Characterization of Essential Oil from Malaysian Curry Leaves

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Characterization of Essential Oil from Malaysian Curry Leaves

JAMIL BIN ROSLAN

Bachelor of Chemical Engineering

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UNIVERSITI MALAYSIA PAHANG

JANUARY 2014

I declare that this thesis entitled "*Characterization of Essential Oil from Malaysian Curry Leaves"* is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : .....

Name : Jamil bin Roslan

Date : .....

To my beloved father and mother, Roslan Bin Ramlee and Mdm Beche Yappe

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ABSTRACT

The objective of this research is to characterize the component in essential oils from M. koenigii leaves by hydro distillation 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 chemical constituent of M.koenigii essential oil that grow in Malaysia. In this research, the methods of grinding, extraction, separation and analysis are used and the sample is separated from water by using a chemical with different polarity 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 optimum time of extraction also determine by using different length of time with correspond to the yield of essential oil. The major component in M. koenigii leaves is caryophyllene with the optimum time of extraction is 9 hour with the yield of essential oil is 0.22%. The compositions of essential oil also show the potential on biological and repellent activity.

ABSTRAK

Objektif kajian ini adalah untuk mencirikan komponen dalam minyak pati dari daun M. koenigii oleh kaedah pengekstrakan hidro penulenan. Konstituen utama M. koenigii telah dilaporkan sebagai caryophyllene dan 3-carene yang bertanggungjawab untuk aroma dan rasa. Kajian ini telah memberi tumpuan kepada konstituen kimia M.koenigii minyak pati yang tumbuh di Malaysia. Dalam kajian ini, kaedah pengisaran, pengekstrakan, pengasingan dan analisis digunakan dan sampel dipisahkan dari air dengan menggunakan bahan kimia dengan polariti yang berbeza untuk mendapatkan minyak pati. Sampel telah dianalisis dengan menggunakan GC-MS untuk mengenal pasti komponen M.koenigii minyak pati. Dalam kajian ini, masa yang paling optimum pengekstrakan juga ditentukan dengan menggunakan panjang masa yang berbeza dengan hasil minyak. Komponen utama dalam M. daun koenigii adalah caryophyllene dengan masa yang optimum pengekstrakan ialah 9 jam dengan hasil minyak adalah 0.22%. Komposisi minyak pati juga menunjukkan potensi aktiviti biologi dan penghalau serangga.

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LIST OF ABBREVIATIONS

GC	-	Gas Chromatograph
MS	-	Mass Spectrometer
FKKSA	-	Fakulti Kejuruteraan Kimia & Sumber Asli
W	-	Watt
mN	-	Millinewton
cm	-	Centimeter
mmHg	-	Mlillimeter of Mercury
cP	-	Centipoise

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of study

Essential oils contain highly volatile substances that are isolated by a physical method or process from plants of a single botanical species. Essential oils are so termed as they are believed to represent the very essence of odor and flavor. Essential oil plants and culinary herbs include a broad range of plant species that are used for their aromatic value as flavorings in foods and beverages and as fragrances in pharmaceutical and industrial products. Some study also found that the essential oil have the properties against various haematophagous arthropods, and some of them being the basis of commercial repellent formulation.

M.koenigii or its common name curry leaf tree is the traditional spices used in south India for all curry preparation. The plant M.koenigii belonging to the family rutaceae is native to India and distributed in most part of southern Asia. Essential oil composition of 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 (Anonymous, 1962; Nigam et al., 1961; Prakash et al., 1974; Macleod et al., 1982; Hiremath et al., 1998). Even though most of the study of the essential oil of curry leaf show the potential in biological activity, but none of them show the potential in the repellent activity.

There are several methods to extraction of essential oil from herb and spices but in this study will use common hydro distillation extraction to extract the oil from the leaves. This extraction method is most used in the industry to obtain the essential oil from the plant. The step required for the preparation of material prior to extraction the essential oil from the leaves and uses the Gas chromatography mass spectrometer (GC-MS) to analyse the composition of essential oil in detail. The analysis of the GC-MS will determine the active compound of the repellent activity in the essential oil.

1.2 Problem statement

The M.koenigii or curry leaves have been used as traditional medicine in eastern Asia as tonics for dysentery, fever, carminative and the bites of poisonous animal and sources of flavors. Many research also show the component of the essential oil such antioxidant, tocopherol, β-carotene and lutein (Palanishwamy,2001) have the pharmaceutical potential such as experimental for treatment of diabetes in rat (Arulselvan and Subramanian, 2006) and antibacterial activity against various human pathogenic bacteria (Ningappa et al., 2009). (citation)

The insect repellent has been introduced in World War 2 as for a formula for clothing in military. In that time, the repellent was develop using a combination of 3 synthetic chemical with formulation of 6-2-2; six parts dimethyl phthalate, two parts Indalone and two parts Rutgers (Peterson and Coats, 2001) but the it fall to provide desired protection of military personnel development around the world. In1956, the insect repellent properties of N, N-diethyl-m-toluamide (DEET) were discover and success to gives a protection from insect. But the problem of this the toxic effect from this synthetic chemical have been recorded, including encephalopathy in children, urticarial syndrome, anaphylaxis, hypotension and decreased heart rate (Peterson and Coats, 2001).

The previous research shows that mostly the constituent of the essential oil consist of the several compound that can be used as the repellent active compound. In this study is conducted in order to characterize the essential oil of the curry leaf using the hydro distillation extraction method and also use the advance equipment for the analysis. It also to find the present of the component inside the essential that can be used as the repellent for the insect.

1.3 Objectives

The objective of this research is to characterize the chemical composition present on the curry leaves essential oil.

1.4 Scope of this research

The following are the scope of this research:

- Preparation and extraction of M.koenigii leaves' essential oil
- Analysis of chemical composition of the essential oil using GC-MS
- Investigate the potential of repellent activity that may have present in essential oil
- Analysis the toxicity on the curry leaves.

1.5 Organisation of this thesis

There are 5 chapters in this thesis. Following the introduction, the remaining chapter in this thesis are organized as follows:

Chapter 2 begins with a description about the curry leaf as well as the composition and the uses of the curry leaves with the previous researches that related to the study. In this chapter also included a general description on the essential oil with a brief explanation about the chemical constituent present in essential oil. The repellent activity of essential oil also discussed in this chapter.

Chapter 3 starts with the methodology to extract the essential oil from the curry leaves. It involve with the pre-treatment on the preparation in the extraction process. After the essential oil successful obtained, it continued to the analysis using GC-MS. Analysis on toxicity of the curry leaves using AAS also covered in this chapter.

Chapter 4 commences with the analysis of the result and discussion

Chapter 5 concludes the overall findings in this work. Some recommendations for future work are presented here.

CHAPTER 2

LITERATURE REVIEW

2.1 MURRAYA KOINIGII (L.) Spreng

M.koenigii (L.) spreng is the aromatic small tree, that belonging to the citrus family, Rutaceae that grow widely in East Asia and in peninsular Malaysia, two species of *Murraya* was found. Originated in Tirai region of Uttar Pradesh, India, it is now widely found in parts of India and also culvative in Sri Lanka, China, Australia and Pacific Island. The plant was spread to Malaysia, South Africa and Reunion Island by south Asian immigrants. The *M.koenigii* plant has been widely used in traditional medicine and as a tonic to treat dysentery, fever, influenza stomachic, stimulants, carminative and bites of poisonous animal. The leaves of *M.koenigii* also may be medicinally useful for the treatment or prevention of diabetes, cancer, and possibly cardiovascular dieses (Dasguptaa, Raoa, & Yadava, 2003). The leaves of *M.koenigii* are widely use in Indian cookery for flavoring food stuff and also became spices after drying. Below is the figure of *M.koenigii* leaves:

Figure 2.1 *M.koenigii* leaves

It is believed that the folks in the rural areas of Malaysia use curry leaves as traditional home remedies for flies' prevention. In Malaysia, it is usual for the local folks to plant the tree at the back yard of their house which makes it more convenient for daily uses. The use of curry leaves, scientifically known as *M.koenigii* and called as daun kari in Malaysia is not new. The leaves have been widely applied in the culinary field due to its aromatic scents and natural flavoring especially in traditional cuisines such as curries and sauces.

2.1.1 Chemical composition of M.koenigii leaves

The curry leaves are rich in minerals, Vitamin A and B, proteins, amino acid and alkaloids (Kong et al., 1986; Tee & Lim, 1991). It also rich with calcium but the present of high concentration of oxalic acid. By the analysis of concentrated essence of *M.koneigii* from Macleod & Pieris (1982) and Quan li et al., (1988) study, they obtain that the component of the essential oil of *M.koenigii* as table below:

Component	Rel. amount [%]	
	Macleod & Pieris (1982)	Quan li et al., (1988)
α-Pinene	-	38.4
Camphene	-	0.5
Sabinene	-	0.3
β-Thujene	4.3	-
β-Pinene	0.7	6.3
α-phellandrene	0.6	-
Limonene	2.1	3.5
β-Phellandrene	6.1	0.5
trans-β-Ocimene	1.9	0.5
τ-Terpinene	-	0.3

α-Cubebene	0.2	-
α-Copaene	0.9	-
β-Elemene	6.8	0.8
β-Caryophyllene	28.7	12.9
Humulene	-	3.5
β-Cubebene	-	2.1
τ-Elemene	-	10.1
δ-Cadinene	-	1.1
β-Gurjunene	21.4	-
ε-Muurolene	0.4	-
β-Bisabolene	2.8	-
τ-Cadinene	2.5	-
α-Selinene	2.9	-

Table 2.1 Components of the essential oil of *M.koenigii*; only those substance are included that make up more than 0.2% of the oil and have been unequivocally

The composition of the essential oil *of M.koenigii* may be different at the different place. Like Sri Lanka, the composition of 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 whereas the composition oil from Chinese was reported contain α- and *β-pinenes*, *β-caryophyllene* and *γ-elemene* as main constituents. For the composition oil from Malaysia, it was shown to be rich in monoterpenes and oxygenated monoterpened (ca. 85%) with *α-pinene, limonene, β-phellandrene*, *terpinen-4-ol* and *β-caryophyllene* as the main contents (Bhattacherya et al., 1982). From Chowdhury study reported that the leaves on hydro distillation gave 0.5% essential oil on fresh weight basis, having dark yellow color, spicy odor and pungent clove- like taste. It has following characteristics:

Table 2.2 Characteristic of curry leaves essential oil

Specific gravity (25^0C)	00.9748
Refractive index (25^0C)	1.5021
Optical rotation (25^0C)	+ 4.8
Saponification value	5.2
Saponification value after acetylation	54.6
	66.3%
Moisture	6.1%
Protein	1.0%
Fat (ether extract)	6.4%
Fiber	4.2%
Mineral matter	801mg/100g of edible portion
Calcium	600 mg/100 g of edible portion
Phosphorus	3.1 mg/100 g of edible portion
Iron	126000 IU/100 g
Carotene (as vitamin A)	2.3 mg/100 g
Nicotinic acid	4 mg/ 100 g
Vitamin C	absent
Thiamine and riboflavin	

2.1.2 Uses of M.koenigii leaves

The different part of the *M.koenigii* plant has been use as a folk medicine in India. The leaves, bark and the root are used in indigenous medicine as tonic, stomachic, anthelmintic, analgesic and as stimulative, appetizing and carminative agent for treating piles, influenza, fever, itching, dropsy, bronchial asthma, eruptions and bites of poisonous animal, dysentery, diarrhea, body aches, fresh cuts, kidney pains and vomiting (Kumar, Sharma, Tiwari &Kumar, 1999; Rana,Juyal,,Rashmi&Blazquez,2004).

Ningappa et al., 2009 reported that the antioxidant protein (APC) that isolated from the *M.koenigii* leaves was exhibited a board spectrum of antibacterial activity against human pathogenic bacterial, compare to the commercial antibiotics in their study. The APC show that the antibacterial activity against Escherichia coli and Staphylococcus aureus. The results show that, the clear zone of inhibition (20-25 mm) was form by using 0.15µg of APC using agar diffusion method.

The other studies also show that the antioxidant properties inside the *M.koenigii* leaf are useful as anti-obesity for the high fatty diet rats (Sachin & Dinesh., 2012). There were lower of hydro peroxides, conjugatedienes and free fatty acid in the liver and hearth of the rats supplemented with *M.koenigii* leaves compared to rats fed on the high fat diet alone. As the *M.koenigii* leaves was supplemented, the activities of superoxide dismutase, catalase and glutathione transferase were increase in the hearth and liver of the rats as well as the increasing activities of glutathione reductase, glutathione peroxidase and glucose-6-phosphate dehydrogenase in the liver. At the same time the concentration of gluthathione was decrease in the liver. Thus the supplementing a high fat diet with 10% of *M.koenigii* leaf can prevent the formation of free radicals and maintain the tissue at normal level

2.2 Essential Oil

Essential oil is the concentrated liquid that generally steam or hydro-distilled from leaves, flowers, roots of plant and trees that containing the volatile aroma compound that can be represent the characteristic flavor to the plant part. These compound usually responsible the aroma and the flavor that associated with the herbs, spices and perfume. The formation and accumulation of essential oil in plant have been thoroughly review by Crotaeu (1986), Guenther (1972) and Runeckles and Mabry (1973). The essential oil also is chemically primarily composed of mono – and sesquiterpense and aromatic polypropanoids synthesized via mevalonic pathway for

terpenes and shikmic acid pathway for aromatic poly – propanoids (Runeckles and Mabry, 1973).

Essential also well known to have the range that useful in biological properties against insect, pests, fungal, bacterial and viral diseases (Ibrahim, 2001). In addition, they are more readily degraded in the environment than synthetic compound. Several report also shown that the essential oil from the plant have control on growth of pathogenic strains (Ruberrto et al.,2000 ; Singh et al.,2002 ; Abed, 2007).

The essential oil that mostly in the aromatic plant are the most part volatile and thus, lend themselves to the several technic or method of extraction such as hydro distillation, water and steam distillation, direct steam distillation, and solvent extraction. The specification of the method depends on the plant material that will be distillate to get the desire end-product. Even though some of the less stable complex mixture of organic constituent inside the essential oil may undergo chemical alteration under the steam distillation process, but it is possible that longer the distillation may give more complete oil. Mostly, essential oil is clear, however there are some exceptions. For the example the essential oil of *M.koenigii* having dark yellow color.

2.2.1 Chemical constituent of Essential Oil

An essential oil contains more than 200 chemical components, but some are many times more complex. Essential oils consist of chemical compounds which have hydrogen, carbon and oxygen as their building blocks. They can be essentially classified into two groups:

- Volatile fraction: Essential oil constituting of 90–95% of the oil in weight, containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.

- Nonvolatile residue: This comprises 1–10% of the oil, containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids.

However the properties of these components can change. For example, the components from the oils extracted from plants can change according to how, when and where these plants are grown and harvested. The constituents can be again subdivided into 2 groups, such as the hydrocarbons which are made up of mostly terpenes and the oxygenated compounds which are mainly alcohols, aldehydes, esters, ketones, phenols and oxides. Some of the common components are listed below along with their properties.

Hydrocarbon:

Building blocks of Essential Oil are hydrogen and carbon. Basic Hydrocarbon found in plants is isoprene having the following structure.

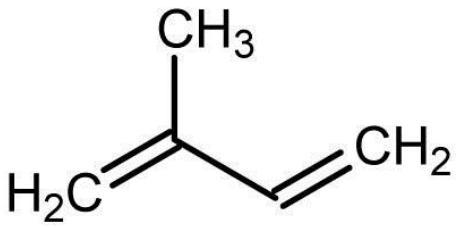

Figure 2.2: Isoprene

- Terpenes:

These components generally have names ending with "ene". Some of them are limonene, pinene, piperene, camphene etc. These components act as an antibacteria l, antiviral, anti-inflammatory, antiseptic, antiviral and bactericidal. These are further categorized intomonoterpene, sesquiterpene and diterpenes. When two of the isoprene units are joined head to tail, the result is a monoterpene, when three are joined, it's a sesquiterpene and similarly four linked isoprene units are diterpenes.

- Monoterpene [$C_{10}H_{16}$]:

Monoterpene are naturally occurring compounds, the majority being unsaturated hydrocarbons (C10).But some of their oxygenated derivatives such as alcohols, Ketones, and carboxylic acids known as monoterpenoids

Two isoprene units are present in these branched-chain C10 hydrocarbons and are widely distributed in nature with more than 400 naturally occurring monoterpenes. Moreover, besides being linear derivatives (Geraniol, Citronellol), the monoterpenes can be cyclic molecules (Menthol – Monocyclic; Camphor – bicyclic; Pinenes (αand β) – Pine genera as well.

Thujone (a monoterpene) is the toxic agent found in Artemisiaabsinthium (wormwood) from which the liqueur absinthe, is made. Borneol and camphor are two common monoterpenes. Borneol, derived from pine oil is used as a disinfectant and deodorant. Camphor is used as a counterirritant, anesthetic, expectorant, and antipruritic, among many other uses.

- Sesquiterpene:

Sesquiterpenes are biogenetically derived from farensyl pyrophosphate and in structure may be linear, monocyclic or bicyclic. They constitute a very large group of secondary metabolites, some having been shown to be stress compounds formed as a result of disease or injury. These are having properties like anti- inflammatory, anti-septic, analgesic and anti-allergic.

- Sesquiterpene Lactones:

These are available as farnesene in chamomile and lavender. They not only have proved to be of interest from chemical and chemotaxonomic point of view, but also possess many antitumor, anti- leukemia, cytotoxic and antimicrobial activities. Chemically the compounds can be classified according to their carboxylic skeletons; thus, guaianolides, pseudoguaianolides, eudesmanolides, eremophilanolides, xanthanolides, etc. can be derived from the germacranolides. Structural features of all these compounds are associated with much of the biological activity. For example beta-caryophyllene in basil and black pepper.

- Diterpenes:

Isoprene has been an integral part in most of the components as there are four isoprene units in diterpenes. By Steam Distillation method we cannot detect diterpenes as this molecule is too heavy to allow for evaporation, so it is rarely found in distilled essential oils. Diterpenes occur in all plant families and consist of compounds having a C20 skeleton.

There are about 2500 known diterpenes that belong to 20 major structural types. Derivatives of diterpenes are plant hormones Gibberellins and phytol occurring as a side chain on chlorophyll. The biosynthesis occurs in plastids and interestingly mixtures of monoterpenes and diterpenes are the major constituents of plant resins. In a similar manner to monoterpenes, diterpenes arise from metabolism of geranyl geranyl pyrophosphate (GGPP). Therapeutically diterpenes have limited importance and are used in certain sedatives (coughs) as well as in antispasmodics and anxiolytics.

- Alcohols:

Naturally Alcohols exist either as a free compound or combined with a terpenes or ester. When terpenes are attached to an oxygen atom, and hydrogen atom, the result is an alcohol. When the terpene is monoterpene, the resulting alcohol is called a monoterpenol. Alcohols are not and are suitable to body or skin. Therefore, they are considered safe to use.Some of these properties are anti-septic, anti-viral, bactericidal and germicidal. Some of the examples are linalool found in ylang-ylang and lavender, geraniol in geranium and rose and nerol in neroli.

- Aldehydes:

Aldehyde containing Essential O ils are effective in treating candida and other fungal infections. Some of these properties are anti- fungal, anti- inflammatory, anti-septic, anti-viral, bactericidal, disinfectant, and sedative. Aldehydes are present as citral in lemon, Citronellal in lemongrass, lemon balm and citrus eucalyptus.

- Acids:

Generally Organic acids are found in very small quantities in their free state within essential oils. Plant acids act as components or buffer systems to control acidity. These also act anti- inflammatory. Examples are cinnamic and benzoic acid in benzoin, citric and lactic.

- Esters:

Esters are formed through the reaction of alcohols with acids. Essential oils containing esters are used for their soothing, balancing effects. Because of the presence of alcohol, they are effective antimicrobial agents. Medicinally, esters are characterized as antifungal and sedative, with a balancing action on the nervous system. They generally are free from precautions with the exception of methyl salicylate found in birch and wintergreen which is toxic within the system. Examples are linlyl acetate in bergamot and lavender and Geranyl formate in geranium.

- Ketones:

Ketones found in plants are used for upper respiratory complaints. They assist the flow of mucus and ease congestion. Essential oils containing ketones are beneficial for promoting wound healing and encouraging the formation of scar tissue. Ketones are (not always) very toxic. The most toxic ketone is Thujone found in mugwort, sage, tansy, thuja and wormwood oils. Other toxic ketones found in essential oils are pulegone in pennyroyal, and pinocamphone in hyssops. Some non-toxic ketones are jasmone in jasmine oil, fenchone in fennel oil, carvone in spearmint and dill oil and menthone in peppermint oil.

- Lactones:

  Lactones are known to be particularly effective for their anti-inflammatory action, possibly by their role in the reduction of prostaglandin synthesis and expectorant actions. Lactones have an even stronger expectorant action than ketones.

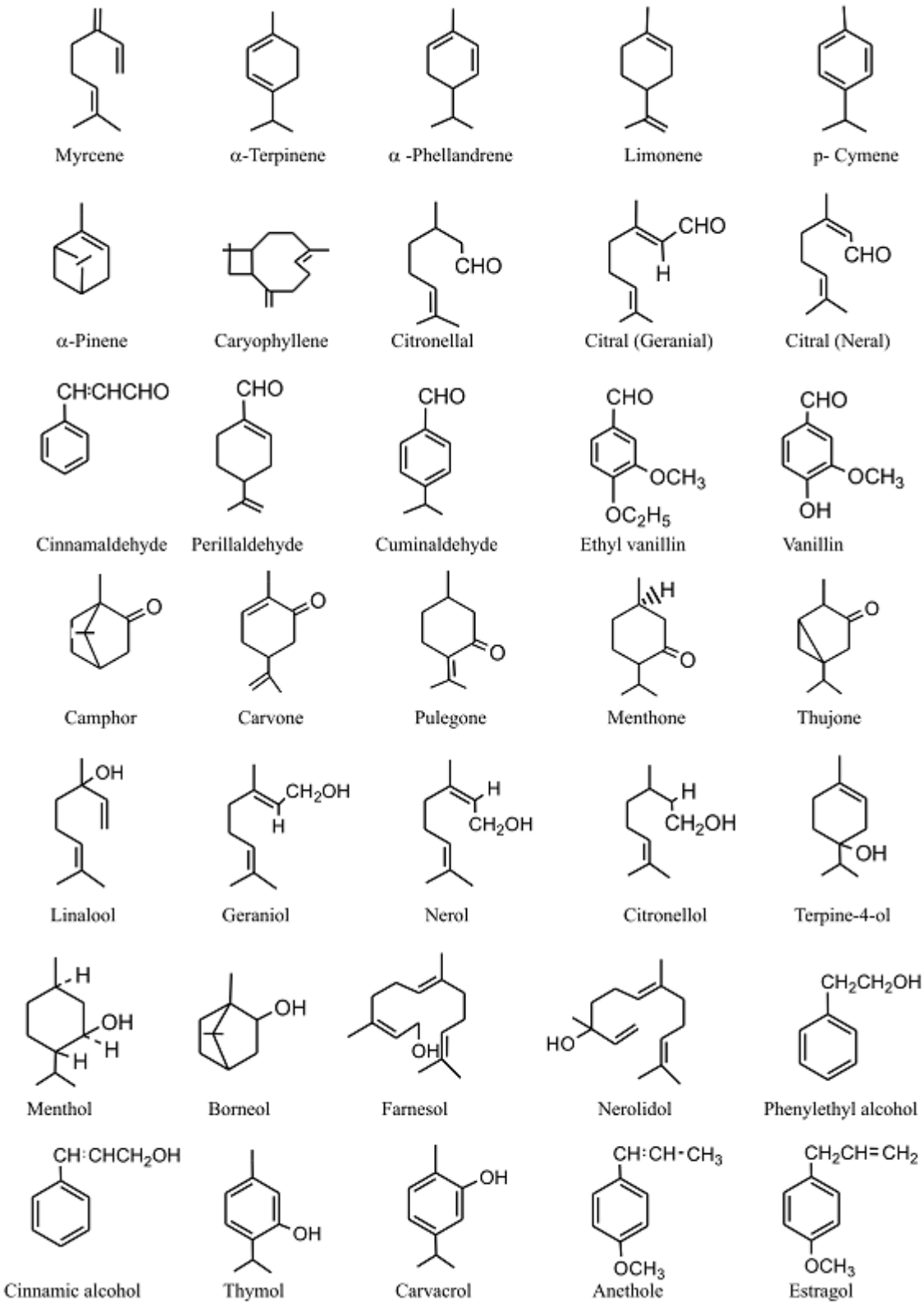

Figure 2-3 (a): Chemical constituent in essential oil

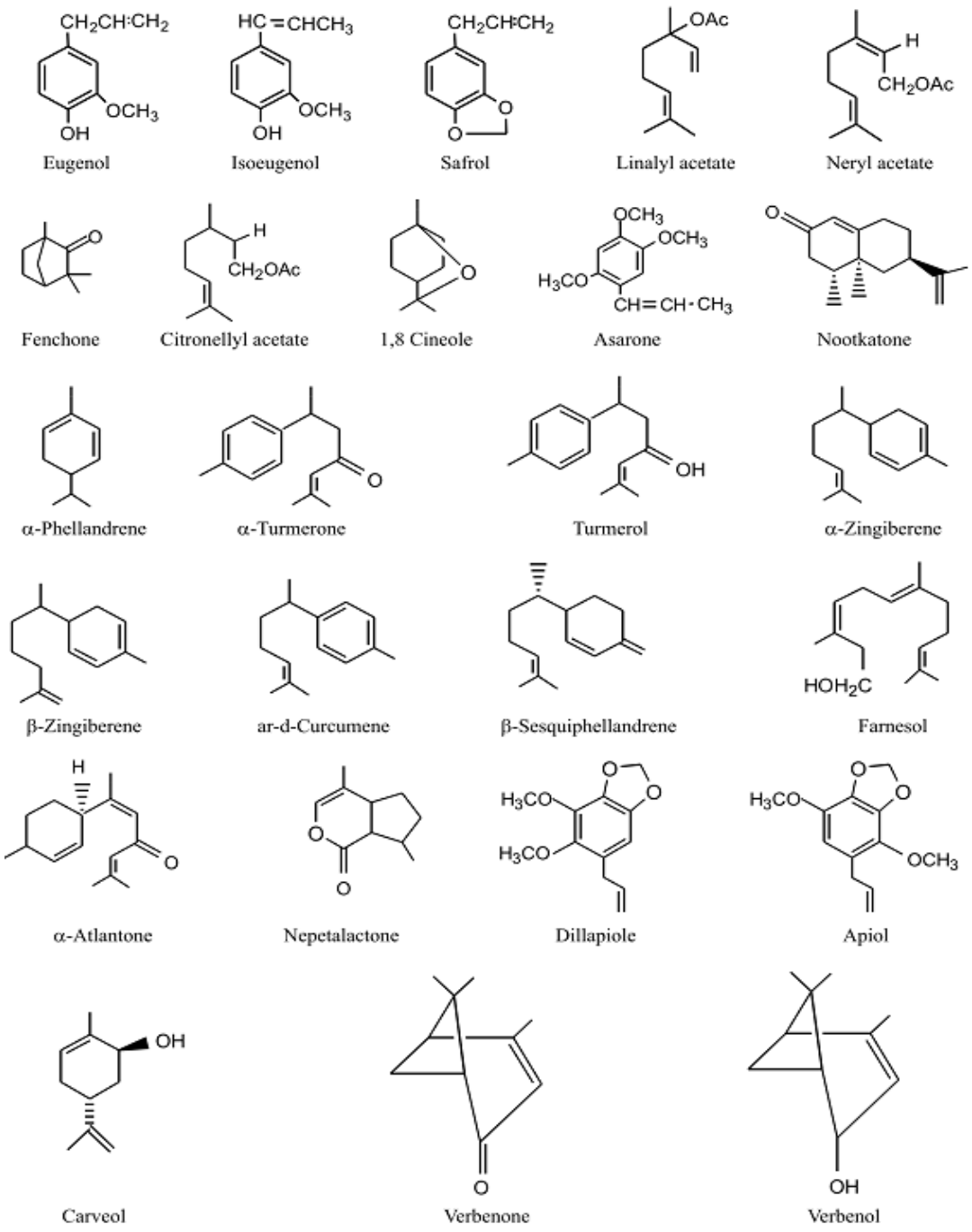

Figure 2-3 (b): Chemical constituent in essential oil

2.3 Insect repellent

From the previous study (Blackwell et al., 2003; Choochote et al., 2007), repellent can be define as are substance that act locally of at a distance, deterring an arthropod from flying to, landing or biting human or animal skin. It shows that, insect repellent work as a vapor barrier in the skin to protect from the insect coming to the surface of the skin (Brown and Hebert, 1997). Before the World War, there are only four repellent principles:

- Oil of citronella- sometimes use as hair dressing for head lice
- Dimethyl phthalate- discover in 1929
- Indalone- which was pattern in 1937
- Rutgers 612- which became available in 1939

In 1953, the insect repellent properties of N, N-diethyl-m-toluamide (DEET) were discovered and at 1956, first DEET product was introduced. Since that time, DEET is still the most widely used mosquito repellent. Even though the DEET is the most efficient as the repellent, but the toxic effect was recorded, including encephalopathy in children, urticarial syndrome, anaphylaxis, hypotension and decreased heart rate. Because of the toxic effect of the DEET, several other compound have been evaluated for the repellent activity as show in Figure 2.3 but none of them have had the commercial success of DEET.

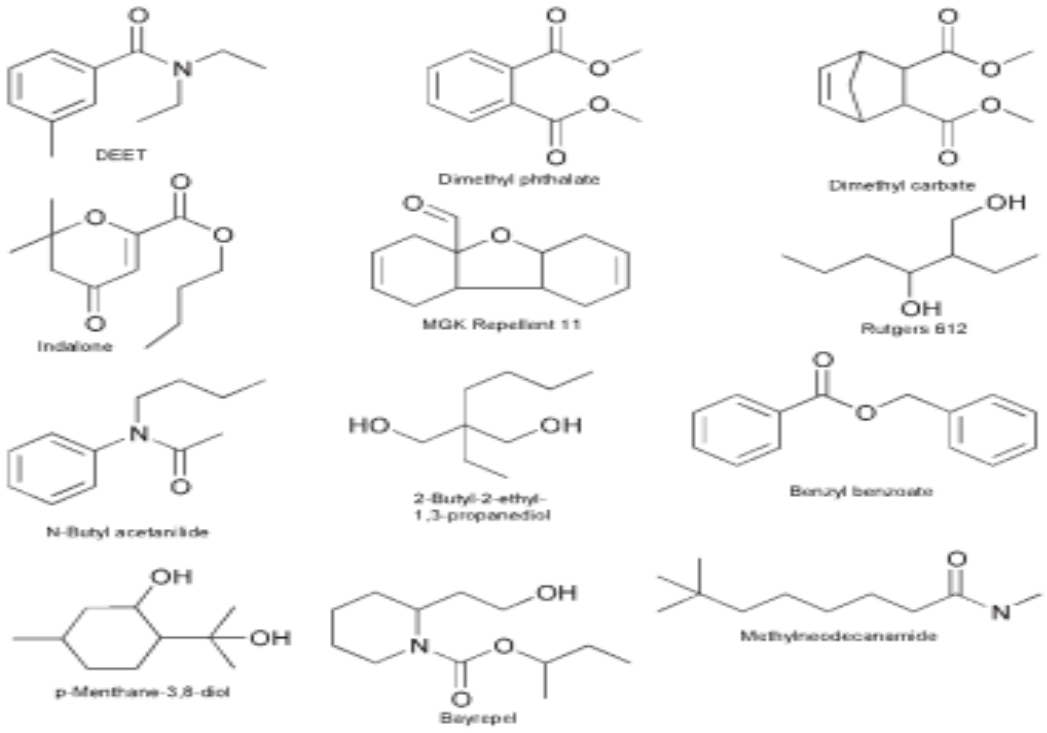

Figure 2-4: Structure of several insect repellent

2.3.1 Repellent activity of essential oil

Synthetic chemicals have been widely used as the main compound in the commercial insect repellent because of the effectiveness and persistent in human skin. It functions as an active ingredient to provide vapor barrier on the surface of the skin as a protection from insect. There are several synthetic chemical compounds have been evaluated for the repellent activity but none of them have had the commercial success as N, N-diethyl-m-toluamide (DEET). To date, DEET is found to be the most efficient material that is being used as repellent, but the toxic effect cause by its application that will contribute to health and environmental risks are intolerable.

Investigation essential oil as insect repellent ingredients for application on human skin has increasing due to their low toxicity, comparable efficacy and customer approval (Katz et al., 2008). Besides that, it also to find the new alternatives to replace the synthetic repellent that higher toxic value even though the effectiveness of their repellent activity to protect from the insect is higher. The

monoterpenes such as α-pinene, cineole, limonene, terpinolene, citronellol, camphor and thymol are the common constituent that in the essential oil as presenting mosquito repellent (Ibrahim and Zaki, 1998; Jaenson et al., 2006; Park et al., 2005; Yang et al., 2004). Even though some plant-based repellent are comparable to or even better than synthetic repellent, however, essential oil repellent tend to being short-lived in their effectiveness, which depends on their volatility.

The monoterpenes such as α-pinene, cineole, limonene, terpinolene, citronellol, camphor and thymol are the common constituent that in the essential oil as presenting mosquito repellent (Ibrahim and Zaki, 1998; Jaenson et al., 2006; Park et al., 2005; Yang et al., 2004). The research that related to the repellency of essential oil has demonstrated on increasing interest of finding the natural alternative to synthetic repellent (Nerio, Olivero-verbel and Stashenko, 2009). The study essential oil of Cymbopogon excavates plant shows that it will give 100% repellency for 2 hour duration against Anapheles arabiensis and the repellency decrease to 59.3% after 4 hour (Govere et al., 2000). Properties of essential oil extracted from genus Eucalyptus are also well documented. These presented high repellent against Ixodes ricinus, Ades albopictus, Mansonia and P.humanus capitis (Jaenson et al., 2006; Yang and Ma. 2005; Hadis et al., 2003; Toloza et al., 2008). Neem oil from Azadirachta indica, when formulated as 2% in coconut oil, it provide a complete protection for 12 hours from Anopheles mosquitos (Sharma et al., 1993).

2.4 Extraction of essential oil

In these days, various methods have being employed for extracting essential oil from different spices and herbs. From journal, hydro distillation, steam-distillation and solvent extraction are common methods being used to extract *M. koenigii* leaves. But in this study, hydro distillation will be used to extract essential oil of *M. koenigii* from their leaves.

In the manufacture of essential oils using the method of hydro distillation or water distillation, the botanic material is completely immersed in water and the still is brought to boil. This method protects the oils extracted to a certain

degree since the surrounding water acts as a barrier to prevent it from overheating. When the condensed material cools down, the water and essential oil is separated and the oil decanted to be used as essential oil. The water that is separated in this process is also used and is marketed as "floral water" (also called hydrosol or sweet water) - such as rosewater, lavender water and orange water (Biljana & Aleksandar, 2004).

Water distillation can be done at reduced pressure (under vacuum) to reduce the temperature to less than 100 degrees, which is beneficial in protecting the botanical material, as well as the essential oils. Neroli oil, which is sensitive to heat, can therefore be successfully extracted using this method.

If extended exposure to hot water is not indicated for a particular plant - such as lavender, it is best to find an extraction method better suited. Any botanical material that contains high amounts of esters do not take well to this extraction method, since the extended exposure to hot water will start to break down the esters to the resultant alcohols and carboxylic acids. Distillation converts the volatile liquid (the essential oils) into a vapor and then condenses the vapor back into a liquid - it is the most popular, and cost effective method in use today in producing essential oils (Biljana & Aleksandar, 2004).
(Gambar hydro distillation)

The downside of distillation is the fact that heat is used in this extraction method, which makes it totally unacceptable for use on very fragile material, or where the oils are extracted with great difficulty. When this method of extraction is applied, great care has to be taken with the temperature and length of exposure of the heat to prevent damage to the oils. Today, most common essential oils, such as lavender, peppermint, and eucalyptus, are distilled. Raw plant material, consisting of the flowers, leaves, wood, bark, roots, seeds, or peel, is put into an alembic (distillation apparatus) over water. As the water is heated, the steam passes through the plant material, vaporizing the volatile compounds. The vapors flow through a coil where they condense back to liquid, which is then collected in the receiving vessel (Biljana & Aleksandar, 2004).

CHAPTER 3

METHODOLOGY

3.1 Material

Fresh leaves of *M.koenigii* were collected from the plants grown in local area during May 2013. The freshly leaf was analyse at the same day of purchase, while the other sample were analysed seven days after purchase when all the treatments were completed. The oven-dried sample was put in glass bottle when completely dried to imitate the traditional method of home storage for culinary use.

3.2 Pre-treatment Method

The pre-treatment methods which were grinding, and weighing should be carry out before proceed to the extraction method. The blender was used to ground the *M.koenigii* leaves to reduce the size into a fine particle. Then the fine particle of *M.koenigii* leaves was weighed by using high precision electronic balance.

3.2.1 Weighing

All the samples were then weight using high precision electronic balance. For this study, the samples were weighed 100 gram for each of extraction process.

3.2.2 Grinding

After the weighing process, the fresh curry leaf was grounded using blander. By this process, it can optimize the extraction process by increase the surface area for contact during the extraction process.

3.3 Hydro distillation extraction method

After the all preparation pre-treatment, the hydro distillation process was carried out. The process was beginning with the place the 100 gram of material into 1000ml of the round flask with 800 ml of deionize water. The apparatus for the hydro distillation was set up as show in figure below. After that the process begins with heat the material sample until it reaches the boiling point of water (100^0C). It took 30 minute for the water and the sample reach the boiling point. After reach the boiling point, the extraction was start with the different parameter that already set.

3.4 Separation method

After extraction process, the mixture of *M. koenigii* essential oil and water need to be separate by using the different chemical solvent. Take oil water mixture and put into separating funnel. Add solvent diethyl ether in it about 100 ml in separating funnel. Shake gently. Separate the water layer and collect the mixture ether and oil into beaker. Ether oil should contain minimum amount of water (less than one drop). Put some anhydrous sodium sulphate in it. It will absorb traces of water. Add only little with spatula. Remove the anhydrous sodium sulphate using filter paper and leave the mixture oil and ether under fume hood to vaporize the ether. (gambar)

3.5 Analysis method

The essential that obtain after separation process will dilute with solvent and placed in clean vial. After that it will be analyze for the characterization for the essential oil and also for the repellent activity.

3.5.1 Gas chromatography- mass spectrometer (GC-MS) analysis

In characterization of essential oil, Gas Chromatography-Mass Spectrometer (GC-MS) will be used to determine the composition of the essential oil that may present as well as the bioactive compound. The essential oil that obtained after separation process will dilute with methanol with the concentration of 1% and the solution is syringe to the clean vial. After that, the sample finally ready to be analyze by using GC-MS Agilent 6890 gas chromatography/mass spectrometry 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 of HP-5MS nonpolar capillary column. The column temperature was at 60 ^{0}C for injection, maintained for 1 minute then heated to 250 ^{0}C at 3 ^{0}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 1l/hr flow rate. Temperature of injection was maintained at 250 ^{0}C. at solvent delay time of 4 min was used.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Introduction

The effect of the operating parameter such as time of extraction due to the quantity and quality of the essential oil extracted by means of hydro distillation extraction was studied using *M.koenigii* leaves as the plant material. To thoroughly investigate the effect of operating parameter on the yield and quality of the oil extract, the focus area in analysis of the result was directed towards the following listed point:

- Quantity of the oil extracted (Yield of the essential oil)
- Quality of the extracted (Characterization of essential oil)
- Potential of repellent activity
- The toxicity of the essential oil
- Potential in biological activity

The results are organized into different and chromatograms with their corresponding mass spectra and raw data.

4.2 Hydro distillation extraction

In this experiment, hydro distillation method was used to extract the essential oil from the curry leave. The purpose of this experiment to determine the highest yield of the essential oil that can be extract from the leave at the most optimum time. The extraction was carried out at a temperature of 100°C and it takes 30 minute to reach the boiling point. This experiment successfully done get the essential oil

4.3 Yield essential oil of curry leaf

The yield of each sample was calculated to obtain the optimum quantitative result based of the rate of the extraction time. This investigation was conducted with eight different extraction time namely : 3 hour, 4 hour, 5hour, 6hour, 7hour, 8hour, 9hour, 10hour. Equation below was used to calculate the percentage of yield of oil.

$$Yield\ of\ oil\ (\%) = \frac{weight\ of\ oil\ obtain\ (g)}{weight\ of\ sample\ used\ (g)} \times 100\ \%$$

Table 4.1: Percentage yield of oil with different time of extraction

Time, hour	Yield, %
3	0.060
4	0.082
5	0.118
6	0.153
7	0.192
8	0.207
9	0.220
10	0.167

From table 4.1, the result show at 3 hour of the extraction process, the yield of essential oil produce is 0.06%. As the process continues for 4 hour extraction, the yield is increase to 0.082%. The result for 5 hour, 6 hour, 7 hour and 8 hour of the

extraction time show the same increasing in the yield of essential oil with 0.118%, 0.153%, 0.192% and 0.207% respectively. At 9 hour of extraction it shows the highest yield of essential oil with 0.22%. At 10 hour of extraction surprisingly shows the yield of essential oil decrease to 0.1678%.

4.3.1 Effect on different time of extraction

The effect of time on the percentage yield of the curry leaves essential oil was determine by using different time of the extraction from 3 hour to 10 hour of extraction. Figure 4.2 below shows the graph of percentage yield of essential oil with different time of extraction.

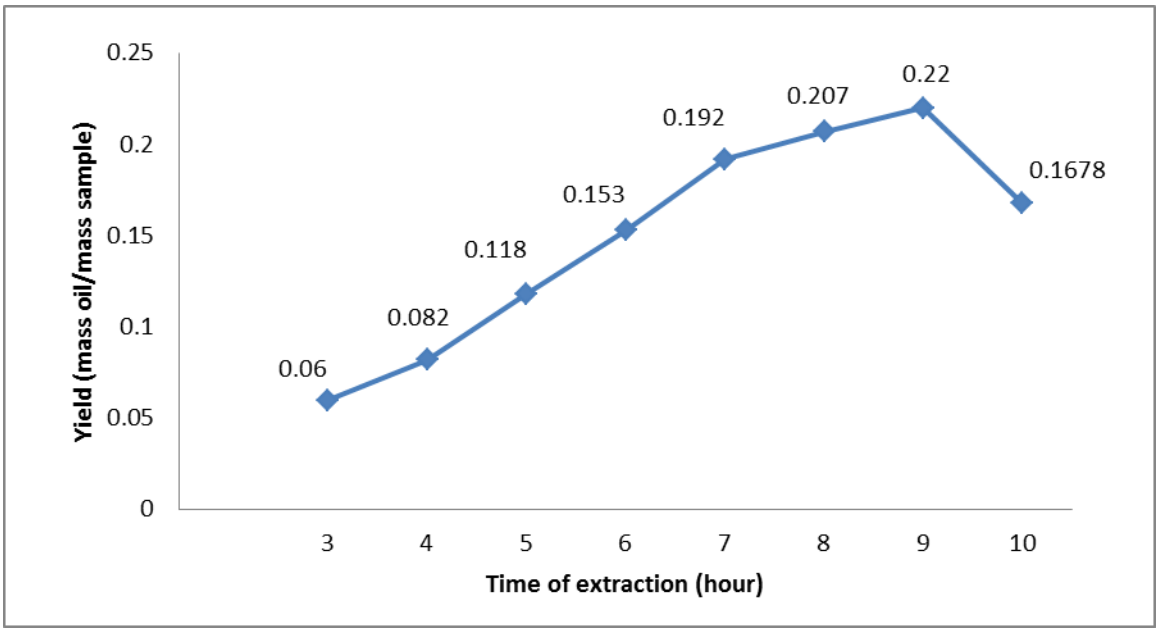

Figure 4-1: Effect of the extraction time on the yield of the curry leaf essential oil

As seen from the result, the percentage yield of the essential oil is increasing as the time increasing from 0.06 % at 3 hour of extraction until the 0.22% yield essential oil at 9 hour of extraction. It shows that the relationship between the time of the extraction is proportional to the percentage yield of the essential oil. The study by Thao-Tran Thi Nguyen et al., (2012) result show that the curry leaf oil obtained is increasing from 0.36% at 3 hour of extraction to 0.83% at 8 hour. It proves that the previous result is agreeable with the finding in this research. Even though the relationship between the time and the yield percent of essential oil is same with the previous study, but the efficiency of the essential oil is not same due to the different technique of the separation between the essential oil and the water. The table below shows the comparison between the current results with the previous study.

Table 4.2: Comparison the yield percent of the essential oil

Extraction time, hour	**Thao-Tran Thi Nguyen et al., (2012)**	**Current result**
3	0.36 %	0.060 %
4	0.52%	0.082 %
5	0.72%	0.118 %
6	0.79%	0.153 %
7	0.80%	0.192 %
8	0.83%	0.207 %
9	0.83%	0.220 %
10	-	0.1678 %

The yield percent of the extraction time at 9 hour show the higher value of 0.22 %. It is show that at the 9 hour of extraction process is the optimum time to get the highest yield of the essential oil. As we compare to the Thao-Tran Thi Nguyen et al., (2012), the optimum time in their study at 8 hour with 0.83 % of yield essential oil.

As the further extraction process for 10 hour show the percentage yield of the essential oil is decreasing to 0.1678%. This result is contradicted with the result by Thao-Tran Thi Nguyen et al., (2012), and Muhammad Hazwan et al., (2012). The study by Muhammad Hazwan et al., (2012) about the Optimization and Kinetics of Essential Oil Extraction from Citronella Grass by Ohmic Heated Hydro Distillation show that the further extraction process after the optimum time of the extraction give no significant to extract more oil. It is because the rate of diffusion strongly relate with the length of time required to achieve equilibrium between two phases (R. T. Toledo, 2007). The result in their experiment has confirmed the Fick's second law of diffusion that is after a certain time, the solute concentrations in the plant matrix and in the solvent achieve final equilibrium (E. M. Silva, 2007).

The increasing of the extraction time also can cause the rate of extraction to decrease. This is because overexposed of the leaves to the heat can may causes the plant cell start to effect and as consequence the essential oil was released to the environment. However prolong the extraction time may causes over heat supplied to the plant material and this lead to the evaporation of the volatile component in the oil.

The grinding process that apply in pre-treatment method in the also may give effect to the extraction yield. Muhammad Hazwan et al., (2012) study shows that as the chopping frequency increase, the extracted oil decrease. It believe that the chopping or grinding the raw material can causes the essential oil or the volatile component may vaporize to the environment. K. K. Singh & T. K. Goswami, (1999) study also find as the temperature rise when chopping or grinding process, sometimes it will affect the product in term of flavor and quality losses.

4.4 Characterization of curry leaf essential oil

In this section intended to study and analyse the characteristic of the essential oil of curry leaves by using the GC-MS to separate the volatile component and to determine the amount of each component present. The component in the sample gets separated in the column because it takes different amount of time to travel through the column depending on how strongly they interact with the stationary phase. As the result, the chemical compositions, the retention times, and the percentage area of the oxygenated compounds of the curry leaf oils obtained are presented in Table 4.4.

From the table 4.4, we find the major composition of the Curry leaves Essential oil as were caryophyllene, naphthalene, globulol, alpha-caryophyllene, pentasiloxane, Cyclohexasiloxane, 4-Cyclohexylidene-n-butanol, and alpha-Pinene. All this component can be group as table below.

Table 4.3: Functional group of the major component

Component	Sesquiterpenes	Monoterpenol	Alcohol	Ester	Aromatic hydrocarbon
Caryophyllene	✓				
Globulol	✓				
alpha-caryophyllene	✓				
alpha-Pinene		✓			
4-Cyclohexylidene-n-butanol			✓		
Pentasiloxane				✓	
Naphthalene					✓
Cyclohexasiloxane					✓

Of all the components the major component is caryophyllene, which is having highest percentage area and retention time as shown in the table. The properties for caryophyllene are:

- Molecular formula : $C_{15}H_{24}$
- Molar mass : 204.36 g/mol
- Density : 905.307 Kg/m³
- Boiling point : 268 °C

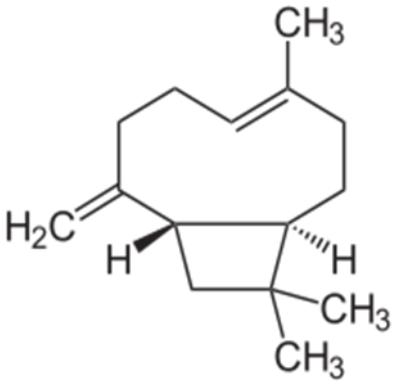

Figure 4-2: Caryophyllene chemical structure

Table 4.4: Chemical constituents, retention times and percentage area of oxygenated compound of curry leaf essential oil.

Compound	**Percentage area %**	**Retention time**
Methanethioamide	0.42	2.504
Furoxan	0.15	3.472
alpha-Pinene	3.44	4.724
Cyclobutane	0.43	6.055
beta-Phellandrene	3.91	6.056
1,3,6-Octatriene	3.39	6.323
2-Propenamide	0.24	7.040

Oxalic acid	0.99	7.238
Cyclohexasiloxane	4.11	10.645
Caryophyllene	32.19	11.640
trans-alpha-Bergamotene	1.53	11.816
beta-Myrcene	0.48	11.992
alpha-Caryophyllene	7.29	12.051
1H-Cycloprop[e]azulene	3.18	12.442
Naphthalene	11.39	12.559
alpha-Farnesene	0.34	12.666
Pentasiloxane	6.34	12.859
Hexasiloxane	1.41	12.859
7,11-Trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate	0.40	13.233
Cyclopentane	0.33	13.501
2(5H)-Furanone	0.28	13.667
Phenol	0.43	13.741
Longipinene epoxide	1.78	13.560
Globulol	2.93	13.870
4-Cyclohexylidene-n-butanol	4.39	14.292
Globulol	10.95	14.298
Benzoic acid	1.93	14.838
Pentadecanoic acid	2.30	17.106

As shown in Table 4.4, 30 components were obtained in the essential oil of curry leaf with the composition was dominated by the terpene hydrocarbon compound with 80%. In further analysis, the major components in the oil were caryophyllene (32.19%), naphthalene (11.39%), globulol (10.95%), alpha-caryophyllene (7.29%), pentasiloxane (6.34%), Cyclohexasiloxane (4.11%), 4-Cyclohexylidene-n-butanol (4.39%), and alpha-Pinene (3.44%). The previous study on curry leaf essential oil that grow in Malaysia by Wong & Tie (1993) shows that the major constituent in the essential oil were identify as beta-phellandrene (24.4%), alpha-pinene (17.5%), beta-caryophyllene (7.3%), terpinene-4-ol (6.1%), limonene (5.1%), gamma-terpinene (4.9%) and alpha-phellandrene (4.8%).

These results show the different of the component as well as amount of the monoterpenes and sesquiterpenes that present in the oil. The composition in the curry leaves may different due to the chemical diversity among populations of species growing in different agro-climatic locations. This is known as owing to anthropological, climatological, and ecological factors. The previous study (Macleod & Piers, 2005; Onayade & Adebajo, 2000; Raina et al., 2002) show the composition of the essential oil of curry leaf in the different region is different. The differences between this analysis and the published information also may be due to differences in GC-MS setting for component identification

As we compare the essential oil of curry leaf from other countries, the major component mainly contained sesquiterpene hydrocarbon. The chemical composition of the essential oil from the Uttaranchal state-Northern India were alpha-pinene (51.7%), sabinene (10.5%), beta-pinene (9.8%), limonene (5.4%) and beta-phellandrene (3.19%). The major component curry leaf oil from Bangladesh reported by Chowdhury et al., (2008) contained 3-carene (54.2%) and caryophyllene (9.5%). It proves that there was a notable variation in the chemical composition of the oil of curry leaves in Northern India and Bangladesh in comparison with those cultivated in other places of the world including Malaysia.

4.4.1 Potential of repellent activity

From Table 4.4, the present of two constituent; α-pinene and β-myrcene show that the essential oil of curry leaf has potential of repellent activity. In recent years, several monoterpenoids have been considered potential alternatives to conventional insecticides as a natural means of pest control. Since oxygenated essential oil constituents are more active, efforts have been made to improve bio efficacy of one such oxygenated essential oil.

The previous study (Ibrahim and Zaki, 1998; Jaenson et al., 2006; Park et al., 2005; Yang et al., 2004) prove that some of monoterpenes such α-pinene, cineole, eugenol, limonene, terpinolene, citronellol, citronellal, camphor and thymol are common constituents in the essential oil that presenting the repellent activity. A research by (W. Thorsell et al., 1998) that study about efficacy of essential oil from various plants as mosquito repellent also show compound β-myrcene can act as repellent for less than 1 hour that refer from repellency USDA. In the USDA-test, a-pinene has a protective time of 1 hour to the mosquito repellency.

Previous study

4.4.2 Biological activity of essential oil

Currently, many researches has proven that the essential oil has many potential that have not discover yet because of the less hazardous properties compare to the synthetic chemical as well as is more environmental friendly, the demand of the essential oil as the alternative source is increasing. The essential oil of the curry leaf also shows some potential that that may had already discover.

In the curry leaf oil, some of the chemical composition shows the potential of the bioactivities. The present of the globulol show that the essential oil of the curry leaf has the potential on the antimicrobial activity. Even though in review of the potential of the *M.koenigii* paper by (Priyanka Gupta et al., 2011) state that the extraction of distillation has no antifungal activity but study by(Thao-Tran Thi Nguyen, 2012) prove that the curry leaf oil can decrease the growing bacterial of *Bacillus subtilis* and *Candida albicans*. As the result, it shows that the essential oil of curry leaf has a potential for using as anti-bacterial. Table below show some of the component in the curry leaf essential oil that has active biological activity.

Table 4.5: Biological activity of the composition of essential oil

Compound	Percentage area %	Retention time	Active biological activity
Furoxan	0.15	3.472	Antioxidant agent
beta-Phellandrene	3.91	6.056	used in fragrances because of their pleasing aromas
2-Propenamide	0.24	7.040	The manufacture of permanent press fabrics. Some 2-Propenamide is used in the manufacture of dyes and the manufacture of other monomers.
Oxalic acid	0.99	7.238	It is used in bleaches, especially for pulpwood. It is also used in baking powder.
Cyclohexasiloxane	4.11	10.645	Antiperspirants
2(5H)-Furanone	0.28	13.667	flavoring agents

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Essential oil of M.koenigii offers a promising potential in the many aspect. The major component of the essential oil of the *M.koenigii* in this study show the different compare to the previous study but some of the component still have the similarity except the percentage area and the retention time due to the differences in the analyzing method. Some of the component in the essential oil shows the potential in the biological activity as well as repellency activity. The present of some repellent active compound in the essential oil prove that the essential oil of curry leaf has a potential in insect repellent.

As for the optimize condition of the extraction in this study, it show that the longer time of extraction may give the highest yield of the essential oil. But if the prolong time the extraction process may causes the essential oil start to vaporize to the environmental as well as the losses of the volatile component in the essential oil.

5.2 Recommendation

It has been proving that the essential oil of curry leaves offer a huge potential that have not discover yet. Nevertheless, there are some recommendations for further improvement in the future research:

I. Future study of the extraction of essential oil should be done in different method of extraction to determine which is the best method to extract the essential oil
II. The parameter of the extraction should be more variety to determine the optimum condition of the extraction of the oil from the leaves
III. For the repellent activity, it should more study on the active compound and also the efficiency repellence to the mosquito.
IV. Further study on the potential of the carbazole alkaloids compound in the essential oil.

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APPENDIX A

Gantt Chart for Undergraduate Research Project I & II

Gantt Chart for Undergraduate Research Project

	PSM 1							PSM 2														
Activities/Week	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

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    GCMSD
Sample Name: 1
Misc Info  :
Vial Number: 2
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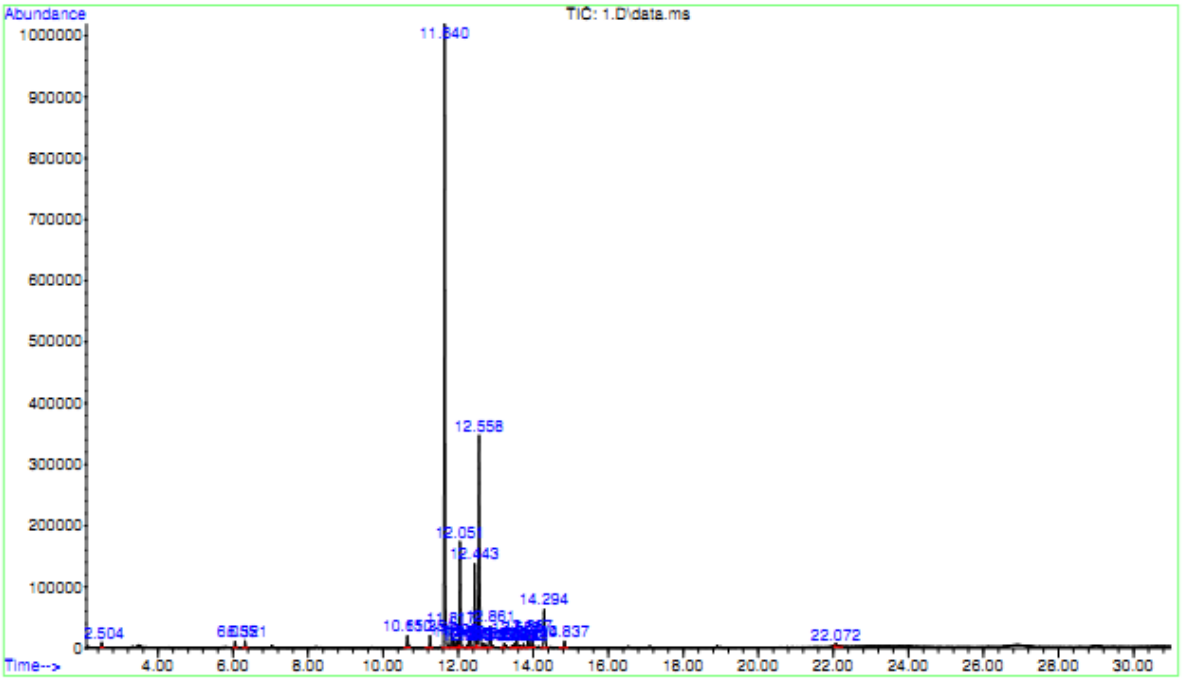

Library Search Report

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Operator :
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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	2.504	0.35	C:\Database\NIST05a.L			
			2,2-Dimethoxybutane	8553	003453-99-4	78
			Methanethioamide, N,N-dimethyl-	2143	000758-16-7	59
			Methanethioamide, N,N-dimethyl-	2144	000758-16-7	38
2	6.056	0.45	C:\Database\NIST05a.L			
			Bicyclo[3.1.0]hexane, 4-methylene- 1-(1-methylethyl)-	15373	003387-41-5	87
			1R-.alpha.-Pinene	15188	007785-70-8	80
			Bicyclo[3.1.0]hexane, 4-methylene- 1-(1-methylethyl)-	15379	003387-41-5	80
3	6.323	0.43	C:\Database\NIST05a.L			
			1,3,6-Octatriene, 3,7-dimethyl-, (E)-	15282	003779-61-1	47
			1-Hexen-3-yne, 5,5-dimethyl-	5332	004911-58-4	47
			1-Methylene-2-vinylcyclopentane	5343	006196-78-7	43
4	10.650	1.05	C:\Database\NIST05a.L			
			Cyclohexasiloxane, dodecamethyl-	179152	000540-97-6	87
			Cyclohexasiloxane, dodecamethyl-	179153	000540-97-6	83
			Cyclohexasiloxane, dodecamethyl-	179151	000540-97-6	50
5	11.255	0.79	C:\Database\NIST05a.L			
			Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-, [1S-(1.al pha.,2.beta.,4.beta.)]-	60003	000515-13-9	58
			1,3-Pentadiene, (Z)-	438	001574-41-0	43
			1,3-Pentadiene	423	000504-60-9	43
6	11.640	47.87	C:\Database\NIST05a.L			
			Caryophyllene	59797	000087-44-5	99
			Caryophyllene	59802	000087-44-5	99
			Bicyclo[7.2.0]undec-4-ene, 4,11,11 -trimethyl-8-methylene-,[1R-(1R*,4 Z,9S*)]-	59970	000118-65-0	90
7	11.816	1.51	C:\Database\NIST05a.L			
			trans-.alpha.-Bergamotene	59863	1000293-01-5	91
			1,3,6,10-Dodecatetraene, 3,7,11-tr imethyl-, (Z,E)-	59891	026560-14-5	90
			(Z,Z)-.alpha.-Farnesene	59857	1000293-03-1	64
8	11.891	0.91	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	96
			Aromadendrene	59796	109119-91-7	90
			1H-Cycloprop[e]azulene, decahydro- 1,1,7-trimethyl-4-methylene-	59928	072747-25-2	70
9	11.993	0.48	C:\Database\NIST05a.L			
			1,6,10-Dodecatriene, 7,11-dimethyl -3-methylene-, (E)-	59899	018794-84-8	72
			(E,E)-7,11,15-Trimethyl-3-methylen e-hexadeca-1,6,10,14-tetraene	107090	070901-63-2	64
			1,6,10-Dodecatriene, 7,11-dimethyl -3-methylene-, (Z)-	59897	028973-97-9	56

AME RSO.M Fri Oct 11 10:21:43 2013

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			.alpha.-Caryophyllene	59848	006753-98-6	99
			.alpha.-Caryophyllene	59847	006753-98-6	93
			.alpha.-Caryophyllene	59846	006753-98-6	91
11	12.249	0.22	C:\Database\NIST05a.L			
			1,3,5-Pentanetrione, 1,5-diphenyl-	102669	001467-40-9	9
			Benzene, (1-nitroethyl)-	23813	007214-61-1	9
			Cyanogen bromide	4820	000506-68-3	5
12	12.303	0.88	C:\Database\NIST05a.L			
			Caryophyllene	59802	000087-44-5	50
			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	43
			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	43
13	12.442	7.20	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	99
			Naphthalene, 1,2,3,5,6,7,8,8a-octa hydro-1,8a-dimethyl-7-(1-methyleth enyl)-, [1S-(1.alpha.,7.alpha.,8a. alpha.)]-	60051	010219-75-7	96
			Eudesma-4(14),11-diene	59851	1000152-04-3	95
14	12.560	18.07	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, 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	81
			Eudesma-4(14),11-diene	59851	1000152-04-3	68
15	12.667	0.35	C:\Database\NIST05a.L			
			Bicyclo[7.2.0]undec-4-ene, 4,11,11 -trimethyl-8-methylene-	59914	013877-93-5	72
			1H-Cycloprop[e]azulene, decahydro- 1,1,7-trimethyl-4-methylene-	59928	072747-25-2	64
			.alpha.-Farnesene	59834	000502-61-4	59
16	12.747	0.20	C:\Database\NIST05a.L			
			(Z,Z)-.alpha.-Farnesene	59857	1000293-03-1	50
			1,3,6,10-Dodecatetraene, 3,7,11-tr imethyl-, (Z,E)-	59891	026560-14-5	25
			1,3,6,10-Dodecatetraene, 3,7,11-tr imethyl-, (Z,E)-	59889	026560-14-5	25
17	12.859	2.06	C:\Database\NIST05a.L			

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			3-Isopropoxy-1,1,1,7,7,7-hexamethy	187800	071579-69-6	47
			l-3,5,5-tris(trimethylsiloxy)tetra			
			siloxane			
			Pentasiloxane, dodecamethyl-	166195	000141-63-9	47
			Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,	177117	000995-82-4	42
			11,11-dodecamethyl-			
18	13.234	0.31	C:\Database\NIST05a.L			
			.beta.-Myrcene	15179	000123-35-3	47
			1,6,10-Dodecatrien-3-ol, 3,7,11-tr	72953	000142-50-7	45
			imethyl-, [S-(Z)]-			
			.beta.-Myrcene	15180	000123-35-3	43
19	13.437	0.21	C:\Database\NIST05a.L			
			Methyl trans-2-(3-cyclopropyl-7-no	62826	1000223-15-6	12
			rcaranyl)acetate			
			Cyclohexanol, 2-methyl-5-(1-methyl	25851	018675-33-7	10
			ethenyl)-, (1.alpha.,2.alpha.,5.be			
			ta.)-			
			Methyl cis-2-(3-cyclopropyl-7-norc	62821	1000223-15-7	10
			aranyl)acetate			
20	13.496	0.27	C:\Database\NIST05a.L			
			10-Undecyn-1-ol	34838	002774-84-7	9
			1,9-Decadiyne	14333	001720-38-3	9
			Bicyclo[2.1.1]hexan-2-ol, 2-etheny	10227	1000221-37-2	9
			l-			
21	13.560	0.86	C:\Database\NIST05a.L			
			1H-Cycloprop[e]azulen-4-ol, decahy	73028	000552-02-3	52
			dro-1,1,4,7-tetramethyl-, [1ar-(1a			
			.alpha.,4.beta.,4a.beta.,7.alpha.,			
			7a.beta.,7b.alpha.)]-			
			Globulol	72897	051371-47-2	49
			Veridiflorol	72904	1000122-17-3	38
22	13.661	0.22	C:\Database\NIST05a.L			
			Bicyclo[4.1.0]heptan-3-ol, 4,7,7-t	25864	054750-08-2	10
			rimethyl-, (1.alpha.,3.beta.,4.bet			
			a.,6.alpha.)-			
			1,3,6-Heptatriene, 2,5,5-trimethyl	15274	029548-02-5	10
			2(5H)-Furanone, 4-methyl-3-(2-meth	24999	089902-23-8	10
			yl-2-propenyl)-			
23	13.742	0.31	C:\Database\NIST05a.L			
			Benzenemethanol, 4-(1,1-dimethylet	32081	000877-65-6	38
			hyl)-			
			9-Azabicyclo[3.3.1]nona-2,6-diene-	22692	034668-91-2	27
			9-carboxaldehyde			
			1,4-Dimethyladamantane, [1.alpha.,	32244	024145-88-8	25
			3.beta., 4.beta., 5.alpha., 7.bet			
			a.]-			
24	13.865	1.07	C:\Database\NIST05a.L			
			2,7-Octadiene-1,6-diol, 2,6-dimeth	36225	075991-61-6	27
			yl-, (E)-			
			Veridiflorol	72904	1000122-17-3	22
			2,7-Octadiene-1,6-diol, 2,6-dimeth	36186	064142-78-5	22
			yl-			

AME RSO.M Fri Oct 11 10:21:43 2013

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			Veridiflorol	72904	1000122-17-3	50
			Ledol	72883	000577-27-5	40
26	14.293	3.67	C:\Database\NIST05a.L			
			(-)-Globulol	72905	000489-41-8	64
			Globulol	72897	051371-47-2	62
			1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [lar-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	73028	000552-02-3	41
27	14.838	0.44	C:\Database\NIST05a.L			
			Cyclopentasiloxane, decamethyl-	161016	000541-02-6	32
			N-Methyladrenaline, tri-TMS	174020	1000071-72-1	23
			N-(Trifluoracetyl)-O,O',O''-tris(trimethylsilyl)epinephrine	184164	054135-51-2	17
28	22.075	0.65	C:\Database\NIST05a.L			
			Heptasiloxane, hexadecamethyl-	186165	000541-01-5	20
			Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	184742	019095-23-9	17
			Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	177117	000995-82-4	10

APPENDIX C

GC-MS analysis: Research 2

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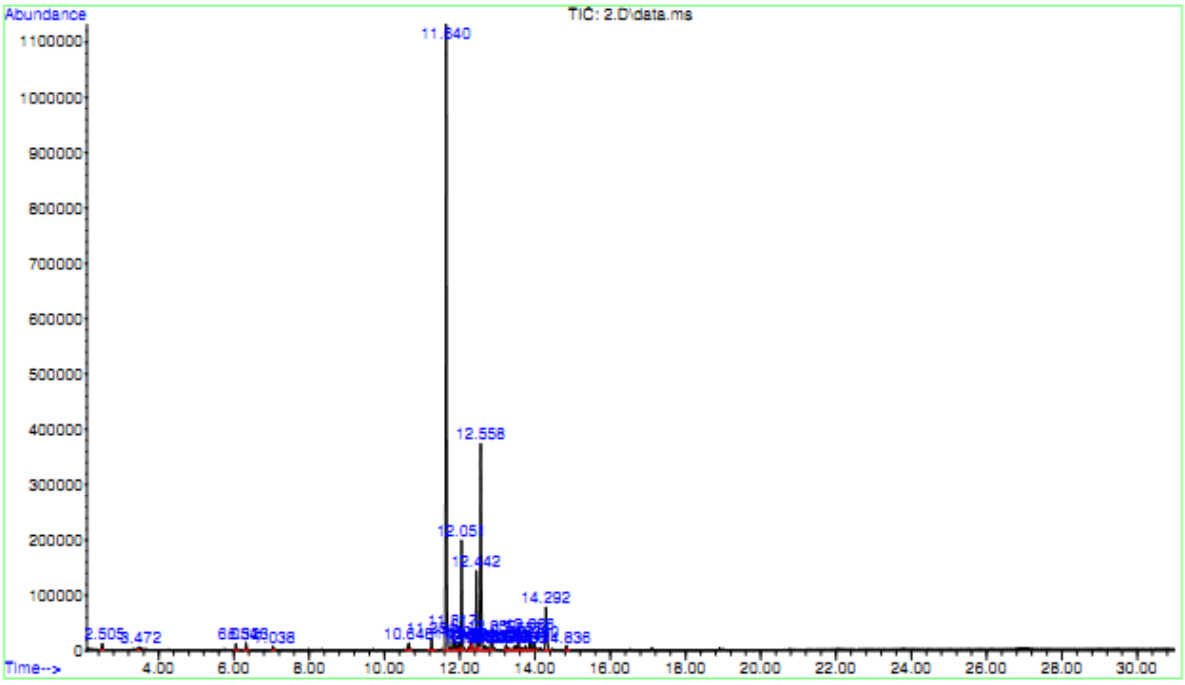

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Integration Events: ChemStation Integrator - autoint1.e

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			Methanethioamide, N,N-dimethyl-	2143	000758-16-7	45
			2,2-Dimethoxypropionamide	14148	1000237-69-7	42
			2,2-Dimethoxybutane	8553	003453-99-4	38
2	3.472	0.15	C:\Database\NIST05a.L			
			Furoxan, 4-nitro-3-phenyl-, 2-oxid	62316	049558-03-4	42
			Methanethioamide, N,N-dimethyl-	2143	000758-16-7	9
			Thiazole, tetrahydro-	2142	000504-78-9	9
3	6.055	0.43	C:\Database\NIST05a.L			
			.beta.-Phellandrene	15198	000555-10-2	80
			Bicyclo[2.2.1]hept-2-ene, 1,7,7-tr imethyl-	15320	000464-17-5	64
			Cyclohexene, 4-methylene-1-(1-meth ylethyl)-	15324	000099-84-3	64
4	6.323	0.43	C:\Database\NIST05a.L			
			Cyclobutane, 2-ethylidene-1-vinyl-	5357	1000150-32-4	64
			1,3,6-Octatriene, 3,7-dimethyl-, (E)-	15280	003779-61-1	53
			Bicyclo[3.2.0]heptane, 6-methylene	5366	003642-22-6	50
5	7.040	0.24	C:\Database\NIST05a.L			
			2-Propenamide	578	000079-06-1	9
			2-Propenamide	577	000079-06-1	9
			2-Propenamide	579	000079-06-1	9
6	10.650	0.69	C:\Database\NIST05a.L			
			Cyclohexasiloxane, dodecamethyl-	179153	000540-97-6	72
			Cyclohexasiloxane, dodecamethyl-	179152	000540-97-6	72
			Cyclohexasiloxane, dodecamethyl-	179151	000540-97-6	50
7	11.254	0.89	C:\Database\NIST05a.L			
			Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-	59911	110823-68-2	87
			Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-, [1S-(1.al pha.,2.beta.,4.beta.)]-	60003	000515-13-9	62
			Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-, (1.alpha. ,2.beta.,4.beta.)-	59995	033880-83-0	49
8	11.639	47.86	C:\Database\NIST05a.L			
			Caryophyllene	59797	000087-44-5	99
			Caryophyllene	59802	000087-44-5	98
			Caryophyllene	59800	000087-44-5	94
9	11.816	1.53	C:\Database\NIST05a.L			
			trans-.alpha.-Bergamotene	59863	1000293-01-5	91
			1,3,6,10-Dodecatetraene, 3,7,11-tr imethyl-, (Z,E)-	59889	026560-14-5	83
			1,3,6,10-Dodecatetraene, 3,7,11-tr imethyl-, (Z,E)-	59891	026560-14-5	80
10	11.891	0.94	C:\Database\NIST05a.L			
			1H-Cycloprop[e]azulene, decahydro- 1,1,7-trimethyl-4-methylene-	59928	072747-25-2	93
			1H-Cycloprop[e]azulene, decahydro-	60075	025246-27-9	86

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Integration Events: ChemStation Integrator - autoint1.e

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			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	60
11	11.992	0.48	C:\Database\NIST05a.L			
			1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-	59899	018794-84-8	59
			(E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene	107090	070901-63-2	53
			.beta.-Myrcene	15179	000123-35-3	40
12	12.051	8.54	C:\Database\NIST05a.L			
			.alpha.-Caryophyllene	59848	006753-98-6	99
			.alpha.-Caryophyllene	59846	006753-98-6	94
			.alpha.-Caryophyllene	59847	006753-98-6	93
13	12.249	0.22	C:\Database\NIST05a.L			
			Cyclohexanol, 3-(aminomethyl)-3,5,5-trimethyl-	37073	015647-11-7	9
			Acetamide, 2-chloro-	2426	000079-07-2	4
			Acetamide, 2-fluoro-	959	000640-19-7	4
14	12.303	0.44	C:\Database\NIST05a.L			
			cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H)benzocycloheptene	59956	1000104-20-1	70
			Humulen-(v1)	59795	1000159-39-4	62
			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	60
15	12.324	0.44	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	94
			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	76
			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.)]-	60096	017334-55-3	76
16	12.442	7.02	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.)]-	60054	000473-13-2	99
			Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.alpha.)]-	60051	010219-75-7	98

Data Path : D:\Data\psm 2_2012\jamil_28may13\10oct\
Data File : 2.D
Acq On : 10 Oct 2013 13:04
Operator :
Sample : 2
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
			Eudesma-4(14),11-diene	59851	1000152-04-3	95
17	12.559	17.44	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	90
			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	90
18	12.666	0.34	C:\Database\NIST05a.L			
			Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	59972	000118-65-0	47
			.alpha.-Farnesene	59834	000502-61-4	43
			(E,Z)-.alpha.-Farnesene	59856	1000293-03-2	42
19	12.747	0.20	C:\Database\NIST05a.L			
			Dispiro[2.0.2.5]undecane, 8-methylene-	30874	051567-09-0	25
			8-Hydroxymethyl-trans-bicyclo[4.3.0]non-3-ene	24216	1000099-21-8	23
			.alpha.-Farnesene	59827	000502-61-4	17
20	12.859	1.41	C:\Database\NIST05a.L			
			3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	187800	071579-69-6	50
			Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	177117	000995-82-4	50
			Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	166198	003555-47-3	43
21	13.233	0.40	C:\Database\NIST05a.L			
			3,7,11-Trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate	113276	1000144-12-7	80
			1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	72942	040716-66-3	80
			1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	72953	000142-50-7	80
22	13.281	0.27	C:\Database\NIST05a.L			
			Longipinene epoxide	71349	142792-93-6	22
			Camphene	15159	000079-92-5	14
			Bicyclo[3.1.1]heptane, 6,6-dimethyl-3-methylene-	15363	016022-04-1	10
23	13.442	0.30	C:\Database\NIST05a.L			
			10,12-Octadecadiynoic acid	109567	007333-25-7	12
			(E,Z)-.alpha.-Farnesene	59856	1000293-03-2	10
			Ledene oxide-(II)	71337	1000159-36-7	10
24	13.501	0.33	C:\Database\NIST05a.L			
			Cyclopentane, 1-ethenyl-3-methylene-	5369	029668-63-1	35

Data Path : D:\Data\psm 2_2012\jamil_28may13\10oct\
Data File : 2.D
Acq On : 10 Oct 2013 13:04
Operator :
Sample : 2
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
			7-Oxabicyclo[4.1.0]heptane, 3-oxiranyl-	17855	000106-87-6	32
			Cyclohexene, 3-ethenyl-	5292	000766-03-0	25
25	13.560	1.05	C:\Database\NIST05a.L			
			1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1ar-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	73028	000552-02-3	60
			1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1ar-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	73026	000552-02-3	49
			Ledol	72883	000577-27-5	43
26	13.667	0.28	C:\Database\NIST05a.L			
			2(5H)-Furanone, 4-methyl-3-(2-methyl-2-propenyl)-	24999	089902-23-8	25
			Ethanone, 2-(1-methylethoxy)-1,2-diphenyl-	94857	006652-28-4	12
			1,2-Cyclobutanedicarboxylic acid, 3-methyl-, dimethyl ester	47909	014132-19-5	9
27	13.741	0.43	C:\Database\NIST05a.L			
			Phenol, 4-(1,1-dimethylethyl)-2-methyl-	32080	000098-27-1	27
			Benzene, 1-(1,1-dimethylethyl)-4-methoxy-	32091	005396-38-3	27
			Benzene, 1-(1,1-dimethylethyl)-4-methoxy-	32092	005396-38-3	27
28	13.864	1.34	C:\Database\NIST05a.L			
			Cis-8-ethyl-exo-tricyclo[5.2.1.0(2.6)]decane	32216	1000215-29-4	25
			2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate, (E,E,E)-	143842	061691-98-3	25
			4-Cyclohexylidene-n-butanol	25606	004441-58-1	22
29	13.982	0.79	C:\Database\NIST05a.L			
			1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-	59928	072747-25-2	50
			Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	59991	000515-17-3	40
			Caryophyllene	59800	000087-44-5	37
30	14.292	4.39	C:\Database\NIST05a.L			
			4-Cyclohexylidene-n-butanol	25606	004441-58-1	27
			9,10-Dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	54297	174226-40-5	25
			1H-3a,7-Methanoazulene, octahydro-1,9,9-trimethyl-4-methylene-, (1.alpha.,3a.alpha.,7.alpha.,8a.beta.)	60016	000508-55-4	18
31	14.838	0.31	C:\Database\NIST05a.L			
			Benzoic acid, 2,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	161138	010586-16-0	28
			N-Methyladrenaline, tri-TMS	174020	1000071-72-1	25

Library Search Report

Data Path : D:\Data\psm 2_2012\jamil_28may13\10oct\
Data File : 2.D
Acq On : 10 Oct 2013 13:04
Operator :
Sample : 2
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
			Phenethylamine, N-methyl-.beta.,3, 4-tris(trimethylsiloxy)-	170946	010538-85-9	23

APPENDIX D

GC-MS analysis: Research 3

Library Search Report

Data Path : C:\msdchem\1\data\13dis13.D\

Data File : 2.D

Acq On : 13 Dec 2013 11:43

Operator :

Sample :

Misc :

ALS Vial : 2 Sample Multiplier: 1

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

Unknown Spectrum: Apex

Integration Events: RTE Integrator - rteint.p

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	20.220	2.33	C:\Database\NIST05a.L			
			Cyclohexane, 1-ethenyl-1-methyl-2,	60003	000515-13-9	94
			4-bis(1-methylethenyl)-, [1S-(1.al			
			pha.,2.beta.,4.beta.)]-			
			Cyclohexane, 1-ethenyl-1-methyl-2,	60001	000515-13-9	68
			4-bis(1-methylethenyl)-, [1S-(1.al			
			pha.,2.beta.,4.beta.)]-			
			Cyclohexane, 1-ethenyl-1-methyl-2,	59995	033880-83-0	53
			4-bis(1-methylethenyl)-, (1.alpha.			

,2.beta.,4.beta.)-

2 21.475 23.28 C:\Database\NIST05a.L

Caryophyllene	59797	000087-44-5 99
Caryophyllene	59802	000087-44-5 99
Caryophyllene	59801	000087-44-5 96

3 22.062 0.85 C:\Database\NIST05a.L

1H-Cyclopenta[1,3]cyclopropa[1,2]b	60103	013744-15-5 97
enzene, octahydro-7-methyl-3-methy		
lene-4-(1-methylethyl)-, [3aS-(3a.		
alpha.,3b.beta.,4.beta.,7.alpha.,7		
aS*)]-		
1,6-Cyclodecadiene, 1-methyl-5-met	59960	023986-74-5 95
hylene-8-(1-methylethyl)-, [s-(E,E		
)]-		
Bicyclo[4.4.0]dec-1-ene, 2-isoprop	59918	150320-52-8 93
yl-5-methyl-9-methylene-		

4 22.223 4.72 C:\Database\NIST05a.L

Bicyclo[3.1.1]hept-2-ene, 2,6-dime	59930	017699-05-7 97
thyl-6-(4-methyl-3-pentenyl)-		
1,3,6,10-Dodecatetraene, 3,7,11-tr	59889	026560-14-5 94
imethyl-, (Z,E)-		
1,3,6,10-Dodecatetraene, 3,7,11-tr	59891	026560-14-5 91
imethyl-, (Z,E)-		

5 22.441 0.75 C:\Database\NIST05a.L

Neoisolongifolene 59831 1000156-12-4 95

.beta.-Neoclovene 59830 056684-96-9 90

(-)-Caryophyllene-(I1) 59854 1000156-13-1 86

6 23.042 23.34 C:\Database\NIST05a.L

.alpha.-Caryophyllene 59848 006753-98-6 96

.alpha.-Caryophyllene 59846 006753-98-6 94

1,4,7,-Cycloundecatriene, 1,5,9,9- 59900 1000062-61-9 93

tetramethyl-, Z,Z,Z-

7 23.437 1.00 C:\Database\NIST05a.L

Azulene, 1,2,3,3a,4,5,6,7-octahydr 60066 022567-17-5 86

o-1,4-dimethyl-7-(1-methylethenyl)

-, [1R-(1.alpha.,3a.beta.,4.alpha.

,7.beta.)]-

Seychellene 59790 020085-93-2 74

Naphthalene, 1,2,4a,5,6,8a-hexahyd 60009 031983-22-9 70

ro-4,7-dimethyl-1-(1-methylethyl)-

, (1.alpha.,4a.alpha.,8a.alpha.)-

8 23.712 1.70 C:\Database\NIST05a.L

Naphthalene, decahydro-4a-methyl-1 59990 000515-17-3 93

-methylene-7-(1-methylethylidene)-

, (4aR-trans)-

Cycloisolongifolene 59842 1000151-99-7 93

2-Isopropenyl-4a,8-dimethyl-1,2,3, 59939 1000192-43-5 90

4,4a,5,6,7-octahydronaphthalene

9 24.257 9.50 C:\Database\NIST05a.L

Naphthalene, decahydro-4a-methyl-1 60025 017066-67-0 99

-methylene-7-(1-methylethenyl)-, [

4aR-(4a.alpha.,7.alpha.,8a.beta.)]

Naphthalene, 1,2,3,4,4a,5,6,8a-oct 60054 000473-13-2 99

ahydro-4a,8-dimethyl-2-(1-methylet

henyl)-, [2R-(2.alpha.,4a.alpha.,8

a.beta.)]-

Eudesma-4(14),11-diene 59851 1000152-04-3 98

10 25.076 1.63 C:\Database\NIST05a.L

Seychellene 59790 020085-93-2 89

1H-Cyclopropa[a]naphthalene, 1a,2, 60096 017334-55-3 81

3,5,6,7,7a,7b-octahydro-1,1,7,7a-t

etramethyl-, [1aR-(1a.alpha.,7.alp

ha.,7a.alpha.,7b.alpha.)]-

Naphthalene, 1,2,3,4,4a,5,6,8a-oct 60004 006813-21-4 70

ahydro-4a,8-dimethyl-2-(1-methylet

hylidene)-, (4aR-trans)-

11 25.419 3.65 C:\Database\NIST05a.L

Butylated Hydroxytoluene 71370 000128-37-0 97

Butylated Hydroxytoluene 71371 000128-37-0 94

Butylated Hydroxytoluene 71369 000128-37-0 91

12 25.699 1.65 C:\Database\NIST05a.L

Naphthalene, 1,2,3,5,6,8a-hexahydr 59980 000483-76-1 98

o-4,7-dimethyl-1-(1-methylethyl)-,

(1S-cis)-

Naphthalene, 1,2,3,5,6,8a-hexahydr 59978 000483-76-1 94

o-4,7-dimethyl-1-(1-methylethyl)-,

(1S-cis)-

Naphthalene, 1,2,3,5,6,8a-hexahydr 59977 000483-76-1 94

o-4,7-dimethyl-1-(1-methylethyl)-,

(1S-cis)-

13 27.411 1.54 C:\Database\NIST05a.L

1,6,10-Dodecatrien-3-ol, 3,7,11-tr 72942 040716-66-3 91

imethyl-, (E)-

1,6,10-Dodecatrien-3-ol, 3,7,11-tr 72952 000142-50-7 91

imethyl-, [S-(Z)]-

1,6,10-Dodecatrien-3-ol, 3,7,11-tr 72930 007212-44-4 91

imethyl-

14 28.210 7.16 C:\Database\NIST05a.L

1H-Cycloprop[e]azulene, 1a,2,3,5,6 60086 021747-46-6 89

,7,7a,7b-octahydro-1,1,4,7-tetrame

thyl-, [1aR-(1a.alpha.,7.alpha.,7a

.beta.,7b.alpha.)]-

Naphthalene, decahydro-4a-methyl-1 60015 017066-67-0 83

-methylene-7-(1-methylethenyl)-, [

4aR-(4a.alpha.,7.alpha.,8a.beta.)]

Cyclopropa[d]naphthalen-2(4aH)-one 59765 004677-90-1 78

, 1,1a,5,6,7,8-hexahydro-4a,8,8-tr

imethyl-, [1aR-(1a.alpha.,4a.beta.

,8aS*)]-

15 28.496 0.77 C:\Database\NIST05a.L

2-Propenal, 3-(2,6,6-trimethyl-1-c 41687 004951-40-0 43

yclohexen-1-yl)-

1,3,4-Trimethyladamantane 41741 1000214-98-3 35

1H-Cycloprop[e]azulene, decahydro- 60075 025246-27-9 25

1,1,7-trimethyl-4-methylene-, [1aR

-(1a.alpha.,4a.beta.,7.alpha.,7a.b

eta.,7b.alpha.)]-

16 28.823 1.01 C:\Database\NIST05a.L

Benzene, 1-(1,1-dimethylethyl)-4-m 32092 005396-38-3 55

ethoxy-

Benzene, 1-butyl-4-methoxy- 32049 018272-84-9 55

1H-Indene, 1-ethylideneoctahydro-7 32229 056362-87-9 50

a-methyl-, cis-

17 29.310 3.59 C:\Database\NIST05a.L

1R,4S,7S,11R-2,2,4,8-Tetramethyltr 59942 1000140-07-6 56

icyclo[5.3.1.0(4,11)]undec-8-ene

Azulene, 1,2,3,3a,4,5,6,7-octahydr 60067 022567-17-5 49

o-1,4-dimethyl-7-(1-methylethenyl)

-, [1R-(1.alpha.,3a.beta.,4.alpha.

,7.beta.)]-

.beta.-Panasinsene 59841 1000159-39-0 47

18 29.814 1.24 C:\Database\NIST05a.L

Cyclohexene, 6-ethenyl-6-methyl-1- 59984 005951-67-7 89

(1-methylethyl)-3-(1-methylethylid

ene)-, (S)-

Naphthalene, 1,2,3,4,4a,5,6,8a-oct 60065 030021-74-0 86

ahydro-7-methyl-4-methylene-1-(1-m

ethylethyl)-, (1.alpha.,4a.alpha.,

8a.alpha.)-

10s,11s-Himachala-3(12),4-diene 59868 060909-28-6 80

19 31.085 6.94 C:\Database\NIST05a.L

.beta.-Panasinsene 59841 1000159-39-0 78

1H-Cycloprop[e]azulen-4-ol, decahy 73029 000552-02-3 70

dro-1,1,4,7-tetramethyl-, [1ar-(1a

.alpha.,4.beta.,4a.beta.,7.alpha.,

7a.beta.,7b.alpha.)]-

Azulene, 1,2,3,3a,4,5,6,7-octahydr 60067 022567-17-5 70

o-1,4-dimethyl-7-(1-methylethenyl)

-, [1R-(1.alpha.,3a.beta.,4.alpha.

,7.beta.)]-

20 45.976 3.33 C:\Database\NIST05a.L

Phytol	122408	000150-86-7 91
Phytol	122409	000150-86-7 91
Phytol	122405	000150-86-7 90

plant1.m Tue Dec 17 10:28:50 2013

APPENDIX E

GC-MS analysis: Research 4

Library Search Report

Data Path : C:\msdchem\1\data\13dis13.D\

Data File : 3.D

Acq On : 13 Dec 2013 15:11

Operator : AIMI

Sample : 2

Misc :

ALS Vial : 3 Sample Multiplier: 1

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

Unknown Spectrum: Apex

Integration Events: RTE Integrator - rteint.p

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	6.226	1.86	C:\Database\NIST05a.L			
			Bicyclo[3.1.0]hex-2-ene, 4-methyl-	15374	028634-89-1	94
			1-(1-methylethyl)-			
			.beta.-Phellandrene	15201	000555-10-2	91
			.beta.-Phellandrene	15198	000555-10-2	91
2	6.797	1.30	C:\Database\NIST05a.L			
			1,3,6-Octatriene, 3,7-dimethyl-, (	15284	003338-55-4	97
			Z)-			

1,3,7-Octatriene, 3,7-dimethyl- 15243 000502-99-8 95

1,3,6-Octatriene, 3,7-dimethyl-, (15281 003338-55-4 93

Z)-

3 20.178 1.23 C:\Database\NIST05a.L

Cyclohexane, 1-ethenyl-1-methyl-2, 60003 000515-13-9 96

4-bis(1-methylethenyl)-, [1S-(1.al

pha.,2.beta.,4.beta.)]-

Cyclohexane, 1-ethenyl-1-methyl-2, 59995 033880-83-0 70

4-bis(1-methylethenyl)-, (1.alpha.

,2.beta.,4.beta.)-

Cyclohexane, 1-ethenyl-1-methyl-2, 60001 000515-13-9 62

4-bis(1-methylethenyl)-, [1S-(1.al

pha.,2.beta.,4.beta.)]-

4 21.548 31.03 C:\Database\NIST05a.L

Caryophyllene 59797 000087-44-5 99

Caryophyllene 59802 000087-44-5 99

Caryophyllene 59801 000087-44-5 98

5 22.036 0.94 C:\Database\NIST05a.L

1H-Cyclopenta[1,3]cyclopropa[1,2]b 60103 013744-15-5 93

enzene, octahydro-7-methyl-3-methy

lene-4-(1-methylethyl)-, [3aS-(3a.

alpha.,3b.beta.,4.beta.,7.alpha.,7

aS*)]-

Bicyclo[4.4.0]dec-1-ene, 2-isoprop 59918 150320-52-8 93

yl-5-methyl-9-methylene-

1,6-Cyclodecadiene, 1-methyl-5-met 59960 023986-74-5 92

hylene-8-(1-methylethyl)-, [s-(E,E

)]-

6 22.228 6.27 C:\Database\NIST05a.L

Bicyclo[3.1.1]hept-2-ene, 2,6-dime 59930 017699-05-7 98

thyl-6-(4-methyl-3-pentenyl)-

Bicyclo[3.1.1]hept-2-ene, 2,6-dime 59933 017699-05-7 95

thyl-6-(4-methyl-3-pentenyl)-

1,3,6,10-Dodecatetraene, 3,7,11-tr 59891 026560-14-5 94

imethyl-, (Z,E)-

7 22.446 1.02 C:\Database\NIST05a.L

Neoisolongifolene 59831 1000156-12-4 89

.beta.-Neoclovene 59830 056684-96-9 89

(-)-Caryophyllene-(I1) 59854 1000156-13-1 83

8 23.027 19.73 C:\Database\NIST05a.L

.alpha.-Caryophyllene 59848 006753-98-6 96

1,4,7,-Cycloundecatriene, 1,5,9,9- 59900 1000062-61-9 95

tetramethyl-, Z,Z,Z-

.alpha.-Caryophyllene 59846 006753-98-6 94

9 23.432 0.95 C:\Database\NIST05a.L

Azulene, 1,2,3,3a,4,5,6,7-octahydr 60066 022567-17-5 91

o-1,4-dimethyl-7-(1-methylethenyl)

-, [1R-(1.alpha.,3a.beta.,4.alpha.

,7.beta.)]-

Naphthalene, 1,2,3,5,6,7,8,8a-octa 60047 004630-07-3 86

hydro-1,8a-dimethyl-7-(1-methyleth

enyl)-, [1R-(1.alpha.,7.beta.,8a.a

lpha.)]-

Naphthalene, 1,2,4a,5,6,8a-hexahyd 60010 031983-22-9 83

ro-4,7-dimethyl-1-(1-methylethyl)-

, (1.alpha.,4a.alpha.,8a.alpha.)-

10 23.707 1.13 C:\Database\NIST05a.L

Naphthalene, decahydro-4a-methyl-1 59990 000515-17-3 95

-methylene-7-(1-methylethylidene)-

, (4aR-trans)-

4,7-Methanoazulene, 1,2,3,4,5,6,7, 60014 000514-51-2 92

8-octahydro-1,4,9,9-tetramethyl-,

[1S-(1.alpha.,4.alpha.,7.alpha.)]-

Cycloisolongifolene 59842 1000151-99-7 92

11 24.246 11.97 C:\Database\NIST05a.L

Naphthalene, decahydro-4a-methyl-1 60025 017066-67-0 99

-methylene-7-(1-methylethenyl)-, [

4aR-(4a.alpha.,7.alpha.,8a.beta.)]

Naphthalene, 1,2,3,4,4a,5,6,8a-oct 60054 000473-13-2 99

ahydro-4a,8-dimethyl-2-(1-methylet

henyl)-, [2R-(2.alpha.,4a.alpha.,8

a.beta.)]-

Eudesma-4(14),11-diene 59851 1000152-04-3 99

12 25.102 1.68 C:\Database\NIST05a.L

Seychellene 59790 020085-93-2 94

Isocaryophillene 59819 1000140-07-2 68

Cyclohexene, 1-methyl-4-(5-methyl- 59932 000495-61-4 64

1-methylene-4-hexenyl)-, (S)-

13 25.367 1.01 C:\Database\NIST05a.L

Bicyclo[3.1.1]hept-2-ene, 2,6-dime 59933 017699-05-7 87

thyl-6-(4-methyl-3-pentenyl)-

1H-Benzocycloheptene, 2,4a,5,6,7,8 60000 003853-83-6 78

,9,9a-octahydro-3,5,5-trimethyl-9-

methylene-, (4aS-cis)-

1H-Benzocycloheptene, 2,4a,5,6,7,8 59961 001461-03-6 74

-hexahydro-3,5,5,9-tetramethyl-, (

R)-

14 25.704 1.87 C:\Database\NIST05a.L

Naphthalene, 1,2,3,5,6,8a-hexahydr 59980 000483-76-1 94

o-4,7-dimethyl-1-(1-methylethyl)-,

(1S-cis)-

Naphthalene, 1,2,3,5,6,8a-hexahydr 59979 000483-76-1 94

o-4,7-dimethyl-1-(1-methylethyl)-,

(1S-cis)-

Naphthalene, 1,2,3,5,6,8a-hexahydr 59977 000483-76-1 93

o-4,7-dimethyl-1-(1-methylethyl)-,

(1S-cis)-

15 27.422 1.58 C:\Database\NIST05a.L

1,6,10-Dodecatrien-3-ol, 3,7,11-tr 72952 000142-50-7 91

imethyl-, [S-(Z)]-

1,6,10-Dodecatrien-3-ol, 3,7,11-tr 72930 007212-44-4 91

imethyl-

1,6,10-Dodecatrien-3-ol, 3,7,11-tr 72953 000142-50-7 90

imethyl-, [S-(Z)]-

16 28.127 3.30 C:\Database\NIST05a.L

Bicyclo[3.1.1]heptane, 6,6-dimethy 15363 016022-04-1 64

l-3-methylene-

Cyclohexene, 1-methyl-4-(5-methyl- 59932 000495-61-4 60

1-methylene-4-hexenyl)-, (S)-

Caryophyllene oxide 71352 001139-30-6 58

17 28.843 1.15 C:\Database\NIST05a.L

Benzene, 1-butyl-4-methoxy- 32049 018272-84-9 60

Phenol, 4-(1,1-dimethylethyl)-2-me 32080 000098-27-1 55

thyl-

1H-Indene, 1-ethylideneoctahydro-7 32242 056324-68-6 53

a-methyl-, (1E,3a.alpha.,7a.beta.)

18 29.316 3.29 C:\Database\NIST05a.L

Azulene, 1,2,3,3a,4,5,6,7-octahydr 60067 022567-17-5 83

o-1,4-dimethyl-7-(1-methylethenyl)

-, [1R-(1.alpha.,3a.beta.,4.alpha.

,7.beta.)]-

2,5,5,8a-Tetramethyl-6,7,8,8a-tetr 59756 124957-09-1 60

ahydro-5H-naphthalen-1-one

Aciphyllene 59792 087745-31-1 49

19 29.793 1.15 C:\Database\NIST05a.L

Cyclohexene, 6-ethenyl-6-methyl-1- 59984 005951-67-7 86

(1-methylethyl)-3-(1-methylethylid

ene)-, (S)-

10s,11s-Himachala-3(12),4-diene 59868 060909-28-6 83

Naphthalene, 1,2,3,4,4a,5,6,8a-oct 60065 030021-74-0 83

ahydro-7-methyl-4-methylene-1-(1-m

ethylethyl)-, (1.alpha.,4a.alpha.,

8a.alpha.)-

20 31.064 7.54 C:\Database\NIST05a.L

.beta.-Panasinsene 59841 1000159-39-0 78

1H-Cycloprop[e]azulen-4-ol, decahy 73029 000552-02-3 70

dro-1,1,4,7-tetramethyl-, [1ar-(1a

.alpha.,4.beta.,4a.beta.,7.alpha.,

7a.beta.,7b.alpha.)]-

Azulene, 1,2,3,3a,4,5,6,7-octahydr 60067 022567-17-5 70

o-1,4-dimethyl-7-(1-methylethenyl)

-, [1R-(1.alpha.,3a.beta.,4.alpha.

,7.beta.)]-

plant1.m Tue Dec 17 10:29:27 2013

APPENDIX F

GC-MS analysis: Research 5

Library Search Report

Data Path : C:\msdchem\1\data\13dis13.D\

Data File : 4.D

Acq On : 13 Dec 2013 16:20

Operator : AIMI

Sample : 3

Misc :

ALS Vial : 4 Sample Multiplier: 1

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

Unknown Spectrum: Apex

Integration Events: RTE Integrator - rteint.p

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	25.818	1.00	C:\Database\NIST05a.L			
			Ethanol, 2,2'-[oxybis(2,1-ethanedi yloxy)]bis-	53221	000112-60-7	83
			Ethanol, 2,2'-[oxybis(2,1-ethanedi yloxy)]bis-	53222	000112-60-7	78
			Pentaethylene glycol	83341	004792-15-8	64
2	30.732	1.02	C:\Database\NIST05a.L			
			1H-Benzocycloheptene, 2,4a,5,6,7,8	60000	003853-83-6	70

,9,9a-octahydro-3,5,5-trimethyl-9-

methylene-, (4aS-cis)-

.beta.-Neoclovene 59830 056684-96-9 64

Guaia-3,9-diene 59814 000489-83-8 55

3 32.496 18.79 C:\Database\NIST05a.L

Octanethioic acid, S-hexyl ester 87932 055590-85-7 38

2-Thiophenecarboxaldehyde, oxime 11367 029683-84-9 38

Propylphosphonic acid, fluoroanhyd 102335 333416-22-1 38

ride, decyl ester

4 33.508 7.19 C:\Database\NIST05a.L

Octanoic acid, 3-chloroprop-2-enyl 69529 1000299-35-0 45

ester

Octanethioic acid, S-hexyl ester 87932 055590-85-7 42

1,3,5-Triazin-2(1H)-one, 4,6-diami 11297 000645-92-1 40

no-

5 36.185 1.44 C:\Database\NIST05a.L

Pentaethylene glycol 83342 004792-15-8 83

Hexagol 112856 002615-15-8 83

Pentaethylene glycol 83339 004792-15-8 78

6 39.610 21.27 C:\Database\NIST05a.L

Decanoic acid, 2,3-dihydroxypropyl 89082 002277-23-8 50

ester

Decanoic acid, 2-hydroxy-1-(hydrox 89085 003376-48-5 50

ymethyl)ethyl ester

1-Octanamine, N-methyl-N-nitroso- 38368 034423-54-6 38

7 40.264 3.98 C:\Database\NIST05a.L

Decanoic anhydride 140216 002082-76-0 53

2,3,4-Trimethylpentan-2-ol decanoa 114859 1000130-74-2 38

te

Decanoic acid, 2,3-dihydroxypropyl 89083 002277-23-8 38

ester

8 41.950 0.74 C:\Database\NIST05a.L

Pentaethylene glycol 83341 004792-15-8 22

15-Crown-5 70735 033100-27-5 22

1,3,5-Triazine, 2,4,6-trimethoxy- 37237 000877-89-4 16

9 45.509 5.34 C:\Database\NIST05a.L

Heptaethylene glycol 139825 005617-32-3 90

Hexagol 112859 002615-15-8 90

Hexagol 112858 002615-15-8 83

10 50.252 5.01 C:\Database\NIST05a.L

2,4-Dichlorobenzonitrile 37270 006574-98-7 38

2,5-Dichlorobenzonitrile 37269 021663-61-6 38

4-Pyrimidinecarboxylic acid, 5-ami 37208 007164-43-4 37

no-1,2,3,6-tetrahydro-2,6-dioxo-

11 53.163 2.33 C:\Database\NIST05a.L

Piperazine, 1-methyl-4-[2-(p-tolyl 113000 016191-68-7 37
sulfonyl)ethyl]-

2-Dimethylisopropylsilyloxybut-3-y 37023 1000299-49-1 33
ne

Xylitol, 1-O-octanoyl- 110383 1000155-88-7 33

12 53.552 6.47 C:\Database\NIST05a.L

2H-Azepin-2-one, hexahydro-1-methy 11498 002556-73-2 38
l-

Pyridin-3-ol, O-acetyl-2-[S-[2-ace 116316 1000211-47-5 38
toxyethyl]dithio]-

Xylitol, 1-O-octanoyl- 110383 1000155-88-7 38

13 55.632 3.19 C:\Database\NIST05a.L

Fluorobenzene, 4,5-dimethoxy-2-met 56728 1000116-17-4 27
henehydroxyimino-

1H-Furo[2,3-c]pyrazol-3-amine, 5-p 56449 210347-43-6 27
henyl-

Fluorobenzene, 2,3-dimethoxy-6-met 56727 1000116-17-6 27
henhydroxyimino-

14 57.708 14.78 C:\Database\NIST05a.L

4-Pyrimidinecarboxylic acid, 5-ami 37206 007164-43-4 38
no-1,2,3,6-tetrahydro-2,6-dioxo-

1-Naphthalenecarbodithioic acid, e 79739 020876-72-6 37

thyl ester

4-[3-(3-Methoxy-phenyl)-4-methyl-5 168658 312282-12-5 32

-thioxo-4,5-dihydro-[1,2,4]triazol

-1-ylmethyl]-piperazine-1-carboxyl

ic acid ethyl ester

15 58.232 1.97 C:\Database\NIST05a.L

4-Cyanocinnoline 27015 016470-90-9 46

Benzenemethanol, .alpha.-methyl-2- 50397 079756-81-3 43

(trifluoromethyl)-

Thiophene-2-carboxamide, 3-ethoxy- 112304 1000267-63-6 43

N-(4-chlorophenyl)-

16 58.600 5.49 C:\Database\NIST05a.L

Thiophene-2-carboxamide, 3-ethoxy- 112304 1000267-63-6 43

N-(4-chlorophenyl)-

4-Cyanocinnoline 27015 016470-90-9 43

Benzene, 2,4-difluoro-1-isocyanato 26787 059025-55-7 43

plant1.m Tue Dec 17 10:29:51 2013

APPENDIX G

GC-MS analysis: Research 5

Library Search Report

Data Path : C:\msdchem\1\data\13dis13.D\

Data File : 5.D

Acq On : 13 Dec 2013 17:28

Operator : AIMI

Sample : 4

Misc :

ALS Vial : 5 Sample Multiplier: 1

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

Unknown Spectrum: Apex

Integration Events: RTE Integrator - rteint.p

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	21.361	40.08	C:\Database\NIST05a.L			
			Caryophyllene	59797	000087-44-5	99
			Caryophyllene	59802	000087-44-5	99
			Caryophyllene	59800	000087-44-5	95
2	21.932	2.98	C:\Database\NIST05a.L			
			Bicyclo[3.1.1]hept-2-ene, 2,6-dime	59933	017699-05-7	94
			thyl-6-(4-methyl-3-pentenyl)-			
			Bicyclo[3.1.1]hept-2-ene, 2,6-dime	59930	017699-05-7	93

thyl-6-(4-methyl-3-pentenyl)-

1,3,6,10-Dodecatetraene, 3,7,11-tr 59891 026560-14-5 93

imethyl-, (Z,E)-

3 22.653 9.03 C:\Database\NIST05a.L

.alpha.-Caryophyllene 59848 006753-98-6 97

.alpha.-Caryophyllene 59846 006753-98-6 95

.alpha.-Caryophyllene 59849 006753-98-6 93

4 23.561 1.27 C:\Database\NIST05a.L

Naphthalene, decahydro-4a-methyl-1 59990 000515-17-3 95

-methylene-7-(1-methylethylidene)-

, (4aR-trans)-

2-Isopropenyl-4a,8-dimethyl-1,2,3, 59939 1000192-43-5 93

4,4a,5,6,7-octahydronaphthalene

Cycloisolongifolene 59842 1000151-99-7 86

5 23.992 9.18 C:\Database\NIST05a.L

Naphthalene, decahydro-4a-methyl-1 60025 017066-67-0 99

-methylene-7-(1-methylethenyl)-, [

4aR-(4a.alpha.,7.alpha.,8a.beta.)]

Naphthalene, 1,2,3,4,4a,5,6,8a-oct 60054 000473-13-2 99

ahydro-4a,8-dimethyl-2-(1-methylet

henyl)-, [2R-(2.alpha.,4a.alpha.,8

a.beta.)]-

Eudesma-4(14),11-diene 59851 1000152-04-3 98

6 24.448 21.07 C:\Database\NIST05a.L

2-Isopropenyl-4a,8-dimethyl-1,2,3, 59944 1000193-57-0 87

4,4a,5,6,8a-octahydronaphthalene

2,10,10-Trimethyltricyclo[7.1.1.0(59752 1000210-81-9 86

2,7)]undec-6-en-8-one

Naphthalene, decahydro-4a-methyl-1 59990 000515-17-3 81

-methylene-7-(1-methylethylidene)-

, (4aR-trans)-

7 28.013 2.05 C:\Database\NIST05a.L

Caryophyllene oxide 71353 001139-30-6 90

Caryophyllene oxide 71352 001139-30-6 52

Cyclohexene, 4-methyl-1-(1-methyle 15312 000586-67-4 50

thenyl)-

8 28.132 2.36 C:\Database\NIST05a.L

Azulene, 1,2,3,3a,4,5,6,7-octahydr 60067 022567-17-5 96

o-1,4-dimethyl-7-(1-methylethenyl)

-, [1R-(1.alpha.,3a.beta.,4.alpha.

,7.beta.)]-

Azulene, 1,2,3,3a,4,5,6,7-octahydr 60064 022567-17-5 96

o-1,4-dimethyl-7-(1-methylethenyl)

-, [1R-(1.alpha.,3a.beta.,4.alpha.

,7.beta.)]-

1H-Cycloprop[e]azulene, 1a,2,3,5,6 60086 021747-46-6 95

,7,7a,7b-octahydro-1,1,4,7-tetrame

thyl-, [1aR-(1a.alpha.,7.alpha.,7a

.beta.,7b.alpha.)]-

9 29.206 2.49 C:\Database\NIST05a.L

1H-3a,7-Methanoazulene, 2,3,6,7,8, 60042 000560-32-7 50

8a-hexahydro-1,4,9,9-tetramethyl-,

(1.alpha.,3a.alpha.,7.alpha.,8a.b

eta.)-

Azulene, 1,2,3,3a,4,5,6,7-octahydr 60067 022567-17-5 49

o-1,4-dimethyl-7-(1-methylethenyl)

-, [1R-(1.alpha.,3a.beta.,4.alpha.

,7.beta.)]-

1H-3a,7-Methanoazulene, 2,3,6,7,8, 60044 000560-32-7 49

8a-hexahydro-1,4,9,9-tetramethyl-,

(1.alpha.,3a.alpha.,7.alpha.,8a.b

eta.)-

10 30.929 7.75 C:\Database\NIST05a.L

.beta.-Panasinsene 59841 1000159-39-0 78

1H-Cycloprop[e]azulene, 1a,2,3,4,4 60090 000489-40-7 60

a,5,6,7b-octahydro-1,1,4,7-tetrame

thyl-, [1aR-(1a.alpha.,4.alpha.,4a

.beta.,7b.alpha.)]-

Azulene, 1,2,3,3a,4,5,6,7-octahydr 60067 022567-17-5 55

o-1,4-dimethyl-7-(1-methylethenyl)

-, [1R-(1.alpha.,3a.beta.,4.alpha.

,7.beta.)]-

11 45.919 1.74 C:\Database\NIST05a.L

Phytol	122408	000150-86-7 91
Phytol	122409	000150-86-7 91
Phytol	122405	000150-86-7 87

plant1.m Tue Dec 17 10:30:15 2013

APPENDIX H

GC-MS analysis: Research 7

Library Search Report

Data Path : C:\msdchem\1\data\13dis13.D\

Data File : 6.D

Acq On : 13 Dec 2013 18:37

Operator : AIMI

Sample : 5

Misc :

ALS Vial : 6 Sample Multiplier: 1

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

Unknown Spectrum: Apex

Integration Events: RTE Integrator - rteint.p

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	21.751	42.42	C:\Database\NIST05a.L			
			Caryophyllene	59797	000087-44-5	99
			Caryophyllene	59802	000087-44-5	98
			Caryophyllene	59801	000087-44-5	97
2	22.140	2.12	C:\Database\NIST05a.L			
			Bicyclo[3.1.1]hept-2-ene, 2,6-dime	59930	017699-05-7	98
			thyl-6-(4-methyl-3-pentenyl)-			
			1,3,6,10-Dodecatetraene, 3,7,11-tr	59889	026560-14-5	93

imethyl-, (Z,E)-

1,3,6,10-Dodecatetraene, 3,7,11-tr 59891 026560-14-5 70

imethyl-, (Z,E)-

3 23.011 14.28 C:\Database\NIST05a.L

.alpha.-Caryophyllene 59848 006753-98-6 97

.alpha.-Caryophyllene 59849 006753-98-6 94

.alpha.-Caryophyllene 59846 006753-98-6 94

4 23.691 1.64 C:\Database\NIST05a.L

4,7-Methanoazulene, 1,2,3,4,5,6,7, 60014 000514-51-2 95

8-octahydro-1,4,9,9-tetramethyl-,

[1S-(1.alpha.,4.alpha.,7.alpha.)]-

Naphthalene, decahydro-4a-methyl-1 59990 000515-17-3 93

-methylene-7-(1-methylethylidene)-

, (4aR-trans)-

Caryophyllene-(I1) 59838 1000158-18-5 90

5 24.231 9.74 C:\Database\NIST05a.L

Naphthalene, decahydro-4a-methyl-1 60025 017066-67-0 99

-methylene-7-(1-methylethenyl)-, [

4aR-(4a.alpha.,7.alpha.,8a.beta.)]

Naphthalene, 1,2,3,4,4a,5,6,8a-oct 60054 000473-13-2 99

ahydro-4a,8-dimethyl-2-(1-methylet

henyl)-, [2R-(2.alpha.,4a.alpha.,8

a.beta.)]-

Eudesma-4(14),11-diene 59851 1000152-04-3 98

6 24.812 21.80 C:\Database\NIST05a.L

2-Isopropenyl-4a,8-dimethyl-1,2,3, 59944 1000193-57-0 90

4,4a,5,6,8a-octahydronaphthalene

Naphthalene, 1,2,3,4,4a,5,6,8a-oct 60063 000473-13-2 76

ahydro-4a,8-dimethyl-2-(1-methylet

henyl)-, [2R-(2.alpha.,4a.alpha.,8

a.beta.)]-

2,10,10-Trimethyltricyclo[7.1.1.0(59752 1000210-81-9 64

2,7)]undec-6-en-8-one

7 28.091 1.34 C:\Database\NIST05a.L

Caryophyllene oxide 71350 001139-30-6 96

Caryophyllene oxide 71352 001139-30-6 93

Caryophyllene oxide 71353 001139-30-6 86

8 29.347 1.94 C:\Database\NIST05a.L

Azulene, 1,2,3,3a,4,5,6,7-octahydr 60067 022567-17-5 86

o-1,4-dimethyl-7-(1-methylethenyl)

-, [1R-(1.alpha.,3a.beta.,4.alpha.

,7.beta.)]-

2-Isopropenyl-4a,8-dimethyl-1,2,3, 59939 1000192-43-5 50

4,4a,5,6,7-octahydronaphthalene

Guaia-3,9-diene 59814 000489-83-8 49

9 31.127 4.73 C:\Database\NIST05a.L

.beta.-Panasinsene 59841 1000159-39-0 78

1H-Cycloprop[e]azulen-4-ol, decahy 73029 000552-02-3 70

dro-1,1,4,7-tetramethyl-, [1ar-(1a

.alpha.,4.beta.,4a.beta.,7.alpha.,

7a.beta.,7b.alpha.)]-

1H-Cycloprop[e]azulene, 1a,2,3,4,4 60090 000489-40-7 55

a,5,6,7b-octahydro-1,1,4,7-tetrame

thyl-, [1aR-(1a.alpha.,4.alpha.,4a

.beta.,7b.alpha.)]-

plant1.m Tue Dec 17 10:30:39 2013

APPENDIX I

GC-MS analysis: Research 8

Library Search Report

Data Path : C:\msdchem\1\data\13dis13.D\

Data File : 7.D

Acq On : 13 Dec 2013 19:45

Operator : AIMI

Sample : 6

Misc :

ALS Vial : 7 Sample Multiplier: 1

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

Unknown Spectrum: Apex

Integration Events: RTE Integrator - rteint.p

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	21.123	63.01	C:\Database\NIST05a.L			
			Caryophyllene	59797	000087-44-5	99
			Caryophyllene	59802	000087-44-5	99
			Bicyclo[7.2.0]undec-4-ene, 4,11,11 -trimethyl-8-methylene-,[1R-(1R*,4 Z,9S*)]-	59971	000118-65-0	94
2	22.529	6.26	C:\Database\NIST05a.L			
			5,7-Octadien-2-ol, 2,6-dimethyl-	25639	005986-38-9	64

Linalyl isobutyrate 74305 000078-35-3 59

1,5-Dimethyl-1-vinyl-4-hexenyl but 74330 000078-36-4 59

yrate

3 23.862 4.90 C:\Database\NIST05a.L

Naphthalene, 1,2,3,4,4a,5,6,8a-oct 60054 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 74

o-1,4-dimethyl-7-(1-methylethenyl)

-, [1S-(1.alpha.,7.alpha.,8a.beta.

)]-

Ledol 72883 000577-27-5 64

4 24.267 18.20 C:\Database\NIST05a.L

Azulene, 1,2,3,5,6,7,8,8a-octahydr 60033 003691-11-0 83

o-1,4-dimethyl-7-(1-methylethenyl)

-, [1S-(1.alpha.,7.alpha.,8a.beta.

)]-

Cycloheptane, 4-methylene-1-methyl 59957 1000159-38-5 64

-2-(2-methyl-1-propen-1-yl)-1-viny

l-

Naphthalene, 1,2,3,4,4a,5,6,8a-oct 60054 000473-13-2 50

ahydro-4a,8-dimethyl-2-(1-methylet

henyl)-, [2R-(2.alpha.,4a.alpha.,8

a.beta.)]-

5 45.867 7.62 C:\Database\NIST05a.L

Cyclohexanol, 3,5-dimethyl- 12210 005441-52-1 42

2,5-Dimethylcyclohexanol 12172 003809-32-3 33

Oxirane, tetradecyl- 85505 007320-37-8 25

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