PATCHOULI OIL EXTRACTION BY USING HYDRO DISTILLATION (LAB SCALE)

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PATCHOULI OIL EXTRACTION BY USING HYDRO DISTILLATION (LAB SCALE)

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Requirement for the award of the degree of

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Faculty of Chemical and Natural Resources Engineering Technology

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MAY 2008

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DEDICATION

Special dedication to my beloved father, mother,

brother and sisters……

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ABSTRACT

Patchouli or its scientific name *Pogostemon cablin,* is used in variety of field. For example it is used in Pharmaceutical industry as effective for fungal or bacterial infection and is of great help for insect bite. Patchouli essential oil is mostly used in perfumery industry as product of cosmetic and fragrance oil. The objectives of this research are to study the effect of extraction time and particle size of the leave on the yield of patchouli essential oil. Effects of extraction time and particle size (grinded and non grinded leave) were studied on the yield of patchouli essential oil. The extraction process is performed by using hydro distillation method. Extraction time is varied at 1, 2, 3 and 4 hours and the extraction process is repeated several times on grinded and non grinded patchouli leave. The essential oil obtained, was analyzed by using GC-MS. From the result, the yield of patchouli essential oil is increased as extraction time increased for both grinded and non grinded leave. The highest yield of grinded and non grinded patchouli leave is at four hours in which 1.32% and 0.89% of yield was obtained respectively. From the result also, grinded patchouli leave produces more oil than non grinded patchouli leave.

ABSTRAK

Patchouli atau nama saintifiknya *Pogostemon cablin*, digunakan di dalam pelbagai bidang. Sebagai contoh ia digunakan dalam industri perubatan dengan berkesan ke atas jangkitan kulat dan bakteria dan membantu mengatasi gigitan serangga. Minyak patchouli digunakan kebanyakannya dalam industri minyak wangi sebagai produk kosmetik dan wangian. Objektif kajian ini adalah untuk mengkaji kesan masa dan ukuran daun ke atas penghasilan minyak patchouli. Kesan masa dan ukuran daun (daun berkisar dan tidak berkisar) dikaji ke atas penghasilan minyak patchouli. Minyak patchouli dihasilkan meggunakan teknik penyulingan air. Masa yang berbeza digunakan iaitu 1, 2, 3, dan 4 jam dan diulang beberapa kali untuk daun berkisar dan tidak berkisar. Minyak yang terhasil akan diuji menggunakan GC-MS. Daripada hasil eksperimen penghasilan minyak patchouli meningkat apabila masa meningkat. Penghasilan tertinggi minyak patchouli bagi daun berkisar dan tak berkisar ialah selama empat jam di mana sebanyak 1.32% dan 0.89% minyak dihasilkan masing-masing. Daripada hasil eksperimen juga daun berkisar menghasilkan lebih banyak minyak daripada daun ttidak berkisar.

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LIST OF SYMBOLS

g	-	Gram
°C	-	Degree Celcius
atm	-	Atmosphere
m	-	Meter
X	-	Multiply
mm	-	Milimeter

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Essential oil or also known as ethereal oil is a concentrated, hydrophobic liquid that contains hundred of aromatic compounds, organic constituents including hormones, vitamins and other natural elements. These compounds are normally found on leaves, stems, flowers, bark, roots or other elements of a plant. Essential oil contains highly volatile components.

From the vast number of species of plant that are known, about 3000 essential oils have been well identified, though only some 150 have been exploited for commercial production. Inside the plant, the oils are stored as micro droplets in glands. The oils needs to diffuse through the wall of the glands, subsequently the droplets spread over the surface of the plant before evaporating and filling the air with perfume.

Essential oil is used for many different reasons and in different ways. They have a profound effect on the central nervous system, relieving depression and anxiety, reducing stress and relaxing. Many essential oils are used in perfumery. It takes many pounds of flowers to construct ounce of essential oil. Moreover essential oil is utilized as aromatherapy which is a form of medicine. Many essential oil often diluted and sometimes the oil is adulterated with synthetic chemicals.

Briefly, essential oil is a liquid that is generally distilled from the leaves, stems flowers, bark roots or other elements of a plant. Most of essential oils are clear and contain the true essence of the plant it was derived from. It is not the same as perfume or fragrance oils as perfume oils are artificially created fragrances or contain artificial substances and do not offer the therapeutic benefits that essential oil offer.

For this particular experiments, patchouli leaves was chosen for extraction of its essential oil. Patchouli essential oil (patchouli oil) obtained from the leaves of a plant of the same name. The scent of patchouli is heavy and strong. Patchouli is characterized as a perennial bushy herb or shrubs with strong large stems and aromatic furry leaves. Its aromatic oils can be found at various parts of the plant including leaves, stalks, branches and roots. Patchouli leaves gives off the characteristic patchouli odor when rubbed. Figure 1.1 below shows patchouli leave.

Figure 1.1 Patchouli (Pogostemon cablin)

The patchouli oil is processed through various methods of extraction. Basically, there are several methods to extract the oil. For example steam distillation, supercritical solvent extraction, ultrasonic extraction, conventional method etc. For this study, hydro distillation extraction method will be used to extract the oil from the dried leaves. Hydro distillation is the most common method of essential oil production. This method is the simplest and cheapest method for ease of use (distillation equipment) and install in the region of patchouli production. Hydro distillation process is chosen in order to maximize the profit and lower the cost, while in the same time to produce a high quality of essential oil.

1.2 Problem Statement

Nowadays, Malaysian Herbal Industry gaining more attention and exposure from various parties following the changes in government policy. The local herbal industry is growing at annual rate of between 15% and 20% and has a market value estimated at RM2 billion (Malaysian Industrial Development Authority, 2005). Nowadays, in Malaysia, essential oil and oleoresin is gaining popularity, as a herbal medication as it gave a lot of benefits to overcome some diseases. Hence, patchouli essential oil appeared to be a clear commercial value with a large opportunity to be developed and distributed.

However extraction of patchouli oil is still new in chemical industries. There are no method that proven has been to be the most efficient to extract the oil from patchouli leave. There is a number of factors determine the rate of extraction and the quality of a hydro distilled patchouli essential oil. Aside from the plant material itself, most important are time, temperature, pressure, particle size, and the efficiency of the distillation equipment. Patchouli essential oil also is a very complex product, made up from numerous of distinct molecules which come together to form the oils aroma and therapeutic properties. Some of these molecules are fairly delicate structures which can be altered or destroyed by adverse environmental conditions.

Therefore, this research intent to improve the feasibility of hydro distillation process as a method of patchouli essential oil extraction.

1.3 The Objective of the Research

The objectives of this research are to study the effect of extraction time and particle size of the leave on the yield of patchouli essential oil.

1.4 The Scope of the Research

The scopes of this research are listed as below:-

1. Patchouli leaves are prepared for the experiment. The leaves are divided into two sample which is non grinded sample and grinded sample (1 mm).
2. A hydro distillation unit is set up and used to extract the essential oils from patchouli leaves. The operating condition temperature is set at $100^{O}C$ and 1atm.
3. In this research, the correlation between the effect of extraction time and surface area is being studied.
4. The essential oil is analyzed by using GC-MS to prove that the oil is patchouli essential oil.

CHAPTER 2

LITERATURE REVIEW

2.1 Essential Oil

Essential oil is a highly volatile substance isolated by a physical process from an odoriferous plant of a single botanical species. The oil is named after the plant from which it is derived like rose oil or peppermint oil. Such oils were called essential oil because they were thought to represent the very essence of odor and flavor of the plant it is extracted from unlike vegetable oils expressed from nuts and seeds, essential oil are not actually oily. Some essential oil are viscous, others are fairly solid and most are somewhat watery (http://www.essentialwholesale.com/aromatherapy.html). The oil is also known as volatile oils, or essential oils because of their properties that easily evaporated at ambient temperature.

These oils can be free from the leaves and extracted by using a few method like hydro distillation. Another extraction process like enfleurage (extraction by using fat), maceration, solvent extraction, high pressure CO2 extraction, and cold pressing extraction are also used, however distillation is by far the most common method for extraction of essential oil. Younger plants produce more oils than older ones, but old plants are richer in more resinous and darker oils because of the continuing evaporation of the lighter fractions of the oil.

The first step in isolation of essential oils is crushing or grinding of the plant material to reduce the particle size and to rupture some of the cell walls of oil bearing glands. Steam distillation is by far the most common and important method of production, while extraction with cold fat (enfleurage) or hot fat (maceration) are chiefly of historical important. Three different methods of steam distillation are practiced. In the oldest and simplest method, a vessel containing water and the chopped or crushed plant material is heated by a direct flame, and water vapor and volatile oil are recovered by a water cooled condenser. The original method is being replaced by a process in which the plant material is suspended on a grid above the water level, and steam from a second vessel is introduced under the grid. The volatiles are condensed and the oil is separated. In the third process, the vessel containing the plant material on a grid is heated to prevent condensation of steam, so that dry distillation is attained.

In southern France, essential oil was extracted with cold fat long before the introduction of extraction with volatile solvents. This process is applied to flowers that do not yield a large quantity of oil by steam distillation or whose odor is change by contact with boiling water and steam. In this process flowers are spread over a highly purified mixture of tallow and lard and are left for a period varying from 24 hours to 72 hours. During this time, most of the flower oil is absorbed by the fat. The petals are then remove (defleurage), and the process is repeated until the fat is saturated with oil. The final product is called pomade.

In most cases, it is possible to shorten the long enfleurage process by extracting the essential oil using molten fat for one to two hours at a temperature ranging from 45^oC to 80^oC (110^oF to 175^oF). The fat is filtered after each immersion and after 10 to 20 extraction cycles, pomade is sold as such, or it may be extracted with alcohol to yield the oil residue.

An extraction procedure called expression is applied only to citrus oils. The outer colored peel is squeezed in process, and the oil is decanted or centrifuged to separate water and cell debris. The method is used for oil of sweet and bitter orange, lemon, lime, mandarin, tangerine, bergamot, and grapefruit. Much oil is produced as a by-product of the concentrated citrus juice industry.

The function of the essential oil in a plant is not well understood. Odors of flowers probably aid in natural selection by acting as attractants for certain insects. Leaf oils, wood oils, and root oils may serve to protect against plant parasites or depredations by animals. Oleoresinous exudations that appear when the trunk of a tree is injured prevent loss of sap and act as a protective seal against parasites and disease organisms. Few essential oils are involved in plant metabolism, and some investigators maintain that many of these materials are simply waste products of plant biosynthesis.

Commercially, essential oil are used in three primary ways: as odorants they are used in cosmetics, perfumes, soaps, detergents, and miscellaneous industrial products ranging from animal feeds to insecticides to paints; as flavours they are present in bakery goods, candies, confections, meat, pickles, soft drinks, and many other food products; and as pharmaceuticals they appear in dental products and a wide, but diminishing, group of medicines.

2.1.1 Chemical Composition of Essential Oil

Terpenes, organic compounds consisting of multiples of isoprene units (containing five carbon atoms), are by far the most dominant constituents of essential oil. Terpenes are built up from units of the simple five-carbon molecule isoprene. Both hydrocarbons and oxygenated compounds such as alcohols, aldehydes, ketones, acids, esters, oxides, lactones, acetals, and phenols are responsible for the characteristic odours and flavours.

Essential oil are generally expensive, with prices ranging from several U.S. dollars per kilogram on the low side to several thousand dollars per kilogram. The high price of the natural oils coupled with their limited availability has encouraged a search for substitutes. Great progress has been made in the synthesis of individual components such as geraniol, citral, linalyl acetate, and the like. These synthetics have been combined with natural oils to extend supplies, and they have also been blended together in an attempt to duplicate the oils themselves. Such reconstituted oils usually lack certain of the odour notes of the natural products, because of absence of trace ingredients, often unidentified, that may be present in the natural oils. They also tend to have a more “chemical” odour, because of trace impurities in the synthetics that are different from the components of natural oils.

2.2 Introduction to Patchouli

Pogostemon cablin (patchouli), like many plants within the Lamiaceae, accumulates large amounts of essential oil. Patchouli oil is unique because it consists of over 24 different sesquiterpenes, rather than a blend of different mono-, sesqui- and di-terpene compounds (Fabienne Deguerry *et al.,* 2006). Patchouli essential oil is very important spiritual oil/root in many countries. Patchouli is a fragrant herb with opposite, egg-shaped leaves, square stems, and famous for its essential oil (patchouli oil). Patchouli oil is obtained by steam distillation or CO_2-extraction of the dried leaves. It has its origin in South East Asia and thrives in tropical climes. Distant relatives of this plant include lavender, rosemary and many other herb plants associated with more temperate regions. Usually, it grows to around 3 feet in height and has a dark brownish appearance and its flowers are whitish in color. Patchouli grows well in partially shaded areas, hence it can be grown as an intercrop in coconut gardens. The crop is cut two or three times a year, the leaves being dried and packed in bales and exported for distillation of the oil. Patchouli oil is one of the volatile oils which have a good prospect. It is used mostly as material of cosmetic especially in perfume industry. Patchouli was first described by botanists in the Philippines in 1845. Today growing interest in its fragrance has led to patchouli's widespread cultivation throughout tropical Asia.

Patchouli essential oil used to give a base and lasting character to a fragrance in perfumery industry. The dry leaves of patchouli are put on steam distillation to 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 world total production (Robbins and Tao, 1983). Currently, India is producing a meagre quantity of patchouli essential oil and most of its domestic requirement is met by importing about 50 tons of pure oil and 100 tons of formulated oil (M. Singh *et al*., 2002). The market world recently needs 1200-1400 tons Patchouli oil per year. 80-90% demand is supplied from Indonesia. The biggest importer is United State of America which needs 210 tons per year. The other importers are United Kingdom, French, Switzerland, Germany, and Dutch. Figure 2.1 shows a patchouli plantation in Indonesia.

Figure 2.1 Pogostemon cablin industry scale plantation in Central Kalimantan, Indonesia

Patchouli oil is an essential ingredient and used as a ‘base’ material in perfumery industry. There is no synthetic substitute for patchouli oil, which increases its value and demand in the perfumery market. Consumption of Patchouli oil in the world is about 2000 tones per annum. In India due to increase in chewing tobacco and pan masala industries, consumption has gone up to about 300 tons per annum while the production is below 50 MT. Hence, the country mostly depends on import mainly from Indonesia and on reconstituted oil.

2.2.1 Physical and Chemical Properties of Patchouli

Several species of the Labiatae family with patchouli-like odour grow in the tropics, but only the *Pogostemon patchouli* is utilised for the commercial distillation of the oil. The scent of the essential oil is partly due to patchouli alcohol which first isolated in its crystalline form by Gal in 1869 and formulated as $C_{15}H_{26}O$ by Montgolfier (Adusumilli and Gedu, 2005). Patchouli oil is obtained by various distillation techniques of the leaves of patchouli, and is widely appreciated for its pleasant characteristic and long lasting woody, earthy, camphoraceous odor. It is generally believed that the odour of the patchouli oil is mainly due to norpatchouli alcohol and a closely related norsesquiterpene alcohol (Adusumilli and Gedu, 2005). Patchouli oil is hence an important ingredient in many fine fragrance products like perfumes, as well as in soaps and cosmetic products (K. Bauer et al., 1997). The composition of the patchouli oil is complex like many essential oils, but distinct because it consists largely of sesquiterpenes. The sesquiterpene (ɣ)-patchoulol (Figure 2.1) is the major constituent and is the primary component responsible for the typical patchouli note. The oil contains a large number of other sesquiterpenes hydrocarbons such as α-/β-/ɣ-patchoulenes, α-bulnesene, α–guaiene and seychellene, with structures clearly related to (ɣ)-patchoulol and sesquiterpenes with unrelated structures like *trans*-β-caryophyllene, α-humulene and ɣ-curcumene (Figure 2.1), (Fabienne Deguerry *et al.*, 2006).

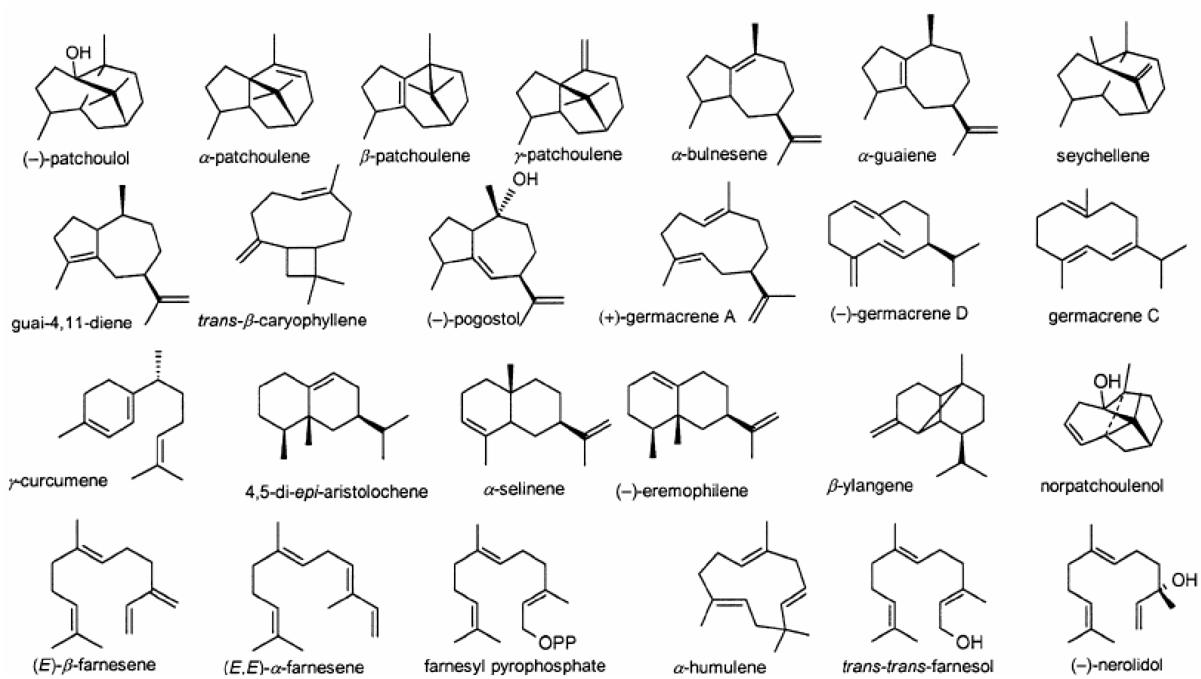

Figure 2.3 Structure of sesquiterpenes identifed in patchouli oil and/or produced by the recombinant sesquiterpene synthases from patchouli.

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 (F. Näf *et al*., 1981). In plants, sesquiterpenes and other volatile secondary metabolites can accumulate on or in the leaves, and often in specialized surface structures such as trichomes. The accumulation and biosynthesis of (¡)-patchoulol and related sesquiterpenes in patchouli leaves has unfortunately received limited attention (W. Henderson *et al*., 1969).

Oil of Patchouli is thick and brownish-yellow tinted green in colour. It contains coerulein,the vivid blue compound found in matricaria, wormwood and other oils. Freshly distilled oil is said to be the best and hence the process is done near the plantations itself. Newly distilled patchouli oil has a fresh, green, slightly harsh aroma. As the oil ages it mellows considerably, becoming sweeter and more balsamic. Other oils sharing this feature are sandalwood and vetiver. Patchouli blends very well with Bergamot, Clary Sage, Geranium, Grapefruit, Lavender, Lemon, Lemongrass, Lime, Litsea Cubeba, Mandarin, Myrrh, Neroli, Olibanum, Orange, Petitgrain, Sandalwood, Tangerine, Tea Tree and Ylang Ylang.

2.2.2 Uses of Patchouli

Patchouli's benefits can be classified as carminative (medication that prevents the formation of gas in the alimentary tract or eases its passing), diaphoretic, alterative, astringent, anti-emetic, anti-fungal, and anti-inflammatory, and it has been shown to have cell-regenerating abilities. The plant has been used as Chinese herbal medicine to remove dampness, relieve summer-heat, exterior syndrome, stop vomiting and stimulate the appetite (Pharmacopeia Commission of PRC, 2000). Essential oil of *patchouli* collected by steam distillation mainly contributes to the pharmacological activities (X.L. Xiao and Y.X. Long, 2004), and the therapeutic properties of the essential oils are directly correlated with their qualitative and quantitative composition which is obviously different derived from various cultivation regions of Patchouli (Y.M. Du *et al.,* 1998). Today it is gaining popularity as an important ingredient in alternative healing. Alternative healing methods like aromatherapy makes use of this fragrant herb in its entirety or in combination with others to achieve various results.

In skin care, the uses of patchouli are manifold. It helps to regenerate skin cells and also helps in reducing cellulite. It is used to treat skin disorders like acne, cracked skin, some eczemas, fungal infections, sores, scars and dandruff. Patchouli also tones and tightens the skin and smoothes rough, dry and cracked skin. It acts as an anti-wrinkle agent and even as a deodorant. Patchouli is a known curative and is used for headaches, muscle spasms, colic and angina. It is also used as a mild sedative and pain-reliever and also for fighting insomnia, anxiety, agitation and depression. It is a venous and lymphatic decongestant, making it useful for varicose veins, hemorrhoids and congestive pelvic conditions.

The emotional effects of Patchouli are numerous. It can ease and diminish anxiety and depression. In high doses it can stimulate, and in lower doses it acts as a sedative. Patchouli helps to sharpen intelligence, improve concentration, and provide insight. Spiritually it is used in incense sticks as it helps to create a calming atmosphere.

Alternative medicine like Ayurveda has always recognized the medicinal and curative features of Patchouli. It served as anti-venom for snakebites and stings of creatures like scorpions and even mosquitoes. Victims of poisonous snakes like cobra are administered pure patchouli oil as a first aid. After that patchouli oil is mixed with any base oil such as cold pressed sesame, coconut, sweet almond, grape seed, wheat germ or sandalwood oil in a 1:1 ratio and applied twice daily until the wound gets completely healed. Stings of other creatures like bugs and mosquitoes are treated with a mixture of patchouli oil and any base oil in a ratio of 1:3.

2.2.3 Planting Patchouli

Patchouli is a hardy plant and adapts itself to a wide range of soil conditions. However, it flourishes best in loose deep loamy soils, rich in humus and nutrients, with a loose friable texture and without impervious layer at the bottom. The pH of the soil from 5.5 to 6.2 is considered to be ideal. The plant flourishes in low altitudes and foothills over slightly moist, well-drained soils in tropical and subtropical conditions. It is also observes that the richest soil produces the best leaf material which gives better yield and better quality oil.

Patchouli grows successfully up to an altitude of 800-1000m above the sea level. It prefers a warm and humid climate. The crop can be grown successfully on a fairly heavy and evenly distributed rainfall ranging from 1500-3000mm per annum. It is also observed that the moderate temperature of 22-28°C and an average humidity of 75% have been found to be ideal for its growth.

Patchouli is propagated vegetatively. Since the crop is highly susceptible to nema tode attack, it is advisable to adopt phytosanitary measures from nursery stage itself. Seed pans or polythene bags are filled with well-heated sand, which can be made by passing steam through it for about one hour. If this is not practicable for a grower, the sand should be treated with suitable nematicide like Furadan @ 20 kg/ha (active ingredient 3%). Dasanit @150 kg/ha (active ingredient 5%) is also very effective.

Nursery that has to be under shade could be raised at any time during the year. Cuttings are prepared preferably in the morning or in the evening to minimise desiccation. Cuttings are taken from healthy stock and as far as possible from the apical region. Cuttings from fairly developed branches, 4-5 nodes in length and with a crown of 2-3 leaves, are ideal for planting in nursery. The basal end of the cutting should be neatly cut in oblique form just about 1 cm below the node. Application of a commercial hormone like Seradix B-2 to the basal end of the cutting encourages early rooting. The cuttings should then be planted in seed pans, nursery beds or in polythene bags with the help of a suitable dibbler at a spacing of about 5 cm. Aeration, partial shade and regular watering are essential for early rooting. The cutting takes about 0-5 days for rooting in nursery.

The main field for transplanting is thoroughly disced and tilled. Suitable nematicide, viz., Furadan at 20 kg/ha.(active ingredient 30%) or Dasanit at 150 kg/ha (active ingredient 5%) is broadcast and mixed well into the soil a few days before transplanting. The plot is then laid out in ridges and furrows. The ridge should be 20-25 cm high and 18-22 cm broad with 60 cm row to row distance. The beds should be irrigated a day before the transplantation.

Patchouli is a shade loving plant. It is felt that patchouli could be tried under coconut in India. It could also be taken up with some suitable crops that provide adequate shade. Gliricidia or Erythrina could be planted well in advance at 5 X 5 m spacing in patchouli field in order to provide the necessary shade.

Rooted cuttings are transplanted generally in the evening in the main field. The planting is done at 60 x 60 cm apart. Normally 28,000 cuttings will be required per hectare. Irrigate the field immediately after transplantation. Planting of 15-20 cm long unrooted cuttings is also practiced in some areas. These cuttings are planted at the rate of 2 to 3 cuttings per hill. During early stages shade and sufficient moisture are most important requirements. Shade can be removed after the plants establish well.

Patchouli requires rich soil in order to obtain proper yield and better quality of oil. Normally, a basal dose of 25 kg N, 50 kg P_2O_5 and 50 kg K_2O per hectare is given in the form of Urea, Superphosphate and Muriate of potash. After about two months, 25 kg N as urea is applied. Likewise, for each harvest 50 kg N is applied in two split doses, the first dose just after the harvest and the other about two months later. In total, 150 kg N per hectare per year is applied to the crop.

For getting good yield of the crop, the area should receive good and evenly distributed rainfall, because it does not do well under rainfed conditions. Immediately after transplanting the field must be irrigated every day for the first 3 to 4 days and subsequently on alternate days for 10 to 15 days. After three weeks irrigation once or twice a week depending on the type of soil and climate is considered sufficient. The crop is highly susceptible to water logging.

The field should be kept weed-free during the first 2 to 3 months of crop growth either by hoeing 2 to 3 times or by hand weeding. Weeding is also necessary after about a month of each of the foliage harvests.

The first harvest of the crop is taken about 5 months after transplanting. The stage at which crop has to be harvested is very important for good yield and better quality of oil. It has to be harvested when the foliage becomes pale green to light brownish and when the stand emits characteristic patchouli odor, which could be easily smelt by a passer-by, especially in the morning hours. Subsequent harvest can be taken after every 3-4 months depending upon the local conditions and management practices. The crop can be maintained for about 3 years. The first 2 or 3 harvests of newly planted plantation give good yield and high quality oil. Harvesting is done with the help of small sharp shear or secateur. Usually the length of the harvested portion ranges from 40-60 cm. It is necessary to leave 4-6 juvenile sprouting buds at the basal region for fast regeneration, while harvesting. The crop should not be harvested prematurely as it gives less yield and oil of inferior quality.

The oil is found mainly in the leaf and small quantity is present in the ender parts of the stem. The yield of fresh leaves/ha/year from three harvests is about 8,000kg which on shade drying reduces to 1600kg and on distillation yields about 40kg of oil. The yield of oil varies from 2.5 to 3.5% on shade dry basis of the leaves and an average yield of 2.5% may be considered satisfactory in commercial distillations.

2.3 Introduction to Distillation

Distillation is a process involving the conversion of a liquid into vapor that is subsequently condensed back to liquid form. Distillation is commonly used to separate liquids from nonvolatile solids, as in the separation of alcoholic liquors from fermented materials, or in the separation of two or more liquids having different boiling points, as in the separation of gasoline, kerosene, and lubricating oil from crude oil. Other industrial applications include the processing of such chemical products as formaldehyde and phenol and the desalination of seawater. The distillation process appears to have been utilized by the earliest experimentalists. Aristotle (384–322 BC) mentioned that pure water is made by the evaporation of seawater. Pliny the Elder (AD 23–79) described a primitive method of condensation in which the oil obtained by heating rosin is collected on wool placed in the upper part of an apparatus known as a still.

Most methods of distillation used by industry and in laboratory research are variations of simple distillation. This basic operation requires the use of a still or retort in which a liquid is heated, a condenser to cool the vapor, and a receiver to collect the distillate. In heating a mixture of substances, the most volatile or the lowest boiling point distills first and the others subsequently or not at all. This simple apparatus is entirely satisfactory for the purification of a liquid containing nonvolatile material and is reasonably adequate for separating liquids of widely divergent boiling points. For laboratory use, the apparatus is commonly made of glass and connected with corks, rubber bungs, or ground-glass joints. For industrial applications, larger equipment of metal or ceramic is employed.

A method called fractional distillation, or differential distillation, has been developed for certain applications, such as petroleum refining, because simple distillation is not efficient for separating liquids whose boiling points lie close to one another. In this operation the vapors from a distillation are repeatedly condensed and revaporized in an insulated vertical column. Especially important in this connection are the still heads, fractionating columns, and condensers that permit the return of some of the condensed vapor toward the still. The objective is to achieve the closest possible contact between rising vapor and descending liquid so as to allow only the most volatile material to proceed in the form of vapor to the receiver while returning the less volatile material as liquid toward the still. The purification of the more volatile component by contact between such countercurrent streams of vapor and liquid is referred to as rectification, or enrichment.

Multiple-effect distillation, often called multistage-flash evaporation, is another elaboration of simple distillation. This operation, used primarily by large commercial desalting plants, does not require heating to convert a liquid into vapor. The liquid is simply passed from a container under high atmospheric pressure to one under lower pressure. The reduced pressure causes the liquid to vaporize rapidly; the resulting vapor is then condensed into distillate.

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A variation of the reduced-pressure process uses a vacuum pump to produce a very high vacuum. This method, called vacuum distillation, is sometimes employed when dealing with substances that normally boil at inconveniently high temperatures or that decompose when boiling under atmospheric pressure. Steam distillation is an alternative method of achieving distillation at temperatures lower than the normal boiling point. It is applicable when the material to be distilled is immiscible (incapable of mixing) and chemically nonreactive with water. Examples of such materials include fatty acids and soybean oils. The usual procedure is to pass steam into the liquid in the still to supply heat and cause evaporation of the liquid. (http://www.britannica.com /eb/ article-9030650)

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2.3.1 Hydro distillation

Hydro distillation is the simplest and usually the cheapest distillation method where the plant material is immersed in water and boiled. The hot water helps to release the aromatic molecules from the plant since the hot water helps to break the pockets in which the oils are kept in the plant material. The molecules of these volatile oils then escape from the plant material and evaporate into the steam. This kind of method protects the oils to a certain degree since the surrounding water acts as a barrier to prevent it from over heating.

The distillation temperature should be about 100^{o}C. Temperature of the process needs to be carefully controlled - just enough to force the plant material to let go off the essential oils. Care needs to be taken to prevent the plant material from being damaged by contacting the overheated still walls.

Hydro distillation can be done at reduced pressure (under vacuum) to reduce the temperature to less than 100^{o}C, which is beneficial in protecting the botanical plant material as well as essential oil (http://www.essentialoils.co.za/water-distillation.htm).

The distillation time depends on the plant material being processed. Prolonged distillation produces only a small amount of extra oil, but does add unwanted high boiling compounds and oxidation products.

The steam which then contains the essential oil passed through a cooling system to condense the steam. When the condensed steam cools down the water and essential oil are separated and the oils is decanted to be used as essential oil.

During distillation only a tiny amount of molecules can evaporate, so they are the only ones which leave the plant. These extremely small molecules make up an essential oil. Oils containing more of the smallest, and therefore most volatile of these tiny molecules, are termed "top notes" in the perfumery world, those containing most of the heaviest and least volatile of the tiny molecules are called "base notes". Those in between are known as "middle notes".

According to Thomas (1992), before distillation the plant material is often field cured, partially dried or disintegrated to some extent. This latter process, commonly reffered to as comminuting or size reduction, is used in the extraction or distillation of herbs or for their incorporation into food products. The reduction in particle size exposes as many oil glands as possible to the solvent or steam. It reduces the thickness of the plant material through which the diffusion must occur, greatly increasing the rate of spread of vaporization and distillation of the essential oils.

The proportion of different essential oil extracted by hydro distillation is 93% and the remaining 7% is extracted by other method (Masango P., 2001). Usually hydro distillation method produces pure quality of essential oil because it only use water rather than the other method that use solvent. Using of solvent in extraction method may lead to produce essential oil and its compound of the composite oil in the waste water (Bohra et. al., 1994). This will be a problem in separating the essential oil but majority cases of steam distillation (or hydro distillation) extraction produce the oil that less dense than the water and so forms the top layer of the distillate. The partition between the water and oil phases of distillate make the separation of the oil is easy and more economically (Masango P., 2001).

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter shows the overall methodology of the research which involves all steps necessary to obtain patchouli essential oil. Hydro distillation is used to extract patchouli essential oil since the method is able to protect the oil and raw material to a certain degree because the surrounding water acts as a barrier to prevent them from overheating. In order to maximize the yield of patchouli essential oil, patchouli leaves need to undergo pre-treatment process or sample preparation which includes drying and comminuting process.

3.2 Sample Preparation of Dried Patchouli Leaves

Drying is an important process before continue with extraction process. Fresh Patchouli leaves were chopped by using scissor or knife into 2 cm size in order to increase the drying rate. Then the leaves were exposed to sunlight for 3 hours, then to ambient temperature for 3 days. This process is important in order to reduce moisture in leaves. Figure 3.1 shows the dried patchouli leave. The percent of moisture lost (ML) of leaves was determined by the following formula:

$$\text{ML (\%)} = \frac{\text{Initial weight of sample (g)} - \text{Current weight of sample (g)}}{\text{Initial weight (g)}} \times 100\%$$

Figure 3.1: Dried patchouli leaves

3.3 Patchouli Oil Extraction

The apparatus of hydro distillation process was set up as shown in Figure 3.2. Patchouli leaves were grinded into 1 mm size by using blender and sieve tray of 1 mm size. Then the mixture of 300 g of grinded patchouli leaves and 3000 ml of water is put into the flask of distillation unit. After that, heating mantle and re-circulating cooler are switched on. The temperature is set at 100 °C and pressure at 1 atm. Aluminum foil need to be wrapped all over the apparatus to make sure there is no heat loss occurs. The essential oil present in the flask is vaporized. Steam and essential oil vapors are passed through a condenser. The condensate which has a mixture of water and essential oil, is collected in a receiving flask. At receiving flask the layer of essential oil is decanted and collected to sample bottle. The same step is then repeated at different extraction time and for non grinded leaves. Figure 3.3 shows a flowchart of extraction process of patchouli leaves as a summary.

Figure 3.2: Hydro distillation units

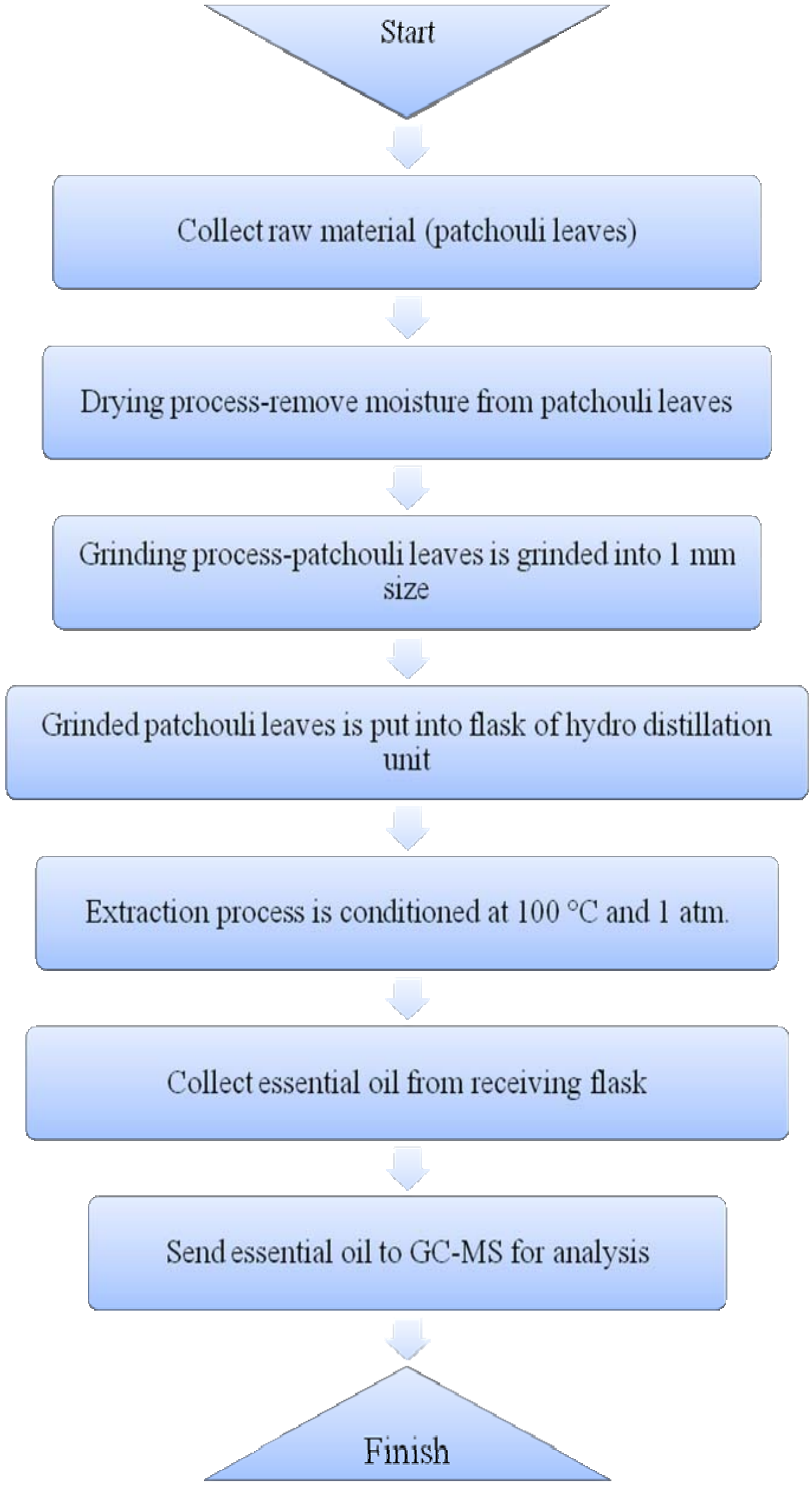

Figure 3.3: Flowchart of extraction process for grinded patchouli leaves

3.4 Data Collection

The data is collected through all parameters that have been studied. Then the yield of patchouli essential oil obtained is calculated by using the following equation:

$$\text{Essential oil yield (\%)} = \frac{\text{amount of essential oil recovered (g)} \times 100\%}{\text{Amount of patchouli distilled (g)}}$$

3.5 Analysis with Gas Chromatography Mass Spectrometer (GC-MS)

The primary goal of GC-MS analysis is to make certain that the oil obtained from hydro distillation extraction process is patchouli essential oil. In this study, gas chromatography mass spectrometer (GCMS) is used to analyze each component of patchouli essential oil. The sample is injected into GC-MS injector port and let it run for a while. During analysis, the chemicals in this essential oil will be separated based on their volatility. In general, small molecules travel more quickly than larger molecules. So if patchouli alcohol for example is more volatile than other compounds, its will reach to detector first and show its peak on computer followed by the other compounds.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Introduction

After completing 5 month of research period, the scheduled experiments were accomplished. Some important data was produced to be analyzed, such as quantity of oil that obtains from grinded (1 mm) and non grinded patchouli leaves. Figure 4.1 below shows some of patchouli essential oil sample obtained from extraction process.

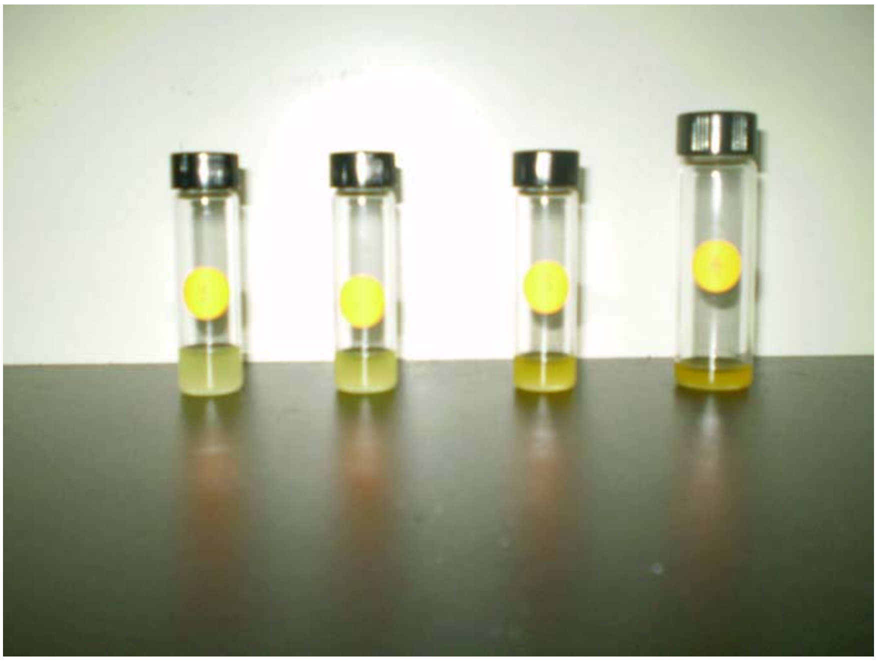

Figure 4.1: Some of patchouli essential oil sample obtained from extraction process

4.2 Experimental Result for Grinded Leaves

The extraction using hydro distillation unit was carried out. About four experiments were done. Table 4.2 shows the experimental result.

Table 4.1: Essential oil obtained for grinded patchouli leaves

Time (h)	Raw Material Quantity (g)	Weight (g)	Percentage yield (%)
1	300g + 3000 ml water	1.385	0.46
2	300g + 3000 ml water	2.529	0.84
3	300g + 3000 ml water	3.412	1.14
4	300g + 3000 ml water	3.952	1.32

From the result, it clearly shows that patchouli essential oil production depend on time of the extraction. The longer the extraction time, the higher yield was obtained. The highest yield of patchouli essential oil was found at four hours in which 1.32% of yield was obtained. Figure 4.3 shows the graph percentage of yield of patchouli essential oil versus time (h).

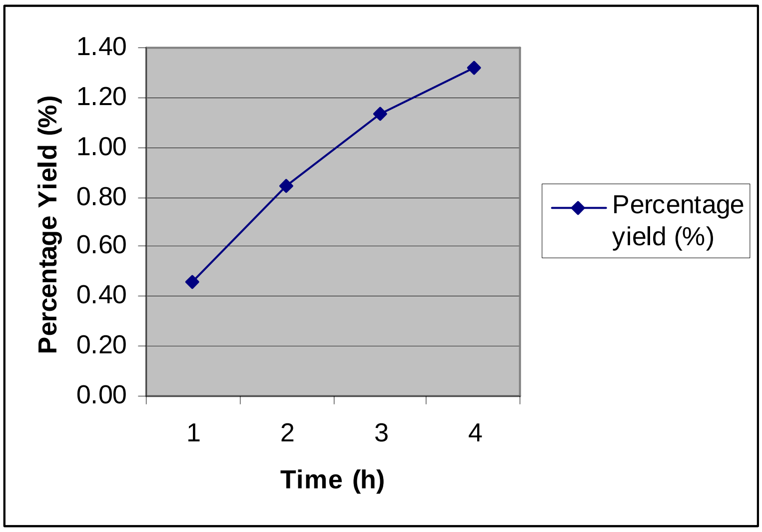

Figure 4.2: Percentage of patchouli oil versus extraction time (hour)

4.3 Experimental Result for Non grinded Leaves

The extraction using hydro distillation unit was carried out. About 4 experiments were done. Table 4.4 shows the experimental result.

Table 4.2 : Essential oil obtained for non grinded patchouli leaves

Time (h)	Raw Material Quantity (g)	Weight (g)	Percentage yield (%)
1	300g + 3000 ml water	0.553	0.18
2	300g + 3000 ml water	1.215	0.41
3	300g + 3000 ml water	1.940	0.65
4	300g + 3000 ml water	2.676	0.89

From the result it clearly shows that patchouli essential oil production depend on time of the extraction. The longer the extraction time, the higher yield was obtained. The highest yield of patchouli oil was obtained at four hours in which 0.89% of yield was obtained. Figure 4.5 shows the graph percentage of yield of patchouli essential oil versus time (h).

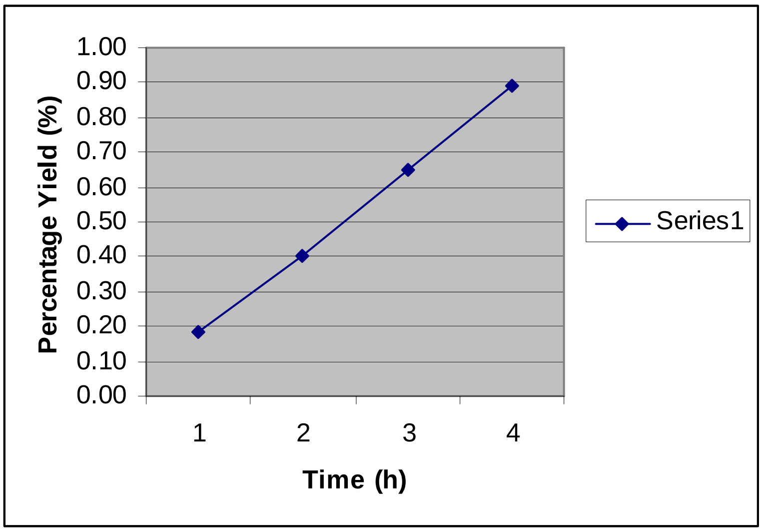

Figure 4.3: Percentage yield of patchouli oil versus extraction time (hour)

4.4 The Effect of Extraction Time on the Yield of Patchouli Essential Oil

From the result (Table 4.1 and Table 4.2), the amount of essential oil extracted increased as the extraction time increased for both grinded and non grinded sample. We also can see that, both grinded and non grinded patchouli leave have shown the same pattern when graph of percentage of yield versus time is drawn (Figure 4.2 and Figure 4.3).

This is because as time increased the contact time between solid and liquid is increased and more oil bearing glands is broken to release essential oil. Moreover larger amount of oils can diffuse through the plant tissue, and more water molecules (steam) can penetrate through the wall of the glands due to the longer extraction time to take place. From the result also, this pattern was continued until the end of experiment.

4.5 Comparison between Grinded Sample (1 mm) and Non Grinded Sample

Figure 4.4 presents the difference percentage of oil yield versus time for grinded and non grinded patchouli leave. From Figure 4.4 we can conclude that grinded patchouli leave produces more essential oil at every extraction time rather than non grinded patchouli leave. This is because the factor of particle size gives a major effect on the yield of patchouli essential oil. Grinded patchouli leave which has smaller particle size has bigger surface area, meanwhile non grinded patchouli leave which has bigger particle size has smaller surface area.

According to Richardson *et al*., (2002), particle size influences the extraction rate in a number of ways. The smaller the size of particle, the greater is the interfacial area between the solid and liquid, and therefore the higher is the rate of transfer of material and the smaller is the distance the solute must diffuse within the solid. Therefore it is desirable to do thorough comminuting or size reduction on plant material in purpose to improve the extraction process.

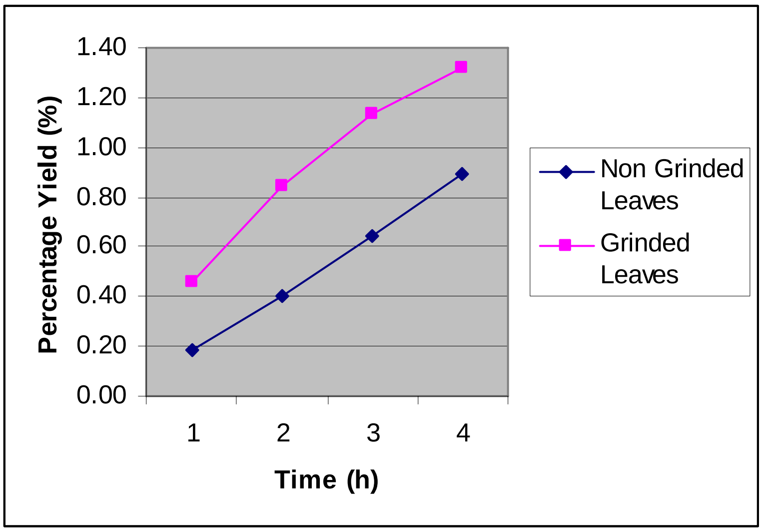

Figure 4.4: Comparison of percentage yield of patchouli oil versus extraction time (hour)

4.6 Result and Discussion of GC-MS Analysis

A sample of patchouli essential oil is analyzed by using GC-MS and the result is shown in Figure 4.5. Figure 4.5 shows the graph analysis of sample of patchouli essential oil, all components in the sample can be search according to the retention time and read in graph style. This result is analyzed from the gas chromatography mass spectrometer (GC-MS) library. When one of the component reach the detector, GC-MS 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 in detail forms. The form is attached in Appendix B.

From the result of analysis that has been done by using GC-MS, one of the patchouli essential oil main component is detected which known as patchouli alcohol. This component is detected at peak 21 where retention time is 14.741 and the quality is equal to 86%. Beside that, some component which commonly found in patchouli essential oil is also present like β-patchoulene, α-guaiene and α-patchoulene. So from this result, we can prove that the oil obtained from hydro distillation extraction process is definitely patchouli essential oil.

The present of many peaks in the Figure 4.5 can be explained by several reasons. Firstly, there are a lot of constituents that made up of patchouli essential oil. Normally, essential oil is made up from several to hundreds of constituent which hard to identify. Each of these constituents will show a peak in graph. Secondly, the present of these peak also due to the fact that the present of contaminate during analyzing processes. The contamination may occur during sample preparation or may be due to the contaminate which already present in GC-MS equipment from other user. So it is desirable that the GC-MS is maintenance frequently.

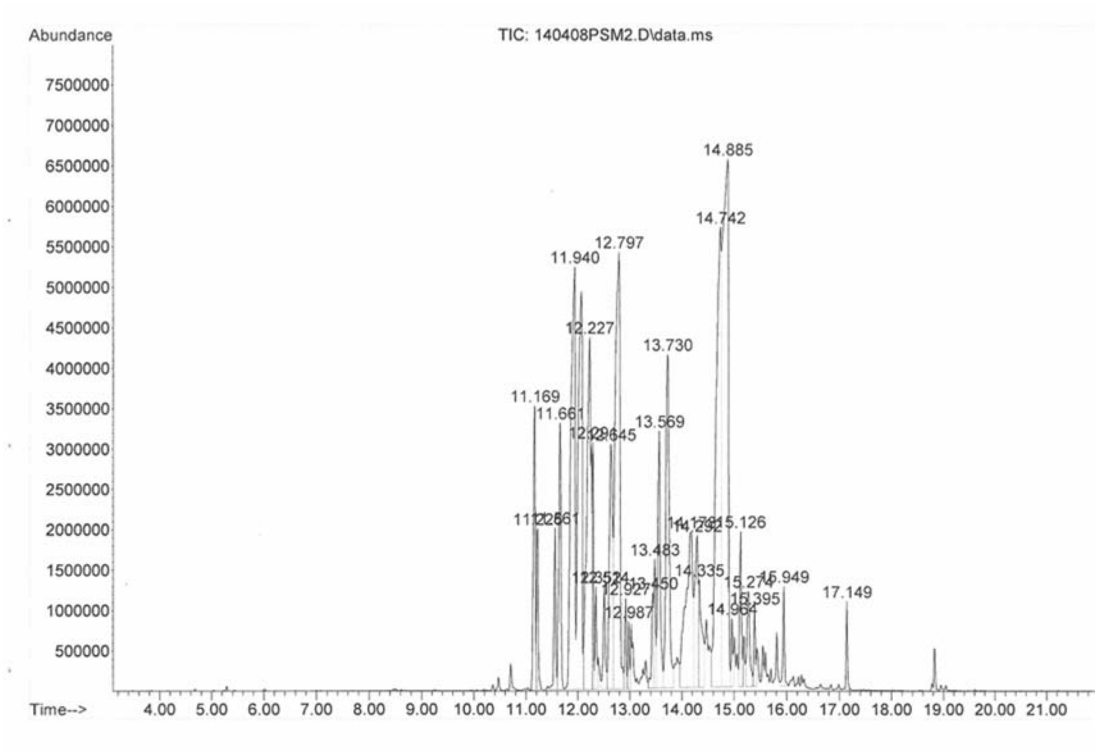

Figure 4.5 Result from analysis on sample of patchouli essential oil

CHAPTER 5

CONCLUSION

5.1 Conclusion

This research, Patchouli Oil Extraction by Using Hydro Distillation (Lab Scale) had been successfully done. The purpose of this research is to study the correlation between extraction time and particle size versus the production of patchouli essential oil. This research also is carried out in order to improve the feasibility of hydro distillation extraction process as patchouli essential oil extraction method. From experiments that have been done, several conclusions had been drawn. The conclusions are as follow.

Firstly, the yield of patchouli essential oil is proportional with extraction time. The yield of patchouli essential oil is increased as extraction time increased. This statement applies to both grinded and non grinded patchouli leave. For both grinded and non grinded patchouli leave the only different is the amount of patchouli essential oil extracted in each of the specific time. The highest yield of grinded and non grinded patchouli leave is at four hours in which 1.32% and 0.89% of yield was obtained respectively.

Secondly, particle size affects the amount of patchouli essential oil production. Grinded patchouli leave which has smaller particle size produces more oil rather than non grinded patchouli leave which has bigger particle size. From the result we can also conclude that thorough comminution or size reduction of plant material before extraction process can improve the production of essential oil.

Lastly, by using hydro distillation, the patchouli essential oil is safe to use for human and easy to commercialize in order to make variety of product like product of cosmetic or aromatherapy. This is because hydro distillation method is free from using other chemical reagent like benzene or hexane during the extraction process. The equipment of hydro distillation also is simple and the price tends to be extremely low that they can operate and maintain in remote locations.

5.2 Recommendation

Patchouli essential oil extraction by using hydro distillation is an important research. The benefits from this research can help local entrepreneur who involve in patchouli essential oil extraction to improve quality and quantity of their production. From the study that has been done, there are several recommendations that should be carried out to improve future research. The recommendations are laid out below.

Firstly, hydro distillation extraction process can be performed at larger scale or pilot scale in order to obtain significant result. This is because the oil from lab scale extraction process is hard to collect and may interfere with the result of the study (in term of accuracy). This is also due to the fact that the yield of patchouli essential oil is too small.

Beside that, hydro distillation process can be carried out in variety of conditions. For example, hydro distillation process can be performed under reduced pressure, atmospheric pressure or excess pressure. These variation of conditions will give effect on the quality and quantity of yield of patchouli essential oil to a certain degree. Therefore these variations could be taken into consideration in future study to find out the best condition to produce patchouli essential oil.

Lastly, extraction of hydro distillation can be extended into 6 to 8 hours to find out the optimum amount of patchouli essential oil. However proper analysis on quality of the oil should be made to prevent some constituent in the oil decomposed or destroyed due to the heating effect at longer time.

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Distillation
http://www.britannica.com/eb/article-9030650. Retrieve on October 15, 2007.

Essential oil Properties
http://www.essentialwholesale.com/aromatherapy.html. Retrieve on December 15, 2007.

Patchouli Uses
http://www.patchoulioilindia.com/uses.htm. Retrieve on December 15, 2007.

Water Distillation
http://www.essentialoils.co.za/water-distillation.htm. Retrieve on December 15, 2007.

APPENDICES

Appendix A

```
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Operator    : FIZA
Acquired    : 14 Apr 2008  11:46     using AcqMethod DIANA PATCHOULI.M
Instrument  :    GCMSD
Sample Name: SAMPLE 2
Misc Info   :
Vial Number: 2
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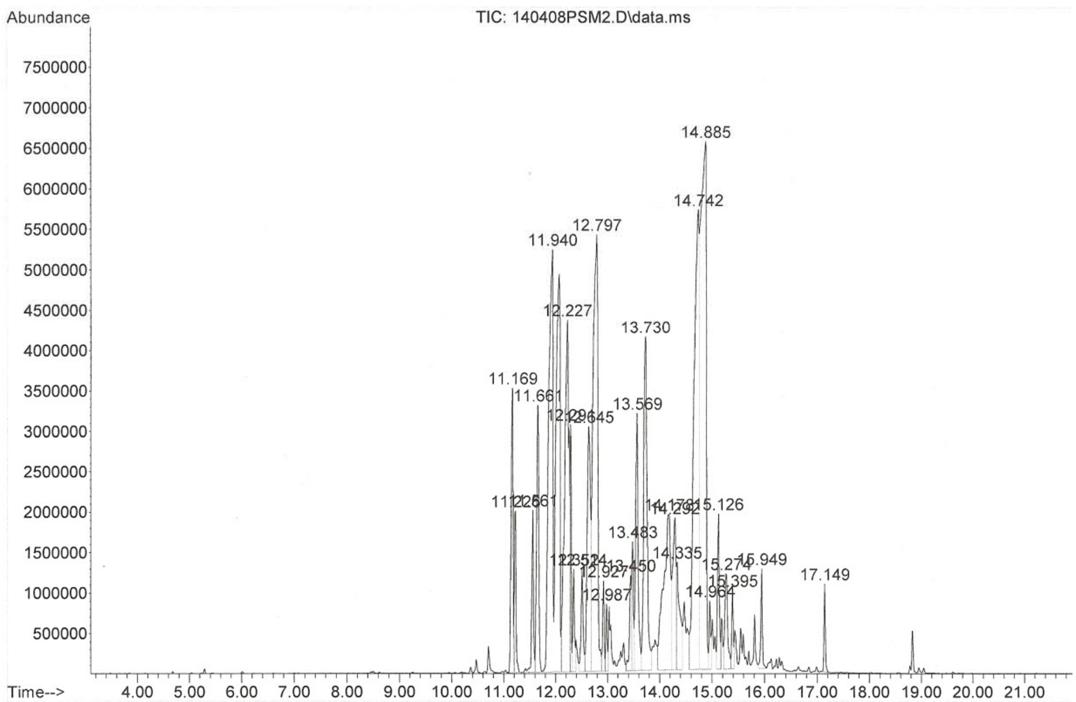

Appendix B

Library Search Report

```
Data Path : D:\Data\Diana Patchouli\PATCHOULI1.D\
Data File : 140408PSM2.D
Acq On    : 14 Apr 2008  11:46
Operator  : FIZA
Sample    : SAMPLE 2
Misc      :
ALS Vial  : 2   Sample Multiplier: 1

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

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

Pk#     RT  Area%          Library/ID                    Ref#     CAS#   Qual
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                 8-octahydro-1,4,9,9-tetramethyl-,
                 [1S-(1.alpha.,4.alpha.,7.alpha.)]-
  β-patchoulene  4,7-Methanoazulene, 1,2,3,4,5,6,7,  60013 000514-51-2 91
                 8-octahydro-1,4,9,9-tetramethyl-,
                 [1S-(1.alpha.,4.alpha.,7.alpha.)]-
                 4,7-Methanoazulene, 1,2,3,4,5,6,7,  60012 000514-51-2 90
                 8-octahydro-1,4,9,9-tetramethyl-,
                 [1S-(1.alpha.,4.alpha.,7.alpha.)]-

 2  11.227  1.30 C:\Database\NIST05a.L
                 Cyclohexane, 1-ethenyl-1-methyl-2,  60003 000515-13-9 60
                 4-bis(1-methylethenyl)-, [1S-(1.al
                 pha.,2.beta.,4.beta.)]-
                 1H-Pyrrole, 1-(2-furanylmethyl)-    21692 001438-94-4 46
                 1,5-Cyclooctadiene, 1,5-dimethyl-   15260 003760-14-3 43

 3  11.558  1.32 C:\Database\NIST05a.L
                 4-Fluorobenzoic acid, pent-2-en-4-  59609 1000299-15-2 47
                 ynyl ester
                 Bioallethrin                       126072 000584-79-2 43
                 2-Cyclohexene-1-carbonyl chloride,  47007 100131-18-8 43
                  1,4,4-trimethyl-

 4  11.665  2.81 C:\Database\NIST05a.L
                 Caryophyllene                       59797 000087-44-5 99
                 Caryophyllene                       59802 000087-44-5 99
                 Bicyclo[5.2.0]nonane, 2-methylene-  59917 242794-76-9 92
                 4,8,8-trimethyl-4-vinyl-

 5  11.942 15.58 C:\Database\NIST05a.L
                 Azulene, 1,2,3,4,5,6,7,8-octahydro  60026 003691-12-1 99
                 -1,4-dimethyl-7-(1-methylethenyl)-
                 , [1S-(1.alpha.,4.alpha.,7.alpha.)
                 ]-
                 Azulene, 1,2,3,4,5,6,7,8-octahydro  60027 003691-12-1 98
                 -1,4-dimethyl-7-(1-methylethenyl)-
                 , [1S-(1.alpha.,4.alpha.,7.alpha.)
                 ]-
                 Azulene, 1,2,3,4,5,6,7,8-octahydro  60028 003691-12-1 87
                 -1,4-dimethyl-7-(1-methylethenyl)-
                 , [1S-(1.alpha.,4.alpha.,7.alpha.)
                 ]-

 6  12.231  7.31 C:\Database\NIST05a.L
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                 8a-hexahydro-1,4,9,9-tetramethyl-,
                  (1.alpha.,3a.alpha.,7.alpha.,8a.b
                 eta.)-
                 1H-3a,7-Methanoazulene, 2,3,6,7,8,  60044 000560-32-7 83
                 8a-hexahydro-1,4,9,9-tetramethyl-,
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                 eta.)-
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                 methylene-, (4aS-cis)-

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Data Path : D:\Data\Diana Patchouli\PATCHOULI1.D\
Data File : 140408PSM2.D
Acq On : 14 Apr 2008 11:46
Operator : FIZA
Sample : SAMPLE 2
Misc :
ALS Vial : 2 Sample Multiplier: 1

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

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

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			Patchoulene	59793	001405-16-9	99
			.beta.-Neoclovene	59830	056684-96-9	94
			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	93
8	12.348	0.75	C:\Database\NIST05a.L			
			Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,4.alpha.,7.alpha.)]-	60027	003691-12-1	78
			.beta.-Humulene	59811	000116-04-1	58
			1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-	60086	021747-46-6	46
9	12.519	1.03	C:\Database\NIST05a.L			
			1H-Cyclopropa[a]naphthalene, decahydro-1,1,3a-trimethyl-7-methylene-, [1aS-(1a.alpha.,3a.alpha.,7a.beta.,7b.alpha.)]-	60072	020071-49-2	90
			Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-	60066	022567-17-5	90
			1H-Cyclopropa[a]naphthalene, 1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl-, [1aR-(1a.alpha.,3a.alpha.,7b.alpha.)]-	60074	000489-29-2	90
10	12.647	3.89	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	96
			1H-Cyclopropa[a]naphthalene, 1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl-, [1aR-(1a.alpha.,3a.alpha.,7b.alpha.)]-	60074	000489-29-2	96
			Cedrene-V6	59786	1000162-76-8	94
11	12.797	10.08	C:\Database\NIST05a.L			
			Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	60033	003691-11-0	99
			Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	60031	003691-11-0	98
			Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	60035	003691-11-0	98
12	12.925	0.67	C:\Database\NIST05a.L			
			(-)-.alpha.-Panasinsen	59853	056633-28-4	86

Library Search Report

Data Path : D:\Data\Diana Patchouli\PATCHOULI1.D\
Data File : 140408PSM2.D
Acq On : 14 Apr 2008 11:46
Operator : FIZA
Sample : SAMPLE 2
Misc :
ALS Vial : 2 Sample Multiplier: 1

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

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

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	59918	150320-52-8	64
			1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*)]-	60103	013744-15-5	62
13	12.989	0.45	C:\Database\NIST05a.L			
			Isoaromadendrene epoxide	71364	1000159-36-6	25
			Caryophyllene oxide	71352	001139-30-6	25
			2,3-Hexadiene, 2-methyl-	2824	029212-09-7	25
14	13.448	0.81	C:\Database\NIST05a.L			
			Phenol, 2-ethyl-	9606	000090-00-6	30
			Phenol, 3,5-dimethyl-	9631	000108-68-9	30
			Phenol, 2,5-dimethyl-	9630	000095-87-4	30
15	13.481	1.08	C:\Database\NIST05a.L			
			Caryophyllene oxide	71352	001139-30-6	42
			2-Isopropylidene-3-methylhexa-3,5-dienal	22844	1000191-76-5	30
			(1H)Imidazole-4-acetonitrile	5005	018502-05-1	30
16	13.566	3.17	C:\Database\NIST05a.L			
			1-Pentene, 5-(2,2-dimethylcyclopropyl)-2-methyl-4-methylene-	32238	1000150-39-5	22
			Phenol, 2-ethyl-	9598	000090-00-6	18
			3,3-Dimethyl-6-methylenecyclohexen	9738	020185-16-4	18
17	13.726	5.47	C:\Database\NIST05a.L			
			Caryophyllene oxide	71352	001139-30-6	91
			Cyclohexene, 3-methyl-6-(1-methylethenyl)-, (3R-trans)-	15383	005113-87-1	55
			Bicyclo[6.1.0]nonane, 9-(1-methylethylidene)-	32218	056666-90-1	53
18	14.175	5.32	C:\Database\NIST05a.L			
			Isoaromadendrene epoxide	71364	1000159-36-6	49
			Alloaromadendrene oxide-(1)	71377	1000156-12-8	43
			Cyclohexane, 1-methyl-2,4-bis(1-methylethenyl)-	41749	061142-58-3	41
19	14.292	2.39	C:\Database\NIST05a.L			
			1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-	60079	000489-39-4	53
			Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]	60015	017066-67-0	49
			1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1.alpha.,3a.beta.,4.alpha.,8a.beta.)]	60020	000475-20-7	49
20	14.335	1.72	C:\Database\NIST05a.L			
			1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,	71465	006750-60-3	64

Data Path : D:\Data\Diana_Patchouli\PATCHOULI1.D\
Data File : 140408PSM2.D
Acq On : 14 Apr 2008 11:46
Operator : FIZA
Sample : SAMPLE 2
Misc :
ALS Vial : 2 Sample Multiplier: 1

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

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

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			7a.beta.,7b.alpha.)]-			
			1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	71464	006750-60-3	64
			Isoaromadendrene epoxide	71364	1000159-36-6	47
21	14.741	11.05	C:\Database\NIST05a.L			
			Patchouli alcohol	72914	005986-55-0	86
			Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-	60067	022567-17-5	83
			Patchouli alcohol	72910	005986-55-0	78
22	14.891	14.58	C:\Database\NIST05a.L			
			Patchouli alcohol	72916	005986-55-0	93
			Patchouli alcohol	72914	005986-55-0	91
			Patchouli alcohol	72910	005986-55-0	90
23	14.965	0.49	C:\Database\NIST05a.L			
			.alpha.-Farnesene	59827	000502-61-4	42
			Cyclohexane, 1,5-diethenyl-2,3-dimethyl-, (1.alpha.,2.alpha.,3.alpha.,5.beta.)-	32246	068779-14-6	35
			Longifolenaldehyde	71344	019890-84-7	30
24	15.126	1.37	C:\Database\NIST05a.L			
			2H-Cyclopropa[a]naphthalen-2-one, 1,1a,4,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, (1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)-	69993	006831-17-0	50
			1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	60077	025246-27-9	48
			2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-4a,5-dimethyl-3-(1-methylethylidene)-, (4ar-cis)-	69988	019598-45-9	46
25	15.275	1.35	C:\Database\NIST05a.L			
			Isoaromadendrene epoxide	71364	1000159-36-6	68
			Ledene oxide-(I)	71329	1000151-93-3	62
			.beta.-Humulene	59811	000116-04-1	60
26	15.393	0.56	C:\Database\NIST05a.L			
			Eudesma-4(14),11-diene	59851	1000152-04-3	83
			1-Cycloheptene, 1,4-dimethyl-3-(2-methyl-1-propene-1-yl)-4-vinyl-	59937	1000159-38-6	70
			Ledol	72882	000577-27-5	64
27	15.948	0.75	C:\Database\NIST05a.L			
			Benzene, 1-isothiocyanato-4-methyl	22616	000622-59-3	38
			6-Methylthieno[2,3-b]pyridine	22608	001759-30-4	38
			N,N,2,4-Tetramethylaniline	22424	000769-53-9	38
28	17.144	0.54	C:\Database\NIST05a.L			
			Pentadecanoic acid, 14-methyl-, methyl ester	105662	005129-60-2	98

Library Search Report

Data Path : D:\Data\Diana Patchouli\PATCHOULI1.D\
Data File : 140408PSM2.D
Acq On : 14 Apr 2008 11:46
Operator : FIZA
Sample : SAMPLE 2
Misc :
ALS Vial : 2 Sample Multiplier: 1

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

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

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			Hexadecanoic acid, methyl ester	105639	000112-39-0	97
			Hexadecanoic acid, methyl ester	105644	000112-39-0	96

Appendix C

7890A
5975C


```
                    INSTRUMENT CONTROL PARAMETERS:    GCMSD
                    --------------------------------------

   D:\METHOD\Diana Patchouli.M
      Fri Apr 04 15:16:15 2008

Control Information
------- -----------

Sample Inlet      :  GC
Injection Source  :  GC ALS
Mass Spectrometer :  Enabled

Oven
Oven                                 On
Equilibration Time                    1 min
Oven Program
    50 degrees C for 0 min
    then 10 °C/min to 240 degrees C for 3 min

Post Run Temperature                  0 degrees C



Front Injector
Syringe Size                         10 µL
Injection Volume                      3 µL
Injection Repetitions                 1
Solvent A Washes (PreInj)             0
Solvent A Washes (PostInj)            3
Solvent A Volume                      8 µL
Solvent B Washes (PreInj)             0
Solvent B Washes (PostInj)            0
Solvent B Volume                      8 µL
Sample Washes                         2
Sample Wash Volume                    8 µL
Sample Pumps                          3
Dwell Time (PreInj)                   0 min
Dwell Time (PostInj)                  0 min
Solvent Wash Draw Speed             300 µL/min
Solvent Wash Dispense Speed        6000 µL/min
Sample Wash Draw Speed              300 µL/min
Sample Wash Dispense Speed         6000 µL/min
Injection Dispense Speed           6000 µL/min
Viscosity Delay                       7 sec
Sample Depth Disabled


Back Injector


Front Inlet SS
Heater                               On    250 °C
Pressure                             On    5.7865 psi
Total Flow                           On    12.24 mL/min
Septum Purge Flow                    On    3 mL/min
Mode                              Split
Gas Saver                            On    20 mL/min After 3 min
Split Ratio                          10 :1
Split Flow                        8.4 mL/min
Injection Pulse Pressure           0 psi Until 0 min


Thermal Aux 2 {MSD Transfer Line}
Heater                               On
Temperature Program
```

280 degrees C for 0 min

Column #1
Agilent 19091S-433: 325 °C: 30 m x 250 µm x 0.25 µm
HP-5MS 5% Phenyl Methyl Silox: 293.38321
In: Front SS Inlet He
Out: Vacuum

Column #2
450 °C: 25 m x 320 µm x 0 µm

Column #3
450 °C: 25 m x 320 µm x 0 µm

Column #4
450 °C: 25 m x 320 µm x 0 µm

Column #5
450 °C: 25 m x 320 µm x 0 µm

Column #6
450 °C: 25 m x 320 µm x 0 µm

Front Detector FID	
Heater	Off
H2 Flow	Off
Air Flow	Off
Makeup Flow	Off
Const Col + Makeup	Off
Flame	Off
Electrometer	Off

Valve 1	
Switching Valve	Off

Signals	
Front Signal	Save Off
Test Plot	Save Off
Test Plot	Save Off
Test Plot	Save Off

MS ACQUISITION PARAMETERS

General Information
------- -----------

Tune File : stune.u
Acquistion Mode : Scan

MS Information
-- -----------

Solvent Delay : 3.00 min

```
EM Absolute              : False
EM Offset                : 0
Resulting EM Voltage     : 847.1

[Scan Parameters]

Low Mass                 : 35.0
High Mass                : 1000.0
Threshold                : 150
Sample #                 : 2       A/D Samples    4
Plot 2 low mass          : 50.0
Plot 2 high mass         : 550.0

[MSZones]

MS Source                : 230 C   maximum 250 C
MS Quad                  : 150 C   maximum 200 C

                     END OF MS ACQUISITION PARAMETERS

                      TUNE PARAMETERS for SN: US73327503
                 -----------------------------

 Trace Ion Detection is OFF.

 EMISSION    :      34.610
 ENERGY      :      69.922
 REPELLER    :      19.904
 IONFOCUS    :      72.125
 ENTRANCE_LE :       0.000
 EMVOLTS     :     847.059
 AMUGAIN     :    1840.000
 AMUOFFSET   :     124.750
 FILAMENT    :       1.000
 DCPOLARITY  :       0.000
 ENTLENSOFFS :      14.306@  3    14.306@ 50    10.541@ 69    12.047@131
11.294@219    14.055@414    14.557@502    14.557@1049
 MASSGAIN    :    -693.000
 MASSOFFSET  :     -36.000

                    END OF TUNE PARAMETERS
               -----------------------------------

               END OF INSTRUMENT CONTROL PARAMETERS
               -----------------------------------
```