EXTRACTION OF ESSENTIAL OIL FROM MURRAYA KOENIGII LEAVES USING ULTRASONIC-ASSISTED SOLVENT EXTRACTION METHOD

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A report submitted in partial fulfillment of the requirement for the award of the degree of Bachelor of Chemical Engineering (Gas Technology)

Faculty of Chemical Engineering & Natural Resources

Universiti Malaysia Pahang

APRIL 2009

I declare that this thesis entitled "*Extraction of Essential Oil from Murraya Koenigii Leaves Using Ultrasonic-Assisted Solvent Extraction Method*" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Name   : Za Iskandar b Mohd Dikui

Date   : ....

To my beloved father and mother, Mr Mohd Dikui Ahang and Mdm Asmah Jelani

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ABSTRACT

The objective of this research is to extract essential oils from *M. koenigii* leaves by using ultrasonic-assisted solvent extraction method. The major constituent of *M. koenigii* has been reported as caryophyllene and 3-carene which is responsible for the aroma and flavor. This research has focused on the influence of ultrasonic, various natures of solvents, sonication times and also drying method towards the extraction of *M.koenigii* essential oil. Two types of solvents are used in this research which is ethanol and hexane. In this research, the methods of drying, grinding, extraction, separation and analysis are used and the sample is separated from solvents by using a rotary evaporator to get the essential oil. The sample was analyzed by using a GC-MS to identify the component of *M. koenigii* essential oil. In this research, the most suitable solvent to produce higher percentage yield is by using ethanol (ultrasonic-assisted solvent extraction of fresh leaves for 30 minutes) and the percentage of oil yield also increased with increasing the time. The major component in *M. koenigii* leaves is caryophyllene and hexane on the other hand is the best solvent to be used to extract caryophyllene

ABSTRAK

Objektif penyelidikan ini adalah bagi mengekstrak minyak pati *M. koenigii* dengan menggunakan ultrasonik bagi membantu pengekstrakan dengan pelarut. Konstituen mejar *M koenigii* telah dilaporkan sebagai kariofilena dan 3-carene yang adalah bertanggungjawab untuk bau dan rasa minyak pati *M. koenigii.* Penyelidikan ini telah tertumpu pada pengaruh ultrasonik, sifat semulajadi pelbagai pelarut, masa pensonikan, dan juga kaedah pengeringan terhadap pengekstrakan minyak pati *M.koenigii.* Dua jenis pelarut adalah digunakan dalam penyelidikan ini yang merupakan etanol dan heksana. Dalam penyelidikan ini, kaedah-kaedah bagi pengeringan, pengisaran, pengekstrakan, pemisahan dan analisis adalah digunakan dan sampel dipisahkan dari pelarut dengan menggunakan satu penyejat berputar untuk mendapat minyak pati. Sampel itu telah dianalisis dengan menggunakan GC-MS untuk mengenal pasti komponen minyak pati *M.koenigii*. Dalam penyelidikan ini, pelarut paling sesuai untuk menghasilkan peratusan lebih tinggi hasil adalah dengan menggunakan etanol (ultrasonik dibantu pengekstrakan pelarut daun segar selama 30 minit) dan peratusan hasil minyak juga bertambah dengan bertambahnya masa. Komponen utama dalam daun *M. koenigii* merupakan kariofilena dan heksana sebaliknya adalah pelarut yang terbaik untuk mengekstrak kariofilena

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LIST OF ABBREVIATIONS

GC   -   Gas Chromatograph

MS   -   Mass Spectrometer

(L.)   -   Linn, Carl Linnaeus

kHz   -   Kilo Hertz

GHz   -   Giga Hertz

FKKSA   -   Fakulti Kejuruteraan Kimia & Sumber Asli

W   -   Watt

mN   -   Millinewton

cm   -   Centimeter

mmHg   -   Mlillimeter of Mercury

cP   -   Centipoise

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CHAPTER 1

INTRODUCTION

1.1   Background of Study

M. koenigii (L.) Spreng (family: Rutaceae) or its common name curry leaf tree is a small strong smelling perennial shrub commonly found in forests as undergrowth, cultivated in India for its aromatic leaves and for ornament. Essential oil composition of the leaves has been studied by various workers. The major constituent responsible for the aroma and flavor has been reported as pinene, sabinene, caryophyllene, cadinol and cadinene [1-5]. Essential oils from *M. koenigii* serves as an important part in soap making ingredients, lotions, massage oils, diffusers, potpourri, scent, air fresheners, body fragrance, perfume oils, aromatherapy products, bath oils, towel scenting, spa's, incense, facial steams, hair treatments, and more [6]. There are several methods to extract essential oil from herb and spices like steam distillation, hydrodistillation, and solvent extraction but this study focus on a new, applicable method of essential oil extraction that is ultrasonic-assisted solvent extraction method. This extraction method is a combination of solvent extraction and ultrasonic extraction method. The steps required for the preparation of the material prior to extraction (including aspects concerning plant selection, collection, identification, drying, grinding and weighing) and analyzing method for the essential oil composition are detailed.

1.2   Problem Statement

The increasing importance of essential oils as pharmaceutical and aromatherapy assist besides their traditional role in cosmetics not only as a potent ingredient but also as a fragrance donor has opened up wide opportunities for global marketing. Essential oils, which are natural volatile extracts of plant material (flowers, leaves, stems, or roots, seeds, or seed peel, depending on the plant) generally distilled by steam, hold high export potential in future. Thus it is crucial that the correct or suitable extraction technique applied to preserve quality of the essential oil extracted.

Common extraction method like steam distillation has the advantage of no solvent residues but it can be a destructive process since heat is used and the oil does go through some chemical change [7]. Solvent extraction is the safest method for extracting high quality oil because some herbs and spices cannot be extracted from distillation method but it also has the disadvantage of having low yield and have remaining of solvents in the essential oils.

This study is conducted in order to introduce ultrasonic extraction as one of applicable method to extract essential oil. Ultrasonic-assisted solvent extraction is a combination of solvent and ultrasonic extraction can be considered an alternative to conventional extraction techniques. This combination can provide more yield compared if we just use only solvent extraction. Even though the effect of ultrasound have been studied in over hundreds of herbal and spice species, to the author's knowledge, its effect to *M. koenigii* plant, has not been investigate yet. Further focus is given on the factors that can influence the selection of the method and suitable solvent for extraction.

1.3   Objective

The objective of this research is to extract essential oils from *M. koenigii* leaves by using ultrasonic-assisted solvent extraction method.

1.4   Scope of Research Work

This research focus on four main scopes:

(i)   Investigate the effect of solvent nature on extraction in terms of percentage yield and extraction of major components in *M. koenigii* leaves.

(ii)   Investigate the effect of ultrasonic-assisted solvent extraction.

(iii)   Investigate the effect of sonication time on extraction.

(iv)   Analyze the product composition from the extraction process to obtain the major component.

CHAPTER 2

LITERATURE REVIEW

2.1   Extraction/leaching – general principles

Figure 2.1 below shows the analytical procedure for extraction/leaching with various solvents in order to isolate the analytes from plant material, as a rule, in order of increasing polarity of the extracting agent [8-9]. By using of various solvents, extracts containing different analytes can be obtained (Extracts P, Q, R, and S). There are procedure should be carried out in several steps so that particular analytes are present in one extract only, while others are present in different extracts - A in Figure 2.1. Application of additional operations, for example extract purification, results in obtaining further fractions (Fractions I, II) - B in Fig. 1. Each of the fractions can then be chromatographically separated into individual components - C in Figure 1.
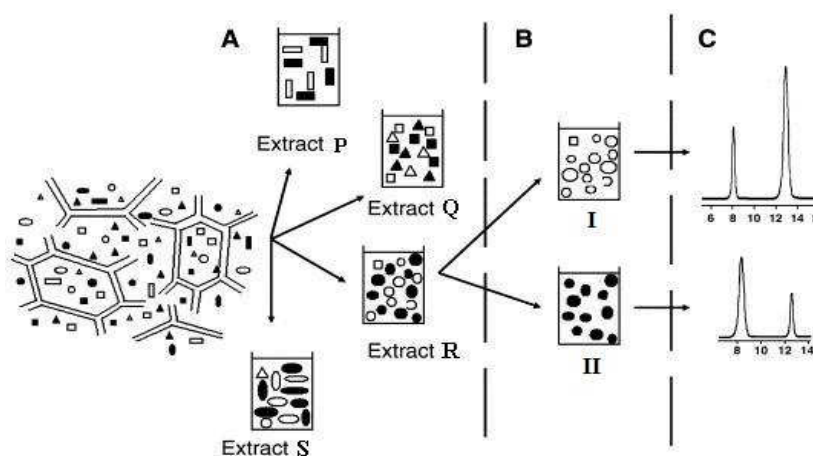
Figure 2.1: Separation steps used for isolation of plant metabolites [20].

2.2   Essential Oil

An essential oil is a liquid that is generally steam or hydro-distilled from flowers, leaves, bark and roots of plants and trees and are the compounds responsible for the aroma and flavor associated with herbs, spices, and perfumes. The formation and accumulation of essential oils in plants have been reviewed by Croteau (1986), Guenther (1972) and Runeckles and Mabry (1973) [10-11]. Chemically, the essential oils are primarily composed of mono- and sesquiterpenes and aromatic polypropanoids synthesized via the mevalonic acid pathway for terpenes and the shikimic acid pathway for aromatic polypropanoids.

The essential oils from aromatic plants are for the most part volatile and thus, lend themselves to several methods of extraction such as hydrodistillation, water and steam distillation, direct steam distillation, and solvent extraction [10, 12-14]. The specific extraction method employed is dependent upon the plant material to be distilled and the desired end-product. The essential oils which impart the distinctive aromas are complex mixtures of organic constituents, some of which being less stable, may undergo chemical alterations when subjected to high temperatures. In this case, organic solvent extraction is required to ensure no decomposition or changes have occurred which would alter the aroma and fragrance of the end-product. Mostly, essential oils are clear, however there are some exceptions. For example the essential oil of *M. koenigii* leaves having dark yellow color.

2.3   *Murraya Koenigii* (L.) Spreng

M. koenigii (L.) Spreng (Rutaceae) is one of the two species of Murraya found in Peninsular Malaysia. The plant usually cultivated for its aromatic leaves is normally used for natural flavoring in curries and sauces [15]. Originated in Tarai regions of Uttar Pradesh, India, it is now widely found in all parts of India and it adorns every house yard of Southern India and is also cultivated in Sri Lanka, China, Australia and the Pacific islands [16]. This plant is also distributed in Andaman

Islands and throughout Central and Southeast Asia [17]. The plant was spread to Malaysia, South Africa and Reunion Island by South Asian immigrants. Parts of the plant have been used as raw material for traditional medicine formulation in India [18]. The plant is used in Indian system of medicine to treat various ailments [19-21]. *M. koenigii* leaves and roots can be used to cure piles and allay heat of the body, thirst, inflammation and itching. The aromatic leaves, which retains their flavour and other qualities even after drying, are slightly bitter, acrid, cooling, weakly acidic in tastes and are considered as tonic, anthelminthic, analgesic, digestive, appetizing and are widely used in Indian cookery for flavouring food stuffs. Below are the figures of *M. koenigii* tree plant (Figure2. 2) and leaves (Figure 2.3):

Figure 2.2: *M. koenigii* tree plant.   **Figure2. 3:** *M. koenigii* leaves.

2.3.1   Chemical Structure of *M. koenigii* leaves

The leaves are reported to have rich source of carbohydrates, proteins, amino acids and alkaloids, and are rich in minerals, vitamins A and B [22-23]. They also a rich source of calcium, but due to the presence of oxalic acid in high concentration (total oxalates, 1.35%; soluble oxalates, 1.15%), its nutritional availability is affected. The leaves also contain a crystalline glucocide, koenigiin and a resin. By analysis of concentrated essence of *M. koenigii* leaf, Macleod and Pieris [4] obtained mainly terpenes. According to them the most important constituent of *M. koenigii* are β-caryophyllene, β-gurjunene, β-elemene, β-phellendrene and β-thujene.

The composition of the essential oil of *M. koenigii* may differ at different places. Earlier investigations on Indian curry leaf oil, hydrodistilled from fresh leaves, led to the identification of α-pinene, β-pinene, β-caryophyllene, isosafrole, lauric and palmitic acids [24]. Later, Sri Lankan oil was reported to contain monoterpenes (15.9%) and sesquiterpenes (80.2%) with β-phellandrene, β-caryophyllene, β-gurjunene, β-elemene, and α-selinene as the main constituents. However, Chinese oil was reported to contain α- and β-pinenes, β-caryophyllene and γ-elemene as main constituents, whereas curry leaf oil from Malaysia was shown to be rich in monoterpenes and oxygenated monoterpenes (ca. 85%) with α-pinene, limonene, β-phellandrene, terpinen-4-ol and β-caryophyllene as the main contents [25]. Chowdhury [26] reported that leaves on hydrodistillation gave 0.5% essential oil on fresh weight basis, having dark yellow color, spicy odor and pungent clove-like taste. It has following characteristics:

- Specific gravity (25°C) 0.9748
- Refractive index (25°C) 1.5021
- Optical rotation (25°C) + 4.8 [6]
- Saponification value 5.2
- Saponification value after after acetylation 54.6
- Moisture 66.3%
- Protein 6.1%
- Fat (ether extract) 1.0%
- Carbohydrate 18.7%
- Fibre 6.4%
- Mineral matter 4.2%
- Calcium 810 mg/100 g of edible portion
- Phosphorus 600 mg/100 g of edible portion
- Iron 3.1 mg/100 g of edible portion
- Carotene (as vitamin A) 12 600 IU/100 g
- Nicotinic acid 2.3 mg/100 g
- Vitamin C 4 mg/100 g
- Thiamine and riboflavin absent

The essential oil of Indian curry leaf collected from two different places in India has been investigated for its composition. The oils from the two places were found to contain mostly monoterpenes and oxygenated monoterpenes. The main items identified are α-pinene (19.0-19.7%), sabinene (31.8-44.85), b-pinene (4.2-4.7%), a-terpinene (1.3-4.3%), β-phellandrene (6.5-7.9%), γ-terpinene (3.9-7.1%) and terpinen-4-ol (5.2-9.9%). Although many of these compounds have already been reported in Malaysian curry leaf oil, there are marked differences between the oils, which suggests that the curry leaf plant exists in different chemical varieties. More recently, Jasim et al. (2008) [27] reported that gas chromatography mass spectroscopy (GC-MS) analysis of the chemical composition of the leaf of *M. koenigii* from Bangladesh were found to contained 39 compounds of which the major is 3-carene (54.2%) followed by caryophyllene (9.5%). The results were completely differ from those reported by previous workers, Raina et al. (2002) [28] and Walde et al. (2005) [29], where pinene, caryophyllenes and phellandrenes were the predominant compounds. Below are the figure of 3-carene (Figure 2.4) and caryophyllene (Figure 2.5):
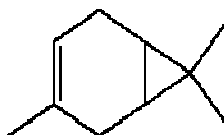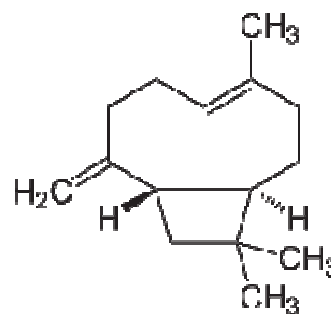
Figure 2.4: 3-carene.   **Figure 2.5:** Caryophyllene.

2.3.2   Uses of *M. koenigii* leaves

M. koenigii leaves are used in traditional medicine, for example ayurvedic and unani medicine [30-32]. The plant is credited with tonic, stomachic and carminative properties. The green leaves are used to treat piles, inflammation, itching, fresh cuts, dysentery, vomiting, burses and dropsy. The green leaves are also eaten raw as a cure for diarrhea and dysentery; bruised and applied externally to cure eruptions; given as a decoction with bitters as a febrifuge; and in snake bite. Moreover its leaves have a potential role in the treatment of diabetes. Hypoglycemic action on carbohydrate metabolism was reported in rats fed with *M. koenigii* leaves [33].

M. koenigii leaf also found to exert antioxidant properties in rats fed a high fat diet [11]. There were lower levels of hydroperoxides, conjugatedienes and free fatty acids in the liver and heart of rats supplemented with *M. koenigii* leaves compared to rats fed on the high fat diet alone. Activities of superoxide dismutase, catalase, and glutathione transferase were increased in the heart and liver of rats supplemented with *M. koenigii* leaves. Activities of glutathione reductase, glutathione peroxidase and glucose-6-phosphate dehydrogenase were also increased in the liver and the concentration of gluthathione was decreased. Thus supplementing a high fat diet with 10% *M. koenigii* leaf can prevent the formation of free radicals and maintain the tissues at normal levels.

The undiluted essential oil exhibited strong antibacterial and antifungal activity when tested with microorganisms [34]. Even the crude leaf extracts of *M. koenigii* leaf plant are reported to possess antibacterial activity [35]. An essential oil, a glucoside and koeinigin are reported from the species. The essential oil is used in soaps and perfume industry.

2.4   Extraction Methods for *M. koenigii* leaves

Various methods have being employed these days for extracting essential oils from different spices and herbs. From the journal, hydrodistillation and solvent extraction are the common extraction methods being used to extract *M. koenigii* leaves.

2.4.1   Hydrodistillation

Hydrodistillation is one of the oldest methods of extraction used. The spice is fully immersed in hot water. The result is a soup, which carries aromatic molecules of the plant. The method is not much in use these days, because of the risk of overheating the plant and subsequent loss of the oil. The method is best suitable for spice in dry and powdered form of roots and barks.

2.4.2   Solvent Extraction

Many herbs and spices cannot be extracted from distillation method, so solvent extraction is the safest method for extracting high quality oil. In this process, the spices or herbs plants are immersed in the solvent and the 'separation' is performed chemically. These include pigments, volatile molecules and non-aromatic waxes. The herbs and spices are then subjected to low pressure distillation and the volatile oil is then separately collected. It should be noted that, even with the most advanced techniques, absolutes extracted in this manner do contain traces of solvent. In solvent extraction, solvent selection is important

2.4.2.1 Solvent

A solvent is a liquid or gas that dissolves a solid, liquid, or gaseous solute, resulting in a solution [36]. Solvents usually have a low boiling point and evaporate easily or can be removed by distillation, leaving the dissolved substance behind. Basically there are two categories of solvent that is organic and inorganic solvent. Organic solvents are commonly used in dry cleaning (e.g. tetrachloroethylene), as paint thinners (e.g. toluene, turpentine) and glue solvents (acetone, methyl acetate, ethyl acetate), in spot removers (e.g. hexane, petrol ether), in detergents (citrus terpenes), in perfumes (ethanol), and in chemical syntheses. The use of inorganic solvents (other than water) is typically limited to research chemistry and some technological processes. The selection of an appropriate solvent is guided by theory and experience. Generally a good solvent should meet the following criteria [37]:

a) It should be inert to the reaction conditions.
b) It should dissolve the reactants and reagents.
c) It should have an appropriate boiling point.
d) It should be easily removed at the end of the reaction.

As a rule of thumb we should know that non-polar reactants will dissolve in non-polar solvents whilst polar reactants will dissolve in polar solvents. There are three measures of the polarity of a solvent:

a) Dipole moment
b) Dielectric constant
c) Miscibility with water

Molecules with large dipole moments and high dielectric constants are considered polar. Those with low dipole moments and small dielectric constants are classified as non-polar. Generally solvents with a dielectric constant of less than 15 are considered non-polar [38]. On an operational basis, solvents that are miscible with water are polar, while those that are not are non-polar. Technically, the dielectric constant measures the solvent's ability to reduce the field strength of the electric field surrounding a charged particle immersed in it. This reduction is then compared to the field strength of the charged particle in a vacuum [39].

2.4.2.2 Solvents Classification

Solvents can be broadly classified into three categories according to their polarity namely polar protic, polar aprotic and non-polar.

Polar Protic Solvents

In this term, protic refers to a hydrogen atom attached to an electronegative atom (oxygen). This means, polar protic solvents are compounds that can be represented by the general formula ROH. The polarity of the polar protic solvents comes from the bond dipole of the O-H bond. The large difference in electronegativities of the oxygen and the hydrogen atom, combined with the small size of the hydrogen atom, warrant separating molecules that contain an OH group from those polar compounds that do not. Examples of polar protic solvents are water (HOH), methanol (CH_3OH), and acetic acid (CH_3CO_2H).

Polar Aprotic Solvents

In this context, aprotic describes a molecule that does not contain an O-H bond. Solvents in this class all contain a bond that has a large bond dipole. Typically this bond is a multiple bond between carbon and either oxygen or nitrogen. Most polar aprotic solvents contain a C-O double bond. Examples are acetone $[(CH_3)_2C=O]$ and ethyl acetate $(CH_3CO_2CH_2CH_3)$.

Non-Polar Solvents

Non-polar solvents are compounds that have low dielectric constants and are not miscible with water. Examples include benzene (C_6H_6), carbon tetrachloride (CCl_4), and diethyl ether $(CH_3CH_2OCH_2CH_3)$.

Table 2.1 presents a list of solvents that are commonly used in chemical reactions. The chemical formula, boiling point, dipole moment, and dielectric constant of each solvent are included. All of these solvents are clear, colorless liquids. The hydrogen atoms of the protic solvents are highlighted in red. The density of nonpolar solvents that are heavier than water is bolded and the solvent used in this study and their dielectric constant is highlighted in blue.

Table 2.1: Properties table of common solvents.

Solvent	Chemical Formula	Boiling point	Dielectric constant	Density
Non-Polar Solvents				
Hexane	$CH_3-CH_2-CH_2-CH_2-CH_2-CH_3$	69 °C	2.0	0.655 g/ml
Benzene	C_6H_6	80 °C	2.3	0.879 g/ml
Toluene	$C_6H_5-CH_3$	111 °C	2.4	0.867 g/ml
Diethyl ether	$CH_3CH_2-O-CH_2-CH_3$	35 °C	4.3	0.713 g/ml
Chloroform	$CHCl_3$	61 °C	4.8	0.713 g/ml
Ethyl acetate	$CH_3-C(=O)-O-CH_2-CH_3$	77 °C	6.0	0.894 g/ml
Polar Aprotic Solvents				
1,4-Dioxane	$/-CH_2-CH_2-O-CH_2-CH_2-O-\backslash$	101 °C	2.3	1.033 g/ml
Tetrahydrofuran (THF)	$/-CH_2-CH_2-O-CH_2-CH_2-\backslash$	66 °C	7.5	0.886 g/ml
Dichloromethane (DCM)	CH_2Cl_2	40 °C	9.1	1.326 g/ml
Acetone	$CH_3-C(=O)-CH_3$	56 °C	21	0.786 g/ml
Acetonitrile (MeCN)	$CH_3-C\equiv N$	82 °C	37	0.786 g/ml
Dimethylformamide (DMF)	$H-C(=O)N(CH_3)_2$	153 °C	38	0.944 g/ml
Dimethyl sulfoxide (DMSO)	$CH_3-S(=O)-CH_3$	189 °C	47	1.092 g/ml
Polar Protic Solvents				
Acetic acid	$CH_3-C(=O)OH$	118 °C	6.2	1.049 g/ml
n-Butanol	$CH_3-CH_2-CH_2-CH_2-OH$	118 °C	18	0.810 g/ml
Isopropanol (IPA)	$CH_3-CH(-OH)-CH_3$	82 °C	18	0.785 g/ml
n-Propanol	$CH_3-CH_2-CH_2-OH$	97 °C	20	0.803 g/ml
Ethanol	CH_3-CH_2-OH	79 °C	24	0.789 g/ml
Methanol	CH_3-OH	65 °C	33	0.791 g/ml
Formic acid	$H-C(=O)OH$	100 °C	58	1.21 g/ml
Water	$H-O-H$	100 °C	80	1.000 g/ml

2.5   Ultrasonic Extraction

The application of ultrasound as a laboratory based technique for assisting extraction from plant material is widely published. Several reviews have been published in the past to extract plant origin metabolites [40], flavonoids from foods using a range of solvents [41] and bioactive from herbs [42]. Ultrasounds are waves with frequencies higher than the audible range to humans ranging from 16 kHz to 1 GHz. Ultrasonic vibrations are the source of energy facilitating the release of some analytes from the sample matrix. The improvement in extraction efficiency due to ultrasound appears at certain values of so-called acoustic pressure [43]. Among the most important phenomena taking place in the acoustic field (cavitation; friction at the boundary and interfacial surfaces; and increase in the diffusion rate), cavitation is the most significant phenomenon, because it has a direct effect on a number of phenomena occurring in a liquid subjected to ultrasound.

Cavitation involves the formation of pulsating bubbles as a result of strong stretching forces, originating from abrupt local pressure drops [43]. Under the ultrasound irradiation, micro-bubbles will be created when the negative pressure is high enough. Once created, the bubbles will grow during the period of negative pressure and compress during the period of positive pressure. The expansion and compression can cause constant pulsating or violent collapsing of micro-bubbles. When the collapse occurred near solid surface, it can damage the cell walls to facilitate the release of contents. Meanwhile, promotion of solvent penetration into cell walls by mechanical function and decrease of solvent viscosity by thermal function can also improve mass transfer [44-46]. These factors, taken together, enhance the efficiency of ultrasonic extraction greatly.

The mechanochemical effect of ultrasound is believed to accelerate the extraction of organic compounds from plant materials due to disruption of cell walls and enhanced mass transfer of the cell contents [47]. Organic compounds can be highly efficiently extracted with ultrasound without observation of substantial changes in their structural, molecular properties and biological activities [48-49]. The extraction conditions can be optimized with respect to time, polarity and amount of

solvent, and the mass and kind of sample. Previous investigations carried out by Melecchi et al. [50] have demonstrated that solvent polarity and extraction time have the greatest effect on the recovery. Examples of substances isolated by ultrasonic extraction along with extraction time and recoveries are presented in Table 2.2. The advantage of this technique is the possibility of extraction of many samples at once in an ultrasonic bath. The extraction is carried out at room temperature, which makes it suitable for the extraction of thermally labile analytes. The need for separation of the extract from the sample following the extraction is a disadvantage of this technique.

Table 2.2: Substances isolated using ultrasonic extraction.

Analyte	Sample	Recovery [%]	Time [min]	Reference
Cobalamins	Biological samples	94.8–101.1	1	[51]
Tartaric and malic acid	Grapes		30	[52]
Isoflavones	Soybeans	100	20	[53]
Isoflavonoids	Root	83	60	[54]
Flavonoids	Plant extract	91.2–95.6	60	[55]
Polysaccharides	Buckwheat hulls	147	70	[56]
Volatile compounds	Citrus flowers and honey		10	[57]
Aroma compounds	Aged brandies	45–113	30	[58]
Steroids and triterpenoids	Stems, leaves and flowers		30	[59]
Antioxidants	Herbs		60/45	

2.5.1 Extraction mechanism

Figure 2.6 shows that vegetal tissue consists of cells surrounded by walls. The extraction mechanism involves two types of physical phenomena: diffusion through the cell walls and washing out (rinsing) the cell contents once the walls are broken. Both phenomena are significantly affected by ultrasonic irradiation. Some

cells exist in the form of glands (external or internal) that are filled with essential oil. A characteristic of such glands (when external) is that their skin is very thin and can be very easily destroyed by sonication. This explains why the extraction of essential oil, as well as fat oil, is facilitated by sonication. For internal glands, it is the milling degree of the vegetal material which plays an important role and this is illustrated in Table 2.3 [61].
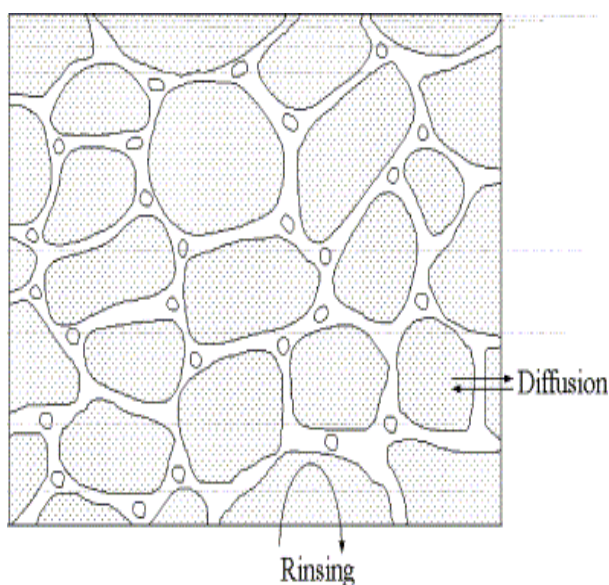
Figure 2.6: Schematic diagram of vegetal cell structures.

Table 2.3: Influence of milling degree on the extraction of clove flower [a]

Extraction time (min)	Extraction technique	Milling Degree	Eugenol extracted (g/100g)
30	Silent	Not milled	4.10
30	Silent	0.1 – 0.5 mm	25.20
30	Ultrasonic	Not milled	4.22
30	Ultrasonic	0.1 – 0.5 mm	32.66

[a] Extraction solvent ethyl alcohol 96%, cleaning bath with stirring. Silent method involves only stirring.

From Table 2.3 it is obvious that by reducing the size of the vegetal material particles will increase the number of cells directly exposed to extraction by solvent and thus exposed to ultrasonically induced cavitation. This effect can be utilized by milling or ground the material before extraction. External essential oil glands are already exposed directly to the cavitating solvent and consequently are readily disrupted.

The ultrasonic breakdown of vegetal cells using normal ultrasonic extraction devices such as a cleaning bath or probe system may not be the only mechanistic hypothesis for extraction improvement, especially when dried vegetal material is used. This is because solvent extraction from dried material is a two stage process involving:

a) steeping vegetal materials in solvent to facilitate swelling and hydration processes
b) mass transfer of soluble constituents from the material to solvent by diffusion and osmotic processes.

2.5.2   Laboratory scale ultrasonic extraction

Laboratory scale ultrasonic extraction can be done using simple cleaning bath (Figure 2.7 and 2.8). Using such equipment it is possible to obtain good extraction yields by direct or indirect extraction [12]. In both cases it is preferable to use a mechanical stirrer and to cool the extraction mixture since the absorption of ultrasonic energy can cause warming. However water in the ultrasonic bath can also be circulated and regulated at constant and desired temperature to avoid temperature rise by exposed to ultrasonic exposure. By indirect sonication, only small amounts of vegetal material can be extracted, whereas using the direct procedure, large amounts of vegetal material can be employed.

Another device that can be used for ultrasonic extraction is the probe system (Figure 2.9). This requires a glass vessel provided with a stirrer and a cooling jacket. But in some cases, it is not possible to perform extractions with such a device using the normal ratio of vegetal material/solvent of 1/10 (for certain herbs this can be 1/5) especially when soft vegetal material is employed. This is due to the vegetal material is too concentrated and dampens the transfer of ultrasonic energy.
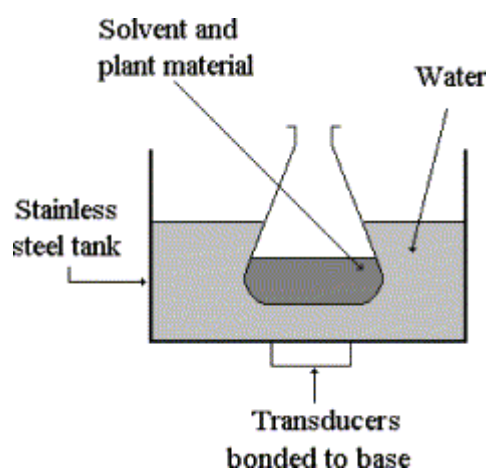
Figure 2.7: Indirect extraction using a cleaning bath.
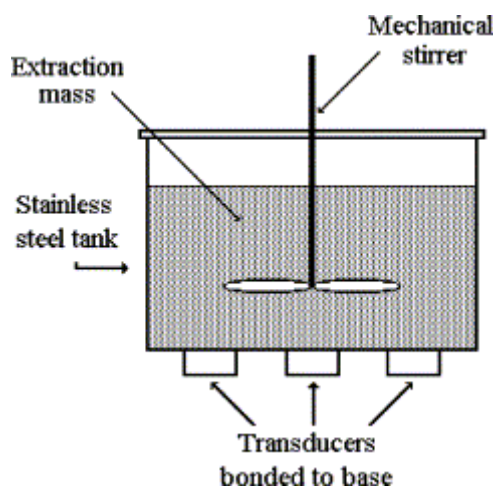
Figure 2.8: Direct extraction using a cleaning bath.
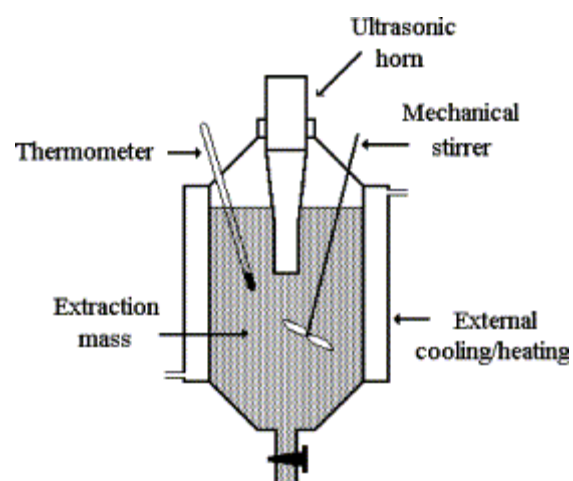
Figure 2.9: Direct extraction using an ultrasonic horn.

CHAPTER 3

METHODOLOGY

3.1 Material and Solvents

Fresh leaves of *M. koenigii* were collected from the plants grown in local area during October 2008. The leaves (plant material) was divided into two lots, and sampled as (1) freshly bought, (2) oven-dried at 60°C for 1 day (24 hours). The freshly bought leaf was analyzed the same day of purchase, while the other samples were analyzed seven days after purchase when all the treatments were completed. The oven-dried samples were put in glass bottles when completely dried to imitate the traditional method of home storage for culinary use.

For this study, organic solvent of various polarity has been used; ethanol (boiling point: 78.4°C) is found to be of advantageous as organic impurities are minimum compare to absolute non-protic organic solvent extracted oil; and hexane (boiling point: 69.0°C) is a common solvent use for extraction of oil have been recommended as solvents for extraction of vegetable oils was used as extracting solvents. The solvents were provided by FKKSA Laboratory Unit.

3.2   Pretreatment Methods

Three pretreatment methods which were drying, grinding and weighing should be carried out before proceed to the extraction method. Drying is a mass transfer process resulting in the removal of water moisture or moisture from another solvent, by evaporation from a solid, semi-solid or liquid (hereafter product) to end in a solid state. After drying process, the blender was used to ground the *M. koenigii* leaves to reduce the size into relatively fine particles. Then the fine particles of the *M. koenigii* leaves were weighed by using high precision electronic balance.

3.2.1   Drying

Plants are usually air dried (Dilika et al, 1996; Baris et al., 2006) to a constant weight but other researchers dry the plants in the oven at about 40ºC for 72h (Salie et al., 1996). Also, plants will have different constituents depending on the climatic conditions in which it is growing. The choice of plant material used in the extract preparation is usually guided by the traditional use of the plant and the ease of handling of the different plant parts like the leaves, stems etc. Drying method carried out in this study was oven drying. Oven drying is air drying with the application of dehumidifier where the temperature and humidity can be set. Drying speed for oven drying is usually increases because of increase air flow and at a low vapor pressure. Optimum air drying also can be achieved because we can control the climate.

3.2.2   Grinding

After drying all four samples was grounded using blender. The extraction period can be shortened by grinding the plant material finer as this will increase the surface area for extraction thereby increasing the rate of extraction. Then the fine particle samples were ready to be weighed.

3.2.3 Weighing

All the samples were then weighed using high precision electronic balance. For this study the samples were weighed 15 gram each and will be place into 250 ml Erlenmeyer flask ready to be extracted.

3.3 Extraction Methods

Two experiments were conducted for the extraction method that is (1) solvent extraction method and (2) ultrasonic extraction method. Solvent extraction method is applied first for all the plant to determine the most suitable solvent can be used to extract essential oil from *M. koenigii* leaves that can produce high percentage of yield

3.3.1 Solvent Extraction

After all the plant material were dried, grounded and weighed, solvent extraction is carried out. Two reagents were used as the solvent for the extraction; Ethanol (b.p. 78.4^{o}C) and Hexane (b.p. 69.0^{o}C) with volume of 150 ml each.

Figure 3.1: Solvent extraction

The extraction is done at room temperature, 1 atm for 1 day (24 hours). After solvent extraction, the essential oil and solvent was separated using rotary evaporator. The essential oil was weighed and then analyzed using GC-MS.

3.3.2   Ultrasonic Extraction

The plant material (15 g) and the extracting solvents were placed in an Erlenmeyer flask (150 ml); the ratio of the plant material and the extracting solvent is 1:10 (w/v). Three samples were prepared (3 samples for ultrasonication; 10, 20 and 30 minutes). The sonication is performed using an ultrasonic cleaning bath (DH.D300H, Daihan Scientific; the capacity of the ultrasonic cleaning bath is 6 Liter with internal dimension of 290 mm x 240 mm x 150 mm; working frequency and power consumption are 40 kHz and 200 W, respectively) as an ultrasound source. The temperature is controlled and maintains at 25 ± 1 °C by periodical replacing the water in the bath with cold one.

Figure 3.2: Ultrasonic-assisted solvent extraction

At the end of the extraction cycle, the liquid extract was separated from the residual plant material by vacuum filtration. The solvent was evaporated using a rotary vacuum evaporator at 78.4 °C for ethanol and 69.0 °C for hexane. The extract was then dried under vacuum at 50 °C to constant weight. The content of essential oil in

the plant material after certain extraction time was calculated from the mass of dry extract and the mass of initial plant material. The essential oil was analyzed using GC-MS.

Figure 3.3: Vacuum pump

3.4   Separation Methods

After extraction process, the mixture of *M. koenigii* essential oil and the solvents need to be separated by using rotary evaporator. Basically, rotary evaporator consists of a heated rotating vessel (usually a large flask) which is maintained under a vacuum though a tube connecting it to a condenser. The rotating flask is heated by partial emersion in a hot water bath. The benefit of the flask's rotation provides improved heat transfer to the contained liquid; the rotation also strongly reduces the occupancies of 'bumps' caused by superheating of the liquid. The solvent vapors leave the flask by the connecting tube and was condensed in the condenser section. The condenser section is arranged so that the condensed vapors drain into another flask where they are collected. The rotary separator speed and temperature is set at 3 rpm and at 78.4 °C for ethanol and 69.0 °C for hexane.

Figure 3.4: Rotary evaporator

3.5   Analysis Methods

The essential oil obtained after separation method is diluted with the solvents and placed in clean vial. Natrium sulphate is also added to the solution in order to remove water. The solution then is syringe from the vial and filtered before transferred to another clean vial. Finally the sample was ready to be analyzed by using GC-MS Agilent 6890 gas chromatography/mass spectrometry (GC/MS) coupled to Agilent 5973 mass spectrometer and Agilent Chem. Station software to determine qualitative analysis of the volatiles. Compounds were separated on a 30 m x 0.25 mm capillary column. The column temperature was at 40 ^{o}C for injection, maintained for 1 minute then heated to 250 ^{o}C at 10 ^{o}C/min where it held for 29 minutes. Split injection (1µl) was conducted with a split ratio of 1:10 and helium was used as the carrier gas of 1 l/hr flow rate. Temperature of injector was maintained at 250 ^{o}C. A solvent delay time of 4 min was used. GC-MS is known as one of the most effective and popular methods of separating, identifying, and quantifying compounds in complex mixtures.

Figure 3.5 shows the summary of methodology in this research.
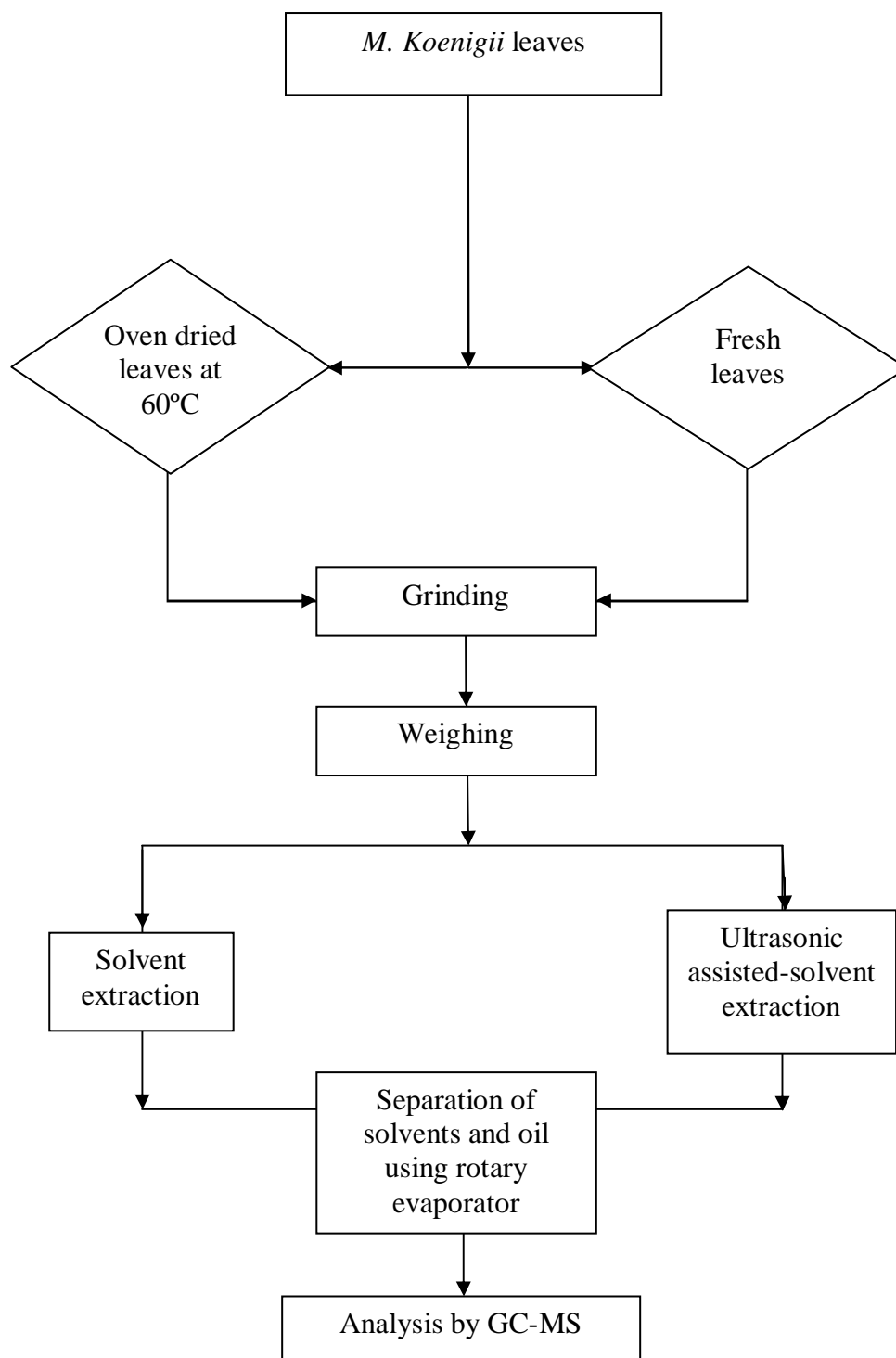
Figure 3.5: Summary of methodology

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1   *M. koenigii* Leaves Essential Oil Yield

The yield for each sample was calculated to obtain the most optimal quantitative result based on the effect of drying method, the effect of solvent used, and the effect of ultrasonic on the extraction and also the effect of time variability during sonication process. Equation below was used to calculate the percentage of yield of oil.

$$\text{Yield of oil (\%)} = \frac{\text{amount of oil obtained (g)}}{\text{amount of sample used (g)}} \times 100\%$$

Figure 4.1: Essential oil dissolved in hexane HPLC grade placed in vial.

4.2   Solvent Extraction

Solvent extraction was the first set of experiments. The purpose of this experiment was to determine the best solvent to extract oil from the leaves that can produce high yield. It was done by soaking oven dried leaves with solvents in Erlenmeyer flask for 24 hours at room temperature (25ºC) without ultrasound being applied.

4.2.1   Effect of type of solvents

The effect of types of solvents on the percentage yield of *M. koenigii* leaves was determined for two commonly used solvents: hexane and ethanol. The solvents used were based on the polarity; hexane (polar) and ethanol (non-polar). Table 4.1 below shows the percentage yield of oil after the extraction.

Table 4.1: Percentage yield of oil for solvent extraction

Weight of oil obtained (g)			
Time	**Sample**	**Extraction using Ethanol**	**Extraction using Hexane**
24 hours	Oven dried	1.4	0.8
Yield (%)		7.0	4.0
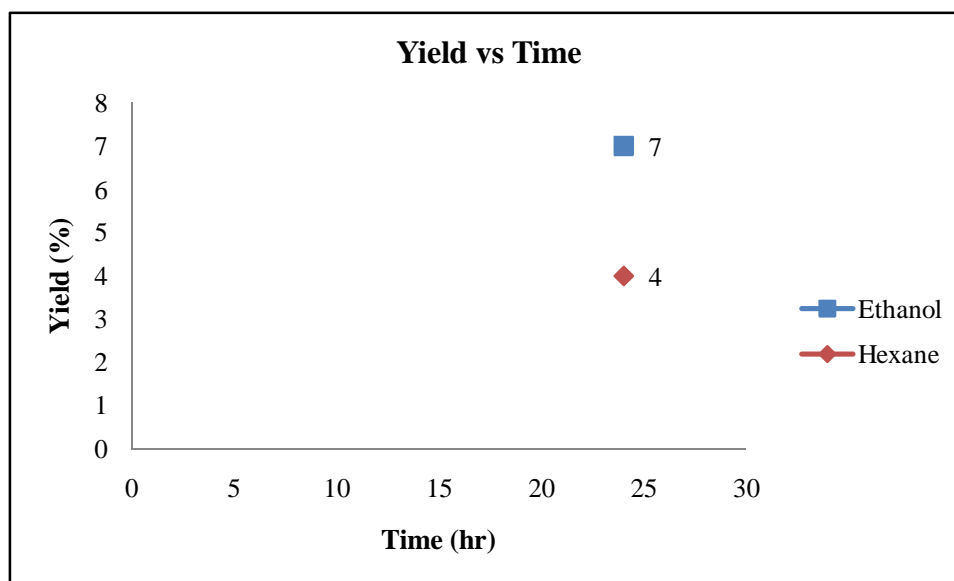
Figure 4.2: Yield (%) against time (hr) for solvent extraction.

As seen in Figure 4.2, for the case of extraction without ultrasound being applied, ethanol gives the highest percent of yield compared to hexane. The different extraction efficiencies of these solvents are attributed to their differing polarities and viscosities. The values for certain properties of hexane and ethanol used in this study are listed in Table 4.2.

Table 4.2: Properties of the solvents used in the research

Type of solvents	Polarity index	Surface tension (mN/cm)	Vapor pressure (mmHg)	Viscosity (cP)
Hexane	0.1	0.1791	150.00	0.294
Ethanol	5.2	23.7	59.02	1.200

Theoretically, solvent with low viscosity should extract more yields from the leaves because by having low viscosity will allow the solvent to easily diffuse into the leaves matrix. However in this case, hexane with the lowest viscosity compared to ethanol gave the lowest percentage of yield. This could be due to the fact that most of the compounds in the oil are more favorable toward polar solvents resulting in higher percentage of yield by ethanol compared to hexane.

4.3   Ultrasonic-assisted Solvent Extraction

In this second set of experiments, solvent extraction was assisted by ultrasound and for these experiments two types of samples were used; fresh leaves and oven dried leaves. The experiment was carried out at 25ºC and at constant power of 100W. The data from this experiment has been used to study effect of ultrasound on the extraction and the effect of time variability during sonication process and also the effect of drying method and solvent used when sonication was applied.

4.3.1   Effect of ultrasonication and its duration on extraction

When ultrasound was applied, ethanol still extracted the highest percentage of yield compared to hexane. This result was to be expected because as seen in Table 4.2, the vapor pressure of ethanol is lower than hexane. The solvent properties that impact the behavior of ultrasonic cavitation include vapor pressure, viscosities, and surface tension. Of these properties, medium vapor pressure is known to be most conducive to ultrasound activity [62]. Ultrasonication in low vapor pressure liquid produces few cavitational bubbles as a result of high cavitation threshold; however, the bubbles implode with relatively greater force, which enhances plant tissue disruption during extraction. High vapor pressure liquid on the other hand is not very effective—more bubbles are created, but they collapse with less intensity due to a smaller internal/external pressure differential. As for the influence of liquid viscosity, acoustic cavitation occurs more easily in the liquid with low viscosity because the ultrasonic intensity applied could more easily exceed the molecular forces of the liquid. Furthermore, the liquid with low viscosity has low density and high diffusivity, and can easily able to diffuse into the pores of the plant materials [63]. Although hexane have low viscosity, which makes cavitation occurs easily, it have high vapor pressure thus resulting in less violent cavitational collapses—more bubbles may be created, but they collapse with less intensity.

Table 4.3 show the percentage yield obtained for ultrasonic-assisted solvent extraction.

Table 4.3: Percentage yield of oil for ultrasonic-assisted solvent extraction

Weight of oil obtained (g)					
Time (min)	Sample	Extraction using Ethanol	Yield (%)	Extraction using Hexane	Yield (%)
10	Fresh	1.1	7.33	0.1	0.67
	Oven dried	0.8	5.33	0.5	3.33
20	Fresh	1.3	8.67	0.2	1.33
	Oven dried	1.1	7.33	0.5	3.33
30	Fresh	2.1	14.00	0.3	2.00
	Oven dried	2.0	13.33	0.6	4.00

Figure 4.3 and 4.4 show the effect of ultrasonication and its durations (10, 20, and 30 min) on the released of the yield.
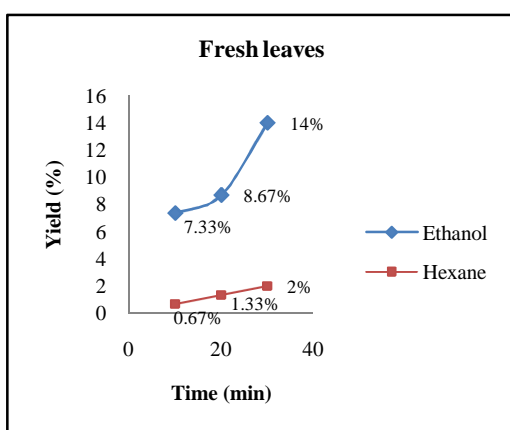
Figure 4.3: Effect of type of solvent for fresh leaves extraction
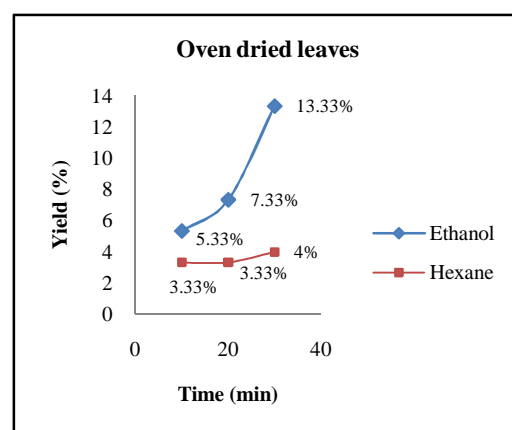
Figure 4.4: Effect of type of solvent for oven dried leaves extraction

Comparing for oven dried leaves extraction based on the extraction technique, Figure 4.4 reveals that the extraction yield could generally be enhanced, for solvent extraction by application of ultrasound. As seen in figure 4.4, the percentage yield for oven dried leaves can achieved 13.33% in just 30 minutes for ultrasonic extraction while for solvent extraction in figure 4.2 the percentage yield reached 7% in 24 hours. This also shows that by applying ultrasound on the solvent extraction can increase the percentage yield of oil and save time.

For the effect of extraction time on extraction, as seen in figure 4.3 and 4.4, when the ultrasound time increased from 10 to 30 min, the extraction yield of oils was increased for both fresh and oven dried leaves due to the mass transfer of oils from cellular material to hexane and ethanol by diffusion and osmosis. During this period, the major action of ultrasound is acoustic cavitations, which disrupted the cell walls and accelerated the washing out of the cell contents. As the time expanding, more and more cells were broken, and oils inside the leaves material were released gradually. However, the optimum ultrasound extraction time for the leaves cannot be determined during this experiment because of the short duration and there are still enhancement when the ultrasound time increasing from 10 min to 30 min.

4.3.2   Effect of drying method on the extraction

Drying method that has been applied this experiment was oven dried. The leaves were dried in the oven at 60ºC and were weighed until the mass was constant. The purpose of this experiment was to observe the effect of drying on the extraction yield. Conceptually, the oven drying process introduced some structural changes in the shape of cells, and even destroyed them, allowing to the cellular substances, including lipids to be easily extracted. From the figures, the results show that the effect of drying was affected by the type of solvent used.

Figure 4.5 and 4.6 show the effect of drying the leaves on the percentage of extraction yield for ethanol and hexane respectively. As seen in figure 4.5, ultrasonic extraction with ethanol as solvent resulting fresh leaves has the higher yield than oven dried leaves, while in figure 4.6, on the other hand has oven dried leaves to produce higher yield than fresh leaves for ultrasonic extraction with hexane as solvent.
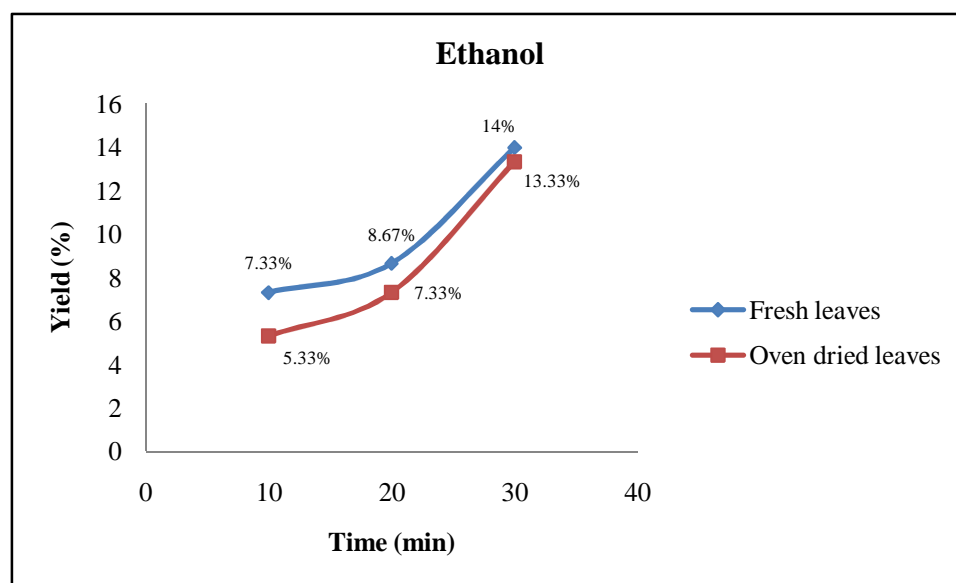
Figure 4.5: Yield (%) against time (hr) using ethanol for ultrasonic-assisted solvent extraction.
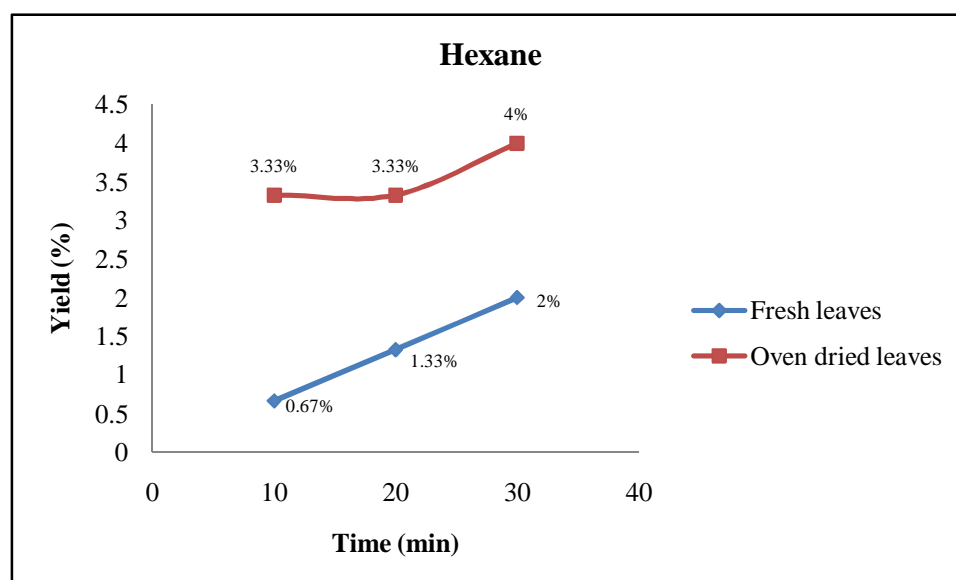
Figure 4.6: Yield (%) against time (hr) using hexane for ultrasonic-assisted solvent extraction

The reason for phenomenon in figure 4.5 to happen was probably due to the water presence in fresh leaves and the effect of sound absorption property of ethanol-water mixture. This was probably due to the relative polarity and the increase in effective swelling of the plant by water presence, which helped increase the surface area for solute-solvent contact [64]. Furthermore, the presence of water lowers the mixture viscosity, thus mass transfer was improved. In the presence of water, the intensity of ultrasonic cavitation in the solvent mixture was also increased as the surface tension increased and the viscosity and the vapor pressure decreased [65]. Table 4.4 shows the percentage yield of oil based on the drying method.

Table 4.4: Percentage yield of oil for ultrasonic-assisted solvent extraction based on effect of drying method.

Weight of oil obtained (g)					
Time	**Solvent**	**Fresh**	**Yield (%)**	**Oven dried**	**Yield (%)**
10 min	Ethanol	1.1	7.33	0.8	5.33
	Hexane	0.1	0.67	0.5	3.33
20 min	Ethanol	1.3	8.67	1.1	7.33
	Hexane	0.2	1.33	0.5	3.33
30 min	Ethanol	2.1	14.00	2	13.33
	Hexane	0.3	2.00	0.6	4.00

4.4   GC-MS Analysis of the Essential Oil Component

The essential oils from the leaves of *M. koenigii* were analyzed by GC-MS to detect 3-carene and caryophyllene substances. From the data obtained, 3-carene was only detected at sample number two for extraction of oven dried leaves by ethanol as solvent without ultrasound being applied. The detection was right after caryophyllene at retention time of 9.806 with percentage peak area of 4.06%. Caryophyllene on the other hand was detected at most of the samples at retention time of 8.973 with percentage of peak area between 8-45%. Table 4.5 shows the retention time of the

caryophyllene in *M. koenigii* essential oils. From the table, there was no detection of caryophyllene in sample number 3, 9, 10, 11, 13, and 14 probably due to technical errors during sample preparation.

Table 4.5: Retention time for caryophyllene in *M. koenigii* essential oil.

Sample				Retention Time (min)	Peak area (%)
No	Solvent	Leaves	Duration		
1	Hexane	Oven dried	24 hours	8.973	8.72
2	Ethanol	Oven dried	24 hours	8.973	13.84
3	Hexane	Fresh	10 minutes	-	-
4	Hexane	Fresh	20 minutes	8.973	15.47
5	Hexane	Fresh	30 minutes	7.648	15.57
6	Hexane	Oven dried	10 minutes	8.973	37.97
7	Hexane	Oven dried	20 minutes	8.973	43.35
8	Hexane	Oven dried	30 minutes	8.973	34.52
9	Ethanol	Fresh	10 minutes	-	-
10	Ethanol	Fresh	20 minutes	-	-
11	Ethanol	Fresh	30 minutes	-	-
12	Ethanol	Oven dried	10 minutes	8.973	32.43
13	Ethanol	Oven dried	20 minutes	-	-
14	Ethanol	Oven dried	30 minutes	-	-

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1   Conclusion

Ultrasonic-assisted solvent extraction offers a promising alternative for efficient extraction of *M. koenigii* essential oil. The application of ultrasound for the extraction offers important advantages over conventional method, namely: shorter extraction time (30 minutes against 24 hours of solvent extraction) and higher percentage yield. The main mechanism during ultrasonic-assisted extraction was acoustic cavitation which causes the leaves tissue disruption, thus enhancing the mass transfer of the solute into the solvents. The percentage of oil yield also increased with increasing the time.

The type and composition of solvents and the method of drying the samples are also important in extraction method. The nature of the solvent like viscosity and vapour pressure towards the condition of the samples also affected the percentage of oil yield. In this research, the most suitable solvent to extract oil from *M. koenigii* leaves in producing higher percentage yield is by using ethanol (ultrasonic-assisted solvent extraction of fresh leaves for 30 minutes). Major component in *M. koenigii* leaves is caryophyllene and hexane on the other hand is the best solvent to be used to extract caryophyllene.

5.2   Recommendation

It has been proven that ultrasonic-assisted solvent extraction offers a huge potential in the area of essential oil extraction. Nevertheless, there are some recommendations for further improvement in future research:

i.   Future study of ultrasonic-assisted solvent extraction should be done in large scale of sample to obtain more of essential oil.

ii.   The sonication time should be increased to determine the optimum time for the extraction of the oil from leaves.

iii.   Other parameters like temperature and liquid-to-solid ratio should be tried to in order to investigate other factors that may affect the extraction.

iv.   Sample preparation to be analyzed using GC-MS is very crucial process. The preparation should be done carefully and follow the procedure to avoid error or inaccuracy.

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APPENDIX A

Gantt Chart for Undergraduate Research Project I & II

Gantt Chart for Undergraduate Research Project I & II

Activities/Week	PSM 1							PSM 2														
	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Supervisor and Title selection	■																						
Discussion with Supervisor		■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■				
Gathering Information/ Journal	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■				
Proposal Preparation	■	■	■	■	■																		
Prepare Slide Presentation				■	■	■																	
Proposal Presentation							■																
Submit Proposal Report							■																
Works																							
Experimental Setup								■	■	■													
Run Experiment										■	■												
Analysis of Result												■	■	■									
Thesis Preparation								■	■	■	■	■	■	■	■	■	■	■	■				
Final Checking																				■	■		
Submit Final Thesis																						■	
Thesis Presentation																							■

APPENDIX B

GC-MS analysis: Research 1

File   :D:\Data\psm curry leaf\curry leaf 1.D
Operator   : HAFIZAH PSM
Acquired   : 19 Mar 2009 17:47   using AcqMethod GAHARU PHD.M
Instrument :   GCMSD
Sample Name: curry leaf 1
Misc Info :
Vial Number: 1

Library Search Report

```
Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 1.D
Acq On    : 19 Mar 2009  17:47
Operator  : HAFIZAH PSM
Sample    : curry leaf 1
Misc      :
ALS Vial  : 1   Sample Multiplier: 1
```

Search Libraries: C:\Database\NIST05a.L   Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	3.279	9.84	C:\Database\NIST05a.L			
			3-Hydroxy-N,N-dimethylpropanamide	8262	029164-29-2	47
			1-Heptadecanamine	95504	004200-95-7	35
			15-Crown-5	70738	033100-27-5	35
2	3.322	7.31	C:\Database\NIST05a.L			
			3-Hydroxy-N,N-dimethylpropanamide	8262	029164-29-2	58
			5-Dimethylamino-1-penten-3,5-dione	18273	063354-23-4	38
			12-Crown-4	40671	000294-93-9	35
3	8.973	8.72	C:\Database\NIST05a.L			
			Caryophyllene	59797	000087-44-5	99
			Bicyclo[7.2.0]undec-4-ene, 4,11,11 -trimethyl-8-methylene-	59912	013877-93-5	93
			Caryophyllene	59800	000087-44-5	93
4	9.806	1.68	C:\Database\NIST05a.L			
			.beta.-Pinene	15175	000127-91-3	43
			Linalyl isobutyrate	74304	000078-35-3	40
			1,6-Octadien-3-ol, 3,7-dimethyl-, acetate	54271	000115-95-7	38
5	10.393	1.55	C:\Database\NIST05a.L			
			Naphthalene, 1,2,3,4,4a,5,6,8a-oct ahydro-4,8-dimethyl-2-(1-methylet henyl)-, [2R-(2.alpha.,4a.alpha.,8 a.beta.)]-	60054	000473-13-2	90
			1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-	59928	072747-25-2	30
			1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetrame thyl-, [1aR-(1a.alpha.,7.alpha.,7a .beta.,7b.alpha.)]-	60087	021747-46-6	25
6	10.425	4.17	C:\Database\NIST05a.L			
			2-Isopropenyl-4a,8-dimethyl-1,2,3, 4,4a,5,6,7-octahydronaphthalene	59939	1000192-43-5	93
			Naphthalene, decahydro-4a-methyl-1 -methylene-7-(1-methylethylidene)- , (4aR-trans)-	59990	000515-17-3	93
			Naphthalene, 1,2,3,4,4a,5,6,8a-oct ahydro-4,8-dimethyl-2-(1-methylet henyl)-, [2R-(2.alpha.,4a.alpha.,8	60063	000473-13-2	91

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Library Search Report

Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 1.D
Acq On   : 19 Mar 2009 17:47
Operator : HAFIZAH PSM
Sample   : curry leaf 1
Misc   :
ALS Vial : 1   Sample Multiplier: 1

Search Libraries: C:\Database\NIST05a.L   Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			a.beta.)]-			
7	15.734	1.21	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl et her	186450	003055-98-9	58
			15-Crown-5	70735	033100-27-5	50
			15-Crown-5	70738	033100-27-5	50
8	16.375	1.47	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl et her	186450	003055-98-9	74
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100938	017455-13-9	72
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	64
9	16.439	1.75	C:\Database\NIST05a.L			
			15-Crown-5	70735	033100-27-5	64
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100938	017455-13-9	64
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	59
10	16.589	3.96	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl et her	186450	003055-98-9	83
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100938	017455-13-9	64
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	59
11	16.696	3.24	C:\Database\NIST05a.L			
			15-Crown-5	70735	033100-27-5	59
			15-Crown-5	70738	033100-27-5	53
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	53
12	16.803	5.15	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl et her	186450	003055-98-9	74
			15-Crown-5	70736	033100-27-5	64
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	53

Library Search Report

```
Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 1.D
Acq On    : 19 Mar 2009  17:47
Operator  : HAFIZAH PSM
Sample    : curry leaf 1
Misc      :
ALS Vial  : 1   Sample Multiplier: 1

Search Libraries:   C:\Database\NIST05a.L          Minimum Quality:   0

Unknown Spectrum:    Apex
Integration Events: ChemStation Integrator - autoint1.e
```

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
13	16.856	3.25	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl ether	186450	003055-98-9	64
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100942	017455-13-9	58
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100939	017455-13-9	53
14	16.909	3.08	C:\Database\NIST05a.L			
			Octaethylene glycol	161142	1000289-34-2	59
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100940	017455-13-9	59
			Heptaethylene glycol	139825	005617-32-3	59
15	16.973	4.09	C:\Database\NIST05a.L			
			15-Crown-5	70738	033100-27-5	59
			15-Crown-5	70737	033100-27-5	59
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100942	017455-13-9	53
16	17.038	5.01	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl ether	186450	003055-98-9	64
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100938	017455-13-9	59
			15-Crown-5	70737	033100-27-5	59
17	17.144	8.07	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100938	017455-13-9	72
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100941	017455-13-9	72
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100939	017455-13-9	64
18	17.208	3.06	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl ether	186450	003055-98-9	64
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100939	017455-13-9	59
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100938	017455-13-9	59

Library Search Report

```
Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 1.D
Acq On    : 19 Mar 2009  17:47
Operator  : HAFIZAH PSM
Sample    : curry leaf 1
Misc      :
ALS Vial  : 1   Sample Multiplier: 1


Search Libraries:   C:\Database\NIST05a.L          Minimum Quality:   0


Unknown Spectrum:   Apex
Integration Events: ChemStation Integrator - autoint1.e
```

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
19	17.273	5.10	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	59
			15-Crown-5	70736	033100-27-5	59
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100938	017455-13-9	53
20	17.337	5.87	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100938	017455-13-9	64
			Heptaethylene glycol	139825	005617-32-3	53
			Octaethylene glycol	161142	1000289-34-2	53
21	18.960	10.90	C:\Database\NIST05a.L			
			Phytol	122409	000150-86-7	42
			15-Crown-5	70735	033100-27-5	35
			15-Crown-5	70736	033100-27-5	35
22	26.309	1.03	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl et her	186450	003055-98-9	86
			15-Crown-5	70738	033100-27-5	53
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	53
23	26.438	0.49	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl et her	186450	003055-98-9	90
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	64
			Octaethylene glycol	161142	1000289-34-2	53

APPENDIX C

GC-MS analysis: Research 2

```
File        :D:\Data\psm curry leaf\curry leaf 2.D
Operator    : HAFIZAH PSM
Acquired    : 19 Mar 2009  18:24      using AcqMethod GAHARU PHD.M
Instrument  :   GCMSD
Sample Name: curry leaf 2
Misc Info   :
Vial Number: 2
```

```
                    Library Search Report

Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 2.D
Acq On    : 19 Mar 2009  18:24
Operator  : HAFIZAH PSM
Sample    : curry leaf 2
Misc      :
ALS Vial  : 2   Sample Multiplier: 1

Search Libraries:   C:\Database\NIST05a.L        Minimum Quality:   0

Unknown Spectrum:   Apex
Integration Events: ChemStation Integrator - autoint1.e
```

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	8.973	13.84	C:\Database\NIST05a.L			
			Caryophyllene	59802	000087-44-5	98
			Caryophyllene	59800	000087-44-5	97
			Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	59971	000118-65-0	96
2	9.806	4.06	C:\Database\NIST05a.L			
			1,5-Dimethyl-1-vinyl-4-hexenyl butyrate	74331	000078-36-4	35
			3-Carene	15151	013466-78-9	27
			Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl-	15345	000488-97-1	27
3	10.394	2.37	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100941	017455-13-9	12
			Octaethylene glycol monododecyl ether	186450	003055-98-9	12
			Ethanol, 1-[2-[2-(1-methylethoxy)ethoxy]ethoxy]-	51683	029681-21-8	12
4	10.426	6.53	C:\Database\NIST05a.L			
			Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	60033	003691-11-0	64
			Humulen-(v1)	59795	1000159-39-4	47
			1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-	60087	021747-46-6	38
5	15.735	2.73	C:\Database\NIST05a.L			
			15-Crown-5	70737	033100-27-5	64
			1-Hexanol, 6-amino-	8295	004048-33-3	47
			12-Crown-4	40671	000294-93-9	47
6	16.108	1.24	C:\Database\NIST05a.L			
			15-Crown-5	70735	033100-27-5	64
			12-Crown-4	40671	000294-93-9	64
			15-Crown-5	70737	033100-27-5	64

FAME CJO.M Mon Mar 23 10:40:52 2009 CHEMSTATION   Page: 1

Library Search Report

```
Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 2.D
Acq On    : 19 Mar 2009  18:24
Operator  : HAFIZAH PSM
Sample    : curry leaf 2
Misc      :
ALS Vial  : 2   Sample Multiplier: 1

Search Libraries:   C:\Database\NIST05a.L            Minimum Quality:   0

Unknown Spectrum:   Apex
Integration Events: ChemStation Integrator - autoint1.e
```

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
7	16.877	3.73	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl et her	186450	003055-98-9	80
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	59
			15-Crown-5	70735	033100-27-5	59
8	16.963	1.81	C:\Database\NIST05a.L			
			15-Crown-5	70736	033100-27-5	72
			15-Crown-5	70735	033100-27-5	64
			12-Crown-4	40671	000294-93-9	59
9	17.016	1.69	C:\Database\NIST05a.L			
			Octaethylene glycol	161142	1000289-34-2	64
			15-Crown-5	70735	033100-27-5	64
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100938	017455-13-9	64
10	17.048	2.02	C:\Database\NIST05a.L			
			Octaethylene glycol	161142	1000289-34-2	59
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	59
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	53
11	17.166	4.80	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl et her	186450	003055-98-9	87
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	64
			15-Crown-5	70736	033100-27-5	64
12	17.251	2.58	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl et her	186450	003055-98-9	86
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	59
			Tetraethylene glycol monododecyl e ther	158005	005274-68-0	53
13	17.305	3.63	C:\Database\NIST05a.L			
			Heptaethylene glycol monododecyl e ther	184119	003055-97-8	58

Library Search Report

Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 2.D
Acq On   : 19 Mar 2009 18:24
Operator : HAFIZAH PSM
Sample   : curry leaf 2
Misc   :
ALS Vial : 2   Sample Multiplier: 1

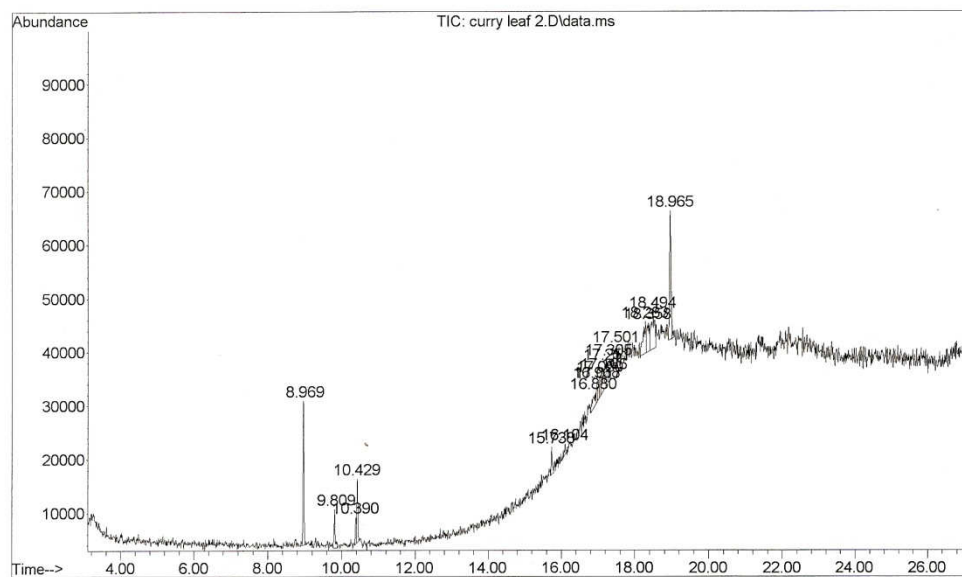Search Libraries: C:\Database\NIST05a.L   Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	53
			Octaethylene glycol	161142	1000289-34-2	53
14	17.497	1.88	C:\Database\NIST05a.L			
			Octaethylene glycol	161142	1000289-34-2	52
			Pentaethylene glycol	83339	004792-15-8	47
			Ether, sec-butyl isopropyl	8159	018641-81-1	43
15	18.288	8.89	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl et her	186450	003055-98-9	72
			Octaethylene glycol	161142	1000289-34-2	53
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	53
16	18.362	7.33	C:\Database\NIST05a.L			
			Ethanol, 2-[2-(2-ethoxyethoxy)etho xy]-	42058	000112-50-5	59
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100941	017455-13-9	53
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	53
17	18.490	12.38	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl et her	186450	003055-98-9	86
			15-Crown-5	70735	033100-27-5	59
			Octaethylene glycol	161142	1000289-34-2	53
18	18.960	18.49	C:\Database\NIST05a.L			
			Ethanone, 1-(3-ethylcyclobutyl)-	11095	056335-71-8	35
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	35
			2-Nonadecanol	114937	026533-36-8	30

APPENDIX D

GC-MS analysis: Research 3

File   :D:\Data\psm curry leaf\curry leaf 3.D
Operator   : HAFIZAH PSM
Acquired   : 19 Mar 2009 19:01   using AcqMethod GAHARU PHD.M
Instrument :   GCMSD
Sample Name : curry leaf 3
Misc Info   :
Vial Number : 3

Library Search Report

Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 3.D
Acq On   : 19 Mar 2009 19:01
Operator : HAFIZAH PSM
Sample   : curry leaf 3
Misc   :
ALS Vial : 3   Sample Multiplier: 1

Search Libraries: C:\Database\NIST05a.L   Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	4.048	2.41	C:\Database\NIST05a.L			
			2H-Benzotriazole, 2-ethyl-	21616	016584-04-6	37
			6-Amino-2-methyl-2H-indazole	21617	050593-30-1	32
			Benzimidazole, 2-amino-1-methyl-	21623	001622-57-7	32
2	8.973	9.35	C:\Database\NIST05a.L			
			Bicyclo[2.2.1]heptane, 2-cycloprop ylidene-1,7,7-trimethyl-	40400	1000159-45-7	37
			1,6,10-Dodecatrien-3-ol, 3,7,11-tr imethyl-, (E)-	72942	040716-66-3	35
			(+)-2-Carene, 4-.alpha.-isopropeny l-	40374	1000151-26-0	35
3	9.806	2.79	C:\Database\NIST05a.L			
			1,6-Octadien-3-ol, 3,7-dimethyl-, acetate	54271	000115-95-7	38
			1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate	107591	007149-26-0	38
			1,6-Octadien-3-ol, 3,7-dimethyl-, propanoate	64225	000144-39-8	38
4	10.383	2.21	C:\Database\NIST05a.L			
			1H-Cycloprop[e]azulene, 1a,2,3,5,6 ,7,7a,7b-octahydro-1,1,4,7-tetrame thyl-, [1aR-(1a.alpha.,7.alpha.,7a .beta.,7b.alpha.)]-	60087	021747-46-6	38
			Cyclopropa[d]naphthalen-2(4aH)-one , 1,1a,5,6,7,8-hexahydro-4a,8,8-tr imethyl-, [1aR-(1a.alpha.,4a.beta. ,8aS*)]-	59765	004677-90-1	35
			1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11, 11-dimethyl-, (1.alpha.,4.alpha.,4 a.alpha.,10a.alpha.)-	59716	1000221-85-9	27
5	10.426	5.85	C:\Database\NIST05a.L			
			Azulene, 1,2,3,5,6,7,8,8a-octahydr o-1,4-dimethyl-7-(1-methylethenyl) -, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	60033	003691-11-0	64
			1R,3Z,9S-2,6,10,10-Tetramethylbicy clo[7.2.0]undeca-2,6-diene	59923	1000140-07-4	35
			Naphthalene, 1,2,3,4,4a,5,6,8a-oct	60054	000473-13-2	35

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Library Search Report

Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 3.D
Acq On   : 19 Mar 2009 19:01
Operator : HAFIZAH PSM
Sample   : curry leaf 3
Misc   :
ALS Vial : 3   Sample Multiplier: 1

Search Libraries: C:\Database\NIST05a.L   Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			ahydro-4a,8-dimethyl-2-(1-methylet henyl)-, [2R-(2.alpha.,4a.alpha.,8 a.beta.)]-			
6	15.734	5.34	C:\Database\NIST05a.L			
			15-Crown-5	70736	033100-27-5	35
			1-Methoxy-3-(2-hydroxyethyl)nonane	58375	070928-44-8	27
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100939	017455-13-9	27
7	16.066	3.51	C:\Database\NIST05a.L			
			1-(3,6,6-Trimethyl-1,6,7,7a-tetrah ydrocyclopenta[c]pyran-1-yl)ethano ne	61393	1000194-97-2	42
			Phenol, 2,6-bis(1,1-dimethylethyl)	61442	000128-39-2	42
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	35
8	16.877	5.88	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100938	017455-13-9	72
			Heptaethylene glycol monododecyl e ther	184119	003055-97-8	72
			Pentaethylene glycol monododecyl e ther	172700	003055-95-6	64
9	17.144	12.40	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	59
			Pentaethylene glycol monododecyl e ther	172700	003055-95-6	59
			3,6,9,12-Tetraoxahexadecan-1-ol	91718	001559-34-8	59
10	17.326	8.45	C:\Database\NIST05a.L			
			15-Crown-5	70736	033100-27-5	72
			Ethanol, 2-[2-(2-ethoxyethoxy)etho xy]-	42058	000112-50-5	64
			Hexaethylene glycol monododecyl et her	179948	003055-96-7	64
11	17.785	15.23	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	80

Library Search Report

Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 3.D
Acq On   : 19 Mar 2009 19:01
Operator : HAFIZAH PSM
Sample   : curry leaf 3
Misc   :
ALS Vial : 3   Sample Multiplier: 1

Search Libraries: C:\Database\NIST05a.L   Minimum Quality: 0
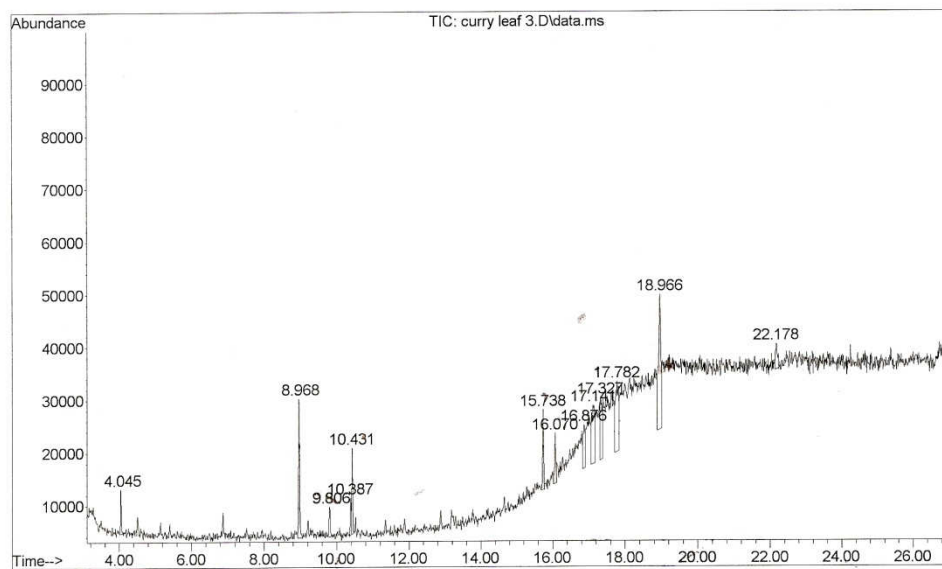
Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			12-Crown-4	40671	000294-93-9	72
			12-Crown-4	40673	000294-93-9	72
12	18.971	23.20	C:\Database\NIST05a.L			
			15-Crown-5	70735	033100-27-5	52
			Octaethylene glycol monododecyl et her	186450	003055-98-9	50
			Octaethylene glycol	161142	1000289-34-2	49
13	22.176	3.38	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	64
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100941	017455-13-9	64
			3,6,9,12,15-Pentaoxanonadecan-1-ol	120685	001786-94-3	59

APPENDIX E

GC-MS analysis: Research 4

File   :D:\Data\psm curry leaf\curry leaf 4.D
Operator   : HAFIZAH PSM
Acquired   : 19 Mar 2009 19:38   using AcqMethod GAHARU PHD.M
Instrument :   GCMSD
Sample Name: curry leaf 4
Misc Info   :
Vial Number: 4

Library Search Report

```
Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 4.D
Acq On    : 19 Mar 2009  19:38
Operator  : HAFIZAH PSM
Sample    : curry leaf 4
Misc      :
ALS Vial  : 4    Sample Multiplier: 1

Search Libraries:   C:\Database\NIST05a.L          Minimum Quality:   0

Unknown Spectrum:   Apex
Integration Events: ChemStation Integrator - autoint1.e
```

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	8.973	15.47	C:\Database\NIST05a.L			
			Caryophyllene	59802	000087-44-5	95
			Caryophyllene	59800	000087-44-5	93
			Bicyclo[7.2.0]undec-4-ene, 4,11,11 -trimethyl-8-methylene-,[1R-(1R*,4 Z,9S*)]-	59971	000118-65-0	90
2	10.383	3.43	C:\Database\NIST05a.L			
			Naphthalene, 1,2,3,4,4a,5,6,8a-oct ahydro-4a,8-dimethyl-2-(1-methylet henyl)-, [2R-(2.alpha.,4a.alpha.,8 a.beta.)]-	60054	000473-13-2	35
			Cyclopropa[d]naphthalen-2(4aH)-one , 1,1a,5,6,7,8-hexahydro-4a,8,8-tr imethyl-, [1aR-(1a.alpha.,4a.beta. ,8aS*)]-	59765	004677-90-1	27
			1H-Cycloprop[e]azulene, 1a,2,3,5,6 ,7,7a,7b-octahydro-1,1,4,7-tetrame thyl-, [1aR-(1a.alpha.,7.alpha.,7a .beta.,7b.alpha.)]-	60087	021747-46-6	25
3	10.436	8.87	C:\Database\NIST05a.L			
			Naphthalene, 1,2,3,4,4a,5,6,8a-oct ahydro-4a,8-dimethyl-2-(1-methylet henyl)-, [2R-(2.alpha.,4a.alpha.,8 a.beta.)]-	60054	000473-13-2	46
			1R,3Z,9S-2,6,10,10-Tetramethylbicy clo[7.2.0]undeca-2,6-diene	59923	1000140-07-4	38
			Tricyclo[4.1.0.0(2,4)]heptane, 3,3 ,7,7-tetramethyl-5-(2-methyl-1-pro penyl)-, (1.alpha.,2.beta.,4.beta. ,5.alpha.,6.alpha.)-	60091	067843-59-8	25
4	15.734	5.03	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl et her	186450	003055-98-9	64
			15-Crown-5	70737	033100-27-5	59
			1,4,7,10,13,16-Hexaoxanonadecane, 18-propyl-	136741	1000163-65-3	50
5	16.963	6.12	C:\Database\NIST05a.L			
			15-Crown-5	70735	033100-27-5	72
			1,4,7,10,13,16-Hexaoxacyclooctadec	100939	017455-13-9	64

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Library Search Report

```
Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 4.D
Acq On    : 19 Mar 2009  19:38
Operator  : HAFIZAH PSM
Sample    : curry leaf 4
Misc      :
ALS Vial  : 4   Sample Multiplier: 1

Search Libraries:   C:\Database\NIST05a.L          Minimum Quality:   0

Unknown Spectrum:   Apex
Integration Events: ChemStation Integrator - autoint1.e
```

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			ane			
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	59
6	17.070	5.15	C:\Database\NIST05a.L			
			15-Crown-5	70737	033100-27-5	72
			Octaethylene glycol monododecyl ether	186450	003055-98-9	72
			Hexaethylene glycol monododecyl ether	179948	003055-96-7	64
7	18.960	18.66	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl ether	186450	003055-98-9	87
			15-Crown-5	70736	033100-27-5	72
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100939	017455-13-9	59
8	21.300	7.83	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	72
			Pentaethylene glycol monododecyl ether	172700	003055-95-6	64
			15-Crown-5	70737	033100-27-5	64
9	21.364	9.79	C:\Database\NIST05a.L			
			Heptaethylene glycol monododecyl ether	184119	003055-97-8	59
			15-Crown-5	70735	033100-27-5	59
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	53
10	21.396	19.65	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100941	017455-13-9	64
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	58
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100939	017455-13-9	53

APPENDIX F

GC-MS analysis: Research 5

File   :D:\Data\PhD SNT.D\KARI180314.D
Operator   : FATIMAH
Acquired   : 18 Mar 2009 10:48   using AcqMethod Gaharu PhD.M
Instrument :   GCMSD
Sample Name: TRAP3
Misc Info   :
Vial Number: 5

Library Search Report

```
Data Path : D:\Data\PhD SNT.D\
Data File : KARI180314.D
Acq On    : 18 Mar 2009  10:48 (#1); 18 Mar 09  10:45 a (#2)
Operator  : FATIMAH
Sample    : TRAP3
Misc      :
ALS Vial  : 5    Sample Multiplier: 1


Search Libraries:   C:\Database\NIST05a.L          Minimum Quality:    0


Unknown Spectrum:   Apex
Integration Events: ChemStation Integrator - autoint1.e
```
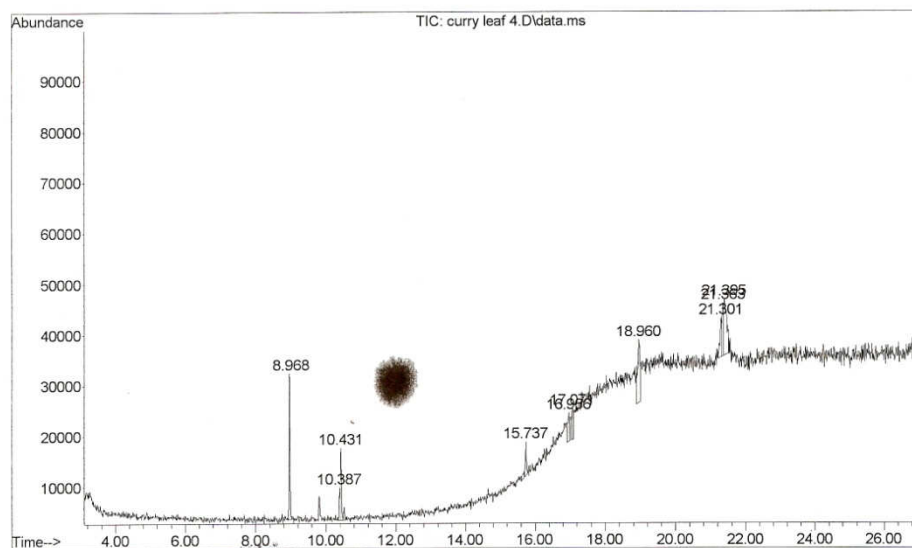
Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	3.514	8.91	C:\Database\NIST05a.L			
			Ethylbenzene	4955	000100-41-4	94
			Ethylbenzene	4956	000100-41-4	91
			Ethylbenzene	4957	000100-41-4	91
2	3.578	2.30	C:\Database\NIST05a.L			
			p-Xylene	4947	000106-42-3	97
			p-Xylene	4946	000106-42-3	97
			o-Xylene	4953	000095-47-6	97
3	3.632	5.60	C:\Database\NIST05a.L			
			o-Xylene	4953	000095-47-6	97
			Benzene, 1,3-dimethyl-	4970	000108-38-3	97
			p-Xylene	4946	000106-42-3	95
4	3.995	3.63	C:\Database\NIST05a.L			
			o-Xylene	4953	000095-47-6	97
			Benzene, 1,3-dimethyl-	4970	000108-38-3	97
			o-Xylene	4945	000095-47-6	94
5	4.337	3.41	C:\Database\NIST05a.L			
			Benzene, 1-ethyl-2-methyl-	9132	000611-14-3	94
			Benzene, 1-ethyl-4-methyl-	9136	000622-96-8	93
			Benzene, 1-ethyl-4-methyl-	9137	000622-96-8	91
6	4.849	1.97	C:\Database\NIST05a.L			
			Benzene, 1,2,3-trimethyl-	9115	000526-73-8	97
			Benzene, 1,3,5-trimethyl-	9124	000108-67-8	95
			Benzene, 1,3,5-trimethyl-	9123	000108-67-8	94
7	7.018	2.48	C:\Database\NIST05a.L			
			Acetamide, N-acetyl-N,N'-1,2-ethan ediylbis-	47788	137706-80-0	38
			Propyl nitrite	2118	000543-67-9	27
			Hexane, 2,3,4-trimethyl-	12311	000921-47-1	27
8	7.648	15.57	C:\Database\NIST05a.L			
			Caryophyllene	59797	000087-44-5	99
			Caryophyllene	59802	000087-44-5	98
			Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	59917	242794-76-9	95
9	8.000	4.71	C:\Database\NIST05a.L			

Gaharu PhD.M Wed Mar 18 11:10:49 2009 CHEMSTATION   Page: 1

```
                         Library Search Report

    Data Path : D:\Data\PhD SNT.D\
    Data File : KARI180314.D
    Acq On    : 18 Mar 2009  10:48 (#1); 18 Mar 09  10:45 a (#2)
    Operator  : FATIMAH
    Sample    : TRAP3
    Misc      :
    ALS Vial  : 5    Sample Multiplier: 1

    Search Libraries:   C:\Database\NIST05a.L          Minimum Quality:   0

    Unknown Spectrum:    Apex
    Integration Events: ChemStation Integrator - autoint1.e

    Pk#    RT  Area%            Library/ID             Ref#     CAS#    Qual
    ──────────────────────────────────────────────────────────────────────────
                 Oxalic acid, cyclohexyl nonyl este 123468 1000309-31-1 50
                 Oxalic acid, cyclohexyl isobutyl e  76950 1000309-30-4 47
                 ster
                 Oxalic acid, cyclohexyl octyl este 114607 1000309-31-0 47

    10   8.225  3.33 C:\Database\NIST05a.L
                 1,4,7,-Cycloundecatriene, 1,5,9,9-  59900 1000062-61-9 98
                 tetramethyl-, Z,Z,Z-
                 .alpha.-Caryophyllene               59848 006753-98-6 97
                 .alpha.-Caryophyllene               59849 006753-98-6 96

    11   8.652 11.36 C:\Database\NIST05a.L
                 Naphthalene, decahydro-4a-methyl-1  59990 000515-17-3 93
                 -methylene-7-(1-methylethylidene)-
                 , (4aR-trans)-
                 Naphthalene, 1,2,3,4,4a,5,6,8a-oct  60063 000473-13-2 91
                 ahydro-4a,8-dimethyl-2-(1-methylet
                 henyl)-, [2R-(2.alpha.,4a.alpha.,8
                 a.beta.)]-
                 Azulene, 1,2,3,5,6,7,8,8a-octahydr  60033 003691-11-0 89
                 o-1,4-dimethyl-7-(1-methylethenyl)
                 -, [1S-(1.alpha.,7.alpha.,8a.beta.
                 )]-

    12   8.706  2.58 C:\Database\NIST05a.L
                 1H-Cycloprop[e]azulene, 1a,2,3,5,6  60086 021747-46-6 90
                 ,7,7a,7b-octahydro-1,1,4,7-tetrame
                 thyl-, [1aR-(1a.alpha.,7.alpha.,7a
                 .beta.,7b.alpha.)]-
                 1,5-Cyclodecadiene, 1,5-dimethyl-8  59934 015423-57-1 87
                 -(1-methylethylidene)-, (E,E)-
                 Bicyclogermacrene                   59828 067650-90-2 86

    13  10.233  2.87 C:\Database\NIST05a.L
                 1-Dodecene                          34946 000112-41-4 96
                 Cyclopropane, nonyl-                34976 074663-85-7 95
                 1-Decene                            17321 000872-05-9 95

    14  10.457  6.94 C:\Database\NIST05a.L
                 Cyclododecane                       34949 000294-62-2 93
                 2-Propenoic acid, tridecyl ester    94777 003076-04-8 91
                 Dodecyl acrylate                    85325 002156-97-0 91

    Gaharu PhD.M Wed Mar 18 11:10:49 2009 CHEMSTATION            Page: 2
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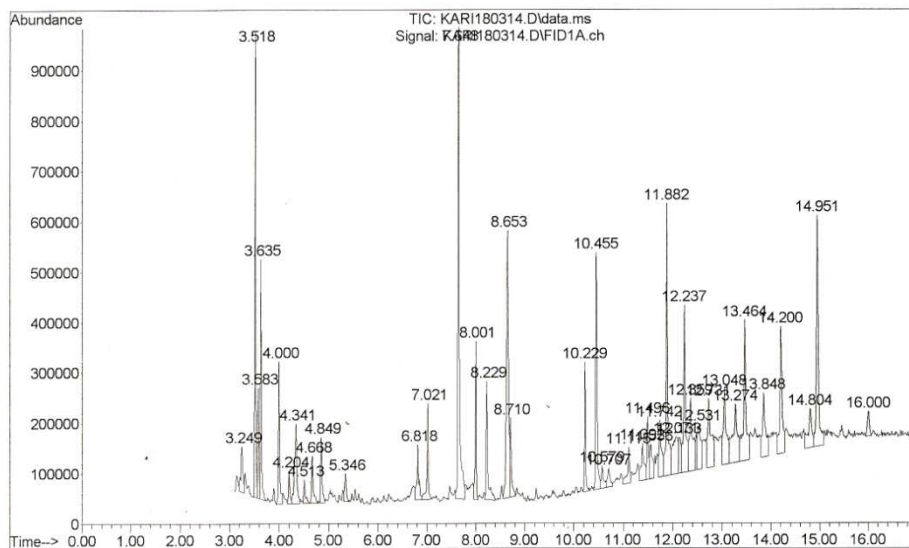
Library Search Report

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Data Path : D:\Data\PhD SNT.D\
Data File : KARI180314.D
Acq On    : 18 Mar 2009  10:48 (#1); 18 Mar 09  10:45 a (#2)
Operator  : FATIMAH
Sample    : TRAP3
Misc      :
ALS Vial  : 5    Sample Multiplier: 1


Search Libraries:   C:\Database\NIST05a.L          Minimum Quality:   0


Unknown Spectrum:   Apex
Integration Events: ChemStation Integrator - autoint1.e
```

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
15	11.878	5.34	C:\Database\NIST05a.L			
			Tridecanoic acid, methyl ester	77300	001731-88-0	95
			Hexadecanoic acid, methyl ester	105646	000112-39-0	95
			Hexadecanoic acid, methyl ester	105644	000112-39-0	94
16	12.241	3.35	C:\Database\NIST05a.L			
			2,3-Dehydro-4-oxo-.beta.-ionone	59625	077503-87-8	42
			15-Crown-5	70735	033100-27-5	38
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	35
17	13.459	3.33	C:\Database\NIST05a.L			
			9-Octadecenoic acid (Z)-, methyl e ster	122323	000112-62-9	97
			9-Octadecenoic acid (Z)-, methyl e ster	122321	000112-62-9	96
			9-Octadecenoic acid, methyl ester, (E)-	122328	001937-62-8	95
18	14.196	3.88	C:\Database\NIST05a.L			
			Triethylene glycol monododecyl eth er	135738	003055-94-5	52
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100938	017455-13-9	38
			Methoxyacetic acid, tetradecyl est er	116037	1000281-82-1	38
19	14.954	8.45	C:\Database\NIST05a.L			
			Phytol	122409	000150-86-7	90
			Phytol	122406	000150-86-7	64
			Phytol	122405	000150-86-7	62

APPENDIX G

GC-MS analysis: Research 6

File   :D:\Data\psm curry leaf\curry leaf 6.D
Operator   : HAFIZAH PSM
Acquired   : 19 Mar 2009 20:52   using AcqMethod GAHARU PHD.M
Instrument :   GCMSD
Sample Name: curry leaf 6
Misc Info :
Vial Number: 6

Library Search Report

Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 6.D
Acq On   : 19 Mar 2009 20:52
Operator : HAFIZAH PSM
Sample   : curry leaf 6
Misc   :
ALS Vial : 6   Sample Multiplier: 1

Search Libraries: C:\Database\NIST05a.L   Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	8.973	37.97	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	96
			Caryophyllene	59802	000087-44-5	95
2	9.806	8.47	C:\Database\NIST05a.L			
			1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	59900	1000062-61-9	87
			.alpha.-Caryophyllene	59848	006753-98-6	87
			.alpha.-Caryophyllene	59846	006753-98-6	83
3	10.383	6.86	C:\Database\NIST05a.L			
			Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]	60025	017066-67-0	95
			1H-3a,7-Methanoazulene, octahydro-1,9,9-trimethyl-4-methylene-, (1.alpha.,3a.alpha.,7.alpha.,8a.beta.)	60016	000508-55-4	95
			Patchoulene	59793	001405-16-9	91
4	10.426	20.08	C:\Database\NIST05a.L			
			Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	59990	000515-17-3	97
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	60063	000473-13-2	93
			(-)-Caryophyllene-(I1)	59854	1000156-13-1	90
5	13.235	3.36	C:\Database\NIST05a.L			
			12-Crown-4	40671	000294-93-9	35
			15-Crown-5	70738	033100-27-5	25
			15-Crown-5	70737	033100-27-5	25
6	15.734	4.91	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100942	017455-13-9	47
			12-Crown-4	40673	000294-93-9	47
			12-Crown-4	40672	000294-93-9	47
7	16.899	3.63	C:\Database\NIST05a.L			

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Library Search Report

```
Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 6.D
Acq On    : 19 Mar 2009  20:52
Operator  : HAFIZAH PSM
Sample    : curry leaf 6
Misc      :
ALS Vial  : 6    Sample Multiplier: 1


Search Libraries:   C:\Database\NIST05a.L          Minimum Quality:   0

Unknown Spectrum:   Apex
Integration Events: ChemStation Integrator - autoint1.e
```
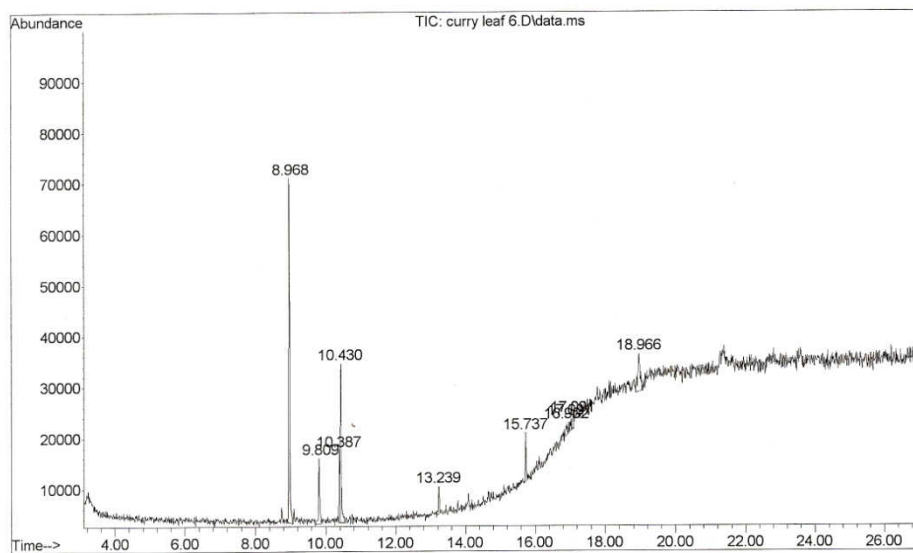
Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	72
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100941	017455-13-9	64
			Octaethylene glycol	161142	1000289-34-2	64
8	17.006	1.59	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100938	017455-13-9	72
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100941	017455-13-9	72
			15-Crown-5	70735	033100-27-5	72
9	17.091	1.97	C:\Database\NIST05a.L			
			15-Crown-5	70735	033100-27-5	80
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	78
			12-Crown-4	40671	000294-93-9	64
10	18.971	11.15	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100938	017455-13-9	72
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	64
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	64

APPENDIX H

GC-MS analysis: Research 7

File   :D:\Data\psm curry leaf\curry leaf 7.D
Operator   : HAFIZAH PSM
Acquired   : 19 Mar 2009 21:30   using AcqMethod GAHARU PHD.M
Instrument :   GCMSD
Sample Name: curry leaf 7
Misc Info   :
Vial Number: 7

Library Search Report

Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 7.D
Acq On   : 19 Mar 2009 21:30
Operator : HAFIZAH PSM
Sample   : curry leaf 7
Misc   :
ALS Vial : 7   Sample Multiplier: 1

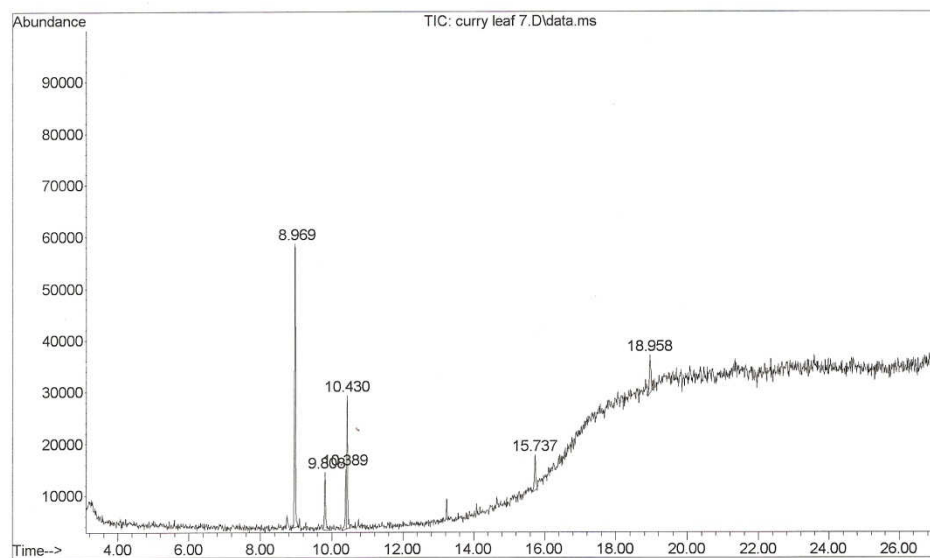Search Libraries: C:\Database\NIST05a.L   Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	8.973	43.35	C:\Database\NIST05a.L			
			Caryophyllene	59797	000087-44-5	98
			Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	59917	242794-76-9	94
			Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-	59912	013877-93-5	86
2	9.806	11.10	C:\Database\NIST05a.L			
			1,3,7-Octatriene, 3,7-dimethyl-	15240	000502-99-8	50
			Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	15352	000508-32-7	47
			3-Cyclohexene-1-methanol, .alpha.,4-dimethyl-	18151	055511-67-6	47
3	10.393	8.49	C:\Database\NIST05a.L			
			Ledol	72883	000577-27-5	35
			Veridiflorol	72904	1000122-17-3	35
			cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H)benzocycloheptene	59956	1000104-20-1	30
4	10.425	21.16	C:\Database\NIST05a.L			
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	60063	000473-13-2	95
			Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	59990	000515-17-3	89
			2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	59944	1000193-57-0	86
5	15.734	7.00	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl ether	186450	003055-98-9	59
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100942	017455-13-9	50
			15-Crown-5	70738	033100-27-5	50
6	18.960	8.90	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100942	017455-13-9	72
			15-Crown-5	70735	033100-27-5	64

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APPENDIX I

GC-MS analysis: Research 8

```
File        :D:\Data\psm curry leaf\curry leaf 8.D
Operator    : HAFIZAH PSM
Acquired    : 19 Mar 2009  22:07      using AcqMethod GAHARU PHD.M
Instrument  :   GCMSD
Sample Name: curry leaf 8
Misc Info   :
Vial Number: 8
```

Library Search Report

```
Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 8.D
Acq On    : 19 Mar 2009  22:07
Operator  : HAFIZAH PSM
Sample    : curry leaf 8
Misc      :
ALS Vial  : 8   Sample Multiplier: 1

Search Libraries:    C:\Database\NIST05a.L           Minimum Quality:   0

Unknown Spectrum:    Apex
Integration Events: ChemStation Integrator - autoint1.e
```

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	8.748	2.26	C:\Database\NIST05a.L			
			Guanidine, N,N-dimethyl-	1828	006145-42-2	9
			Acetonitrile, bromo-	8722	000590-17-0	9
			15-Crown-5	70738	033100-27-5	9
2	8.973	34.52	C:\Database\NIST05a.L			
			Caryophyllene	59797	000087-44-5	99
			Bicyclo[7.2.0]undec-4-ene, 4,11,11 -trimethyl-8-methylene-	59912	013877-93-5	94
			Bicyclo[5.2.0]nonane, 2-methylene- 4,8,8-trimethyl-4-vinyl-	59917	242794-76-9	91
3	9.806	6.96	C:\Database\NIST05a.L			
			.alpha.-Caryophyllene	59848	006753-98-6	97
			1,4,7,-Cycloundecatriene, 1,5,9,9- tetramethyl-, Z,Z,Z-	59900	1000062-61-9	97
			.alpha.-Caryophyllene	59849	006753-98-6	97
4	10.383	5.75	C:\Database\NIST05a.L			
			Naphthalene, 1,2,3,4,4a,5,6,8a-oct ahydro-4a,8-dimethyl-2-(1-methylet henyl)-, [2R-(2.alpha.,4a.alpha.,8 a.beta.)]-	60054	000473-13-2	96
			Azulene, 1,2,3,4,5,6,7,8-octahydro -1,4-dimethyl-7-(1-methylethenyl)- , [1S-(1.alpha.,4.alpha.,7.alpha.)]-	60026	003691-12-1	94
			1H-Cycloprop[e]azulene, decahydro- 1,1,7-trimethyl-4-methylene-, [1aR -(1a.alpha.,4a.beta.,7.alpha.,7a.b eta.,7b.alpha.)]-	60073	025246-27-9	93
5	10.425	15.02	C:\Database\NIST05a.L			
			Naphthalene, 1,2,3,4,4a,5,6,8a-oct ahydro-4a,8-dimethyl-2-(1-methylet henyl)-, [2R-(2.alpha.,4a.alpha.,8 a.beta.)]-	60063	000473-13-2	95
			Naphthalene, decahydro-4a-methyl-1 -methylene-7-(1-methylethylidene)- , (4aR-trans)-	59990	000515-17-3	95
			Azulene, 1,2,3,5,6,7,8,8a-octahydr o-1,4-dimethyl-7-(1-methylethenyl) -, [1S-(1.alpha.,7.alpha.,8a.beta.	60033	003691-11-0	93

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```
                        Library Search Report

   Data Path : D:\Data\psm curry leaf\
   Data File : curry leaf 8.D
   Acq On    : 19 Mar 2009  22:07
   Operator  : HAFIZAH PSM
   Sample    : curry leaf 8
   Misc      :
   ALS Vial  : 8    Sample Multiplier: 1


   Search Libraries:   C:\Database\NIST05a.L            Minimum Quality:    0

   Unknown Spectrum:   Apex
   Integration Events: ChemStation Integrator - autoint1.e

   Pk#    RT  Area%            Library/ID            Ref#     CAS#    Qual
   ─────────────────────────────────────────────────────────────────────────
                    )]-

    6  13.235  2.03 C:\Database\NIST05a.L
                    2-Pentanol, formate                 7859 058368-66-4 12
                    sec-Butyl nitrite                   4435 000924-43-6 12
                    2-Propanol, 1-(1-methylethoxy)-     8588 003944-36-3 12

    7  14.655  2.12 C:\Database\NIST05a.L
                    12-Crown-4                         40673 000294-93-9 64
                    12-Crown-4                         40672 000294-93-9 64
                    15-Crown-5                         70736 033100-27-5 64

    8  15.734  3.83 C:\Database\NIST05a.L
                    Octaethylene glycol monododecyl et 186450 003055-98-9 47
                    her
                    1,4,7,10,13,16-Hexaoxacyclooctadec 100939 017455-13-9 43
                    ane
                    Ethanol, 2-[2-(2-ethoxyethoxy)etho  42057 000112-50-5 43
                    xy]-

    9  16.802  4.88 C:\Database\NIST05a.L
                    1,4,7,10,13,16-Hexaoxacyclooctadec 100942 017455-13-9 86
                    ane
                    1,4,7,10,13,16-Hexaoxacyclooctadec 100938 017455-13-9 78
                    ane
                    15-Crown-5                         70737 033100-27-5 78

   10  16.888  5.38 C:\Database\NIST05a.L
                    Octaethylene glycol monododecyl et 186450 003055-98-9 64
                    her
                    1,4,7,10,13,16-Hexaoxacyclooctadec 100940 017455-13-9 59
                    ane
                    3,6,9,12,15-Pentaoxanonadecan-1-ol 120685 001786-94-3 53

   11  17.005  1.87 C:\Database\NIST05a.L
                    15-Crown-5                         70735 033100-27-5 80
                    1,4,7,10,13,16-Hexaoxacyclooctadec 100940 017455-13-9 72
                    ane
                    1,4,7,10,13,16-Hexaoxacyclooctadec 100942 017455-13-9 64
                    ane

   12  17.166  6.23 C:\Database\NIST05a.L
                    Octaethylene glycol monododecyl et 186450 003055-98-9 64
```
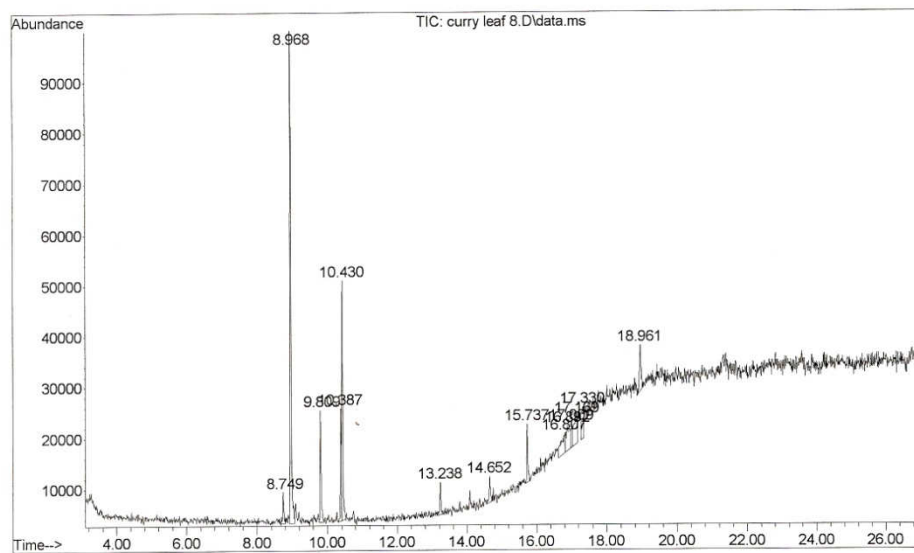
Library Search Report

```
Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 8.D
Acq On    : 19 Mar 2009  22:07
Operator  : HAFIZAH PSM
Sample    : curry leaf 8
Misc      :
ALS Vial  : 8   Sample Multiplier: 1

Search Libraries:   C:\Database\NIST05a.L              Minimum Quality:   0

Unknown Spectrum:   Apex
Integration Events: ChemStation Integrator - autoint1.e
```

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			her			
			Hexaethylene glycol monododecyl et	179948	003055-96-7	64
			her			
			Octaethylene glycol	161142	1000289-34-2	59
13	17.326	4.57	C:\Database\NIST05a.L			
			15-Crown-5	70735	033100-27-5	72
			12-Crown-4	40671	000294-93-9	64
			Hexaethylene glycol monododecyl et	179948	003055-96-7	59
			her			
14	18.960	4.57	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadec	100941	017455-13-9	72
			ane			
			1,4,7,10,13,16-Hexaoxacyclooctadec	100940	017455-13-9	64
			ane			
			1,4,7,10,13,16-Hexaoxacyclooctadec	100942	017455-13-9	64
			ane			

APPENDIX J

GC-MS analysis: Research 9

```
File       :D:\Data\psm curry leaf\curry leaf 9.D
Operator   : HAFIZAH PSM
Acquired   : 19 Mar 2009  22:44     using AcqMethod GAHARU PHD.M
Instrument :    GCMSD
Sample Name: curry leaf 9
Misc Info  :
Vial Number: 9
```

Library Search Report

Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 9.D
Acq On   : 19 Mar 2009 22:44
Operator : HAFIZAH PSM
Sample   : curry leaf 9
Misc   :
ALS Vial : 9   Sample Multiplier: 1

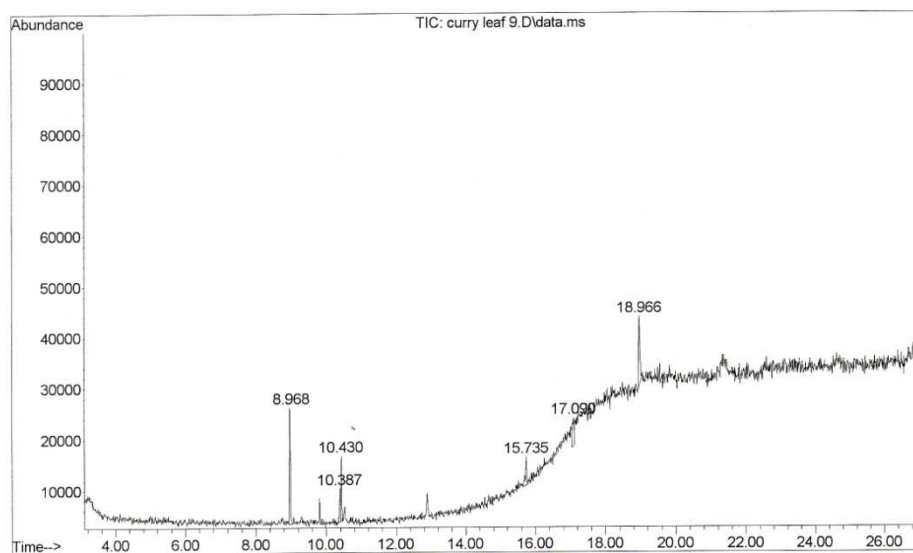Search Libraries: C:\Database\NIST05a.L   Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	8.973	29.18	C:\Database\NIST05a.L			
			1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-	59890	026560-14-5	43
			1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	72942	040716-66-3	38
			Spiro[4.4]nona-1,3-diene, 1,2-dimethyl-	21932	1000163-57-6	38
2	10.383	6.73	C:\Database\NIST05a.L			
			cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H)benzocycloheptene	59956	1000104-20-1	14
			2,3-Dimethylamphetamine	31416	075659-60-8	14
			15-Crown-5	70735	033100-27-5	10
3	10.426	16.35	C:\Database\NIST05a.L			
			Humulen-(v1)	59795	1000159-39-4	81
			cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H)benzocycloheptene	59956	1000104-20-1	58
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	60054	000473-13-2	55
4	15.735	10.16	C:\Database\NIST05a.L			
			15-Crown-5	70738	033100-27-5	64
			15-Crown-5	70736	033100-27-5	53
			15-Crown-5	70737	033100-27-5	53
5	17.091	12.43	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100942	017455-13-9	80
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100940	017455-13-9	72
			15-Crown-5	70735	033100-27-5	72
6	18.971	25.15	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100942	017455-13-9	72
			15-Crown-5	70738	033100-27-5	64
			15-Crown-5	70735	033100-27-5	64

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APPENDIX K

GC-MS analysis: Research 10

File   :D:\Data\psm curry leaf\curry leaf 10.D
Operator   : HAFIZAH PSM
Acquired   : 19 Mar 2009 23:21   using AcqMethod GAHARU PHD.M
Instrument :   GCMSD
Sample Name: curry leaf 10
Misc Info   :
Vial Number: 10

Library Search Report

Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 10.D
Acq On   : 19 Mar 2009 23:21
Operator : HAFIZAH PSM
Sample   : curry leaf 10
Misc   :
ALS Vial : 10   Sample Multiplier: 1

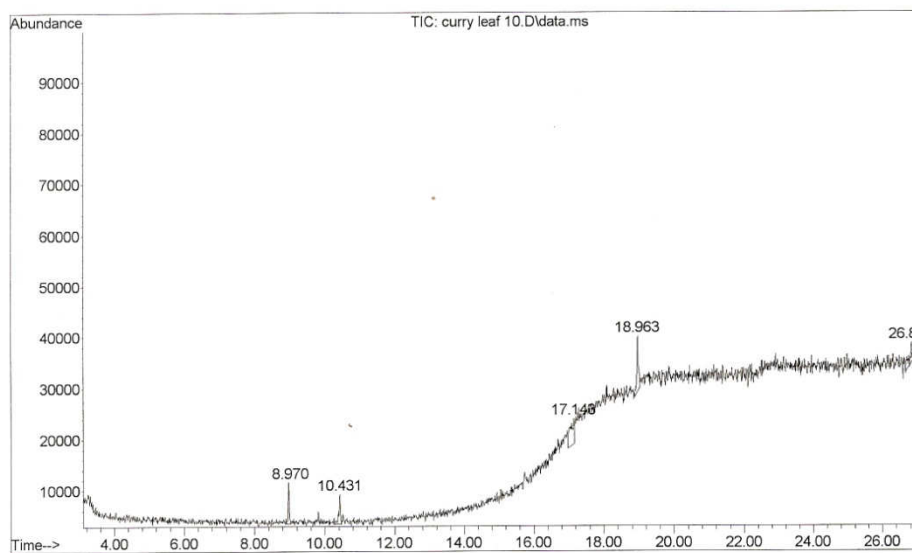Search Libraries: C:\Database\NIST05a.L   Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	8.973	14.25	C:\Database\NIST05a.L			
			Cyclohexene, 4-isopropenyl-1-metho xymethoxymethyl-	54294	1000195-93-2	12
			exo-7-(trans-1-Propenyl)bicyclo[4. 2.0]oct-1(2)-ene	21961	107983-42-6	10
			Cyclopropane, 1-(2-methylene-3-but enyl)-1-(1-methylenepropyl)-	30916	051567-07-8	10
2	10.436	13.50	C:\Database\NIST05a.L			
			2,4,6-Octatrien-1-ol, 3,7-dimethyl -(E,E)-	24183	089155-85-1	10
			Toluene, 4-chloro-2-fluoro-5-nitro	49485	018349-11-6	9
			Pyrido[2,3-d]pyrimidin-5(8H)-one, 2,4,7-trimethyl-	49175	161465-97-0	9
3	17.144	31.04	C:\Database\NIST05a.L			
			15-Crown-5	70738	033100-27-5	78
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	72
			15-Crown-5	70735	033100-27-5	64
4	18.960	25.98	C:\Database\NIST05a.L			
			15-Crown-5	70735	033100-27-5	53
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	47
			Heptaethylene glycol	139825	005617-32-3	47
5	26.822	15.22	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100938	017455-13-9	72
			Octaethylene glycol	161142	1000289-34-2	64
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100940	017455-13-9	64

APPENDIX L

GC-MS analysis: Research 11

File   :D:\Data\psm curry leaf\curry leaf 11.D
Operator   : HAFIZAH PSM
Acquired   : 19 Mar 2009   23:58   using AcqMethod GAHARU PHD.M
Instrument   :   GCMSD
Sample Name: curry leaf 11
Misc Info   :
Vial Number: 11

Library Search Report

Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 11.D
Acq On   : 19 Mar 2009 23:58
Operator : HAFIZAH PSM
Sample   : curry leaf 11
Misc   :
ALS Vial : 11   Sample Multiplier: 1

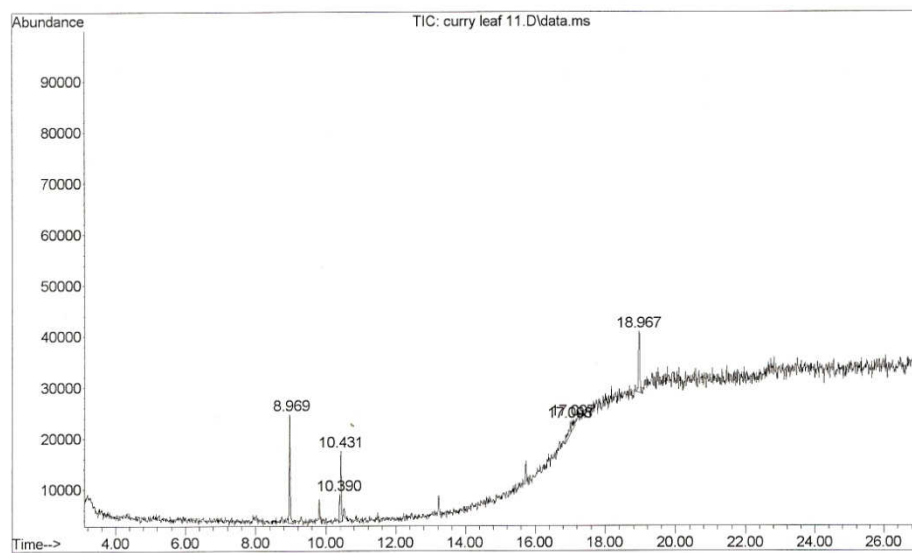Search Libraries: C:\Database\NIST05a.L   Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	8.973	32.26	C:\Database\NIST05a.L			
			Tricyclo[3.2.2.0]nonane-2-carboxyl ic acid	33248	033101-05-2	35
			Cyclopropane, 1-(2-methylene-3-but enyl)-1-(1-methylenepropyl)-	30916	051567-07-8	27
			6,6-Dimethyl-2-vinylidenebicyclo[3 .1.1]heptane	21955	039021-75-5	22
2	10.393	6.88	C:\Database\NIST05a.L			
			1H-Cycloprop[e]azulene, decahydro- 1,1,7-trimethyl-4-methylene-, [1aR -(1a.alpha.,4a.beta.,7.alpha.,7a.b eta.,7b.alpha.)]-	60076	025246-27-9	11
			Butanoic acid, heptafluoro-, 4-but oxy-4-oxobutyl ester	155049	055649-46-2	10
			Naphthalene, 1,2,3,4,4a,5,6,8a-oct ahydro-4a,8-dimethyl-2-(1-methylet henyl)-, [2R-(2.alpha.,4a.alpha.,8 a.beta.)]-	60054	000473-13-2	10
3	10.436	19.69	C:\Database\NIST05a.L			
			1R,3Z,9S-2,6,10,10-Tetramethylbicy clo[7.2.0]undeca-2,6-diene	59923	1000140-07-4	50
			Cyclopropa[d]naphthalen-2(4aH)-one , 1,1a,5,6,7,8-hexahydro-4a,8,8-tr imethyl-, [1aR-(1a.alpha.,4a.beta. ,8aS*)]-	59765	004677-90-1	35
			1H-Cycloprop[e]azulene, 1a,2,3,5,6 ,7,7a,7b-octahydro-1,1,4,7-tetrame thyl-, [1aR-(1a.alpha.,7.alpha.,7a .beta.,7b.alpha.)]-	60087	021747-46-6	35
4	17.005	8.42	C:\Database\NIST05a.L			
			15-Crown-5	70735	033100-27-5	72
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100938	017455-13-9	64
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100939	017455-13-9	64
5	17.102	5.56	C:\Database\NIST05a.L			
			15-Crown-5	70735	033100-27-5	80
			15-Crown-5	70738	033100-27-5	78
			15-Crown-5	70736	033100-27-5	64

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Library Search Report

```
Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 11.D
Acq On    : 19 Mar 2009  23:58
Operator  : HAFIZAH PSM
Sample    : curry leaf 11
Misc      :
ALS Vial  : 11   Sample Multiplier: 1

Search Libraries:   C:\Database\NIST05a.L          Minimum Quality:   0

Unknown Spectrum:   Apex
Integration Events: ChemStation Integrator - autoint1.e
```

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
6	18.971	27.18	C:\Database\NIST05a.L			
			Octaethylene glycol monododecyl et her	186450	003055-98-9	59
			15-Crown-5	70735	033100-27-5	58
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100938	017455-13-9	52

APPENDIX M

GC-MS analysis: Research 12

File   :D:\Data\psm curry leaf\curry leaf 12.D
Operator   : HAFIZAH PSM
Acquired   : 20 Mar 2009 00:35   using AcqMethod GAHARU PHD.M
Instrument :   GCMSD
Sample Name: curry leaf 12
Misc Info   :
Vial Number: 12

Library Search Report

Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 12.D
Acq On   : 20 Mar 2009 00:35
Operator : HAFIZAH PSM
Sample   : curry leaf 12
Misc   :
ALS Vial : 12   Sample Multiplier: 1

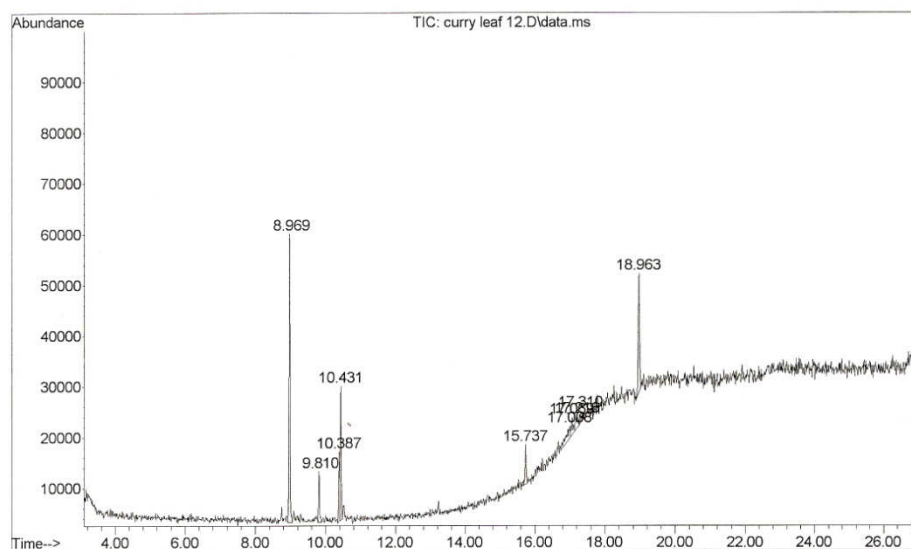Search Libraries: C:\Database\NIST05a.L   Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	8.973	32.43	C:\Database\NIST05a.L			
			Caryophyllene	59797	000087-44-5	99
			Caryophyllene	59802	000087-44-5	91
			Bicyclo[7.2.0]undec-4-ene, 4,11,11 -trimethyl-8-methylene-	59912	013877-93-5	90
2	9.806	6.36	C:\Database\NIST05a.L			
			1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	59900	1000062-61-9	96
			.alpha.-Caryophyllene	59848	006753-98-6	95
			.alpha.-Caryophyllene	59849	006753-98-6	94
3	10.383	6.46	C:\Database\NIST05a.L			
			1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	60076	025246-27-9	83
			1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]-	60094	017334-55-3	60
			1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-	60087	021747-46-6	49
4	10.426	15.60	C:\Database\NIST05a.L			
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	60063	000473-13-2	96
			2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	59944	1000193-57-0	91
			Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	59990	000515-17-3	90
5	15.735	5.34	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100938	017455-13-9	53
			15-Crown-5	70736	033100-27-5	53
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100941	017455-13-9	53

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```
                        Library Search Report

   Data Path : D:\Data\psm curry leaf\
   Data File : curry leaf 12.D
   Acq On    : 20 Mar 2009  00:35
   Operator  : HAFIZAH PSM
   Sample    : curry leaf 12
   Misc      :
   ALS Vial  : 12   Sample Multiplier: 1


   Search Libraries:   C:\Database\NIST05a.L          Minimum Quality:   0

   Unknown Spectrum:   Apex
   Integration Events: ChemStation Integrator - autoint1.e

   Pk#    RT  Area%          Library/ID          Ref#     CAS#    Qual
   ─────────────────────────────────────────────────────────────────────

    6  17.006  6.56 C:\Database\NIST05a.L
                     15-Crown-5                        70735 033100-27-5 80
                     15-Crown-5                        70737 033100-27-5 72
                     1,4,7,10,13,16-Hexaoxacyclooctadec 100938 017455-13-9 59
                     ane

    7  17.070  3.27 C:\Database\NIST05a.L
                     1,4,7,10,13,16-Hexaoxacyclooctadec 100941 017455-13-9 72
                     ane
                     15-Crown-5                        70735 033100-27-5 72
                     1,4,7,10,13,16-Hexaoxacyclooctadec 100942 017455-13-9 72
                     ane

    8  17.219  3.06 C:\Database\NIST05a.L
                     1,4,7,10,13,16-Hexaoxacyclooctadec 100942 017455-13-9 72
                     ane
                     15-Crown-5                        70736 033100-27-5 64
                     Octaethylene glycol              161142 1000289-34-2 59

    9  17.305  2.91 C:\Database\NIST05a.L
                     15-Crown-5                        70735 033100-27-5 86
                     1,4,7,10,13,16-Hexaoxacyclooctadec 100938 017455-13-9 86
                     ane
                     1,4,7,10,13,16-Hexaoxacyclooctadec 100942 017455-13-9 78
                     ane

   10  18.960 18.02 C:\Database\NIST05a.L
                     15-Crown-5                        70738 033100-27-5 38
                     12-Crown-4                        40671 000294-93-9 38
                     Octaethylene glycol monododecyl et 186450 003055-98-9 38
                     her
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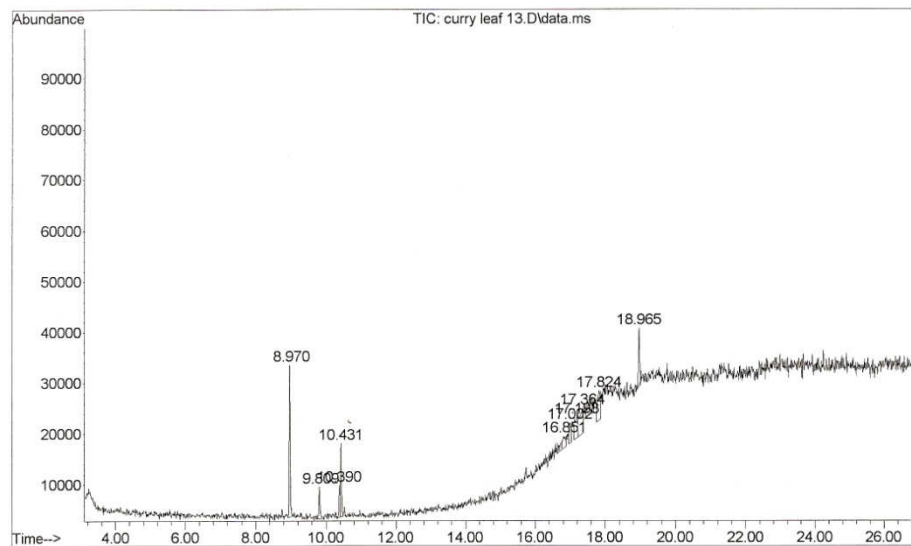
APPENDIX N

GC-MS analysis: Research 13

File   :D:\Data\psm curry leaf\curry leaf 13.D
Operator   : HAFIZAH PSM.
Acquired   : 20 Mar 2009   1:12   using AcqMethod GAHARU PHD.M
Instrument :   GCMSD
Sample Name: curry leaf 13
Misc Info   :
Vial Number: 13

Library Search Report

Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 13.D
Acq On   : 20 Mar 2009   1:12
Operator : HAFIZAH PSM
Sample   : curry leaf 13
Misc   :
ALS Vial : 13   Sample Multiplier: 1

Search Libraries: C:\Database\NIST05a.L   Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	8.973	21.99	C:\Database\NIST05a.L			
			Ethanone, 1-cyclopropyl-2-(3-pyridinyl)-	30169	057276-33-2	35
			2-Butenamide,N-phenyl-	30128	001733-40-0	35
			.beta.-Myrcene	15180	000123-35-3	25
2	9.806	5.69	C:\Database\NIST05a.L			
			1,5-Dimethyl-1-vinyl-4-hexenyl butyrate	74331	000078-36-4	27
			1,3,7-Octatriene, 3,7-dimethyl-	15240	000502-99-8	25
			Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl-	15356	000488-97-1	22
3	10.393	4.05	C:\Database\NIST05a.L			
			2,4,6-Octatrien-1-ol, 3,7-dimethyl-(E,E)-	24183	089155-85-1	17
			Cyclohexene, 4-isopropenyl-1-methoxymethoxymethyl-	54294	1000195-93-2	16
			Bicyclo[4.3.0]nonane, 7-methylene-2,4,4-trimethyl-2-vinyl-	59915	1000156-11-9	12
4	10.436	10.15	C:\Database\NIST05a.L			
			1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-	59928	072747-25-2	64
			1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	60075	025246-27-9	55
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	60054	000473-13-2	43
5	16.856	8.08	C:\Database\NIST05a.L			
			15-Crown-5	70735	033100-27-5	64
			15-Crown-5	70738	033100-27-5	59
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100940	017455-13-9	59
6	17.005	4.44	C:\Database\NIST05a.L			
			15-Crown-5	70735	033100-27-5	72
			15-Crown-5	70738	033100-27-5	64
			1,4,7,10,13,16-Hexaoxacyclooctadec	100941	017455-13-9	64

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Library Search Report

```
Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 13.D
Acq On    : 20 Mar 2009    1:12
Operator  : HAFIZAH PSM
Sample    : curry leaf 13
Misc      :
ALS Vial  : 13    Sample Multiplier: 1

Search Libraries:    C:\Database\NIST05a.L              Minimum Quality:    0

Unknown Spectrum:    Apex
Integration Events: ChemStation Integrator - autoint1.e
```

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			ane			
7	17.198	8.65	C:\Database\NIST05a.L			
			15-Crown-5	70735	033100-27-5	80
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100939	017455-13-9	80
			Octaethylene glycol monododecyl et her	186450	003055-98-9	80
8	17.369	13.84	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	80
			12-Crown-4	40671	000294-93-9	72
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100941	017455-13-9	64
9	17.828	12.75	C:\Database\NIST05a.L			
			15-Crown-5	70735	033100-27-5	64
			15-Crown-5	70737	033100-27-5	64
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	64
10	18.960	10.35	C:\Database\NIST05a.L			
			15-Crown-5	70735	033100-27-5	64
			Ethanol, 1-[2-[2-(1-methylethoxy)e thoxy]ethoxy]-	51683	029681-21-8	59
			1,4,7,10,13,16-Hexaoxacyclooctadec ane	100942	017455-13-9	50

APPENDIX O

GC-MS analysis: Research 14

File   :D:\Data\psm curry leaf\curry leaf 14.D
Operator : HAFIZAH PSM
Acquired : 20 Mar 2009   1:49   using AcqMethod GAHARU PHD.M
Instrument :   GCMSD
Sample Name: curry leaf 14
Misc Info :
Vial Number: 14
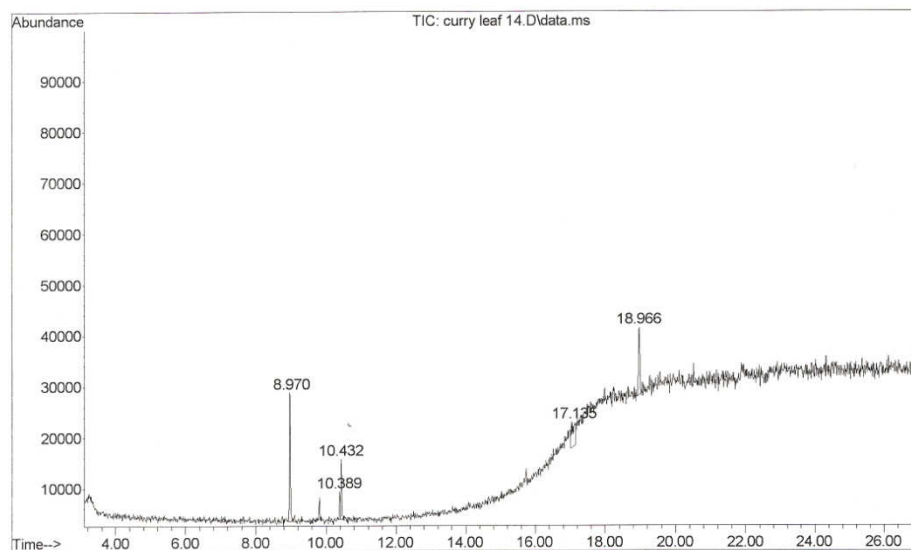
Library Search Report

Data Path : D:\Data\psm curry leaf\
Data File : curry leaf 14.D
Acq On   : 20 Mar 2009   1:49
Operator : HAFIZAH PSM
Sample   : curry leaf 14
Misc   :
ALS Vial : 14   Sample Multiplier: 1

Search Libraries: C:\Database\NIST05a.L   Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	8.973	33.59	C:\Database\NIST05a.L			
			Cyclohexane, 1,5-diethenyl-3-methyl-2-methylene-, (1.alpha.,3.alpha.,5.alpha.)-	30919	074742-35-1	46
			1,7-Octadiene, 2,7-dimethyl-3,6-bis(methylene)-	30903	016714-60-6	30
			3-Butenamide, 2-methyl-N-phenyl-	39715	080188-12-1	22
2	10.394	5.37	C:\Database\NIST05a.L			
			3-Hydroxy-N,N-dimethylpropanamide	8262	029164-29-2	17
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100938	017455-13-9	10
			2,4,6-Octatrien-1-ol, 3,7-dimethyl-(E,E)-	24183	089155-85-1	10
3	10.436	15.58	C:\Database\NIST05a.L			
			1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	60075	025246-27-9	38
			1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-	60087	021747-46-6	18
			1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-	59928	072747-25-2	18
4	17.134	20.05	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100942	017455-13-9	64
			12-Crown-4	40673	000294-93-9	59
			Hexaethylene glycol monododecyl ether	179948	003055-96-7	59
5	18.971	25.41	C:\Database\NIST05a.L			
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100938	017455-13-9	62
			1,4,7,10,13,16-Hexaoxacyclooctadecane	100939	017455-13-9	58
			15-Crown-5	70736	033100-27-5	53