EXTRACTION OF GAHARU ESSENTIAL OIL USING ULTRASONIC ASSISTED STEAM DISTILLATION

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EXTRACTION OF GAHARU ESSENTIAL OIL USING ULTRASONIC ASSISTED STEAM DISTILLATION

AHMAD JUNAIDY BIN JAAPAR

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

Faculty of Chemical and Natural Resources Engineering
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APRIL 2008

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

Signature	:	……
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Special dedication to my family members that always inspire, love and stand besides me, my supervisor, my beloved friends especially the one who always help me, my fellow colleagues,
and all faculty members

For all your love, care, support, and believe in me. Thank you so much.

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ABSTRACT

Aquilaria species from the family of Thymelaeceae are the main source of gaharu, which has been classified as one of the most highly valuable, non-timber products in the world market. Its distinctive fragrance has been valued in many cultures and it is widely used in religious ceremonies, medication, incense, perfume and toiletry products. Currently, the method used for extracting gaharu essential oil is by using hydrodistillation. However, this method is inefficient which it produced low yield of oil and longer time of extraction and thus increasing the production cost. To overcome those problems, this study will be conducted to improve existing method of extraction by using ultrasonic assisted steam distillation. Parameters involve in this study is pretreatment time and gaharu-to-water ratio and both are manipulated to gain high yield of oil with optimum and maximum. The results from this study is the gaharu essential oil yield is increasing with the increment of both pretreatment time and gaharu-to water ratio until it reached a condition where the yield of oil become constant. The best pretreatment time obtained is at 9 hours with oil yield of 0.1276% and the gaharu to water ratio of 1:20.which gave 0.1295% oil yield. It is approved that ultrasonic assisted steam distillation is feasible to improve current method of gaharu essential oil extraction by gaining high oil yield and saving production cost.

ABSTRAK

Spesis *Aquilaria* dari keluarga Thymelaeceae adalah sumber utama penghasilan gaharu yang telah diklasifikasikan sebagai hasil bukan kayu paling berharga di pasaran dunia. Harumannya yang unik telah dihargai oleh banyak kebudayaan and ia telah digunakan secara meluas di dalam upacara keagamaan, perubatan, setanggi, wangian dan barangan pembersih. Pada masa ini, kaedah yang digunakan untuk pengekstrakan minyak pati gaharu adalah dengan menggunakan penyulingan air. Tetapi, kaedah ini tidak efisien kerana ia menghasilkan hasil minyak yang sedikit serta masa pengekstrakan yang panjang sekaligus meningkatkan kos penghasilan. Untuk mengatasi masalah tersebut, kajian ini akan dijalankan untuk meningkatkan kaedah asal pengekstrakan dengan menggunakan penyulingan wap dibantu *"ultrasonic"*. Parameter yang terlibat dalam kajian ini adalah masa pra-rawatan dan kadar kayu gaharu per isipadu air dan kedua-duanya dimanipulasi untuk mendapat hasil minyak pati yang banyak dengan pengekstrakan minyak yang optimum.dan maksimum. Keputusan yang dijangkakan dari kajian ini adalah hasil minyak pati gaharu meningkat dengan peningkatan masa pra-rawatan serta kadar kadar kayu gaharu per isipadu air sehingga ia mencapai keadaan di mana hasil minyak menjadi tetap. Masa prarawatan yang terbaik diperoleh pada 9 jam dengan menghasilkan 0.1276% pengeluaran minyak dan kadar kayu gaharu per isipadu air terbaik adalah pada nisbah 1:20 yang menghasilkan 0.1295% pengeluaran minyak. Adalah diharapkan bahawa penyulingan wap dibantu "*ultrasonic*" akan memperbaik kaedah pada masa ini untuk mengekstrak minyak pati dengan memperoleh hasil minyak yang tinggi dan menjimatkan kos penghasilan.

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CHAPTER 1

INTRODUCTION

1.1 Background of Research

Gaharu is the resinous, fragrant and highly valuable heartwood produced by *Aquilaria malaccensis* and other species of the Indomalesian tree genus *Aquilaria*, from the family of Thymelaeceae. There are fifteen species in the *Aquilaria g*enus and eight are known to produce gaharu. In Malaysia, gaharu is primarily produced from *A.malaccensis, A.hirta, A.microcarpa, A.rostrata* and *A.beccariana* (Chang *et al.*, 2002) and they are large evergreen trees growing over 15-30 m tall and 1.5-2.5 m in diameter, and has white flowers (Chakrabarty *et al.*, 1994). The taxonomy of gaharu tree is shown below in Table 1.1.

Table 1.1 : The taxonomy of gaharu tree

KINGDOM	Plantae
PHYLUM	Tracheophyta
CLASS	Magnoliopsida
ORDER	Myrtales
FAMILY	Thymelaeaceae
GENUS	*Aquilaria*
SPECIES	*malaccensis*
SPECIES AUTHORITY	Lamk.

(Source: International Union for Conservation of Nature and Natural Resources)

Gaharu is known under many names in different cultures: Gaharu in Malaysia and Indonesia, Chen-xiang in Chinese and Jin-koh in Japanese that both literally mean “sinking incense” due to its high density of oleoresin, Oudh in Middle East, Agarwood, Aloeswood and Eaglewood.

Figure 1.1 *Aquilaria malaccensis* Plantation in Thailand
(Source: Robert A. Blanchette)

The formation of gaharu is a natural process in response to a parasitic ascomycetous mould, *Phialophora parasitica*, a dematiaceous (dark-walled) fungus attacks (Gibson, 1977 cited in Ng *et al.*, 1997) and the aromatic resin is usually formed in the bark and the roots as well as the heartwood of the trees (Jalaluddin, 1977 cited in Donovan and Puri, 2004). As a response, the tree produces a resin high in volatile organic compounds that aids in suppressing or retarding fungal growth. While the unaffected wood of the tree is relatively light in colour, the resin dramatically increases the mass and density of the affected wood, changing its colour from pale beige to dark brown or black (Rahman and Basak, 1980 cited in Blanchette and van Beek, 2005). The uninfected gaharu has no scented value. In natural forest only 10% of the trees are infected by the fungus. A common method in artificial forestry is to inoculate, a forced method where the trees are deliberately wounded, leaving them more susceptible to a fungal attack.

Gaharu has been known to possess medicinal properties as well. Its use as a medicinal product has been recorded in the *Al-Hadith Al-Sahih Muslim,* which dates back to approximately the eighth century, and in the Ayurvedic medicinal text the *Susruta Samhita*. It is used in Ayurvedic, Tibetan and traditional East Asian medical practices (Barden *et al.,* 2000) as an alternative to modern pharmaceutical products.

The high value of gaharu makes it become the good choice for collection and investment. The larger piece of some gaharu which possesses special artistic shape or natural carving is precious and its value is exceptional.

The odour of gaharu is complex and pleasing, with few or no similar analogues. Due to its aromatic properties, gaharu has been widely used for thousand of years in Middle East, China, Japan, India and Indochina, notably in religious purposes by Buddhists, Hindus and Muslims either in the form of essential oil or incense sticks.

Its distinctive fragrance has been valued in many cultures, thus it is used by direct burning the chips or pieces of gaharu, slow-burning incense sticks and perfumes. Moreover, its use as a perfume has been recorded in the *Old Testament.* In East Asia, Indochina and Tibet, gaharu is used extensively by the monks as an aphrodisiac and calming of minds.

Majority of genuine essential oils are extracted by distillation method. The production of gaharu essential oil has become the cottage industry in East of Peninsular Malaysia and also traditionally distilled by the indigenous people mainly by using water distillation or hydrodistillation. It is the oldest form of essential oil extraction and is believed by many to be the only way oils should be extracted.

Nowadays, the research on gaharu and the extraction of essential oil have been done actively by research institutes and local universities in order to find the best method that yield the maximum essential oils with best quality and high value with energy and costs savings. In Malaysia, the scientific works have been done by

Forest Research Institute of Malaysia (FRIM) and several public higher learning institutions.

1.2 Problem Statement

Currently, the method of extracting gaharu essential oil is using traditional water distillation method (Chang *et al.*, 2002) or hydro distillation. This method involves submerging the desired raw material (gaharu chips or powders) in water in the still and brought to boil, the oil that evaporates is lost in the water in the still as well as in the aqueous phase of the distillate. The residual oil dissolved in the water usually causes odour nuisance when it degrades and is also a waste of the valuable product in the water stream (Masango, 2005).

Besides, this extraction method acquires long extraction times that consume a lot of fuel for heating purposes. The extraction process did not produce the maximum yield of oil because the efficiency of the method itself is relatively low. All this will result in higher operating cost especially for heating process (A. Fadzli, 2006).

Extraction process using ultrasonic assisted steam distillation method may contribute to improve the efficiency and capacity of gaharu essential oil extraction. This approach will be applied in this research to examine the feasibility of ultrasonic assisted steam distillation as an improved method for gaharu essential oil extraction process.

1.3 Objective of Research

To examine the feasibility of ultrasonic assisted steam distillation as an improved method for gaharu essential oil extraction process.

1.4 Scopes of Research

In order to achieve the objective, these following scopes have been identified and to be applied:

i. To study the effect of ultrasonic pre-treatment time on the gaharu essential oil yield.
ii. The study the effect of solid to solvent ratio on the gaharu essential oil yield.

CHAPTER 2

LITERATURE REVIEW

2.1 Gaharu

2.1.1 Overview of Gaharu

Aquilaria spp. (Thymelaeaceae) are the principal source of Gaharu (Soehartono and Newton, 2001), a resin-impregnated heartwood that is fragrant and highly valuable. Other names used by both collectors and traders of the fragrant wood are agar, aloeswood, eaglewood, kalambak or gaharu depending on the country and generally encompass the fragrant wood produced by most species of *Aquilaria* (Ng *et al.*, 1997).

In Malaysia, the tree of *Aquilaria* is called karas and its fragrant wood is known as gaharu. Five species of *Aquilaria* are recorded for Peninsular Malaysia and all are believed to be able to produce oleoresins. The most popular species generally associated with gaharu is *A.malaccensis* (Chang *et al.*, 2002).

A.malaccensis is widely distributed in south and south-east Asia. According to Oldfield *et al.* (1998), *A.malaccensis* is found in 10 countries: Bangladesh, Bhutan, India, Indonesia, Iran, Malaysia, Myanmar, Philippines, Singapore and Thailand. *Aquilaria* species have adapted to live in various habitats, including those that are rocky, sandy or calcareous, well-drained slopes and ridges and land near

swamps. They typically grow between altitudes of 0-850 m, in locations with average daily temperatures of 20-22 °C (Wiriadinata, 1995).

A.malaccensis is distributed throughout Peninsular Malaysia, except in the states of Perlis and Kedah (Whitmore, 1990), and is known to produce medium-quality grade gaharu (Burkill, 1966). Gaharu is frequently found as irregular patches or streaks in the wood of about 20 years old trees. Very often, the quantity and quality of gaharu produced increase with age, with the best yields occurring in trees aged 50 and above (Sadgopal, 1960).

2.1.2 Formation of Gaharu Resin

Research conducted so far has focused mainly on the following three hypotheses exist regarding gaharu formation, namely that it is the result of diseased or pathological, wounding/ pathological and/ or non-pathological processes (Ng *et al.*, 1997). The pathological condition was first hypothesized by fungal infections that lead to diseased wood. Moreover, the wounding/ pathological condition considered that wounding has a primary effect on gaharu formation with fungal infection as a secondary influence (Gibson, 1977; Rahman and Basak, 1980). Ng *et al.* (1997) suggested the non-pathological condition is a defensive response of the tree towards wounding, therefore release the gaharu resin.

The infection of fungi occurs when branch and stem injured by larvae of mainly a parasitic ascomycetous mould, *Phialophora parasitica*, a dematiaceous (dark-walled) fungus (Gibson, 1977 cited in Ng *et al.*, 1997). It is seen that the larvae of *P. parasitica* bore the standing tree trunk of *A.malaccensis* and make tunnels inside the tree trunks. Fungus enters the plant through this vertical hollow sometimes-zigzag tunnel inside the stem, which serves the initial sites of infections. Later on infections spread an all sides slowly and gradually and ultimately a larger wood volume gets infected. More insect infestation in the infected area more is the chances to form gaharu in 7-8 years time after infection. Gaharu formation is the resinification of accumulated oleoresin due to the action of microorganisms. Figure 2.1 shows the scanning electron microscopy of a cross section of decayed tree which

contain abundant amounts of resin formed in the wood cells. *Aquilaria* has an unusual anatomy and specialized cells within the xylem produce the gaharu resin.

Infections may also occur due to mechanical or natural injuries on the stem, for example following wind or storm damage. Due to infections, oleoresins are accumulated in the infected wood and later become odoriferous. At the initial stage infections appear as brown streaks in the tissue. Accumulation of oleoresins goes on increasing with the increase of infection rate as well as aging of the infection. As more of oleoresins are deposited the intensity of colour of the infected wood increases and finally it becomes black due to increase in concentration. Figure 2.2 shows the cross section cut of the tree showing dark regions of gaharu formed in the heartwood.

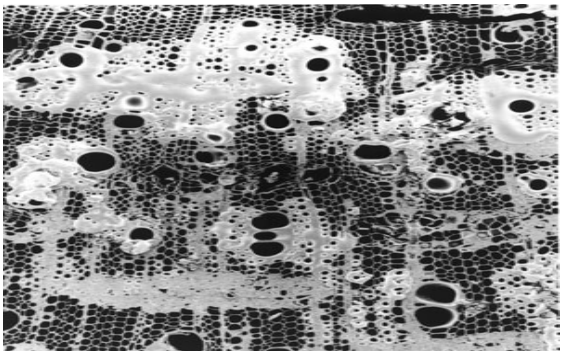


Figure 2.1 Abundant amounts of resin formed in the wood cells
(Source: Robert A. Blanchette)

The fungal infection takes long time to mature and trees about 50 years old have the highest concentration between 2.5-5.0 kilogram/ tree. Furthermore, other factors such as environmental variation, within-tree seasonal variation in responsiveness and the possible existence of two or more varieties or genetic strains in *Aquilaria spp*. may also play an important role in the formation of gaharu (Ng ***et al.***, 1997).

Figure 2.2 Cross section cut shows gaharu formed in the heartwood
(Source: Robert A. Blanchette)

However, the Tropical Rainforest Project (TRP) in Vietnam has found that *Aquilaria malaccensis* can be artificially induced to yield gaharu at a ten times faster than in natural formation (Barden *et al.*, 2003). Figure 2.3 shows the artificial wounding made to the trunk of *Aquilaria* tree in order to generate more gaharu resin production.

Figure 2.3 Artificial wounding to generate the production of gaharu resin
(Source: Yip and Lai, 2005)

2.1.3 Harvesting of Gaharu

Since gaharu is located in the heartwood of the *Aquilaria spp.*, its detection from outer appearance is not easy. Generally, such trees are distinguished by certain

external features whether or not the tree harbours precious gaharu oil or gaharu deposits. These include:

i. a poor crown, decayed branches, and uneven bole;
ii. swelling or depressions and cankers on the bole;
iii. the appearance of hordes of ants in the fissures;
iv. a distinctly yellowish to brownish trace in the wood under the outer bark; and
v. signs of ill-health particularly a die-back symptom of the top and outer branches and a yellow tint to the woody tissues.

The visible wounds, cankers on the bole, stem distortions, smaller leaves and the rotten branches provide evidences of gaharu deposits within a tree. Wood assumes distinctly yellowish trace when gaharu formation takes place. The normal wood in the healthy trees is of pale brown-beige colour. The change can be observed by removing the bark of the tree. Sometimes screw augers are driven inside at various depth and samples are drawn for examination. Finally the odour on the examination by drawing samples with the help of screw augers. The disease or the fungal infection usually takes some time to make it manifest, hence gaharu is hardly found in young shrubs (Anonymus, Hand Book On Medicinal & Aromatic Plants).

In Peninsular Malaysia, Orang Asli is the traditional harvester of gaharu. Over in East Malaysia, Penans are the traditional gatherers of gaharu. Gaharu harvesting is a destructive process; however they will not fell a tree unless he is certain of the presence of good quality gaharu in the tree. Slash marks on standing karas trees made by the harvesters to check for the presence of gaharu (Hansen, 2000 cited in Chang *et al.*, 2002).

Dayak communities in Indonesia believe that dying seedlings and saplings (indicated by yellowish leaves) testify to infection of the mother tree. They appear to be able to identify infected trees by differentiating between the sound made by knocking on the infected trunks and the sound made by knocking on non-infected trunks (Soehartono and Mardiastuti, 1997).

2.1.4 Grading and Pricing of Gaharu

There are five major criteria to grade the gaharu which are the buoyancy, fragrance and medicinal property, colour and reasons of forming (http://www.jdcorp.com). The detailed descriptions as shown as below:

1. Buoyancy

The most important and objective criterion to grade the genuine *Aquilaria* is to measure its buoyancy. *Materia Medica* and *Description of the Plants in Southern China* stated that there are three grades for *A.agallocha* according to its buoyancy whether they are complete sinking, half sinking (floating under the surface of water), and floating kinds.

The density of *Aquilaria* tree without oleoresin is a mere 0.4. However, when the percentage of containing oleoresin is over 25%, gaharu in any forms (chips, powders, or larger pieces) will sink into water. The Chinese name of gaharu is originated from this characteristic.

In Japan and Korea, only *Aquilaria* that contains more than 25% of the oleoresin can be used as medicine. This regulation is based on the fact that the only sinking *Aquilaria* can be used as medicine. In China, 15% is the minimum requirement.

2. Fragrance and Medicinal Potential

The characteristics of Aquilaria are acrid, bitter and warm. These characteristics can also apply its medicinal potency and fragrance. One important characteristic of gaharu is that it does not have noticeable fragrance before burning.

Different from most raw materials, gaharu possesses strong ‘natural’ antibiotic function. The higher the grade of gaharu, the more effective and ‘warmer’ the curing process, and the warmer and the richer flavor it has.

Generally, gaharu with different colors has different fragrance. However, grading gaharu by its fragrance is quite difficult as individual preferences of fragrance differ. Therefore, judging grade simply by its fragrance inevitably will result in subjective judgment.

3. Colour

Gaharu oleoresin presents in several different colors. Examining the color of gaharu should be done under natural sun light. There were various researches about the color of gaharu.

There are five colour grading for gaharu. The highest grade is Green; Dark Green comes second, and then Golden (light yellow), Yellow and Black.

It's generally believed that the color gaharu oleoresin is black. In reality, gaharu containing higher percentage of oleoresin usually shows green or dark green luster.

4. Gaharu’s reason of formation

There are four reasons of gaharu oleoresin's formation shows in most studies. Different formation results in differences on colors, fragrances, and containing of gaharu resin.

The best gaharu is formed naturally inside the live *Aquilaria* without any wounds. The second grade gaharu is formed after the tree is died. The third

grade is caused by man-made wounds by cutting on the surface of *Aquilaria* tree. The forth grade is formed by the wounds caused by a kind of insect.

Even though there are four major criterions as mention above, the grading system for gaharu is always a subjective and complicated process, the allocation of grades varies from countries and from buyers and traders. Irregular shapes with angled features fetch more attention than regular shaped pieces because of the greater ease of lighting them to burn. Chips a few centimeters long are likely to fetch a higher price per kilogram compared with larger pieces, even though their colour and odour may indicate a lower grade.

Burning gaharu chips is one indication of resin content. Brown chips burn with strong flame while the more desirable black gaharu burns for a shorter time before the flame dies and incense from the smoke is produced for an extended period of time. Burning can be used to identify Phaleria as the smoke smells bitter and unpleasant. Another way of separating high grade from low grade is to place the wood in water to determine whether it floats or sinks. High grade gaharu will sink and low grade gaharu floats (http://www.forestpathology.coafes.umn.edu).

In Papua New Guinea, grading of gaharu is based on color, shape, and density of the wood. At present, there are five grades; Super A, A, B, C and D. According to Chang ***et al.***, 2002, prices of gaharu could range from 60 sen per kilogram for the low and mixed grades to more than RM 2000 per kilogram for the high grade. Table 2.1 shows the grade the grade of gaharu with value per kilogram.

Table 2.1 The grade of gaharu with the value per kilogram

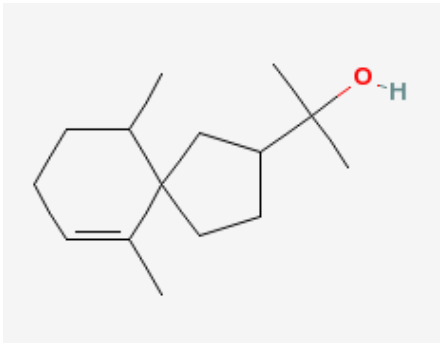
Grade of Gaharu	Value (USD/kg)
Super A	560
A Grade	420
B Grade	280
C Grade	140
D Grade	14

(Source: http://www.forestpathology.coafes.umn.edu)

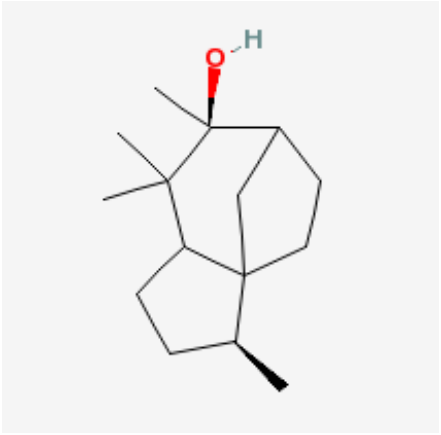
2.1.5 Chemical Compositions of Gaharu

The first investigation on the chemical components of gaharu was reported by Kafuku and Ichikawa in 1935 (Ng *et al.*, 1997). Important chemical components and the number of components that contribute the characteristics of gaharu aroma will determined the quality of gaharu essential oil in all samples for every process of extraction. According to Chang *et* al. (2002), gaharu oils are generally mixtures of sesquiterpenes, sesquiterpene alcohols, oxygenated compounds, chromone derivatives and resins.

Some of the more important compounds are agarospirol, jinkohol-eremol, jinkohol and kusenol that may contribute to the characteristic aroma of gaharu (Ishihara *et* al., 1993). Chang et al. (2002) reported that several chemical compounds such as agarospirol, guaiene, jinkohol, and jinkohol II have been detected in Malaysian gaharu oil. Figure 2.4, shows the major components structure of agarospirol, jinkohol , kusenol and jinkoh-eremol. Ng *et al.*, (1996) cited that 2-[2-(4'-methoxyphenyl)ethyl] chromone and 2-(2-phenylethyl) chromone or flidersiachromone, through pyrolysis at 150°C produces 4-methoxybenzaldehyde and benzaldehyde respectively (Hashimoto *et al.*, 1985). These molecules are odorless at room temperature but produce a long lasting fragrance upon burning.



(a)



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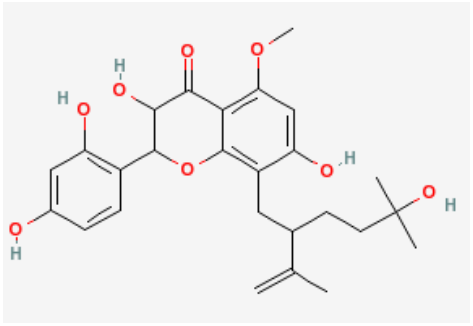
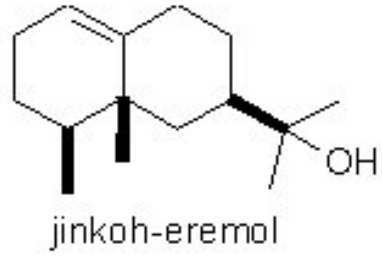


(c)



(d)

Figure 2.4 Major chemical component structures in the gaharu which are (a) agarospirol (b) jinkohol (c) kusenol (d) jinkoh-eremol
(Source: http://www.pubchem.com)

2.2 Essential Oil

2.2.1 Overview of Essential Oil

The International Organization of Standardization (ISO) defines essential oil as “products obtained from natural raw materials by distillation with water or steam or from the epicarp of citrus fruits by a mechanical process or by dry distillation. The essential oil is subsequently separated from the aqueous phase by physical means”.

Commonly, essential oils, or ‘essences’ as they are also called, are highly concentrated substances extracted from various parts of odoriferous plants and trees (heartwood, bark, leaves, stems, flowers, stigmas, reproductive parts and rhizomes) at concentrations ranging from thousandths of a percent to one or several percent. They are also produced by the distillation of oleoresins and absolutes. Essential oils are products of the secondary metabolism of plants, and generally are highly volatile materials. They evaporate on contact with air unlike ordinary vegetable oils, such as corn and olive oils.

Oil is often contained in specialised secretory structures which include secretory cells, ducts, cavities and glandular trichomes. The yield of essential oils from seeds can often be high - in the several tens of percentage - but for the majority of other materials, the main range is 0.1% to 1%.

Note that essential oils from different parts of the same plant may have completely different scents and properties. Although known as 'oils' these compounds are chemically completely unrelated to fatty oils such as olive oil. The chemistry of essential oils is very complicated. Chemically they belong to the huge family of terpenes, which are ubiquitous in the plant world. Terpenes are very complex chemicals and some form enormously long chained molecules. Essential oils tend to consist of rather shorter sequences known as monoterpenes and sesquiterpenes or ring-like structures called 'benzene rings'.

2.2.2 Physical Properties of Essential Oil

Each essential oil has its own blueprint that is absolutely unique. The combination of the plants blueprints, the energy of the sun, soil, air and water gives each of the oil its individual viscosity, colour, clarity, odour and beneficial healing properties. The same species of plant can produce an essential oil with different properties depending on whether it was grown in dry or damp earth, at high or low altitude, or even in hot or cold climate.

During the distillation process, the essential oil can be continually separated off usually by gravity in a purpose-built separating vessel (traditionally a Florentine flask) which can be modified to isolate oils lighter or heavier than water.

Essential oils are very sensitive to heat and light. Both factors can damage the quality of the valuable essential oils in terms of decolourisation and losing its odour. To prevent that from happen, it should be stored in a medium ambient temperature and in dark bottles.

Besides, they are highly volatile and will evaporate when on contact with air. Therefore a small amount of oil in large container should be transferred in to a small bottle to minimize the air exposure. For this reason single oil can help a wide variety of disorders. Lavender, for instance, is endowed with antiseptic, antibacterial, antibiotic, antidepressant, analgesic, decongestant and sedative properties. Moreover, due to their tiny molecular structure, essential oils applied to the skin can be absorbed into the bloodstream.

They also reach the blood as a result of the aromatic molecules being inhaled. In the lungs, they pass through the tiny air sacs to the surrounding blood capillaries by the process of diffusion. Once in the bloodstream the aromatic molecules interact with the body's chemistry.

2.3 Separation Process

Many chemical process materials and biological substances occur as mixtures of different components in the gas, liquid, or solid phase. In order to separate or remove one or more of the components from its original mixture, it must be contacted with another phase. The two phases are brought into more or less intimate contact with each other so that a solute or solutes can diffuse from one to the other. The two-phase pair can be gas-liquid, gas-solid, liquid-liquid, or liquid-solid. During the contact of the two phases the components of the original mixtures redistribute

themselves between the two phases. The phases are then separated by simple physical methods. By choosing the proper conditions and phases, one phase is enriched while the other is depleted in one or more components (Geankoplis, 2003).

Separation process is defined as a process that transforms a mixture of substances into two or more compositionally-distinct products. It is also defined as any sets of operations that separate of two or more components into two or more products that differ in composition. Separation is attained by exploiting the differences between chemical and physical properties of the substances through the use of separating agent (mass or energy). There are a few types of separation process:

1. Absorption

When the two contacting phases are a gas and liquid, this operation is called absorption. A solute or several solutes are absorbed from the as into the liquid phase in absorption.

2. Distillation

In the distillation process, a volatile vapor phase and a liquid phase that vaporizes are involved.

3. Liquid-liquid extraction

When the two phases are liquids, where a solute or solutes are removed from one liquid phase to another liquid phase, the process is called liquid-liquid extraction.

4. Leaching

If a fluid is being used to extract a solute from a solid, the process is called leaching. Sometimes this process is also called extraction.

5. Membrane processing

Separation of molecules by the use of membranes is a relatively new separation process and becoming more important. The relatively thin, solid membrane controls the rate of movement of molecules between two phases.

6. Crystallization

Solute components soluble in a solution can be removed from a solution by adjusting the conditions, such as temperature ar concentrations, so that the solubility of one or more of the components is exceeded and they crystallize out a solid phase.

7. Adsorption

In an adsorption process, one or more components of a liquid or gas stream are adsorbed on the surface or in the pores of a solid adsorbent and a separation are obtained.

8. Ion exchange

In an ion exchange process, certain ions are removed by an ion-exchange solid. This separation process closely resembles adsorption.

Separation process is done for its own function. There are three primary functions of separation processes:

1. Purification

It is used to remove undesired components in a feed mixture from the desired species.

2. Concentration

It is used to obtain a higher proportion of desired components that are initially dilute in a feed stream.

3. Fractionation

Fractionation is a separation process in which a certain quantity of a mixture (solid, liquid, solute or suspension) is divided up in a large number of smaller quantities (fractions) in which the changes according to a gradient.

The analysis of separation processes are divided into two fundamental categories:

i. Equilibrium-based processes
ii. Rate-based processes

For equilibrium-based processes, the degree of separation process in each stage is governed by a thermodynamic equilibrium relationship between the phases. Examples of separation processes in this category are:

i. Distillation
ii. Extraction and leaching

In distillation, the liquid is partially vaporized to create another phase, which is a vapor. The separation of the components depends on the relative vapor pressures of the substances. In distillation also, a different temperature at each stage alters the vapor phase equilibrium between typically binary mixtures.

The desire of a new equilibrium between the two phases at the temperature of each stage is the driving force for separation. The end result is the separation of two liquids with different boiling point temperatures.

Extraction is a process where a species is removed from a liquid in which it is dissolved by means of another liquid for which it has higher affinity. While for leaching, a species is removed from a solid phase by means of another liquid for which it has a stronger affinity.

Rate-based processes are mainly about the limited of the processes by the rate of mass transfer of individual components from one phase into another under the influence of physical stimuli (such as concentration, temperature, pressure, external force). Under this category, there are a few types of processes:

i. Gas absorption
ii. Desorption or stripping
iii. Adsorption
iv. Ion exchange
v. Membrane separations

2.3.1 Extraction

An extraction is the process to remove one or more compounds from a liquid (usually water) by transferring the solute into a second liquid phase which is immiscible and usually organic, for which the solute has higher affinity (Noble and Terry, 2004). Other than that, it can involve the selective transfer from solid to a liquid. The former process is called a liquid-liquid extraction and the latter is called a

liquid-solid extraction. More recently, with the development of reactive solid phases, materials are extracted from liquids by a solid. This is called solid phase extraction. This type of separation process depends on the differences in both solute solubility and density of the two phases.

In the process, there will be the advantages and disadvantages. One of the advantages is extraction can be performed at ambient temperature. Thus, it is relatively energy efficient and can be applied to separations involving thermally unstable molecules.

2.3.2 Distillation

Distillation is one of the separation processes. Distillation is defined as a process in which a liquid or vapor mixture of two or more substances is separated into its component fractions of desired purity, by the application and removal of heat. Besides that, extraction processes can accommodate changes in flow rates and the solvent can be recovered and recycled for reuse. It offers greater flexibility in terms of operating conditions too, since the type, amount of solvent and operating temperature can be varied.

On the other hand, one of the disadvantages is, in this process, the solvent must be recovered for reuse (usually by distillation), and the combined operation is more complicated and often more expensive than ordinary distillation without extraction (McCabe, Smith & Harriott, 2001).

2.3.3 Availability of Extraction Methods

There are various methods to extract the essential oils from the botanical materials. The availability of the extraction methods used is mainly depends on the

suitability of the raw materials to be extracted. Some method only work best with soft part of material such as flowers, leaves and stigmas while other suit best with harder part such as woods, heartwood, stems and roots of the plant.

Currently, the most popular method used to extract essential oils is by using hydro distillation. It is the most ancient method and still be used even though it is very time and cost consuming. New technologies have been developed to improvise the yield and quality of essential oil produced. Beside that, the most economical and cleaner production method is the next requirement in choosing the best extraction method in order not to pollute the environment. For example, extraction can be done using steam or water distillation as only water is used as the solvent instead of using chemical solvent extraction that will release to the surroundings.

The integration of several methods also can improve the yield of essential oil. For example by using microwave assisted steam distillation and ultrasonic extraction. The advantage of using these methods are the extraction time will be reduced and give higher yield. Besides, several pre-treatment have been developed in order to optimize the essential oils production.

2.3.3.1 Steam Distillation

Steam distillation is a special type of distillation or separation process for temperature sensitive materials like natural aromatic compounds. The majority of essential oils available in the market are extracted using this kind of method. The fundamental nature of steam distillation is that it enables a compound or mixture of compounds to be distilled and subsequently recovered at a temperature substantially below that of the boiling points of the individual constituents.

Essential oils contain substances with boiling points up to 200°C or higher, including some that are solids at normal temperatures. In the presence of steam or boiling water, however, these substances are volatilized at a temperature close to 100°C at atmospheric pressure. The process is quite simple and as long as the extraction process is closely monitored, the steam will remain at a temperature and pressure that will not damage the plant material.

The steam distillation process for the extraction of essential oils from plant materials consists of three basic parts which are the still, the condenser and the separator. Figure 2.5 shows the overview of the three main components.

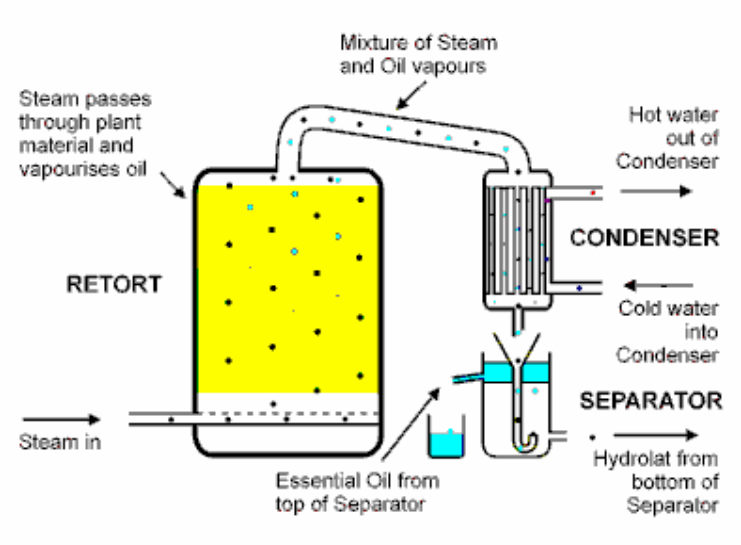


Figure 2.5 The three main components in steam distillation
(Source: http://www.distillation.co.uk)

First, the desired plant material is placed onto a still. A still is a specialized piece of equipment that is used in the distillation process. It consists of a vessel into which high pressure steam is passed in at the base into a void beneath a perforated grid which supports the plant materials. The heat from the steam helps to open the pockets of the plant that contain the aromatic molecules or oils of the plant material. Once open, it releases these aromatic molecules and at this state, the odoriferous molecules are able to rise along with the steam. Figure 2.6 below shows the inner side of the still in which the plant material is located on a perforated grid.

The vapors carrying these molecules travel within a closed system towards the cooling device or condenser. Cold water is used to cool the vapors. As they cool, they condense and transform into a liquid state.

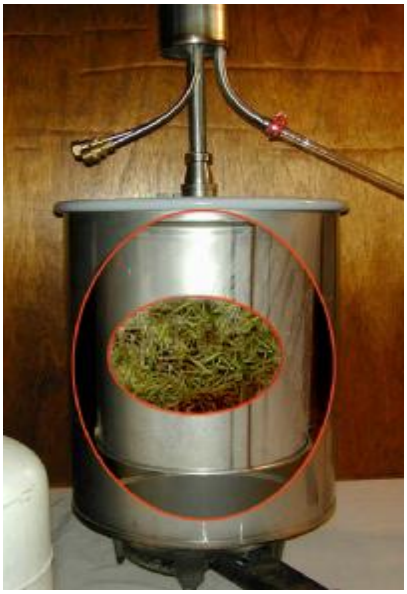


Figure 2.6 An overview of the inner side of the still
(Source: http://www.heartmagic.com)

The liquid is collected in a container and as with any type of oil/water mixture, it comprise two distinct layers. The oils float towards the top while the water settles below. From there, it is a simple matter of removing the oils that have been separated. These are the highly condensed, aromatic oils desired product. Figure 2.7 shows the receiver with two distinct layers of essential oil and hydrosol.

However, the water is not discarded. The water, which also contains the plant's aroma along with the other parts of the plant that are water soluble, are the hydrosols - a milder form of the essential oils. The hydrosols have market value and can be sold as floral water and used in aromatherapy industry. When steam is used, it is created at a pressure higher than that of the atmosphere. The boiling point is above 100 degrees Celsius and creates an extraction process that is safe and fast. If the temperature is allowed to become too hot, however, the botanical material as well as its essential oils can easily become damaged. The use of steam generated within the vessel requires that the leaf be supported above some boiling water by a grid. The water is heated either directly using a fire or by heat exchanger coils. The simplicity of the method makes it suitable for small-scale distillation of essential oils.

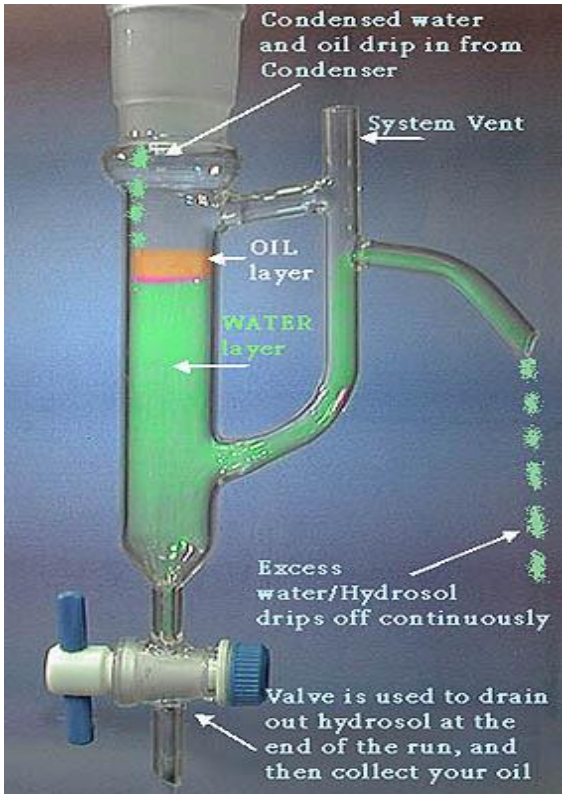


Figure 2.7 Two distinct layers of essential oil and hydrosol exist in the receiver (Source: http://www.scientific-glass.com)

The steam that is used for the distillation is generated either within the steel vessel that contains the plant material (by boiling water contained at the base) or by an external boiler. The use of steam generated within the vessel requires that the leaf be supported above some boiling water by a grid. The water is heated either directly using a fire or by heat exchanger coils. The simplicity of the method makes it suitable for small-scale distillation of essential oils.

If steam is generated, instead, by an external boiler it is introduced into the base of the vessel via an open coil, jets or similar device. The advantages of this type of distillation are that it is relatively rapid and capable of greater control by the operator. The vessel can be emptied and recharged quickly and with the immediate reintroduction of steam there is no unnecessary delay in the commencement of the distillation process. Oils produced by this means are more likely to be of acceptable quality than those produced using the more direct method.

The disadvantage about steam distillation this is the best method for distilling leaf or flower materials, but it does not work very well for woods, roots, seeds and barks.

2.3.3.2 Hydro Diffusion

Hydro diffusion is another method to extract the essential oil from plant materials. It is similar to the steam distillation process. The only difference is that instead the heat is supplied from the bottom and up through the still, the heat passes into the still from the top. It is cooled from below, which makes collection of the essential oils a lot easier. This method actually results in a higher yield of essential oils because less steam and consequently less processing time are involved.

However, most people mistaken this method with water distillation. Water distillation is the most ancient method of distillation and the most versatile. It involves placing the desired plant material in a still and then fully immersed in water. The water is then brought to the boil. The heat helps open the pockets containing the plant's aromatic molecules so they can be extracted. The vapors cool and condense the essential oils which are then separated from the water and they are collected in the receiver. Figure 2.8 shows the lab-scale water distillation apparatus to extract essential oil.

The water in this case provides protection for the plant because it acts as a barrier. Less pressure is used as well as a lower temperature than that which is used in the steam distillation method. This extraction method works well with plants that cannot tolerate high heat.

Figure 2.8 Lab-scale water distillation apparatus for essential oil extraction (Source: http://www. aromatherapy-essential-oils.org)

The risk, however, is that the still can run dry, or overheated which burning the aromatic compounds and resulting in an essential oil with a burnt smell. Any botanical material that contains high amounts of esters do not take well to this extraction method, since the extended exposure to hot water will start to break down the esters to the resultant alcohols and carboxylic acids.

2.3.3.3 Microwave-Assisted Extraction

Microwaves are electromagnetic radiations with a frequency from 0.3 to 300 GHz. Domestic and industrial microwaves generally operate at 2.45 GHz, and occasionally at 0.915 GHz in the USA and at 0.896 GHz in Europe. Microwaves are transmitted as waves, which can penetrate biomaterials and interact with polar molecules such as water in the biomaterials to create heat. Consequently, microwaves can heat a whole material to penetration depth simultaneously.

Microwave-assisted extraction (MAE) offers a rapid delivery of energy to a total volume of solvent and solid plant matrix with subsequent heating of the solvent and solid matrix, efficiently and homogeneously. Because water within the plant

matrix absorbs microwave energy, cell disruption is promoted by internal superheating, which facilitates desorption of chemicals from the matrix, improving the recovery of nutraceuticals (Kaufmann, Christen, & Veuthey, 2001). Kratchanova, Pavlova, and Panchev (2004) observed using scanning electron micrographs that microwave pretreatment of fresh orange peels led to destructive changes in the plant tissue. These changes in the plant tissue due to microwave heating gave a considerable increase in the yield of extractable pectin. Furthermore, the migration of dissolved ions increased solvent penetration into the matrix and thus facilitated the release of the chemicals. The effect of microwave energy is thus strongly dependent on the dielectric susceptibility of both the solvent and the solid plant matrix.

There are two types of commercially available MAE systems: closed extraction vessels under controlled pressure and temperature, and focused microwave ovens at atmospheric pressure (Kaufmann & Christen, 2002). The closed MAE system is generally used for extraction under drastic conditions such as high extraction temperature. The pressure in the vessel essentially depends on the volume and the boiling point of the solvents. The focused MAE system can be operated at a maximum temperature determined by the boiling point of the solvents at atmospheric pressure. Ericsson and Colmsjo (2000) introduced a dynamic MAE system, which was demonstrated to yield extract equivalent to yield of extract from Soxhlet extraction, but in a much shorter time.

MAE has been considered as a potential alternative to traditional solid-liquid extraction for the extraction of metabolites from plants. It has been used to extract nutraceuticals for several reasons:

i. reduced extraction time,
ii. reduced solvent usage,
iii. improved extraction yield.

MAE is also comparable to other modern extraction techniques such as supercritical fluid extraction (SFE) due to its process simplicity and low cost. By

considering economical and practical aspects, MAE is a strong novel extraction technique for the extraction of nutraceuticals.

However, compared to SFE, an additional filtration or centrifugation is necessary to remove the solid residue during MAE. Furthermore, the efficiency of microwaves can be very poor when either the target compounds or the solvents are non-polar, or when they are volatile.

2.3.3.4 Solvent Extraction

Another method of extraction used on delicate plants is solvent extraction, which yields a higher amount of essential oils at a lower cost. A hydrocarbon solvent is added to the plant material to help dissolve the essential oil. When the solutions are filtered and concentrated by distillation, a substance containing resin, or a combination of wax and essential oil (concrete) remains. From the concentrate, pure alcohol is used to extract the oil and when the alcohol evaporates, the oils are left behind. This is not considered the best method for extraction as the solvents can leave small amount of residue behind which could cause allergies and affect the immune system

2.3.3.5 Supercritical Carbon Dioxide Extraction

The end result of super critical carbon dioxide extraction - one of the newest extraction technologies - is a super-concentrated, high-quality version of essential oil. This rapid extraction method uses lower temperatures and higher pressure to transform carbon dioxide, a gas, into a liquid. It is an inert solvent meaning that it is non-reactive and therefore cannot form another chemical compound. When the extraction process is complete, the carbon dioxide is returned back to a gaseous state therefore, no residual remains. All that is left is pure essential oil.

Although this technology produces one of the purest forms of essential oil, it is not yet widely used. The equipment needed for this extraction process is very expensive,

which keeps production costs high. Because production costs are high, the costs of the essential oils that are produced via carbon dioxide extraction become expensive.

2.4 Pretreatment Methods for Essential Oil Extraction

In order to enhance the oil yield of essential oil extraction, the pretreatment is highly recommended to apply during early steps before distillation is occurred. The need for pretreatment steps is related to the location of the essential oils within the herbaceous matrix. Essential oils are produced in glandular cells and stored in the subcuticular space formed at the gland apex. These gland develop on the surface of the leaves, bracts, petals, roots, stems, heartwood, and other organs of aromatic plants (Gaspar, Santos & King, 2000).

To disrupt these glands and liberate the entrapped oils, a pre-treatment of the matrix is need to perform before start the extraction process. Pre-treatments applied to herbaceous matrixes are normally restricted to particle size reduction by mechanical means. However, during the mechanical pre-treatments, losses by degradation (oxidation and thermal degradation) and by evaporation of volatiles are observed, leading to a discrepancy between the essential oil composition of the plants and that of the extract.

There are a few pre-treatment techniques already applied in essential oil extraction. The pretreatment usually done by enzymatic pre-treatment and soaking with ultrasonic bath.

2.4.1 Enzymatic Pretreatment

This technique using enzyme derived from bacteria such as cellulase which derived from *Trichoderma reesei*. It main function in the essential oil extraction is to develop micro fracture and disruption of the parenchymatous cell and oil gland

(Chang *et al.*, 2002). Enzyme will react with the plant's cell wall and disrupt the oil glands to release the oil particle. More concentrate the enzyme with the plants material, more oil yield will be obtain as more direct contact between the internal part of the plants (cell wall and oil glands) with the enzyme.

In order to successfully use the enzymatic pre-treatment, the enzyme itself should be in their optimum condition (referring to their origin) with the addition of buffer solution. Usually the optimum temperature for these enzymes is at 50 ± 5℃ and pH 4.5 – 5.0.

2.4.2 Ultrasonic Bath Pretreatment

It is an active enhancement to be applied in distillation process to reduce the time consuming in the reaction and to increase the percentage of yield of gaharu essential oil.

Ultrasonic enhancement in distillation will improve the mixing and chemical reactions in the material plant that will undergo the process. It will generates alternating low pressure and high pressure waves in liquid, leading the formation and violent collapse of small vacuum bubbles that was called cavitations that causes high speed impinging liquid jets and strong hydrodynamic shear-forces. This process will be use for the disintegration of cells or the mixing of reactants.

This active enhancement is use as an alternative to high-speed mixers and agitator bead-mills. Ultrasonic gives a benefited in chemical reactions from free radicals created by cavitations that will leads to a substantial reduction in reaction time.

The enhancement by ultrasonic cavitation is mainly contributed by acoustically excited bubble break-ups on the membrane surface. Those bubble break-

ups result in the hydraulic pressure impulses by the impinging actions similar to the water hammer effect. The hydraulic pressure impulses further lead to a corresponding impulsive elevation of vapor partial pressure in the dissolved vapor phase. As a result, an increase in vapor pressure difference over the membrane is obtained provided that the vapor pressure on the other side of the membrane remains unchanged. Consequently, the vapor permeated through the membrane is increased or enhanced (C. Zhu, 2000).

Figure 2.9 below showed the ultrasonic irradiation for modeling system on the porous membrane for the membrane distillation.

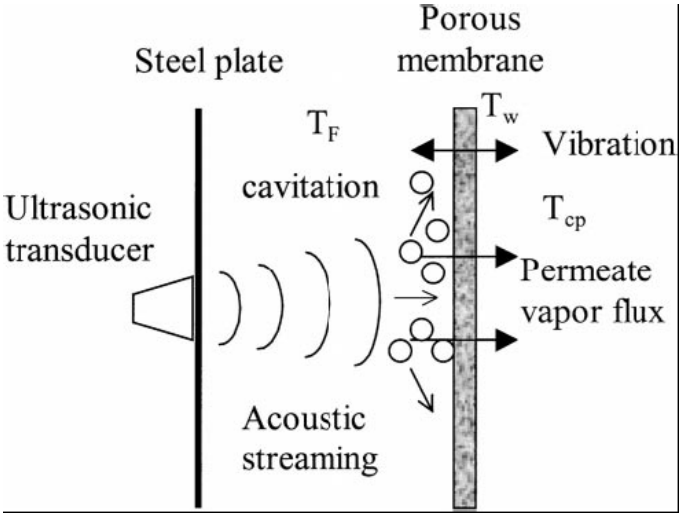


Figure 2.9 Ultrasonic irradiation modelling systems
(Source: Chao Zhu, 2000)

CHAPTER 3

METHODOLOGY OF RESEARCH

3.1 Introduction

In extracting the gaharu essential oil, the best alternative technique is by using steam distillation unit. It is because this technique is effective which produces higher yield of oil (Chang *et* al., 2002) than any conventional and traditional methods. Besides, steam distillation is very efficient as not a single drop of essential oil is waste into the water such in the case of water distillation. This is because the high pressure steam supplied directly in contact with the botanical materials and this lead to open up the oil pouch inside the plant's cell that contain aromatic compounds. Furthermore, only steam is used as the carrier and no solvent is applied in the process. Therefore, steam distillation is the cleaner approaches of extraction method towards the environment as the steam will condensate into water after being cooled.

To extract the essential oil, there are a few steps that must be done to complete the whole procedure. The steps include preparation of gaharu sample, ultrasonic pretreatment, steam distillation extraction process, essential oil recovery and data analysis. To maximize the yield of essential oils, pretreatment of gaharu is applied during soaking process in order to achieve higher amount of oil.

3.2 Sample Preparation

As in the literature, gaharu can be divided into five gades which are Super A, A, B, C and D. The grading system is based on the quality of gaharu. In common, higher grade of gaharu is used as incense while the lesser grade is used to make the essential oil. In this research, grade C gaharu is used to extract the essential oil as at this grade the wood able to produce essential oil. However, it is expected that the oil yield is not as much as predicted because grade C gaharu only contain small amount of resin The plant material is bought from traditional distiller in Gua Musang in the state of Kelantan with the price of RM 30 per kilogram. Figure 3.1 shows the grade C gaharu wood that will be used in this research.

Figure 3.1 Grade C Gaharu Wood

3.2.1 Drying

Drying is defined as the final removal of water, or another solute. It is an important process before grinding process take place. First, the wood is chopped into small pieces in order to increase the surface area of the wood. The main purpose of this procedure is for the convenience of the drying process as small pieces will be easier to dry than the original shape of gaharu. Besides drying will remove the moisture content and to avoid any blockage when grinding. Other than that, it is

crucial to dry the wood to prevent the growth of insects' larvae that might contain in the wood during soaking process.

3.2.2 Grinding

After the drying process is completed, the next step is to grind the dried gaharu wood. The pieces of gaharu wood will be grind into fine sawdust with the particle size of 1 millimeter. The purpose of reducing its size is mainly because the rate of extraction is directly proportional with the contact surface per unit area of plant material. Therefore, the higher the surface area in contact, more gaharu essential oil will be extracted. The grinder model that will be used is "Disk Mill FFC23" as shown below in Figure 3.2.

Figure 3.2 Grinder: Disk Mill FFC23 Type

3.2.3 Sample Weighing

The ground gaharu with 1mm particle size is then been weighed on the analytical balance with the desired amount as in Table 3.1. Later, the gaharu sawdust is then been put into muslin bag (or any loose cloth such as "*kain kasa*") for next procedure. Figure 3.3 shows the ground gaharu sample after being weighed.

Figure 3.3 The ground gaharu sample after being weighed

3.3 Ultrasonic Bath Pretreatment

In order to maximize the yield of essential oil, ultrasonic pretreatment of the gaharu is applied by soaking the wood samples in the ultrasonic bath for a vary period of time as stated in Table 3.1.

The ground sample of gaharu that has been weighed is then been put into a bag made by loose material such as muslin cloth or "*kain kasa*". The purposes of the cloth are for easy penetration of the water and the gaharu sample and also as strainer. During soaking in the ultrasonic bath, the high and low pressure ultrasound waves will disrupt and rupture the cell walls and oil glands that contain aromatic compounds of essential oil. Thus, the used of loose material is crucial to allow the aromatic compound to flow out with the water and not trapped within the cloth.

The pretreatment process is done by leave a bag of 500 grams of gaharu sample with 4000 mL of dionized into an ultrasonic bath. The ratio of solid to solvent is 1:8. The sample is sonicated in ultrasonic bath for pretreatment time that has been set which is 3, 6, 9, and 12 hours. Figure 3.4 shows the ultrasonic bath with gaharu sample during pretreatment.

(a) (b)

Figure 3.4 The ultrasonic pretreatment process (a) ultrasonic bath equipment (b) the gaharu sample inside loose cloth during pretreatment process

Later the mixture is going through a cooling step and the water with traces of essential oil compound is collected and been transferred into round flask for distillation purpose. The gaharu sample in strained by lifted up to drain the excess water. After some water been drained, the gaharu sample is then been put into second round flask for distillation purposes. Then the experiment is repeated with the manipulated variable of solid to solvent ratio of 1:8, 1:12, 1:16, and 1:20 with the time taken during pretreatment is applied using the best time that obtained maximum oil yield percentage in the previous experiment.

3.4 Gaharu Oil Extraction Process

The concept of steam distillation is the same as usual kitchen steamer utensil. First, the gaharu sample is being taken out from the muslin bag and is been put into an upper round flask with the help of muslin cloth at the base of the flask's mouth to avoid the gaharu sample from falling into the lower round flask. The water with traces of oil compounds that are collected from the ultrasonic pretreatment is been put into the lower round flask. Both the lower and upper flasks is installed together and the distillation apparatus such as condenser and separating funnel is been set up. Figure 3.5 shows the steam distillation unit after being installed. The heating mantle is switched on and let the water been boil until the temperature reached 100 °C. When the water starts to vaporize, the time is recorded and let the

vaporized oil and steam being chilled with condenser. The distillate is collected in a separating funnel and collected in a sample bottle. A small amount of hexane is pipette into the separating funnel which it functioned as a medium to separate the oil from water. The distillation process is run for two days. Then the experiment is repeated with the manipulate variable of solid to solvent ratio of 1:8, 1:12, 1:16, and 1:20. The time for distillation process is constant that is two days

Figure 3.5 The steam distillation unit

The summary of the experimental works matrix with those manipulated variables is shown as in Table 3.1 below.

Table 3.1 Experimental matrix for ultrasonic assisted steam distillation

Variable	Sample	Pretreatment Time (h)	Gaharu to Water Ratio	Sample Mass (g)	Water Volume (mL)
Pretreatment Time	T1	3	1 : 8	500	4000
	T2	6	1 : 8	500	4000
	T3	9	1 : 8	500	4000
	T4	12	1 : 8	500	4000
Gaharu to Water Ratio	R1	Optimum Data	1 : 8	500	4000
	R2	Optimum Data	1 : 12	350	4200
	R3	Optimum Data	1 : 16	250	4000
	R4	Optimum Data	1 : 20	200	4000

The high pressure heat will contact with the gaharu sample and in the process, it will crush the plants cells and oil glands and oil vaporized together with the steam. The vaporized steam and oil will pass the condenser and it cools until they become a distinct two layers of immiscible liquids in the separating. Water at room temperature is used as cooling agent.

Later, the separation took place after a day of settling in the separating funnel where the less density high concentration of essential oil will float above the high dense water. The water in the distillate also is known as hydrosol. It contains a very little amount of aromatic compound that soluble in water. Thus, it makes the water have the odor property of the essential oil but in small concentration. The essential oil is decanted, cleaned and dry by using anhydrous sodium sulphate. Later, the essential oils collected must be transfer into a translucent glass bottle and be stored in cool and dark place. This is because direct light and heat will ruin the quality of the essential oils.

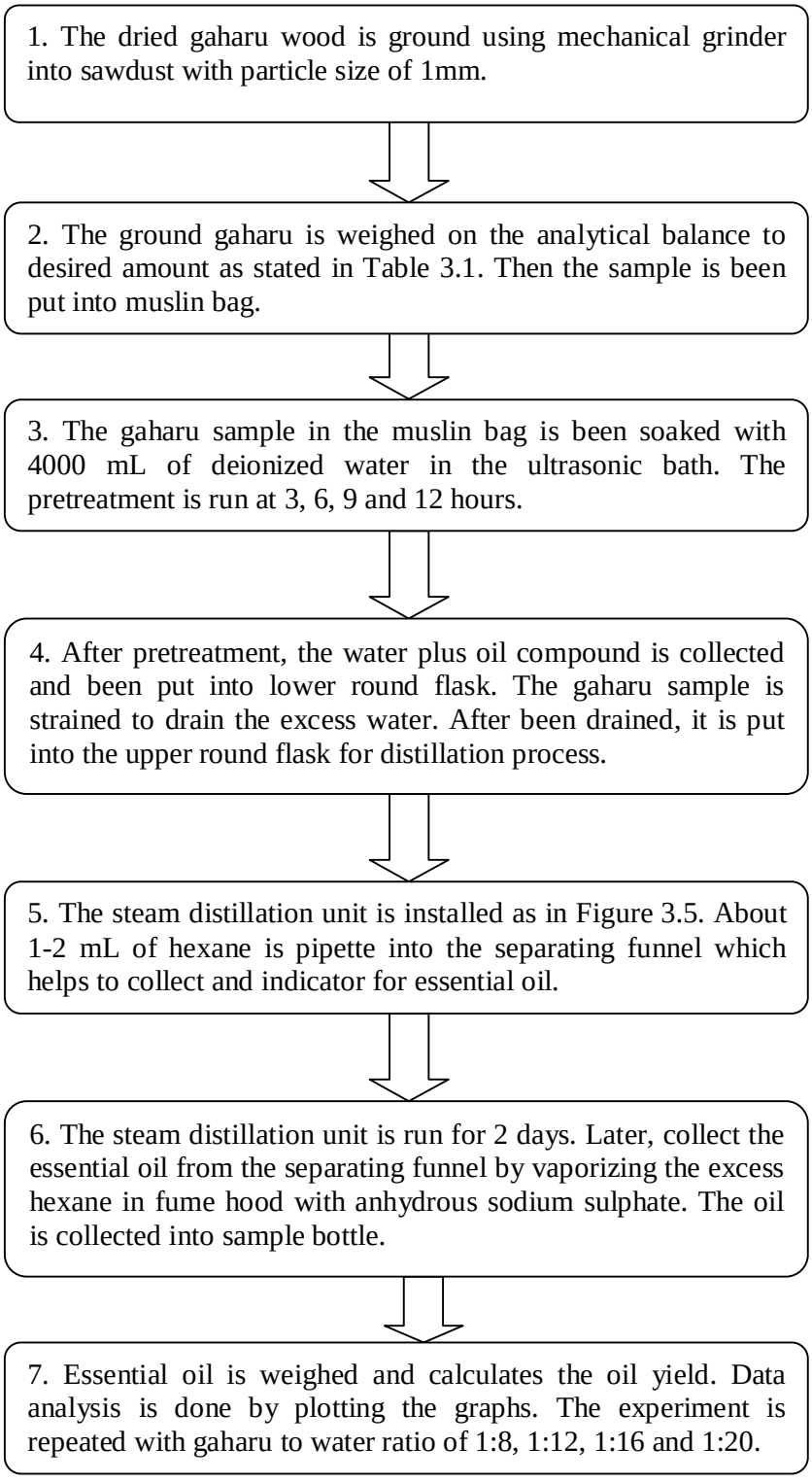
3.5 Data Collecting

For every experiment with manipulated variables (T1, T2, T3, T4, R1, R2, R3 and R4), the yield of essential oil will be collected in every parameters that been studied. The yield of gaharu essential oils extracted will be calculated using equation as shown below.

$$\text{Essential Oil Yield (\%)} = \frac{\text{Amount of Essential Oil Recovered (g)}}{\text{Amount of Gaharu Wood Distilled (g)}} \times 100\%$$

Graph of essential oil yield (g) versus sample collected also can be plotted to show at what condition in term of steam flow rate and temperature that give high yield.

3.6 Flow Method of the Experiment



CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Introduction

In this research, the ultrasonic assisted steam distillation method is developed to study the feasibility of this technique to produce more oil yield than the conventional method of extracting the gaharu essential oil. This method is also to get the alternative method that is more effective and producing a higher yield of essential oil. In the process, the consuming time for the process is expected to be shorter than other extraction method namely hydro distillation which usually takes about 1 week to get the essential oil; the yield that produced is higher because it is improved with the ultrasonic pretreatment. The oil yield will increase along with the increasing time until it reaches a time where its oil yield increment will be constant. It is because the entire sample has been completely extracted. The solid to solvent ratio affecting the oil yield with increasing oil yield due to increase of the solid to solvent ratio. The higher solvent ratio that been used, the higher product obtained (Bulyamin, 2005).

4.2 Results

After conducting the experiments for both manipulated variables which are the pretreatment time and gaharu to water ratio, the results are obtained as in Table 4.1 below and brief data can be found in the Appendix A, B and C.

Table 4.1 Experimental results for (a) effect of pretreatment time (b) effect of gaharu-to-water ratio

(a)

Sample	Pre-treatment Time (h)	Gaharu-to-Water Ratio	Sample Mass (g)	Water Volume (mL)	Mass of Oil Recovered (g)	Essential Oil Yield (%)
T1	3	1:8	500	4000	0.407	0.0814
T2	6	1:8	500	4000	0.511	0.1022
T3	9	1:8	500	4000	0.638	0.1276
T4	12	1:8	500	4000	0.685	0.1370

(b)

Sample	Pre-treatment Time (h)	Gaharu-to-Water Ratio	Sample Mass (g)	Water Volume (mL)	Mass of Oil Recovered (g)	Essential Oil Yield (%)
R1	9	1:8	500	4000	0.638	0.1276
R2	9	1:12	350	4200	0.449	0.1283
R3	9	1:16	250	4000	0.322	0.1288
R4	9	1:20	200	4000	0.259	0.1295

In this study, there two major process of extraction involved; the pretreatment time and gaharu-to-water ratio. Four durations of pretreatment time (3, 6, 9 and 12 hours) an four gaharu-to-water ratio (1:8, 1:12, 1:16 and 1:20) were investigated for both processes. The experiments were performed at 100 °C and room temperature of about 1 atm and the steam distillation is run constantly for 2 days.

4.3 Discussions

Table 4.1 (a) and (b) shows the data collected from extraction processes, with vary pretreatment times and with vary gaharu-to-water ratio. In extraction process with variation of pretreatment times in ultrasonic bath, there are small quantities of essential oil can be recovered in two days of steam distillation. For further detail, the data in Table 4.1 (a) is tabulated into graphic plotting as shown in Figure 4.1 below.

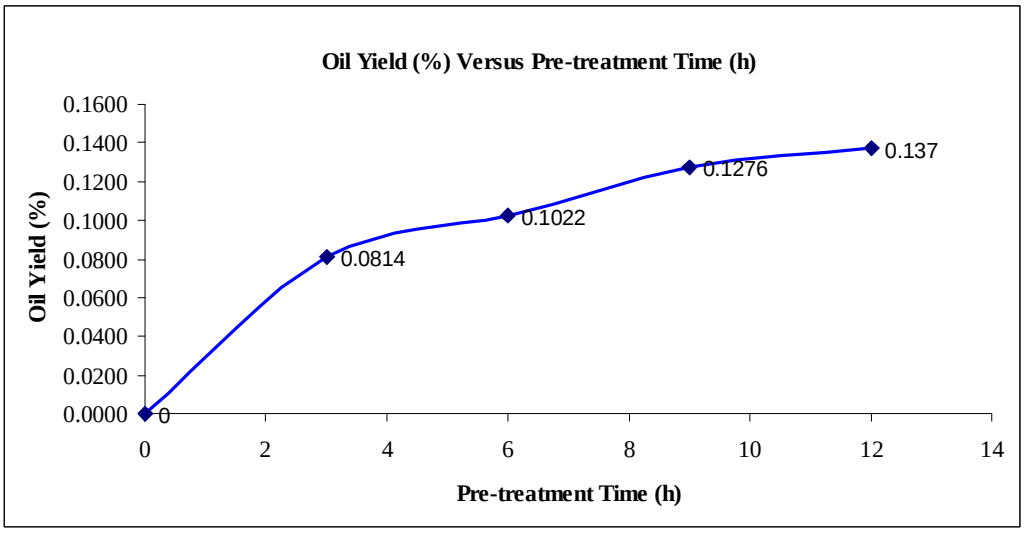


Figure 4.1 Graphical plotting of oil yield percentage versus pretreatment time

As seen in the Figure 4.1, the amount of essential oil extracted is increased with the increment of ultrasonic pretreatment time. The increment of pretreatment time was intended to increase the amount of essential oil due to the reaction of alternating high and low pressure-ultrasound waves that lead to formation and violent collapse of small vacuum bubbles that was also called cavitations. It will disrupt and rupture the cell walls and oil glands of the internal part of gaharu sample and hence the aromatic oil compounds is released and mix together with water during soaking process. The longer the pretreatment time, the higher the chances of oil compound to be released and mix with water.

From the graph, the amount of oil recovered from extraction process is increasing rapidly for the first 3 hours. It is shown from the steep gradient obtained in the plotting. However, this is a normal process of extraction when the oil recovered rapidly in the early period of time as it slowly increased for every 3 hours interval namely at 3, 6, 9 and 12 hours.

The steepest gradient among those 4 periods of time intervals is in between 6 to 9 hours. Therefore, it can be concluded that the optimum value of pretreatment time that yield the largest amount of essential oil yield is at 9 hours

The amount of essential oil yield will keep increasing until it reaches a constant value at a given time. When the time becomes constant, this is an indication that quantitative recoveries were achieved and that extending the pretreatment time is unnecessary.

The optimum ultrasonic pretreatment time obtained from the first part of research is at 9 hours is being applied in the next study of the effect of gaharu-to-water ratio of essential oil extraction. The data for the effect of gaharu-to-water ratio to essential oil yield in Table 4.1 (b) is tabulated into graphical plotting as can be seen in Figure 4.2 below.

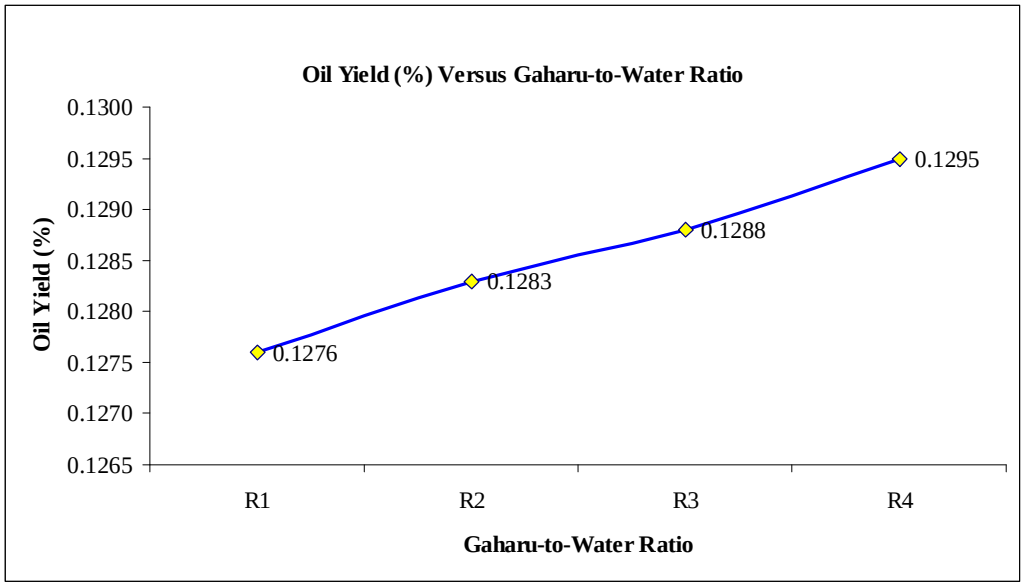


Figure 4.2 Graphical plotting of oil yield percentage versus gaharu-to-water ratio

As seen in the Figure 4.2, the amount of essential oil extracted is also increased with the increment of gaharu-to-water ratio even though the amount difference is very small indeed The increment of gaharu-to-water ratio from R1 (1:8), R2 (1:12), R3 (1:16) and R4 (1:20) was intended to increase the amount of essential oil due to large amount of water per mass gaharu sample that act as medium of reaction of alternating high and low pressure-ultrasound waves that lead to formation and violent collapse of small vacuum bubbles that was also called cavitations. The kinetic energy from the ultrasonic waves is transferred to water; which makes the energy absorbed increased tremendously and hence it will violently disrupt and

rupture the cell walls and oil glands of the internal part of gaharu sample and hence the aromatic oil compounds is released and mix together with water during soaking process. Therefore, the larger the amount of water per mass sample (water-to-gaharu ratio), the higher the chances of oil compound to be released and mix with water.

In general, a larger solvent volume can dissolve constituents more effectively leading to an enhancement of the extraction yield. Theoretically, for a fixed amount of solid matrix, the more quantity of solvent used, the more dilute effect in the solvent side. This gave a larger concentration difference between interior of the plant cells and the external solvent, thus a faster extraction rate could be obtained. But if the solution was very dilute, an extra solvent increase would not lead to a sufficient increase in the concentration difference, the increase in extraction yield would be limited (Shuna Zhaoa, Kin-Chor Kwoka & Hanhua Liang, 2006).

However, the increment of oil yield percentages is not that significant as they only increased a small amount of essential oil. Thus, it can be concluded that to increase the gaharu-to-water ratio is really unnecessary as it gave a small difference in terms of oil yield. But, the process might show slightly bigger difference if the experiment is done in pilot scale as more quantity of gaharu sample which is rich with resin can be pull out from the internal part of the plants.

From the graph, the steepest gradient among those 4 variant of gaharu-to water ratio R3 and R4 which is from 1:16 ratio to 1:20 ratio. Therefore, it can be concluded that the optimum value of gaharu-to-water ratio that yield the largest amount of essential oil yield is at 1:20 ratio.

It is also crucial to make sure that no heat loss is occurred during steam distillation. To prevent such case to happen, the distillation unit must be wrapped with aluminum foil especially at both the upper and lower flasks and also at the joints. Besides, over pressure might occur if not control the heat mantle properly. It is important to set the power of heating mantle at medium and to let the steam come out for every 30 minutes interval in order to release some pressure.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

This study on Extraction of Gaharu Essential Oil using Ultrasonic Assisted Steam Distillation had been successfully done. The purpose of this study is to evaluate the correlation between the times of pretreatment versus the production of gaharu essential oil. The study also is made to correlate the gaharu-to-water ratio with the percentage of gaharu essential oil yield. Thus, by doing this research, several conclusions can be made which are:

i. The ultrasonic assisted steam distillation can be consider as an improved method for the extraction of gaharu essential oil where it can produce a higher yield of oil compared to the traditional and conventional technique that still applied by small scale industry. Hence, the ultrasonic assisted steam distillation method is a feasible method to be commercialized to extract gaharu essential oil.

ii. The application of ultrasonic bath as a pretreatment will reduce the time consumed for extracting gaharu essential oil because the sonification and cavitations mechanism will disintegrate the cell walls and oil glands of gaharu sample and making the process a lot more easier.

iii. In order to achieve the research objective, the parameters which are ultrasonic pretreatment time and gaharu-to-water ratio are needed to manipulate according to the experimental matrix in the research methodology.

iv. Based form the data and graphical plotting obtained, the optimum condition for the experiments which gave highest yield of gaharu essential oil are at ultrasonic pretreatment time at 9 hours with the gaharu-to-water ratio at 1:20. The optimum value is taken at the point where it gave the largest gradient value in graphical plotting.

5.2 Recommendations

Extraction of gaharu essential oil using ultrasonic assisted steam distillation is an important study that has continuation of research for the time being as the value of gaharu essential oil and demands for it is increasing days by days. This applied technique is proven to improve the traditional and conventional method of gaharu essential oil extraction and thus local distiller and entrepreneurs must take this opportunity to enhance the method by doing some modifications that can yield the oil more efficiently either in terms of cost, time and energy savings.

After doing the experimental works, these are several recommendations to improve the efficiency of the applied method:

i. Modification must be develop to enhance the efficiency of the method by combining other pretreatment techniques or by addition some apparatus or equipments that are relevant to be applied.

ii. In order to obtain the high quality of gaharu essential oil, it is advisable to do qualitative analysis by running some sample of essential oil yield into GC-MS to trace the major constituents that give the distinctive

odour of gaharu. It is impossible to guess the quality of gaharu essential oil based from physical traits such as color and odour. A standard and high quality of gaharu essential oil sample must be purchased and make comparison

iii. In order to recover the oil in bulk, it is better to run the experiment in lab scale size as the amount oil yield would be significant in difference. This is because by doing in lab scale, the oil yield cannot be fully collected as some of them stick to the glass surface of the separating funnel

iv. Make sure selection of the raw materials (gaharu wood) must be carefully choose as the gaharu wood used in this experiment is not fully comes from the resin. It is a combination of the highly valuable resin and useless gaharu bark that do not contain any traces of oil compound.

v. Avoiding doing error such as parallax error or whiling handling the equipment or apparatus as these errors can affect oil yield.

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APPENDIX A

OIL YIELD PERCENTAGE OF ESSENTIAL OIL

Result on extraction process with varies ultrasonic pre-treatment time

Sample	Pre-treatment Time (h)	Gaharu-to-Water Ratio	Sample Mass (g)	Water Volume (mL)	Mass of Oil Recovered (g)	Essential Oil Yield (%)
T1	3	1:8	500	4000	0.407	0.0814
T2	6	1:8	500	4000	0.511	0.1022
T3	9	1:8	500	4000	0.638	0.1276
T4	12	1:8	500	4000	0.685	0.1370

Result on extraction process with varies gaharu-to-solvent ratio

Sample	Pre-treatment Time (h)	Gaharu-to-Water Ratio	Sample Mass (g)	Water Volume (mL)	Mass of Oil Recovered (g)	Essential Oil Yield (%)
R1	9	1:8	500	4000	0.638	0.1276
R2	9	1:12	350	4200	0.449	0.1283
R3	9	1:16	250	4000	0.322	0.1288
R4	9	1:20	200	4000	0.259	0.1295

APPENDIX B

RESULT ON EXTRACTION PROCESS WITH VARIES ULTRASONIC PRE-TREATMENT TIME

Sample T1

Date of Experiment = 15 Mar 2008

Amount of gaharu (g) = 500

Ratio of gaharu to water is **1:8**

Amount of water (mL)= 4000

Time period for pre-treatment by soaking in ultrasonic bath (hrs) = 3

Steam Distillation Process

Setting Temperature = 100 °C

Time of Experiment Date = 15 Mar 2008 – 17 Mar 2008

From 12.00 pm to 12.00 pm

Observation = Oil layer can be seen formed at top layer of the water in separating funnel. The color of hexane changed from colorless to dark greenish indicates the existence of essential oil. Smell of gaharu in hydrosol, essential oil seem to dilute in water

Oil recovered → Empty sample bottle = 4.725 g

Sample Bottle + Oil = 5.132 g

Weight of Gaharu Oil = 0.407 g

Yield = 0.0814 %

Sample T2

Date of Experiment = 17 Mar 2008

Amount of gaharu (g) = 500

Ratio of gaharu to water is **1:8**

Amount of water (mL)= 4000

Time period for pre-treatment by soaking in ultrasonic bath (hrs) = 6

Steam Distillation Process

Setting Temperature = 100 °C

Time of Experiment Date = 17 Mar 2008 – 19 Mar 2008

From 4.30 pm to 4.30 pm

Observation = Oil layer can be seen formed at top layer of the water in separating funnel. The color of hexane changed from colorless to dark greenish indicates the existence of essential oil. Smell of gaharu in hydrosol, essential oil seem to dilute in water

Oil recovered → Empty sample bottle = 4.638 g

Sample Bottle + Oil = 5.149 g

Weight of Gaharu Oil = 0.511 g

Yield = 0.1022 %

Sample T3

Date of Experiment = 19 Mar 2008

Amount of gaharu (g) = 500

Ratio of gaharu to water is **1:8**

Amount of water (mL)= 4000

Time period for pre-treatment by soaking in ultrasonic bath (hrs) = 9

Steam Distillation Process

Setting Temperature = 100 °C

Time of Experiment Date = 20 Mar 2008 – 22 Mar 2008

From 8.30 am to 8.30 am

Observation = Oil layer can be seen formed at top layer of the water in separating funnel. The color of hexane changed from colorless to dark greenish indicates the existence of essential oil. Smell of gaharu in hydrosol, essential oil seem to dilute in water

Oil recovered → Empty sample bottle = 4.713 g -

Sample Bottle + Oil = 5.351 g

Weight of Gaharu Oil = 0.638 g

Yield = 0.1276 %

Sample T4

Date of Experiment = 21 Mar 2008

Amount of gaharu (g) = 500

Ratio of gaharu to water is **1:8**

Amount of water (mL)= 4000

Time period for pre-treatment by soaking in ultrasonic bath (h) = 12

Steam Distillation Process

Setting Temperature = 100 °C

Time of Experiment Date = 22Mar 2008 – 24 Mar 2008

From 12.45 pm to 12.45 pm

Observation = Oil layer can be seen formed at top layer of the water in separating funnel. The color of hexane changed from colorless to dark greenish indicates the existence of essential oil. Smell of gaharu in hydrosol, essential oil seem to dilute in water

Oil recovered → Empty sample bottle = 4.722 g

Sample Bottle + Oil = 5.407 g

Weight of Gaharu Oil = 0.685 g

Yield = 0.137 %

APPENDIX C

RESULT ON EXTRACTION PROCESS WITH VARIES GAHARU TO WATER RATIO

Sample R1

Date of Experiment = 19 Mar 2008

Amount of gaharu (g) = 500

Ratio of gaharu to water is **1:8**

Amount of water (mL)= 4000

Time period for pre-treatment by soaking in ultrasonic bath (h) = 9

Steam Distillation Process

Setting Temperature = 100 °C

Time of Experiment Date = 20 Mar 2008 – 22 Mar 2008

From 8.30 am to 8.30 am

Observation = Oil layer can be seen formed at top layer of the water in separating funnel. The color of hexane changed from colorless to dark greenish indicates the existence of essential oil. Smell of gaharu in hydrosol, essential oil seem to dilute in water

Oil recovered →

Empty sample bottle = 4.713 g

Sample Bottle + Oil = 5.351 g

Weight of Gaharu Oil = 0.638 g

Yield = 0.1276 %

Sample R2

Date of Experiment = 23 Mar 2008

Amount of gaharu (g) = 350

Ratio of gaharu to water is **1:12**

Amount of water (mL)= 4200

Time period for pre-treatment by soaking in ultrasonic bath (h) = 9

Steam Distillation Process

Setting Temperature = 100 °C

Time of Experiment Date = 24 Mar 2008 – 28 Mar 2008

From 4.35 pm to 4.35 pm

Observation = Oil layer can be seen formed at top layer of the water in separating funnel. The color of hexane changed from colorless to dark greenish indicates the existence of essential oil. Smell of gaharu in hydrosol, essential oil seem to dilute in water

Oil recovered → Empty sample bottle = 4.741 g

Sample Bottle + Oil = 5.190 g

Weight of Gaharu Oil = 0.449 g

Yield = 0.1283 %

Sample R3

Date of Experiment = 28 Mar 2008

Amount of gaharu (g) = 250

Ratio of gaharu to water is **1:16**

Amount of water (mL)= 4000

Time period for pre-treatment by soaking in ultrasonic bath (h) = 9

Steam Distillation Process

Setting Temperature = 100 °C

Time of Experiment Date = 29 Mar.2008 – 31 Mar 2008

From 8.40 am to 8.40 am

Observation = Oil layer can be seen formed at top layer of the water in separating funnel. The color of hexane changed from colorless to dark greenish indicates the existence of essential oil. Smell of gaharu in hydrosol, essential oil seem to dilute in water

Oil recovered → Empty sample bottle = 4.697 g

Sample Bottle + Oil = 5.019 g

Weight of Gaharu Oil = 0.322 g

Yield = 0.1288 %

Sample R4

Date of Experiment = 2 Apr 2008

Amount of gaharu (g) = 200

Ratio of gaharu to water is **1:20**

Amount of water (mL)= 4000

Time period for pre-treatment by soaking in ultrasonic bath (h) = 9

Steam Distillation Process

Setting Temperature = 100 °C

Time of Experiment Date = 3 Apr.2008 – 5 Apr 2008

From 9.00 am to 9.00 am

Observation = Oil layer can be seen formed at top layer of the water in separating funnel. The color of hexane changed from colorless to dark greenish indicates the existence of essential oil. Smell of gaharu in hydrosol, essential oil seem to dilute in water

Oil recovered → Empty sample bottle = 4.771 g

Sample Bottle + Oil = 5.03 g

Weight of Gaharu Oil = 0.259 g

Yield = 0.1295 %

APPENDIX D

Gantt Chart For PSM 1 & 2

Month	July		August		September		October		November		January		February		March		April	
Planning / Week	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**	**26**	**28**	**30**	**32**	**34**	**36**
Learning and discussing topics																		
Reviewing Literature																		
Proposal preparation																		
Submit first draft (PSM 1)																		
Presentation(Seminar 1)																		
First draft correction																		
Final draft submission																		
PSM 2 preparation																		
Method analyzing																		
Running experiments																		
Data collection																		
Data Analysis																		
Thesis writing																		
Final thesis submission																		

Gantt Chart Work Flow for PSM 2

Month	January		February		March		April	
Planning / Week	**26**	**28**	**30**	**32**	**34**	**36**	**38**	**40**
Reviewing Literature	√	√	√	√	√	√	√	√
Draft 1 Correction	√	√	√	√				
Method Analyzing	√	√	√		√	√		
Troubleshooting Equipment	√	√	√	√	√	√	√	
Running Experiment					√	√	√	√
Data Collection					√	√	√	√
Progress Report Presentation			√					
Data Analysis					√	√	√	
Submission of Draft 2							√	
Seminar 2 Presentation								√
Draft 2 Correction							√	√
Thesis Writing	√	√	√	√	√	√	√	√
Final Thesis Submission								√
Technical Paper Writing& Poster								√