EXTRACTION OF ESSENTIAL OILS FROM PATCHOULI LEAVES USING ULTRASONIC-ASSISTED SOLVENT EXTRACTION METHOD

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Thesis submitted to the Faculty of Chemical and Natural Resources Engineering in
Partial Fulfillment of the Requirement for the
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Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

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

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Date : 14 MAY 2008

Special Dedication of This Grateful Feeling to My…

Beloved father and mother;
Mr. Shukor Mohd Nordin and Mrs. Zaitun Ibrahim

Loving brothers and sisters;
Zuhairi, Fadhli, Aiman, Faiz, Fahmi, Syazwani

Supportive families;
Uncles and Aunties

For Their Love, Support and Best Wishes.

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ABSTRACT

Essential oil is an aromatic liquid that is extracted from various parts of the plants. It contains the true essence of the plant and has many therapeutic benefits. Patchouli essential oil from the extraction of dried Patchouli (*Pogostemon Cablin*) leaves is the important ingredient in many fragrance products like perfumes and also use widely in medical field. This experiment use ultrasonication-assisted solvent extraction method that comprises two set of experiments in order to investigate the effect of ultrasonic and type of solvent on extraction process. Ethanol, hexane and acetone are the solvents used for the first experiment. The best solvent among three is chosen to be used in second experiment. In the second experiment, ultrasound is used in order to investigate its effect compared to the experiment without using ultrasonic. The qualitative and quantitative analysis has been done in order to show the objectives were achieved. Qualitative analysis involved the chromatogram analysis from GCMS while quantitative analysis is based on the percent yield. From qualitative analysis, ethanol gives the highest peak area (27.92%) than hexane (20.01%) and acetone (20.42%). In addition, average peak area for ultrasonic method (50.18%) is better than without using ultrasonic (42.40%). Meanwhile, for qualitative analysis, ethanol can extract highest yield (2.87%) compared to hexane (2.53%) and acetone (2.00%). Then, by using ultrasonic, it gives higher average yield (2.27%) than without using ultrasonic (1.67%). Therefore, from these analyses, the best solvent used for solvent extraction is ethanol because it produced highest quality and most yields of patchouli oil. This experiment also has the better result when it involves the ultrasonication method.

ABSTRAK

Pati minyak adalah cecair aroma yang boleh diekstrak daripada banyak bahagian tumbuhan. Ia mengandungi pati asli tumbuhan dan mempunyai banyak kelebihan untuk terapi. Minyak patchouli yang terhasil daripada pengekstrakan daun (*Pogostemon cablin)* kering merupakan bahan ramuan dalam banyak produk wangian seperti minyak wangi dan juga banyak digunakan dalam bidang perubatan. Eksperimen ini menggunakan kaedah penggunaan pelarut dengan bantuan ultrasonik yang melibatkan dua set eksperimen dalam usaha untuk mengkaji kesan ultrasonik dan pelarut dalam proses pengekstrakan. Etanol, heksane dan aseton adalah tiga pelarut yang digunakan dalam eksperimen ini. Pelarut yang terbaik daripada tiga pelarut tersebut akan dipilih untuk digunakan dalam eksperimen yang kedua. Dalam eksperimen yang kedua, ultrabunyi digunakan untuk mengkaji kesannya jika dibandingkan dengan eksperimen yang tidak menggunakan ultrasonik. Analisis kualitatif dan kuantitatif telah dibuat untuk menunjukkan objektif tercapai. Analisis kualitatif melibatkan analisis kromatogram daripada GCMS manakala analisis kuantitatif pula berdasarkan peratus hasil. Daripada analsis kualitatif, etanol memberikan luas puncak yang paling besar (27.92%) berbanding heksane (20.01%) dan aseton (20.42%). Selain itu, purata luas puncak kaedah penggunaan ultrabunyi (52.18%) lebih baik daripada tidak menggunakan ultrabunyi (42.40%). Manakala daripada analisis kuantitatif, etanol boleh mengektrak hasil yang paling banyak (2.87%) berbanding heksane (2.53%) dan aseton (2.00%). Kemudian, penggunaan ultrabunyi memberikan lebih purata hasil (2.27%) berbanding tidak menggunakan ultrabunyi (1.67%). Maka, daripada analisis ini etanol adalah pelarut terbaik kerana ia menghasilkan hasil minyak patchouli yang paling berkualiti dan paling banyak. Daripada eksperimen ini juga, hasil minyak adalah lebih baik sekiranya melibatkan penggunaan ultrasonik.

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LIST OF SYMBOLS

%	- Yield percentage
°C	- Degree Celsius
µ	- Micro

LIST OF ABBREVIATIONS

g	- Gram
ml	- Mililiter
GCMS	- Gas Chromatography Mass Spectrometer
Min	-Minute

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CHAPTER 1

INTRODUCTION

1.1 Background

Essential oil is a concentrated liquid that contains various elements such as aromatic compounds, organic constituents, including hormones, vitamin and other natural elements. These compounds are extracted from various parts of a plant and are highly volatile. In the plant, essential oils are produced inside the protoplasm of the cells and stored as micro droplets in the glands of the plant. The oil needs to diffuse through the wall of the glands and spread over the surface of the plant before evaporating and filling the air with perfume.

The oils are rich in energy and chemically active. As such, essential oils are used for many different reasons and in different ways. Commercially, essential oils are used extensively in three main industries which are food, pharmaceutical and fragrance industries. Modern scientific research leads to production synthetic essential that creates the fragrances. However, they are dissimilar from natural fragrance oils or perfume as essential oils are derived from the true plants, as such it may also not contain the therapeutic benefit same like the natural essential oil does.

Patchouli essential oil is obtained from the leaves of *Pogostemon cablin* (Patchouli), a plant from the *Lamiaceae* family, and is widely acknowledged for its characteristic pleasant and long lasting woody, earthy, camphoraceous odor. It is one of the important natural essential oils used to give a base and lasting character to a fragrance in perfumery industry. Patchouli oil is an important ingredient in many fragrance products like perfumes, as well as in soaps and cosmetic products (Bauer *et al.*, 1997) as well as for aromatherapy, spiritual use and medicinal field.

The composition of the patchouli oil is complex but distinct because it consists largely of sesquiterpenes (Buré *et al.*, 1719; Dung *et al.*, 1989; Lawrence, 1990). Specifically, the main constituent of patchouli oil is patchoulol. At present, patchouli plants are the only commercial source of patchoulol and cost-effective synthetic routes for enantiomeric pure patchoulol have yet to be developed (Näf *et al.*, 1981; Srikrishna and Satyanarayana, 2005).

From the researches that have been done before, there are many methods to extract essential oil including conventional and modern techniques but each of them has its own advantages and disadvantages. The suitable method depends on the plant that we want to extract. So, the selection of the best method is crucial to ensure the best quality of essential oil obtained. Most oils are extracted using steam distillation, which is one of the conventional methods.

In this experiment, we study on two methodologies which are the solvent extraction and ultrasonication. These two methods are independent and have the different scope of experiment. Solvent extraction uses the solvent as the medium extractor while ultrasonication use wave to ease the extraction of essential oils from patchouli leaves. Then, the yield from both experiments will be analyzed to obtain the compound in the patchouli oil. Finally, qualitative and quantitative analysis will be done to show the objectives are achieved.

1.2 Problem Statement

In this study, the patchouli leaf is being used as the raw material. Currently, the demand of patchouli oil is very high almost every year (2000 tonnes per annum) because it has wide range of usages. Therefore, it is a big potential to make the profit from this patchouli oil extraction as the growth of market demand. Even many aromatics chemicals have been produced, many people still prefer to the true botanical aroma.

Patchouli is commonly extracted for its essential oils using methanol or ethanol as solvent. This time we are using other solvents which are ethanol, hexane and acetone to make comparison and choose the best solvent in extracting patchouli essential oils. Conventional steam distillation method use a large amount of heat in the process which can cause the thermal degradation of many compounds contained in the patchouli leaf. Compared to steam distillation method, solvent extraction method is more suitable to be used on delicate plants because it uses very little heat that makes it able to produce essential oils in higher amounts and at lower cost. Other than that, it is important to improve the existing products of fragrance and also try to encourage the development of local technologies to take advantage of market opportunities.

Meanwhile, ultrasonication is the new method in essential oil extraction. The wave used will penetrate the cell walls and facilitates the transfer from the cell into the solvent. Therefore, the extraction becomes easier. So, this research is important in comparing the effect of using solvent extraction and ultrasonic extraction method to the extraction process of patchouli oil.

1.3 Objective

The aim of this project is to extract essential oils from patchouli leaves by using ultrasonic-assisted solvent extraction method.

1.4 Scope

This research is based on experimental studies of solvent extraction and ultrasonication. In order to achieve the objectives mentioned above, three scopes have been identified:

i. To investigate the effect of solvents on extraction.
ii. To investigate the effect of ultrasonic on extraction.
iii. To analyze the product compounds from the extraction process.

CHAPTER 2

LITERATURE REVIEW

2.1 Essential Oils

2.1.1 Introduction

Essential oil is known as volatile or ethereal oils, or simply as the oil of the plant material from which they were extracted. The term essential shows that the oil carries distinctive scent (essence) of the plant. Essential oils do not as a group needs to have any specific chemical properties in common, beyond conveying characteristic fragrances. They are 75 to 100 times more concentrated than the oils in dried herbs. The use of volatile plant oil, including essential oils, for psychological and physical well being has dated back thousands of years. The ancient Chinese are generally acknowledged as the founders of aromatherapy from essential oils and it was used by the ancient Egyptians and ancient Greeks as medicinal perfumes.

Essential oil can be generally distilled from the leaves, stems, flowers, bark, roots or other elements from various parts of plants. They are not true oils in the manner of lubricant vegetable oils, but highly fluid and exceptionally volatile. However, most of essential oils are clear and contain the true essence of the plant it was derived from. Experts recognize an essential oil by its aroma and check its composition by a process called Gas Liquid Chromatography. Colour can also be an indicator; eucalyptus is colourless, chamomile varies from white to blue and others,

like basil and sandalwood (both light greenish-yellow), are in pastel shades. Yet others are richly pigmented, like jasmine, a deep reddish-brown, patchouli, brown, and rose, orange-red.

There are only about 700 plants which considered aromatic among all types of plants in the world that can be used for the production of essential oils and hundreds of other essential oils available for use, many with known antibacterial properties. Table 2.1 shows the parts of various plants that can be extracted.

Table 2.1: Parts of various plants for extraction

Berries • Allspice • Juniperkejut Seeds • Almond • Anise • Celery • Cumin • Nutmeg oil Bark • Cassia • Cinnamon • Sassafras Wood • Camphor • Cedar • Rosewood • Sandalwood Rhizome • Ginger	Leaves • Basil • Bay leaf • Cinnamon • Common sage • Eucalyptus • Lemon grass • Melalueca • Oregano • Patchouli • Peppermint • Pine • Rosemary • Spearmint • Tea tree • Thyme • Wintergreen Resin • Frankincense • Myrrh	Flower • Chamomile • Clary sage • Clove • Geranium • Hyssop • Jasmine • Lavender • Manuka • Marjoram • Orange • Rose • Ylang-ylang Peel • Bergamot • Grapefruit • Lemon • Lime • Orange • Tangerine Root • Valeria

2.1.2 Essential Oils Constituents

Essential oils contain numerous constituents that contribute to the characteristic odour and medicinal effects. The major chemical components that account for the pleasant aromatic odours are primarily terpenenes, monoterpenes and linalool (Williams, 1997). The presence and quantity of the various components varies between oils and determines the individuality of the oil (Tisserand and Balacs, 1996).

Essential oils represent a small fraction of a plant's composition but confer the characteristic for which aromatic plants are used in the pharmaceutical, food and fragrance industries. Essential oils have a complex composition, containing from a few dozen to several hundred constituents, especially hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, aldehydes, ketones, acids, phenols, oxides, lactones, acetyls, ethers and esters). Both hydrocarbons and oxygenated compounds are responsible for the characteristic odors and favors. The proportion of individual compounds in the oil composition is different from trace levels to over 90%. The aroma's oil is the result of the combination of the aromas of all components. Trace components are important, since they give the oil a characteristic and natural odor. Thus, it is important that the natural proportion of the components is maintained during extraction of the essential oils from plants by any procedure (Anitescu *et al.*, 1997).

2.1.3 Uses of Essential Oils

Various essential oils have been used medicinally at different periods in history. Medical applications proposed by those who sell medicinal oils vary from skin treatments to remedies for cancer, and are often based on historical use of these oils for these purposes. Interest in essential oils has revived in recent decades, with the popularity of aromatherapy, a branch of alternative medicine which claims that the specific aromas carried by essential oils have curative effects. Oils are volatilized or diluted in carrier oil and used in massage, or burned as incense, for example.

Furthermore, these aromatic characteristics of essential oils may provide various functions for the plants itself including; attracting or repelling insects (odors of the flowers); while in plant metabolism, a few essential oils might involved in this process; Leaf oils, wood oils, and root oils may serve to protect against plant parasites or depredations by animals as well as anti-bacterial agent which is utilizing the hormone in the oil.

2.1.4 Essential Oil versus Synthetics Oils

Nowadays, essential oils are subjected to more scientific investigation and it was discovered that some of them could be synthesized from other materials. Essential oils are not the same as fragrance oils or perfume where essential oils are derived from the true plants.

Synthetic essential oils are produced by blending aromatic chemicals mostly derived from coal tar which can duplicate the smell of the pure essential oils while the essential oil have the complex chemical components which created in nature that can determine its true aromatic benefits. Synthetic essential oils are unnaturally created fragrances since it contains artificial substances, and it may also do not offer the therapeutic benefit that essential oils does. This is how we can easily compare the differences between synthetic and pure essentials oils. Although synthetic essential oils are not suitable for aromatherapy, they still can be used as the scent to crafts, potpourri, soap and perfume at a fraction of the cost. The reason of these synthetic products is mainly to reduce the cost of production. As it is always quicker and cheaper to produce the laboratory versions than natural plant extracts, true essential oils began to fall from favor.

There is no synthetic substitute for patchouli oil until today, which increases its value and demand in the perfumery market. Currently, the consumption of patchouli oil in the world is about 2000 tonnes per annum (Amir H. Barati *et al.*, 2007).

2.1.5 Hazardous of Essential Oils

Although essential oils are known for their antimicrobial properties, medical teams rarely use them. This is primarily due to lack of scientific evidence of their efficacy, toxicity issues and the availability of conventional therapy. Most readily available essential oils are safe if used in small doses, and side effects are generally rare. Possible side effects include rashes, itching, and irritation on the skin. Allergic reactions include watery eyes, sneezing, and inflammation. Some essential oils may cause nausea, dizziness, or gastrointestinal discomfort when used in excess or by those with allergic reactions. Some essential oils, particularly those derived from citrus fruit plants, can cause increased sensitivity to sunlight and increased risk of sunburn. In addition, some essential oils have not been thoroughly tested and may be toxic. Therefore, any essential oils that have not been tested or lack adequate information should be avoided.

2.2 Patchouli

2.2.1 Patchouli Plant

Patchouli is an aromatic herb plant. Patchouli is native to The Philippines and grows wild and also cultivated in Malaysia, Indonesia, Singapore, China and India. This fragrant herb is a bush with furry leaves and purplish white flowers. It can grow up to three and half feet tall and it has large fragrant leaves. True patchouli has hairy stems, flowers only reluctantly, and is usually propagated by cuttings. Figure 2.1 and Table 2.2 show the images and the scientific classification of patchouli plant.

Figure 2.1: Patchouli *(Pogostemon cablin)*

Table 2.2: Scientific classification of patchouli

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Lamiaceae
Genus	*Pogostemon*
Species	*P. cablin*

Patchouli is a plant which has good economic prospect. Patchouli leaves are the economic part that contains the oil gland to be extracted out. The leaves of the patchouli bush are first dried in the sun, and then distilled to produce thick oil, amber to dark orange in colour (Fang Chen *et al.*, 2007). The yield of oil from the dried leaves is about 3%. Natural fragrances like sandalwood, rose, jasmine, vetiver, agarwood and patchouli are complex mixtures of organic molecules, which cannot be reproduced in the laboratory. Thus, patchouli enjoys an additional importance as aromatic oil. Patchouli alcohol will have long-lasting fragrant aroma when blended with other aroma chemicals.

The world production is estimated to be more than 500 tonnes/year. A small number of companies have specialized in the manufacture of refined qualities of patchouli oil for the perfume industry, where it finds extensive use in modern high-

class perfumes. There are no synthetic equivalents of the patchouli scent. The shade dry leaf upon steam distillation yields the patchouli oil of commerce, which is used in perfumery, cosmetics, processed food and is imported into India every year in large quantities. The essential oil is one of the best fixatives for heavy perfumes, which imparts strength, strong character, alluring notes and lasting patchouli qualities.

2.2.2 Patchouli Essential Oil

Patchouli oil is one of the important natural essential oils used to give a base and lasting character to a fragrance in perfumery industry. The dry leaves of patchouli when extracted using steam distillation yield an essential oil called the oil of patchouli. Indonesia is the major producer of patchouli oil in the world with an estimated 550 tons per year, which is more than 80% of the total (Robbins, 1983; Tao, 1983).

The essential oil of patchouli is extracted from the leaves. The leaves need to be shade dried and partially fermented before distilling. Fresh patchouli essential oil has a sharp, green fragrance, and needs to age to develop the deeper, earthier aroma of good patchouli oil. Patchouli essential oil should always be aged and will continue to improve the longer it sits. The color of the oil will deepen from a light yellowish, pale red to deep, dark amber upon aging, and the oil will become more and more viscous. It is non-toxic, non-irritant and non-sensitizing, but the smell of patchouli oil may be a little persistent for some people and large doses may cause loss of appetite in some individuals. There is no synthetic substitute for patchouli oil until today, which increases its value and demand in the perfumery market.

2.2.2.1 Patchouli Essential Oil Constituents

The chemical components of Patchouli oil are b-patchoulene, a-guaiene, caryophyllene, a-patchoulene, seychellene, a-bulnesene, norpatchoulenol, Patchouli alcohol and pogostol. The constituents of the oil include: Patchoulol (25-35%), Alpha-Bulnesene (12-20%), Alpha-Guaiene + Seychellene (15-25%), and Alpha-Patchoulene (5-9%) (Srikrishna and Satrayanarayana, 2005). However, it is maintained that norpatchoulenol, present in only 0.3-0.4 %, is playing a principal part in the overall odor picture. Natural patchouli oil contains numerous other compounds, including a wide variety of hydrocarbons, epoxides, alcohols and sesquiterpene ketones many of which are also odoriferous compounds. Figure 2.2 shows the structures of nine identified compounds in patchouli oil.

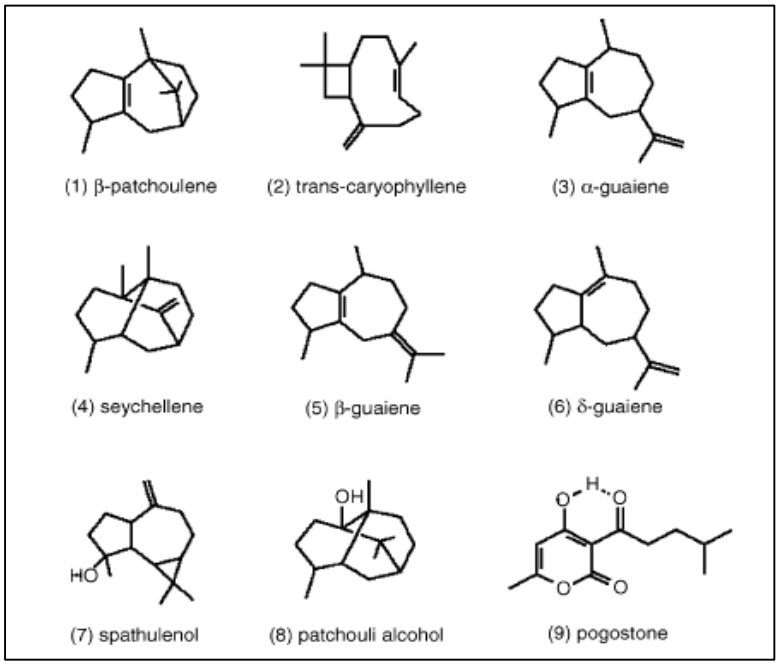

Figure 2.2: The structures of nine identified compounds in *Pogostemon cablin*.

2.2.2.2 Uses of Patchouli Essential Oil

Patchouli oil has therapeutic properties, namely antidepressant, anti-inflammatory, antiseptic, aphrodisiac, astringent, carminative, diuretic, febrifuge, fungicide, insecticide, sedative and tonic. Patchouli can also act as an anti-depressant and can work well on all stress related conditions and anxiety. Patchouli oil mixes well with many essential oils including vetiver, sandalwood, frankincense, bergamot, cedarwood, myrrh, jasmine, rose and the citrus oils (Saligov/t *et al.,* 1996).

Hence, the oils have numerous of uses. It is widely used in soaps, cosmetics, tobacco and in scents (Singh *et al.,* 2002)**.** It has been used medicinally at different periods in history. On the skin, this oil is one of the most active and is a superb tissue regenerator, which helps to stimulate the growth of new skin cells. In wound healing, it not only promotes faster healing, but also helps to prevent ugly scarring when the wound heals. Patchouli oil is very effective in sorting out rough, cracked and overly dehydrated skin and is used to treat acne, acne, eczema, sores, ulcers, any fungal infections, as well as scalp disorders.

Patchouli oil has grounding and balancing effect on the emotions and banishes lethargy, while sharpening the wits, fighting depression and anxiety. The aroma assists the body to relax and promotes a feeling of peace (Fang Chen et *al.*, 2007). It is also said to create an amorous atmosphere. It is effective for fungal and bacterial infection and is of great help for insect bites. As an antifungal, patchouli oil has been used to treat athlete's foot. For the hair, patchouli oil has been used for dandruff and to aid oily hair. For the nervous system, patchouli essential oil helps to reduce tension, insomnia and anxiety. It is also known as uplifting fragrance that helps to soothe away everyday cares, and to bring about a sense of nourishment. As a fixative, it slows the evaporation of other, more volatile oils so that their aroma may be released over a longer period of time (Saligov/t *et al.,* 1996). In this way, and due to its wine-like intoxicating aroma, patchouli oil is also known as an aphrodisiac.

It could also be used as an insect repellant and is also used as a support for dealing with any substance addiction. With its excellent diuretic properties, it is effective in fighting water retention and to break up cellulite, easing constipation and helping to reduce overweight. Furthermore, it has a great deodorizing action, and helps when feeling hot and bothered, while cooling down inflammations and assisting with wound healing.

2.3 Extraction of Essential Oils

2.3.1 Introduction

Extraction is phenomenon that can be defined as the process of separating desired components from a material. Fragrance extraction refers to the extraction of aromatic compounds from raw materials. Nowadays, more extraction on essential oil has been done in extraction field.

There are many methods of essential oil extraction, the most popular being used is steam distillation which is practiced thousands years ago and remains until today. Extraction techniques have been widely investigated to obtain such valuable natural compounds from plants for commercialization. Traditional methods, such as Soxhlet extraction, which have been used for many decades, are very time-consuming and require relatively large quantities of solvents (Luque and Garcia-Ayuso, 1998). There is an increasing demand for new extraction techniques with shortened extraction time, reduced organic solvent consumption, and increased pollution prevention. Novel extraction methods including ultrasound-assisted extraction (Vinatoru, 2001), microwave-assisted extraction (Kaufmann & Christen, 2002), supercritical fluid extraction (Marr and Gamse, 2000; Lang and Wai, 2001; Meireles and Angela, 2003), and accelerated solvent extraction (Kaufmann and Christen, 2002; Smith, 2002) are fast and efficient for extracting chemicals from solid plant matrixes.

Each technique has its own merits and the choice of extraction depends on several factors including capital cost, operating cost, simplicity of operation, amount of organic solvent required, sample through put and the availability of a standardized method (Budzinski *et al.*, 1999b).

The selection of appropriate extraction method will determine the quality and quantity of essential oils. Other factors such as types of plant, chemical make up of oils, and location of oils within the plant (root, bark, wood, branch, leaf, flower, fruit and seed) are also needed to be considered prior to the extraction. The results of the extracts are either essential oils, absolutes, concretes, or butters, depending on the amount of waxes in the extracted product. To a certain extent, all of these techniques tend to distort the odor of the aromatic compounds obtained from the raw materials. Heat, chemical solvents, or exposure to oxygen in the extraction process denature the aromatic compounds, either changing their odor character or rendering them odorless.

In this chapter, the focuses are only given to the solvent extraction and ultrasonic extraction because both of these methods are used in this study.

2.3.2 Solvent Extraction

Solvent extraction is the process of transferring a substance from any matrix to an appropriate liquid phase. If the substance is initially present as a solute in an immiscible liquid phase the process is synonymous with liquid-liquid extraction. The extraction of essential oil components using solvents at high pressure, or supercritical fluids, has received much attention in the past several years, especially in food, pharmaceutical and cosmetic industries, because it presents an alternative to conventional processes such as organic solvent extraction and steam distillation (Eikani *et al.*, 1999).

During extraction, a component is separated from its mixture by selective solubility. If a substance is in contact with two different phases then, in general, it will have a different affinity for each phase. Part of the substance will be absorbed or dissolved by one and part by the other; the relative amounts depend on the relative affinities. The substance is said to be partitioned between the two phases. For example, if two immiscible liquids are taken and a third compound is shaken up with them, then equilibrium is reached in which the concentration in one solvent differs from that in the other. The ratio of the concentrations is the partition coefficient of the system. The partition law states that this ratio is a constant for a given liquid. Factors such as the nature of the solvent used, the temperature etc. would affect the efficiency of extraction.

This method is used for delicate flowers whose odors are damaged by the high heat needed to boil water. The oils are extracted using solvents which have lower boiling points than water. Various substances such as ether or high-grade petroleum, which evaporate rapidly, are used in modern perfumery to dissolve essential oils from fragrant plant and animal materials. The usual method involves placing the fragrant material on perforated metal plates in a container (the extractor); the solvent is passed over them and led into a still, where it evaporates, leaving a semi-solid mass known as concrete, which contains the essential oil together with stearoptene. The oil can then be separated from the stearoptene by extraction with alcohol in a 'batteuse', producing the substance called absolute, which is the purest and most concentrated form of essential oil known.

This is one of a very fast and effective essential oil extraction method. In addition, it yields a higher amount of essential oil at a lower cost. However, the disadvantage of this method is the residual solvent remains in the essential oil that can cause the essential oil become impure.

2.3.3 Ultrasonic Extraction

The use of ultrasound to enhance the extraction yield is a technique that stated in the 1950s with the laboratory scale experiments. (Schmall, *et al*., 1953). A number of papers have been published dealing with ultrasonically assisted extraction of different vegetal materials. One of the first citations concerning ultrasonic extraction (1952) was related to hop extraction in aqueous medium and showed that ultrasonic reaction was comparable with the boiling extraction process. (Specht and Forrsch, 1952).

It is known that the use of ultrasound-assisted extraction (USE) aids extraction by signicantly reducing extraction times (Rezic *et al.*, 2005) in comparison with traditional methods (exp. shake-ask extraction). The mechanical effect of Ultrasound provides a greater penetration of solvent into matrix, via cavitation effects, and improves extraction. Cavitation is usually considered playing the most important role in ultrasonic enhancement of membrane process for liquid–liquid and liquid–solid system. Ultrasonic enhancement on mass transfer has been realized in such unit operations as leaching, extraction, adsorption and desorption (Haizhou *et al.,* 2003). Therefore, efficient cell disruption and effective mass transfer are cited as two major factors leading to the enhancement of extraction with ultrasonic power (Mason *et al.,* 1996). Qualitative and quantitative composition of the volatiles was determined by GC and GCMS.

To perform ultrasonically assisted extraction is not difficult on a laboratory scale using a simple cleaning bath (Figure 2.3 and 2.4). Using such equipment it is possible to obtain good extraction yields by direct or indirect extraction. In both cases it is preferable to use a mechanical stirrer and to cool the extraction mixture since the absorption of ultrasonic energy can cause warming. By indirect sonication, only small amounts of vegetal material can be extracted whereas using the direct procedure, large amounts of vegetal material can be employed.

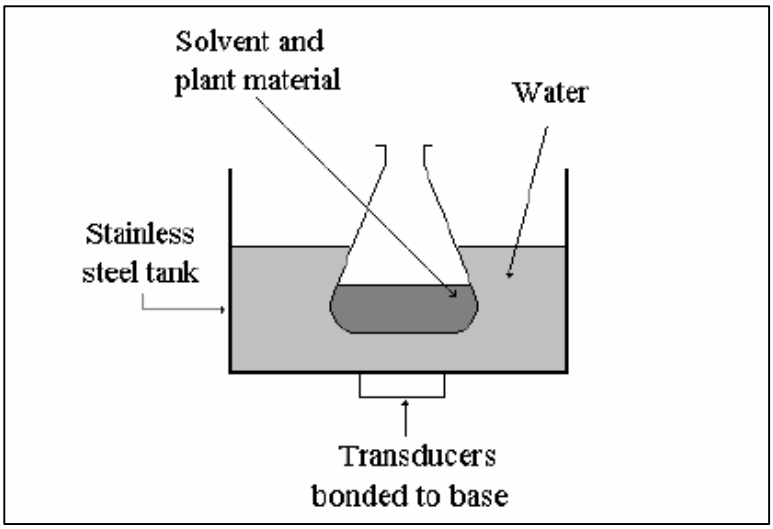

Figure 2.3: Experimental setup for indirect using a cleaning bath

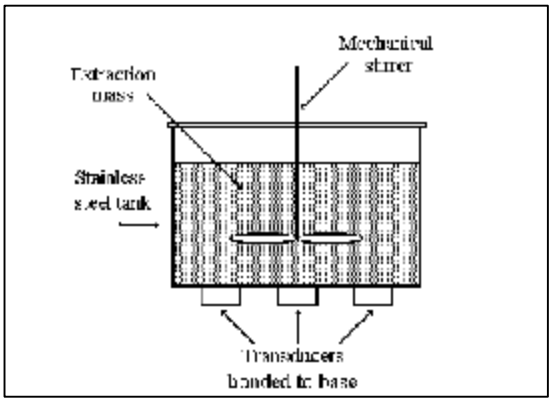

Figure 2.4: Experimental setup for direct extraction using a cleaning bath

Ultrasonication offers several advantages that make it an ideal method for analyzing a large number of samples. These include high extraction efficiency, lower equipment costs and ease of operation, little or no sample preparation, lower extraction temperatures and the ability to process batches of samples makes ultrasonic extraction an ideal method for laboratories analyzing large numbers of samples. In oil extraction research from ginseng showed that the used of ultrasound in the extraction process increased the yield and quality of the oil better than other methods such as maceration and stirring (Hui *et al.*, 1994).

There are some factors that affect ultrasound cavitation such as ultrasonic intensity, ultrasonic frequency, mass to solvent ratio, liquid properties, extraction

temperature and hydrostatic pressure. In general, this research will be focus more on the ultrasonication time (extraction time) and temperature factors. The manipulated variables in this research will be the extraction times. The dependent variable is the oil yield while the independent variables are volume of the solvent, solvent polarity, solvent concentration, size and amount of patchouli leaves, ultrasonic intensity and frequency.

2.4 Extraction Principles and Mechanisms

2.4.1 Extraction Principles and Mechanisms of Solvent

Solvent extraction is a process of diffusion of solvent into the oil bearing cells of the raw material resulting a solution of the oil in the solvent. Various solvents can be used for extraction. The separation of materials of different chemical types and solubilities by selective solvent action; that is, some materials are more soluble in one solvent than in another, hence there is a preferential extractive action.

For thorough and efficient extraction, it is necessary that each and every oil-bearing cell of the material is brought in contact with the solvent. Therefore, proper preparation of materials prior to extraction is very important to ensure this contact. The smaller the material size, the better is the penetrating of the solvent into the oil-bearing cells; but too fine a size will prevent the solvent from percolating through the mass. Therefore an optimum size is to be maintained for best extraction. Hence material preparation methods vary from material to material depending on its oil content, size and physical properties.

Solvent extraction is carried out regularly in the laboratory by the chemist as a commonplace purification procedure in organic synthesis, and in analytical separations in which the extraordinary ability of certain solvents preferentially to remove one or more constituents from a solution quantitatively is exploited.

2.4.2 Extraction Principle and Mechanism of Ultrasonic

Sound waves, which have frequencies higher than 20 kHz, are mechanical vibrations in a solid, liquid and gas. Unlike electromagnetic waves, sound waves must travel in a matter and they involve expansion and compression cycles during travel in the medium. Expansion pulls molecules apart and compression pushes them together. The expansion can create bubbles in a liquid and produce negative pressure. The bubbles form, grow and finally collapse. Close to a solid boundary, cavity collapse is asymmetric and produces high-speed jets of liquid. The liquid jets have strong impact on the solid surface (Luque and Castro, 2003).

Vegetal tissue consists of cells surrounded by walls (Figure 2.5). The extraction mechanism involves two types of physical phenomena: diffusion through the cell walls and washing out (rinsing) the cell contents once the walls are broken. Both phenomena are significantly affected by ultrasonic irradiation. Some cells exist in the form of glands (external or internal) that are filled with essential oil. A characteristic of such glands (when external) is that their skin is very thin and can be very easily destroyed by sonication. This explains why the extraction of essential oil, as well as fat oil is facilitated by sonication. For internal glands, it is the milling degree of the vegetal materials which plays an important role (Badica, 2000). It is obvious that reducing the size of the vegetal material particles will increase the number of cells directly exposed to ultrasonically induced cavitation. This effect can be utilized by milling the material before extraction. External essential oil glands are already exposed directly to the cavitating solvent and consequently are readily disrupted.

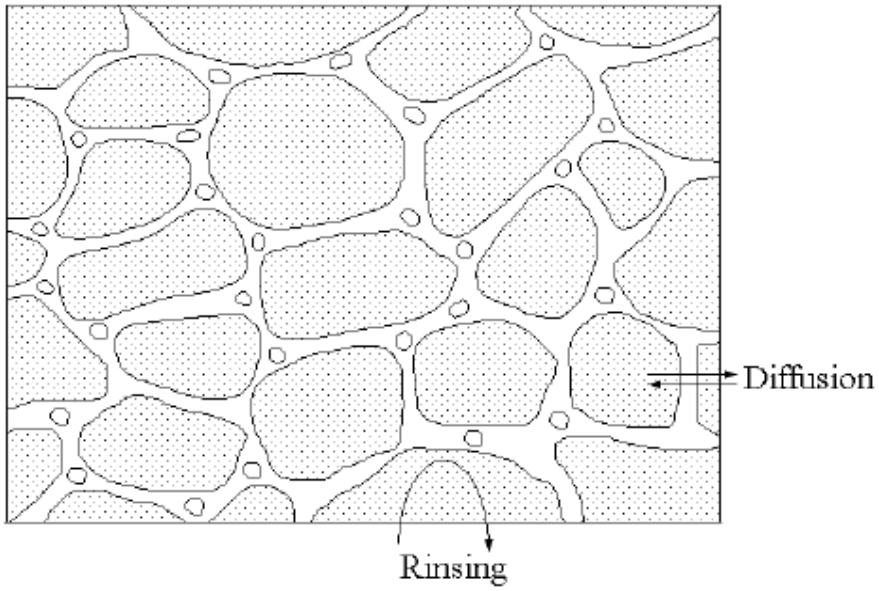

Figure 2.5: Schematic diagram of vegetal cell structures

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

i. Steeping vegetal materials in solvent to facilitate swelling and hydration processes.
ii. The mass transfer of soluble constituents from the material to solvent by diffusion and osmotic processes.

Ultrasound can facilitate swelling and hydration and so cause an enlargement in the pores of the cell wall. This will improve the diffusion process and therefore enhancing mass transfer.

2.5 Solvent Used

In this experiment, there are three solvents used in order to extract patchouli essential oil. These solvents are commonly chosen in the extraction process. Table 2.3 shows the comparison between the properties of these solvents.

Table 2.3: The comparison of solvent properties used.

Solvent	Formula	Boiling point (^{o}C)	Miscibility	Polarity Index	Dielectric properties
Ethanol	C_2H_5OH	78.5	Miscible in water and organic solvent	5.2 (polar)	24
Hexane	C_6H_{14}	69	Immiscible in water but miscible in organic solvent	0.1 (non-polar)	2.0
Acetone	CH_3COCH_3	56.29	Miscible in water and organic solvent	5.1 (polar)	20.7

2.5.1 Ethanol

Ethanol is a monohydric primary alcohol. Its boiling point is 78.5°C. Ethanol is a versatile solvent, miscible in all proportions with water and many organic solvents. Ethanol, like most short-chain alcohols, is flammable, colorless, has a strong odor, and is volatile. The polar nature of the hydroxyl group causes ethanol to dissolve many ionic compounds. In addition, the ethanol molecule also has a nonpolar end, make it dissolves nonpolar substances, including most essential oils, as well as numerous flavoring, coloring, and medicinal agents.

Ethanol burns in air with a blue flame, forming carbon dioxide and water. It reacts with active metals to form the metal ethoxide and hydrogen, e.g., with sodium it forms sodium ethoxide. It reacts with certain acids to form esters, e.g., with acetic

acid it forms ethyl acetate. It can be oxidized to form acetic acid and acetaldehyde. It can be dehydrated to form diethyl ether or, at higher temperatures, ethylene.

Ethanol is used extensively as a solvent in the manufacture of varnishes and perfumes; as a preservative for biological specimens; in the preparation of essences and flavorings; in many medicines and drugs; as a disinfectant and in tinctures (e.g., tincture of iodine); and as a fuel and gasoline additive.

2.5.2 Hexane

Hexane is a very volatile aliphatic hydrocarbon. The boiling point is 69^{o}C. Laboratory grade hexane contains approximately 99% hexane. Hexane isomers are largely unreactive, and are frequently used as an inert solvent in organic reactions because they are very non-polar. Hexane is immiscible in water but miscible in organic solvent.

The acute toxicity of hexane is relatively low, although it is a mild anesthetic. Inhalation of high concentrations produces first a state of mild euphoria, followed by somnolence with headaches and nausea.

It is a constituent in the paraffin fraction of crude oil and natural gas and is also used as an industrial chemical and laboratory reagent. They are also common constituents of gasoline and glues used for shoes, leather products and roofing. Additionally, it is used in solvents to extract oils for cooking and as a cleansing agent for shoe, furniture and textile manufacturing.

2.5.3 Acetone

Acetone (also known as propanone, dimethyl ketone, 2-propanone, propan-2-one and β-ketopropane) is a colorless, mobile, flammable liquid. It is the simplest example of the ketones. Acetone is miscible with water, ethanol, ether, etc., and itself serves as an important solvent. The boiling point of acetone is 56.93^{o}C

Acetone has many uses such as in automotive fuel additive, a cleaning agent and removes residues from glass and porcelain. In the laboratory, acetone is used as a polar aprotic solvent in a variety of organic reactions, such as S_N2 reactions. Because of acetone's medium polarity, it dissolves a wide range of compounds. Thus, it is commonly loaded into squeezebottles and used as a general solvent in rinsing laboratory glassware.

Acetone is an irritant and inhalation may lead to hepatotoxic effects (causing liver damage). The vapors should be avoided. In no circumstance should it be consumed directly or indirectly. Kidney, liver, and nerve damage, increased birth defects, and lowered reproduction ability of males (only) occurred in animals exposed long-term to acetone.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This research works focus on the achievement of the conceptual study, laboratory experimental work, analyzing and the completion of the project. The detailed experimental work procedures are discussed through out this chapter. This study consists of two independent experiments; the first experiment, to investigate the effect of different solvents to the extraction process and the second, to investigate the effect of ultrasonic to the extraction process. The yields from both experiments were analyzed with Gas Chromatography Mass Spectrometer (GCMS).

In the first experiment, three different solvents were used; ethanol, hexane and acetone, to investigate the effect of solvents used to the extraction process. During the extraction process, components were selectively separated from the mixture by selective solubility. The oils were extracted using solvents which have lower boiling points than water. From the result of the experiments, the best solvent was chosen.

Then, the second experiment of investigating the effect of ultrasonic extraction was carried out. Through this method, patchouli leaves could be mixed with the solvent and thereafter exposed to the ultrasonic wave for certain duration. The result of this experiment was compared with the experiment without using ultrasonic.

3.2 Experimental Methodology

3.2.1 Methodology

Figure 3.1 shows the overall methodology of this study. It consists of two different sets of experiments. The main activities in this research are the experimental study and the subsequent analysis.

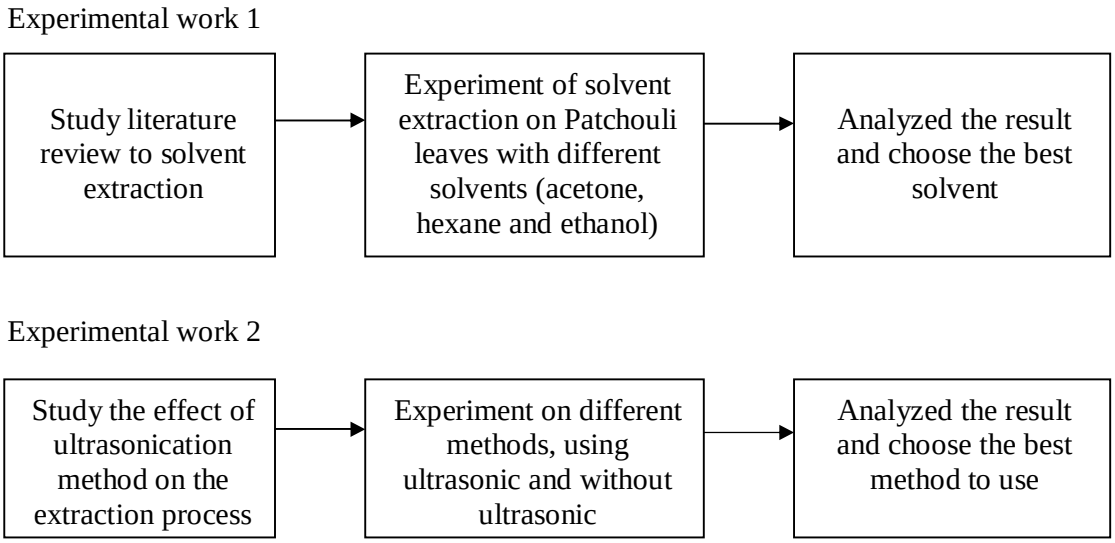

Figure 3.1: Overall methodology

Two different sets of experiments were carried out during the extraction of essential oil from patchouli leaves which comprised of:-

i. Solvent extraction method by using ethanol, hexane and acetone solvent to investigate the best solvent.
ii. Ultrasonication method to investigate its effect to the extraction process.

In the first experiment, we studied about the solvent extraction method, the solvents used and the effect of the solvents to the yield of the extraction. Hence, extraction time, the amount of solvents and sample used, temperature and pressure

are the fixed parameters in order to see the different amount of yield produced. Therefore, the solvent that can extract most oils and give the high concentration based on analysis from the sample was chosen as the best solvent to be used in this study.

Meanwhile, the second experiment was quite similar to the first one including the parameters, solvent and sample used, but in this experiment ultrasonication method was used to investigate its effect towards extraction process. Then, both of the methods were compared based on the yield got and the result from the analysis. After that, the best method was chosen.

3.3 Experimental Works

3.3.1 Solvent Extraction Experimental Works

Raw materials of patchouli leaves were collected and dried to a constant mass. It was to get free of any substance that can influence the impurities of oil when it has been extracted (Norazlina, 2005).

The patchouli leaves were grinded to maximize the contact surface between the leaves and solvent. Finely ground sample is more easily extracted than large particles.

Next, dried patchouli leaves were mixed with solvent in the beaker. This process was called as soaking to break down the cells and oil glands. This would help in the extraction process and make the process faster. Three samples were prepared. Each sample contains 15 gram of patchouli leaves mixed with 150 ml solvent in a ratio of 1:10. The samples were soaked in one day for extraction process. After one day, the samples were filtered using filter and the oil was later separated from the solvent using rotary evaporator. It was then analyzed using Gas Chromatography Mass Spectrometer (GCMS). Table 3.1 shows the condition of this

experiment while Figure 3.2 shows the flow chart summarizing the method employed in the experiment.

Table 3.1: Experiment condition of solvent extraction.

Reagent used (Boiling point)	Sample 1: 150ml Ethanol (78.4 °C) Sample 2: 150ml Hexane (69 °C) Sample 3: 150ml Acetone (56.3 °C)
Weight of Patchouli leaves in each sample	15 gram
Pressure	1 atm
Temperature	Room temperature
Extraction time (soaking)	24 hours

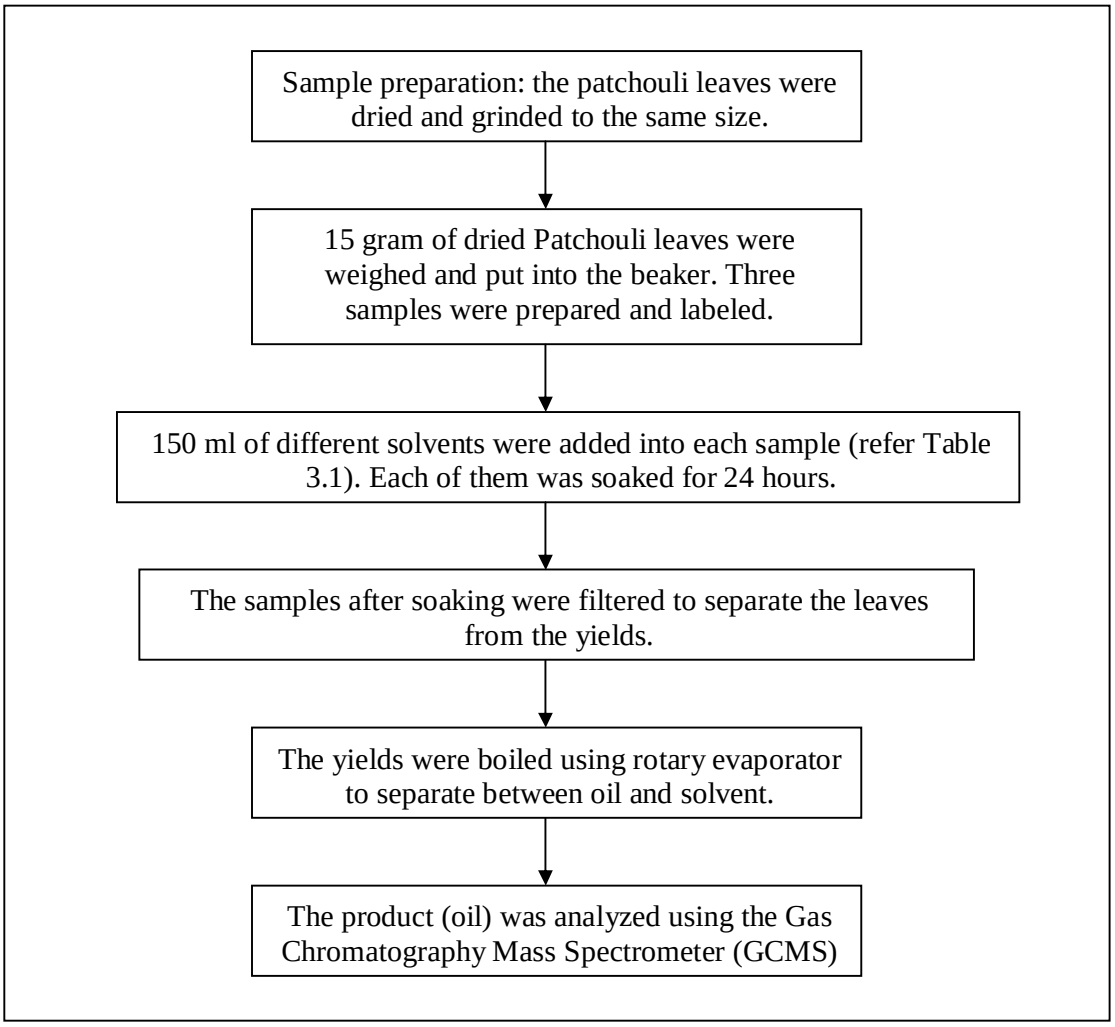

Figure 3.2: Flow chart detailing solvent extraction experiment

3.3.2 Ultrasonic Extraction Experimental Works

For ultrasonic extraction, the preparation of samples was same with solvent extraction except the solvent was fixed and set up of the ultrasonic bath/cleaner. In this experiment, the objective was to investigate the influence of the ultrasonic to the extraction process and comparing with the method without using ultrasonic. The manipulated variables in this research was the extraction time while the dependent variable was the oil yield and the independent variables were volume of the solvent,

solvent polarity, solvent concentration, size and amount of patchouli leaves, ultrasonic intensity and frequency.

Six samples were been prepared in this experiment. Three samples were used in the ultrasonic method while the other three samples were used in experiment without ultrasonic. Ethanol was used in the entire samples because it was the best solvent investigated from the first experiment. After the samples were prepared, both of the experiments were run at the same duration. The first three samples were partially immersed into the ultrasonic bath which contains water. The mixture was ultrasonicated for 10, 20 and 30 minutes with constant ultrasonic wave intensity in the bath. With the same time interval, the other three samples were let to soaking without ultrasonic. After that, all the samples were filtered using the filter and the oil was separated from the solvent using rotary evaporator. The product (oil) was analyzed using the Gas Chromatography Mass Spectrometer (GCMS). Table 3.2 shows the condition of this experiment while the Figure 3.3 shows the flow chart of ultrasonic-assisted solvent extraction experiment.

Table 3.2: Experiment condition of ultrasonic and without ultrasonic

Reagent used in each sample (Boiling point)	150 ml ethanol (78.4 °C)
Weight of Patchouli leaves in each sample	15 gram
Method	Sample 1: Ultrasonic (10 min)
	Sample 2: Ultrasonic (20 min)
	Sample 3: Ultrasonic (30 min)
	Sample 4: Without ultrasonic (10 min)
	Sample 5: Without ultrasonic (20 min)
	Sample 6: Without ultrasonic (30 min)
Pressure	1 atm
Temperature	Room temperature

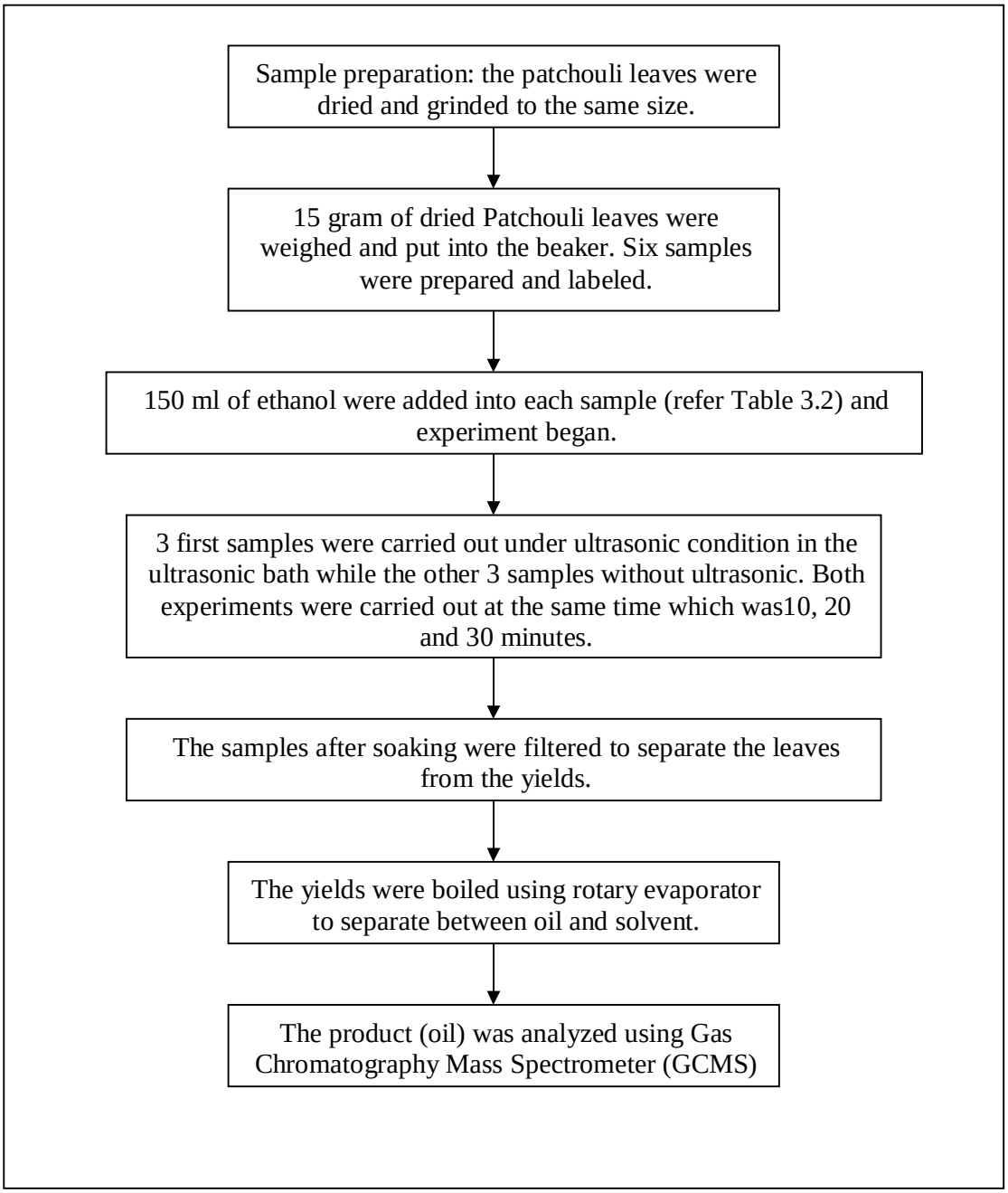

Figure 3.3: Flow chart showing ultrasonic-assisted solvent extraction experiment

3.3.3 Preparation of Sample for Analysis

The next step after extraction process is analysis. The samples for analysis were prepared according certain steps. Based on two different experiments, nine samples must be prepared, three samples for first experiment and six others for second experiment. The method of preparing all the samples is same.

Firstly, 10 µl oil was sucked by micropipette and put in the clean vial. Then, the oil was mixed with 1000 µl solvent. The colour of the solution must be light and not muddy. After that, the solution was added with sodium acetate (15% of the volume solution) in order to remove water in the solution. Water must be removed to prevent damage to the GCMS. Natrium sulphate became solid if there were water appearance in the solution. The water was assumed to be all removed when excess sodium sulphate was added and its does not turn into solid. Then by using syringe, needle and small filter, the solution was transferred again into the other vial. This step was to make sure no disturbance appeared during the analysis process.

3.4 Analysis

3.4.1 Analysis of Essential Oils Using GCMS

GCMS was used to analyze the patchouli essential oil. GCMS can also be used to separate small amounts of materials and determine whether a desired component was present.

In this part, GCMS was performed with an Agilent 7890A type model 5975C, with the GC system of Agilent Technologies. Compounds were separated on a 30m x 0.25mm id capillary column coated with 0.25µm film 5% phenyl methyl siloxane. The column temperature was at 50^{o}C for injection, temperature program began at 10oC/min until 240^{o}C. Split injection was conducted with a split ratio of 1:10 and

helium was used as carrier gas of 1.0 ml/min flowrate. The inlet ionization source temperatures were 250^{o}C and 280^{o}C respectively.

3.4.2 Identification of Essential Oil Constituents

Essential oil constituents were identified by comparing retention times of chromatogram with reference compound run under identical condition. Analytical results were obtained using the reference compounds based on the area ratio between the target components and the reference compound. The percentage composition of the identification compounds were computed from the GC peak area without any correction factor. The compounds were identified by comparing their retention times and mass spectra with those of pure reference compounds. Mass spectra were also compared with those in the NIST and WILEYS mass spectra libraries. Figure 3.4 shows computerized GCMS system available in the lab.

Figure 3.4: Computerized GCMS system

3.5 Calculation of Yield of Extraction Process

Below is the formula on how the yield of the extraction process was calculated.

$$\text{Yield of the essential oil (\%)} = \frac{\text{Amount of essential oil (g) obtained}}{\text{Amount of jasmine flower (g) used}} \times 100$$

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

Both of the experiments were completed. This chapter discussed based on the data from the experiment that had been carried out. Therefore, from the result, qualitative and quantitative analysis was done in order to show the objectives of this study were achieved.

4.2 Qualitative Analysis

Qualitative analysis involved the analysis from the Gas Chromatography Mass Spectrometer (GCMS) library. When one of the component reach the detector, GCMS will search for the component in library and show the detail for the component including the retention time, purity, area and component name in IUPAC and detail forms. The results describe on the analysis of the patchouli essential oil component based on the literature and comparing the purity of this oil using different solvent. The analysis was carried out from the chromatogram by comparing the retention time and peak area in order to determine the best sample used. From the chromatogram also, the compound that was detected in the sample can be determined. Based on this result, it was compared to the literature that has been made by previous researcher.

4.2.1 GCMS Analysis of Patchouli Essential Oil Using Solvent Extraction Method

From this experiment, three samples of patchouli essential oil were analyzed using GCMS to determine the component of the essential oil. This experiment was conducted using 150 ml of different solvent; ethanol, hexane and acetone, and 15 gram of dried patchouli. The extraction time was 24 hours.

i- Sample 1

Sample 1 was conducted using ethanol as the solvent. Figure 4.1 shows the GCMS analysis for sample 1. Only patchouli alcohol can be detected in this sample at retention time of 14.644 min.

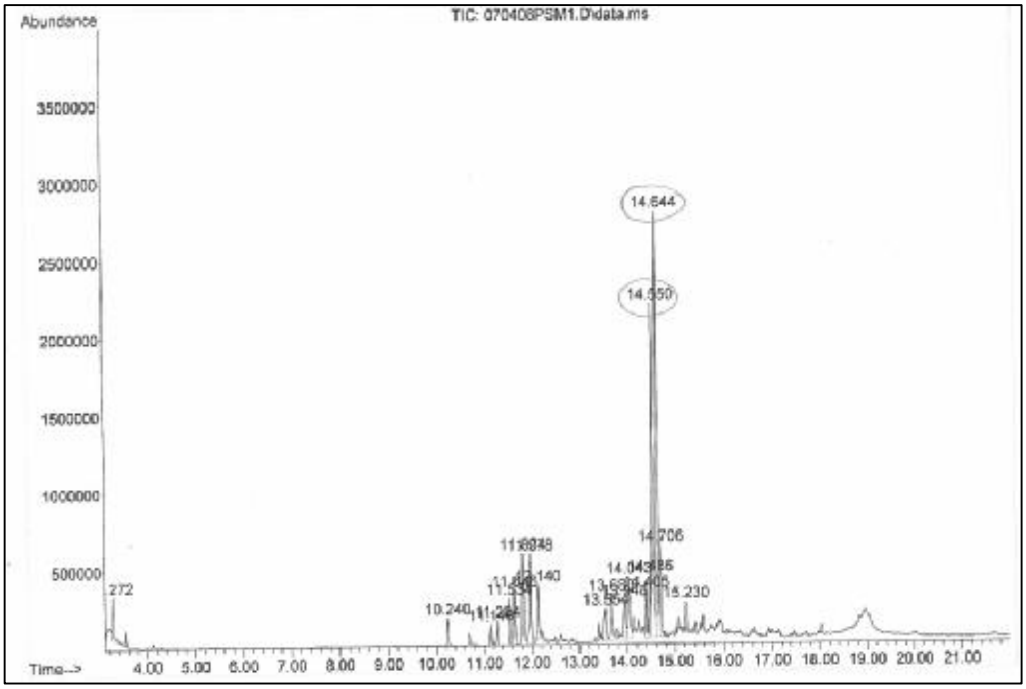

Figure 4.1: Analysis of sample 1 (ethanol)

ii- Sample 2

Sample 2 was conducted using hexane as the solvent. Figure 4.2 shows the GCMS analysis for sample 2. Only patchouli alcohol can be detected in this sample at retention time of 14.737 min.

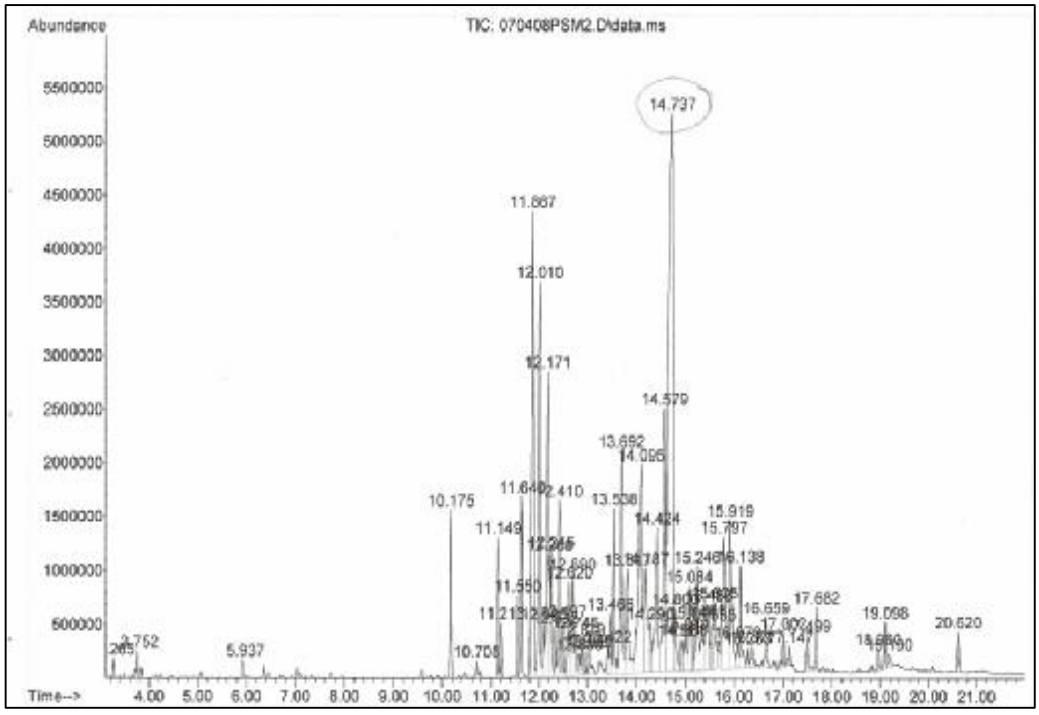

Figure 4.2: Analysis of sample 2 (hexane)

iii- Sample 3

Sample 3 was conducted using acetone as the solvent. Figure 4.3 shows the GCMS analysis for sample 3. Only patchouli alcohol can be detected in this sample at retention time of 14.638 min.

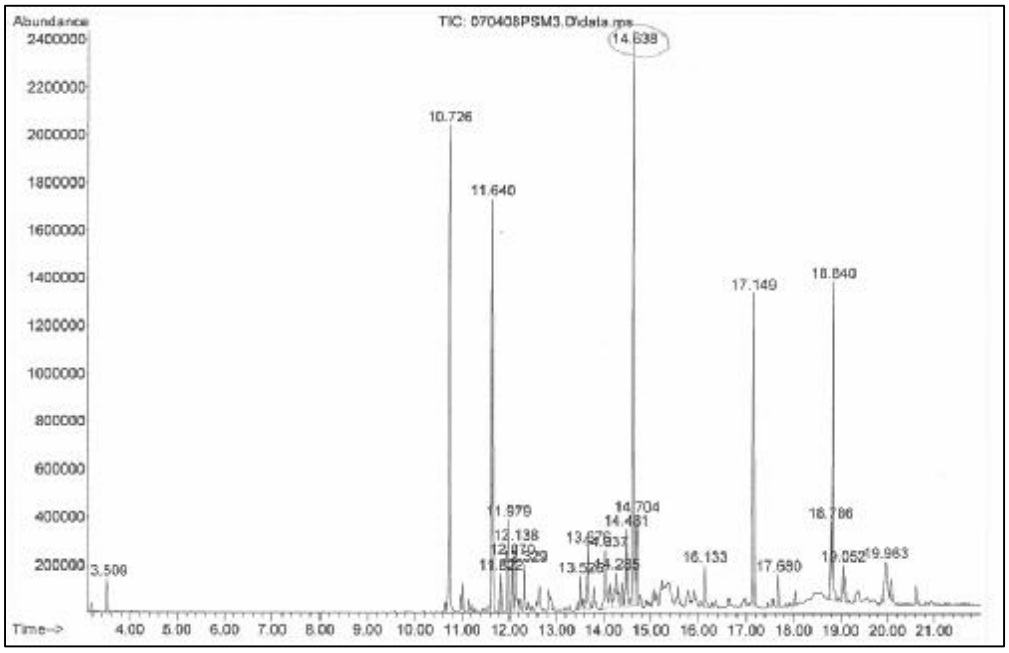

Figure 4.3: Analysis of sample 3 (acetone)

Table 4.1 shows the summary of qualitative analysis of three samples above.

Table 4.1: Summary of qualitative analysis of experiment 1

Sample (Solvent used)	Peak	Retention time (minute)	Peak Area (%)
1 (Ethanol)	16	14.645	27.92
2 (Hexane)	32	14.737	20.01
3 (Acetone)	14	14.638	20.42

From this qualitative analysis, patchouli alcohol was detected at 14 minutes and above in all samples. The peak of patchouli alcohol was the highest peak among the other compound peak in the chromatogram. From GCMS library, the compound can be easily detected including its retention time and peak area. The peak area shows how much the compound was present in the sample. When the peak area is

large, its mean the compound is present much in the sample and give the high quality. In this case, sample 1 which using ethanol as the solvent give the largest peak area (27.92%) of patchouli alcohol compared to other two samples. Therefore, ethanol can give the highest quality of patchouli alcohol. Hence, ethanol was used in the second experiment.

4.2.2 GCMS Analysis of Patchouli Essential Oil Using Ultrasonic and Without Ultrasonic Extraction Method

4.2.2.1 Using Ultrasonic Extraction Method

i- Sample 1

Sample 1 was carried out for 10 min in ultrasonic bath. Figure 4.4 shows the GCMS analysis for sample 1. Patchouli alcohol was the only compound that can be detected in this sample at retention time of 14.632 min.

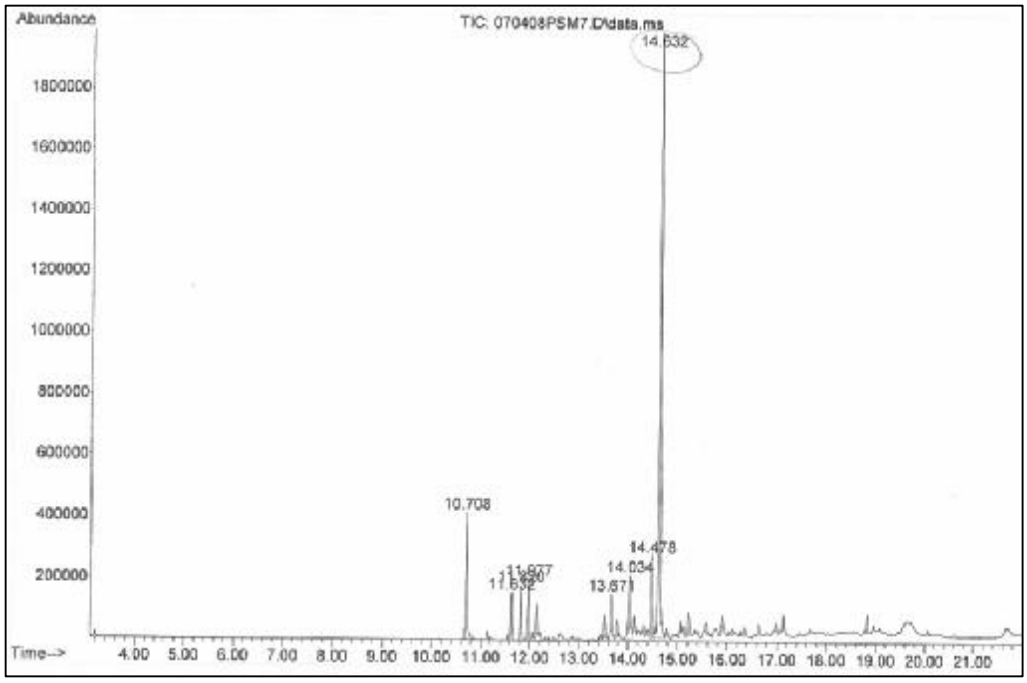

Figure 4.4: Analysis of sample 1 (ultrasonic-10 min)

ii- Sample 2

Sample 2 was carried out for 20 min in ultrasonic bath. Figure 4.5 shows the GCMS analysis for sample 2. Patchouli alcohol was the only compound that can be detected in this sample at retention time of 14.636 min.

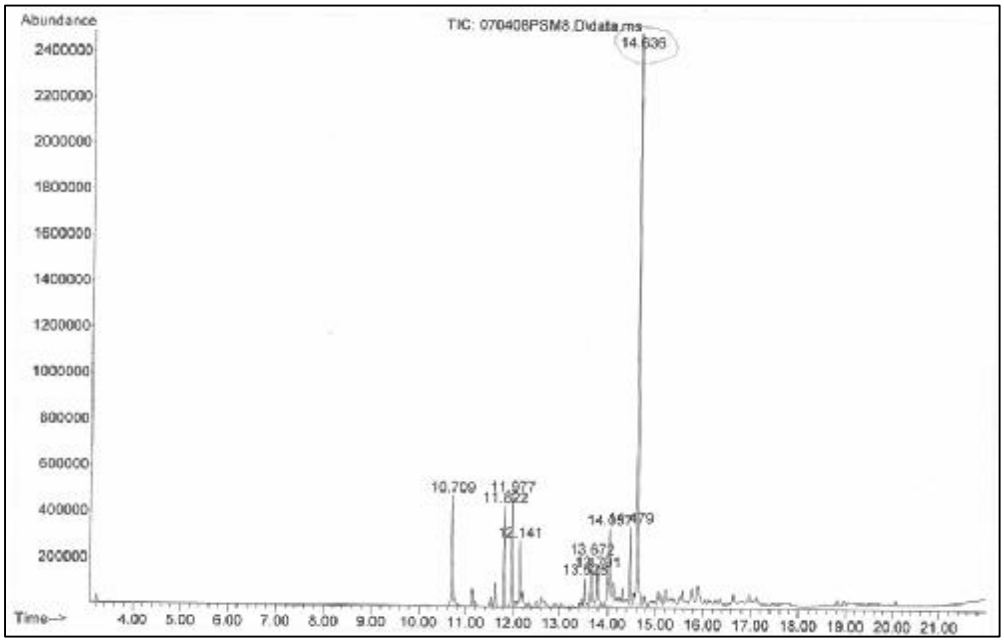

Figure 4.5: Analysis of sample 2 (ultrasonic-20 min)

iii- Sample 3

Sample 3 was carried out for 30 min in ultrasonic bath. Figure 4.6 shows the GCMS analysis for sample 3. Patchouli alcohol was the only compound that can be detected in this sample at retention time of 14.644 min.

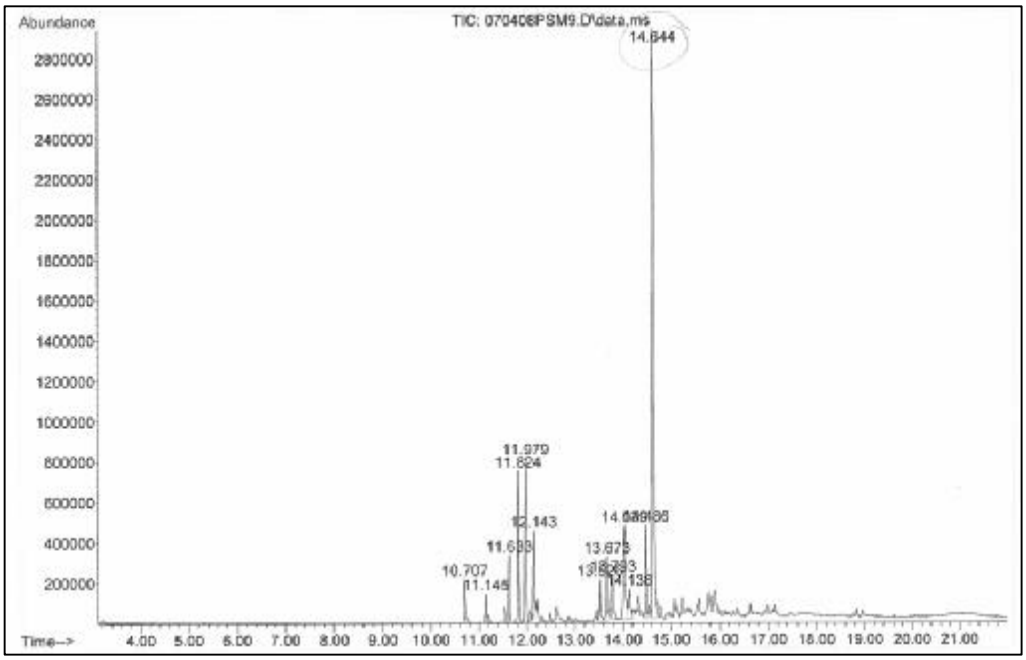

Figure 4.6: Analysis of sample 3 (ultrasonic-30 min)

4.2.2.2 Without Ultrasonic Extraction Method

i- Sample 4

Sample 4 was soaked for 10 minute without using ultrasonic bath. Figure 4.7 shows the GCMS analysis for sample 4. Patchouli alcohol was the only compound that can be detected in this sample at retention time of 14.646 min.

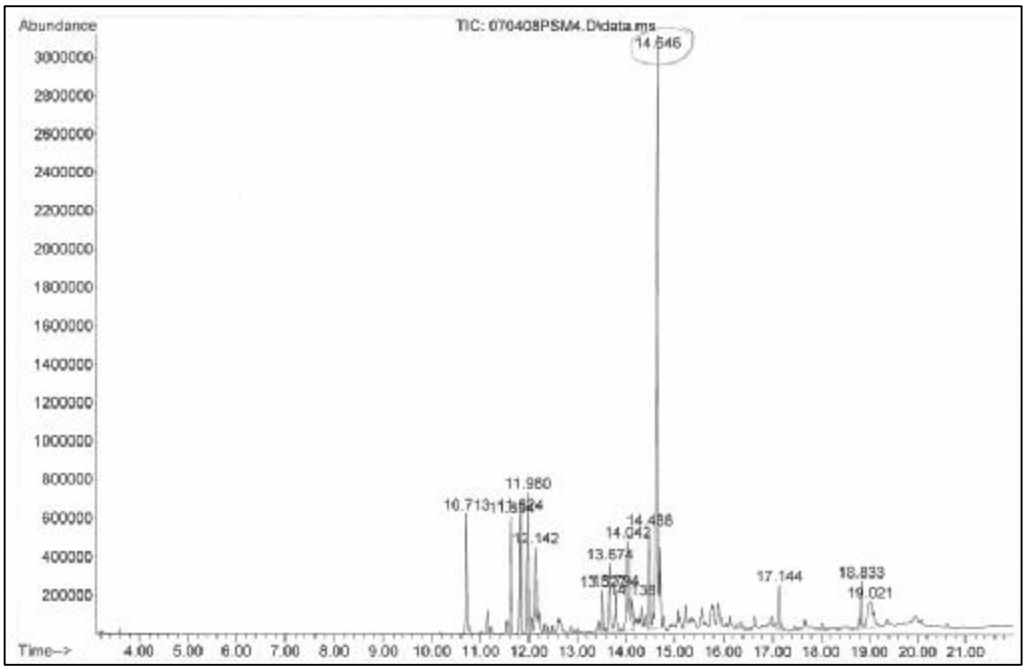

Figure 4.7: Analysis of sample 4 (without ultrasonic-10 min)

ii- Sample 5

Sample 5 was soaked for 20 minute without using ultrasonic bath. Figure 4.8 shows the GCMS analysis for sample 5. Patchouli alcohol was the only compound that can be detected in this sample at retention time of 14.648 min.

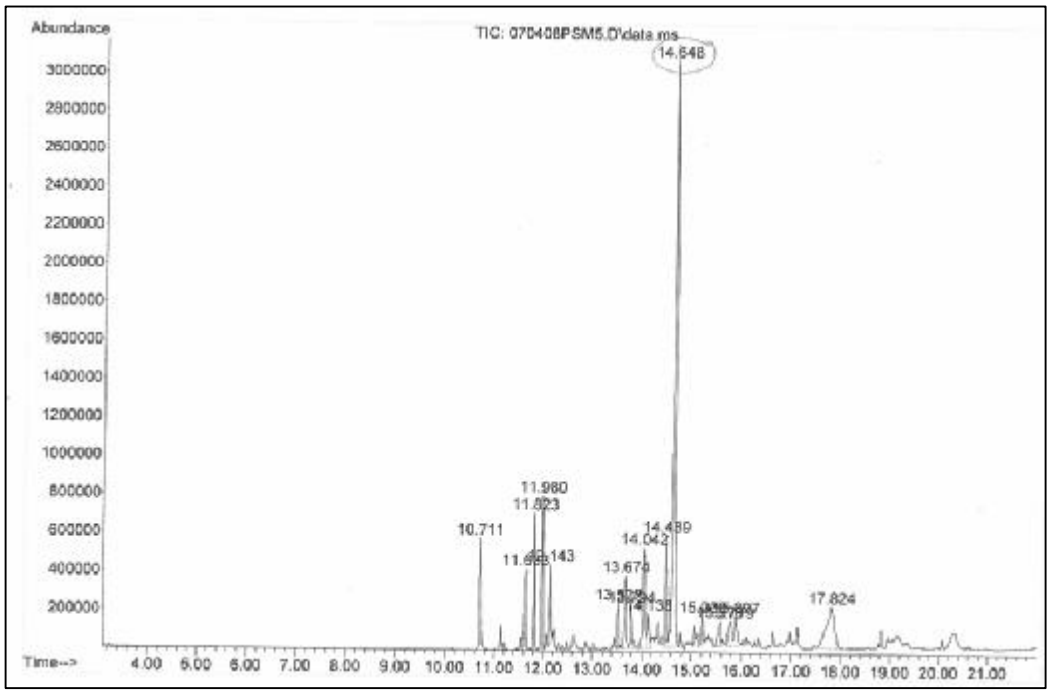

Figure 4.8: Analysis on sample 5 (without ultrasonic-20 min)

iii- Sample 6

Sample 6 was soaked for 30 minute without using ultrasonic bath. Figure 4.9 shows the GCMS analysis for sample 6. Patchouli alcohol was the only compound that can be detected in this sample at retention time of 14.634 min.

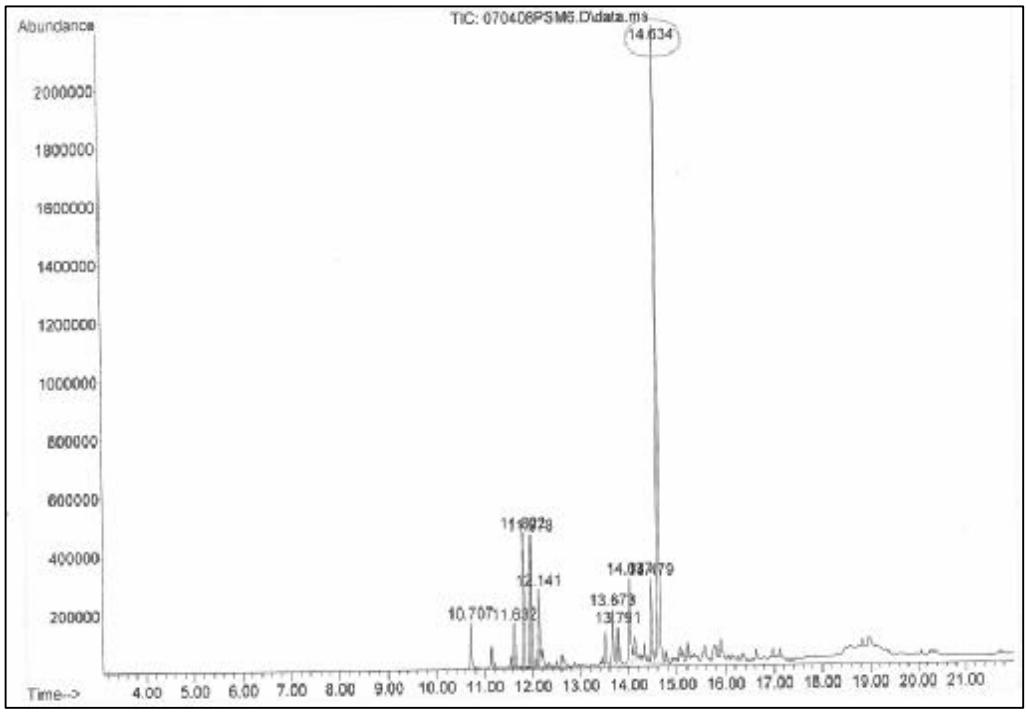

Figure 4.9: Analysis on sample 6 (without ultrasonic-30 min)

Table 4.2 shows the summary of qualitative analysis of six samples above.

Table 4.2: Summary of qualitative analysis of experiment 2

Method	Sample (duration)	Peak	Retention time (minute)	Peak Area (%)
Ultrasonic	1 (10 minute)	8	14.632	58.06
	2 (20 minute)	10	14.636	48.33
	3 (30 minute)	13	14.644	44.15
			Average	50.18
Without ultrasonic	4 (10 minute)	12	14.646	37.20
	5 (20 minute)	12	14.648	38.71
	6 (30 minute)	9	14.634	51.30
			Average	42.40

From the qualitative analysis of these six samples, patchouli alcohol was detected at 14 minutes and above in all samples. In this case, average peak area (50.18%) of patchouli alcohol of the experiment that assisted by ultrasonic was larger compared to experiment without assisted by ultrasonic. Therefore, the quality of extraction is much better when assisted by ultrasonic.

4.3 Quantitative Analysis

4.3.1 Yield of Patchouli Oil from Solvent Extraction Method

Quantitative analysis was done in order to calculate the yield of essential oil produced. The percent yield shows the pure essential oil that produced during the experiment. It can be calculated using formula that was shown in chapter 3. Table 4.3 shows the result of the yield of patchouli essential oil for three samples in the first experiment.

Table 4.3: Table yield of oil essential oil from solvent extraction method

Sample	Amount of Patchouli used (g)	Amount of oil recovered (g)	Yield of the essential oil (%)
1	15	0.43	2.87
2	15	0.38	2.53
3	15	0.30	2.00

From the quantitative analysis of these three samples, all solvents used can extract the patchouli oil. All the oils extracted are below 3% which is the maximum value of patchouli oil that can be extracted from the amount of raw material used. However, ethanol extracts the highest percent of yield among the others. Ethanol is polar solvent while patchouli alcohol is also polar compound. Polar substances tend to be soluble in polar liquids. Therefore, ethanol can extract most polar compound such patchouli alcohol compared to hexane which is non-polar solvent. Ethanol is

also better than acetone although both are polar solvents but the polarity index of ethanol is higher than acetone. Solvent with a higher polarity extracted a significantly higher amount of total desired compound. Therefore, from this quantitative analysis, ethanol is the best solvent.

4.3.2 Yield of Patchouli Oil from Ultrasonic and Without Ultrasonic Extraction Method

Table 4.4: Table yield of oil essential oil from ultrasonic and without ultrasonic method

Method	Sample	Amount of Patchouli used (g)	Amount of oil recovered (g)	Yield of the essential oil (%)
Ultrasonic	1	15	0.28	1.87
	2	15	0.31	2.07
	3	15	0.43	2.87
			Average	2.27
Without ultrasonic	4	15	0.20	1.33
	5	15	0.25	1.67
	6	15	0.30	2.00
			Average	1.67

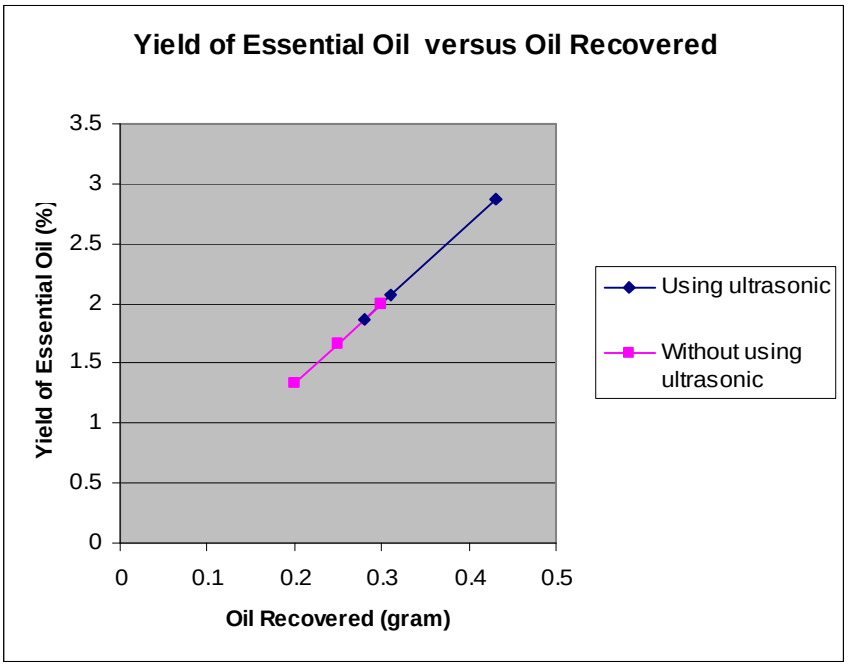

Figure 4.10: Graph of yield of patchouli oil versus oil recovered

Based on Table 4.4, the average value of the yield from the method assisted by ultrasonic are more than the yield from method without assisted by ultrasonic. All the oils extracted are also below 3%. From the table, it shows that the amount of yield is proportional to time in both experiments. When time is increase, the amount of yield is also increase.

The ultrasonic wave formed bubble and produced great force to penetrate deep into the cells. Ultrasound will help to break the cell wall and the oil is diffuse quicker. It has been anticipated that the introduction of the ultrasonic wave in this method would increase the yield and quality of the oil extracted.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Patchouli oil is widely use for many purposes. Therefore, the best method is needed in order to extract the high quality of patchouli oil. Based on the result produced, it shows all the objectives of this study are achieved which are to investigate the effects of solvent and ultrasonication method to the extraction process. From the qualitative and quantitative analysis, it can be concluded that ethanol is the best solvent to extract highest quality and most yields compared to hexane and acetone. Properties of solvent used can affected the extraction. In addition, ultrasonic extraction is better than without ultrasonic in producing higher quality and better yields as the ultrasound can help to break the cell wall and improve the diffusion process and therefore enhancing mass transfer.

5.2 Recommendations

In order to preserve and maintain the high quality of essential oils; the essential oils should be kept in a dark place or dark container to avoid contact with light. It also should be kept in a closed container and at a cold place to avoid quality reduced because of heat.

Research must be carry out in clean condition. This reason is to avoid other derivatives interrupt the experiment especially in analysis process which GCMS will detect other compound that didn't have any related with the gaharu essential oil. All appliances must be clean up perfectly before running another experiment and decon usually used as cleaning solution.

The raw materials amount used should be in the larger quantitiy in order to extract more oil. The mixture between solvent and oil should be filtered by filter paper or filtered syringe to reduce the green colour of chlorophyll in the mixture and to remove any unwanted substances.

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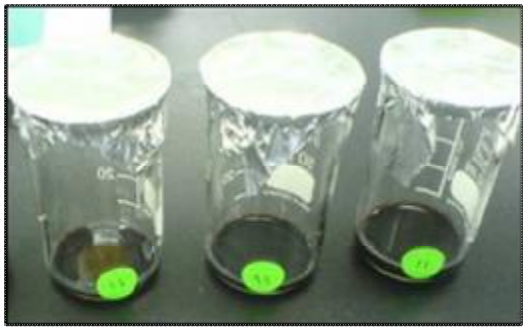
APPENDIX A

Picture of experiments

Dried Patchouli

Sample preparation

Soaking



Patchouli oil after concentrated

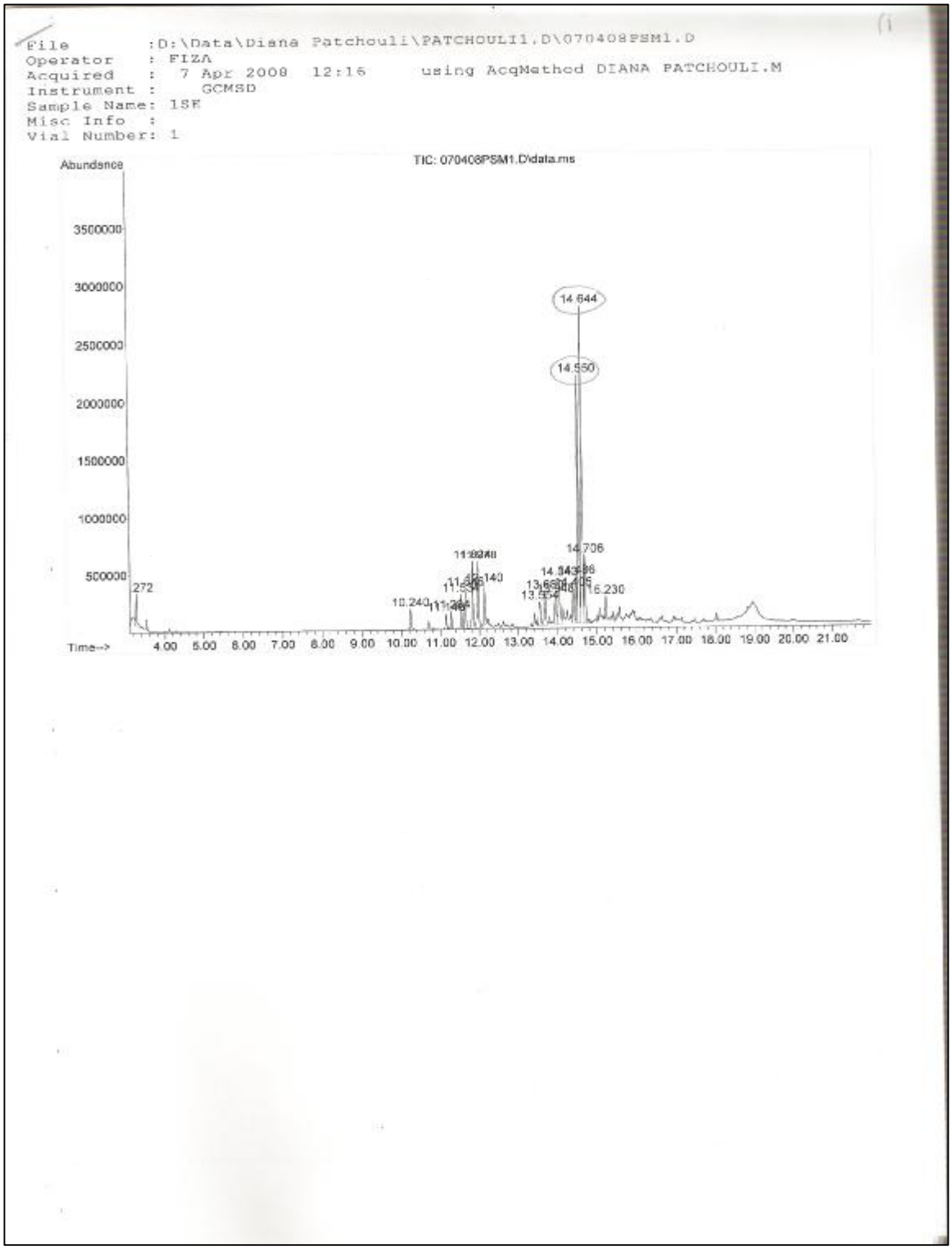
Ultrasonication process

Ultrasonic bath

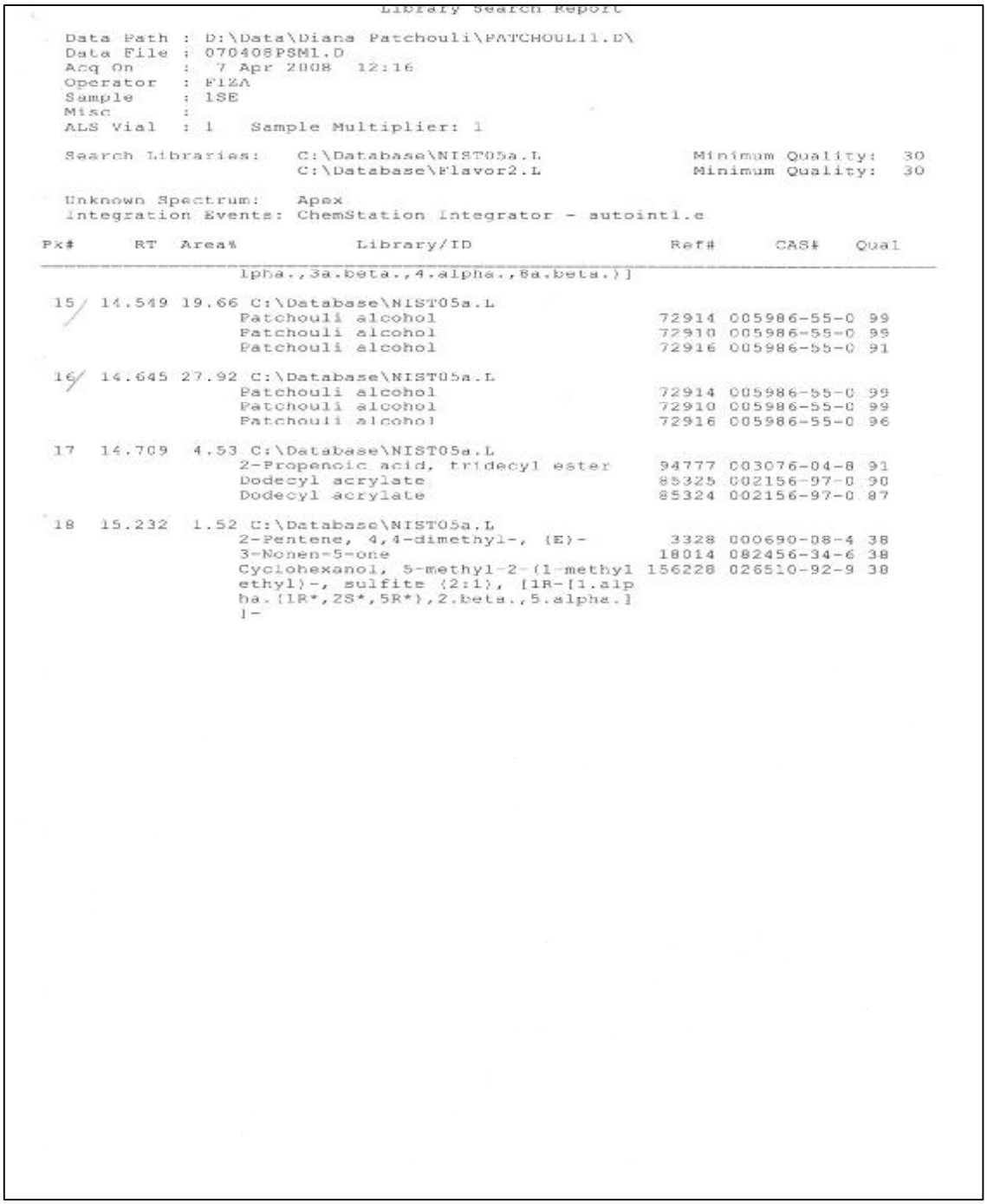
Rotary evaporator

APPENDIX B

Result of chromatogram



Experiment 1-Analysis of sample 1 (ethanol)



Library Search Report

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Data File : 070408PSM1.D
Acq On : 7 Apr 2008 12:16
Operator : FIZA
Sample : 1SE
Misc :
ALS Vial : 1 Sample Multiplier: 1

Search Libraries: C:\Database\NIST05a.L Minimum Quality: 30
C:\Database\Flavor2.L Minimum Quality: 30

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

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
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			Patchouli alcohol	72914	005986-55-0	99
			Patchouli alcohol	72910	005986-55-0	99
			Patchouli alcohol	72916	005986-55-0	91
16	14.645	27.92	C:\Database\NIST05a.L			
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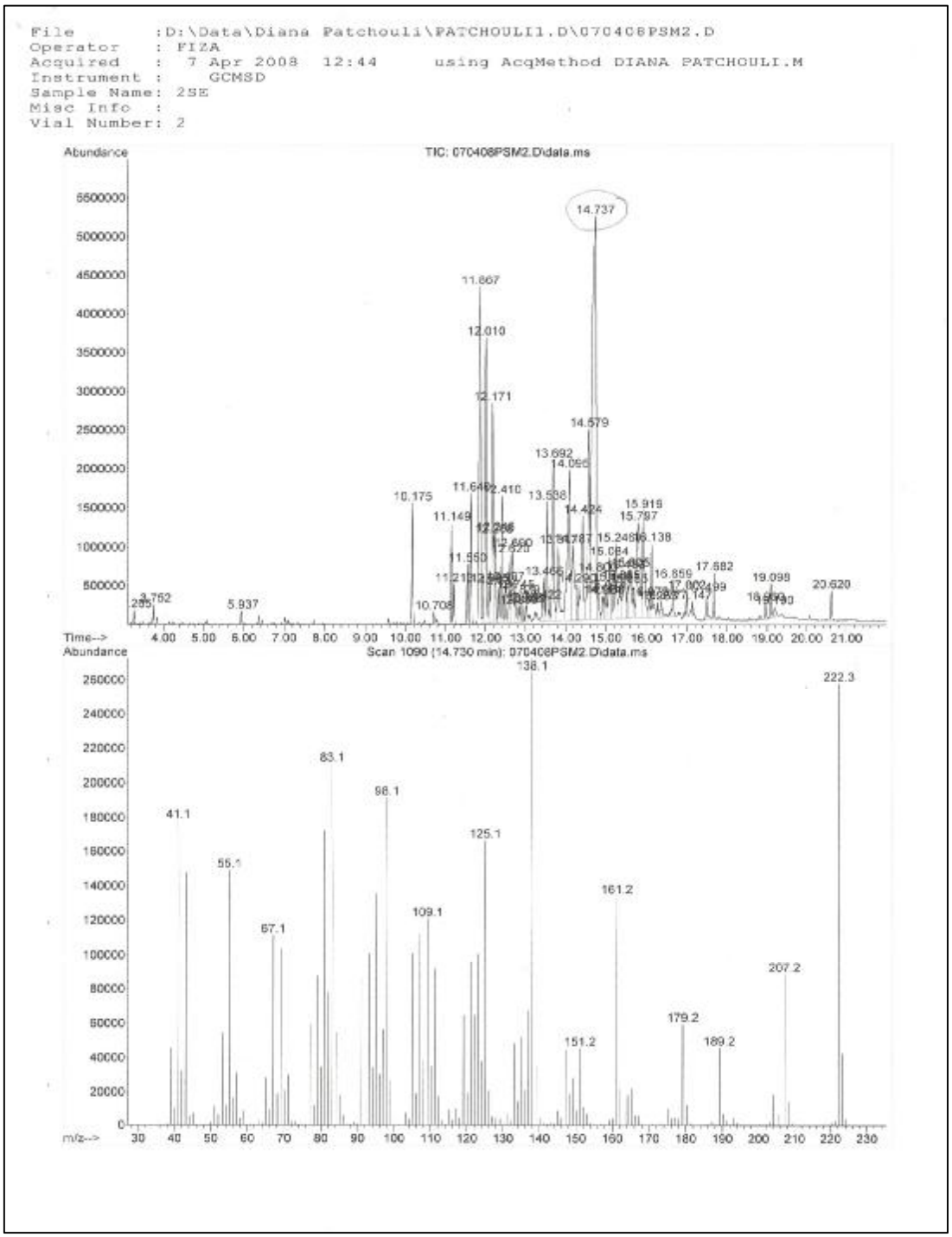
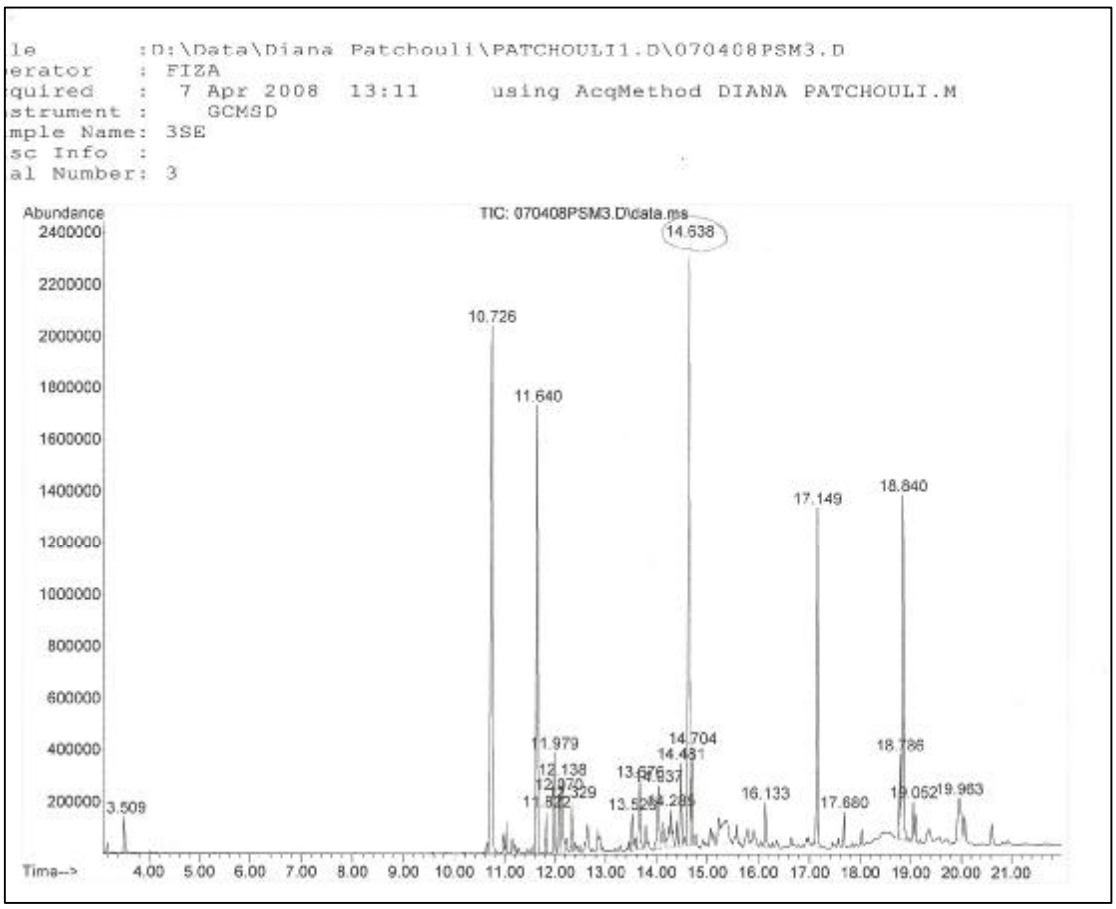
			Patchouli alcohol	72910	005986-55-0	99
			Patchouli alcohol	72916	005986-55-0	96
17	14.709	4.53	C:\Database\NIST05a.L			
			2-Propenoic acid, tridecyl ester	94777	003076-04-8	91
			Dodecyl acrylate	85325	002156-97-0	90
			Dodecyl acrylate	85324	002156-97-0	87
18	15.232	1.52	C:\Database\NIST05a.L			
			2-Pentene, 4,4-dimethyl-, (E)-	3328	000690-08-4	38
			3-Nonen-5-one	18014	082456-34-6	38
			Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-, sulfite (2:1), [1R-[1.alp ha.(1R*,2S*,5R*),2.beta.,5.alpha.]]-	156228	026510-92-9	38

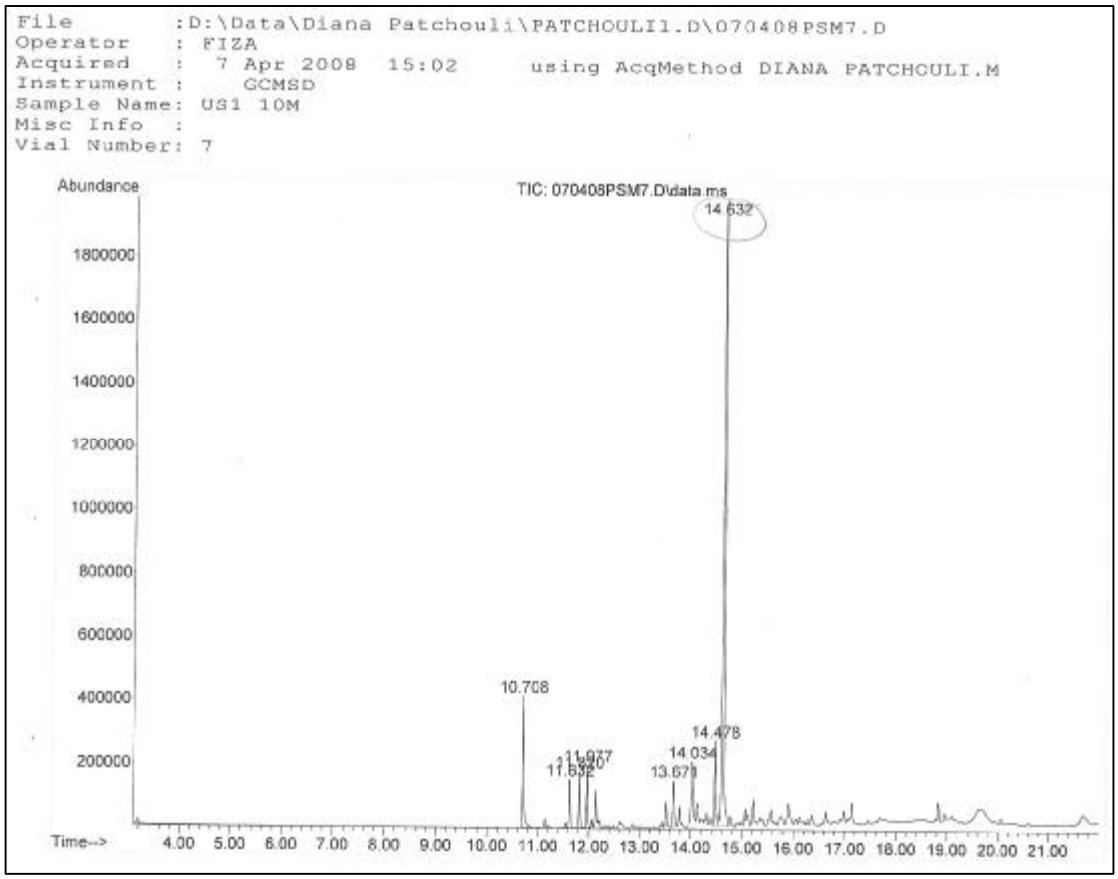
Sample of GCMS library of sample 1 in experiment 1



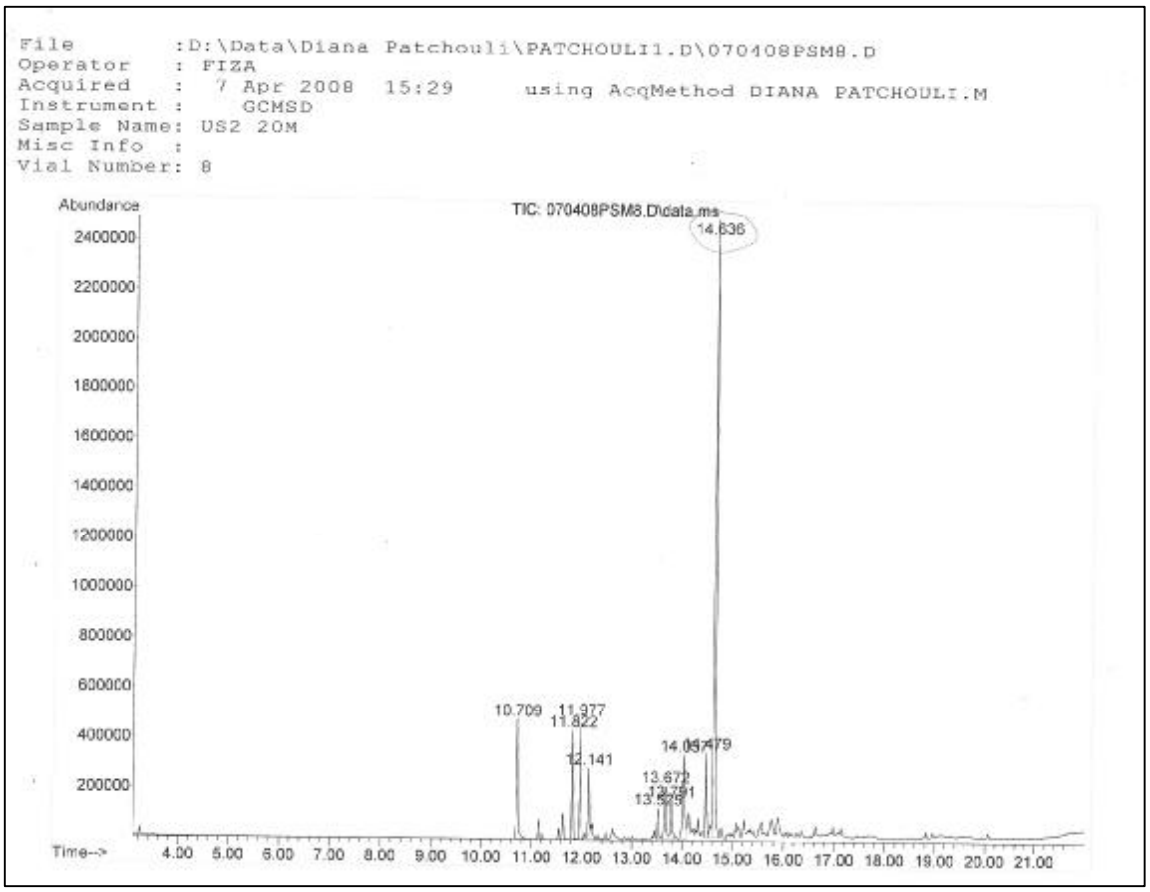
Experiment 1-Analysis of sample 2 (hexane)



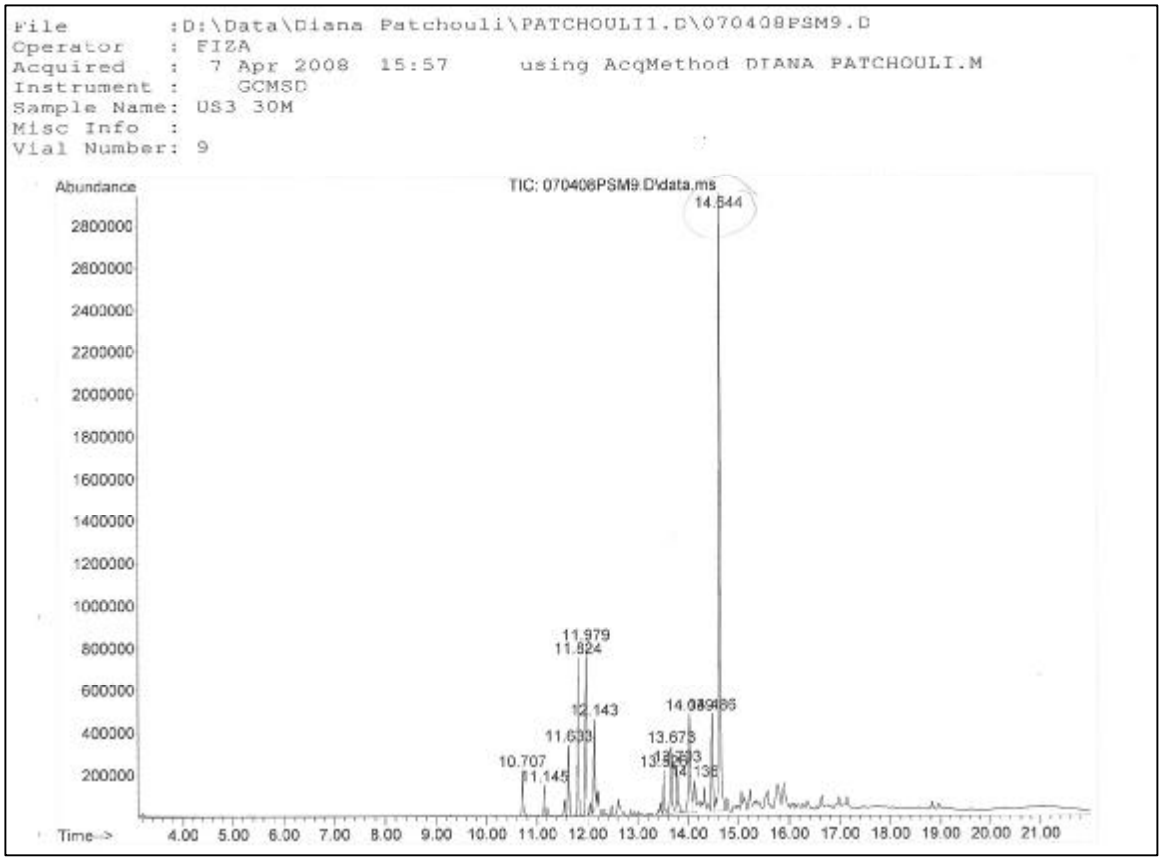
Experiment 1-Analysis of sample 3 (acetone)



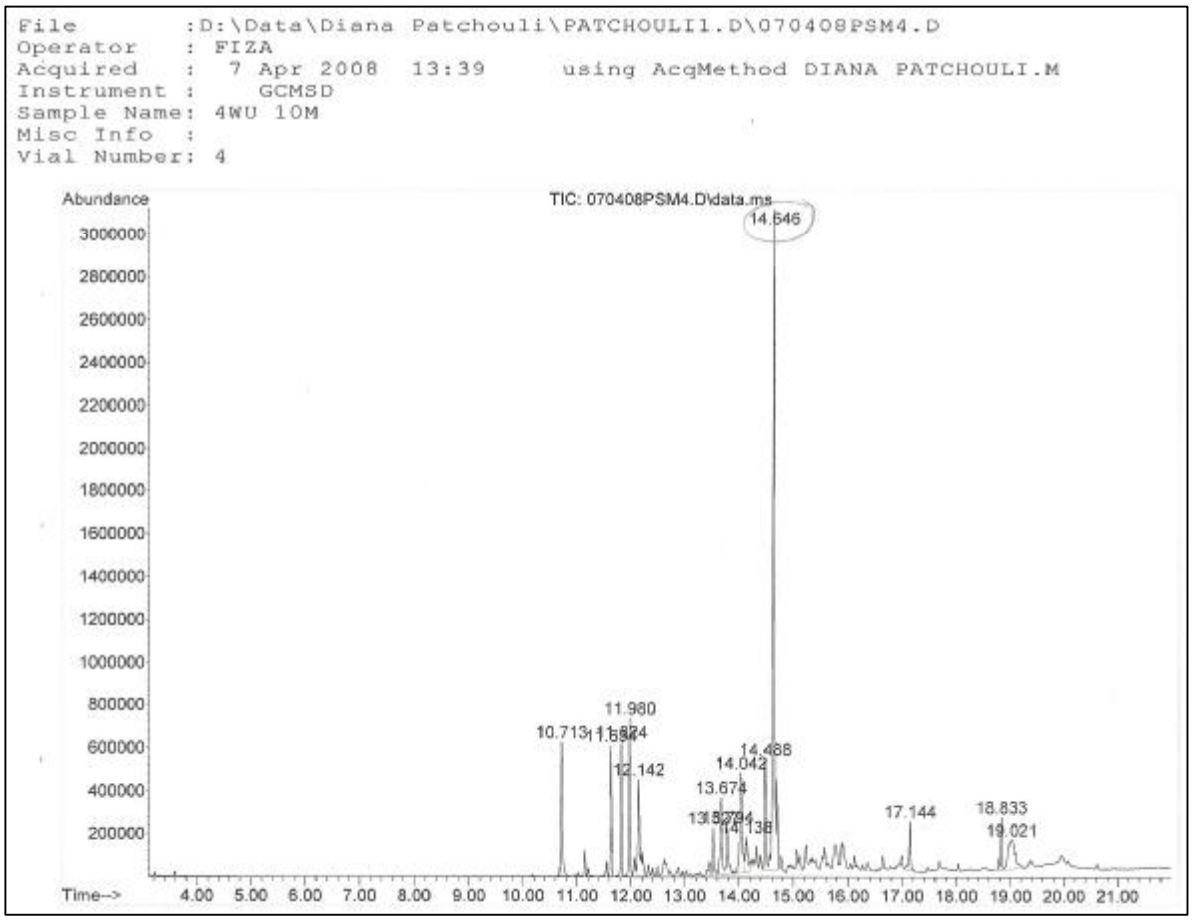
Experiment 2-Analysis of sample 1 (ultrasonic-10 min)



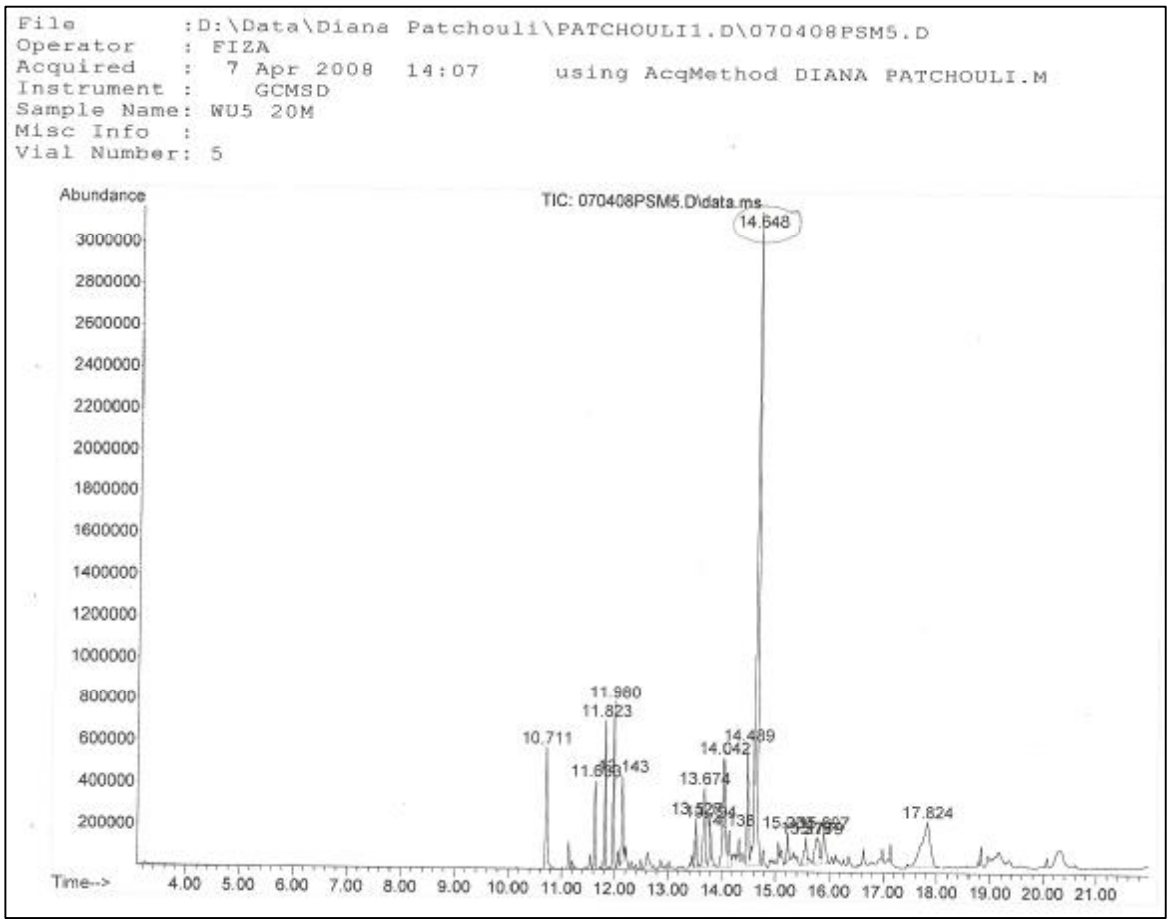
Experiment 2-Analysis of sample 2 (ultrasonic-20 min)



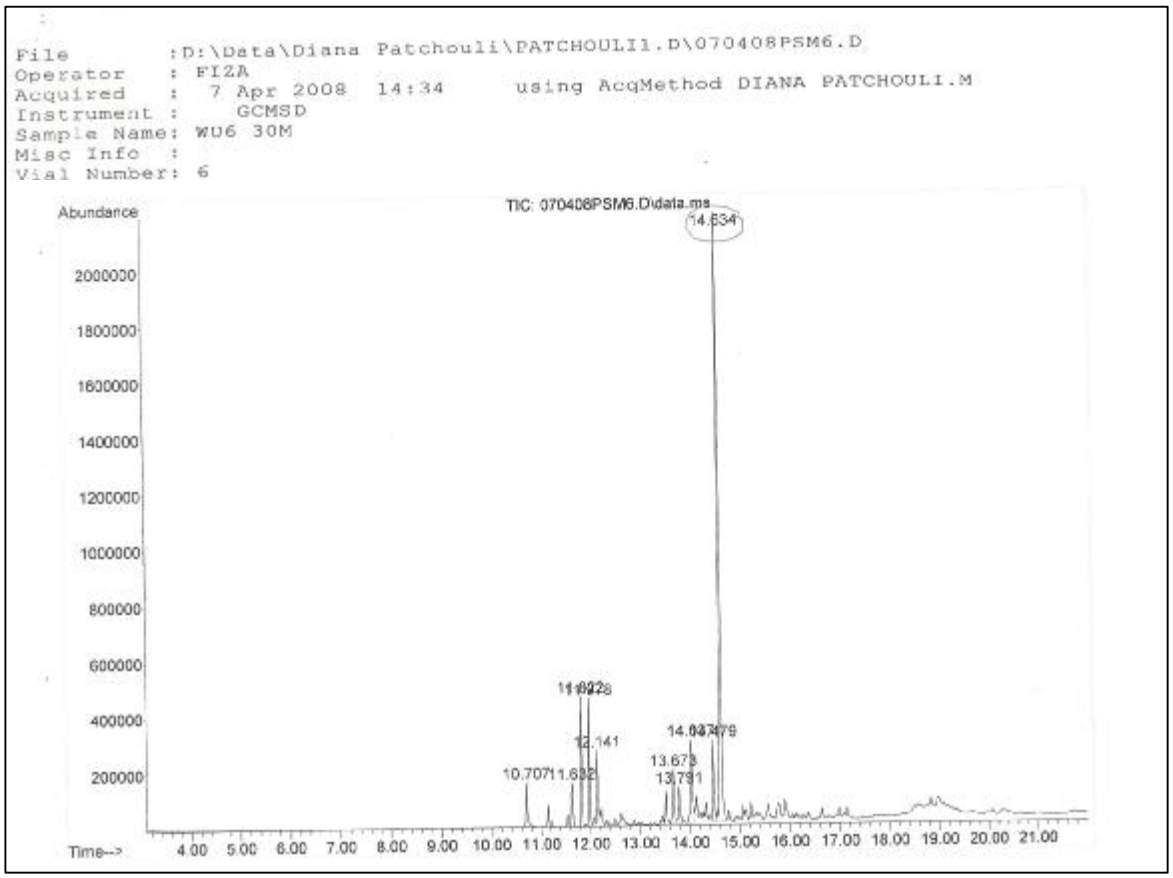
Experiment 2-Analysis of sample 3 (ultrasonic-30 min)



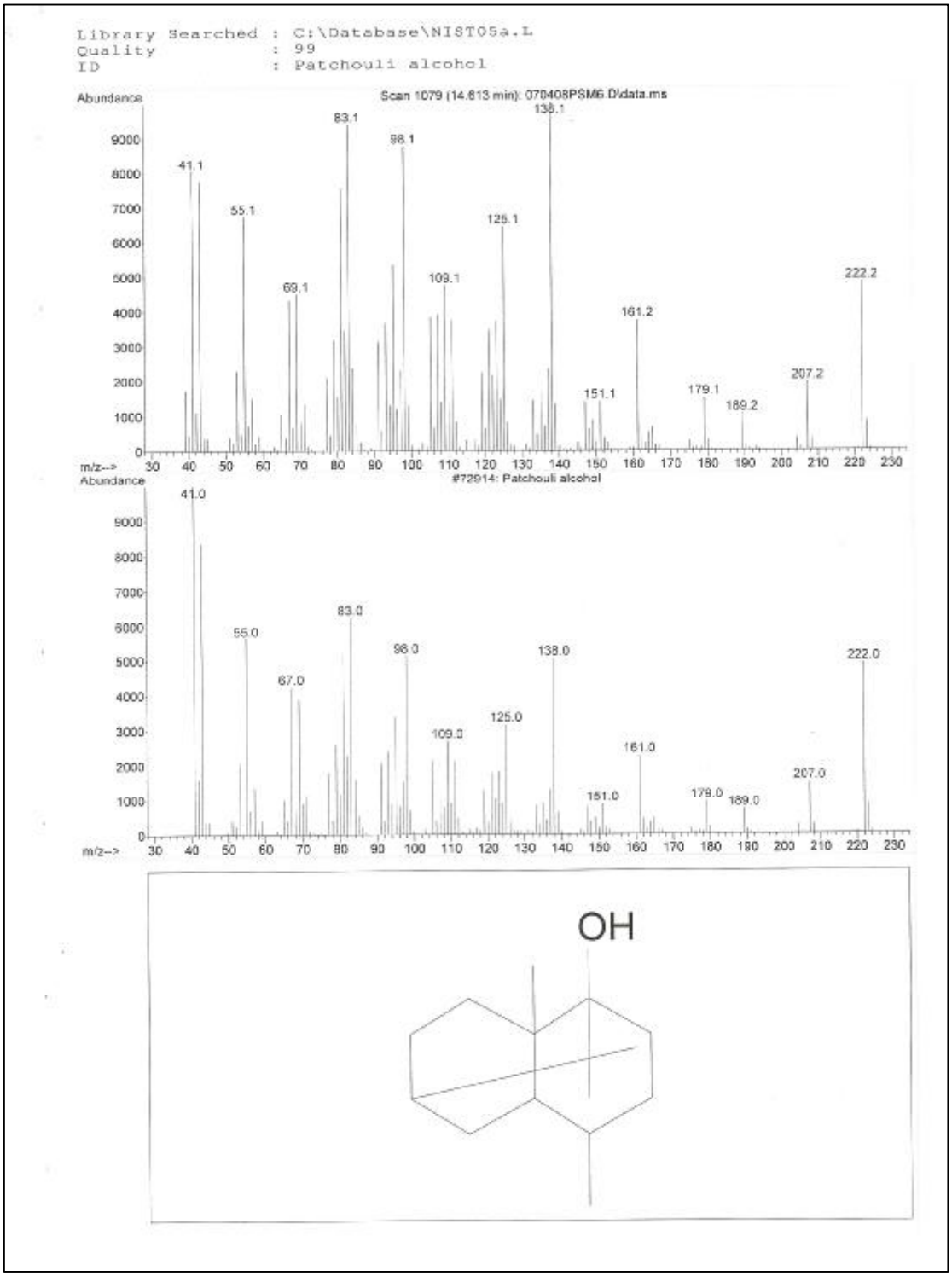
Experiment 2-Analysis of sample 4 (without ultrasonic-10 min)



Experiment 2-Analysis of sample 5 (without ultrasonic-20 min)



Experiment 2-Analysis of sample 6 (without ultrasonic-30 min)



Sample identification of Patchouli alcohol