COMPARISON OF MICROWAVE-ASSISTED HYDRODISTILLATION WITH THE CONVENTIONAL HYDRODISTILLATION METHOD IN THE EXTRACTION OF ESSENTIAL OIL (LEMONGRASS AND STAR ANISE)

TAN KIM PIEU

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

FACULTY OF CHEMICAL AND NATURAL RESOURCES ENGINEERING
UNIVERSITI MALAYSIA PAHANG

DECEMBER 2010

I declare that this thesis entitled "Comparison of Microwave-Assisted Hydrodistilaltion with the Conventional Hydrodistillation method in the Extraction of Essential Oil (Lemongrass and Star Anise)" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

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Special thanks to my beloved father and mother for their constant encouragement and motivation and also to my supervisor and friends for their inspiration.

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ABSTRACT

Microwave-assisted hydrodistillation (MAHD) has recently been developed for the extraction of essential oils from plant materials. In this study, microwave-assisted hydrodistillation was investigated for the extraction of essential oils from lemongrass and start anise and the results were compared with those of the conventional hydrodistillation in terms of extraction time, extraction yield/efficiency and chemical composition. Microwave-assisted hydrodistillation was efficient in extraction in terms of extraction time and energy saving. Lemongrass and star anise was in the ratio of 1:10 with water and the essential oils components were identified using GCMS. There were significant different in the extraction yield of essential oils from both of the method and higher yield were obtained from MAHD method. Results of analysis from gas chromatography-mass spectrometry indicated that the use of microwave in hydrodistillation did not adversely influence the composition of essential oils. Microwave-assisted hydrodistillation was found to be environmentally friendly due to its shorter extraction time and therefore lower energy consumption.

ABSTRAK

Pergabungan microwave dengan penyulingan berasaskan air (MAHD) telah diaplikasikan dalam penghasilan minyak asli daripada tumbuhan sejak kebelakangan ini. Dalam kajian ini, MAHD digunakan untuk menghasilkan minyak asli daripada serai dan bunga lawang dan hasilnya dibandingkan dengan penyulingan tradisional berasaskan air (HD) dari segi jangka masa proses, kadar penghasilan dan komposisi minyak asli. MAHD efisien dalam penghasilan minyak asli dari segi jangka masa proses dan menjimatkan tenaga. Serai dan bunga lawang yang digunakan adalah dalam nisbah 1:10 dengan air dan komposisi minyak asli dikenalpasti dengan menggunakan menggunakan alat GC-MS. Perbezaan jangka masa proses yang ketara didapati daripada dua cara tersebut dan penghasilan minyak asli yang lebih tinggi didapati dalam cara MAHD. Data analisis yang diperoleh daripada GC-MS menunjukkan penggunaan microwave dalam penyulingan berasaskan air tidak mempengaruhi komposisi minyak asli. MAHD tidak memberi kesan negatif terhadap alam sekeliling kerana jangka masa proses yang rendah dan justeru penggunaan tenaga yang rendah.

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LIST OF ABBREVIATIONS

HD	=	Hydrodistillation
MAHD	=	Microwave-Assisted Hydrodistillation
GC-MS	=	Gas Chromatography-Mass Spectrometer
SFE	=	Supercritical Fluid Extraction
MAE	=	Microwave-Assisted Extraction
MASD	=	Microwave Accelerated Steam Distillation
MASE	=	Microwave-Assisted Solvent Extraction
SFME	=	Solvent-Free Microwave Extraction
MHG	=	Microwave Hydrodiffusion and Gravity
SPME	=	Solid Phase Micro Extraction

LIST OF SYMBOLS

˚ C	=	Degree Celsius
%	=	Percentage
kPa	=	Kilo-Pascal
Hz	=	Hertz
MHz	=	Mega-Hertz
GHz	=	Giga-Hertz
W	=	Watts
mL	=	Mili-Liter
g	=	Grams
L	=	Liter
min	=	Minutes
hr	=	Hours
m	=	Meter
mm	=	Mili-Meter
µL	=	Micro-Liter
µm	=	Micro-Meter
mL/min	=	Mili-Liter Per Minute
w	=	Weight
w/w	=	Weight of Oil/Weight of Plant Materials

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

In food industry, the use of herbs and plants in the production of essential oils becomes significance because of their use in many applications including flavours and fragrances as well as in medicine. Essential oils contain the DNA of the plant or herb they are extracted from. They are complex mixtures of volatile compounds such as terpenes (mostly monoterpenes and sesquiterpenes), phenolics and alcohols (*Lucchesi et al., 2004*), which gives the characteristic odour and flavor closely associated with the vegetative matter they are obtained.

Essential oils can be isolated using a number of isolation methods such as hydrodistillation, steam distillation and organic solvent extraction. The conventional method for extraction of essential oils is hydrodistillation. In this method, a mixture of water and plant materials are heated and followed by liquefaction of the vapors in a condenser to evaporate the essential oils. However, this method resulted in several disadvantages including losses of volatile compounds and long extraction time (*Khajeh et al., 2004*). Recently, microwave-assisted hydrodistillation (MAHD) has gained attention and widely used to obtain essential oils from plant materials. Plant

material placed in a Clevenger apparatus and heated inside microwave oven for a short period of time. In this study, MAHD was applied as a new technology for the extraction of essential oils from lemongrass and star anise.

1.2 Problem Statement

The worldwide market for essential oils due to its increasing importance in pharmaceutical, fragrances and food industry trigger the research on new techniques for a better extraction. Conventional hydrodistillation involves distillation of plant material in water for long period. Hydrodistillation is economically viable and safe and it is the most common method for extraction of essential oils. However, the market values of essential oils lead to the application of microwave energy in the extraction to obtain higher quality oils and effective extraction.

Conventional hydrodistillation method is time consuming and low efficiency. In conventional hydrodistillation, heat transfer depends on thermal conductivity. Heat is transferred from the heating medium to the interior of the sample (*Bousbia et al., 2009*) resulted in the slowly increase in temperature. In microwave-assisted hydrodistillation, microwaves are volumetrically distributed (*Bousbia et al., 2009*). Due to the volumetric heating effect, a faster increase in temperature can be obtained depending on the microwave power and the dielectric loss factor of the material being irradiated. Extraction of essential oils from plant materials started right after the sample achieved boiling point. This causes an important difference in extraction time between the conventional and microwave-assisted hydrodistillation.

Instead of extraction time, losses of volatile compounds are another problem that arises when using conventional hydrodistillation method. In microwave-assisted hydrodistillation heat energy is produced by microwave energy. The efficiency of

MAHD depends strongly on the dielectric constant of water and the matrix (*Brachet et al., 2002*). It caused the rapid delivery of energy to the total volume of solvent/sample in which the sample reaches its boiling point rapidly. Heat is originated through the molecular motions (*Brachet et al., 2002*) and the rise in temperature within the plant cells is similar to that occurring outside the cells. The external cell walls break apart once the pressure within the glands reaches certain level (*Chemat et al., 2005*) to release the essential oil. The rapid heating of plant materials by the microwave energy minimizes the losses of volatile compounds from the plant materials.

1.3 Objectives

The objective of this project is:

i) Using a new technology, the microwave-assisted hydrodistillation as an alternative method to extract essential oil from plant materials.

ii) Extract essential oil from plant materials using conventional hydrodistillation method.

iii) Analyze the overall performances of microwave-assisted hydrodistillation and make a comparison between microwave-assisted hydrodistillation and conventional hydrodistillation method.

1.4 Scope of study

There are some important tasks to be carried out in order to achieve the objectives of this study. The important scopes have been identified and all the research works will be base on the scopes throughout the study.

i) In this study, we have been restricted the raw materials (Lemongrass and Star Anise). The extraction of essential oil will be carried out on these raw materials using conventional hydrodistillation and microwave-assisted hydrodistillation.

ii) Analysis on the essential oil will be carried out using Gas Chromatography – Mass Spectrometer (GC-MS) to determine the components of the essential oil for particular raw material.

iii) The comparison on both of the method will be in terms of :

a) Extraction time
b) Extraction yield/efficiency
c) Chemical composition
d) Cost of operation

1.5 Rationale and Significance

The rationale and significance of this study is:

i) The market value of essential oils increases due to its importance in pharmaceutical, fragrances, and food industry. There is a need to explore new technique on extraction to replace conventional extraction method to get a better extraction in terms of time, cost, and quality of essential oils.

ii) Extraction of essential oil using microwave-assisted hydrodistillation involved short extraction time, high extraction efficiency, and minimize the losses of volatile compounds in the plant materials.

CHAPTER 2

LITERATURE REVIEW

2.1 Essential Oils

Essential oils are the volatile fraction of the secondary metabolites produced plants (*Ramanadhan et al., 2005*). The essential oils extracted from the plant materials contain the DNA of the plant. It is normally very concentrated that it gives 100 times the flavoring strength of the parent plant (*Mohamed, 2005*). Essential oils bearing plants have their value in food industry, fragrance and pharmaceutical.

Essential oils are highly complex compounds and their constituents included oxygenated compounds. They are a group of natural organic compounds that are predominantly composed of terpenes (hydrocarbons) and terpenoids (oxygen containing hydrocarbons). Essential oils also contain simple phenols, sulphur containing mustard oils, methyl anthranilate and coumarins. Majority of them are fairly stable and soluble in high strength alcohol but have poor water solubility.

Terpenes and terpenoids in the plant were built from the basic 3-methyl-3-butenyl pyrophosphate. The 5-carbon unit of this molecule is the source of the

isoprene unit, and combination two of these units give rise to geranyl pyrophosphate to form the skeleton of monoterpenes (10 carbons). Subsequently combination of 3 of these units gives rise to farnesyl pyrophosphate to form the skeleton of the sesquiterpenes (15 carbons). These complex mixtures of volatile compounds give the characteristic odour and flavor associated with the vegetative matter.

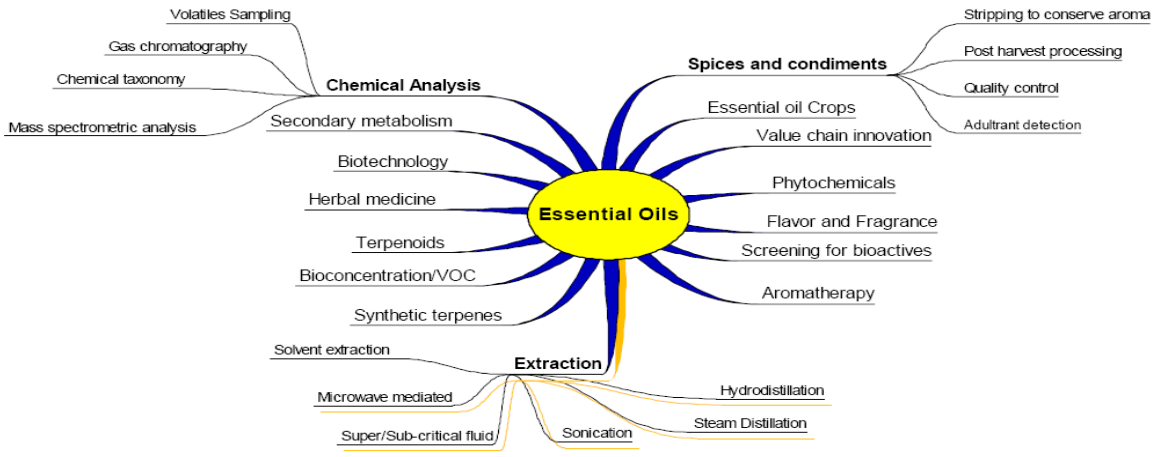


Figure 2.1: Tree diagram showing the wide branching of specializations in the field of essential oils (*Ramanadhan et al., 2005*)

In order to obtain the essentials oils from the plant materials, the isolation by physical means have to be carried out. The physical methods are direct distillation of essential oils, water steam distillation of essential oils or organic solvent extraction of organic compounds. The conventional method for the extraction of essential oils such as Soxhlet extraction method, liquid-liquid, and solid-liquid extraction are characterized by lengthy extraction procedures, consumption of large amount of solvent and energy and losses of some volatile compounds.

In the recent years, several studies have been conducted on new techniques to extract essential oils from plant materials. Among the techniques introduced was ultrasonic extraction, supercritical fluid extraction (SFE), extraction with subcritical or critical water, and application of microwave technique such as microwave-assisted extraction (MAE), solvent-free microwave extraction (SFME), microwave accelerated steam distillation, microwave hydrodiffusion and gravity (MHG), and

microwave-assisted hydrodistillation. These new techniques is proved to be effective in the extraction of essential oils in which they involved shorter extraction time compared with the conventional method, higher yield and better quality of essential oils, minimize the consumption of solvent, energy saving and therefore environmentally friendly.

Table 2.1: Important essential oils (*Gunther, 1994*)

Name of oil	Method of production	Part of plant used
Almond	Steam distillation	Kernels
Bay	Steam distillation	Leaves
Bergamot	Expression	Peel
Caraway	Steam distillation	Seed
Cassia	Steam distillation	Leaves and twigs
Cedarwood	Steam distillation	Red core wood
Cinnamon	Steam distillation	Bark
Citronella	Steam distillation	Grass
Clove	Steam distillation	Buds
Coriander	Steam distillation	Fruits
Eucalyptus	Steam distillation	Leaves
Geranium	Steam distillation	Leaves
Jasmine	Cold pomade	Flowers
Lavender	Hydro-distillation	Flowers
Lemon	Expression	Peel
Orange	Expression, distillation	Peel
Peppermint	Steam distillation	Leaves and tops
Rose	Steam distillation, solvent, enfleurage	Flowers
Sandalwood	Steam distillation	Wood
Spearmint	Steam distillation	Leaves
Tuberose	Solvent, enfleurage	Flowers
Wintergreen	Steam distillation	Leaves
Ylang-ylang	Steam distillation, solvent extraction	Flowers

2.2 Plant Materials

2.2.1 Agarwood

Agarwood (Gaharu) is a dark resinous heartwood that forms in *Aquilaria* trees when they become infected with a type of mold. There are 25 species of *Aquilaria* and 15 species are reported to form agarwood (*Barden et al., 2002*). In the Malaysia forests, the main species producing agarwood is *A. malaccensis* as it is commonly known (*Nor Azah et al., 2008*).

Agarwood is the resin impregnated, fragrant and highly valuable heartwood found in species of *Aquilaria*. It ranks among the most highly valuable traded forest products world-wide (*Wollenberg, 2001*). The wood released fragrance that is considered as scent when it is burnt.

Formation of agarwood occurs in the trunk and roots of trees that have been infected by a dematiaceous (dark-walled) fungus. As a response, the tree produces a resin high in volatile organic compounds to suppress or retarding the fungal growth. The resin dramatically increases the mass and the density of the affected wood, while the unaffected wood of the tree is relatively light in colour. The affected wood changing its colour to dark brown or black.

A common method in artificial forestry is to inoculate all the trees with the fungus. High quality resin produced when a tree's natural immune response to fungal attack. Agarwood from this process is commonly regarded as first quality agarwood. When trees are deliberately wounded, leaving them more susceptible to a fungal attack to create an inferior resin, it is commonly called second quality agarwood.

Agarwood oil made from the agarwood is very tenacious and the colour of the oils may vary from greenish brown to dark reddish brown. The tiniest drops is needed to fill the air with its soul evoking aroma. Regarding the distinctive fragrance, it is used as perfumes, an essential oil and aroma therapy. Agarwood oil consists of complex mixtures such as sesquiterpene, hydrocarbons, sesquiteterpene alcohols, and aliphatic hydrocarbons in which to be identified using Gas Chromatography-Mass Spectrometer (GC-MS).

Table 2.2: Chemical compounds in Malaysia Agarwood oils (*Nor Azah et al., 2008*).

Chemical compounds	RI	Selangor (%)	Kelantan (%)	Pahang (%)	Terengganu (%)
3-phenyl-2-butanone	1249	1.50	5.77	7.80	0.79
α-guaiene	1448	-	0.67	-	-
β-agarofuran	1477	1.69	1.98	0.69	0.50
α-agarofuran	1553	4.83	2.96	1.48	1.57
Nor-ketoagarofuran	1557	2.09	-	-	-
10-epi-γ-eudesmol	1618	11.54	9.03	8.10	3.32
Agarospirol	1631	14.86	5.49	7.11	18.86
β-eudesmol	1649	-	-	-	5.74
Jinkoh-eremol	1650	10.62	7.70	6.31	-
kusunol	1659	18.94	-	-	-
Jinkohol II	1751	4.71	-	-	-

2.2.2 Lemongrass

Lemon grasses (*Cymbopogon Citratus*) are a group of commercially important tropical grasses. The leaves of lemon grasses contain up to 1.5 % essential oils with a typical lemon-like aroma (*Lewinsohn et al., 1997*). Lemon grasses are indigenous in tropical and semi-tropical areas of Asia, and are cultivated in South and Central America, Africa and other tropical countries (*Weiss, 1997*).

Lemon grass is a perennial fast-growing aromatic grass, growing to about 1 meter high with long and thin leaves. It produces a network of roots and rootlets that rapidly exhausted the soil. The main chemical components of lemon grass oil are myrene, citronellal, geranyl acetate, nerol, geraniol, nearl and traces of limonene and citral. Citral is the name given to a natural mixture of two isomeric acyclic monoterpene aldehydes, geranial (trans-citral, citral A) and neral (cis-citral, citral B) (*Lewinsohn et al., 1997*).

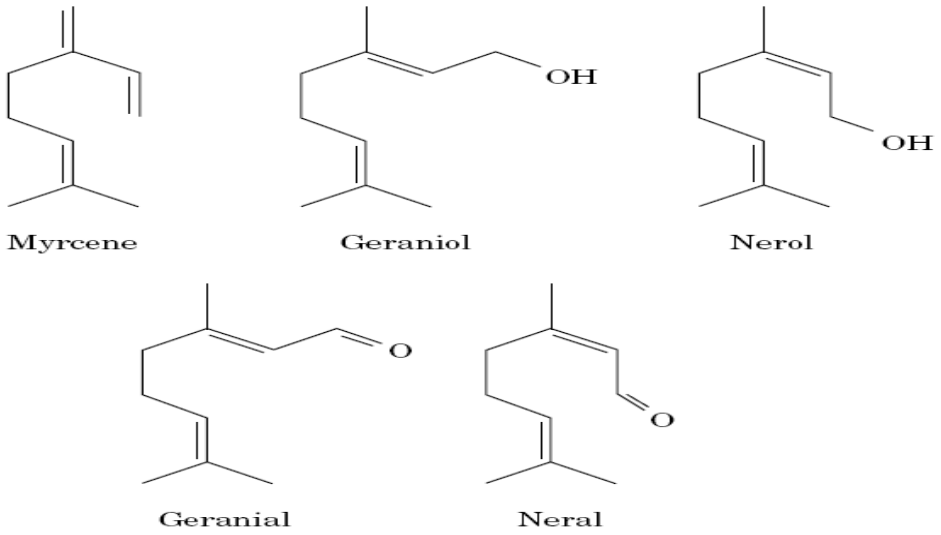


Figure 2.2: Chemical structure of the major constituents of lemongrass essential oil (*Lewinsohn et al., 1997*).

The medicinal part of lemon grass is the leaves, in which the lemon grass oil is extracted from the fresh or partly dried leaves by distillation. Lemon grass oil has a

lemony, sweet smell and is dark yellow to amber and reddish in colour. Lemongrass oil can irritate a sensitive skin, so care should be taken. It is used in soaps, cosmetics, perfumes flavours and pharmaceutical products.

Table 2.3: Chemical constituents of Lemongrass oil determined by GC-MS (*Chimmalee et al., n.d.*)

Compound no.	Retention time (minute)	Compound	Relative amount (%)
1	5.28	Linalool	2.78
2	6.08	Citronellal	1.10
3	6.25	Verbenol	3.42
5	6.36	α-phellandren-8-ol	0.23
6	6.51	trans-Caran, 4, 5,	6.06
7	6.94	epoxi	0.33
8	7.17	cis-Caveol	0.96
9	7.21	β-Citronellol	0.32
10	7.41	cis-Geraniol	45.75
11	7.82	β-Citral	30.42
12	8.22	α-Citral	0.12
13	9.32	Geranyl formate	4.34
14	9.96	Geranyl acetate	0.37
15	12.45	trans-Caryophyllene	0.59
		Junipercamphor	2.83
		Unknown	

2.2.3 Star Anise

Star anise is defined as the dried, star-shaped multiple fruit of the tree of Illicium verum Hook., which is a member of the magnolia family (Magnoliaceae). Star anise fruits are produced on a medium-sized evergreen tree (Illicium verum

Hook), native to southern China and northern Vietnam that is the most important essential oil tree cultivated in Vietnam (*Dang & Ilangantileke, 1997*).

The small, red-brown, star-shaped fruits contain 6-8 unevenly sized, boat-shaped individual fruits 12-17 mm in length, each containing a glossy brown, egg-shaped seed. The fruit is picked unripe, then dried in the sun to a brown colour. The fruit is always dried, and never eaten fresh.

True star anise (Illicium verum) which is native to southern China may be confused with the aniseed star (Illicium anisatum) which is found in Japan. Aniseed star from Japan is known as shikimi. It is toxic for human consumption, and has been associated with serious adverse effects including emesis and diarrhea, bradycardia, hallucinations, rhabdomyolysis, and convulsions (*Howes et al., 2009*). Instead it is used as one of the ingredients for making incense.

Figure 2.3: Star Anise

The essential oil from Star anise fruits is traditionally extracted by steam distillation. In the steam distillation process, the relatively high temperature of steam, combined with the hydrolytic influence of water, may cause degradation of the essential oil components (*Stahl & Gerard, 1985*).

The main component in Star anise essential oil is trans-anethole, a paramethoxyphenyl propene which accounts for about 85-90% (by weight). The rest includes terpene, pinene, I-phellandrene, cr-diterpene, limonene, estragole, safrol and terpineol etc (WHO & Institute of Materia Medica, 1990). 25 volatile compounds were identified in star anise essential oil, accounting for 99.9% of total oil. 15 components were identified in the acetone extract (80.27% of total oil). Major compounds in the essential oil were *trans*-anethole (94.37%) followed by methyl chevicol and *cis*-anethole (1.2 and 1.59%, respectively) (*Singh et al., 2006*).

In traditional Chinese medicine, star anise is prescribed as a digestive aid and to help cure colic in babies. It is well known for its effect on the digestive system and may have a good effect on asthma and breathing difficulties. More recently, Shikimic Acid, extracted from star anise, is one of the chief ingredients in the antiviral Tamiflu drug used to fight avian influenza.

Other than that, the essential oil from Star anise fruits is used in the confectionery trade to flavor licorice and other candies, in the baking trade to flavour cakes, cookies and biscuits, and in the liqueur industry for flavoring anisette (*Dang & Ilangantileke, 1997*). Star anise is one of the spices in five-spice powder. It is also the secret ingredient in many Indian stews and curries. Star anise can replace regular anise in western recipes.

2.3 Steam Distillation

Steam distillation is a commonly used method in the extraction of essential oils for food industry and perfumery. A lot of studies have been conducted on steam distillation and compared it with the new techniques to figure out the advantages of

new technique over conventional method. Steam distillation is widely used because the water as the solvent consists of high latent heat of vaporization. The solvent, water is easier, cheaper and widely available. There are two types of steam distillation, the water/steam distillation and steam distillation.

In steam distillation, the process uses the steam to percolate and vaporize out the essential oils from the plant materials. The extract is then being condensed in the condenser at the top of the apparatus. The separation can be carried out to separate the desired compounds from the condensed materials. During distillation, only the molecules with size that tiny enough for diffusion can be evaporated. The tiny molecules that evaporate and condense make up essential oils.

The water/steam distillation is an improved method of steam distillation. In this method, plant materials are placed on the grid plate that just above the water level. The water is being heated to produce wet steam and percolate through plant materials to extract essential oils.

The conventional steam distillation method has several drawbacks in which it could result in economic lose for the extraction. The method is time consuming, thermal degradation and losses of volatile compounds from the plant materials, large solvent consumption and not energy saving render it to be not environmental friendly.

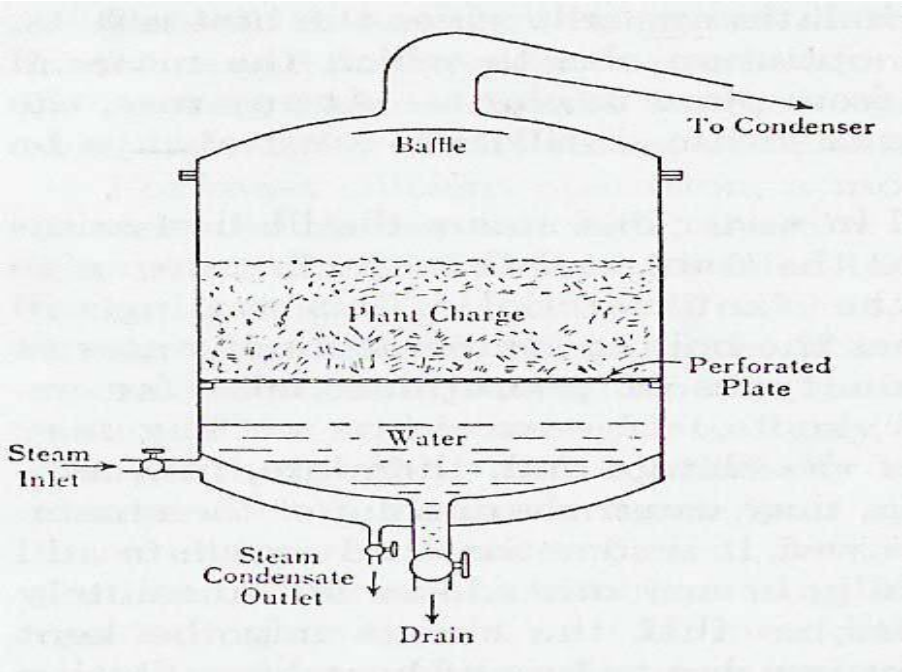


Figure 2.4: Water/steam distillation unit (*Guenther, 1972*).

2.4 Soxhlet Extraction

A Soxhlet extractor is a laboratory apparatus invented in 1879 by Franz von Soxhlet. The method was invented to extract lipid from a solid material. The basic procedure is a solid sample to be placed in a porous container and the condensed solvent will extract the desired compound continuously. Nowadays, this method no longer limited to extraction of lipids and is used to extract desired compound from the sample. The simplest form of solid-liquid extraction is the treatment of solid with solvent. Soxhlet extraction is applicable when the desired compound is insoluble in solvent used or it has a limited solubility in a solvent. In the case the desired compound soluble in a solvent, a simple filtration can be carried out to separate the desired compound.

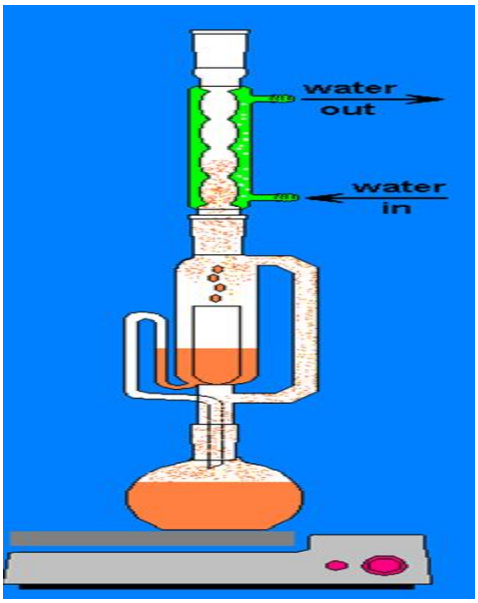


Figure 2.5: Schematic diagram of Soxhlet Extraction apparatus

The apparatus of Soxhlet extraction consists of three components which are condenser, porous container and distillation pot. The condenser is to cool the solvent vapor and condense it back into a liquid. It normally has a large surface area for an efficient condensation. The porous container is used to hold the solid sample. The condensed solvent vapor will pass through the porous container and thereby extract desired compound. The distillation pot is function as a solvent pool and it also collect the concentrated material from the porous container.

The operation of Soxhlet extraction begins with boiling solvent vapors rise up through the larger side-arm in the schematic diagram above. The condensed solvent vapors fall back into porous container and dissolving out the desired compound from the sample. When the smaller side-arm fills to overflowing, it initiates a siphoning action. The solvent containing the dissolved compound is siphoned into the distillation pot. The residual solvent then drains out of the porous container as fresh condensed solvent vapors continue to fall into the porous container. The process is repeated until a concentrated material is collected.

Soxhlet extraction is a conventional extraction method in which it is time-consuming and large solvent consumption. This method is slowly replaced by enhanced extraction method such as supercritical fluid extraction and microwave-assisted extraction.

2.5 Ultrasound Extraction

The using of ultrasound in the plant extraction has already been demonstrated for a number compound of interest in the food industries and pharmaceutical. There was several studies have been conducted regarding the use of ultrasound-assisted extraction. Among the studies were the ultrasound-assisted extraction of polysaccharides from Salvia Officinalis L. (*Hromadkova et al., 1999*) and extraction of bioactive principles from plant materials (*Vinatoru et al., 1997*).

The application of ultrasound significantly accelerates the analyte extraction, reduction of the sample preparation time, usage of small amounts of material and minimum solvents consumption. Specific examples include the extraction of tea from dried leaves with water using ultrasound and the yield was up to 20% at 60 ˚C approaching the efficiency of thermal extraction at 100 ˚C (*Mason, 1994*). In pharmaceuticals, the use of ultrasound provide an economic process and improved environmental and health and safety considerations (*Ishtiaq et al., 2009*).

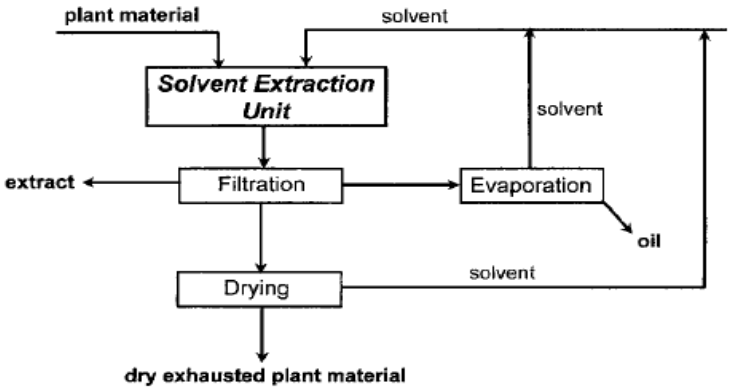


Figure 2.6: General scheme for solvent unit extraction (*Vinatoru et al., 1997*)

The solvent unit extraction is a suitable place for the installation of an ultrasonic devices. The plant material mix with the solvent at the solvent extraction unit. In the case an alcoholic or volatile solvent is used, an ultrasonic cleaning bath or a closed reactor fitted with an horn transducer ultrasonic device should be applied. The product from the solvent extraction unit will undergoes filtration and

evaporation to obtain oil and the solvent is recycled in the process. Recycling of solvent accounts for the minimization of solvent consumption for this method.

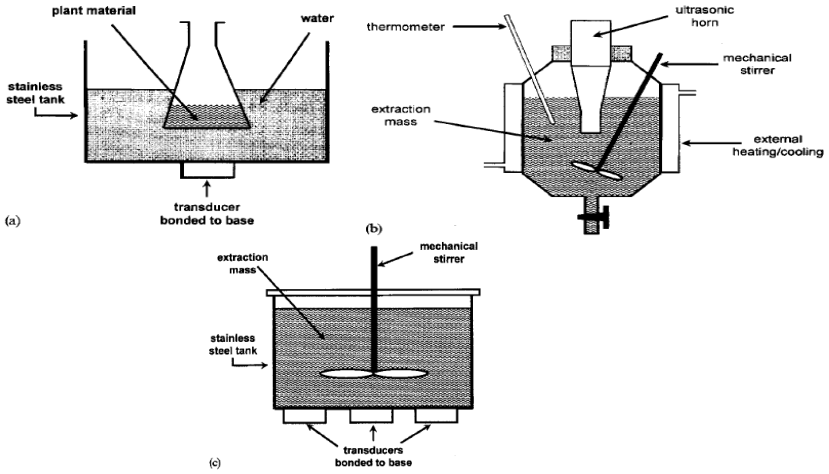


Figure 2.7 (a) Indirect sonification using an ultrasonic bath (b) Direct sonification using an ultrasonic horn (c) Direct sonification using an ultrasonic bath (*Vinatoru et al., 1997*)

2.6 Supercritical Fluid Extraction (SFE)

A supercritical fluid is any substance above its critical temperature and critical pressure. In the supercritical area there is only one state-of-the-fluid and it possesses both gas and liquid-like properties. Since 1980, there has been rapid development of SFE for the extraction of hops, cholesterol from butter, perfumes and flavors from natural products, residual solvents and monomers from polymers, unsaturated fatty acids from fish oils. Recently, studies have been conducted on this technique in the extraction of essential oils on different plant materials, such as palm kernel (*Hassan et al., 2000*), hiprose seeds (*Reverchon et al., 2000*), sunflower seeds (*Kiriamiti et al., 2002*), lemon grass (*Huynh et al., 2008*), and clove buds (*Baseri et al., 2008*).

A supercritical fluid is made from a gas or a liquid but not from solid. When a gas or liquid is compressed under pressure and heated above its critical point, it enters a phase called supercritical phase. The temperature and pressure at which this happen are unique to each pure substance. A supercritical fluid exhibits physicochemical properties intermediate between those of liquids and gasses. Characteristics of a supercritical fluid are it is a dense gas, solubility approaching liquid phase and diffusivities approaching gas phase. The most important properties of a supercritical fluid are its density, viscosity, diffusivity, heat capacity and thermal conductivity. Manipulating the temperature and pressure above the critical points affects these properties and therefore enchances the ability of the supercritical fluid to penetrate and extract the plant materials.

Supercritical fluid extraction is commonly used to extract chemicals or flavors from products such as coffee, tea, hops, herbs, and spices. It has the potential to be an environmentally friendly green processing technique to replace the traditional organic solvent base extraction techniques with more benign solvents such as supercritical carbon dioxide, ethanol and water.

Supercritical fluid extraction offers many advantages over the conventional extraction techniques. The conventional extraction process such as solvent extraction, hydrodistillation, steam distillation have many drawbacks such as solvent elimination after extraction, thermal degradation, hydrolysis and solubilization in water of compounds (*Baseri et al., 2008*). The supercritical fluid extraction has not any of these problems.

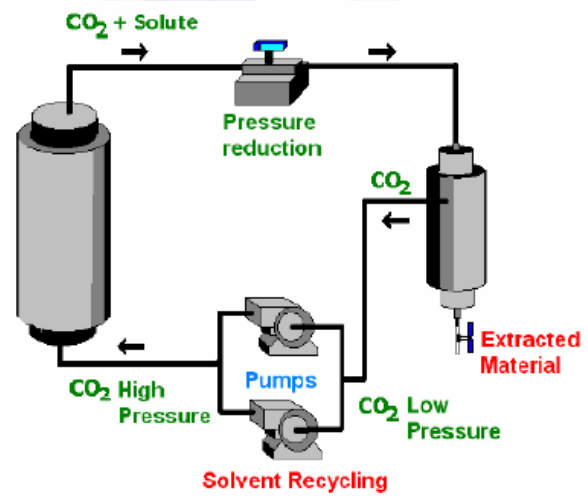


Figure 2.8: Simple scheme of supercritical fluid extraction (*Baseri et al., 2008*)

A supercritical fluid extraction unit consists of a series extraction column that used to do some fractionation of the extract, a pump to compress the solvent, a solvent reservoir, a solvent condenser and a heater to set the solvent temperature prior to the extraction column entrance. The solvent used after the extraction is recycled back to the process and this accounts for the minimization of the solvents used.

Supercritical fluid extraction needs to be more fully developed in few areas to be successful in future environmental lab. The higher investment in supercritical fluid extraction compared with the other conventional atmospheric pressure extraction techniques is the serious drawback from this method. The other prospects regarding the use of SFE would be the ease of use of the method, the ability of the method to interface with the existing lab instrumentation and computer systems and the enhanced understanding of the method and firmer theoretical base for sample preparation.

2.7 Microwave

Microwaves are non-ionizing electromagnetic fields in the frequency range of 300 MHz to 300 GHz and wavelengths within 1 cm and 1 m. The position of microwaves is between X-ray and infrared rays in the electromagnetic spectrum. Microwaves spreads as a harmonic wave through space, it composed of two oscillating perpendicular fields, an electric field which is responsible for heating and a magnetic field. Nowadays, microwaves are used for the major purpose of communication and as an energy sources. Heat produced by microwave energy is an alternative for the thermal application due to its efficient volumetrically heating. The interaction of microwaves and the matter resulted in energy transfer in which part of the electromagnetic energy is converted to heat energy.

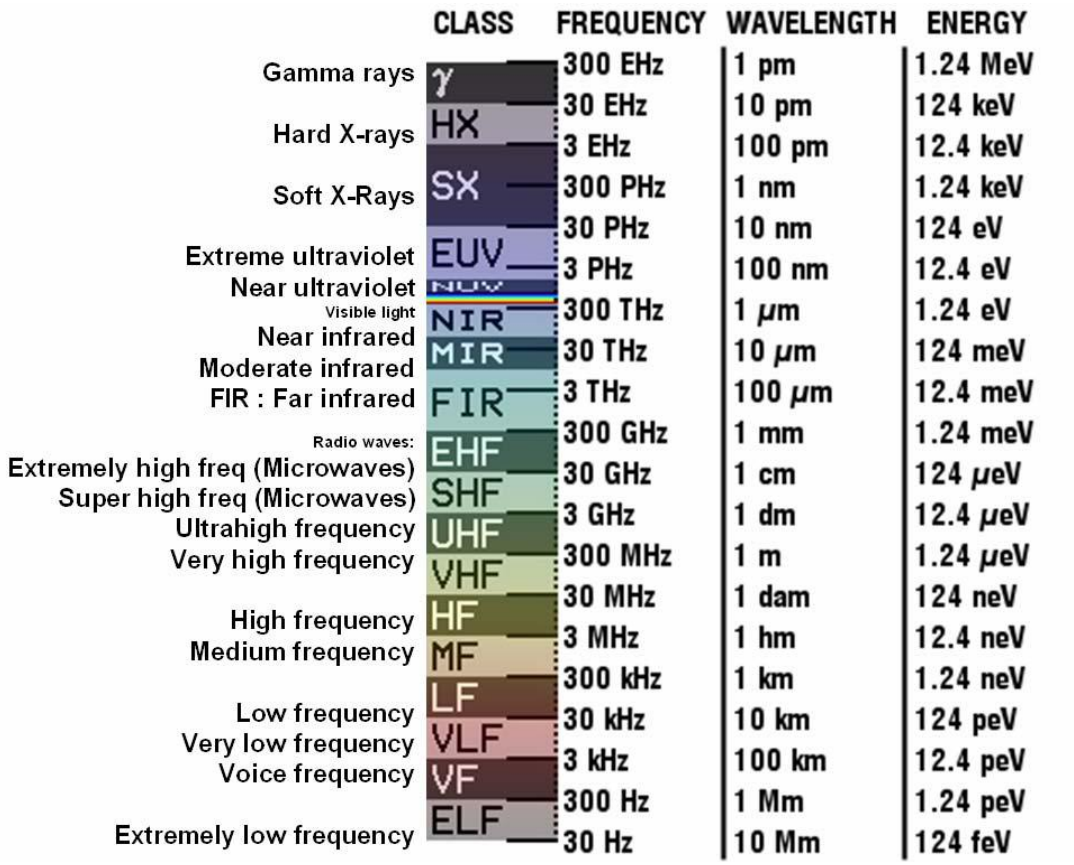


Figure 2.9: The electromagnetic spectrum (*GNU 2005*)

Electromagnetic fields have an orienting effect on polar molecules. If a polar molecule such as water present in the microwave susceptible materials, disturbance of polar molecules by an impressed electric field resulted an orienting effect experienced. There are two phenomenon occurred when heating using microwave, the ionic conduction and dipole rotation. Ionic conduction is the electrophoretic migration of ions when it is influenced by the changing electric field. When this phenomenon occurred, there is friction generated because the solution offered resistance to the migration of ions. As a result, the heat generated and heats up the solution. In dipole rotation, the polar molecules experience a force that will orient the permanent dipole and realignment of the dipole with the rapidly changing electric field. If an alternating field continuously propagates, the individual molecules will be introduced to rotate in an oscillatory manner about an axis through their centers (*Ramanadhan, 2005*), associated with energy absorption in the quantum rotational band.

In conventional heating, the heat transfer is occurred through conduction and convection phenomenon. The heating is depends on the thermal conductivity and heat transfer from heating medium to the interior of the sample and much of the heat energy being lost to the environment, which eventually resulted in slowly increase in temperature. In heating of materials using microwaves, heat energy produced by microwave energy. The heat transfer occurred through conduction, convection and irradiation. In this case, the microwaves are volumetrically distributed and heat is originated through molecular motion. As a result, the sample achieves the boiling point faster due to the volumetrically heating. This in return will cause the external cell walls of the sample break apart because the glands were subjected to the more severe thermal stresses and high pressures. When the pressure build-up within the glands reached certain level or exceeded their capacity for expansion, the cell walls will rupture more rapidly than in conventional heating.

Table 2.4: Comparison of conversion efficiencies of various heating sources (*Wilson, 2003*)

Appliance	Temp (°C)	Appliance rating (W)	Time	Energy used (kWh)	Energy cost US $
Electric oven	177	2000	1hr	2	0.17
Convection oven	163	1853	45 min	1.39	0.12
Gas oven	177	36	1 hr	3.57	0.07
Frying pan	216	900	1 hr	0.9	0.07
Toaster oven	218	1140	50 min	0.95	0.08
Crockpot	93	100	7 hr	0.7	0.06
Microwave oven	High	1440	15 min	0.36	0.03

Microwaves are generated by applying alternating current to a domestic oven. The microwaves generated inside the oven are at frequency of 60 Hz and stepped up to 2450 million Hz accomplished by a device called magnetron operates at 4000 to 8000 volts inside microwave oven (*Ramanadhan, 2005).* The electromagnetic waves channeled through a conduit by a waveguide into the cavity of microwave oven that holds the samples for heating. Microwave oven consists of highly reflecting cavity walls and a rotating reflector or fan works to maximizing the effect of microwave on heating.

Microwave ovens applied by industrial, scientific and medical microwave application operate at specific frequencies, the most favored frequency being 2.45 GHz. The orientation of solvent molecule swing by the electric field 2.45 x 10^9 times every second at this frequency. Solvent molecule tries to align itself with the electric field to keep itself in the same phase. The electric component of the wave that changing at such a rapid speed cause the solvent molecules failed to align themselves and generate heat through frictional force when they vibrating. At the frequency greater than 2.45 GHz, the solvent molecules do not even have sufficient time to align themselves because the electric component of wave changing at the much higher speed. At the frequency less than 2.45 GHz, the solvent molecules have sufficient time to align themselves with the electric field because the electric component of wave changing at the lower speed. As a result, there is no heating for both the frequency greater and less than 2.45 GHz.

The efficiency of microwave heating depends strongly on the dielectric constant of solvent and the matrix. Solvent with high dielectric constants can absorb more microwave energy. The dielectric constant is a measure of extent that heating can be achieve by the absorption of microwaves. The dielectric loss constant is the indicator of the efficiency of converting microwave energy into heat.

Table 2.5: Dissipation factor and dielectric constants of solvents (*Mandal et al., 2007*)

Solvent	Dielectric constant[a] (ε')	Dielectric loss (tanδ) × 10^{-4}
Acetone	20.7	
Acetonitrile	37.5	
Ethanol	24.3	2500
Hexane	1.89	
Methanol	32.6	6400
2- propanol	19.9	6700
Water	78.3	1570

a: determined at 20°C

The polarity of solvent is important in microwave extraction because polar solvent with high dielectric constant influences the extent of microwave absorbed. The polar solvent is believed to be better than the non polar solvent. However, there exists an opposite opinion, the 'broken cell-wall theory' (*Pare et al., 1991*). According to the theory, microwave transparent solvents are better than microwave absorbing ones. If we use microwave-transparent solvent, the solvent itself does not absorb microwaves. As a result, the plant materials will absorb microwave energy without any hindrance. Besides that, the glands inside cell walls contain much water. Water has high dielectric constant that it absorbs microwave energy rapidly. Thus, there is sudden increase in temperature inside the cells and eventually break the cell wall to release its constituent. Water is an ideal solvent for absorbing microwaves energy because it boils without explosiveness and inflammability.

The microwave heating technique have been widely used analytical chemistry include sample digestion for elemental analysis, sample drying, measurement of moisture, analyte desorption and adsorption, sample clean-up, chromogenic reaction, speciation and nebulization of sample solutions (*Jin et al., 1999*). Microwave applications in agriculture and food technology such as tempering, freeze drying,

blanching, baking, sterilization and extraction (*Ramanadhan, 2005).* In recent years, the application of microwave technique is increasingly finding in natural product areas such as pharmaceutical as well as flavor and fragrances industry.

2.7.1 Microwave Accelerated Steam Distillation (MASD)

Microwave accelerated steam distillation is a technique that combine both microwave technique and conventional steam distillation method. In the recent study, the microwave accelerated steam distillation was used to extract essential oil from lavender (*Chemat et al., 2006*).

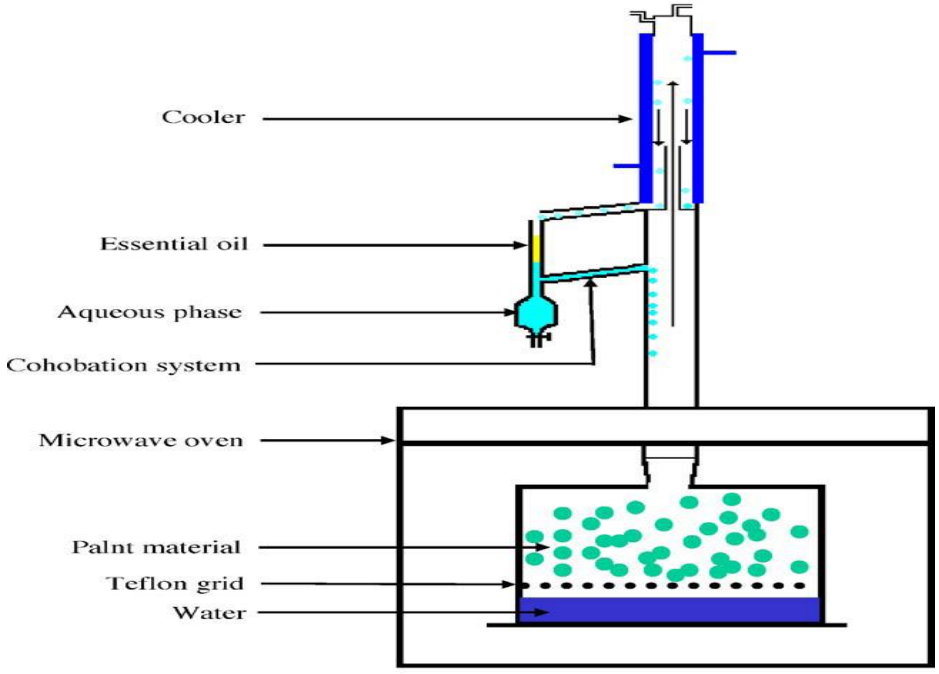


Figure 2.10: Microwave Accelerated Steam Distillation (*Chemat et al., 2006)*

A schematic diagram for the MASD apparatus is shown above. The apparatus has a cylindrical Pyrex body with a Teflon grid. The plant materials are placed on the Teflon grid to discard it from mixing with water the bottom. Steam is produced by heating the water using microwave energy. The steam produced will pass through the sample, evaporating and carrying the essential oil in the vapour form. The vapours

are then condensed and collected at specific glassware. The excess of water is refluxed back to restore water for steam production. The essential oil obtained can be simply separate from water using decantation.

Microwave accelerated steam distillation was better than conventional steam distillation in terms of extraction time, energy saving, product yield, cleanliness and product quality. In the study of extraction essential oils, MASD was better than SD in terms of rapidity (6 min versus 30 min for lavender flowers), thereby allowing substantial savings of costs in terms of time and energy (*Sahraoui et al., 2008*).

2.7.2 Microwave-Assisted Solvent Extraction (MASE)

Microwave-assisted solvent extraction (MASE), also called microwave extraction, is a new extraction technique, which combines microwave and traditional solvent extraction. Study shows that microwave-assisted extraction has many advantages, such as shorter time, less solvent, higher extraction rate, better products with lower cost. The apparatus of microwave-assisted extraction is simpler and cheaper and can be used to more materials with less limit of the polarity of extractant.

The applications of microwave technique in microwave-assisted solvent extraction as an alternative to the conventional solvent extraction have been introduced. This method was used as a new method for extraction of artemisinin from *Artemisia annua* L (*Hao et al., 2002*). Recently, the microwave-assisted solvent extraction was used to extract thymol from seeds of *Trachyspermum ammi* (TA) (*Gujar et al., 2009*) and oil from olive cake using hexane as solvent (*Armani and Kadi, 2010*).

The conventional solvent extraction (CSE) method that used to extract oil from vegetal materials is not efficient due to the long extraction time and solvent consumption. The microwave-assisted solvent extraction (MASE) system consisted of a domestic microwave oven, a reactor and a stirrer.

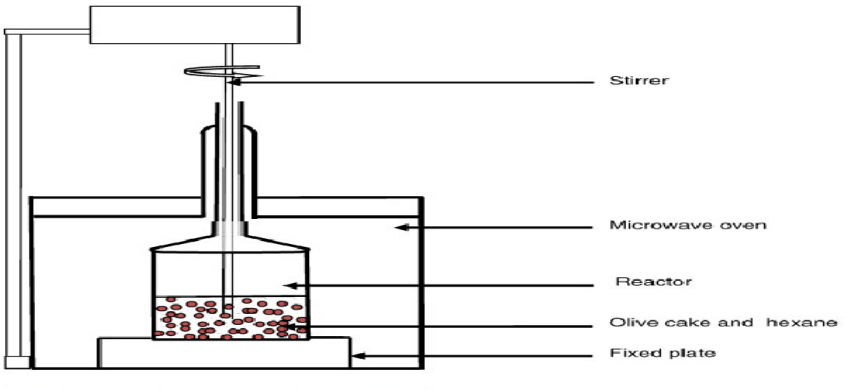


Figure 2.11: Schematic diagram of microwave-assisted extraction system (MASE) (*Armani and Kadi, 2010).*

The conventional solvent extraction system was the same as that used in the MASE system but a thermostated bath was used for the immersion of the reactor to control the temperature.

By applying microwave technique in the microwave-assisted extraction system, there was significance difference in the extraction time. The conventional solvent extraction needs a longer extraction time and thus a low efficiency. The enhanced extraction in microwave-assisted solvent extraction is due to the internal heating of the in situ water within the sample and cause the temperature rise rapidly and eventually accelerates cell rupture to release constituents.

The use of microwave as heating source also prevents the losses of volatile compounds of the sample that undergoes degradation reactions. This in return gives higher quality oil compared to the conventional method. In addition, the yield of obtained oils increased with the power radiation of microwave. This is due to the rise in power increases the temperature in which eventually accentuate the destruction of cells and cause the sample to releases more oil.

2.7.3 Solvent-Free Microwave Extraction (SFME)

Solvent-free microwave extraction (SFME) is a new technique that combined microwave heating and dry distillation. It is performed at an atmospheric pressure without presence of any solvent or water. SFME has already been applied to extraction of essential oil from fresh plant materials or dried materials prior moistened. A few studies have been conducted using this technique, among them was the solvent-free microwave extraction of essential oil from aromatic herbs (*Lucchesi et al., 2004*) and solvent-free microwave extraction of essential oil from oregano (*Bayramoglu et al., 2008*).

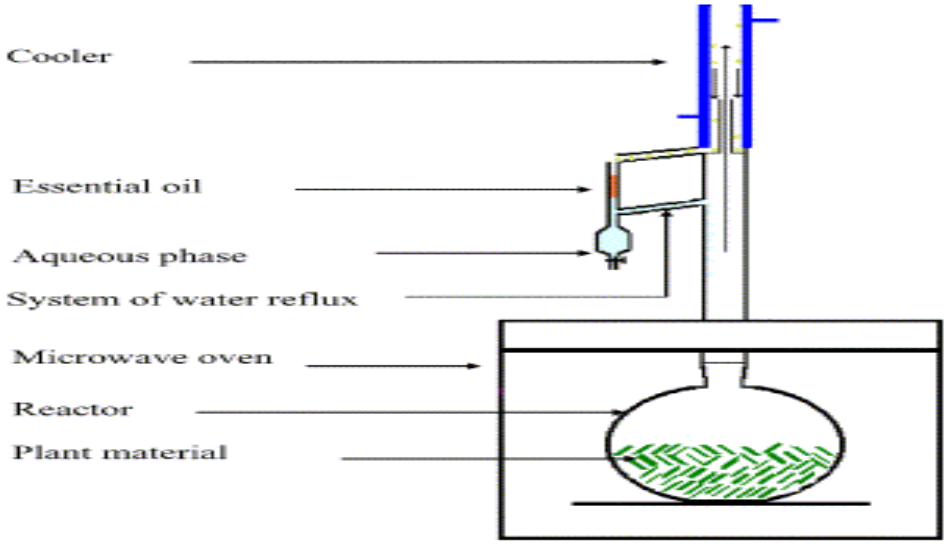


Figure 2.12: Solvent-free microwave extraction system (*Lucchesi et al., 2004)*

In solvent-free microwave extraction, the samples are placed in a flask without mix with the solvent. The internal heating of the water within the sample will cause the gland of cells rupture and therefore release essential oils. The oils are then undergoes evaporation by the in-situ water of the plant material. A cooler is placed outside the microwave oven to condense the vapours and the products are collected in glassware. There is a system of water reflux to reflux back the excess water to restore the in-situ water to the sample. Collection of the essential oil can be separated from the water simply by decantation.

The solvent-free microwave extraction method significantly involved shorter extraction time compared with the conventional distillation method such as hydrodistillation and steam distillation. The method is substantially energy saving and considered as a new green technique in which it do not involved any solvent. The solvent-free microwave extraction of *Origanum vulgare* L. (*Bayramoglu et al., 2008)* was found to be reducing the time significantly and higher essential oil yields can be obtained at higher power levels of SFME.

Table 2.6: Extraction of essential oil from aromatic herbs (*Lucchesi et al., 2004*)

	NEOS (SFME)		Hydro Distillation	
	Time (minutes)	*Yield (%)*	*Time (minutes)*	*Yield (%)*
Basil *(Ocimum Basilicum L.)*	30	0,029	270	0,028
Garden mint *(Mentha Crispa L.)*	30	0,095	250	0,095
Thyme *(Thymus Vulgaris L.)*	30	0,160	250	0,161

Recently, an improved method of solvent-free microwave extraction of essential oil from dried *Cuminum cyminum L*. and *Zanthoxylum bungeanum Maxim* (*Wang et al., 2006*) has been introduced. In this method, a kind of microwave absorption solid medium, such as carbonyl iron powders (CIP) was added and mixed with the sample. Improved solvent-free microwave extraction offered the advantage on even shorter time of extraction time compared to the conventional SFME at the same power levels. In addition, there need no pretreatment on the sample of improved SFME.

In the conventional SFME, the heating of in-situ water in the sample can be carried out because the fresh sample contains water that can absorb microwave. There is not easy to preserve the fresh plant material and therefore the plant materials we obtained usually are dried. There is no much water inside the sample available for absorb the microwave. Thus, adding of CIP on the dried sample can simplified the sample preparation and the extraction process becomes simple and rapid.

2.7.4 Microwave Hydrodiffusion and Gravity (MHG)

Microwave hydrodiffusion and gravity is a new and green technique in which it combine microwave heating technique and application of earth gravity at atmospheric pressure to extract essential oils. In microwave hydrodiffusion and gravity method, there is no any solvent or water used in which it is much more similar to SFME. The application of earth gravity in this method gives an advantage on separation of essential from other component.

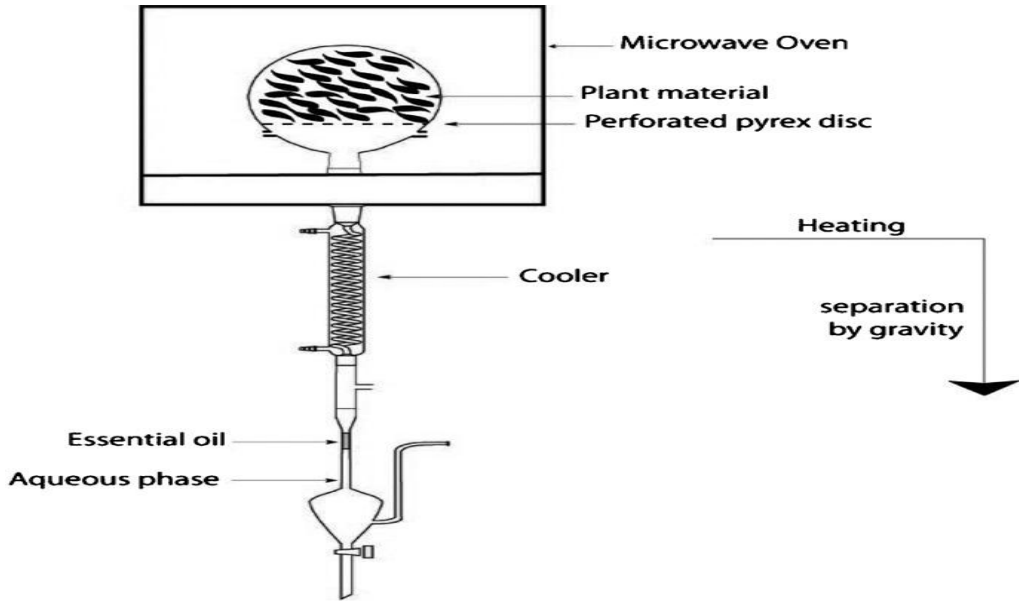


Figure 2.13: Microwave hydrodiffusion and gravity (*Vian et al., 2008*)

Microwave hydrodiffusion and gravity method based on a relatively simple principle. The sample is placed in perforated pyrex disc without mixing with any solvent or water. The direct interaction of microwaves with biological water caused the steam produced from the water present in the fresh plant material in which favours the release of essential oils trapped inside the cells of plant tissues (*Bousbia et al., 2009*). The mixture of essential oils and steam is therefore diffuse out from the sample and move downwards naturally by earth gravity to the spiral condenser outside the microwave oven where it start to condense collected in a vessel. The essential oils that lighter than the water will floats on the top of water and this makes the separation process become easier.

In microwave hydrodiffusion and gravity method, it should be noted that there are no distillation and evaporation process which are time-consuming. The energy cost is lower in MHG compare with conventional hydrodistillation renders this technology being more environmentally friendly than hydrodistillation.

2.7.5 Microwave-Assisted Hydrodistillation (MAHD)

In an attempt to apply the microwave heating in the conventional hydrodistillation, microwave-assisted hydrodistillation has recently been attended for the extraction of essential oil. There were a few studies conducted on the comparison between conventional hydrodistillation and microwave-assisted distillation method. For example, the extraction of essential oils from *Thymus vulgaris L* (*Golmakani & Rezaei, 2008*) and *Pimpinella anisum L* (*Kurkcuoglu et al., n.d.*) were carried out to compare both of the method.

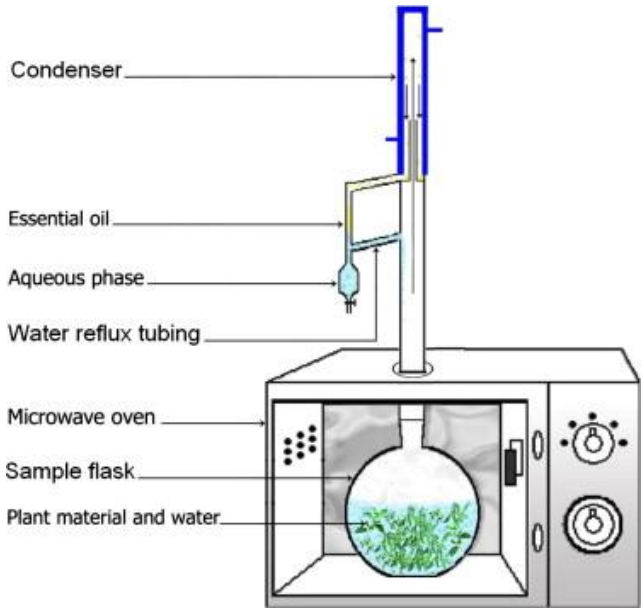


Figure 2.14: Microwave-assisted Hydrodistillation apparatus (*Golmakani & Rezaei, 2008*)

In the microwave hydrodistillation, a modified microwave oven used as a device to provide the microwave energy as heating source. A Clevenger apparatus placed in the modified microwave oven and the plant materials is placed in the sample flask. During distillation time, temperature, pressure and power were monitored and controlled.

The comparison for both the method will be in terms of extraction time, extraction yield, chemical composition, quality of the essential oils and cost of operation. In microwave-assisted hydrodistillation, the extraction time was shorter than the conventional hydrodistillation and the sample reach boiling point faster because effect of the volumetrically heating by the microwave energy. The extraction of essential oils started when the sample reached its boiling point.

From the results of the previous study, there is no significant different in the essential oils yield obtained by both of the method. In the study of extraction of essential oils from *Satureja hortensis* and *Satureja Montana*, the extraction yield obtained by microwave-assisted hydrodistillation and conventional hydrodistillation was almost equal (*Rezvanpanah et al., 2008*).

2.8 Gas Chromatography-Mass Spectrometer (GC-MS)

Gas chromatography coupled to mass spectrometer is a versatile tool to separate, quantify and identify unknown volatile organic compounds. It is normally use for determination of molecular weight and elemental compositions of unknown organic compounds in complex mixture (*Hites, n.d.*). In addition, the structure of unknown organic compounds can be determined by matching their spectra with the reference spectra.

Gas chromatography is used to separate volatile compounds in a mixture. The separated compounds can then be identified and quantified. There are three steps in a GC-MS system, injection, separation and detection.

In injection part, the sample injected depending on its property (gas, liquid or solid). Compounds in a mixture need to be volatized or extracted from the matrix for the injection. There are several injection techniques such as thermal desorption, split, headspace and solid phase micro extraction (SPME).

After the injection of mixture, separation is achieved in a capillary column. The column is coated with a fluid or solid support as the stationary phase. The mobile phase is an inert gas in which it flowing through the column. Compounds travel with different velocities through the column depending on the phase equilibrium between the stationary and mobile phase. As a result, the mixture is being separated and the individual compounds reach the detector at a different retention time. A wide range of compounds can be separated if we choosing a column which separates on boiling point, polarity, size or stereochemistry.

The detector used is mass spectrometer in which compounds enter the ion source and are ionized and fragmented by using a high-energy electron bombardment. The ions are extracted from the source with an electric field and fed into the mass analyzer. By applying electric fields, ions with a certain mass to charge ratio can reach the electron multiplier. The fragmentation pattern measured is characteristic for each molecule making identification possible.

There are limitations for using GC-MS. The GC-MS is applicable only for the compounds with vapor pressures exceeding 10^{-10} torr (*Hites , n.d.*). Compounds with lower pressures but are chemically derivatized can be analyzed using GC-MS. The isomeric compounds such as naphthalene versus azulene can not be identified using

GC-MS. However, the chromatography analysis on such isomeric compounds is possible.

The common application of GC-MS is the identification and quantification of pollutants in drinking and waste water. It is also used in the quantification of drugs and their metabolites in blood and urine.

CHAPTER 3

MATERIALS AND METHODS

3.1 Plant Materials

3.1.1 Lemongrass

Lemongrass (*Cymbopogon Citratus)* leaves was obtained from the residents around Gambang housing area in Kuantan, Pahang. The leaves were first chopped into small pieces to increase the surface area expose for distillation. In the process extraction of essential oil, the rate of extraction increase when the surface area of materials increased. The plant materials were then kept in a dark and cold refrigerator until it used for the experiments.

During the experiments, the small pieces lemongrass leaves was mixed with the water in the ratio of 1:10, in which 50 grams of lemongrass leaves was mixed with 500 mL of water in a 1 liter round bottom flask.

3.1.2 Star Anise

Star anise was purchased from the supplier in Kuantan, Pahang. The star anise purchased was in the powder form in which the supplier grinded it and sold it in prices per kilo. The powdered star anise was then kept in a dark and dry plastic box for the experiment purpose.

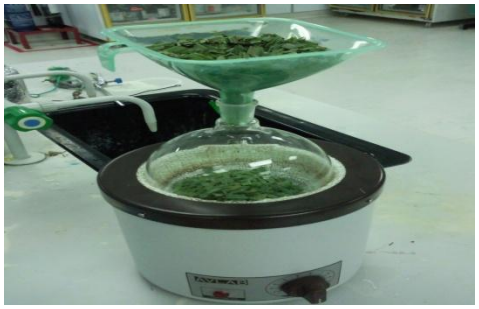
During the experiments, the powder of star anise was mixed with water in the ration of 1:10, in which 40 gram of star anise powder was mixed with 400 mL water in a 1 liter round bottom flask.

3.2 Conventional Hydrodistillation

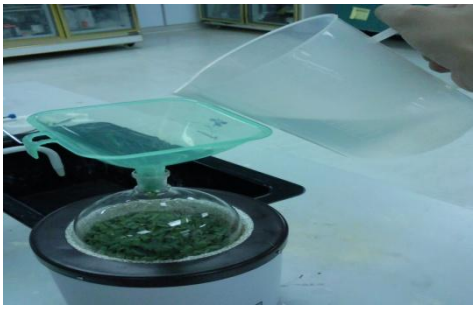
The conventional method for extraction of essential oil is hydrodistillation in which heating of a mixture of water and plant materials to achieve boiling point followed by the liquefaction of the vapors in the condenser to get the essential oils. Fifty grams of lemongrass leaves and forty grams of star anise powder in the ratio of 1:10 with water were submitted to a Clevenger apparatus for distillation as in Figure 3.1. A heating mantle model of AV-HM-04 (1000 mL capacity, 230 volts, 300 watts) was used as a heat source.

After the mixture of water and plant materials achieved boiling point, the time of extraction started when there was first drop of condensed vapors. The sample collection interval was 30 minutes in which sample were collected at 30 minutes, 60 minutes, 90 minutes and 120 minutes time of extraction. The condensate which consists of mixture of water and essential oil was collected in a vial with the water

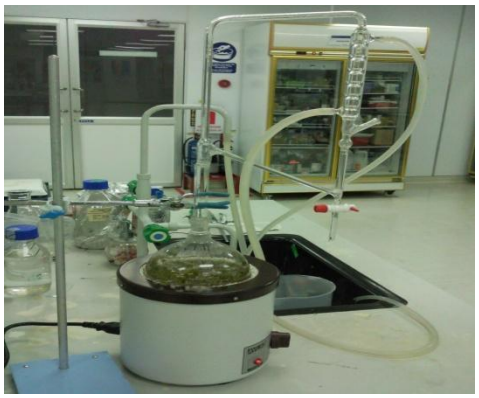
discarded. The extracted essential oils is dried over anhydrous sodium sulfate, weighed and stored in amber vials at 4℃ for the use of analysis.



1. 50 grams of lemongrass leaves put into 1L round bottom flask.

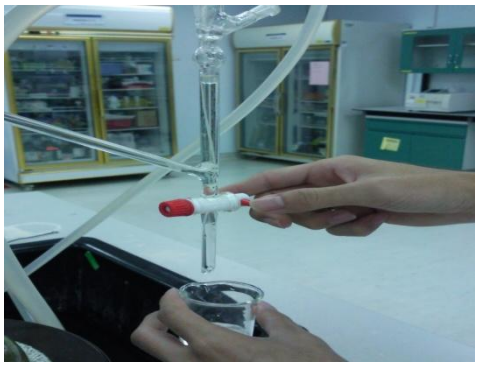


2. 500 mL of water was added to the round bottom flask.

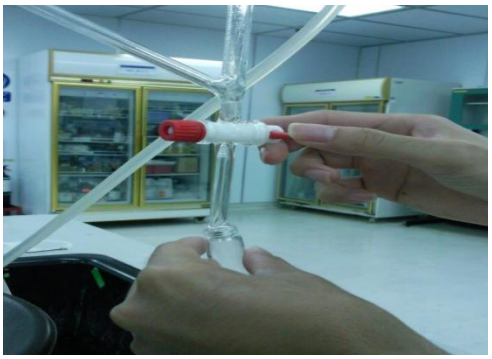


3. Apparatus setup for conventional hydrodistillation.

4. Condensate consists of mixture of water and lemongrass essential oil.

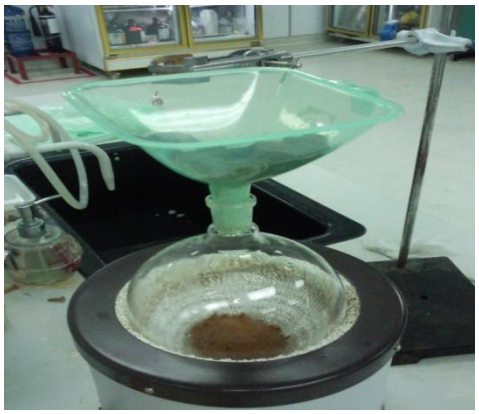


5. Water discarded from the condensate.

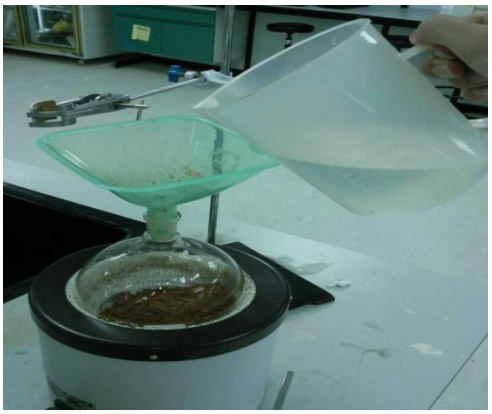


6. Sample collected at 30 minutes interval, dried, weighed and stored at 4℃ in vial

Figure 3.1: Flow process of Lemongrass Hydrodistillation

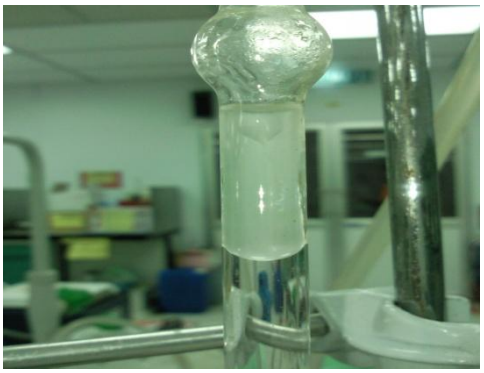


1. 40 grams of star anise powder put into 1L round bottom flask.



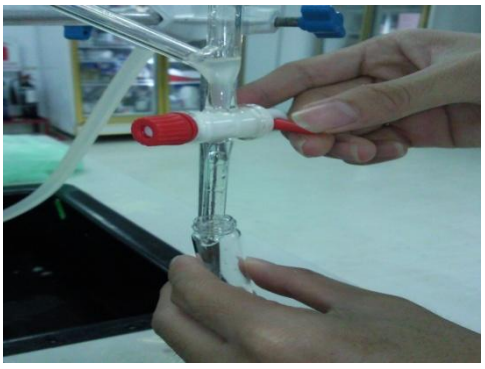
2. 400 mL of water was added to the round bottom flask.

3. Apparatus setup for conventional hydrodistillation.



4. Condensate consists of mixture of water and star anise essential oil.

5. Water discarded from the condensate.



6. Sample collected at 30 minutes interval, dried, weighed and stored at 4℃ in vial.

Figure 3.2: Flow process of Star Anise Hydrodistillation

3.3 Microwave-Assisted Hydrodistillation

In microwave-assisted hydrodistillation, a domestic microwave oven (Samsung, 250v-50Hz, maximum: 800 watts) was modified for the distillation. A round bottom flask is set up within the microwave oven cavity. Fifty grams of lemongrass leaves and forty grams of star anise powder were placed in the flask containing water in the ratio of 1:10. A hydrodistillation set was used (outside the oven) to collect the extracted essential oils.

Microwave powers of 200 W were used in the experiments. After the mixture of water and plant materials achieved boiling point, the time of extraction started when there was first drop of condensed vapors. The sample collection interval was 30 minutes in which sample were collected at 30 minutes, 60 minutes, 90 minutes and 120 minutes time of extraction. The condensate which consists of mixture of water and essential oil was collected in a vial with the water discarded. The extracted essential oils is dried over anhydrous sodium sulfate, weighed and stored in amber vials at 4℃ for the use of analysis.

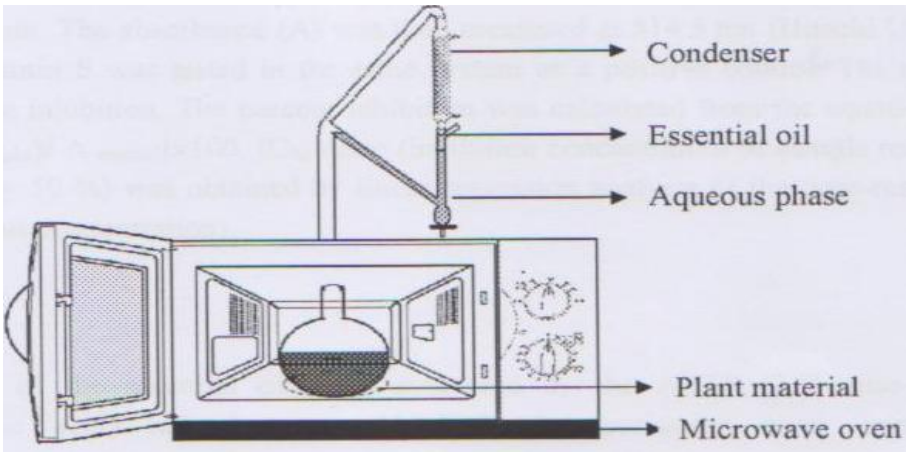
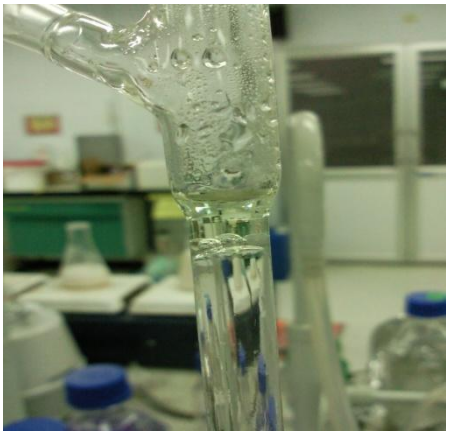


Figure 3.3: Microwave-Assisted Hydrodistillation method

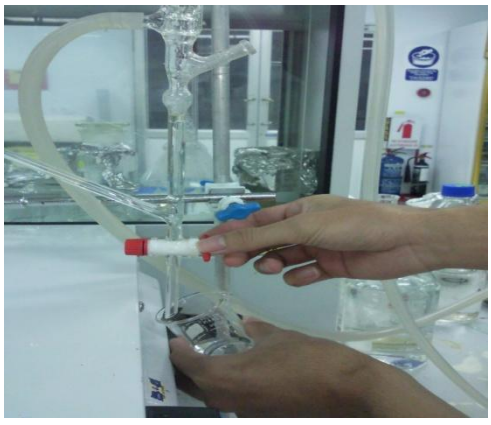
1. 50 grams of lemongrass leaves put into 1L round bottom flask that set inside microwave oven cavity.

2. 500 mL water was added to the round bottom flask.

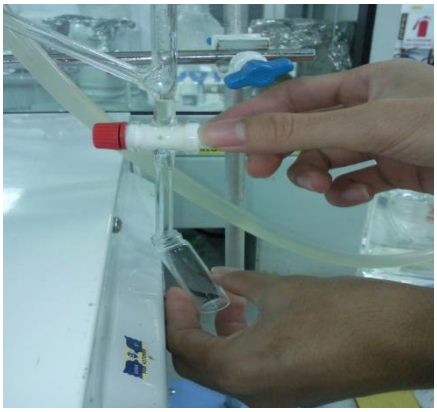
3. Apparatus setup for microwave-assisted hydrodistillation.



5. Condensate consists of mixture of water and lemongrass essential oil.



5. Water discarded from the condensate.



6. Sample collected at 30 minutes interval, dried, weighed and stored at 4 ℃ in vial.

Figure 3.4: Flow process of Lemongrass Microwave-Assisted Hydrodistillation

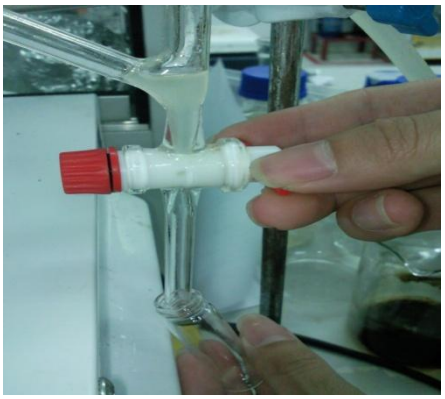
1. 40 grams of star anise powder put into 1L round bottom flask that set inside microwave oven cavity.

2. 400 mL water was added to the round bottom flask.

3. Apparatus setup for microwave-assisted hydrodistillation.

5. Condensate consists of mixture of water and star anise essential oil.

5. Water discarded from the condensate.



6. Sample collected at 30 minutes interval, dried, weighed and stored at 4 ℃ in vial.

Figure 3.5: Flow process of Star Anise Microwave-Assisted Hydrodistillation

3.4 Gas Chromatography-Mass Spectrometer (GC-MS)

A GC-MS instrument equipped with a mass selective detector operating in the electron impact mode is used to study the compositions of the extracted essential oils. The GC part is equipped with a capillary column. GC-MS analyses were performed using an Agilent 5975C Series GC/MSD and a DB-WAX fused silica column (30m x 0.25mm x 0.25μm film thickness). Temperature-programming of the oven included an initial hold at 50℃ for 10 minutes and a rise to 230℃ at 3 ℃ per minutes.

The essential oil for both lemongrass and star anise from HD and MAHD were first filtered using syringes and 0.25μm PFTE filters. Both the samples for lemongrass and star anise were then diluted with dichloromethane to a concentration of 2% by adding 20μL of pure essential oil to 980μL of dichloromethane to prepare 1mL samples. All the diluted samples were then put on the vortex mixer for 2 minutes to make the samples in homogeneous form.

A volume of 1.0 μL was injected to the GC with the injector in the same split ratio. Carrier gas, He, is adjusted to a linear velocity of 1.0 ml min^{-1}. The compounds of the extracted essential oils are identified by comparing their mass spectral fragmentation patterns with those of similar compounds from a database. For each compound on the chromatogram, the percentage of peak area relative to the total peak areas from all compounds is determined and reported as relative amount of that compound.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results Overview

In this research, experiments were carried out using Hydrodistillation and Microwave-Assisted Hydrodistillation method on the extraction of essential oil from lemongrass and star anise in order to identify the effect of variation of extraction parameters such as extraction time, extraction yield/efficiency and chemical composition of the essential oil. These findings will give some important information on the best technique application in the natural product extraction.

4.2 Extraction Time and Yield

Extraction of lemongrass and star anise essential oil using hydrodistillation and microwave-assisted hydrodistillation methods were done to study the effect of extraction time on the yield of the lemongrass and star anise essential oil. The yield of essential oil was constructing by measure the weight of essential oil from the

extraction per weight of lemongrass leaves and star anise powder, in which 50 grams of lemongrass and 40 grams of star anise powder was used. The extraction time started when the mixture of water and plant material in the ratio of 1:10 achieved boiling point and first drop of condensed vapor was obtained.

Extractions of essential oil with MAHD at 200 W took 25 minutes for lemongrass leaves and 20 minutes for star anise to achieve boiling point. On the other hand, HD method took 45 minutes for lemongrass leaves and 40 minutes for star anise mixtures to achieve boiling point. The longer time taken for the mixture in HD method to achieve boiling point is due to the fact that heat transfer in HD method occurred through conduction and convection phenomenon. Thus, the heating is depend on thermal conductivity and heat transfer from heating medium to the interior of the sample which eventually resulted slower increase in temperature. In MAHD method, heat transfer through conduction, convection and irradiation. The microwaves are volumetrically distributed and heat is originated through molecular motion. As a result, the mixture achieves boiling time faster.

From the results of Table 4.1, the extraction yield for lemongrass essential oil was 0.3754 % for HD method and the yield for MAHD method is higher which was 0.4678 %. At the time of 60 minutes, the yield for HD method was 0.4736 % and the yield for MAHD method increased to 0.549 %. The yield at time of 90 minutes was 0.517 % for HD method and 0.5664 % for MAHD method. Lastly, the highest yield of essential oil obtained from HD method was 0.5518 % while the highest yield of MAHD method was 0.593 %. The optimum yield for MAHD is at 60 minutes which was 0.549% and yield for HD is optimum at 120 minutes which was 0.5518%.

From the results of Table 4.2, the extraction yield for star anise essential oil obtained was 1.3095 % for HD method and 1.8948 % for MAHD method. This yield was increase to 2.1433 % and 2.5855 % for HD and MAHD method at time of 60 minutes. At time of 90 minutes, the yield for HD method was 2.7955 % and 3.301 % for MAHD method. Lastly, the highest yield for both method occurred at 120

minutes in which 3.1368 % for HD method and 3.5345 % for MAHD method. The optimum yield for MAHD is at 90 minutes which was 3.301 % and yield for HD is optimum at 120 minutes which was 3.1368 %.

Observation on the results of extraction time and extraction yield showed that the longer the extraction time, the higher the yield of essential oil for HD and MAHD method on both lemongrass and star anise. Other than that, the yield of essential oil obtained from MAHD method is higher than HD method for both lemongrass and star anise. The difference in yield of essential oil for both methods is significant. The increase in yield of essential oil for both lemongrass and star anise slowly reduced when the time of extraction is longer. This is because the amount of essential oils in 50 grams of lemongrass leaves and 40 grams of star anise powder is limited. As a result, the increase in the yield will become slower when the time is longer.

The difference in the yield of essential oil for both the HD and MAHD methods is largely relate to the way of heat transfer in both methods. In HD method, there was only two ways heat transfer and heat may lose to the environment. In MAHD method, heat was transferred in three ways. The efficiency of microwaves heating depends strongly on the dielectric constant of the solvent. Solvent with high dielectric constants can absorb more microwave energy. The volumetrically heating of microwaves will cause the external cell walls of the sample break apart. When the pressure build-up within the glands reached certain level or exceeded their capacity for expansion, the cell walls will rupture more rapidly than in HD because the gland undergo more severe thermal stresses and pressures.

Table 4.1: Essential oil yield of Lemongrass for HD and MAHD method

Extraction Time (Min)	Lemongrass							
	HD				MAHD			
	Wt of Vial	Wt of Vial + Oil	Wt of oil	Yield %	Wt of Vial	Wt of Vial + Oil	Wt of oil	Yield %
30	7.4826	7.6703	0.1877	0.3754	10.8034	11.0373	0.2339	0.4678
60	7.4575	7.6943	0.2368	0.4736	11.2798	11.5543	0.2745	0.549
90	7.5395	7.7980	0.2585	0.517	7.5408	7.8420	0.2832	0.5664
120	7.4683	7.7442	0.2759	0.5518	15.4832	15.7797	0.2965	0.593

Table 4.2: Essential oil yield of Star Anise for HD and MAHD method

Extraction Time (Min)	Lemongrass							
	HD				MAHD			
	Wt of Vial	Wt of Vial + Oil	Wt of oil	Yield %	Wt of Vial	Wt of Vial + Oil	Wt of oil	Yield %
30	7.5938	8.1176	0.5238	1.3095	8.1643	8.9222	0.7579	1.8948
60	7.3828	8.2401	0.8573	2.1433	7.4096	8.4438	1.0342	2.5855
90	7.5258	8.6440	1.1182	2.7955	7.5264	8.8468	1.3204	3.301
120	7.5125	8.7672	1.2547	3.1368	7.4882	8.9020	1.4138	3.5345

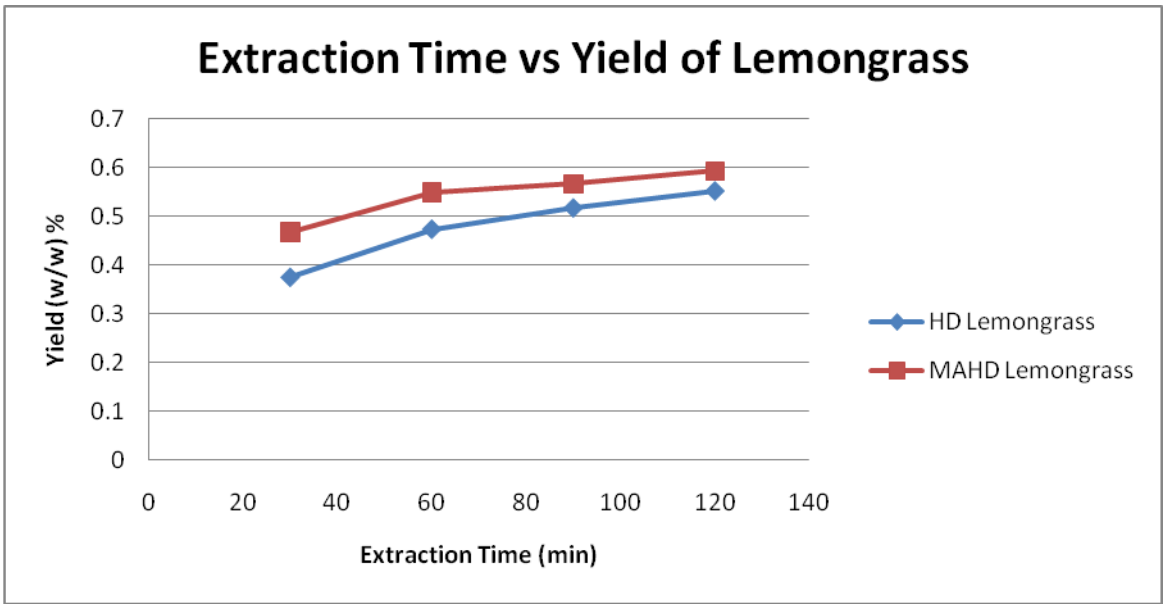


Figure 4.1: Yield profile as a function of extraction time for HD and MAHD of essential oil from Lemongrass.

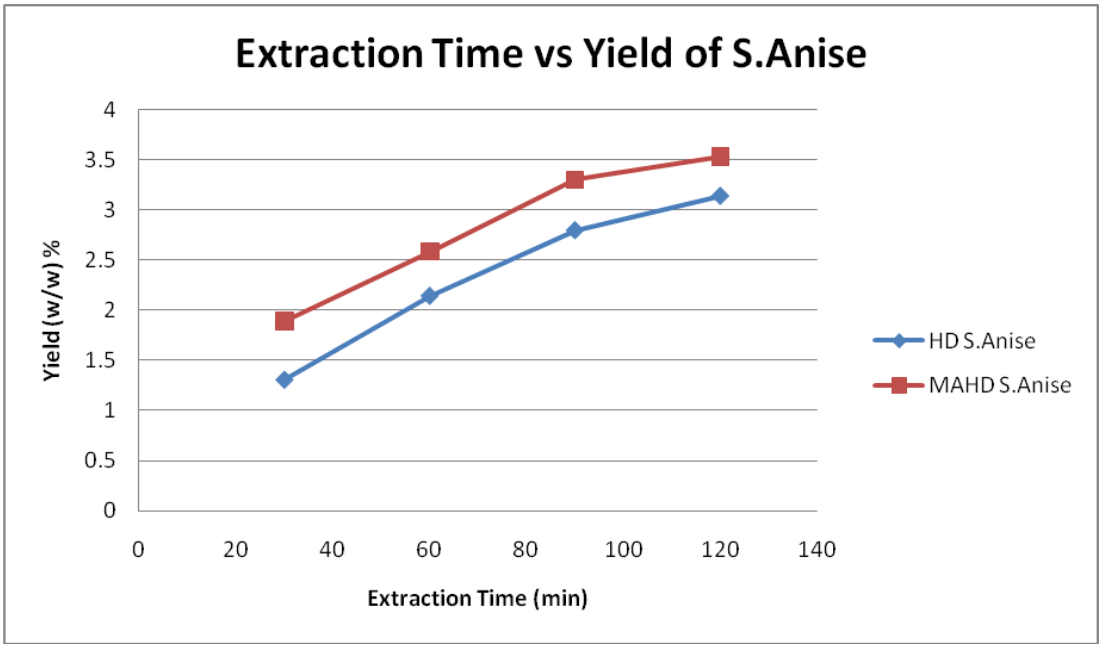


Figure 4.2: Yield profile as a function of extraction time for HD and MAHD of essential oil from Star Anise.

4.3 Analysis of Essential Oils by GC-MS

In the analysis of GC-MS on Lemongrass and Star Anise, there were total 67 components detected in essential oils of Lemongrass and 27 components detected in the essential oils of Star Anise. In the essential oil of Lemongrass, the main components obtained from GC-MS analysis were Citral, (Z)-Citral, β-Myrcene, β-Thujene, Linalool, Geranic Acid, Geranyl Acetate, and Neric Acid. Citral and (Z)-Citral were the dominant components in the essential of lemongrass.

In the essential oil of Star Anise, the main components obtained were Anethole, Estragole, Para-Anisaldehyde, α-terpineol, Eugenol, Linalool, (D)-Limonene, β-Caryophyllene, and α-Bergamotene. Anethole and Estragole were the dominant components in the essential oil of star anise.

4.3.1 GC-MS Analysis of Lemongrass Essential Oil

4.3.1.1 Analysis of Lemongrass Essential Oil at 30 Minutes

At 30 minutes conventional hydrodistillation of lemongrass leaves, there were total 11 components obtained from the analysis as listed in the Table 4.3. The main components for the essential oil at this extraction time were Citral (47.15%), (Z)-Citral (35.40%), and β-Myrcene (11.62%).

For MAHD, there were 19 components obtained from the result of analysis. The main components for the essential oil at this extraction time were Citral (46.95%), (Z)-Citral (34.20%), β-Thujene (6.80%), Geranic acid (1.74%), 3-propyl-Cyclohexene (1.58%), and n-Heptadecylcyclohexane (1.21%).

4.3.1.2 Analysis of Lemongrass Essential Oil at 60 Minutes

In the conventional hydrodistillation of lemongrass for 60 minutes, there were 21 components obtained from the result analysis. The main components for the essential oil at this extraction time were Citral (46.15%), (Z)-Citral (32.92%), β-Thujene (6.80%), and Vinylcyclohexane (1.41% and 1.12%).

At 60 minutes MAHD of lemongrass, there were total 29 components found in the essential oil of lemongrass. The main constituents consist of Citral (44.24%), (Z)-Citral (32.96%), β-Thujene (9.44%), Geranic acid (1.53%), and Vinylcyclohexane (1.22%).

4.3.1.3 Analysis of Lemongrass Essential Oil at 90 Minutes

Conventional hydrodistillation of lemongrass for 90 minutes showed that there were 16 components found in the essential oil. Among the main constituents for the essential oil were Citral (43.79%), (Z)-Citral (28.86%), 2,3-dimethyl-3-Buten-2-ol (6.20%), Geranic acid (4.51%), β-Phellandrene (4.06%), n-Pentadecylcyclohexane (3.79%), (-)-Lavandulol (2.30%), and Neric acid (1.61%).

For 90 minutes MAHD of lemongrass, there were also 16 components found in the analysis of essential oil. The main constituents were Citral (48.16%), (Z)-Citral (34.64%), β-Thujene (7.45%), Geranyl acetate (1.56%), Glycine, furan-2-yl-methyl ester (1.45%), and Geranic acid (1.05%).

4.3.1.4 Analysis of Lemongrass Essential Oil at 120 Minutes

At time of 120 minutes conventional hydrodistillation of lemongrass, the results obtained showed that only 9 components found in the essential oil. Among the main components were Citral (50.06%), (Z)-Citral (32.67%), β-Thujene (9.11%), and Geraniol (3.57%).

At 120 minutes MAHD of lemongrass, there were total 13 components found in the essential oil. The main constituents of the essential oil were Citral (50.45%), (Z)-Citral (33.67%), β-Thujene (9.37%), Vinylcyclooctane (1.34%), and Geranyl acetate (0.99%).

4.3.2 GC-MS Analysis of Star Anise Essential Oil

4.3.2.1 Analysis of Star Anise Essential Oil at 30 Minutes

At 30 minutes HD of star anise, there were total 13 components found in the essential oil. Among the components of the essential oil were Anethole (91.37% and 0.97%), Estragole (3.43%), Gingerol (1.01%), Para-Anisaldehyde (0.67%), (D)-Limonene (0.57%) and Linalool (0.47%).

There were 10 components found in the analysis of star anise essential oil at 30 minutes MAHD. The main constituents were Anethole (91.36% and 0.78%), Estragole (3.12%), Thymol (2.32%), Para-Anisaldehyde (0.76%), Foeniculin (ether) (0.58%), and Linalyl anthranilate (0.45%).

4.3.2.2 Analysis of Star Anise Essential Oil at 60 Minutes

At 60 minutes HD on star anise, the results of analysis showed that 14 components were found from the essential oil. Among the components were Anethole (88.08% and 1.02%), 1-Oxaspiro[2.5] oct-5-ene, 8,8-dimethyl-4-methylene (4.78%), Estragole (3.27%), (D)-Limonene (0.65%), Para-Anisaldehyde (0.76%), and Linalyl acetate (0.65%).

The results obtained from analysis of essential oil using MAHD at 60 minutes showed that there were total 11 components found in the essential oil. There components were Anethole (91.79% and 1.13 %), Estragole (3.32%), Para-Anisaldehyde (1.16%), Foeniculin (ether) (0.95%), and Linalyl anthranilate (0.47%).

4.3.2.3 Analysis of Star Anise Essential Oil at 90 Minutes

At 90 minutes HD of star anise, there were total 13 components found in the results of analysis. The main components of essential oil at this timing were Anethole (91.48% and 1.04 %), Estragole (3.27%), Para-Anisaldehyde (0.86%), (D)-Limonene (0.73%), Nutty quinoxaline (0.72%), and Linalyl anthranilate (0.42%).

MAHD of star anise essential oil for 90 minutes showed that there were total 14 components found in the essential oil. Among the constituents were Anethole (86.53% and 0.92%), 2-methoxy-benzeneacetaldehyde (5.13%), Estragole (2.80%), Nutty quinoxaline (1.40%), Para-Anisaldehyde (0.86%), and Eugenol (0.48%).

4.3.2.4 Analysis of Star Anise Essential Oil at 120 Minutes

At 120 minutes HD of star anise, the results of analysis showed that 15 components were found in the essential oil. These components were Anethole (89.81% and 1.05 %), Estragole (3.02%), Pyridine,4-(4-methyl-5-trans-phenyl-1,3-oxazolidin-2-yl) (1.79%), Para-Anisaldehyde (1.20%), (D)-Limonene (0.62%), Eugenol (0.46%), Linalool (0.40%).

The results obtained from analysis of essential oil using MAHD at 120 minutes showed that there were total 14 components found in the essential oil. Among the components were Anethole (90.21% and 0.98 %), Estragole (3.01%), Nutty quinoxaline (1.60%), Para-Anisaldehyde (1.48%), Chavibetol (0.63%), (D)-Limonene (0.73%), and Linalool (0.39%).

4.3.3 Comparison on Composition of Essential Oil for HD and MAHD

Essential oil is a group of natural organic compounds that are predominantly composed of terpenes (hydrocarbons) and terpenoids (oxygen containing hydrocarbons). Analysis of chromatogram by GC-MS showed that the components extracted from both the lemongrass and star anise were mainly monoterpenes (10 carbons) and sesquiterpenes (15 carbons). Both HD and MAHD methods gave the same main components on essential oil of lemongrass and star anise. There were some minor components that found on HD method but not on MAHD method and vice versa. Observation on the peak area of the chromatogram showed that there were slightly fewer compounds presents in essential oil that extracted by HD compared with those extracted by MAHD method. The observation showed that MAHD is more efficient in the extraction of essential oil.

Some new compounds were found from extraction at certain timing and some compounds were loss during the extraction process. This is because reduction amount of water content with additional time that finally affect reduction of the degradation of compounds by hydrolysis, trans-esterification or oxidation. Thus, there was fewer degradation production noted in the analysis (Lucchesi et al., 2004).

4.3.3.1 Comparison on Lemongrass Essential Oil Composition

From the results of GC-MS analysis of essential oil on lemongrass, both HD and MAHD methods gave the same main components. The main components of lemongrass essential oil were Citral, (Z)-Citral, β-Myrcene, β-Thujene, Linalool, Geranic Acid, Geranyl Acetate, and Neric Acid.

As listed in the table 4.3, there were some compounds found in HD extraction of lemongrass essential oil that not occurred in MAHD method and vice versa. Among the compounds that presents in the HD method and not occurred in the MAHD method were compound (No 9) Cyclofenchene, (No 13) (E,Z)-2,6-nonadien-1-ol, (No 15) 4,6-dimethyl-2-Pyrimidinamine, (No 17) 3,3-dimethyl-1,5-Heptadiene, (No 20) (R)-Citronellal, (No 23) Cis-Verbanol, (No 31) (-)-Lavandulol, (No 32) Tetrahydromyrcemol, (No 33) Epoxy-linalool oxide, (No 36) Acetic acid, cyanohydroxyimino-,ethyl ester, (No 39) n-Pentadecylcyclohexane, (No 40) 13-Heptadecyn-1-ol, (No 41) Methanamine, N-[3-methyl-2-butenylidene], (No 45) Cyclohexene,3-(1-methylethyl)-, (No 48) 2,3-dimethyl-3-Buten-2-ol, (No 49) Geraniol, (No 57) 4-Acetylocta-1,2-diene, (No 62) 1,2,4,8-Tetramethylbicyclo[6.3.0] undeca-2,4-diene, (No 64) 3,4 -dihydro-6-methyl-2H-Pyran, and (No 67) N-(E-2-buten-1-one-1-yl)-Succinimide.

Among the compounds that presents in the MAHD method and not occurred in the HD method were compound (No 8) 1,2,3,3a,4,6a-Hexahydropentalene, (No 11) 1,2-Dihydropyridine, 1-(1-oxobutyl)-, (No 12) 1-methylpyrazole, (No 14) 1,1-Dimethyl-2-(1-methyl-2-propenyl)cyclopropane, (No 16) Trans-Chrysanthemal, (No 18) 1,5-Heptadiene, 2,6-dimethyl-, (No 19) 6-methyl-1,5-heptadiene, (No 21) Bicyclo[2.2.2] octane, (No 22) Citronellal, (No 24) Vinylcyclooctane, (No 26) Bicyclo[4.1.0]heptane, 3-methyl-, (No 27) Isopulegone, (No 28) (1R)-(+)-Neocarvomenthol, (No 34) (E)-1-(1-methoxyethoxy)-3-hexene, (No 35) Acetic acid, 2-acetoxymethyl-1,2,3-trimethylbutyl ester, (No 37) 1,3-Propanediol, 2,2-diethyl-, (No 38) n-Heptadecylcyclohexane, (No 43) Nerol, (No 44) (E)-isoeugenol, (No 46) Cyclohexene, 3-propyl-, (No 52) Geranyl Acetone, (No 53) Methyl-methacrylate, (No 54) (E)-2-Tetradecene, (No 55) Glycine, furan-2-yl-methyl ester, (No 56) Bicyclo[5.3.1] undecan-11-one, (No 58) β-Caryophyllene, (No 59) β-Caryophyllene oxide, (No 61) Selina-6-en-4-ol, (No 63) 3-Ethoxy-5-methyl-1H-pyrazole, (No 65) 2-buten-1-one, 1-(2,2,5a-trimethyl perhydro-1-benzoxiren-1-yl, and (No 66) 2-Methyl 6-methylene 2-octene.

At 30 minutes of MAHD, there was β-Thujene present in the essential oil and this compounds presents in essential oil of HD at the time of 60 minutes. Besides that, compound (No 7) Bicyclo[3.1.0]hexane, 6-methylene- present in essential oil of MAHD at 30 minutes and it only present in essential oil of HD at time of 60 minutes. The same ouucred for The compound (No 10) Linalool and compound (No 42) Neric acid in which they present in essential oil of MAHD at 30 minutes and only present in essential oil of HD at time of 60 minutes. Lastly, compound (No 60) 1-(3,7-Dimethyl-1-octenyl) cyclopropanol present in essential oil of MAHD at time of 30 minutes but only present in essential oil of HD at time of 90 minutes. From the analysis of results, it is clear that extraction of essential oil using MAHD method is more efficient in terms of compounds extracted.

Table 4.3: Chemical composition of Lemongrass essential oil

NO	Component	30 Min				60 Min				90 Min				120 Min			
		HD		MAHD		HD		MAHD		HD		MAHD		HD		MAHD	
		RT	Area%	RT	Area%	RT	Area%	RT	Area%	RT	Area%	RT	Area%	RT	Area%	RT	Area%
1	5-Hepten-2-one, 6-methyl-	-	-	-	-	10.269	0.37	10.280	0.64	-	-	-	-	-	-	-	-
2	β-Phellandrene	-	-	-	-	-	-	-	-	11.775	4.06	-	-	-	-	-	-
3	β-Myrcene	11.808	11.62	-	-	-	-	18.975	0.71	-	-	-	-	-	-	-	-
4	β-Thujene	-	-	11.797	6.80	11.861	12.22	11.872	9.44	-	-	11.807	7.45	11.786	9.11	11.829	9.37
5	(E)-β-Ocimene	-	-	-	-	16.273	0.50	16.283	0.36	-	-	-	-	-	-	-	-
6	(Z)-β-Ocimene	-	-	-	-	16.914	0.24	-	-	-	-	-	-	-	-	-	-
7	Bicyclo[3.1.0]hexane, 6-methylene-	-	-	18.623	0.46	18.623	0.41	-	-	18.623	0.75	18.622	0.45	-	-	-	-
8	1,2,3,3a,4,6a-Hexahydropentalene	-	-	-	-	-	-	18.623	0.46	-	-	-	-	-	-	-	-
9	Cyclofenchene	18.975	0.61	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Linalool	-	-	18.975	0.66	18.975	0.61		-	-	-					18.975	0.59
11	1,2-Dihydropyridine, 1-(1-oxobutyl)-	-	-	-	-	-	-	-	-	-	-	18.975	0.71	-	-	-	-
12	1-methylpyrazole	-	-	-	-	-	-	19.189	0.24	-	-	-	-	-	-	-	-
13	(E,Z)-2,6-nonadien-1-ol	-	-	-	-	19.402	0.18	-	-	-	-	-	-	-	-	-	-

14	1,1-Dimethyl-2-(1-methyl-2-propenyl)cyclopropane	-	-	-	-	-	-	19.413	0.27	-	-	-	-	-	-	-	-
15	4,6-dimethyl-2-Pyrimidinamine	-	-	-	-	20.001	0.25	-	-	-	-	-	-	-	-	-	-
16	Trans-Chrysanthemal	-	-	-	-	-	-	20.011	0.24	-	-	-	-	-	-	20.011	0.14
17	3,3-dimethyl-1,5-Heptadiene	-	-	-	-	20.054	0.35	-	-	-	-	-	-	-	-	-	-
18	1,5-Heptadiene, 2,6-dimethyl-	-	-	-	-	-	-	20.065	0.37	-	-	-	-	-	-	-	-
19	6-methyl-1,5-heptadiene	-	-	-	-	-	-	-	-	-	-	20.065	0.31	-	-	-	-
20	(R)-Citronellal	-	-	-	-	20.289	0.17	-	-	-	-		-	-	-	-	-
21	Bicyclo[2.2.2] octane	-	-	20.567	0.66	-	-	-	-	-	-	-	-	-	-	-	-
22	Citronellal	-	-	-	-	-	-	20.300	0.22	-	-	-	-	-	-	-	-
23	Cis-Verbanol	20.567	0.78	-	-	-	-	-		-	-	-	-	-	-	-	-
24	Vinylcyclooctane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20.567	0.91
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	20.983	1.34
25	Vinylcyclohexane	-	-	-	-	20.567	1.12	20.577	0.87	-	-	20.567	0.70	20.567	0.68	-	-
		-	-	-	-	20.983	1.41	20.983	1.22	-	-	-	-	20.983	0.92	-	-
26	Bicyclo[4.1.0]heptane, 3-methyl-	-	-	-	-	-	-	-	-	-	-	20.983	1.04	-	-	-	-
27	Isopulegone	-	-	20.983	1.03	-	-	-	-	-	-	-	-	-	-	-	-

28	(1R)-(+)-Neocarvomenthol	-	-	-	-	-	-	21.539	0.19	-	-	-	-	-	-	-	-
29	(Z)-Citral	22.158	35.40	22.158	34.20	22.201	32.92	22.212	32.96	22.137	28.86	22.169	34.64	22.137	32.67	22.180	33.67
30	Citral	22.703	47.15	22.703	46.95	22.757	46.15	22.756	44.24	22.692	43.79	22.724	48.16	22.682	50.06	22.735	50.45
31	(-)-Lavandulol	-	-	-	-	-	-	-	-	22.586	2.30	-	-	-	-	-	-
32	Tetrahydromyrcemol	-	-	-	-	-	-	-	-	22.788	0.53	-	-	-	-	-	-
33	Epoxy-linalool oxide	22.799	0.81	-	-	-	-	-	-	-	-	-	-	22.788	1.10	-	-
34	(E)-1-(1-methoxyethoxy)-3-hexene	-	-	-	-	-	-	-	-	-	-	22.821	0.44	-	-	-	-
35	Acetic acid, 2-acetoxymethyl-1,2,3-trimethylbutyl ester	-	-	22.810	1.15	-	-	-	-	-	-	-	-	-	-	-	-
36	Acetic acid, cyanohydroxyimino-,ethyl ester	-	-	-	-	22.842	0.22	-	-	-	-	-	-	-	-	-	-
37	1,3-Propanediol, 2,2-diethyl-	-	-	-	-	-	-	22.853	1.05	-	-	-	-	-	-	22.821	0.38
38	n-Heptadecylcyclohexane	-	-	23.611	1.21	-	-	23.622	0.88	-	-	23.611	1.01	-	-	-	-
39	n-Pentadecylcyclohexane	-	-	-	-	-	-	-	-	23.622	3.79	-	-	-	-	-	-
40	13-Heptadecyn-1-ol	23.611	0.48	-	-	-	-	-	-	-	-	-	-	-	-	-	-

41	Methanamine, N-[3-methyl-2-butenylidene]	-	-	-	-	23.622	0.58	-	-	-	-	-	-	-	-	-	-
42	Neric Acid	-	-	23.793	0.66	23.782	0.24	23.793	0.62	23.793	1.61	23.782	0.54			23.782	0.26
		-	-	32.691	0.40	-	-	32.691	0.31	32.691	0.47			-	-	-	-
43	Nerol	-	-	-	-	-	-	24.530	0.58	-	-	-	-	-	-	-	-
44	(E)-isoeugenol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24.017	0.79
45	Cyclohexene,3-(1-methylethyl)-	24.134	0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	3-propyl-Cyclohexene	-	-	24.145	1.58	-	-	-	-	-	-	-	-	-	-	-	-
47	2,4-Dimethyl-1-hepten-4-ol	-	-	-	-	24.145	0.86	24.145	1.19	-	-	-	-	-	-	-	-
48	2,3-dimethyl-3-Buten-2-ol	-	-	-	-	-	-	-	-	24.156	6.20	-	-	-	-	-	-
49	Geraniol	-	-	-	-	-	-	-	-	-	-	-	-	22.586	3.57	-	-
50	Geranic Acid	24.231	0.76	24.273	1.74	24.252	0.50	24.327	1.53	24.327	4.51	24.263	1.05	24.220	0.91	-	-
51	Geranyl Acetate	24.519	0.52	24.519	0.82	24.519	0.55	-	-	24.519	0.82	24.519	1.56	24.519	0.97	24.519	0.99
52	Geranyl Acetone	-	-	-	-	-	-	26.698	0.13	-	-	-	-	-	-	-	-
53	Methyl-methacrylate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24.263	0.88
54	(E)-2-Tetradecene	-	-	24.914	0.37	-	-	-	-	-	-	-	-	-	-	-	-
55	Glycine, furan-2-yl-methyl ester	-	-	-	-	-	-	-	-	-	-	24.145	1.45	-	-	-	-
56	Bicyclo[5.3.1] undecan-11-one	-	-	-	-	-	-	26.143	0.19	-	-	-	-	-	-		--
57	4-Acetylocta-1,2-diene	-	-	-	-	-	-	-	-	26.687	0.39	-	-	-	-	-	-

58	β-Caryophyllene	-	-	25.235	0.39	-	-			-	-			-	-		
59	β-Caryophyllene oxide	-	-	27.200	0.30	-	-	27.200	0.22	-	-	-	-	-	-	-	-
60	1-(3,7-Dimethyl-1-octenyl) cyclopropanol	-	-	27.029	0.30	-	-	-	-	27.029	0.62	-	-	-	-	-	-
61	Selina-6-en-4-ol	-	-	-	-	-	-	27.606	0.14	-	-	27.606	0.25	-	-	27.606	0.24
62	1,2,4,8-Tetramethylbicyclo[6.3.0] undeca-2,4-diene	-	-	-	-	27.606	0.16	-	-	-	-	-	-	-	-	-	-
63	3-Ethoxy-5-methyl-1H-pyrazole	-	-	-	-	-	-	28.535	0.20	-	-	-	-	-	-	-	-
64	3,4 -dihydro-6-methyl-2H-Pyran	-	-	-	-	-	-	-	-	28.535	0.44	-	-	-	-	-	-
65	2-buten-1-one, 1-(2,2,5a-trimethyl perhydro-1-benzoxiren-1-yl	-	-	32.787	0.26	-	-	-	-	-	-	32.691	0.23	-	-	-	-
66	2-Methyl 6-methylene 2-octene	-	-	-	-	-	-	32.787	0.18	-	-	-	-	-	-	-	-
67	N-(E-2-buten-1-one-1-yl)-Succinimide	-	-	-	-	-	-	-	-	32.787	0.47	-	-	-	-	-	-

4.3.3.2 Comparison on Star Anise Essential Oil Composition

From the results of GC-MS analysis of essential oil on star anise, both HD and MAHD methods gave the same main components. the main components obtained were Anethole, Estragole, Para-Anisaldehyde, α-terpineol, Eugenol, 4-Carvomenthol, Linalool, (D)-Limonene, β-Caryophyllene, and α-Bergamotene.

As listed in the Table 4.4, among the compounds that presents in the HD method and not occurred in the MAHD method were compound (No 3) Linalyl acetate, (No 14) 1-Oxaspiro[2.5] oct-5-ene, 8,8-dimethyl-4-methylene, (No 20) α-zingiberene, (No 21) (-)-β-bisabolene, (No 23) Pyridine,4-(4-methyl-5-trans-phenyl-1,3-oxazolidin-2-yl), (No 24) Chavicol, and (No 27) Gingerol.

The compounds that presents in the MAHD method and not occurred in the HD method were compound (No 8) (-)-α-terpineol, (No 12) Thymol, (No 13) 2-methoxy-Benzeneacetaldehyde, (No 17) Para-methoxypropiophenone, and (No 26) Methyl isoeugenol.

At 60 minutes of MAHD extraction, there was compound Linalyl anthranilate present in the essential oil. This compound only presents in the essential oil of HD method at time of 90 minutes. Other than that, compound (-)-Terpinen-4-ol present at essential of MAHD method at time of 60 minutes and this compound only presents in essential oil of HD method at time of 90 minutes.

Table 4.4: Chemical composition of Star Anise essential oil

No	Component	30 Min				60 Min				90 Min				120 Min			
		HD		MAHD		HD		MAHD		HD		MAHD		HD		MAHD	
		RT	Area%	RT	Area%	RT	Area%	RT	Area%	RT	Area%	RT	Area%	RT	Area%	RT	Area%
1	(D)-Limonene	15.108	0.57	-	-	15.140	0.65	-	-	15.140	0.73	-	-	15.140	0.62	15.140	0.52
2	Linalool	18.975	0.47	-	-	-	-	-	-			18.975	0.37	18.975	0.40	18.975	0.39
3	Linalyl acetate	-	-	-	-	18.975	0.43	-	-	-	-	-	-	-	-		
4	Linalyl anthranilate	-	-	18.975	0.45	-	-	18.975	0.47	18.975	0.42	-	-	-	-	-	-
5	4-Carvomenthol	20.898	0.24	20.898	0.22	20.898	0.22	-	-	-	-	20.898	0.20	20.898	0.22	20.898	0.22
6	(-)-Terpinen-4-ol	-	-	-	-	-	-	20.898	0.25	20.898	0.22	21.186	0.22	21.186	0.24	21.186	0.27
7	α-terpineol	21.186	0.22	21.176	0.19	21.186	0.23	-	-	21.186	0.23	-	-	-	-	-	-
8	(-)-α-terpineol	-	-	-	-	-	-	21.286	0.26	-	-	-	-	-	-	-	-
9	Estragole	21.250	3.43	21.250	3.12	21.261	3.27	21.261	3.32	21.261	3.27	21.261	2.80	21.261	3.02	21.261	3.01
10	Para-Anisaldehyde	22.009	0.67	22.009	0.76	22.009	0.50	22.019	1.16	22.020	0.86	22.019	1.39	22.019	1.20	22.020	1.48
11	Anethole	22.319	0.97	22.319	0.78	22.319	1.02	22.319	1.13	22.319	1.04	22.318	0.92	22.319	1.05	22.319	0.98
		23.013	91.37	23.013	91.36	23.013	88.08	23.023	91.79	23.024	91.48	23.023	86.53	23.024	89.81	23.013	90.21
12	Thymol	-	-	23.301	2.32	-	-	-	-	-	-	-	-	-	-	-	-
13	2-methoxy-Benzeneacetaldehyde	-	-	-	-	-	-	-	-	-	-	23.301	5.13	-	-	-	-
14	1-Oxaspiro[2.5] oct-5-ene, 8,8-dimethyl-4-methylene	-	-	-	-	23.312	4.78	-	-	-	-	-	-	-	--	-	-

15	Eugenol	24.017	0.21	24.017	0.22	-	-	24.017	0.43	24.017	0.28	24.017	0.48	24.017	0.46	-	-
16	Chavibetol	-	-	-	-	24.017	0.13	-	-	-	-	-	-	-	-	24.017	0.63
17	Para-methoxypropiophenone	-	-	-	-	-	-	25.181	0.10	-	-	25.181	0.16	-	-	25.181	0.19
18	β-Caryophyllene	25.235	0.28	-	-	25.235	0.20	-	-	25.235	0.27	25.235	0.14	25.235	0.30	25.235	0.19
19	α-bergamotene	25.470	0.19	-	-	25.181	0.11	25.470	0.13	25.181	0.18	25.470	0.14	25.181	0.23	25.470	0.18
		-	-	-	-	25.470	0.21	-	-	25.470	0.29	-	-	25.470	0.33	-	-
20	α-zingiberene	-	-	-	-	-	-	-	-	-	-	-	-	26.185	0.20	-	-
21	(-)-β-bisabolene	-	-	-	-	-	-	-	-	-	-	-	-	26.367	0.13	-	-
22	Foeniculin (ether)	28.151	0.35	28.151	0.58	-	-	28.151	0.95	-	-	-	-	-	-	-	-
23	Pyridine,4-(4-methyl-5-trans-phenyl-1,3-oxazolidin-2-yl)	-	-	-	-	-	-	-	-	-	-	-	-	28.151	1.79	-	-
24	Chavicol	-	-	-	-	28.151	0.16	-	-	-	-	-	-	-	-	-	-
25	Nutty quinoxaline	-	-	-	-	-	-	-	-	28.151	0.72	25.181	1.40	-	-	28.151	1.60
26	Methyl isoeugenol	-	-	-	-	-	-	-	-	-	-	25.886	0.11	-	-	25.886	0.13
27	Gingerol	31.890	1.01	-	-	-	-	-	-		--	-	-	-	-	-	-

4.4 Composition Analysis of Essential Oil

4.4.1 Composition Analysis of Lemongrass Essential Oil

Table 4.5: Essential oil compounds found in Lemongrass

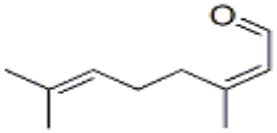
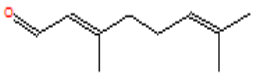
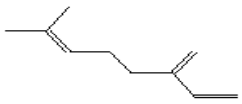
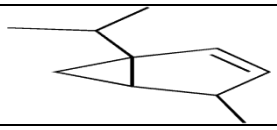
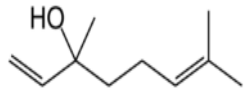
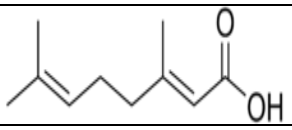
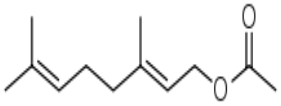
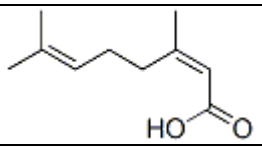
No	Compound	Formula	Symbol
1	Citral	$C_{10}H_{16}O$	
2	(Z)-Citral	$C_{10}H_{16}O$	
3	β-Myrcene	$C_{10}H_{16}$	
4	β-Thujene	$C_{10}H_{16}$	
5	Linalool	$C_{10}H_{18}O$	
6	Geranic Acid	$C_{10}H_{16}O_2$	
7	Geranyl Acetate	$C_{12}H_{20}O_2$	
8	Neric Acid	$C_{10}H_{16}O_2$	

Table 4.5 showed the main compounds that found in the essential oil of lemongrass. The main components of lemongrass oil consist of Citral, (Z)-Citral, β-Myrcene, β-Thujene, Linalool, Geranic Acid, Geranyl Acetate, and Neric Acid. Citral and (Z)-Citral were the dominant components in the essential of lemongrass.

Citral a (trans form) and citral b (cis form) are terpene aldehydes, the E-isomer is known as geranial or citral A. The Z-isomer is known as neral or citral B. It was colorless liquids with Boiling point of 229 ℃. They are components of many essential oils that have a strong lemon and verbena odor. Citral, or 3,7-dimethyl-2,6-octadienal or lemonal, is either of, or a mixture of, a pair of terpenoids. Neral has a lemon odor that is less intense than geranial that has a strong lemon odor, but Neral is sweeter. Citral is therefore an aroma compound used in perfumery for its citrus effect. Other than that, Citral is also used as a flavor and for fortifying lemon oil. It also has strong anti-microbial qualities, and pheromonal effects in insects. Citral is used in the synthesis of vitamin A, ionone, and methylionone, and to mask the smell of smoke.

β-myrcene, is an olefinic natural organic compound. It is classified as a hydrocarbon, but more precisely as a monoterpene with a boiling point of 166-168 ℃. It is a component of the essential oil of the several plants and mainly semi-synthetically from myrcia, from which it gets its name. It is also unstable in air, tending to polymerize. It is thus more highly valued as an intermediate for the preparation of flavor and fragrance chemicals such as menthol, citral, citronellol, citronellal, geraniol, nerol, and linalool.

Thujene (or α-thujene) is a colorless clear liquid with a boiling point of 150-152 ℃ .Thujene is a natural organic compound classified as a monoterpene. It is found in the essential oils of a variety of plants. The term thujene usually refers to α-thujene. A less common chemically-related double-bond isomer is known as β-thujene (or 2-thujene).

Linalool is a naturally occurring terpene alcohol chemical with a boiling point of 198-199 ℃. Linalool is a water-soluble organic compound, liquid at room temperature. It is a natural substance, a terpenoid alcohol that is biosynthesised as d-,

l- or dl-linalool by a host of plants, specifically many herbs, spices and fruits. It is used in vitamin E synthesis, added to processed food and beverages, to perfumes, cosmetics and soaps as well as to household detergents and waxes for its flavouring and fragrant properties. It is also used traditionally for stored-food pest control and some mosquito repellent products.

Geranic acid, or 3,7-dimethyl-2,6-octadienoic acid, is a pheromone with oily appearance that has a boiling point of 249-251 ℃. A pheromone is a secreted or excreted chemical factor that triggers a social response in members of the same species. Geranic acid has two stereoisomers, which are responsible for the trans and cis geometry on the conjugated double bond. Both isomers are present in the active ethyl acetate-soluble extract of the lemongrass (*Masuda et al., 2008*). Their use among insects has been particularly well documented.

Geranyl acetate is a clear colorless liquid with a pleasant floral or fruity rose aroma found in many essential oil. It is Insoluble in water but soluble in some organic solvent such as alcohol & oil and reacts with strong oxidizing agents. It is a natural organic compound that is classified as a monoterpene. Geranyl acetate is generally used as components of perfumes for creams and soaps as well as flavouring ingredient.

4.4.2 Composition Analysis of Star Anise Essential Oil

Table 4.6: Essential oil compounds found in Star Anise

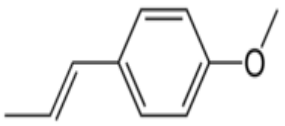
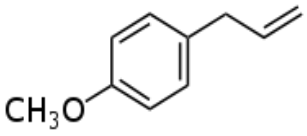
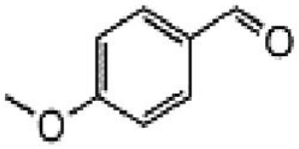
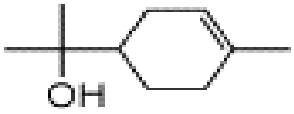
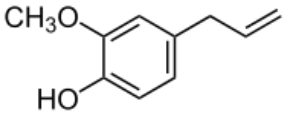
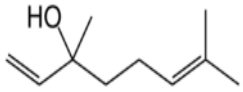
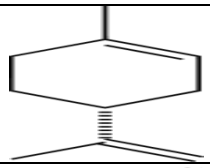
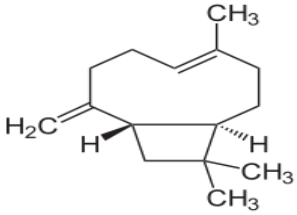
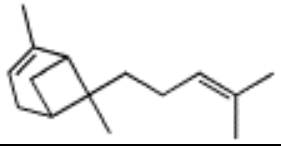
No	Compound	Formula	Symbol
1	Anethole	$C_{10}H_{12}O$	
2	Estragole	$C_{10}H_{12}O$	
3	Para-Anisaldehyde	$C_8H_8O_2$	
4	α-terpineol	$C_{10}H_{18}O$	
5	Eugenol	$C_{10}H_{12}O_2$	
6	Linalool	$C_{10}H_{18}O$	
7	(D)-Limonene	$C_{10}H_{16}$	
8	β-Caryophyllene	$C_{15}H_{24}$	
9	α-Bergamotene	$C_{15}H_{24}$	

Table 4.6 showed the main compounds that found in the essential oil of lemongrass. The main components of star anise consists of Anethole, Estragole, Para-Anisaldehyde, α-terpineol, Eugenol, 4-Carvomenthol, Linalool, (D)-Limonene, β-Caryophyllene, and α-Bergamotene. Anethole and Estragole were the dominant components in the essential oil of star anise.

Anethole is an aromatic compound that occurs widely in nature, in essential oils. Although two isomers exist, only the trans-isomer is commonly found in plants. The cis-isomer does not have the anise-like flavour. Anethole is a clear, colourless liquid with boiling point of 234 ℃ and is not or poorly soluble in water, but readily soluble in alcohol or oils. . Anethole is a flavoring substance of commercial value. In addition, it is distinctly sweet, measuring 13 times sweeter than sugar. Thus, it is used in flavours for chewing gum and anise flavour alcoholic drinks. Besides that, it is used in liquorice confectionery and in honey, nut, sarsaparilla and various spice flavours. It is also used to sweeten perfumes for soaps and detergents, household products and industrial deodorants.

Estragole is a colourless liquid, insoluble in water, with an anise odour. It has a specific gravity of 0.965 g/L, a boiling point of 215-216 ℃, and a flash point of 81 ℃. It is a natural organic compound with chemical structure consists of a benzene ring substituted with a methoxy group and a propenyl group. Estragole is a double-bond isomer of anethole and occurs naturally in many common plants. Flavor and fragrances containing estragole are used in numerous foods and food products, perfumes, soaps, and detergents.

Para Anisaldehyde is a clear to slight yellow liquid with a strong aroma and boiling point of 248 to 249 ℃. It is an organic compound that consists of a benzene ring substituted with an aldehyde and a methoxy group. It comes in 3 varieties, ortho, meta, and para and the unmodified term anisaldehyde generally refers to the para

isomer. Anisaldehyde is an intermediate in the synthesis of other compounds important in pharmaceuticals and perfumery. Other applications of para-anisaldehyde are in flavouring, electroplating and used in UV absorber.

Terpineol is a nearly colourless liquid that very slightly soluble in water, soluble in alcohol and has a boiling point of 219 ℃. It has a pleasant odour similar to lilac and stable under ordinary conditions of use and storage. Prolonged or excessive heat and/or exposure to air may cause decompostion or oxidation of the material. Terpineol is a naturally occurring monoterpene alcohol that usually a mixture of three isomers, alpha-, beta-, and gamma-terpineol, with alpha-terpineol as the major constituent. It is a common ingredient in perfumes, cosmetics, and flavours.

Eugenol appears as a clear or pale yellow oily liquid with a boiling point of 256 ℃. It is an allyl chain-substituted guaiacol, that slightly soluble in water and soluble in organic solvents and has a spicy, clove-like aroma. Eugenol has some interesting properties. Its specific gravity is slightly more than 1.06 at room temperature, making it heavier than water. It has about a 2 year shelf life before its potency begins to seriously degrade. Overdose of Eugenol is possible, causing a wide range of symptoms. Some of the symptoms are shallow and rapid breathing, coughing up blood, blood in urine, burns in mouth and throat, abdominal pain, nausea, rapid heartbeat, dizziness, seizures, and even coma. Eugenol is used in perfumeries, flavorings, essential oils and in medicine (local antiseptic and analgesic). It is used in the production of isoeugenol for the manufacture of vanillin. Eugenol has wide application in dentistry for analgesic and antiseptic properties. Eugenol is found in insect attractants as well as UV absorbers, analgesics, biocides and antiseptic, it is used in manufacturing of stabilizers and antioxidants for plastics and rubbers.

D-Limonene is a colourless liquid hydrocarbon classified as a cyclic terpene that has strong smell of oranges and boiling point of 176 ℃. D-Limonene is biodegradable, but due to its low flash point (50 ℃), it must be treated as hazardous waste for disposal. The usage of Limonene is common in cosmetic products. It is also used in is used in food manufacturing and some medicines, as a solvent for cleaning purposes, such as the removal of oil from machine parts. Besides that, Limonene can be used to dissolve polystyrene, and is a more ecologically friendly substitute for acetone.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this research, experiments were carried out for comparison between Hydrodistillation (HD) and Microwave-Assisted Hydrodistillation (MAHD) methods on the extraction of essential oil from lemongrass and star anise. Comparison between two methods would be in terms of extraction parameters such as extraction time, extraction yield/efficiency and chemical compositions of the essential oil. GS-MS analysis was the analysis method used to identify the chemical compositions of the essential oil.

From the results obtained from Hydrodistillation and Microwave-Assisted Hydrodistillation, higher yield of essential oil was obtained in MAHD method. The optimum yield for lemongrass essential oil was optimum in MAHD at 60 minutes with 0.549 % and in HD at 120 minutes with 0.5518 %. For star anise essential oil, the optimum yield occurred at 90 minutes in MAHD with 3.301 % and at 120 minutes in HD with only 3.1368 %.

When the time of extraction is longer, the increase in yield was not significant due to the limited essential oils in amount of 50 grams lemongrass and 40 grams star anise. Extraction time in the research started when the mixture achieved boiling point. In fact, MAHD method took an average of 20 minutes to achieve boiling point and HD method took about 45 minutes. As a result, there was significant difference in extraction time and extraction yield between MAHD and HD method.

In the GC-MS analysis on chemical compositions of lemongrass and star anise essential oil, there were total 67 components identified in lemongrass essential oil and 27 components in star anise essential oil. The same main components were identified in the essential oil using HD and MAHD method. There were total 8 main components identified in lemongrass essential oil with Citral and Z-Citral as the dominant components. In star anise essential oil, there were total 9 main components obtained from results of analysis with Anethole and Estragole as the dominant components.

In overall, there was no significant difference in dominant components percentages obtained from HD and MAHD methods. The percentages of Citral in HD and MAHD methods ranged from 43-50% and for Z-Citral ranged from 28-35%. The percentages of Anethole in HD and MAHD methods ranged from 86-92% and for Estragole ranged from 2.8-3.4%.

As a conclusion for GC-MS analysis, the main components obtained from both HD and MAHD methods were same. There were no considerable changes in main components percentages for HD and MAHD methods. The losses of volatile compounds can be observed significantly from HD & MAHD of Lemongrass at 30 & 60 min.

Based on the results obtained, it can be concluded that MAHD offered substantial advantages over conventional HD. A higher extraction yield was achieved at shorter extraction time when using MAHD. Microwave accelerated extraction rate without causing considerable changes in main composition of essential oil. Shorter extraction time, lower energy consumption with MAHD renders this technology being more environmentally friendly than HD.

5.2 Recommendations

For the time I carried out experiments on my research, the experiments opened new possibilities as well as new problems. In this section, some of the ideas towards improvements of experiments were given for future reference.

The first and most important events would be the optimization of the extraction parameters such as microwave power, heating level of heating mantle and ratio of sample used. In the research, optimum condition was used for the operation of microwave oven and heating mantle. The optimum condition means that the microwave power and heating level used were at its minimum for the condensation of the vapours to occur. This is important in terms it might affect the extraction yield and cost of operation between HD and MAHD methods. From previous researches, power consumption was determined with a wattmeter at the microwave generator entrance and the electrical heater power supply. The microwave oven and heating mantle were operated at optimum condition in the experiments. From the researches, it proved that application of microwave in extraction can save the cost on electricity in which 1.5 kWh was used for steam distillation (SD), and 0.13 kWh was used for Microwave-Accelerated Steam Distillation (MASD) in the extraction of Lavender (*Chemat et al., 2006*) and 2.00 kWh was used for HD and 1.24 kWh was used for MAHD in the extraction of Thymus Vulgaris (*Golmakani & Rezaei, 2008*).

Other than optimization of extraction parameters, soaking of raw materials would be another factor that will definitely increase the yield of essential oil in experiments. Plant materials can be subjected for constant soaking time before used for the extraction in HD and MAHD. The soaking of plant materials will caused the plant materials undergoes hydrolysis and experienced changes in structure that eventually give higher yield of essential oil when submitted for extraction.

Date of harvest of plant materials should be same if the samples were collected for comparison. The plants materials should be submitted for extraction as soon as possible once it been harvested. If the plant materials were kept for long period, there will be a reduction in the yield of essential oil because the structure of glands undergoes changes and lesser essential oil contained within the plant cell.

Lastly, the plant materials used for extraction should be grinded or cut into smaller and constant pieces to increase the surface area of plant materials. When the surface area of plants materials increased, there will be a more efficient heating in HD and MAHD. This in returns will give a higher yield of essential oil.

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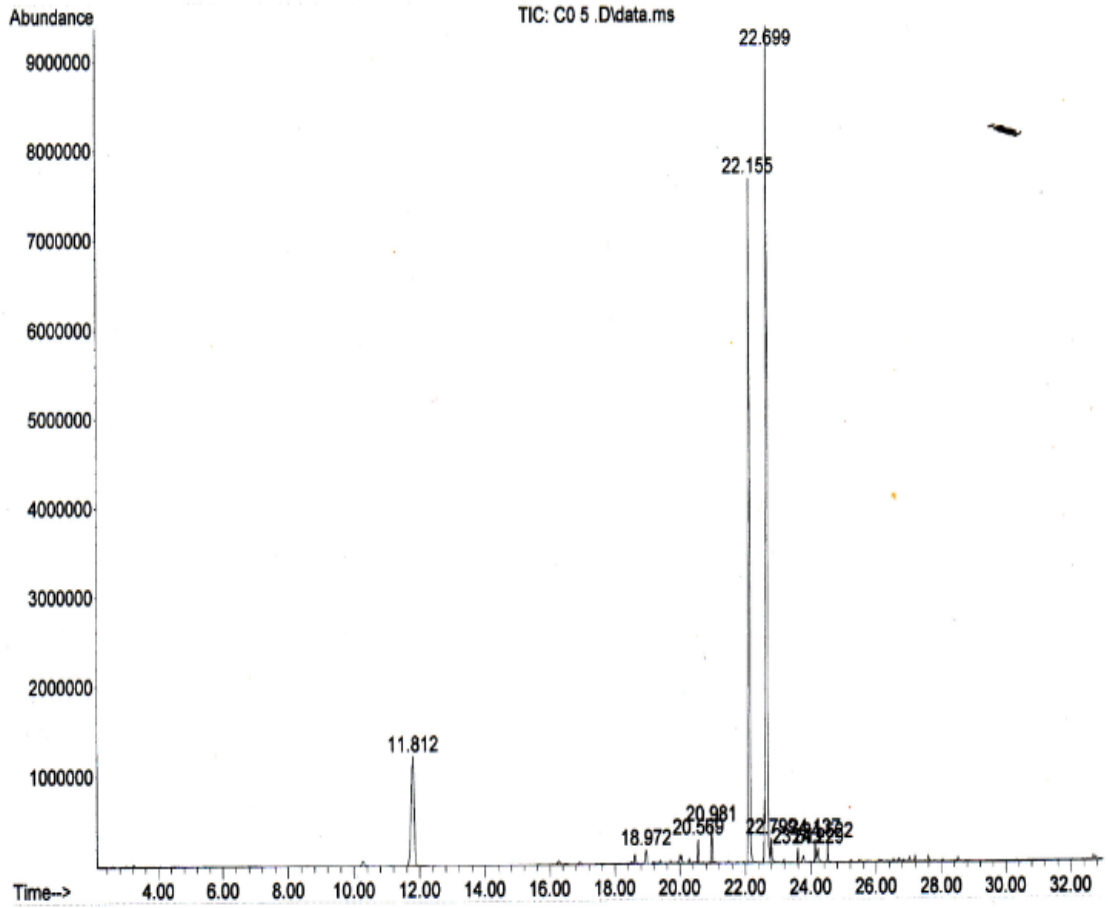
APPENDICES

A1: Spectrum and compound of Lemongrass for 30 minutes HD

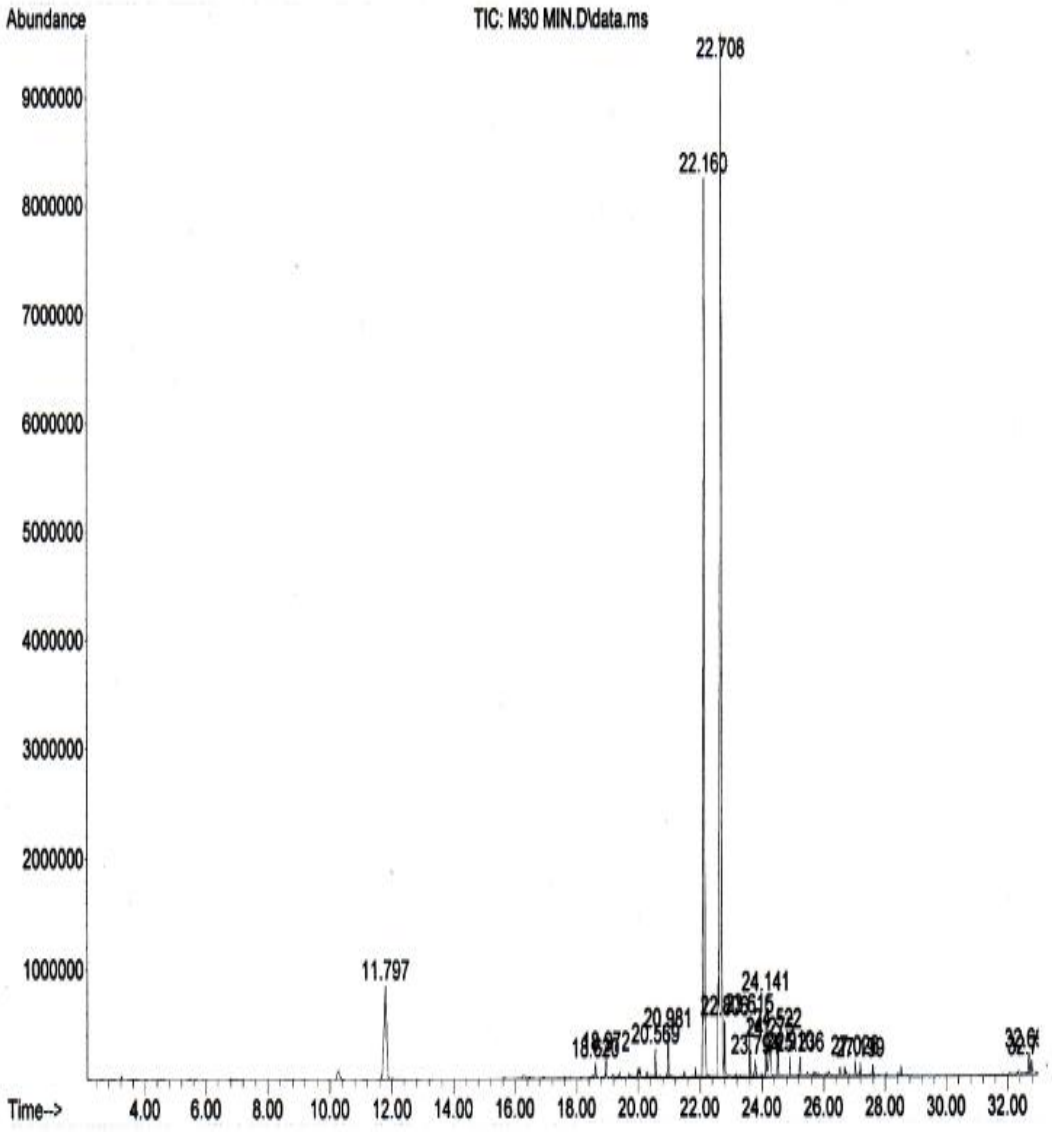
File :D:\Data\psm Tan kim Pieu\C0 5 .D
Operator : FIZA05OCT
Acquired : 5 Oct 2010 15:53 using AcqMethod GINGER OIL.M
Instrument : GCMSD
Sample Name: C0.5H
Misc Info :
Vial Number: 4



Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	11.808	11.62	C:\Database\NIST05a.L			
			.beta.-Myrcene	15180	000123-35-3	87
			Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl-	15314	004889-83-2	59
			.beta.-Myrcene	15177	000123-35-3	58
2	18.975	0.61	C:\Database\NIST05a.L			
			Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl-	15345	000488-97-1	55
			1,6-Octadien-3-ol, 3,7-dimethyl-, acetate	54271	000115-95-7	52
			1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate	107591	007149-26-0	52
3	20.567	0.78	C:\Database\NIST05a.L			
			Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl-	24230	000473-67-6	45
			Cyclohexane, ethenyl-	5785	000695-12-5	41
			Bicyclo[4.1.0]heptane, 3-methyl-	5859	041977-47-3	41
4	20.983	1.21	C:\Database\NIST05a.L			
			Bicyclo[4.1.0]heptane, 3-methyl-	5859	041977-47-3	41
			Cyclohexane, ethenyl-	5785	000695-12-5	38
			Methyl ethyl cyclopentene	5808	019780-56-4	38
5	22.158	35.40	C:\Database\NIST05a.L			
			2,6-Octadienal, 3,7-dimethyl-, (Z)	24148	000106-26-3	95
			2,6-Octadienal, 3,7-dimethyl-	24106	005392-40-5	64
			2,6-Octadienal, 3,7-dimethyl-, (Z)	24150	000106-26-3	59
6	22.703	47.15	C:\Database\NIST05a.L			
			2,6-Octadienal, 3,7-dimethyl-	24109	005392-40-5	96
			2,6-Octadienal, 3,7-dimethyl-, (E)	24151	000141-27-5	94
			2,6-Octadienal, 3,7-dimethyl-, (E)	24141	000141-27-5	94
7	22.799	0.81	C:\Database\NIST05a.L			
			Epoxy-linalooloxide	47012	1000007-96-5	47
			2-Octanol, 2,6-dimethyl-	28380	018479-57-7	35
			Hexylene Glycol	8546	000107-41-5	27
8	23.611	0.48	C:\Database\NIST05a.L			
			13-Heptadecyn-1-ol	93520	056554-77-9	45
			Methanamine, N-[3-methyl-2-butenylidene]	2943	1000196-86-4	38
			Cyclohexaneethanol, acetate	36087	021722-83-8	38
9	24.134	0.67	C:\Database\NIST05a.L			
			Cyclohexene, 3-(1-methylethyl)-	10370	003983-08-2	22
			Cyclohexene,3-propyl-	10311	003983-06-0	16
			Cyclohexene,3-propyl-	10313	003983-06-0	16
10	24.231	0.76	C:\Database\NIST05a.L			
			Geranic acid	34553	000459-80-3	90
			4(1H)-Pyrimidinone, 6-ethoxy-2-methyl-	26178	038249-34-2	47
			1,5-Heptadiene, 3,4-dimethyl-	10351	1000061-77-9	47
11	24.519	0.52	C:\Database\NIST05a.L			
			2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (E)-	54284	000105-87-3	91
			2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	54270	016409-44-2	91
			2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	54279	000141-12-8	91

A2: Spectrum and compound of Lemongrass for 30 minutes MAHD

File :D:\Data\psm Tan kim Pieu\M30 MIN.D
Operator : FIZA05OCT
Acquired : 5 Oct 2010 18:32 using AcqMethod GINGER OIL.M
Instrument : GCMSD
Sample Name: M30 MIN
Misc Info :
Vial Number: 8



Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	11.797	6.80	C:\Database\NIST05a.L			
			Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	15374	028634-89-1	80
			.beta.-Myrcene	15177	000123-35-3	70
			Pyridine, 2-propyl-	9271	000622-39-9	59
2	18.623	0.46	C:\Database\NIST05a.L			
			Bicyclo[3.1.0]hexane, 6-methylene-	2573	054211-16-4	46
			Pentalene, 1,2,3,3a,4,6a-hexahydro	5360	005549-09-7	32
			Bicyclo[3.2.1]octan-2-ol, exo-	11079	001965-38-4	25
3	18.975	0.66	C:\Database\NIST05a.L			
			1,6-Octadien-3-ol, 3,7-dimethyl-	25643	000078-70-6	64
			1,6-Octadien-3-ol, 3,7-dimethyl-	25636	000078-70-6	64
			.beta.-Myrcene	15180	000123-35-3	50
4	20.567	0.66	C:\Database\NIST05a.L			
			Bicyclo[2.2.2]octane	5774	000280-33-1	38
			Bicyclo[4.1.0]heptane, 3-methyl-	5859	041977-47-3	38
			Bicyclo[2.2.2]octane	5771	000280-33-1	35
5	20.983	1.03	C:\Database\NIST05a.L			
			Cyclohexanone, 5-methyl-2-(1-methylethenyl)-, trans-	24303	029606-79-9	58
			Cyclooctane, ethenyl-	16310	061142-41-4	47
			Cyclohexane, ethenyl-	5785	000695-12-5	46
6	22.158	34.20	C:\Database\NIST05a.L			
			2,6-Octadienal, 3,7-dimethyl-, (Z)	24148	000106-26-3	95
			2,6-Octadienal, 3,7-dimethyl-	24106	005392-40-5	64
			2,6-Octadienal, 3,7-dimethyl-, (Z)	24150	000106-26-3	59
7	22.703	46.95	C:\Database\NIST05a.L			
			2,6-Octadienal, 3,7-dimethyl-	24109	005392-40-5	96
			2,6-Octadienal, 3,7-dimethyl-, (E)	24151	000141-27-5	94
			2,6-Octadienal, 3,7-dimethyl-	24102	005392-40-5	91
8	22.810	1.15	C:\Database\NIST05a.L			
			Acetic acid, 2-acetoxymethyl-1,2,3-trimethylbutyl ester	78402	1000187-50-7	25
			Epoxy-linalooloxide	47012	1000007-96-5	16
			Ethanol, 2-ethoxy-	2251	000110-80-5	14
9	23.611	1.21	C:\Database\NIST05a.L			
			n-Heptadecylcyclohexane	138126	019781-73-8	43
			8-Azabicyclo[3.2.1]octan-3-one, 8-methyl-	17253	000532-24-1	38
			Methanamine, N-[3-methyl-2-butenylidene]	2943	1000196-86-4	38
10	23.793	0.66	C:\Database\NIST05a.L			
			Neric acid	34552	004613-38-1	87
			Geranic acid	34553	000459-80-3	64
			2-Propenoic acid, 2-methyl-, methyl ester	3671	000080-62-6	53
11	24.145	1.58	C:\Database\NIST05a.L			

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			Cyclohexene,3-propyl-	10313	003983-06-0	22
			Cyclopentanol, 2,4,4-trimethyl-	12234	056470-83-8	12
			Cyclohexene,3-propyl-	10311	003983-06-0	12
12	24.273	1.74	C:\Database\NIST05a.L			
			Geranic acid	34553	000459-80-3	72
			2-Butenoic acid, methyl ester, (E)	3665	000623-43-8	59
			2-Propenoic acid, 2-methyl-, methy l ester	3671	000080-62-6	59
13	24.519	0.82	C:\Database\NIST05a.L			
			2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (E)-	54284	000105-87-3	91
			2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	25690	000106-25-2	91
			2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	54270	016409-44-2	90
14	24.914	0.37	C:\Database\NIST05a.L			
			2-Tetradecene, (E)-	54521	035953-53-8	97
			1-Tetradecene	54512	001120-36-1	96
			Cyclotetradecane	54516	000295-17-0	94
15	25.235	0.39	C:\Database\NIST05a.L			
			Caryophyllene	59797	000087-44-5	99
			Bicyclo[5.2.0]nonane, 2-methylene- 4,8,8-trimethyl-4-vinyl-	59917	242794-76-9	98
			Caryophyllene	59802	000087-44-5	94
16	27.029	0.35	C:\Database\NIST05a.L			
			Cyclopropanol, 1-(3,7-dimethyl-1-o ctenyl)-	54447	065147-72-0	50
			1,7-Octadiene, 2,3,3-trimethyl-	24409	1000150-47-7	35
			Cyclopentanone, 2,4,4-trimethyl-	11098	004694-12-6	30
17	27.200	0.30	C:\Database\NIST05a.L			
			Caryophyllene oxide	71353	001139-30-6	58
			Cyclohexene, 5-methyl-3-(1-methyle thenyl)-, trans-(-)-	15382	056816-08-1	46
			Cyclohexene, 3-methyl-6-(1-methyle thenyl)-, (3R-trans)-	15383	005113-87-1	46
18	32.691	0.40	C:\Database\NIST05a.L			
			Neric acid	34551	004613-38-1	43
			Butane, 1,3-dibromo-3-methyl-	77498	024443-15-0	38
			2,6-Octadiene, 2,7-dimethyl-	16336	016736-42-8	32
19	32.787	0.26	C:\Database\NIST05a.L			
			2-Buten-1-one, 1-(2,2,5a-trimethyl perhydro-1-benzoxiren-1-yl)	62863	1000196-77-5	43
			4-Methyl-1,5-Heptadiene	5794	000998-94-7	30
			Neric acid	34551	004613-38-1	25

A3: Spectrum and compound of Star Anise for 30 minutes HD

```
File       :D:\Data\psm Tan kim Pieu\eeooil 11oct\AS C30min.D
Operator   : FIZA11OCT
Acquired   : 12 Oct 2010  11:02     using AcqMethod GINGER OIL.M
Instrument :    GCMSD
Sample Name: A.S C30min
Misc Info  :
Vial Number: 1
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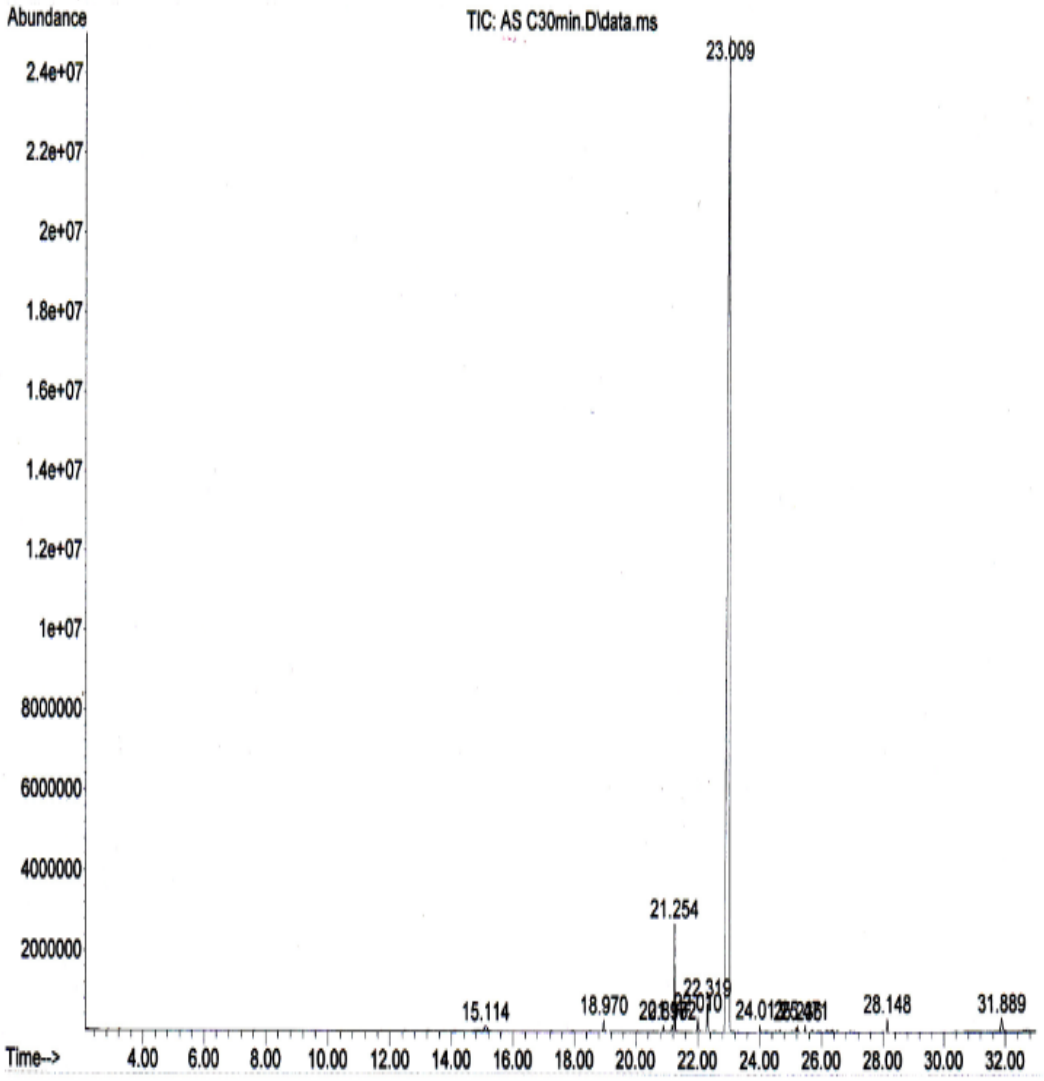

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	15.108	0.57	C:\Database\NIST05a.L			
			D-Limonene	15164	005989-27-5	94
			D-Limonene	15162	005989-27-5	93
			Limonene	15153	000138-86-3	93
2	18.975	0.47	C:\Database\NIST05a.L			
			1,6-Octadien-3-ol, 3,7-dimethyl-	25636	000078-70-6	76
			1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate	107591	007149-26-0	58
			1,5-Dimethyl-1-vinyl-4-hexenyl butyrate	74331	000078-36-4	52
3	20.898	0.24	C:\Database\NIST05a.L			
			3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	25751	000562-74-3	96
			3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	25750	000562-74-3	96
			3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	25784	020126-76-5	96
4	21.186	0.22	C:\Database\NIST05a.L			
			3-Cyclohexene-1-methanol, .alpha., .alpha.4-trimethyl-	25797	000098-55-5	91
			3-Cyclohexene-1-methanol, .alpha., .alpha.4-trimethyl-	25798	000098-55-5	90
			3-Cyclohexene-1-methanol, .alpha., .alpha.,4-trimethyl-, (S)-	25843	010482-56-1	90
5	21.250	3.43	C:\Database\NIST05a.L			
			Estragole	21721	000140-67-0	98
			Estragole	21723	000140-67-0	98
			Benzene, 1-methoxy-4-(1-propenyl)-	21815	000104-46-1	97
6	22.009	0.67	C:\Database\NIST05a.L			
			Benzaldehyde, 4-methoxy-	15757	000123-11-5	94
			Benzaldehyde, 4-methoxy-	15756	000123-11-5	91
			Benzaldehyde, 4-methoxy-	15753	000123-11-5	91
7	22.319	0.97	C:\Database\NIST05a.L			
			Benzene, 1-methoxy-4-(1-propenyl)-	21817	000104-46-1	98
			Benzene, 1-methoxy-4-(1-propenyl)-	21815	000104-46-1	98
			Benzene, 1-methoxy-4-(1-propenyl)-	21816	000104-46-1	97
8	23.013	91.37	C:\Database\NIST05a.L			
			Benzene, 1-methoxy-4-(1-propenyl)-	21815	000104-46-1	98
			Benzene, 1-methoxy-4-(1-propenyl)-	21817	000104-46-1	97
			Benzene, 1-methoxy-4-(1-propenyl)-	21816	000104-46-1	94
9	24.017	0.21	C:\Database\NIST05a.L			
			Eugenol	31714	000097-53-0	97
			3-Allyl-6-methoxyphenol	31757	000501-19-9	97
			Eugenol	31716	000097-53-0	97
10	25.235	0.28	C:\Database\NIST05a.L			
			Caryophyllene	59797	000087-44-5	99
			Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	59917	242794-76-9	93

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-	59914	013877-93-5	93
11	25.470	0.19	C:\Database\NIST05a.L			
			Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	59930	017699-05-7	93
			trans-.alpha.-Bergamotene	59863	1000293-01-5	59
			Bicyclo[3.1.1]hept-3-ene, 4,6,6-trimethyl-2-vinyloxy-	41693	1000163-23-1	50
12	28.151	0.35	C:\Database\NIST05a.L			
			1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene	58493	078259-41-3	72
			5,6,7,8-Tetrahydroquinoxaline	14719	034413-35-9	64
			Cyclohexanol, 2-[2-pyridyl]-	40968	099858-60-3	50
13	31.890	1.01	C:\Database\NIST05a.L			
			Gingerol	120912	023513-14-6	98
			Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-	52494	303187-89-5	93
			2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)-	52491	000122-48-5	93

A4: Spectrum and compound of Star Anise for 30 minutes MAHD

```
File       :D:\Data\psm Tan kim Pieu\eeooil 11oct\AS M30MINT.D
Operator   : FIZA11OCT
Acquired   : 12 Oct 2010  16:33     using AcqMethod GINGER OIL.M
Instrument :    GCMSD
Sample Name: AS M30MINT
Misc Info  :
Vial Number: 1
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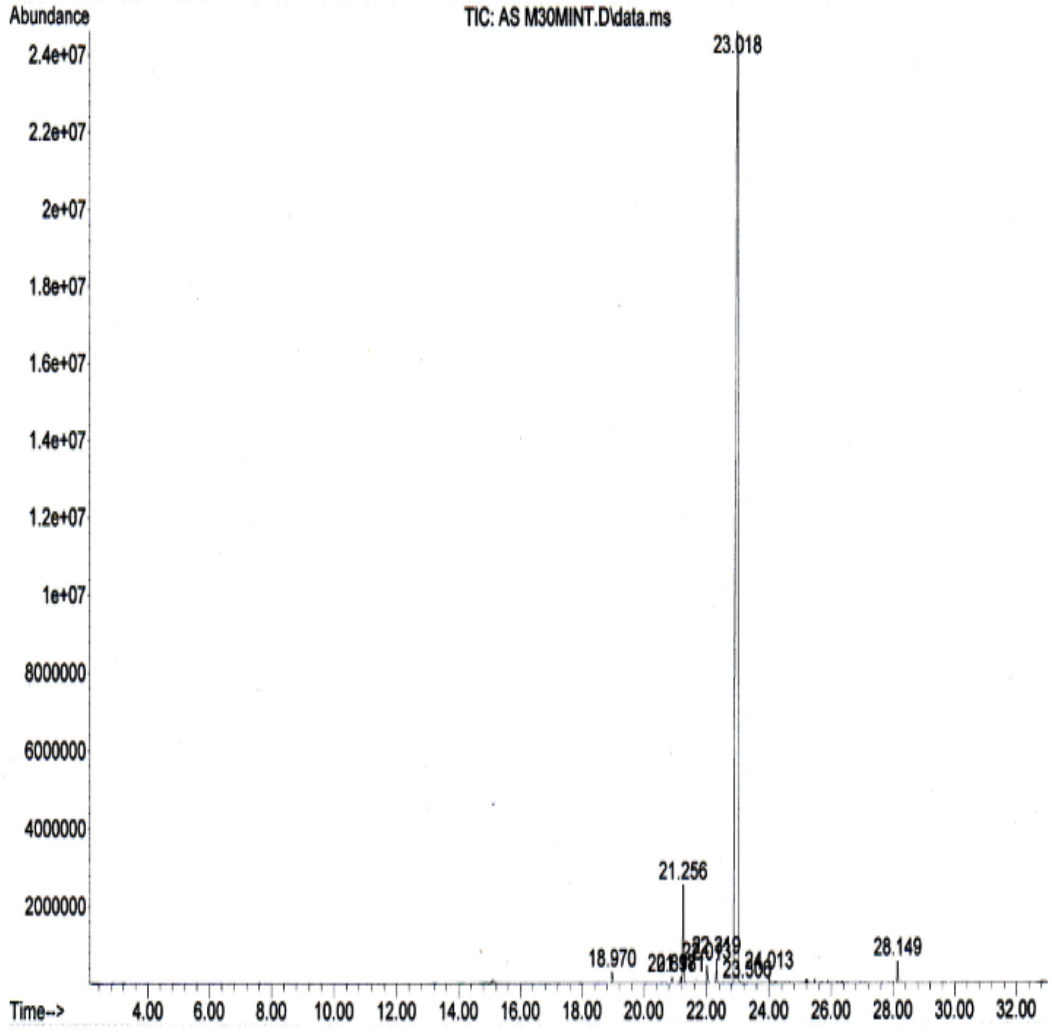

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	18.975	0.45	C:\Database\NIST05a.L			
			1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate	107591	007149-26-0	62
			1,5-Dimethyl-1-vinyl-4-hexenyl butyrate	74332	000078-36-4	52
			Linalyl isobutyrate	74304	000078-35-3	52
2	20.898	0.22	C:\Database\NIST05a.L			
			3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	25751	000562-74-3	96
			3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	25784	020126-76-5	95
			3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	25750	000562-74-3	94
3	21.176	0.19	C:\Database\NIST05a.L			
			3-Cyclohexene-1-methanol, .alpha., .alpha.4-trimethyl-	25797	000098-55-5	86
			3-Cyclohexene-1-methanol, .alpha., .alpha.,4-trimethyl-, (S)-	25843	010482-56-1	72
			Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	15346	000508-32-7	60
4	21.250	3.12	C:\Database\NIST05a.L			
			Estragole	21723	000140-67-0	98
			Estragole	21721	000140-67-0	98
			Benzene, 1-methoxy-4-(1-propenyl)-	21815	000104-46-1	97
5	22.009	0.76	C:\Database\NIST05a.L			
			Benzaldehyde, 4-methoxy-	15756	000123-11-5	96
			Benzaldehyde, 4-methoxy-	15757	000123-11-5	94
			Benzaldehyde, 4-methoxy-	15753	000123-11-5	86
6	22.319	0.78	C:\Database\NIST05a.L			
			Benzene, 1-methoxy-4-(1-propenyl)-	21817	000104-46-1	98
			Benzene, 1-methoxy-4-(1-propenyl)-	21815	000104-46-1	98
			Estragole	21721	000140-67-0	94
7	23.013	91.36	C:\Database\NIST05a.L			
			Benzene, 1-methoxy-4-(1-propenyl)-	21815	000104-46-1	98
			Benzene, 1-methoxy-4-(1-propenyl)-	21817	000104-46-1	97
			Benzene, 1-methoxy-4-(1-propenyl)-	21816	000104-46-1	94
8	23.301	2.32	C:\Database\NIST05a.L			
			Thymol	22702	000089-83-8	49
			Phenol, 2-methyl-5-(1-methylethyl)	22821	000499-75-2	49
			Phenol, 2-methyl-5-(1-methylethyl)	22815	000499-75-2	49
9	24.017	0.22	C:\Database\NIST05a.L			
			Eugenol	31714	000097-53-0	98
			Phenol, 2-methoxy-4-(1-propenyl)-, (Z)-	31881	005912-86-7	97
			Eugenol	31715	000097-53-0	96
10	28.151	0.58	C:\Database\NIST05a.L			
			1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene	58493	078259-41-3	64
			Cyclohexanol, 2-[2-pyridyl]-	40968	099858-60-3	50
			5,6,7,8-Tetrahydroquinoxaline	14719	034413-35-9	50