COMPARISON OF MICROWAVE-ASSISTED HYDRODISTILLATION WITH THE CONVENTIONAL HYDRODISTILLATION METHOD IN THE EXTRACTION OF ESSENTIAL OILS

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PSZ 19:16 (Pind. 1/97)

BORANG PENGESAHAN STATUS TESIS

JUDUL: COMPARISON OF MICROWAVE-ASSISTED HYDRODISTILLATION WITH THE CONVENTIONAL HYDRODISTILLATION METHOD IN THE EXTRACTION OF ESSENTIAL OILS

SESI PENGAJIAN:2009/2010

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

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APRIL 2010

I declare that this thesis entitled *"Comparison of Microwave-Assisted Hydrodistillation with the Conventional Hydrodistillation Method in the Extraction of Essential Oils"* is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Date : 30 April 2010

To my adorable mother, Salmah Binti Haji Abdul Aziz,
my handsome father, Mohd Sadek Bin Supah, my dear grandma, Asiah Hj Omar,
my naughty brothers and sisters,
and to my beloved special friend, Mohd Rahimi Bin A. Rahman.

Thanks for being too supportive and always be my side.

ACKNOWLEDGEMENT

Praise is to Allah S.W.T for His help and guidance that I finally able to complete this thesis. In addition, I would like to express my gratitude to all those who gave me the possibility to complete this thesis.

In particular, I wish to express my sincere appreciation to my thesis supervisor, Associate Professor Dr. Abdurahman Hamid Nour, for encouragement, guidance, critics and friendship. I am also very thankful to Dr Azahari Hamid Nour for his willingness helping me. Additionally, I would like to thank to the FKKSA Lab for giving me help for technical needed. Without their continued support and interest, this thesis would not have been the same as presented here.

My sincere appreciation also extends to all my colleagues and others who have provided assistance at various occasions. Their views and tips are useful indeed. Unfortunately, it is not possible to list all of them in this limited space. I am grateful to all my family members. I can never thank you enough for their love, and for supporting me throughout my studies in Universiti Malaysia Pahang (UMP). Last but not least, a big thank you to my supportive special friend.

ABSTRACT

The production of essential oils from natural sources is highly profitable nowadays. People worldwide start to realize the important of healthy awareness. Highly demand of ginger *(Zingiber officinale Roscoe)* oil and citronella grass *(Cymbopogon nardus)* oil due to its uses. Here, a modern and an alternative method, microwave-assisted hydrodistillation (MAHD) is approach. The purpose of this study is to investigate the performance of MAHD on the extraction of essential oils. The essential oils from ginger and citronella grass were studied and results were compared with the conventional HD in terms of extraction yield, extraction time, cost of operation and chemical composition. MAHD was provided higher quantitative in short of time. MAHD resulted in similar citronella oil recovery for 60 min to that obtained by 90 min of HD (1.17%, v/w). Same as ginger essential oil, MAHD required extraction time period of 30 min for recovering 0.88% yield of essential oil of ginger, instead of HD required 90 min of extraction time for recovering same percentage yield. Gas chromatography-mass spectrometry analysis of the extracted essential oils indicated that the use of microwave irradiation recover high total peak area (%). MAHD was found to be a green technology.

ABSTRAK

Pengeluaran minyak pati daripada sumber semulajadi pada masa kini menjanjikan pulangan yang lumayan kepada para pengusaha. Ramai yang telah mula menyedari kepentingan penjagaan kesihatan. Permintaan yang tinggi bagi minyak pati halia *(Zingiber officinale Roscoe)* dan juga serai wangi *(Cymbopogon nardus)* berikutan penggunaanya yang semakin meluas. Bagi memenuhi permintaan tersebut, satu kaedah alternatif dan moden, hydrodistillation dengan bantuan gelombang mikro diperkenalkan. Dengan itu, kajian ini dijalankan bertujuan untuk mengenalpasti prestasi MAHD pada pengekstrakan minyak pati. Minyak-minyak pati daripada halia dan serai wangi telah dikaji dan dibandingkan dengan HD konvensional dari segi hasil pengekstrakan, masa penghasilan, kos operasi dan komposisi kimia. MAHD telah menghasilkan lebih tinggi kuantitatif dalam waktu yang singkat. MAHD mampu mengekstrak minyak serai wangi sebanyak 1.17 % pada minit ke 60 berbanding HD yang memerlukan 90 minit untuk menghasilkan jumlah minyak yang sama. Begitu juga bagi minyak pati halia, pada minit ke 30, MAHD telah mengekstrak seabanyak 0.88% hasil minyak pati halia berbanding HD pada minit ke 90. Hasil analisis *gas chromatography-mass spectrometry*, minyak pati yang dihasilkan dengan penggunaan penyinaran gelombang mikro mencapai jumlah puncak yang tinggi (%). MAHD merupakan satu teknologi yang mesra alam.

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LIST OF ABBREVIATIONS

bp	-	Boiling point
DEET	-	(N,N-diethyl-m-toluamide)
DNA	-	Deoxyribonucleic acid
etc	-	Et cetera (and so forth)
g	-	Gram
GC	-	Gas chromatography
GC-FID	-	Gas chromatography-flame ionization detector
GC-MS	-	Gas chromatography- mass spectrometry
GHz	-	Gigahertz
HD	-	Hydrodistillation
HPLC	-	High performance liquid chromatography
i.e.	-	Id est (that is)
MAE	-	Microwave-assisted extraction
MAHD	-	Microwave-assisted hydrodistillation
MHz	-	Megahertz
min	-	Minute
mL	-	Milliliter
Mw	-	Molecular weight
SFE	-	Supercritical fluid extraction
TLC	-	Thin layer chromatography
UV	-	Ultraviolet

LIST OF SYMBOLS

^{o}C	-	Degree Celsius
η	-	Refractive index
%	-	Percent
&	-	And
×	-	Multiply
v/w	-	Volume per weight
W	-	Watt

CHAPTER 1

INTRODUCTION

1.1 Research Background

Nowadays, people were eager to find something new, especially from herbs or medicinal plant. It is because people worldwide start to realize the important of healthy awareness. By using product base natural sources, they believe and realize there are no side effect to society and environment. Essential oil or also known as ethereal oil is a concentrated, hydrophobic liquid that contains hundreds of aromatic compounds, organic constituents, including hormones, vitamins and other natural elements. These compounds are extracted from leaves, stems, flowers, bark, roots or other elements of a plant; essential oil contains highly volatile components. These essential oils have many uses. Besides being medicinal herbs, most of them are produced for the perfume industry, handmade soap, bath salts, lotions and other natural bath and body products.

There are a lot of plants that can extract the essential oils from. For example, Ginger (*Zingiber officinale Roscoe),* Citronella Grass *(Cymbopogon nardus),* Lavender flowers (*Lavandula angustifolia Mill*, Lamiaceae), Gaharu (*Agarwood*), Thyme (*Thymus vulgaris L.),* and Misai Kucing (*Orthosiphon Stamineus)*.

Zingiber officinale Roscoe (common ginger), a member of *Zingiberaceae* family is an aromatic/medicinal plant. It is indigenous to the Asia South East. Ginger products such as essential oil and oleoresin, are internationally commercialized for use in food and pharmaceutical processing (C. Z. Kelly et al., 2002). The name Ginger is derived from the Sanskrit word Sringavera (meaning shaped like a horn) and being commonly found in South East Asia. It is highly beneficial in combating depression. It has also proved its effectiveness in relieving pain. Ginger essential oil is sought after due to its aphrodisiac and stimulant properties. Ginger herb is extensively brought to use for its moisture retention characteristics. It helps a great deal in toning the body. It serves as the best remedy to fight against Malaria.

Cymbopogon nardus also known as citronella grass is a perennial of the *Poaceae* grass family, originating in tropical Asia. It is a genus of about 55 species of grasses, native to warm temperate and tropical regions of the Old World and Ocenia. It is a perennial tufted grass with long, sharp-edged blades. The fresh stalks and leaves have a clean lemonlike odour because they contain an essential oil, which is also present in lemon peel. Citronella grass cannot be eaten because of its unpalatable nature. This plant is an invasive species that renders pastureland useless as cattle will starve even in its abundance.

Orthosiphon stamineus, better known as Misai Kucing by the locals is rich in flavonoids. Most flavonoids are bioactive compounds due to the presence of phenolic group in their molecule (Khamsah et al., 2006). *Orthosiphon Stamineus* (Misai Kucing) is a medicinal herb found mainly throughout South East Asia. It is believed to have anti allergic, antihypertensive, anti-inflammatory and diuretic properties. It is used as a remedy for arteriosclerosis (capillary and circulatory disorders), kidney stones and nephritis.

Thymus vulgaris L. (thyme), a member of the Labiatae family, is an aromatic/medicinal plant of increasing economic importance for North America, Europe, North Africa and Asia (Letchamo & Gosselin, 1996). Thyme is one of many aromatic plants that have been utilized in variety of food products to provide a flavour specific to this herb.

The main methods to obtain essential oils from the plant materials are hydro distillation (HD), steam distillation, steam and water distillation maceration, empyreumatic (or destructive) distillation, and expression (Stahl-Biskup & Saez, 2002). Among these methods, HD has been the most common approach to extract the essential oils from the medicinal herbs/plants (Stahl-Biskup & Saez, 2002). However, in order to reduce the extraction time and possibly improve the extraction yield, to enhance the quality of the extracts and also to reduce the operation costs, new approaches such as microwave-assisted extraction (MAE), pressurized solvent extraction, supercritical fluid extraction, and ultrasound-assisted extraction have also been sought (Kaufmann & Christen, 2002; Wang & Weller, 2006).

1.2 Problem Statement

Currently, in Malaysia, essential oil and oleoresin is gaining popularity as herbal medication as it gave a lot of benefit to overcome the disease. Most of the essential oil has medicinal properties and it had been used since thousand years ago. Ginger is a well known as marketable spice. Today, the essential oil from ginger is widely used and the most important is that the ginger oil is used in medical field for a few sicknesses. Same as citronella oil, people widely used as insect repellent, aromatherapy, astringent and also perfume industry.

By using the conventional hydrodistillation, it was prolong the extraction time. For example, it was needed 7 days continuous heating in order to extract volatile material. So, in order to fulfil the demand and commercialize the product, Microwave-assisted hydrodistillation is an alternative and cost effective method to extract essential oil from ginger. This method will be useful in order to overcome the disadvantages of conventional hydrodistillation. Besides, this method is environmental friendly and cleaner process for isolation of essential oil using microwave energy. Regarding environmental impact, the calculated quantity of carbon dioxide rejected in the atmosphere is lower. The waste water also reduced (Asma Farhat et al., 2009).

1.3 Objectives

The objectives of this study are:-

1) To identify the chemical composition of the essential oil extracted from ginger *(Zingiber officinale Roscoe)* and citronella grass *(Cymbopogon nardus)* by using microwave-assisted hydrodistillation (MAHD) and conventional hydrodistillation (HD).
2) To investigate the performance of potential microwave-assisted hydrodistillation (MAHD) on the yield of essential oil.
3) To identify the effect of extraction time and operational cost.

1.4 Scopes of Study

The scopes of this research are listed as below:-

1) Study the effect of extraction time to the yield of essential oil. Every method consumed different time of extraction.
2) Comparing the extraction yield and efficiency between microwave-assisted hydrodistillation MAHD and conventional hydrodistillation HD method.
3) Study performance of microwave-assisted hydrodistillation MAHD in extraction of essential oil.
4) Analyze the chemical composition of essential oil by using Gas Chromatography-Mass Spectrometry (GC-MS)
5) Study the operational cost based on extraction time and yield.

CHAPTER 2

LITERATURE REVIEW

2.1 Essential Oils

An essential oil is a concentrated, hydrophobic liquid containing volatile aroma compounds from plant. Essential oils contain the DNA of the plant of herb they are extracted from. Essential oils or as they are sometimes called volatile or ethereal oils are believed to be that small portion of the plant material, which imparts the characteristic odour and flavour most closely associated with the vegetative matter which they are obtained. Essential oils do not as a group need to have any specific chemical properties in common, beyond conveying characteristic fragrances.

The advantages of essential oils are their flavour concentrations and their similarity to their corresponding sources. The majority of them are fairly stable (notable exception is the citrus oil) and contain a few natural antioxidants. Essential oils are usually colorless, particularly when fresh. Nevertheless with age essential oil may oxidize and resinify, which resulting the colour becomes darker. Therefore, essential oil need to be stored in a cool, dry place tightly stoppered and preferably full in amber glass containers.

The choice of a particular process for the extraction of essential oil is generally dictated by the following considerations:

1) Sensitivity of the essential oils to the action of heat and water.
2) Volatility of the essential oil.

3) Water solubility of the essential oil.

As most of the essential oils of commerce are steam volatile, reasonably stable to action of heat and practically insoluble in water hence are suitable for processing by distillation. They are used in perfumes, cosmetics and bath products, for flavouring food and drink, and for scenting incense and household cleaning products. Essential oils are derived from various sections of plants. For example, Lavender flowers (*Lavandula angustifolia Mill*, *Lamiaceae)*, Gaharu *(Agarwood)*, Misai Kucing *(Orthosiphon Stamineus)*, Thyme *(Thymus vulgaris L.)*, Lime *(Citrus latifolia Tanaka)*, Citronella grass *(Cymbopogon nardus)* and Ginger (*Zingiber officinale Roscoe).*

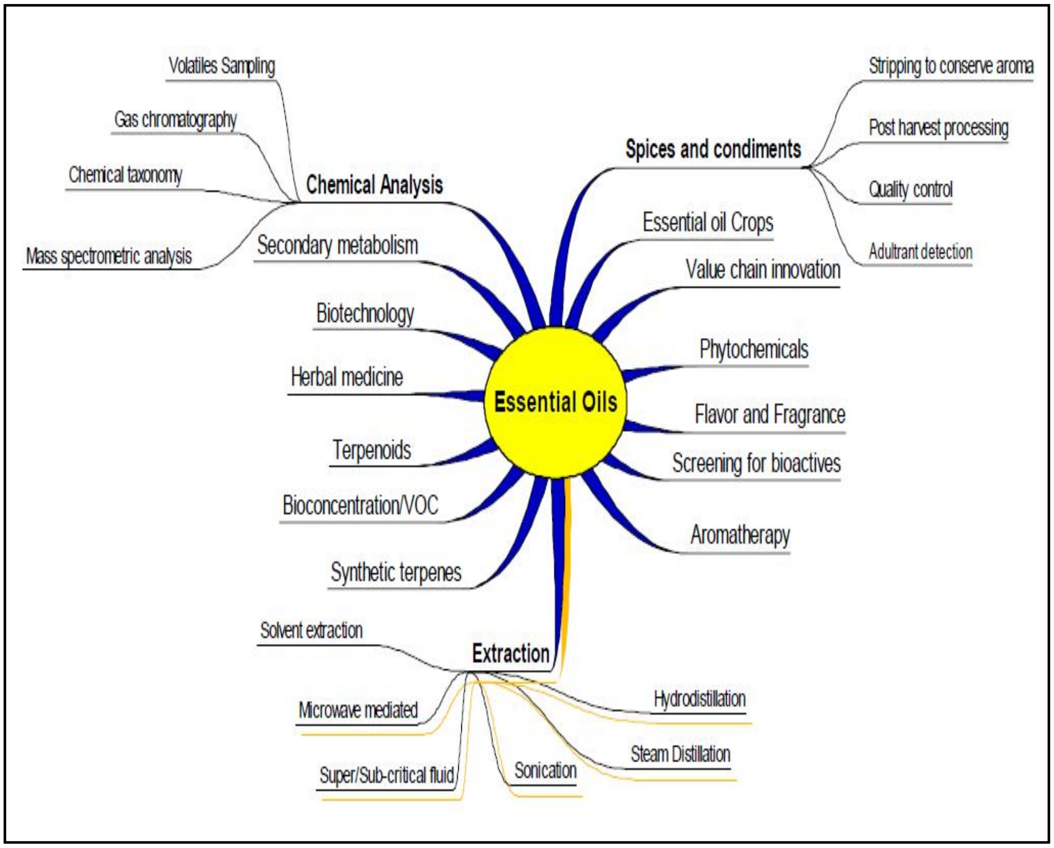

Figure 2.1: Tree diagram showing the wide branching of specializations in the field of essential oils

2.1.1 Lavender Flowers

The name lavender (*Lavandula angustifolia Mill*, Lamiaceae) comes from the Latin root *lavare*, which means "to wash." Lavender is one of the most useful medicinal plants. Commercially, the lavender is an important source of essential oil that is widely used in fragrance industry including soaps, colognes, perfumes, skin lotions and other cosmetics (Paul et al., 2004). In food manufacturing, lavender essential oil is employed in flavouring beverages, ice-cream, candy, baked goods and chewing gum. Recently, uses of lavender in aromatherapy as relaxant are becoming increasingly popular (Fakhari et al., 2005). The scent has a calming effect which may aid in relaxation and the reduction of anxiety.

Lavender is native to the mountainous zones of the Mediterranean where it grows in sunny, stony habitats. Today, it flourishes throughout southern Europe, Australia, and the United States. Lavender is a heavily branched short shrub that grows to a height of roughly 60 centimetres. Its broad rootstock bears woody branches with erect, rod-like, leafy, green shoots. A silvery down covers the gray-green narrow leaves, which are oblong and tapered, attached directly at the base, and curled spirally. Figure 2.2 below shows the lavender plant.

Human clinical studies have reported that lavender essential oil may be beneficial in a variety of conditions, including insomnia, alopecia (hair loss), anxiety, stress, postoperative pain, and as an antibacterial and antiviral agent. Lavender oil is also used together with other forms of integrative medicine, such as massage, acupuncture, and chiropractic manipulation. It may also help to relieve pain from tension headache when breathed in as vapor or diluted and rubbed on the skin. When added to a vaporizer, lavender oil may aid in the treatment of cough and respiratory infection.

Figure 2.2: Lavender plant

2.1.2 Gaharu

Gaharu or also known as 'Agarwood' 'Aloeswood', 'Eaglewood' in English and 'Jinko' in Japanese is a fragrant wood product that usually obtained from a pathological condition of the wood of standing trees of *Aquilaria (Thymelaeceae)* species and one of the most valuable non-timber products in Asian tropical forest. *Aquilaria* is a fast-growing, archaic tropical forest tree, which occurs in South and Southeast Asia, from the foothills of the Himalayas to the rainforests of Papua New Guinea. The tree grows in natural forests at an altitude of a few meters above sea level to about 1000 meters, and it grows best around 500 meters. It can grow on a wide range of soils, including poor sandy soil. Seedlings need a lot of shade and water. According to Dato Dr Abdul Rashid Abdul Malik there are 25 species of Agarwood worldwide and only about 12 of it were able to produce gaharu (Borneo Post, 2009). The major source of agarwood is Aquilaria Malaccensis.

The production of gaharu resins have a several potential such as used primarily in traditional Chinese and Korean medicines. The oil also used in perfumes and cosmetic product. Besides that the chips of agarwood are ground into powder can be used for special cigarettes. Its ethereal fragrance is demanding for incense which used in religious and spiritual ceremonies of Islam or Buddhism especially in Asia such as Thailand, Malaysia and Indonesia. Agarwood might have some effects towards central nervous system (CNS) such as higher brain function, from traditional use as a sedative (Ueda J. et al, 2006). The odor of agarwood is complex and pleasing, with few or no similar natural analogues.

Formation of agarwood occurs in the trunk and roots of trees that have been infected by a parasitc ascomycetous mold, *Phaeoacremonium parasitica*, a dematiaceous (dark-walled) fungus. As a response, the tree produces a resin high in volatile organic compounds that aids in suppressing or retarding the fungal growth. While the unaffected wood of the tree is relatively light in colour, the resin dramatically increases the mass and density of the affected wood, changing its colour from pale beige to dark brown or black. In natural forest only about 7% of the trees are infected by the fungus. Figure 2.3 shows the picture of agarwood trees and Figure 2.4 shows pieces of agarwood.

Figure 2.3: Agarwood trees

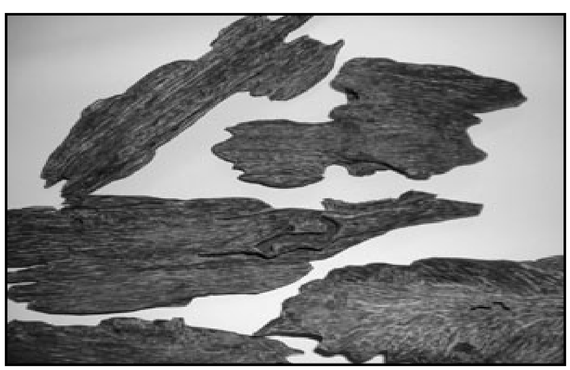

Figure 2.4: Pieces of agarwood

2.1.3 Thyme

Thyme (*Thymus vulgaris L.)* is a low growing herbaceous plant, sometimes becoming somewhat woody. Figure 2.5 shows thyme plant. It is native to southern Europe. It is much cultivated as a culinary herb. Thyme is one of many aromatic plants that have been utilized in variety of food products to provide a flavor species to this herb (Golmakani & Rezaei, 2008). Thyme is a good source of iron and is used widely in cooking. Thyme is often used to flavour meats, soups and stews. It has a particular affinity to and is often used as a primary flavour with lamb, tomatoes and eggs.

From the medicinal point of view, the essential oil of thyme is made up of 20-54% thymol. Thymol, an antiseptic, is the main active ingredient in Listerine mouthwash. Before the advent of modern antibiotics, it was used to medicate bandages. It has also been shown to be effective against the fungus that commonly infects toenails. Medicinally thyme is used for respiratory infections. In traditional Jamaican childbirth practice, thyme tea is given to the mother after delivery of the baby.

Figure 2.5: Thymus vulgaris L.

2.1.4 Misai Kucing

One of the local herbs which is scientifically known as *Orthosiphon stamineus* Benth (*O. stamineus*) or locally called as Misai Kucing is rich in flavonoids (Surmayono *et al*., 1991). Most flavonoids are bioactive compounds due to the presence of phenolic group in their molecule (Apati, 2003: Havsteen, 2002). The leaves of *O. stamineus* are arranged in opposite pairs. The petiole is relatively short, about 0.3 cm in length and reddish purple in colour. The flowers are borne on verticals about 16 cm length, white to bluish in colour with long far-exerted filaments, making it look like cat's whispers (Wong FK, 2002). Figure 2.6 below shows how *Orthosiphon stamineus* look like.

Orthosiphon stamineus Benth (Lamiaceae) is a popular medicinal plant in Southeast Asia. It is widely used for the treatments of many diseases, especially those affecting the urinary tract, diabetes mellitus, hypertension, rheumatism, tonsillitis and menstrual disorder (Awale et al., 2003a,b). The methanolic extracts of this plant have shown the inhibitory activity on nitric oxide production in macrophage like cells (Awale et al., 2003a,b). *O. stamineus* is also found in other countries such as Thailand, Indonesia and Europe. In these countries, Misai Kucing is also known as Yaa Nuat Maeo, Rau Meo or Cay Bac (Thailand), Kumis Kucing or Remujung (Indonesia), moustaches de chat (French) and Java Tea (European) (Indubala & Ng, 2000).

Figure 2.6: *Orthosiphon stamineus* plants

2.1.5 Lime

The lime tree is a member of the Rutaceae family. The essential oil from the lime has a sweet, tangy scent. It is also used now medicinally to help enhance the nervous system. The oil also has antiseptic properties. They are very well known across the world and are extensively used in pickles, jams, marmalades, sauces, confectionaries, squashes, sorbets, desserts, beverages, cosmetics and a numerous other industrial products.

Citrus oils are mixtures of very volatile components as terpenes and oxygenated compounds. Limonene, a monoterpene, is the major component of lime and other related citrus essential oils. These oils are used in the pharmaceutical, perfumery and food industries, and the quality of the oils is related to the value of total aldehydes, basically citral content, which is between 4-5%. The common commercial methods to produce the oils from citrus fruits and peels are machine cold pressing and distillation (Atti-Santos, 2005).

2.1.6 Ginger

Zingiber officinale Roscoe also known as ginger consists of the fresh or dried roots of *Zingiber officinale*. The English botanist William Roscoe (1753-1831) gave the plant the name *Zingiber officinale* in an 1807 publication. The ginger family is a tropical group especially abundant in Indo-Malaysia, consisting of more 1200 plant species in 53 genera. The genus *Zingiber* includes about 85 species of aromatic herbs from East Asia and tropical Australia. The name of the genus, *Zingiber*, derives from a Sanskrit word denoting "horn-shaped," in reference to the protrusions on the rhizome (Foster, 2009). Today, it is cultivated all over tropic and subtropaic Asia (50% of the world's harvest is produced in India), in Brazil, Jamaica (whence the best quality is exported) and Nigeria, whose ginger is rather pungent, but lacks the fine aroma of other (Arnould, 1981). Figure 2.7 shows a picture of the fresh ginger rhizome and Figure 2.8 shows the ginger plant.

Ginger is a perennial herb and grows to about 3 - 4 feet high with thick spreading tuberous rhizome. Every year it shoots up a stalk with narrow spear-shaped leaves as well as white or yellow flowers growing directly from the root (Lawrence and Reynolds, 1984). The ginger of commerce consists of the thick scaly rhizomes (underground stems) of the plant. They branch with thick thumb-like protrusions, thus individual divisions of the rhizome are known as "hands."

Figure 2.7: Fresh ginger

Figure 2.8: Ginger plant

2.1.6.1 Historical Use

Ginger had a reputation for being the remedy that could cure some of the most difficult and chronic ailments. Some physicians regularly used it as a remedy to counter violent temper and hysteria and the treatment for such conditions involved making the patients smoke without their knowledge, dried, finely shredded ginger roots out of a hollowed reed instead of tobacco. Along with Cinnamon, Ginger was high on the list of culinary ingredients and was and still is a much appreciated addition to food in England. Although not quite as popular as it used to be, the consumption of ginger is still fairly high in the UK and this is reflected in traditional ginger beer, ginger brandy, ginger champagnes, ginger wines and gingerbread men (Perez, 2005).

Ginger is very widely used in Traditional Chinese Medicine. It is said to be a Yang/spleen remedy and is supportive to the spleen, stomach and kidneys (the latter is specially indicated for men and it is classified as an aphrodisiac and considered a good remedy for impotence). Both fresh and dried roots are official drugs of the modern Chinese pharmacopoeia, as is a liquid extract and tincture of ginger. Ginger is used in dozens of traditional Chinese prescriptions as a "guide drug" to "mediate" the effects of potentially toxic ingredients. In fact, in modern China, Ginger is believed to be used in half of all herbal prescriptions (Foster, 2009).

Meanwhile, in Arabic medicine, ginger is said to be hot in the second degree and moist in the first. It is warming and has a softening effect on the belly; it is beneficial to the body against digestive ailments such as flatulence, food poisoning and constipation (Perez, 2005).

Ginger holds just as an important place in Western medicine as it does in Eastern medicine (China, Japan and India to name but a few). It has been used on its own or included as an ingredient in specific herbal formula and also used as a ‘corrective remedy’ against the unwanted effects of other plants. It has been verified in recent research.

2.1.6.2 The Composition of Ginger Oil

Figure 2.9 shows the structures of zingiberene and β-sesquiphellandrene for ginger oil constituents. Ginger essential oil mostly consists of zingiberene, dexto-camphene, beta-sesquiphellandrene, bisabolene, dexto-phellandrene, betaphellandrene and 1,8-cineole. Ginger oil owes its aroma to bisabolene, zingiberene and zingiberol. The physical properties and characteristics of some of the constituents of the ginger oil are shown in Table 2.1 (Nurul Azlina, 2005).

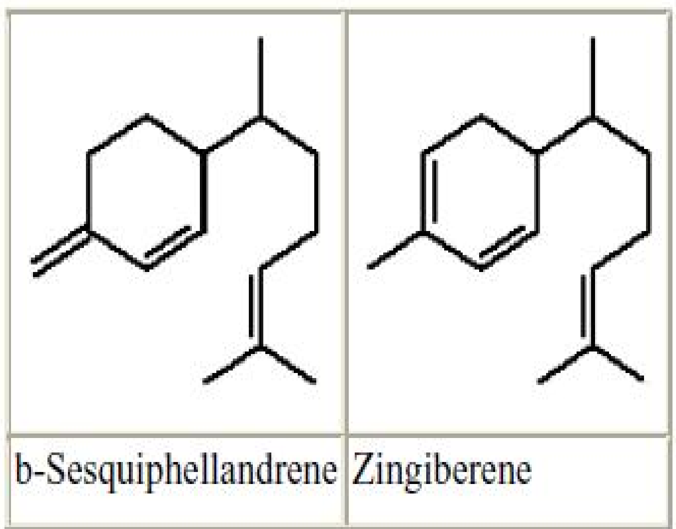

Figure 2.9: Structures of ginger oil constituents (Lawrence and Reynolds, 1984)

Table 2.1: Properties data for the prominent constituents in ginger oil

Name of Constituents and Characteristics	Molecular Formula	Melting point (^{0}C)	Boiling point (^{0}C)	Density	UV Maxima	Refractive index	Flash Point (^{0}C)	Mw
Oil of ginger -yellowish -viscid liquid -almost insole in water -sparingly soluble in alcohol, soluble in ether,carbon disulfide				d1515 0.875- 0.885 d2515 0.870- 0.885		ηD20 1.4880- 1.4950 ηD20 1.4880- 1.4940 ηD20 1.4900- 1.8920		
***d*-Camphene** -colourless -used in perfumery -cubic crystals from alcohol -volatilizers on exposure to air -insipid odor	C10 H6	51	158- 160 bp17 52	d504 0.8486		ηD20 1.4605	36	136
Zingiberene -constituents of ginger oil -natural oil always contaminated with bisabolene	C15H24		bp14 134			ηD20 1.4956		204
β-bisabolene -occurs in opaponax and bisabol myrrh oils	C15H24	79-80	bp12 155 -157			ηD20 1.4917		204
Ar-curcumene -oil of ginger -liquid	C15H22		bp17 137			ηD20 1.4989		202

The ginger oil has many uses. Table 2.2 shows some of the important uses of ginger oil in the industry (Lawrence and Reynolds, 1984).

Table 2.2: The constituents and uses of ginger oil

Constituents	Uses
Linalool, β-bisabolene, geraniol	Flavourist
Zingiberene, δ-camphene, borneol, phellandrene	Mouth washes, ginger beverages, liqueurs
α-pinene	Manufacture of camphor, insecticides, solvents, plasticisers, perfume bases, synthetic pine
Zingiberene, β-bisabolene	Aromatic principles
Zingiberene, δ-camphene, cineol, borneol, α-pinene, linalool, β-bisabolene	Perfumer

2.1.7 Citronella Grass

Cymbopogon nardus also known as citronella grass is a perennial of the Poaceae grass family, originating in tropical Asia. It is a genus of about 55 species of grasses, native to warm temperate and tropical regions of the Old World and Ocenia. Citronella grass is a tall tropical grass. It is a perennial tufted grass with long, sharp-edged blades. The fresh stalks and leaves have a clean lemonlike odour because they contain an essential oil, which is also present in lemon peel. As a spice, fresh citronella grass is preferred for its vibrant flavour, but is also sold in dried form. The dried spice is available in several forms: chopped in slices, cut and sifted, powdered, or as oil can be extracted from the plant. Citronella grass cannot be eaten because of its unpalatable nature. This plant is an invasive species that renders pastureland useless as cattle will starve even in its abundance.

2.1.7.1 Historical Use

Cittronella grass is originally from Sri Lanka and it became popular all over the world in the 19th century. The lemongrass oil was known as Oleum Siree when it was exported for the first time to Europe. Sri Lanka was the main center of citronella grass oil production until 1890 when Java started production of this oil of better quality. In 1900s, citronella grass that was exported to other countries contained more of geraniol (natural alcohol) and had much stronger smell, but then the lemongrass oil was adulterated and Sri Lanka has to reduce its price. Use of citronella grass oil in wax candles has been very common because it repels insects and mosquitoes. Citronella oil is largely used in soaps, perfumes, skin lotions, detergents, deodorants, cosmetic products and also paints. This citronella oil uses can be classified as:

1) **Insect Repellent:**

   Citronella oil repels insects such as mosquitoes, black flies, fleas and ticks, therefore, preventing its bites. It is used on humans and their clothing – in the form of oil, liquid and patch. Citronella oil is a natural, non-toxic alternative to chemical insect repellents such as DEET, therefore, is usually the preferred choice. Also available are solid products such as citronella oil insect repelling candles and cartridges. Citronella oil is also used in a tablet or pellet form in recreational or outdoor household areas and around trees and shrubs. In addition, there are animal collars and tags containing citronella oil for pets and other domestic animals to repel fleas. A combination of the citronella oil and cedarwood virginian oil also helps to repel mosquitoes.

2) **Aromatherapy:**

   Citronella oil, an essential oil, possesses activating and warming qualities both physically and mentally. The aroma is said to be like its relative's lemongrass and palmarosa. However, citronella oil is overlooked in aromatherapy because of its association with insect repellency. When citronella oil is diluted properly in base oil and is applied to skin, it produces a mild sensation of warmth – which relieves painful muscles and joints. Mentally, the aroma of citronella oil may help with nervous fatigue due to its clarifying properties. It can also ease pressure of migraines and headaches.

3) **Astringent:**

Citronella oil is an astringent and if used correctly it may help with oily skin areas. Use a single drop of citronella oil on the skin (usually inner forearm) to test for irritation. Apply two or three drops of citronella oil to a cotton ball and gently wipe off the excess oil.

4) **Perfume Industry:**

The Java citronella oil is one of the most widely used perfume oils and is often used as a starting point for perfume materials, as its constituent's citronellal and geraniol are important and widely used perfume building blocks.

2.1.7.2 The Composition of Citronella Oil

Citronella oil is obtained from the leaves of the oil grasses *Cymbopogon nardus*. Two derivatives of citronella oil include the alcohol citronellol and the aldehyde citronellal (Brown, 2010). Figure 2.10 shows both citronellol and citronella structure. Citronella oil contains a number of fragrant fractions of which citronellal, geraniol, and citronellol are the major components. Citronella oil indicates that it contains large amounts of monoterpenes mostly in the form of limonene. The chemical composition of essential oil can vary tremendously. Other compounds predominant in citronella oil include citronellyl acetate, β bourbonene, geranyl acetate, elemol, L-borneol, and nerol. A geraniol-rich mutant of citronella has been developed; it is reported to have a geraniol content as high as 60% (Ranaweera et al., 1996).

Monoterpene fractions containing mycrene were very lethal to late third instar Culex quinquefasciatus larvae. Elemol and methyl iso-eugenol were responsible for larvicidal activity in other fractions (Pattnaik et al., 1996).

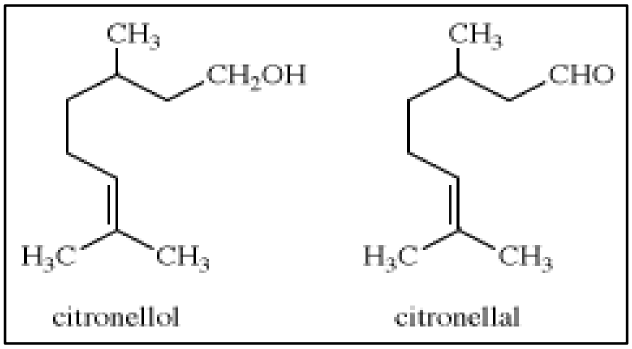

Figure 2.10: Structure of citronellol and citronella

2.2 Extraction Processes of Essential Oil

Extraction is phenomenon that can be defined as the process of separating desired components from a material. In order to separate one or more of the components in a mixture, the mixture is brought into contact with another phase (Geankoplis, 2003). There are a few conventional and modern methods of extracting essential oils. A wide variety of analytical methods is used to extract the volatile compounds from plant material. Techniques commonly used to extract the essential oils include cold pressing, enfleurage, hydro-diffusion, supercritical fluid extraction, steam distillation and hydrodistillation.

2.2.1 Cold Pressing

The term cold pressed theoretically means that the oil is expeller-pressed at low temperatures and pressure. Cold pressed expression, or scarification, is used to obtain essential oils for the peels and seeds of citrus's, such as bergamot, grapefruit, lemon, lime, mandarin, orange, and tangerine oils (Arnould, 1981). The spray of orange essential oil that can be released by scoring or zesting the skin of the fruit is familiar. The cold pressed citrus oils are commercial produced just this way, by machines which score the rind and capture the resulting oil.

In this process, the outer layer of the fruit peel contains the oil are removed by scrubbing. Then the whole fruit is pressed to squeeze the juice from the pulp and to release the essential oil from the pouches. The essential oil rises to the surface of the juice and is separated from the juice by centrifugation.

2.2.2 Enfleurage

Enfleurage is an expensive process and is rarely used today except in a few places in France and India (Arnould, 1981). Some flowers, such as jasmine or tuberose, have very low contents of essential oil and are extremely delicate; heating them would destroy the blossoms before releasing the essential oils. In such cases, enfleurage is sometimes used to remove the essential oils (Guenther, 1972). Flower petals are placed on solid sheets of warm fat as shown in Figure 2.11, which will absorb the flowers' essential oils. When all the fragrance is transferred from the flowers to the fat, they are removed and replaced with fresh ones (Billot and Wells, 1975). This process is repeated several times until the fat becomes saturated with the essential oil. A solvent, most of the time alcohol, is then added which separates the essential oil from the fatty substance. The alcohol will then evaporate leaving only the essential oil. This method is no longer commercially viable (Poucher, 1974).

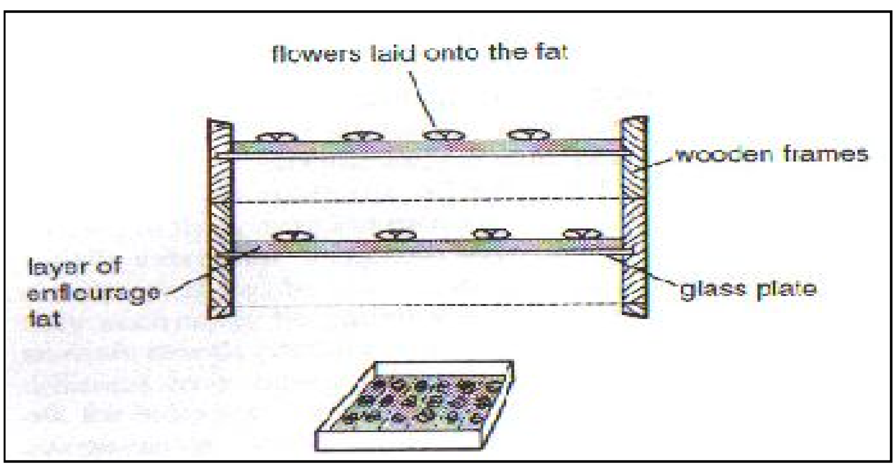

Figure 2.11: Schematic representation of the enfleurage method of extraction (Poucher, 1974)

2.2.3 Hydro-diffusion

Although introduced more recently than carbon dioxide extraction, hydrodiffusion is similar to steam distillation except that the steam is produced above the plant material and percolates down through it (Chrissie, 1996). The advantage of hydrodiffusion over distillation is that the process is quicker, especially for fibrous material such as woods and barks. The resultant oils are reported to have a superior aroma and a richer colour obtained by ordinary distillation. Nevertheless, oils captured by hydrodiffusion process are not widely available. Figure 2.12 shows the process of hydrodiffusion.

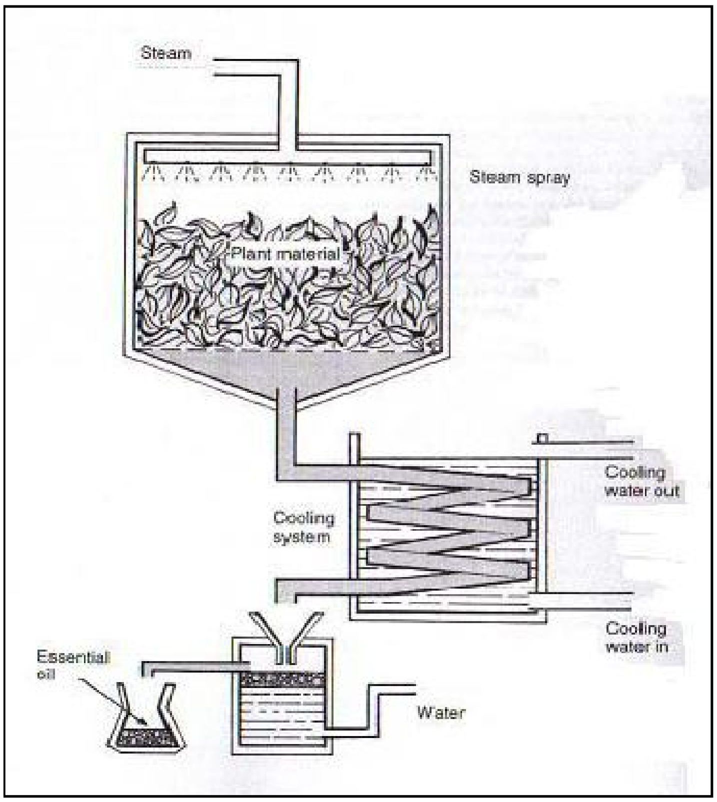

Figure 2.12: Schematic representation of the process of hydro-diffusion (Chrissie, 1996)

2.2.4 Supercritical Fluid Extraction

Supercritical fluids have been used as solvents for a wide variety of applications such as essential oil extraction, metal cation extraction, polymer synthesis and particle nucleation. In practice, more than 90% of all analytical supercritical fluid extraction (SFE) is performed with carbon dioxide (CO_2) for several practice reasons. Apart from having relatively low critical pressure (74 bar) and temperature (32oC), CO_2 is relatively non-toxic, non-flammable, available in high purity at relatively low cost and is easily removed from the extract. In the supercritical state, CO_2 has a polarity comparable to liquid pentane and is, therefore, best suited for lipophilic compounds. The main drawback of CO_2 is its lack of polarity for the extraction of polar analytes (Pourmortazavi et al., 2007).

Supercritical fluid extraction is an advantageous alternative process for refining citrus oils due to its low operating temperature and the absence of solvent residues. Figure 2.13 below shows the laboratory supercritical fluid extraction apparatus.

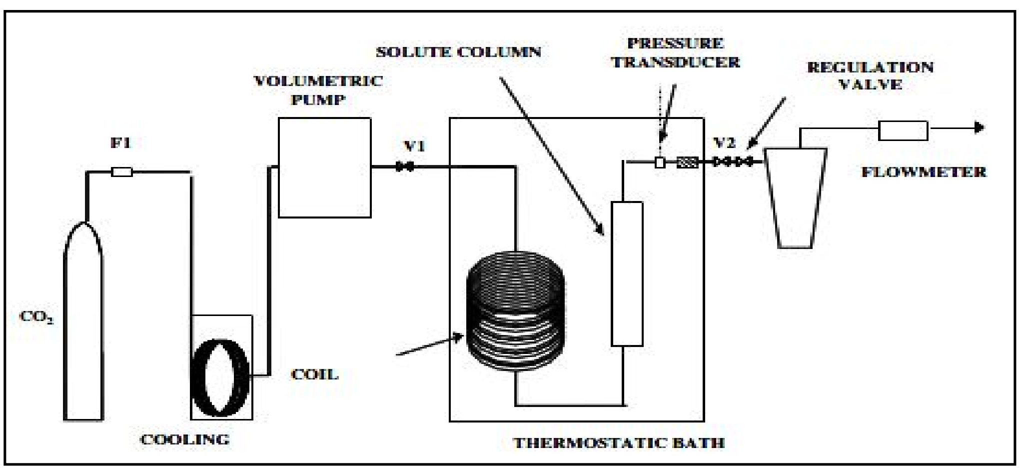

Figure 2.13: Laboratory supercritical fluid extraction apparatus (Porto et al., 2009)

2.2.5 Solvent Extraction

Solvent extraction is used on delicate plants to produce higher amounts of essential oils at a lower cost (Chrissie, 1996). An extracting unit is loaded with perforated trays of raw material. The raw materials are washed repeatedly with a solvent (usually hexane). The solvent dissolve all extractable matter from the plant which includes non-aromatic axes, 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 concentrated concretes are processed further to remove the waxy materials which dilute the pure essential oil.

2.2.6 Steam Distillation

Steam distillation is one of the separation processes that used solid-liquid extraction theory. Liquid will be used to extract the solid. It means the essential oil will be removed from its raw material. This process involves the use of steam to percolate and vapourise out the essential oils from the plant material, with the subsequent condensation of steam and essential oil prior to their separation. It can be seen from the experimental work done that there is an art to distillation and that, especially for low yield plants, much skill is needed. The role of the distiller is to achieve oil as close as possible to the oil as it exists in the plant.

Steam distillation is a method where steam is flowed through the material as shown in Figure 2.14. This steam functions as agents that breaks up the pores of the raw material and release the essential oil from it. The system yields a mixture of a vapour and desired essential oil. This vapour is then condensed further and the essential oil is collected (Rai R. & Suresh B., 2004).

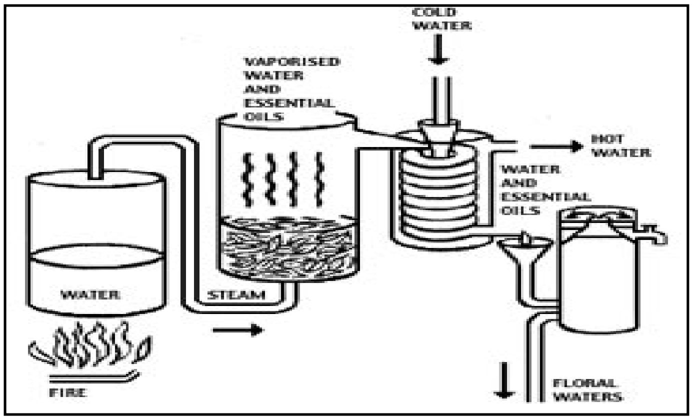

Figure 2.14: Schematic representation of the steam distillation apparatus

2.2.7 Hydrodistillation (HD)

Hydrodistillation is a traditional method for removal of essential oils. Water or hydro distillation is one of the oldest and easiest methods being used for the extraction of essential oils. There are three types of hydrodistillation: with water immersion, with water immersion and vapor injection, and with direct vapor injection. It is a versatile process that can be employed for small or large industries. The distillation time depends on the plant material being processed. Prolonged distillation produces only a small amount of extra oil, but does add unwanted high boiling compounds and oxidation products. Figure 2.15 below shows the schematic set up apparatus for hydrodistillation.

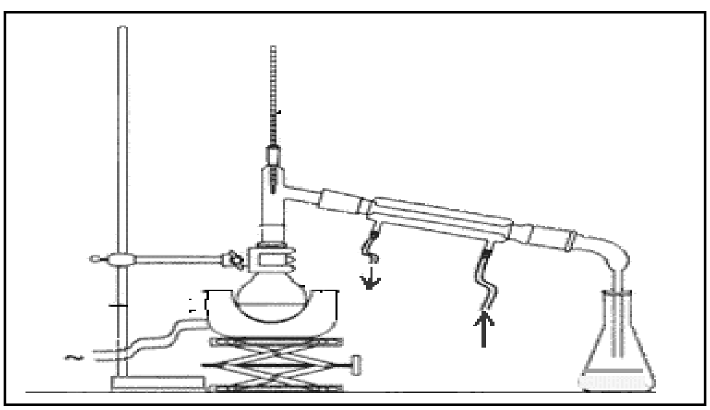

Figure 2.15: Schematic representation of the simple hydrodistillation apparatus

2.2.8 Microwave-assisted Hydrodistillation (MAHD)

In recent years, the use of microwave for extraction of constituents from plant material has shown tremendous research interest and potential. Conventional techniques for the extraction of active constituents are time and solvent consuming, thermally unsafe and the analysis of numerous constituents in plant material is limited by the extraction step (Mandal, 2007). The use of microwaves in industrial materials processing can provide a versatile tool to process many types of materials under a wide range of conditions.

Microwave-assisted hydrodistillation is an advanced hydrodistillation technique utilizing a microwave oven in the extraction process. Golmakani *et al.*, (2008) reported some recently published studies have successfully utilized a microwave oven for the extraction of active components from medical herbs/plants. The efficiency of MAHD is strongly dependent on the dielectric constant of water and the matrix (Brachet *et al.*, 2002). MAHD causes the rapid delivery of energy to the total volume of solvent/ sample leading to a rapid rise in the temperature. Heat is originated through the molecular motions within the polar components or ionic species. That is, the rise in temperature within the plant cells is similar to that occurring outside the cells. Once the pressure within the glands reaches above certain level, the external cell walls break apart and as a consequence the essential oils are re-leased to the environment. From this point on, the essential oils are carried away by water vapor, liquefied in the condenser on the top of the main apparatus and collected in the receiving flask. Because of different densities and also due to their immis-cibilities, water and essential oils are separated from each other and the excess water is refluxed to the extraction vessel (Rezvanpanah *et al.*, 2008). Figure 2.16 shows the schematic set up apparatus for MAHD.

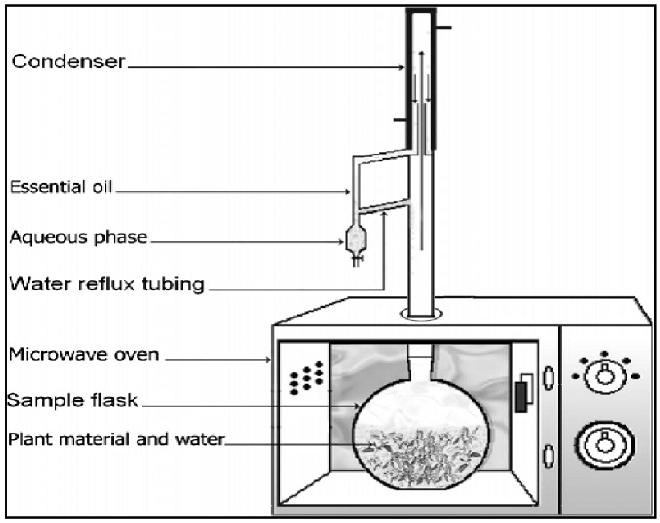

Figure 2.16: Schematic representation of the microwave-assisted hydrodistillation apparatus (Golmakani & Rezaei, 2008)

2.3 Microwave theory

Microwave is non-ionizing electromagnetic waves of frequency between 300MHz to 300GHz and positioned between the x-ray and infrared rays in the electromagnetic spectrum (Lettellier & Budzinski, 1999). Apparatus and techniques may be described qualitatively as microwave when the wavelengths of signals are roughly the same as the dimensions of the equipment, so that lumped-element circuit theory is inaccurate. As a consequence, practical microwave technique tends to move away from the discrete resistors, capacitors, and inductors used with lower frequency radio waves. Instead, distributed circuit elements and transmission-line theory are more useful methods for design and analysis.

The latter application is the direct action of waves on materials that has the ability to convert a part of the absorbed electromagnetic energy to heat energy. Microwaves are made up of two oscillating perpendicular field's i.e. electric field and magnetic field and the former is responsible for heating (Lettellier & Budzinski, 1999).

The principle of heating using microwave is based upon its direct impact with polar materials/solvents and is governed by two phenomenon’s: ionic conduction and dipole rotation, which in most cases occurs simultaneously (Letellier et al., 1999). Ionic conduction refers to the electrophoretic migration of ions under the influence of the changing electric field. The resistance offered by the solution to the migration of ions generates friction, which eventually heats up the solution. Dipole rotation means realignment of the dipoles of the molecule with the rapidly changing electric field. Heating is affected only at a frequency of 2450 MHz. The electric component of the wave changes 4.9 × 104 times per second (Zuloaga et al., 1999).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

In this study, the materials used were ginger, citronella grass and deionized water. Ginger and citronella grass were used as the raw materials that essential oils extracted from. Deionized water was used for immersed the ginger and citronella grass in both methods (HD and MAHD).

The ginger family is a tropical group especially abundant in Indo-Malaysia, consisting of more 1200 plant species in 53 genera. The genus *Zingiber* includes about 85 species of aromatic herbs from East Asia and tropical Australia. This plant was bought from market in Kuantan.

Citronella grass is a perennial of the *Poaceae* grass family, originating in tropical Asia. It is a genus of about 55 species of grasses, native to warm temperate and tropical regions of the Old World and Ocenia. Leaves of this citronella grass were cropped from open experimental areas of Universiti Malaysia Pahang.

3.1.1 Preparation of the Ginger Rhizomes

On receipt, the ginger rhizomes are store under refrigerated and wash prior to use. The ginger then, slices it longitudinally to an averages thickness. After that, the sliced ginger rhizomes are solar dried for a few days and finally grind it into ground ginger. Figure 3.1 below shows sliced ginger and Figure 3.2 shows the ground ginger.

Figure 3.1: Sliced ginger

Figure 3.2: Ground ginger

3.1.2 Preparation of Citronella Grass

Process of preparing the citronella grass begins with cropped the leaves of the citronella grass early in the morning. Before start the experiment, the leaves of citronella grass had been chopped into small pieces.

3.2 Methods and Procedure

3.2.1 Hydrodistillation (HD)

Hydrodistillation has been the most common approach to extract the essential oils from the medicinal herbs/plants. In this method, the plant materials are fully submerged in water. It's the method most often used in primitive countries. HD seems to work best for powders (ie, spice powders, ground wood, etc.) and very tough materials like roots, wood or nuts.

HD is carry out by using the conventional Clevenger apparatus and using an electromantle in order to gain heat. Apparatus used in this research is the heating mantel, condenser, and round-bottom flask 1000mL and collection flask. A "water to plant ratio of 15:1 (v/w) was considered for ginger and 10:1 (v/w) for citronella grass. 40g ground ginger is submerged in 600mL of deionized water and 60g chopped citronella grass is submerged in 600mL of deionized water. The extraction was performed for a different extraction time; 30 min, 60 min, 90 min and 120 min. Figure 3.3 below shows the set up apparatus for HD.

Figure 3.3: Hydrodistillation apparatus.

3.2.2 Microwave-Assisted Hydrodistillation (MAHD)

The efficiency of MAHD is strongly dependent on the dielectric constant of water and the matrix. The use of microwaves in industrial materials processing can provide a versatile tool to process many types of materials under a wide range of conditions. MAHD is carrying out in a similar manner as the one explained for MAHD. However, an electromantle is replaced by microwave oven. A domestic microwave oven was modified for MAHD operation. The maximum power operation for this microwave is 800W. But, for this extraction, microwave oven is operating at 200W. Figure 3.4 represented the schematic set up apparatus for MAHD. Figure 3.5 shows the procedure for both methods.

Figure 3.4: Microwave-assisted hydrodistillation apparatus

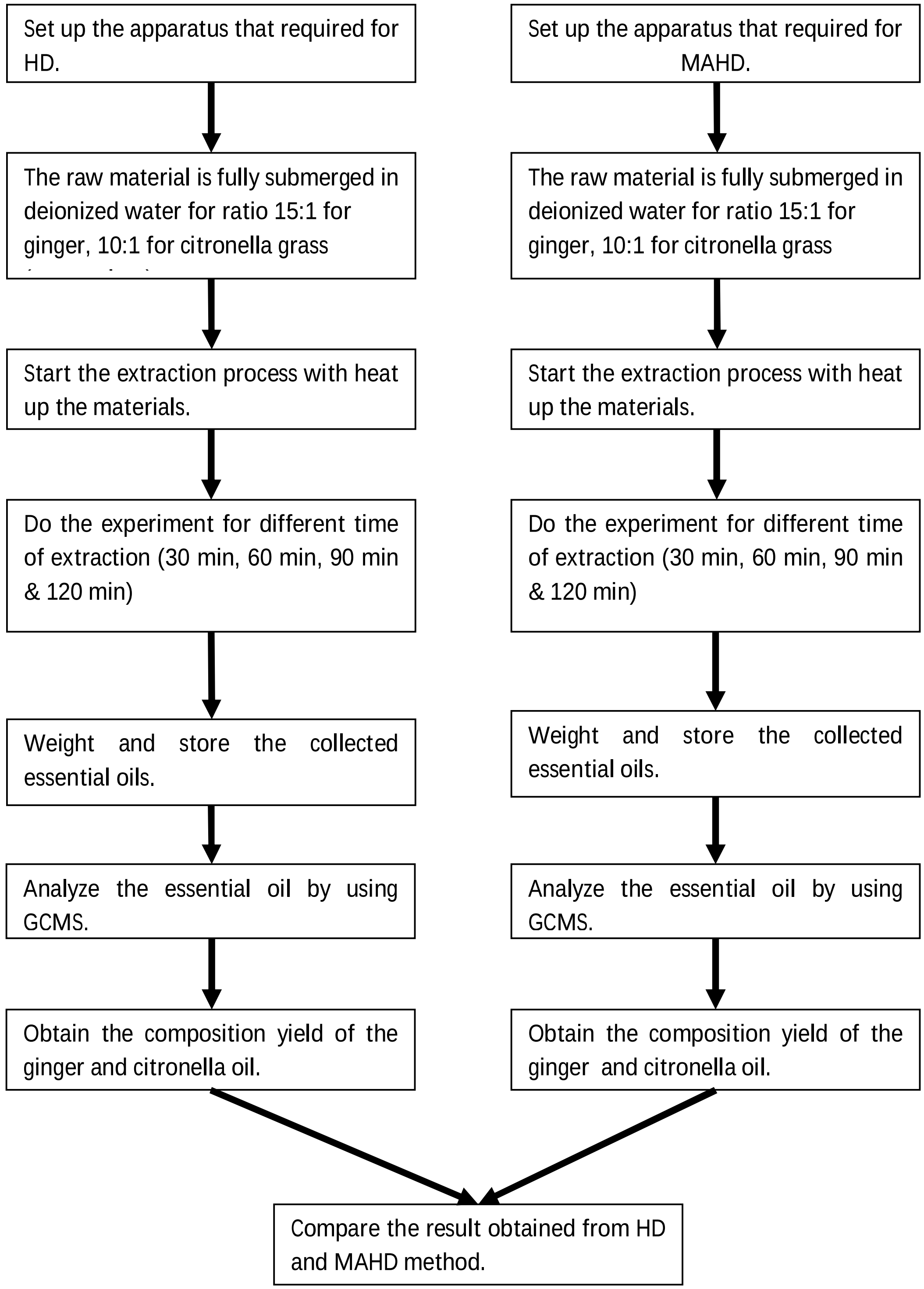

Figure 3.5: Hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD) flow diagram

3.2.3 Analysis with Gas Chromatography-mass spectrometry (GC-MS)

As shown in Figure 3.6 below, gas chromatography-mass spectroscopy (GC-MS) is one of the so-called hyphenated analytical techniques. As the name implies, it is actually two techniques that are combined to form a single method of analyzing mixtures of chemicals. Gas chromatography separates the components of a mixture and mass spectroscopy characterizes each of the components individually. By combining the two techniques, an analytical chemist can both qualitatively and quantitatively evaluate a solution containing a number of chemicals. A GC-MS instrument equipped with a mass selective detective operating n the electron impact mode is use to study the composition of the extracted essential oils. The compounds of the extracted essential oils are identifying by comparing their mass spectral fragmentation patterns. Gas Chromatography-mass spectrometry was used to analyze the chemical composition of the extracted essential oil. This analysis was accomplished on DB-WAX MS column (30 m x 0.25 mm i.d and 0.25 µm film thickness). The chromatographic system used was an Agilent 5975C Series GC/MSD, with injection volume 1 µL at 3 mL/min. The injector was set at 250°C. For each compound on the chromatogram, the percentage of peak area relative to the total peak areas from all compounds was determined and reported as relative amount for that compound.

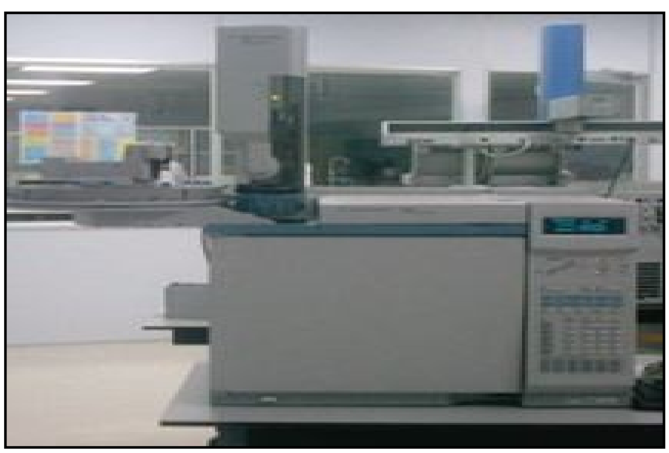

Figure 3.6: Gas chromatography-mass spectrometry (GC-MS)

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Quantitative Analysis

4.1.1 Comparison of Extraction Yield

The extraction yield of essential oil extraction from ginger and citronella grass using MAHD has been compared with that of HD on Figure 4.1 and 4.2. The extraction of essential oil for both ginger and citronella grass by using MAHD gives high percentage yield rather than using HD. This is due to ion rotation that happened in microwave units. Each microwave oven contains a magnetron, which generates microwaves at just the right frequency to interact with the molecules in material and heat it directly. In microwave heating, the energy is highly distributed from all of directions. The electromagnetic energy is directly transferred into the material, absorbed by molecules and converted into heat energy; as a consequence the temperature raises much faster compared to conventional method (Bartholme, 2009).

The radio waves penetrate the dipole material and exciting atoms together to convert the energy into heat. No heat has to migrate toward the interior by conduction. There is heat everywhere all at once because the molecules are all excited together or can say as bulk heating. The microwaves make the water molecules rotate. Each individualmolecule of water is rotating. There are electric forces acting on the water molecule that cause it to rotate when the negative charge is brought near it. Since the water molecule slows down in its rotation, there must be friction forces. These forces produce heat energy. As a simplified, it can say motion leads to friction, and friction leads to heating. For this study, the extraction starts for both methods (MAHD and HD) at the boiling point of water (100 ^{o}C if performed at atmospheric pressure). The extraction time is setting as manipulative variables. The experiments were conducted only four times (30 min, 60 min, 90 min and 120 min) to compare the extraction yield for both ginger and citronella grass.

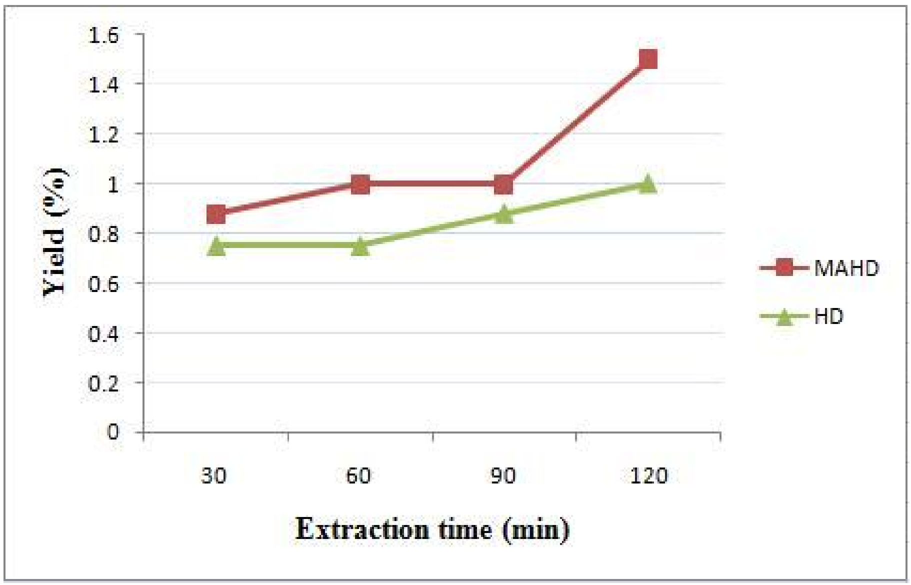

Figure 4.1: Extraction yield of essential oil as a function of time for microwave-assisted hydrodistillation and hydrodistillation from ginger (*Zingiber officinale Roscoe)*

From Figure 4.1 above, the graph shows the extraction yield from ginger for both MAHD and HD gradually increase. After 30 min of extraction, MAHD resulted in similar oil recovery to that obtained by 90 min of HD (0.88%, v/w). By the maximum time of operation (120 min), the ultimate yield of essential oils from ginger extracted by MAHD recover the greater percentage yield (1.5%, v/w), compared to HD (1.0%, v/w). These results mean a substantial saving in time and highly quantitative.

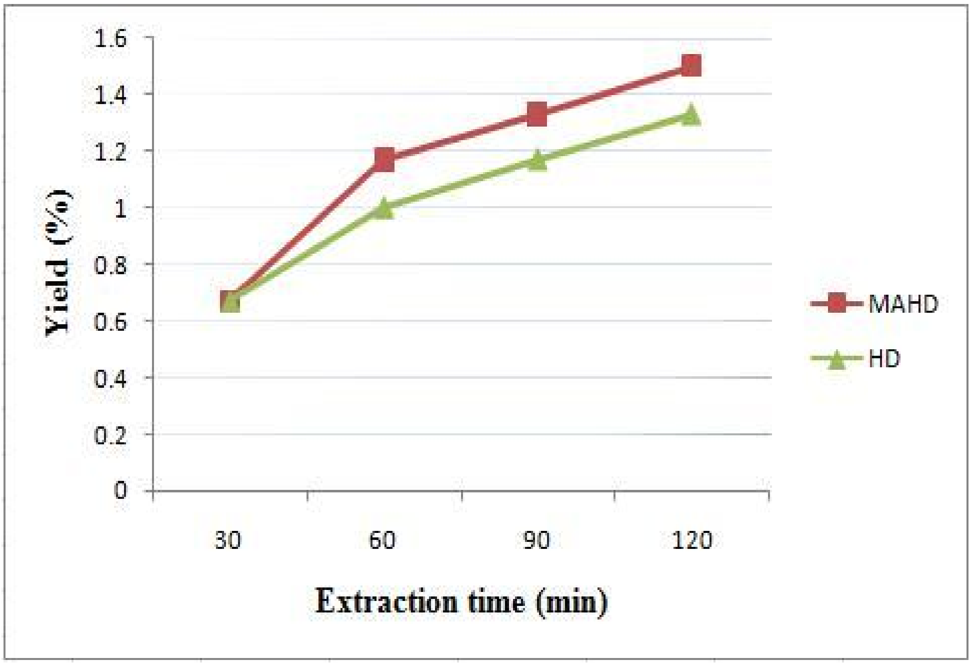

Figure 4.2: Extraction yield of essential oil as a function of time for microwave-assisted hydrodistillation and hydrodistillation from citronella grass (*Cymbopogon nardus)*

From Figure 4.2 above, the graph shows the extraction yield from citronella grass for both MAHD and HD gradually increase. After 60 min of extraction, MAHD resulted in similar oil recovery to that obtained by 90 min of HD (1.17%, v/w). By the maximum time of operation (120 min), the ultimate yield of essential oils from citronella grass extracted by MAHD recover the greater percentage yield (1.5%, v/w), compared to HD (1.33%, v/w). These results mean same as the previous discussed.

4.1.2 Chemical Composition of the Extracts

To quantitatively analyze the ginger and citronella grass extract would require a combination of analytical tools, such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), GC-MS and GC-FID. But in this study, GC-MS are used. An analytical gas chromatograph ultimately produces a plot of detector signal intensity versus time. The plot of peaks versus time describes the elution of the sample components and is called a chromatogram. Each peak ideally represents a single, pure compound. The identities of the extracted essential oils from both methods (HD and MAHD) and both materials (ginger and citronella grass) for several of operating time are represented in Table 4.1-4.8. Total peak area for all different operating time by using MAHD shows the higher value compared to HD.

Table 4.1: Chemical composition of essential oils from ginger (*Zingiber officinale Roscoe)* for 30 min

No.	**Compound**	**Relative peak area (%)**	
		HD	**MAHD**
1	α-Pinene	1.52	-
2	Camphene	7.23	3.61
3	β-Phellandrene	-	9.64
4	Limonene	2.42	-
5	α-Terpinolene	0.64	-
6	Linalool	1.16	1.25
7	Citronellal	0.52	0.53
8	Isogeraniol	1.2	1.43
9	α-Terpineol	1.91	2.51
10	Citral	10.39	21.73
11	Bornyl acetate	0.24	0.29
12	Geranyl acetate	0.37	0.46
13	β-Cubebene	-	1.51
14	β-Elemene	0.54	0.71
15	ar-Curcumene	7.11	7.42
16	α-Zingiberene	13.61	25.57
17	β-Bisabolene	4.46	5.96
18	β-Sesquiphellandrene	6.82	9.24
19	Nerolidol	0.88	0.94
20	α-Bisabolol	-	0.56
21	β-Mycrene	1.09	-
22	β-Farnesene	0.52	-
23	α-Farnesene	4.12	-

24	β-Linalool	0.51	-
25	Ledol	0.74	-
26	Borneol	4.66	5.83
27	α-Longipenene	1.23	-
Total peak area (%)		**73.89**	**99.19**

The 27 components shown in Table 4.1 consist of about 99% of total GC peak area. α-Zingiberene was the most abundant component in ginger essential oil (25.57% for MAHD and 13.61% for HD) followed by Citral, β-Phellandrene and β-Sesquiphellandrene. From Table 4.1 above shows by using MAHD discovered higher total peak area for essential oil (99.19%) compared to HD (73.89%).

Table 4.2: Chemical composition of essential oils from ginger (*Zingiber officinale Roscoe)* for 60 min

No.	**Compound**	**Relative peak area (%)**	
		HD	**MAHD**
1	α-Pinene	0.62	1.27
2	Camphene	2.91	5.95
3	β-Phellandrene	7.14	12.05
4	Limonene	1.2	2
5	α-Terpinolene	0.5	0.61
6	Linalool	0.74	0.95
7	Borneol	4.23	4.89
8	Terpinen-4-ol	0.61	-
9	β-Eudesmol	0.97	0.65
10	Cycloisosativene	0.29	-
11	α-Terpineol	2.1	2.15
12	Citral	3.63	18.5
13	Bornyl acetate	0.28	0.33
14	2-Undecanone	0.35	0.29
15	Geranyl acetate	0.26	0.39

16	α-Cubebene	0.57	0.36
17	β-Elemene	1.04	0.67
18	Citronellal	-	0.46
19	β-Bergamotene	0.29	-
20	γ-Elemene	0.9	0.31
21	β-Farnesene	0.96	0.58
22	α-Aromadendrene	0.28	-
23	α-Curcumene	12.11	7.55
24	α-Zingiberene	28.35	19.19
25	β-Bisabolene	7.32	6.08
26	β-Sesquiphellandrene	11.59	7.7
27	α-Elemol	0.98	-
28	Nerolidol	0.9	1.34
29	β-Bisabolene	0.65	-
30	β-Linalool	1.13	-
31	Valencene	1.7	-
32	Methyl heptenone	-	0.45
33	β-Mycrene	-	0.94
34	Camphor	-	0.31
35	β-Guaiene	-	1.2
36	α-Cedrene	1.38	1.08
Total peak area (%)		**95.98**	**98.25**

The 36 components shown in Table 4.2 consist of about 98% of total GC peak area. The predominent sesquiterpene hydrocarbon in ginger essential oil is α-Zingiberene, other sesquiterpenes present in the oil are α-Curcumene, β-Sesquiphellandrene and relatively smaller amounts of α-Cubebene, β-Elemene, and α-Cedrene. The data from Table 4.2 shows the highest total peak area for MAHD (98.25%) compared to HD (95.98%).

Table 4.3: Chemical composition of essential oils from ginger (*Zingiber officinale Roscoe)* for 90 min

No.	Compound	Relative peak area (%)	
		HD	MAHD
1	α-Pinene	0.66	-
2	Camphene	3	1.78
3	β-Phellandrene	6.41	2.69
4	Limonene	1.08	-
5	α-Terpinolene	0.44	-
6	Linalool	0.63	0.74
7	Neral	-	1.53
8	γ-Gurjunene	-	0.4
9	β-Eudesmol	-	1.06
10	Geranyl formate	-	0.6
11	α-Terpineol	1.88	2.03
12	Geranial	5.32	4.48
13	Geraniol	0.3	0.66
14	Bornyl acetate	0.23	0.35
15	2-Undecanone	0.3	-
16	Geranyl acetate	0.23	-
17	α-Cubebene	0.53	0.63
18	β-Elemene	0.96	1.28
19	Citronellol	-	0.39
20	γ-Muurolene	-	1
21	δ-Cadinene	-	0.35
22	Epizonarene	0.27	-
23	α-Zingiberene	26.89	36.51
24	β-Bisabolene	7.03	-
25	β-Cedrene	11.01	-
26	α-Elemol	0.92	0.7
27	1-8 Cineole	-	2.77
28	β-Farnesene	-	1.65
29	α-Farnesene	-	7.96

30	Nerolidol	0.9	1.23
31	α-Aromadendrene	0.48	-
32	Thujopsene	0.64	1.04
33	α-Bisabolol	1.12	0.87
34	Ledol	1.77	0.41
35	α-Cadinol	1.02	-
36	α-Cedrene	1.57	1.43
37	*trans*-α-Bergamotene	0.26	-
38	γ-Elemene	0.37	0.42
39	α-Curcumene	11.29	18.81
40	Citronellal	0.28	-
41	Borneol	3.86	3.53
42	Cadinene	0.56	-
43	Terpinen-4-ol	0.7	-
Total peak area (%)		**92.91**	**97.3**

The 43 components shown in Table 4.3 consist of about 97% of total GC peak area. The major components in ginger essential oil that obtained from Table 4.3 above were α-Zingiberene (26.89% for HD and 36.51% for MAHD) followed by α-Curcumene (11.29% for HD and 18.81% for MAHD), β-Cedrene and α-Farnesene. From Table 4.3 above represented by using MAHD discovered higher total peak area (97.30%) compared to HD (92.91%).

Table 4.4: Chemical composition of essential oils from ginger (*Zingiber officinale Roscoe)* for 120 min

No.	**Compound**	**Relative peak area (%)**	
		HD	**MAHD**
1	α-Pinene	0.9	0.61
2	Camphene	4.1	2.82
3	β-Phellandrene	7.93	3.68
4	Limonene	1.46	-
5	Eucalyptol	-	3.44

6	Linalool	0.63	0.76
7	α-Cubebene	0.51	0.63
8	α-Zingiberene	19.32	34.71
9	Bornyl acetate	0.22	0.36
10	β-Elemene	0.91	2.54
11	γ-Elemene	0.45	1.91
12	β-Farnesene	0.84	1.64
13	Citral	11.6	4.38
14	α-Terpineol	1.81	1.94
15	Borneol	3.77	3.32
16	α-Farnesene	6.32	7.83
17	α-Curcumene	11	19.1
18	δ-Cadinene	-	0.35
19	Geraniol	-	0.59
20	Ledol	1.75	0.4
21	Nerolidol	1.76	1.18
22	α-Elemol	-	0.7
23	Thujopsene	1.64	0.95
24	β-Linalool	-	1.47
25	γ-Selinene	-	0.43
26	α-Cedrene	-	1.04
27	β-Eudesmol	-	0.47
28	Di-epi α-Cedrene	-	1.02
29	Geranyl formate	-	0.79
30	Ocimene	0.46	-
31	Citronellal	0.29	-
32	Isopulegol	0.83	-
33	2-Undecanone	0.27	-
34	α-Elemene	0.26	-
35	α-Bergamotene	0.24	-
36	α-Bisabolol	1.1	-
Total peak area (%)		**80.31**	**99.06**

The 36 components shown in Table 4.4 were found in ginger essential oil consist of about 99% of total GC peak area. Similarly, from Table 4.1-4.3, major components that obtained from Table 4.4 above were α-Zingiberene (19.32 % for HD and 34.71% for MAHD) followed by α-Curcumene (11% for HD and 19.10% for MAHD), β-Sesquiphellandrene and Citral. From Table 4.4 above represented by using MAHD discovered higher total peak area (99.06%) compared to HD (80.31%).

Table 4.5: Chemical composition of essential oils from citronella grass (*Cymbopogon Nardus)* for 30 min

No.	Compound	Relative peak area (%)	
		HD	**MAHD**
1	Limonene	3.24	2.94
2	Linalool	0.82	0.89
3	Isopulegol	0.69	0.42
4	Citronellal	40.02	42.78
5	Citronellol	14.44	-
6	Geraniol	18.66	17.96
7	Citronellyl propionate	3.43	13.1
8	Geranyl acetate	4.28	5.89
10	β-Elemene	1.08	0.72
11	β-Cubebene	0.8	0.65
12	δ-Cadinene	1.65	1.14
13	α-Elemol	4.91	5.01
14	Germacrene D-4-ol	2.77	2.8
15	Copaene	1.54	-
16	α-Cadinol	1.2	0.84
17	Citronellyl acetate	-	4.08
18	γ-Cadinene	-	0.77
Total peak area (%)		**99.53**	**99.99**

The 18 components shown in Table 4.5 consist of about 99% of total GC peak area. The major components from citronella essential oil that obtained from Table

4.5 above were Citronellal (40.02% for HD and 42.78% for MAHD) followed by Geraniol and Citronellol. The data represented MAHD discovered higher total peak area (99.99%) compared to HD (99.53%).

Table 4.6: Chemical composition of essential oils from citronella grass (*Cymbopogon Nardus)* for 60 min

No.	Compound	Relative peak area (%)	
		HD	**MAHD**
1	Limonene	2.91	2.46
2	Linalool	0.82	0.8
3	Isopulegol	0.63	0.7
4	Citronellal	34.24	31.54
5	Citronellyl propionate	11.62	12.21
6	Geraniol	18	20.4
7	Citronellyl acetate	4.8	4.01
8	Geranyl acetate	6.6	4.74
9	β-Elemene	1.43	1.19
10	β-Cubebene	1.2	0.88
11	α-Muurolene	0.46	0.46
12	δ-Cadinene	2.15	1.97
13	α-Elemol	6.87	8.76
14	Germacrene D-4-ol	2.96	2.65
15	Nerolidol	-	0.86
16	γ-Eudesmol	1.08	-
17	γ-Cadinene	1.34	3.41
18	α-Bisabolol	0.63	-
19	τ-Muurolol	1.9	-
20	Isolongifolene	-	2.96
Total peak area (%)		**99.64**	**100**

The 20 components shown in Table 4.6 consist of about 100% of total GC peak area. The major components from citronella essential oil that obtained from Table 4.6 above were Citronellal followed by Geraniol and Citronellyl Propionate.

The data represented MAHD discovered higher total peak area (100%) compared to HD (99.64%).

Table 4.7: Chemical composition of essential oils from citronella grass (*Cymbopogon Nardus)* for 90 min

No.	Compound	Relative peak area (%)	
		HD	**MAHD**
1	Limonene	2.43	2.06
2	Linalool	0.67	0.64
3	Isopulegol	0.67	0.3
4	Citronellal	29.38	28.4
5	Citronellol	-	10.56
6	β-Citronellol	11.75	-
7	Geraniol	17.72	16.23
8	Citronellyl acetate	4.06	3.64
9	Geranyl acetate	4.59	3.84
10	β-Elemene	1.94	1.93
11	β-Cubebene	1.65	1.58
12	α-Muurolene	0.55	0.48
13	δ-Cadinene	2.54	2.16
14	α-Elemol	9.96	12.5
15	Germacrene D-4-ol	2.6	2.5
16	Nerolidol	-	1.45
17	γ-Eudesmol	-	2.6
18	γ-Cadinene	4.06	3.03
19	α-Bisabolol	1.07	-
20	γ-Elemene	-	5.55
21	Farnesol	0.33	0.54
Total peak area (%)		**95.97**	**99.99**

The 21 components shown in Table 4.7 consist of about 99% of total GC peak area. The predominent smonoterpene hydrocarbon in citronella essential oil is Citronellal; other monoterpenes present in the oil are Citronellol, Geraniol, β-

Citronellol and relatively smaller amounts of Linalool and Isopulegol. The data from Table 4.7 shows the highest total peak area for MAHD (99.99%) compared to HD (95.97%).

Table 4.8: Chemical composition of essential oils from citronella grass (*Cymbopogon Nardus)* for 120 min

No.	**Compound**	**Relative peak area (%)**	
		HD	**MAHD**
1	Limonene	2.39	1.43
2	Linalool	0.74	0.74
3	Isopulegol	0.37	0.41
4	Citronellal	25.9	24.87
5	Citronellol	11.18	11.54
6	Geraniol	16.78	18.72
7	Citronellyl acetate	5.01	4.22
8	Geranyl acetate	-	4.74
9	β-Elemene	2.71	1.87
10	β-Cubebene	2.31	1.43
11	α-Muurolene	0.7	0.5
12	δ-Cadinene	3.13	2.31
13	α-Elemol	10.49	12.04
14	Germacrene D-4-ol	1.94	2.38
15	Nerolidol	1.39	1.59
16	α-Eudesmol	-	1.01
17	γ-Eudesmol	2.11	1.46
18	γ-Cadinene	2.69	2.97
19	γ-Elemene	4.59	5.22
20	Farnesol	0.43	0.54
21	Citronellyl butyrate	4.51	-
Total peak area (%)		**99.37**	**99.99**

The 21 components shown in Table 4.8 consist of about 99% of total GC peak area. Similarly, from Table 4.5-4.7, the major components from citronella essential oil that obtained from Table 4.8 above were Citronellal followed by Geraniol and Citronellal. The data represented MAHD discovered higher total peak area (99.99%) compared to HD (99.37%). The result obtained from GC-MS shows that the major chemical compositions for citronella grass are Citronellal, Geraniol and Citronellol while for ginger are α-Zingiberene, α-Curcumene and β-Sesquiphellandrene. From the result approach, total peak area obtained for both methods represented how many percentages of compounds of essential oils are recovered. From result, MAHD gives greater total peak area.

4.2 Qualitative Analysis

4.2.1 Cleanliness and Cost

As shown in Figure 4.1, MAHD required extraction time period of 30 min for recovering 0.88% yield of essential oil of ginger instead of HD required 90 min of extraction time for recovering same percentage yield. Same as shown in Figure 4.2, after 60 min of extraction, MAHD resulted in similar citronella oil recovery to that obtained by 90 min of HD (1.17%, v/w). Therefore, MAHD can result in significant saving in the extraction time. Furthermore, since MAHD can save the extraction time, the energy requirements to MAHD perform the extraction are lower compared to HD. This indicates a saving in the extraction cost when using MAHD instead of HD.

Regarding environmental impact, the calculated quantity of carbon dioxide rejected in the atmosphere is lower. According to Asma Farhat et al., in order to produce of 1g essential oils will be released equivalent of carbon dioxides to the atmosphere were estimated 1600g for HD and 990g for MAHD. The waste water also reduced by referring the water flow for distillate must be continuously added to avoid overheating and volatile compound losses. Hence, MAHD can be suggested as a green technology.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

MAHD presented significant advantages over conventional HD. This method can be considered as an innovative and cleaner design process for extraction of essential oils. With respect to the percentage of extraction yield, a similar extraction yield was achieve at significantly shorter extraction time when used MAHD instead of HD. For the maximum operation time (120 min), MAHD achieved a greater extraction yield (1.55% for both materials). It was significant in saving the extraction time. Therefore, the operation cost for MAHD would be inferior rather than HD. GC-MS results indicated that MAHD recover almost 100% total peak area. Finally, compared to many solvent extraction techniques, MAHD is modern, green and rapid.

5.2 Recommendations

There are several recommendations that can be recommended:

1) The water flow for distillate must be continuously added to avoid overheating.
2) Better to cover the distillation column to evade volatile oil evaporate.

3) Carefully collect the essential oils from separator funnel to separate the oil from water.
4) Cleaned the apparatus cautiously for the first time uses by using chromic acid to make sure there is no other chemical from previous user to avoid any contaminant.

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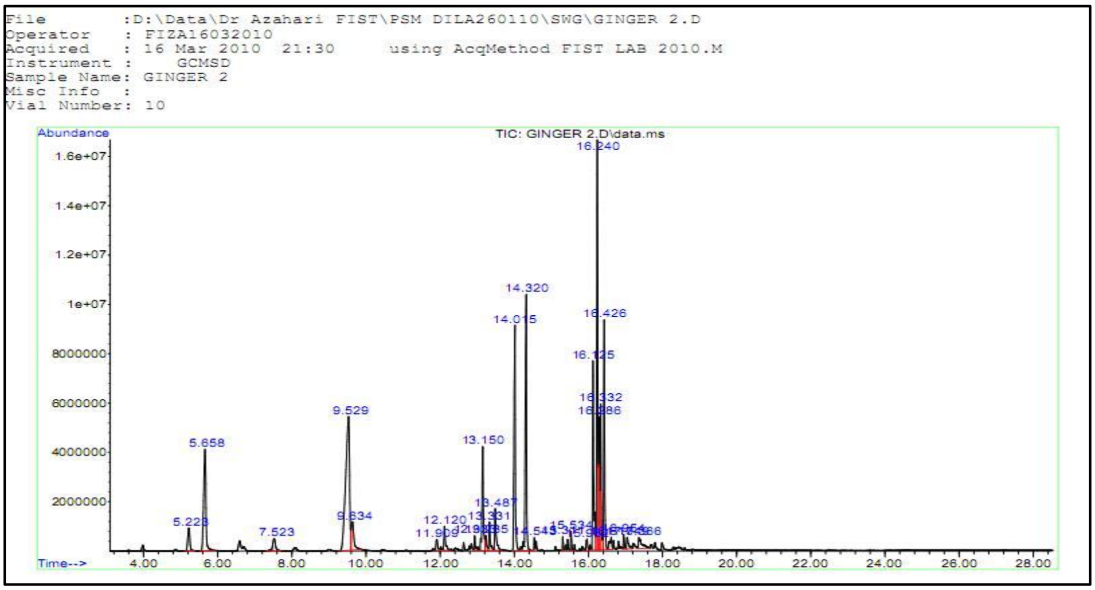
APPENDIX A

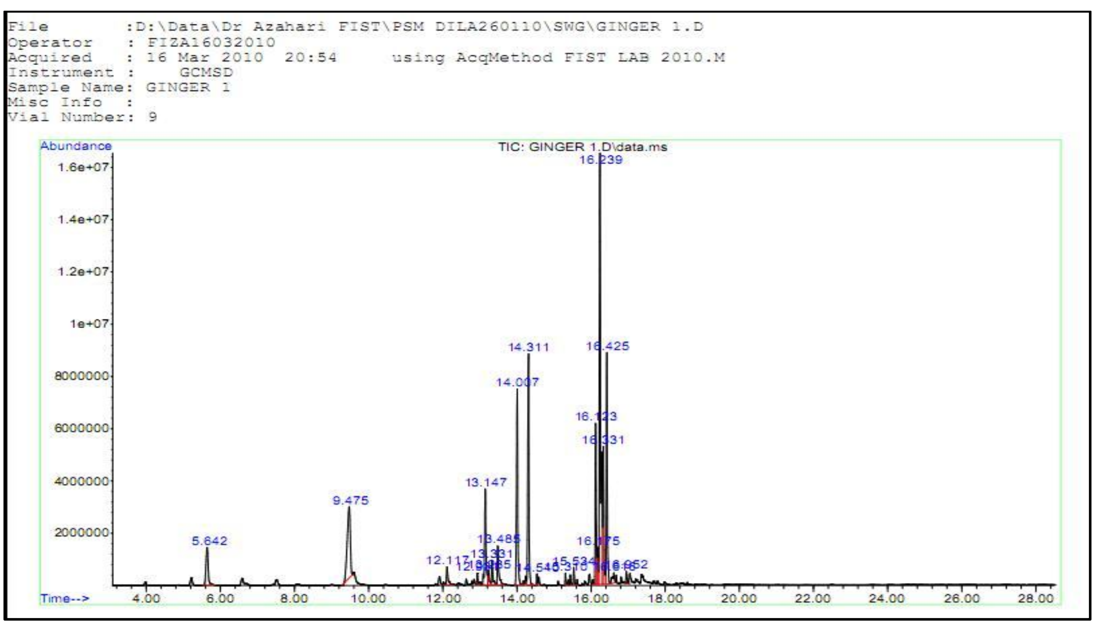
CHROMATOGRAMS

A.1 Chromatograms of Ginger Oil

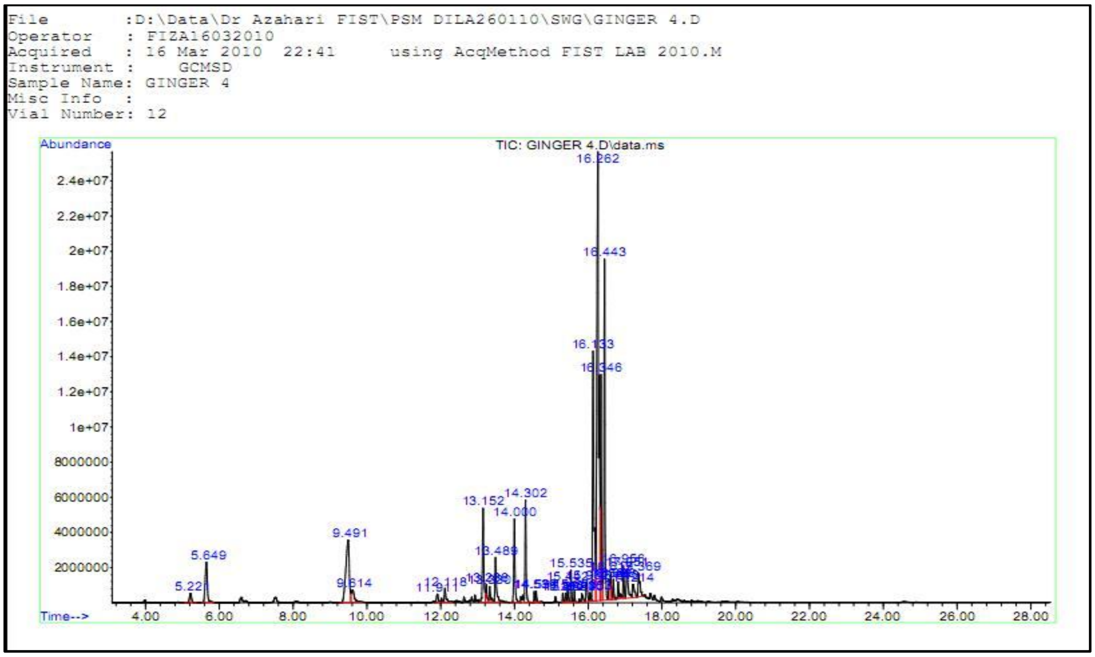
A.1.1 Hydrodistillation 30min



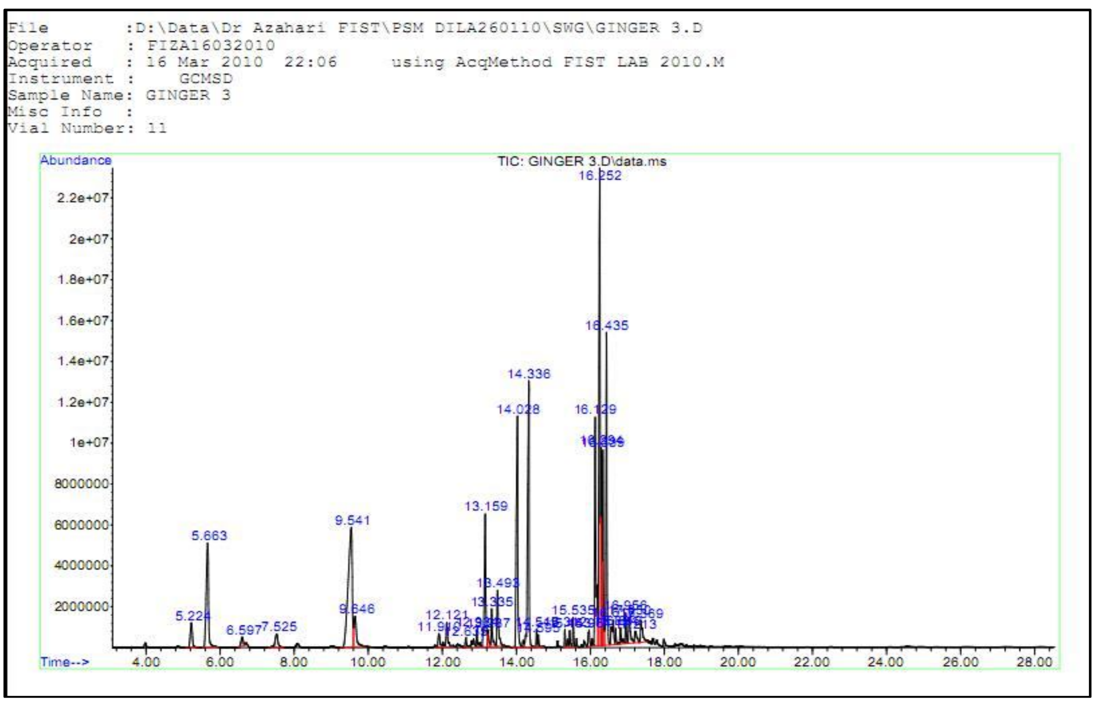
A.1.2 Microwave-Assisted Hydrodistillation 30min



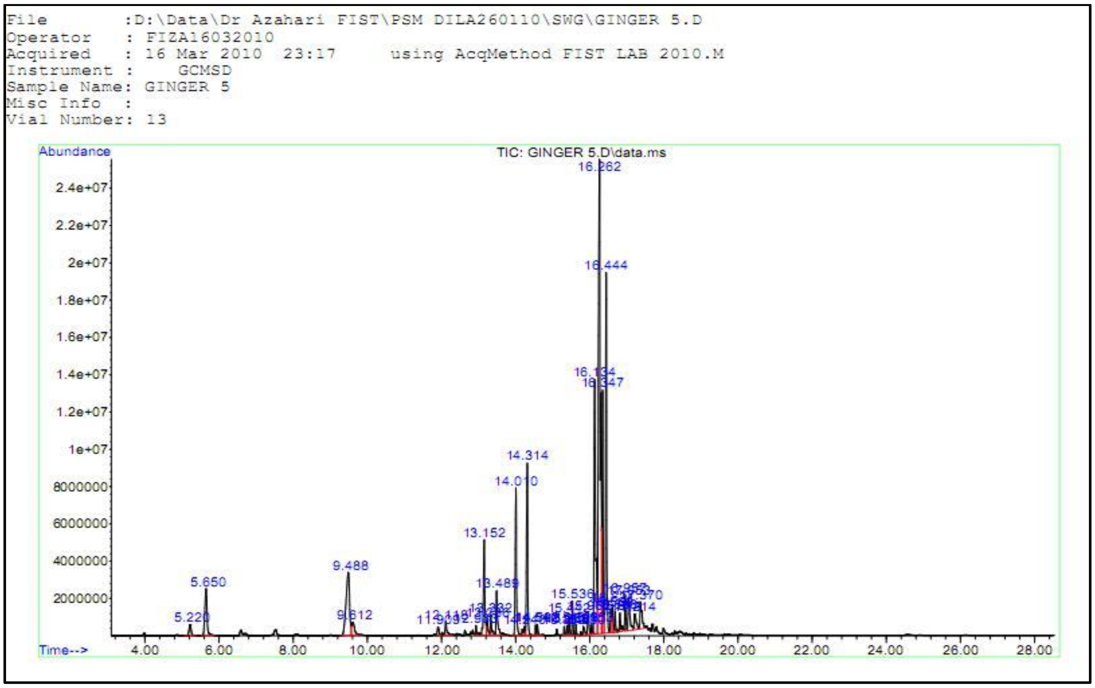
A.1.3 Hydrodistillation 60min



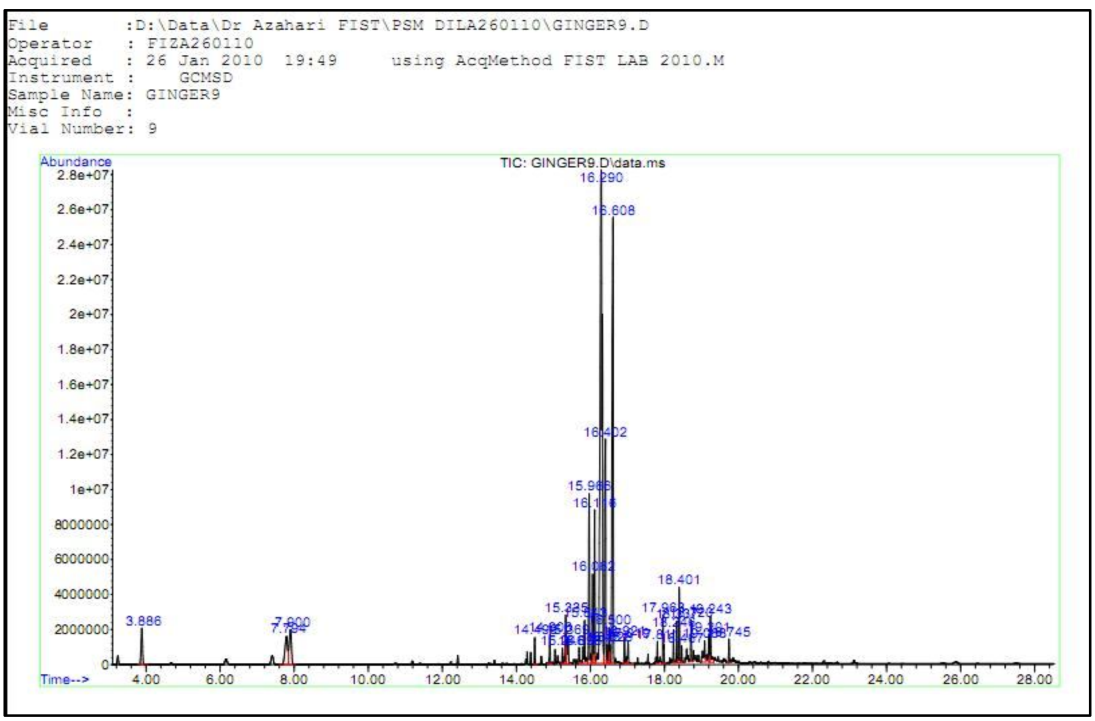
A.1.4 Microwave-Assisted Hydrodistillation 60min



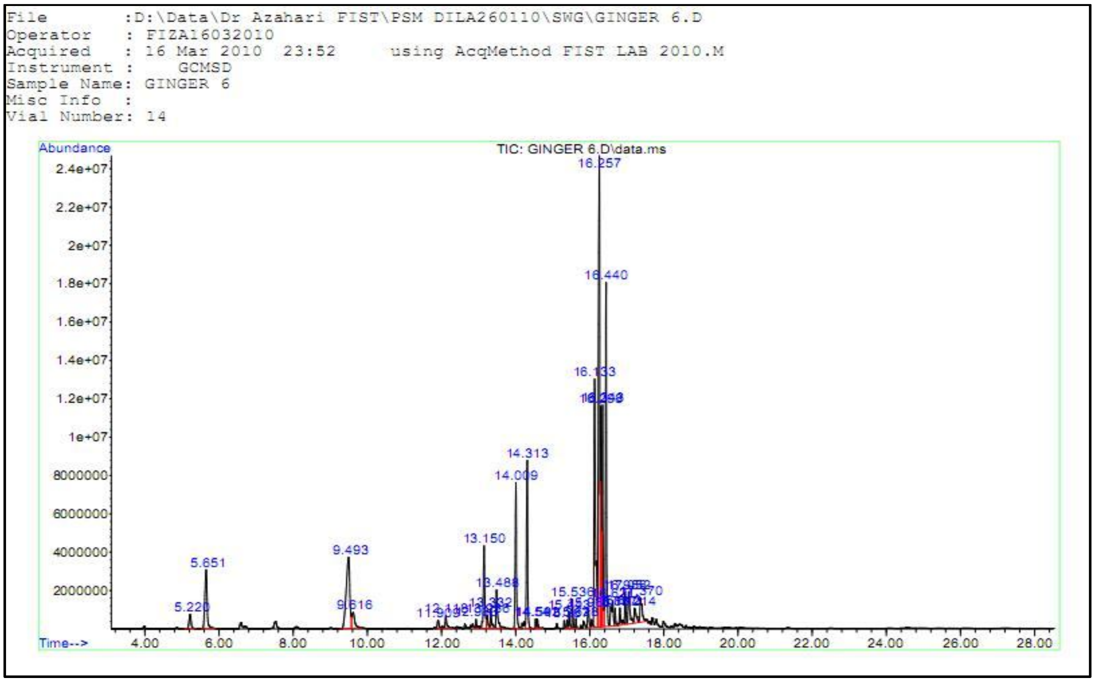
A.1.5 Hydrodistillation 90min



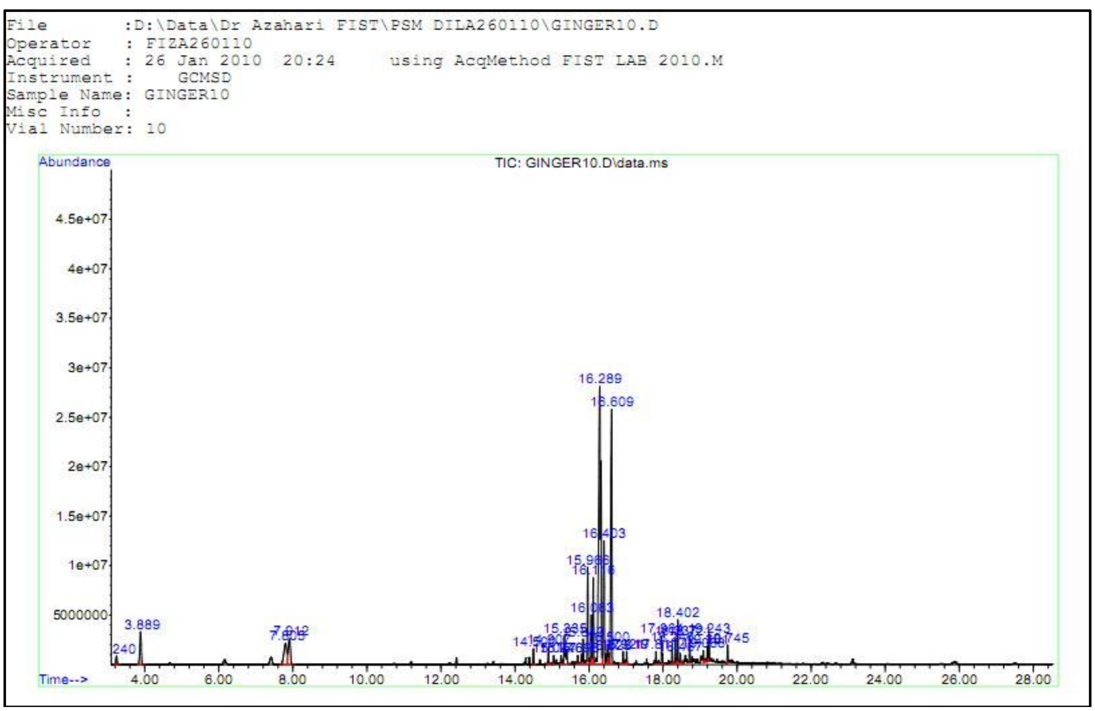
A.1.6 Microwave-Assisted Hydrodistillation 90min



A.1.7 Hydrodistillation 120min

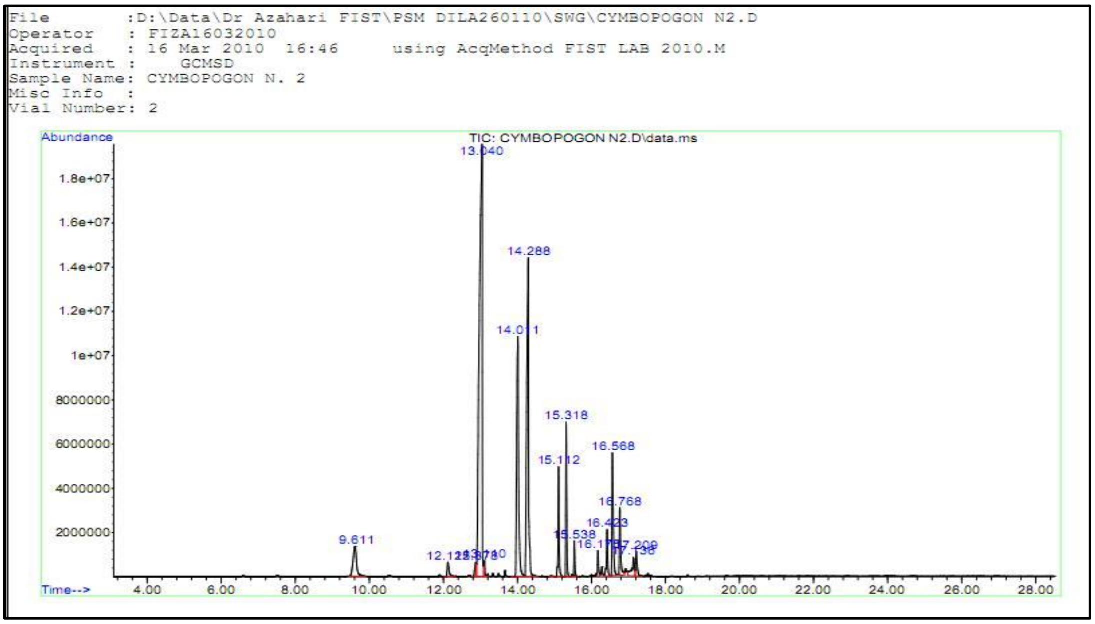


A.1.8 Microwave-Assisted Hydrodistillation 120min

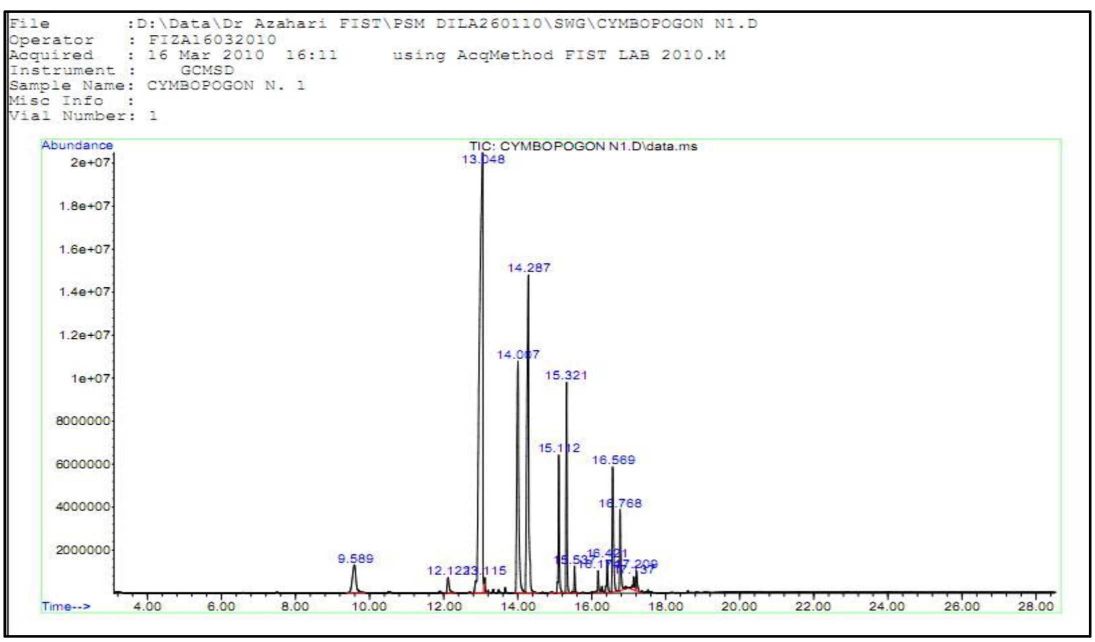


A.2 Chromatograms of Citronella Oil

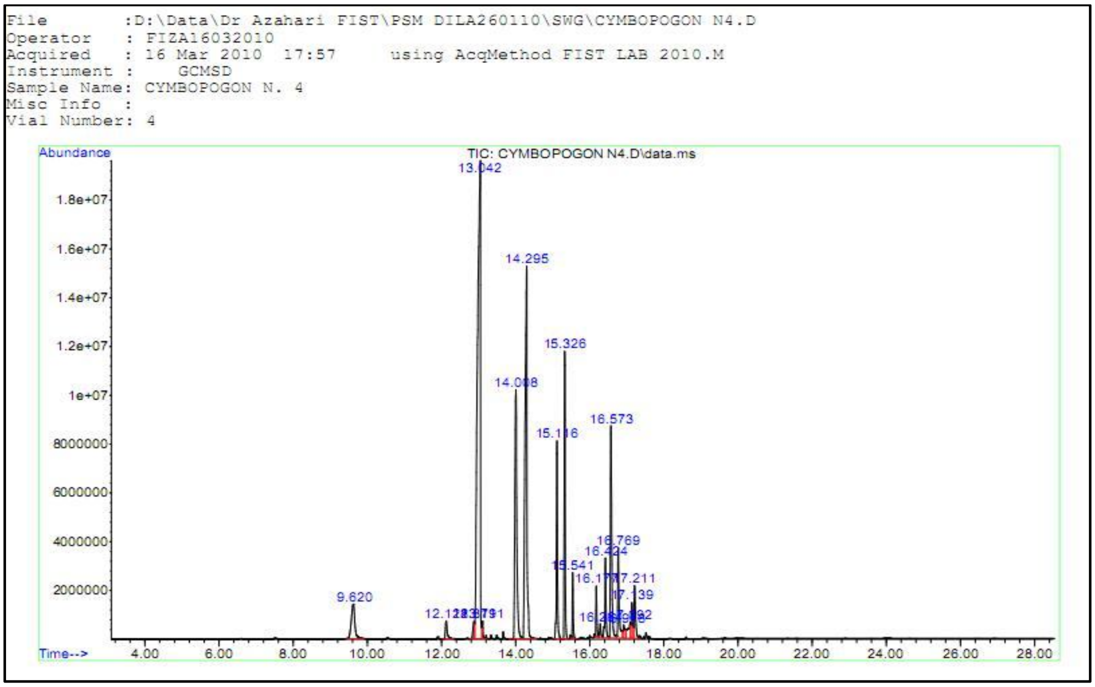
A.2.1 Hydrodistillation 30min



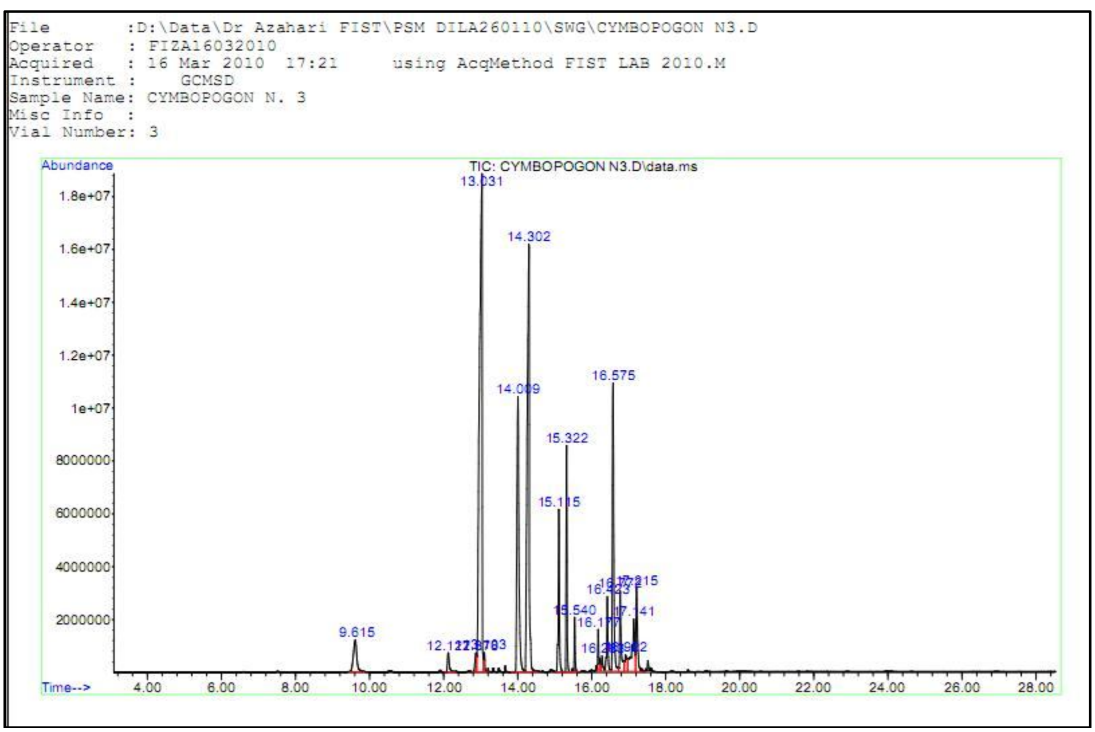
A.2.2 Microwave-Assisted Hydrodistillation 30min



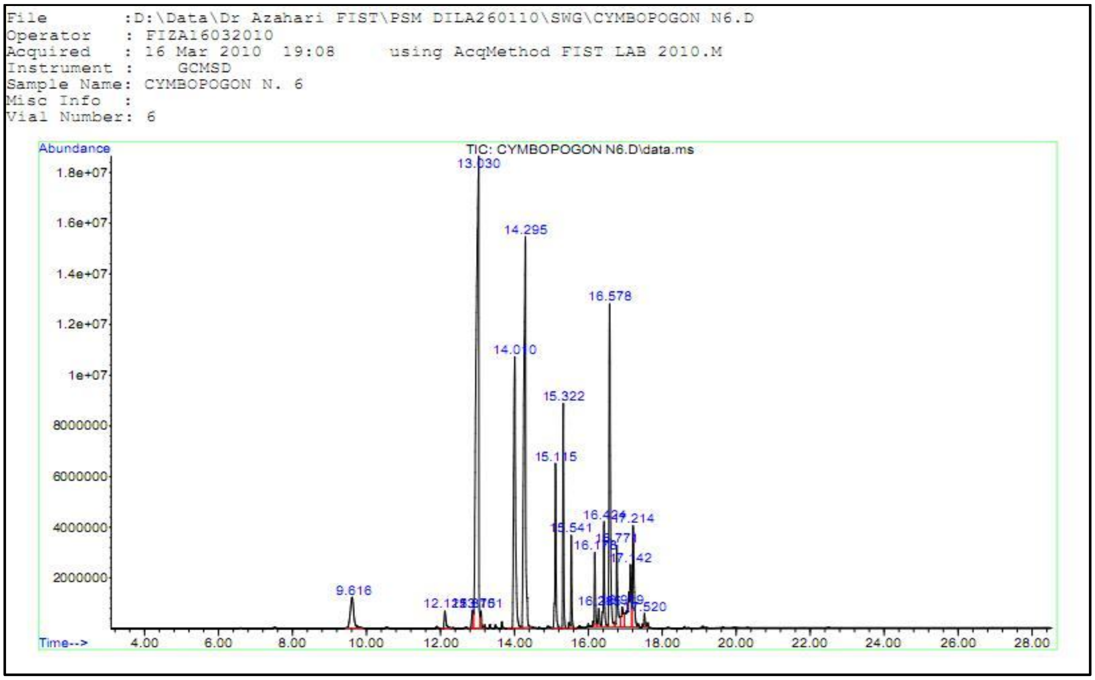
A.2.3 Hydrodistillation 60min



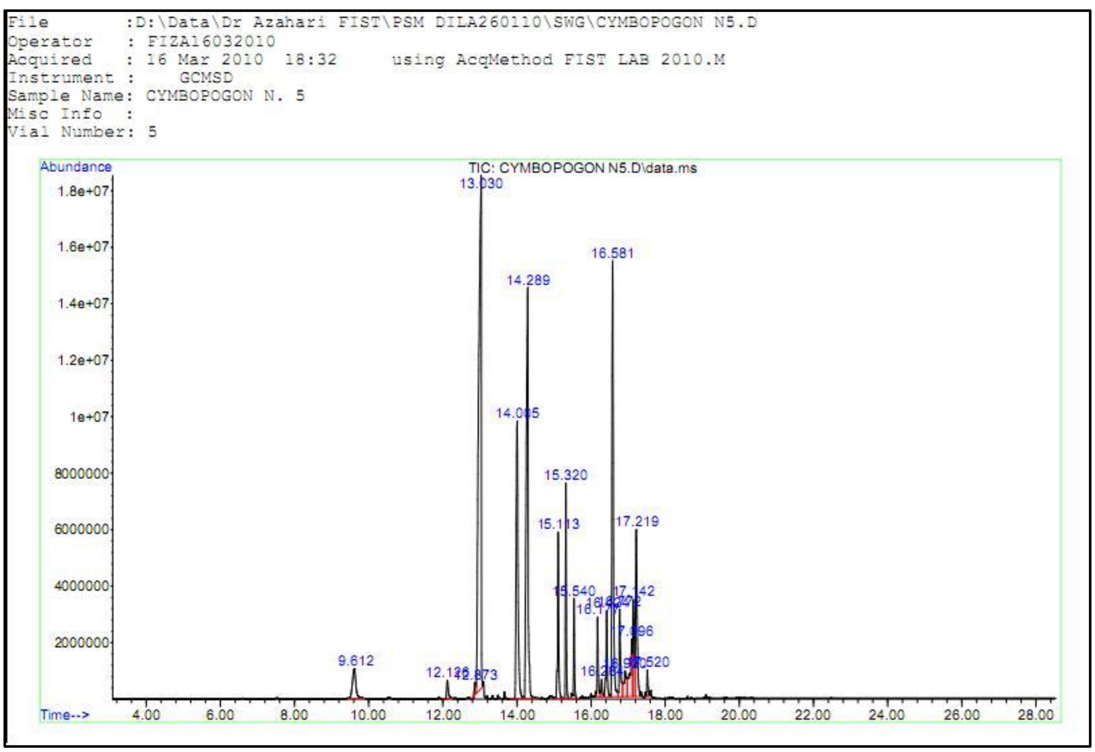
A.2.4 Microwave-Assisted Hydrodistillation 60min



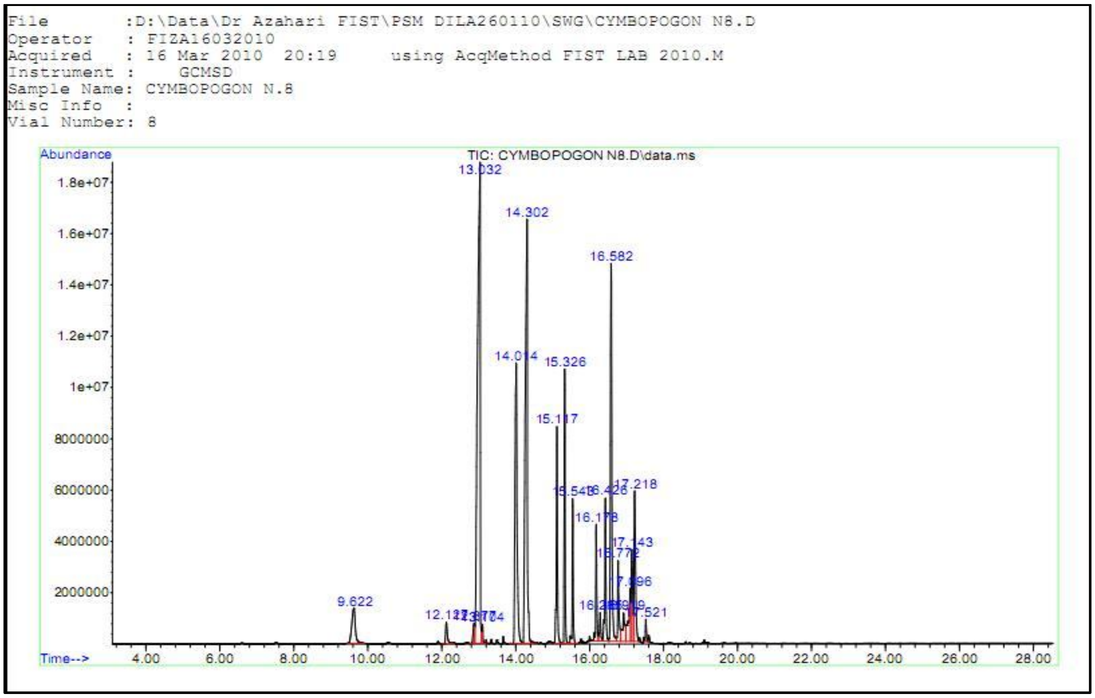
A.2.5 Hydrodistillation 90min



A.2.6 Microwave-Assisted Hydrodistillation 90min



A.2.7 Hydrodistillation 120min



A.2.8 Microwave-Assisted Hydrodistillation 120min