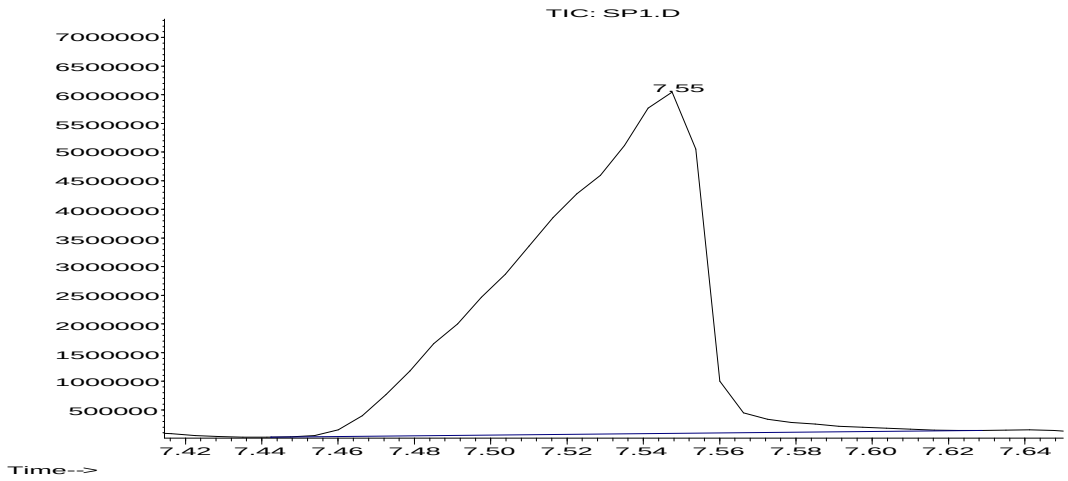
4.1.3.1 GC-MS Results



Scan 943 (5.973 min): SP1.D
SP1

1	Decanoic acid (CAS) $$ Capric acid $$ Decoic acid $$ Decylic acid $$ Caprynic acid $$ Caprinic acid $$ n-Decoic acid $$ n-Capri			172	C10H20O2		99	*		
	000334-48-5	76310	125	0	0	84	0	80	0	99
	9983									
2	Decanoic acid (CAS) $$ Capric acid $$ Decoic acid $$ Decylic acid $$ Caprynic acid $$ Caprinic acid $$ n-Decoic acid $$ n-Capri			172	C10H20O2		95	*		
	000334-48-5	76306	112	10	0	71	14	74	53	95
	9426									
3	Decanoic acid (CAS) $$ Capric acid $$ Decoic acid $$ Decylic acid $$ Caprynic acid $$ Caprinic acid $$ n-Decoic acid $$ n-Capri			172	C10H20O2		93	*		
	000334-48-5	76314	80	39	0	85	20	66	0	93
	9678									
4	Decanoic acid (CAS) $$ Capric acid $$ Decoic acid $$ Decylic acid $$ Caprynic acid $$ Caprinic acid $$ n-Decoic acid $$ n-Capri			172	C10H20O2		72			
	000334-48-5	76318	88	31	1	90	20	42	0	47
	9861									
5	Decanoic acid (CAS) $$ Capric acid $$ Decoic acid $$ Decylic acid $$ Caprynic acid $$ Caprinic acid $$ n-Decoic acid $$ n-Capri			172	C10H20O2		64	*		
	000334-48-5	76315	74	46	0	69	34	37	0	81
	9664									

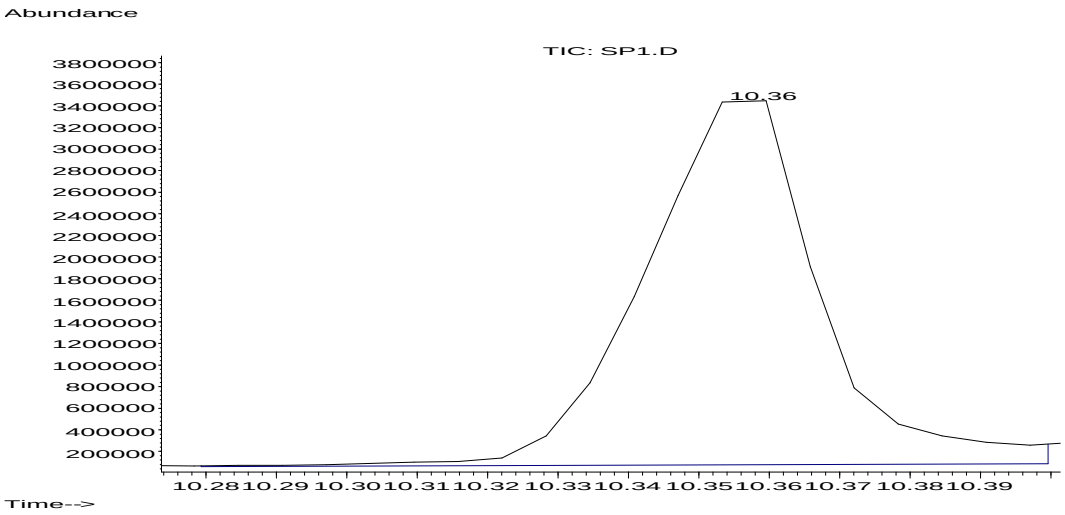
Figure 4.3: Chromatogram and data of 5.973 min



Scan 1195 (7.548 min): SP1.D
SP1

1	Dodecanoic acid (CAS) $$ Lauric acid $$ Abl $$ Neo-fat 12 $$ Vulvic acid $$ Univol u-314 $$ Aliphat no. 4 $$ Neo-fat 12-43 $$ D			200	C12H24O2		99	*		
	000143-07-7	115375	147	0	0	80	0	80	27	99
	9917									
2	Dodecanoic acid			200	C12H24O2		97	*	000143-07-7	115373
	130	17	0	66	8	78	33	97	9762	
3	Dodecanoic acid $$ n-Dodecanoic acid $$ Neo-fat 12 $$ Aliphat no. 4 $$ Abl $$ Dodecylic acid $$ Lauric acid $$ Laurostearic aci			200	C12H24O2		93	*		
	000143-07-7	115374	119	23	1	75	7	68	12	93
	9515									
4	Lauric anhydride			382	C24H46O3		93		000645-66-9	321635
	141	35	0	85	14	66	0	93	9849	
5	Dodecanoic acid (CAS) $$ Lauric acid $$ Abl $$ Neo-fat 12 $$ Vulvic acid $$ Univol u-314 $$ Aliphat no. 4 $$ Neo-fat 12-43 $$ D			200	C12H24O2		92	*		
	000143-07-7	115378	81	53	0	75	28	64	0	93
	9441									

Figure 4.4: Chromatogram and data of 7.548 min



Scan 1644 (10.353 min): SP1.D
SP1

1	n-Hexadecanoic acid	256	C16H32O2	99	*	000057-10-3	195432				
	157	9	0	72	7	80	4	99	9908		
2	Hexadecanoic acid (CAS) $$ Palmitic acid $$ Palmitinic acid $$ n-Hexadecoic acid $$ n-Hexadecanoic acid $$ Pentadecanecarboxyli	256	C16H32O2	99	*						
	000057-10-3	195440	151	13	0	72	0	80	61	99	
	8966										
3	Hexadecanoic acid (CAS) $$ Palmitic acid $$ Palmitinic acid $$ n-Hexadecoic acid $$ n-Hexadecanoic acid $$ Pentadecanecarboxyli	256	C16H32O2	98	*						
	000057-10-3	195439	132	30	0	81	21	79	59	98	
	7582										
4	Hexadecanoic acid (CAS) $$ Palmitic acid $$ Palmitinic acid $$ n-Hexadecoic acid $$ n-Hexadecanoic acid $$ Pentadecanecarboxyli	256	C16H32O2	97	*						
	000057-10-3	195444	132	27	0	68	21	78	1	97	
	9517										
5	Hexadecanoic acid (CAS) $$ Palmitic acid $$ Palmitinic acid $$ n-Hexadecoic acid $$ n-Hexadecanoic acid $$ Pentadecanecarboxyli	256	C16H32O2	94	*						
	000057-10-3	195435	106	46	0	67	21	70	53	94	
	9067										

Figure 4.5: Chromatogram and data of 10.353 min

4.1.3.2 GC Results

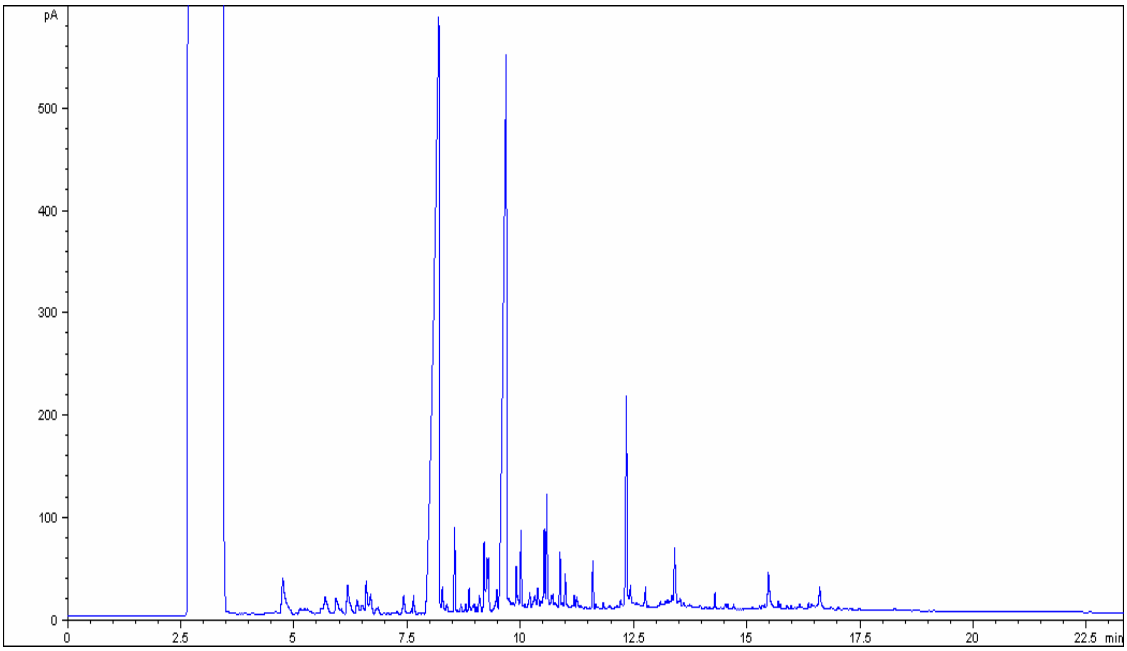


Figure 4.6: GC-FID analysis for fresh *Etlingera* sp.1 essential oil

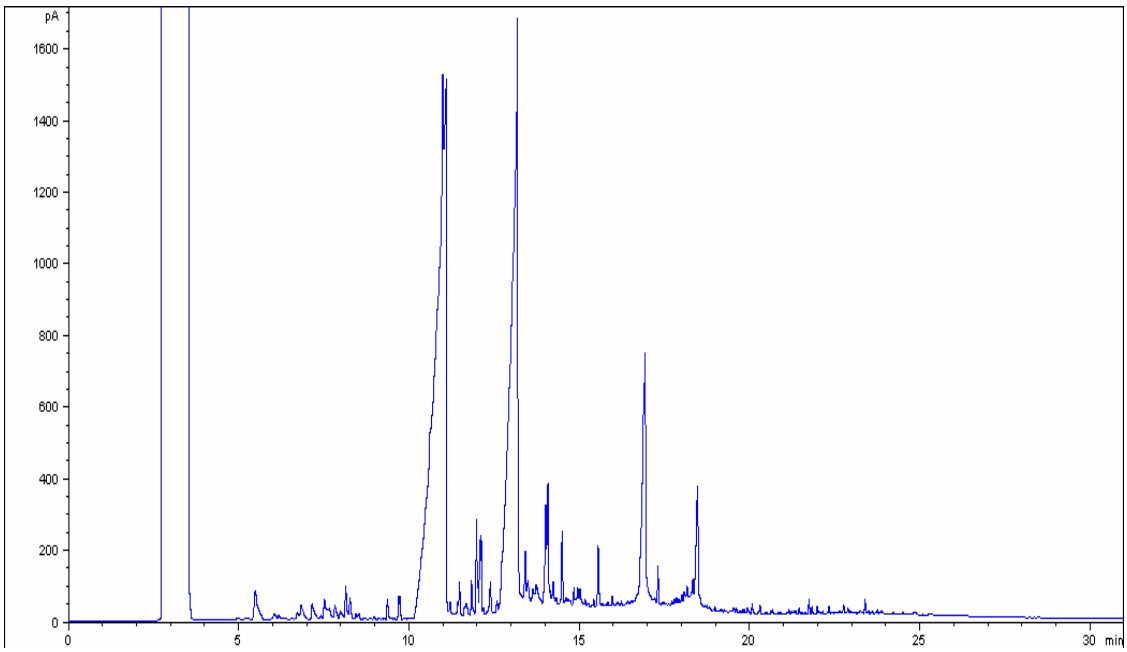


Figure 4.7: GC-FID analysis for test of temperature week 1

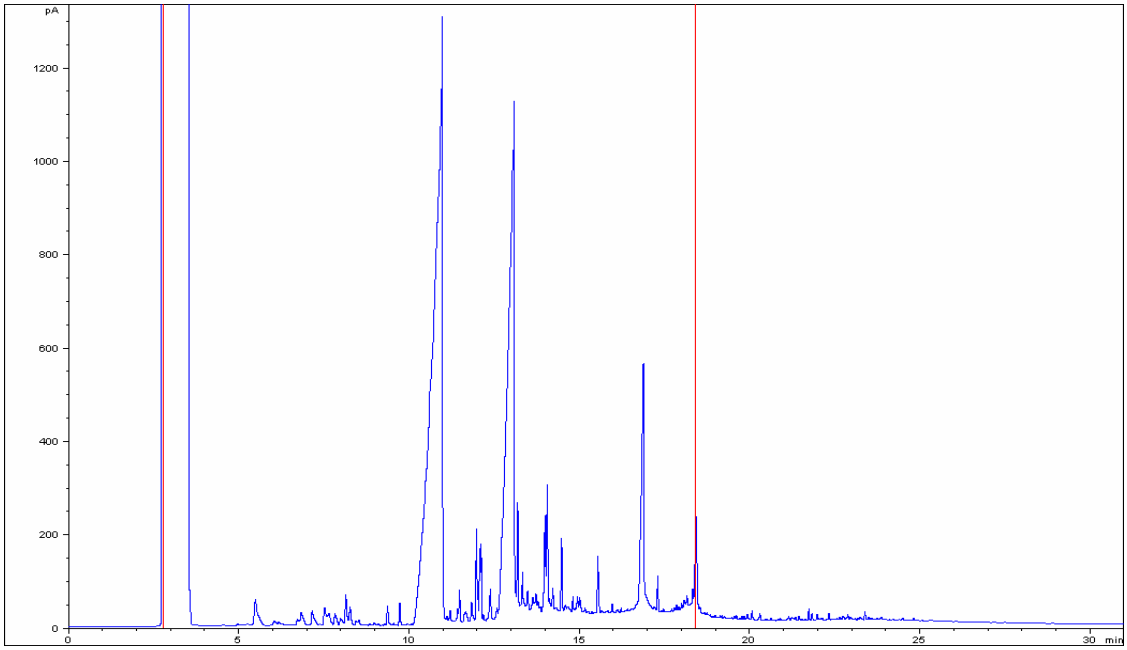


Figure 4.8: GC-FID analysis for test of temperature week 2

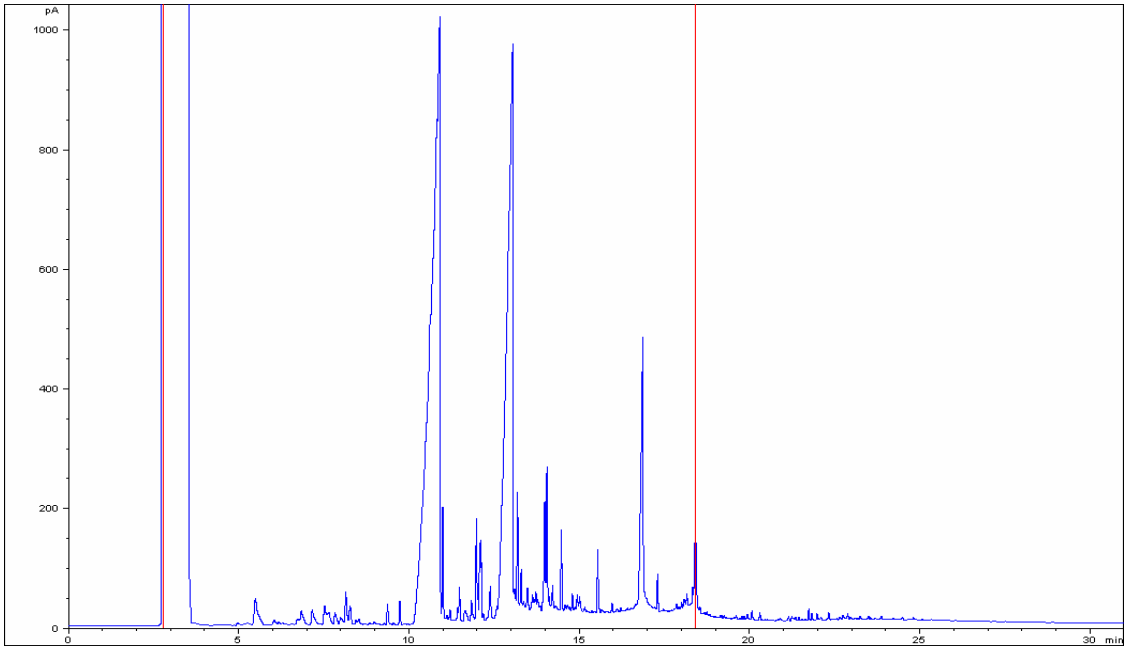


Figure 4.9: GC-FID analysis for test of temperature week 3

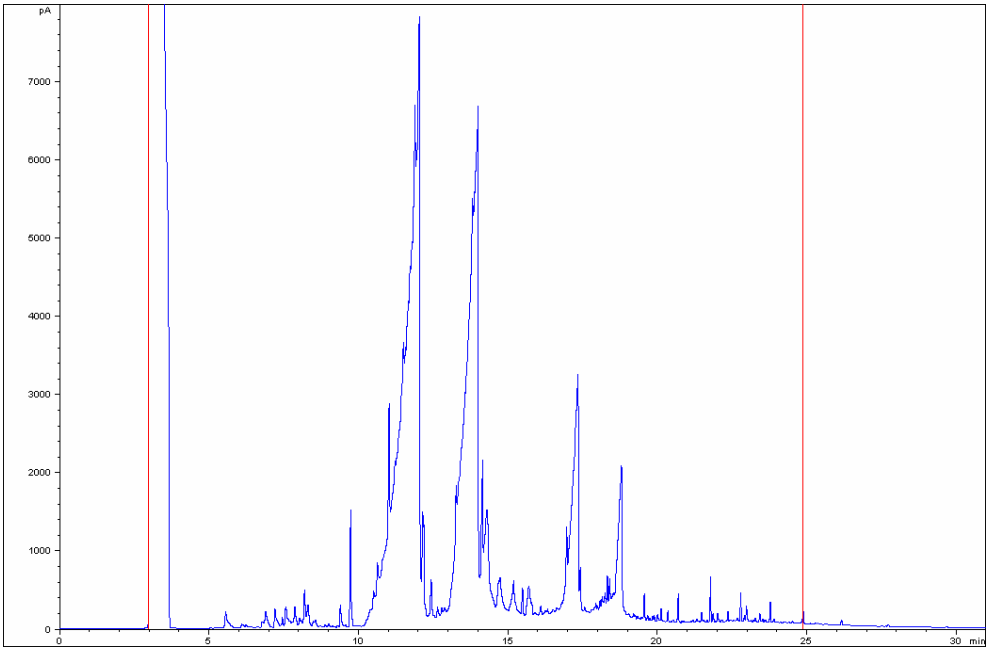


Figure 4.10: GC-FID analysis for test of light week 1

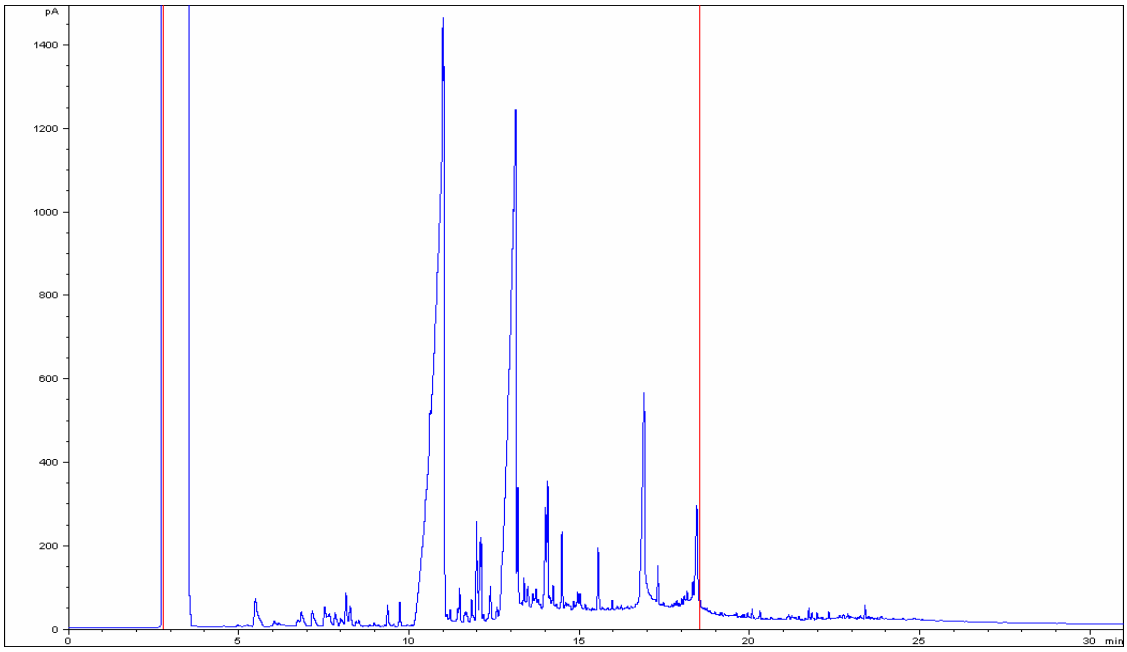


Figure 4.11: GC-FID analysis for test of light week 2

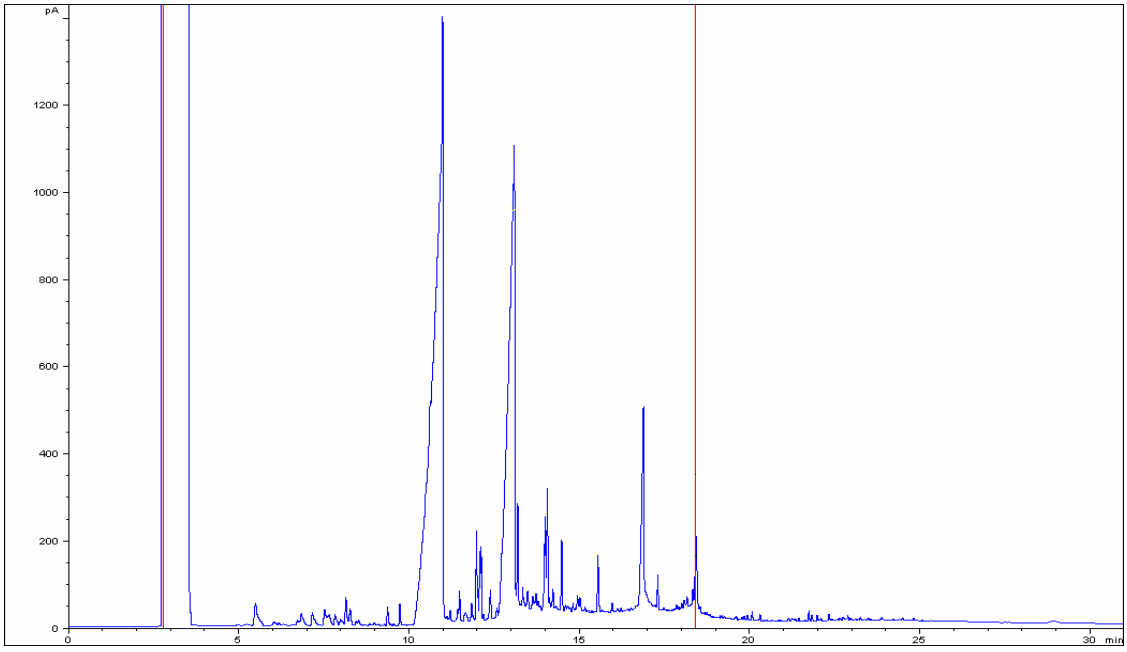


Figure 4.12: GC-FID analysis for test of light week 3

From the study, three viable peaks were chosen to compare and to study the effect of the heat and light on essential oil quality.

4.1.4 Chromatogram Comparison Analysis

To compare the clear peaks from the GC-FID analysis, a table is constructed. The tables represent the area of some peaks that are evidently seen in the analysis and consist of the area from week 1 to week 3 and also from the fresh essential oil. The retention time of the weekly test is slightly different to one another but the difference is negligible since the peaks are the same.

Table 4.3 Fresh essential oil peak area

Retention time (min)	Area (pA*s)
4.745	0.00192
6.177	0.00112
8.188	0.04544
8.539	0.00143
9.677	0.02686
9.900	0.00077
10.003	0.00154
10.518	0.00160
10.573	0.00220
10.867	0.00108
11.591	0.00095
12.334	0.00468
13.398	0.00167
15.465	0.00161

Table 4.4 Light test peak area

	Area (pA*s)		
Retention time (min)	**Week 1**	**Week 2**	**Week 3**
5.546	1399.16150	488.51300	380.397
8.184	1323.43103	254.42249	211.40729
9.381	929.20056	146.00201	108.80592
9.727	3706.09009	140.21576	106.99607
10.623	3950.14746	20262.7	17701.7
11.881	36979.4	700.97266	496.90445
13.992	59319.24	16497.8	14091.0
14.282	15156.6	660.05511	570.49109
17.336	37485.1	4511.77490	3512.17578
17.414	1607.47681	484.47079	379.68100
18.786	16919.4	1461.59338	963.67529

From the area, we can compare the differences between the chosen peaks as the percentage of the area will show us that the peaks have increased or decreased during the test period. There will be 3 considerable peaks that will be analyzed for both light and temperature test. The 3 significant peaks consists of the highest peak which is Peak 1 at the retention time of 8.188 minute, the second clear peak which is Peak 2 with retention time of 9.677 minute, and Peak 3 by 12.334 minute. These 3 peaks will be studied for its area percentage difference linking each week.

Table 4.5 Temperature test peak area

	Area (pA*s)		
Retention time (min)	**Week 1**	**Week 2**	**Week 3**
5.479	640.27454	410.27591	345.57211
9.355	166.26608	116.38616	87.19778
10.982	27119.9	23970.4	19420.5
11.973	8368.25519	471.11426	395.79941
13.175	22213.5	14326.6	1.1668.5
14.005	1207.3715	808.86066	665.92218
14.486	899.87109	523.35547	436.49185
15.544	790.59601	474.99557	382.50754
16.924	6226.10303	3658.11475	2766.01758
17.300	500.24533	308.88736	245.54234
18.459	2186.39697	1070.46667	767.31329

To compare the peak from one week to the other, the area percentage will be calculated.

4.1.4.1 Comparison for Light Test

For the 3 sets of peaks in the light test, the area percentage obtained from GC-FID analysis report is as below: -

Peak 1:

Area percentage fresh = 0.04544 %

Area percentage week 1 = 2.27292 %

Area percentage week 2 = 0.22071 %

Area percentage week 3 = 0.19110 %

Peak 2:

Area percentage fresh = 0.062686 %

Area percentage week 1 = 3.64603 %

Area percentage week 2 = 0.17970 %

Area percentage week 3 = 0.15212 %

Peak 3:

Area percentage fresh = 0.00468 %

Area percentage week 1 = 2.30400 %

Area percentage week 2 = 0.04914 %

Area percentage week 3 = 0.03792 %

4.1.4.2 Comparison for Temperature Test

For the 3 sets of peaks in the temperature test, the area percentage obtained from GC-FID analysis report is as below: -

Peak 1:

Area percentage fresh = 0.04544 %

Area percentage week 1 = 0.29925 %

Area percentage week 2 = 0.26158 %

Area percentage week 3 = 0.21586 %

Peak 2:

Area percentage fresh = 0.062686 %

Area percentage week 1 = 0.24511 %

Area percentage week 2 = 0.15634 %

Area percentage week 3 = 0.12969 %

Peak 3:

Area percentage fresh = 0.00468 %

Area percentage week 1 = 0.06870 %

Area percentage week 2 = 0.03992 %

Area percentage week 3 = 0.03074 %

4.1.5 Compounds Identification

From the GC-FID analysis and confirmed by GC-MS three evident compounds identified. Those three are closely connected to each other and identified to be Decanoic acid, Dodecanoic acid and n-Hexadecanoic acid.

4.1.6 Decanoic Acid

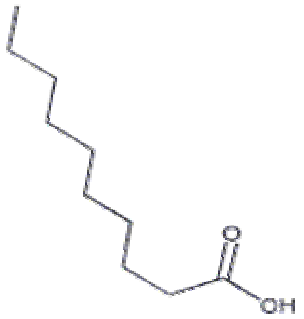


Figure 4.13: Decanoic acid $C_{10}H_{20}O_2$

Decanoic acid is a type of carboxylic acid with its molecular formula $CH_3(CH_2)_8COOH$. Other common names are Capric acid, *n*-Decanoic acid, Decylic acid and *n*-Decylic acid.Salts and esters of decanoic acid are called decanoates. Commercially used in organic synthesis, manufacture of perfumes, lubricants, greases, rubber, plastics, food additives and pharmaceuticals. (http://en.wikipedia.org/wiki/ Decanoic acid).

4.1.7 Dodecanoic Acid

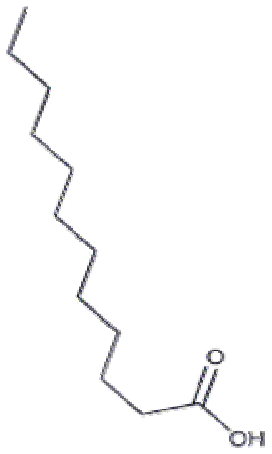


Figure 4.14: Dodecanoic acid $CH_3(CH_2)_{10}COOH$

Dodecanoic acid also known as Lauric acid, is mainly found in coconut oil and palm kernel oil, is believed to have antimicrobial properties. It is a white, powdery solid with a faint odor of bay oil or soap. Dodecanoic acid, slightly irritating to mucous membranes, has low toxicity and used in soaps and shampoos. It is also found in human milk(5.8% of total fat), cows milk(2.2%), and goat milk(4.5%). Lauric acid has a non-polar hydrocarbon tail and a polar carboxylic acid head, it can interact with polar solvents as well as fats, allowing water to dissolve fats. This accounts for the abilities of shampoos to remove grease from hair. (http://en.wikipedia.org/wiki/Dodecanoic acid).

4.1.8 n-Hexadecanoic Acid

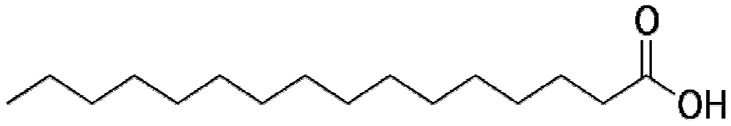


Figure 4.15: n-Hexadecanoic acid $C_{16}H_{32}O_2$

n-Hexadecanoic acid or palmitic acid is one of the most common saturated fatty acids found in animals and plants. As its name indicates, it is also a major component of palm oil and palm kernel oil. The word palmitic is from the French "palmitique", the pith of the palm tree.

It is widely used as a lubricant and as an additive in industrial preparations. It is used in the manufacture of metallic stearates, pharmaceuticals, soaps, cosmetics, and food packaging. It is also used as a softener, accelerator activator and dispersing agent in rubbers. Oleic acid (systematic chemical name is cis-octadec-9-enoic acid) is the most abundant of the unsaturated fatty acids in nature. (http://en.wikipedia.org/wiki/Hexadecanoic acid).

Table 4.6 Summary of compound properties

Properties	**Decanoic acid**	**Dodecanoic acid**	**n-Hexadecanoic acid**
Molecular formula	$C_{10}H_{20}O_2$	$C_{12}H_{24}O_2$ or	$C_{16}H_{32}O_2$
Molar mass	172.26 g/mol	200.31776	256.42 g/mol
Density	0.893 g/cm^3	0.880 g/cm³	0.853 g/cm^3
Melting point	31 °C	44-46 °C	60 °C
Boiling point	269 °C	298.9 °C	351-352 °C
Solubility in water	Immiscible	Insoluble	Insoluble
Appearance	White crystals with strong smell	White solid	White crystals

CHAPTER 5

CONCLUSION & RECOMMENDATION

5.1 Conclusion

In this study on the objective of the project achieved, where it is proved that there are changes over time in the *Etlingera* sp.1 essential oil samples via scientific methods and justified that essential oils degrade upon exposure to light and temperature. From the chromatogram of samples it is also understood that denaturalization of compounds occur upon exposure to light and temperature higher than the standard room temperature.

5.2 Recommendation

For future research purpose, it is recommended monitoring period extended to 3 months to demonstrate a more obvious difference in terms of oil quality. Hydrodistillation method should be substituted with a more productive method. Analysis parameter can be extended to humidity.

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APPENDIX A

(GC-MS Datasheet)

APPENDIX B

(GC-FID Datasheet)

APPENDIX C

Figure 6.0: Grinding of the rhizomes

Figure 6.1: The drying process

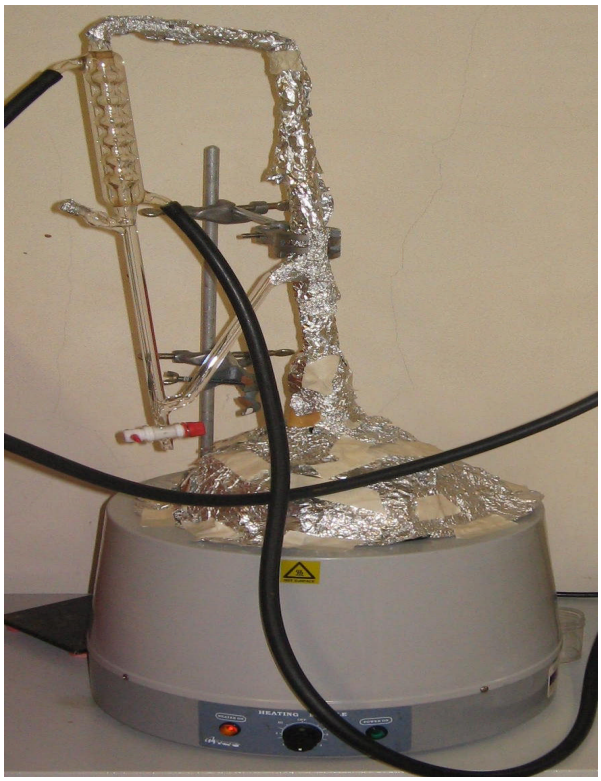

Figure 6.2: Hydrodistillation

Figure 6.3: Light test

Figure 6.4: Temperature test

Figure 6.5: Light test samples for GC analysis

Figure 6.6: Temperature test samples for GC analysis