STUDY OF *CYMBOPOGON CITRATUS* (LEMONGRASS) ESSENTIAL OIL EXTRACTION TECHNIQUE

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

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APRIL 2010

ABSTRACT

The Lemongrass (*Cymbopogon Citratus*) essential oil was a significant interest as a new high-value essential oil especially in pharmaceutical, aromatherapy aid and cosmetics industries which give large opportunities for global marketing. This study obtained to identify the effect of the retention time and particle size on production of Lemongrass essential oil by using Soxhlet extraction method. Soxhlet extraction method used to obtain essential oil processes are not expensive but can induce thermal degradation, hydrolysis and water solubilization of some fragrance constituents. Ratio of Lemongrass and ethanol use was 1:3 were placed in the Soxlet extraction setup. The essential oil components were identified by using GC-MS. The result shows stem can obtained higher yield at highest retention time and lowest particle size. The dominated compound from leaves, stem and mixture was Citral (1.27%, 0.86% and 2.74%) follow by Geraniol (0.58%, 0.61% and 0.88%) an oxygenated compound for effect of particle size. The compound from effect of retention time for leaves, stem and mixture is Neral (33.95%, 2.11% and 3.17%) followed by Geranial (40.64%, 3.98% and 2.98%). As a conclusion stem obtained greater yield with longest time and lowest particle size and also high percentage of oxygenated compounds compare with leaves and mixture. Furthermore stem shows a good alternative part of Lemongrass to produce essential oil.

ABSTRAK

Minyak asli serai (*Cymbopogon Citratus*) adalah amat bermanfaat sebagai minyak asli baharu yang mendapat permintaan tinggi terutamanya dalam industri perubatan, aromaterapi dan kosmetik yang mempunyai nilai pasaran dunia. Penelitian ini diperoleh untuk mengetahui pengaruh masa dan saiz bahan pada pengeluaran minyak asli serai dengan menggunakan kaedah ekstraksi Soxhlet. Kaedah ekstraksi Soxhlet yang digunakan untuk mendapatkan proses minyak asli serai tidak mahal, tetapi boleh menyebabkan degradasi terma, hidrolisis dan solubilization air dari beberapa unsur aroma. Nisbah serai dan etanol 1:3 ditempatkan di pemasangan ekstraksi Soxlet. Komponen minyak asli dikenalpasti dengan menggunakan GC-MS. Keputusan kajian menunjukkan batang boleh mendapat hasil yang lebih tinggi pada masa yang lama dan pada saiz bahan yang kecil. Sebatian yang didominasi dari daun, batang dan campuran ialah Citral (1,27%, 0,86% dan 2,74%) diikuti dengan Geraniol (0,58%, 0,61% dan 0,88%) sebagai sebatian oksigen untuk pengaruh saiz zarah. Kompleks dari pengaruh masa untuk daun, batang dan campuran adalah Neral (33,95%, 2,11% dan 3,17%) diikuti oleh Geranial (40,64%, 3.98% dan 2,98%). Sebagai kesimpulan batang memperoleh hasil yang lebih besar dengan waktu terpanjang dan ukuran saiz bahan terendah dan peratusan sebatian oksigen juga tinggi berbanding dengan daun dan campuran. Seterusnya batang menunjukkan bahagian alternatif yang baik untuk mengeluarkan minyak asli serai.

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^{o}C	= Degree Celsius
%	= Percentage
CO_2	= Carbon Dioxide
mL	= Mili-Liter
g	= Gram
L	= Liter
min	= Minutes
hr	= Hours
m	= Meter
μm	= Micron- meter
mm	= Mili-Meter
cm/s	= Centi-Meter Per Second
^{o}C/min	= Degree Celcius Per Minutes
μL	= Micro-Liter
V	= Volume
RT	= Retention Time
A	= Area

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Nowadays, people worldwide are looking towards natural base products since there are no side effects when taken accordingly. Historically, the first essential oil encountered was the oil of rose. It was discovered by the Chinese prior to the Christian era. A layer of this oil was found on a pool that was filled with rose water. Essential oils contain DNA of the plant of herbs they are extracted from. Essential oils or sometimes called volatile oils are believed to be that small portion of the plant material, which imparts the characteristics odors and flavor most closely associated with the vegetative matter which they are obtained. Most of the essential oils are used at about a level of 0.01-0.1 percent in the finished product (Ibrahim, 2006). Furthermore, there is also an interest in the production of functional, high value, natural products without chemical modification and residues of solvents or additives. This trend in consumer preference increases the demand tremendously with variety products range from essential oils (Mohamed, 2005).

Essential oil is a volatile compound in botanical material which has been used in many applications since long time ago (Rahiman et al., 2008). Essential oil comes from every part of the plant including leaf, stems, flower, seed, branch, and root, additionally essential oil is commonly used in cosmetics, drugs, and perfume (Mindaryani and Sri Rahayu, 2007). This essential oil is composed of different chemical group of terpenic hydrocarbons and their oxidized derivatives such as aldehydes, ester, ketones, and alcohols.Terpenes represent a large group of natural compounds that do not contribute much to flavor, fragrance or odour of the oil (Makgwane, 2005). The advantages of essential oils are their flavor concentrations and their similarity to their corresponding sources. The majority of them is fairly stable and contains a few natural antioxidants (Ibrahim, 2006).

Lemongrass (*Cymbopogon citrates*) is an herb that grows in most tropical countries. It belongs to the genus *Cymbopogon* of aromatic grasses and contains essential oil with fine lemon flavor. Its major constituent is citral which is more than 75% by weight of the essential oil. Citral is the name given to a natural mixture of two isomeric acyclic monoterpene aldehydes, geranial (*trans* citral, citral A) and neral (*cis*-citral, citral B) at the ratio of 4:1 by weight (Huynh et al., 2008). The oil quality is judged by its citral content and its solubility in alcohol. It bears reddish-yellow to reddish-brown colour, with strong, lemon odour properties. It is used in the perfume, soap and cosmetics industries and forms the starting material in the manufacture of synthetic Vitamin A. Further the oil serves as an input to pharmaceutical preparations, such as pain balm, disinfectants, and mosquito-repellent creams (Prommegger et al., 2005).

The essential oil of aromatic herbs is traditionally obtained by hydro distillation, steam distillation, or solvent extraction. These processes are not expensive but can induce thermal degradation, hydrolysis and water solubilization of some fragrance constituents. Solvent extraction is simpler in terms of the number of steps employed but there are still important choices to be made when designing a process. Solvent extraction of solid sample is commonly known as solid-liquid extraction. In physicochemical terminology, it is referred as leaching or lixiviation and is one of the oldest ways of solid sample pretreatment. In this study, chemical constituent extraction from Lemongrass with Soxhlet extraction process has been studied experimentally. Two extraction conditions including the size of lemongrass and length of extraction, on the yield from chemical constituent extraction have been investigated. Compositions of Lemongrass were analyzed by gas chromatography–mass spectrometry (GC–MS) (Ahmad et al., 2010).

The quality of the essential oils produced by both of the above methods can vary greatly depending again on various factors. Two of the main factors are the quality of the starting plant material and process control during extraction of the essential oil. In some distillation units where the plant material and water are heated together, the plant material may get damaged by overheating or localized charring. During solvent extraction, if the temperature and pressure are not controlled properly, some of the solvent may remain behind in the absolute causing a foul odor (Anonymous, 2008).

Now days, it has been conducting about the operating condition to produce source of material and methods essential oil for lemongrass. Therefore, it will be investigated which one is the best methods and the source of materials (leaves, stem and mixture) to find the essential oil. So that, it could be improve the yield and quality of the lemongrass essential oil.

1.2 Problem Statement

Generally, there are a few problems that arise in lemongrass extraction. There are many types of extraction. The extraction can be conducted with or without solvent. But, to get the essential oil, extraction through solvent extraction is the most used method. Solvent extraction is usually used to recover a component from either a solid or liquid. The sample is contacted with a solvent that will dissolve the solutes of interest. Solvent extraction is of major commercial importance to the chemical and biochemical industries, as it is often the most efficient method of separation of valuable products from complex feed stocks or reaction products.

Some extraction techniques in involve partition between two immiscible liquids, others involve either continuous extractions or batch extractions. Because of environmental concerns, many common liquid/liquid processes have been modified to either utilize benign solvents, or move to more frugal processes such as solid phase extraction.

The other problem is the quality and percentage yield in essential oil are lower. In some distillation units where the plant material and water are heated together, the plant material may get damaged by overheating or localized charring. During solvent extraction, if the temperature and pressure are not controlled properly, some of the solvent may remain behind in the absolute causing a foul odor. So, it can reduce the quality and percentage yield in essential oil.

1.3 Research Objective

Thus, the objectives of this research are:

i. To study of *Cymbopogon citratus* (Lemongrass) essential oil extraction technique using extraction solvent methods.
ii. To obtain the effect size of lemongrass stem, leaves, mixture and retention time on production of lemongrass essential oil.
iii. To find the optimum yield of lemongrass essential oil.
iv. To analyze which part of lemongrass will produce the most essential oil.

1.4 Scope of Research

The important scopes have been identified for this research in achieving the objective:

i. Study essential oil extraction technique using solvent extraction method. The solvent used for this research is ethanol.

ii. The effect of size and retention time in production of lemongrass essential oil. Four different size and retention time are used for this research to get the best essential oil.

iii. The optimum condition for production of lemongrass essential oil. The optimum condition means the part can produce more percentage yield.

iv. Analyze the lemongrass essential oil by using GC-MS at optimum condition. After analyze the sample, we can see the types of essential oil in the lemongrass essential oil.

CHAPTER 2

LITERATURE REVIEW

2.1 Lemongrass

According to Dichoso et al (2000), Lemongrass is an aromatic grass about 210 to 315 cm tall. The leaves are linear, lanceolate (125 x 1.7 cm); panicle very large (30 to 80 cm long), drooping and lax. The color is grayish or grayish green, rarely with a tingle of purple. It is decompounds with receme pairs in dense masses, spreading and slightly hairy. It has low glumes of the sessile spikelets with 1 to 3 define nerves, shallowly concave with 1 or 2 depressions.

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Citral is also an important raw material used in the pharmaceutical, perfumery and cosmetics industries, especially for the synthesis of Vitamin A and ionones; synthetic citral, derived from conifer turpentine is normally used for those purposes. Citral possesses antifungal activity against plant and human pathogens inhibit seed germination, and have bactericidal and insecticidal properties. Their chemical structures are shown in Figure 1 (Lewinsohn et al., 1998).

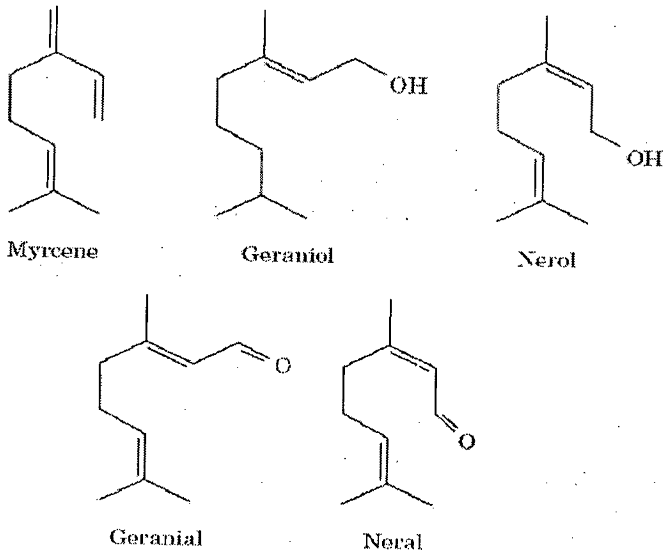

Figure 2.1: Chemical structure of major constituents of lemongrass essential oil (Source: Lewinsohn *et. al* 1998)

2.2 Essential oil

The essential oils which were regularly used in ancient Rome, Greece, and Egypt and through out the Middle and Far East had, as a common feature, the essence of a plant; an identifiable aroma, flavor, or other characteristic that was of some practical use. They were used as perfumes, food flavors, deodorants, pharmaceuticals, and embalming antiseptics. Usually, plant material was steeped in a fatty oil or wine that acted as a solvent for the desired flavor or aroma. The extracts (usually impure and dilute) were used as oils or creams. They were introduced into Europe, without further development, to become the subject of specialist craftsmen (the English Guild of Pepperers and the French court perfumers of the 12th Century) and early publications ("The Book of Nurture", 1430) (Porter et al., 2001).

According Phineas et al (2004), although essential oils are produced by different methods such as solvent extraction, expression and critical fluid extraction, most are produced by steam distillation. The proportion of different essential oils extracted by steam distillation is 93% and the remaining 7% is extracted by the other methods. Essential oils are multi-component chemicals. The mixture of oil compounds that constitute the essential oil comprises polar and non-polar compounds. The very important compounds that make up the chemicals usually referred to, as the top notes, in the fragrance industry are the polar compounds. This polarity makes the compounds soluble in water and this solubility is a function of the physical properties of the system such as pressure, temperature and chemical potential.

Essential oils are volatile, natural, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites. Known for their antiseptic, i.e. bactericidal, virucidal and fungicidal, and medicinal properties and their fragrance, they are used in embalment, preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and locally anesthesic remedies. In nature, essential oils play an important role in the protection of the plants as antibacterials, antivirals, antifungals, insecticides and also against herbivores by reducing their appetite for such plants. They also may attract some insects to favors the dispersion of pollens and seeds, or repel undesirable others. Essential oils are extracted from various aromatic plants generally localized in temperate to warm countries like Mediterranean and tropical countries where they represent an important part of the traditional pharmacopoeia. They are liquid, volatile, limpid and rarely colored, lipid soluble and soluble in organic solvents with a generally lower density than that of water (Bakkali et al., 2008).

2.3 The Constituents of Lemongrass

According Katzer et.al (2007), the essential oil of lemon grass (0.2 to 0.5%, “West Indian lemon grass oil”) consists mainly of citral. Further terpenoids in lemon grass oil are nerol, limonene, linalool and β-caryophyllene. The content of myrcene is low, but still enough to make the oil susceptible to oxidative polymerization. Citral is a mixture of two stereoisomeric monoterpene aldehydes; in lemon grass oil, the trans isomer geranial (40 to 62%) dominates over the cis isomer neral (25 to 38%).

Its major constituents are different from East Indian types by the occurrence of substantial quantities of myrcene (12%-15%). The myrcene may undergo diene-condensation and polymerization on aging, and hence losses its solubility in 70% alcohol. The lemongrass is in important crop in Ethiopia and its analysis has shown geraniol (40%), geranial and neral (13%-15%) and alpha- oxobisabole (12%) as major constituents, which are different from usual West Indian lemongrass oil (Akhila et al., 2010).

2.4 Extraction Method

2.4.1 Soxhlet Extraction

The principle of Soxhlet is initially, the sample is placed in a thimble-holder, and during operation gradually filled with condensate fresh solvent from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates the solute of the thimble-holder and unloads it back into the distillation flask, carrying the extracted analytes into the bulk liquid. This operation is repeated until complete extraction is achieved. This performance makes Soxhlet a hybrid continuous–discontinuous technique. In as much as the solvent acts stepwise, the assembly can be considered as a batch system; however, since the solvent is recirculated through the sample, the system also bears a continuous character. The most significant drawbacks of Soxhlet extraction, as compared to the other conventional are the long time required for the extraction and the large amount of solvent wasted, which is not only expensive to dispose off but which can itself cause additional environmental problems (Halim et al., 2010)

Samples are usually extracted at the boiling point of the solvent for a long period of time and the possibility of thermal decomposition of the target compounds cannot be ignored, when thermolabile analytes are involved. The conventional Soxhlet device is unable to provide agitation, which would accelerate the process. Due to the large amount of solvent used, an evaporation/concentration step after the extraction is mandatory. The technique is restricted to solvent selectivity and is not easily automated.

2.4.2 Steam Distillation Method

Many of the essential oils presently used in perfumery are obtained by steam distillation of flowers, leaves, bark, etc. Steam is widely used because of its high latent heat of evaporation, relatively cheaper and widely available. There are two types of steam distillation: water / steam distillation and steam distillation. Steam distillation is still considered to be the most economical method of extracting essential oils. This process involves the use of steam to percolate and vaporized out the essential oils from the plant material, with the subsequent condensation of steam and essential oil prior to their separation. It can be seen from the experimental work done that there is an art to distillation and that, especially for low yield plants, much skill is needed. The role of the distiller is to achieve oil as close as possible to the oil as it exists in the plant (Mohamed et al., 2005)

Steam distillation is a special type of distillation process (separation process) for temperature sensitive materials like natural aromatic compounds. Through this process the botanical material is placed in a still and steam is forced over the material. The hot steam will help to release the aromatic molecules from the plant material. The molecules of these volatile oils are then escape from the plant material and evaporate into the steam. The temperature of the steam therefore needs to be carefully controlled. The temperature should be just high enough to force the plant material to release the essential oils, yet not too hot as it can degrade the plant material or the essential oils. The steam containing the essential oil is passed through a cooling system to condense the steam, which then form a liquid from which the water and the essential oils is then separated. The steam is produced at greater pressure than the atmosphere and therefore it boils at above $100^{o}C$ which facilitates the removal of the essential oil at a faster rate. By doing so, it could prevent damage to the oil as well (Ibrahim et al., 2006)

2.4.3 Supercritical Fluid Extraction

According Rozzi et.al (2001), supercritical fluid extraction (SFE) utilizes the ability of certain gases to behave as non-polar solvents once a certain temperature and pressure combination has been reached. In addition, the solvating power of the supercritical fluid can be manipulated by changing the temperature and/or pressure, allowing extractions to be tailored to extract desired compounds while leaving undesirable compounds behind. The most popular gas to be used as a solvent in SFE is carbon dioxide (CO2), because it is nonflammable, noncorrosive, inexpensive, and has generally recognized as safe (GRAS) status. In addition, CO2 extraction can minimize the potential hydrolysis and isomerization during extraction due to the low temperatures needed for SFE (generally$<100^{o}C$) and since water is not used as part of the extraction process. Other advantages of using supercritical fluids as a solvent include: increased diffusion coefficient and lower viscosity, lack of surface tension allowing for a more rapid diffusion, and lack of a chemical residue.

The Supercritical Fluid Extraction (SFE) process is a powerful technique developed in the field of separation technology. It has become popular as attested by the numerous studies and investigations in recent years, especially on its probable industrial application. It is the preferred choice for separating products with high value added. The process operates at low temperature, produces extracts free from solvent contamination, prevents thermal decomposition especially of the oil's light components, has high product recovery and the facility does not require a large area compared with the conventional methods of separation such as solvent extraction or distillation. SFE is non-toxic, non-explosive and the CO_2 solvent used is relatively cheap. SFE was used in the past to extract essential oil from different feed materials such as palm kerne, hiprose seeds, sunflower seeds, chamomile and hazelnut (Huynh et al., 2008).

2.4.4 Hydro-distillation

Hydro-distillation is one of the most frequently used methods for volatiles isolation. Obtained isolates, called essential oils, according to international definition are distillation products that are not soluble in water and can be easily separated from distillation water. However, some compounds of the essential oils are water-soluble, especially at elevated temperature that can produce losses and smaller oil yields during hydro-distillation (Halim et al., 2010).

2.5 Usage of lemongrass oil

Lemongrass is originally from India and lemongrass oil comes from the grasses themselves. The color of the oil is yellow to reddish (depending on where the grasses were grown) and has a citrus scent similar to lemons. Like lemon and eucalyptus essential oils, lemongrass will also keep biting insects away. It also smells nicer than many commercial repellents (Halvorsen et al., 2008).