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UNIVERSITI MALAYSIA PAHANG

# ESSENTIAL OIL EXTRACTION FROM GAHARU

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## ACKNOWLEDGEMENT

We are grateful to Universiti Malaysia Pahang (UMP) for funding this research under short-term research grant. We owe special thanks to Forest Research Institute of Malaysia (FRIM) and University Kebangsaan Malaysia (UKM) for the collaboration and helpful guidelines. Sincere appreciation also extends to all group member in producing a resourceful research.

## ABSTRACT

Gaharu is one of the most valuable incenses in the world. Gaharu trees form resins that can then produce some of the highest quality gaharu oils. This resinous material, produced by tropical rainforest trees is extremely valuable and has been used for centuries as incense, perfumes, traditional medicine and etc. Currently, other uses of this product are restricted by limited supply and high prices. Due to it rarity and high demand for it, agarwood extract (gaharu oil) bring high prices. The price is around RM 30 000 per liter for lower grade and superior grades could be priced up to RM 60 000 per liter. It is anticipated that the prices of gaharu will remain high in the future because the high demand for gaharu material in Arabic countries, introduction of new applications for gaharu materials in the cosmetical industry and the traditional users of gaharu in China, Japan and India for manufacturaing joss-sticks and other. Given the tremendous demand and diverse applications of gaharu, the economic potential of this product is substantial. Thus, an effective extraction method for recovery the gaharu oil product would be desirable. Currently, the gaharu oil is extracted from the agaharu chips by heating it through hydro distillation process. The drawbacks associated with the hydro distillation method include long hours required to complete the extraction process and produce very low yield. In this research, an enzymatic pretreatment step is introduced in order to speed up the breakage agarwood cell's wall, hence reduce the mass transfer resistances and releases the cell's contents into the extraction medium during the hydro distillation process. Thereby; the oil is released easier in the subsequent separation process. Two process variables, namely extraction time and temperature were varied in order to observe the overall performance of the improved extraction technique and to find the optimum operating condition within the explored range. Similar study was also conducted without enzymatic pretreatment as the basis of comparison for the enzymatic pretreatment hydro distillation. Compared to the conventional method (without enzymatic pretreatment), the improved method (with enzymatic pretreatment) provides high extraction yield (an increase of up to 59%)

# ABSTRAK

Gaharu merupakan salah satu komoditi perhutanan yang berharga dalam pasaran dunia. Pokok gaharu menghasilkan resin yang menyumbang kepada kualiti minyak yang tinggi. Resin yang dihasilkan sangat berharga dan ianya digunakan sebagai kemenyan, bahan pewangi, perubatan traditional dan sebagainya. Bekalan minyak gaharu yang terhad dan harga yang tinggi di pasaran menghadkan penggunaan minyak secara lebih meluas. Bekalan minyak gaharu yang terhad juga menyumbang kepada harga minyak yang tinggi. Harga minyak gaharu adalah antara RM 30 000 sehingga RM 60 000 bagi satu liter. Adalah dianggarkan bahawa harga minyak gaharu akan sentiasa meningkat memandangkan permintaan yang tinggi terutama di semenanjung Arab, China, Jepun dan India. Oleh itu proses pengekstrakan yang effektif sangat diperlukan untuk memenuhi permintaan pasaran yang tinggi. Dewasa ini, minyak gaharu di ekstrak menggunakan kaedah penyulingan. Kaedah penyulingan memerlukan masa yang panjang dan menghasilkan minyak gaharu yang sedikit. Dalam kajian ini, langkah pra rawatan menggunakan enzim di jalankan ke atas kaedah penyulingan untuk mempercepatkan proses dan meninggikan hasil pengekstrakan melalui pemecahan dinding sel sampel gaharu. Pemecahan dinding sel gaharu oleh enzim akan memudahkan pemindahan minyak daripada kayu kepada pelarut. Dalam kajian ini dua parameter iaitu masa dan suhu telah dikaji untuk melihat kesan langkah pra rawatan enzim ke atas hasil pengekstrakan. Daripada kajian ysng dijalankan didapati pra rawatan enzim dapat meningkatkan hasil pengekstrakan sebanyak 59%.

# TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF SYMBOLS

| | | |
|---|---|---|
| $c$ | - | Concentration |
| $c_A$ | - | Concentration of $A$ |
| $c_{Li}$ | - | Bulk fluid concentration |
| $c_{Li}$ | - | Concentration in the fluid next to the surface of the solid |
| $D_{AB}$ | - | Molecular diffusivity of the molecule $A$ in $B$ |
| $J^*_{AZ}$ | - | Molar flux of component $A$ in the z direction |
| $k_c$ | - | Mass transfer coefficient |
| $N_A$ | - | Rate of convective mass transfer |
| $z$ | - | Distance of diffusion |
| $\varepsilon_M$ | - | Turbulent or mass eddy diffusivity |

# CHAPTER 1

# INTRODUCTION

## 1.1 Research Background

*Aquilaria malaccensis* or gaharu is a resin impregnated heartwood of Aquilaria species. This wood is able to gives unique aromatic scent and is categorized as one of the most expensive and highly prized commodities.

Several of compounds such as agarospirol, jinkohol-eremol and kusenol have been reported to possibly contribute to the characteristic aroma of gaharu (Nakanishi *et al*., 1984; Ishihara *et al*., 1993).

Gaharu is divided into several grades in the market. The higher quality gaharu wood can be recognized by its dark colour and strong aroma released upon burning its chips or quality incense. However, there is very little information on the quality of different gaharu essential oils produced (Chang *et al*., 2002).

The best and darkest gaharu woods are used in incense mixtures while the lower grades are extracted to produce gaharu oil used in perfumery. On average, the oil represent 1% of the total weight of the lower grades gaharu wood.

Gaharu is traditionally used to produce incense for rituals and religious ceremonies in the Far East. Gaharu is also believed to have tonic and therapeutic properties (Burkill, 1966). In Asia, gaharu is used to treat smallpox, rheumatism, to heal wound and illness during and after birth. Based on a researcher from Forest Research Institute of Malaysia (FRIM) gaharu oil contains the anti-cancer agent. For the time being FRIM also conducting the research of gaharu which has a potential to become an insect repellent.

Its essential oil is in heavy demand in the perfume industry as evidenced by the recent expansion of the range of uses for gaharu to include new products such as gaharu essence, soap and shampoo (Chakrabarty *et al*., 1994). These products are marketed at prices about ten times more expensive than the common brands of toilet soaps and shampoos. With advancing technology, it is expected that in future more new products derived from gaharu will appear in the market.

## 1.2 Problem Statement

The extraction of essential oils from plant material can be achieved by a number of different methods. The choice of extraction method will depend on the nature of the material, the stability of the chemical components and the specification of the targeted product.

Currently, gaharu oils are obtained by distillation. The main advantage of distillation is that it can generally be carried out with very simple equipment. Using distillation method, the plant material is mixed directly with water in a still pot. A perforated grid was inserted above the base of the still pot to prevent the plant material settling on the bottom and coming in direct contact with the heated base of the still and charring (Figure 1.1).

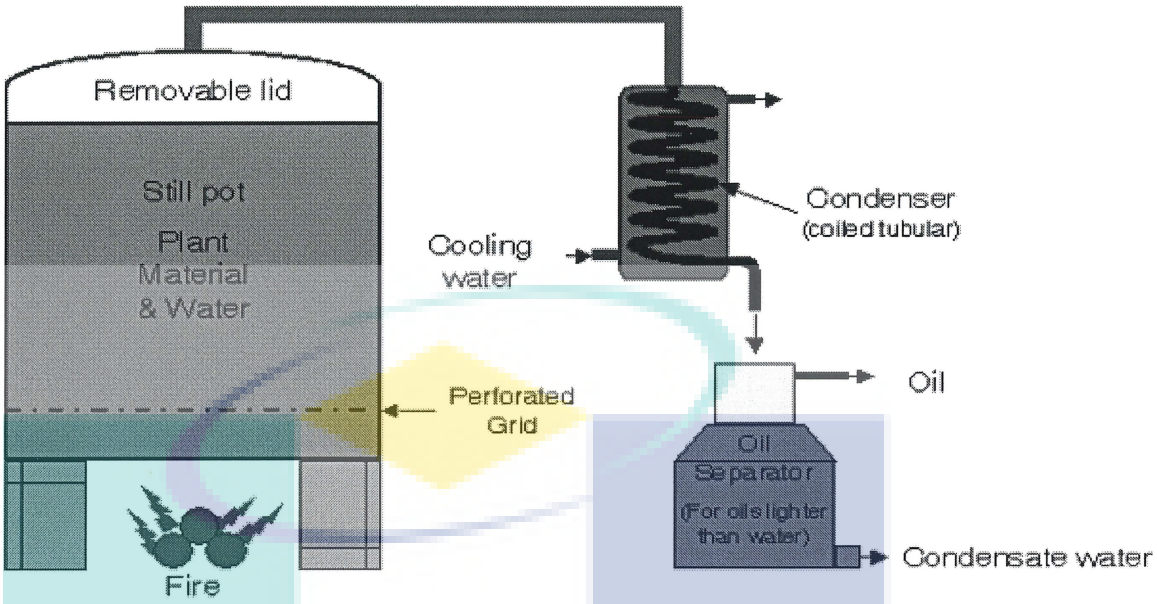



Figure 1.1: Hydro distillation schematic diagram

Water distillation is probably the simplest and cheapest method of extracting essential oils. Adopting the simplest or cheapest extraction method however, may prove to be false economy because of low yield, poor or highly variable oil quality and low market value. Therefore, the quality of the oil has the greatest potential to be modified due to the effects of direct heating and the water contact.

Figure 1.2 and figure 1.3 shows the conventional extraction method which could not extract and separate the oil from solid particle very effectively. Thus, the improvement of the gaharu oil extraction method should be carried out in order to get a better quality and quantity of the gaharu oil extracted.

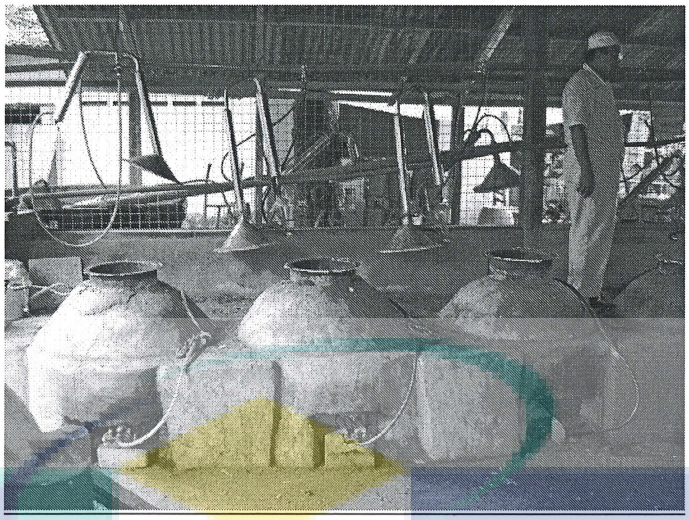


Figure 1.2: Conventional method of gaharu extraction.

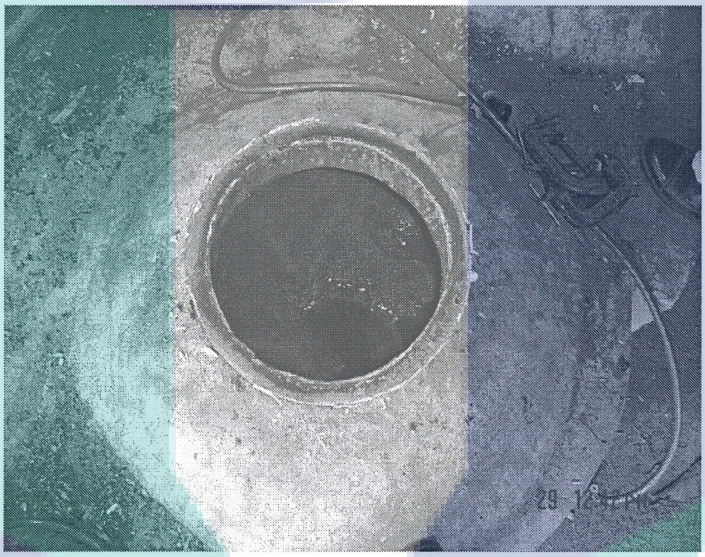


Figure 1.3: The remains oil of gaharu that can not be separated effectively.

Currently, other uses of gaharu oil product are restricted by limited supply and high prices. Due to it rarity and high demand for it, gaharu extract (gaharu oil) bring high prices. The price is around RM 30 000 per liter for lower grades and superior grades could be priced up to RM 60 000 per liter (Ng et al., 1997).

It is anticipated that the prices of gaharu will remain high in the future because the high demand for gaharu material in Arabic countries, introduction of new applications for gaharu materials in the cosmetical industry and the traditional users of gaharu in China, Japan and India for manufacturaing joss-sticks and other products

(Chang *et al.*, 2002). Given the tremendous demand and diverse applications of gaharu, the economic potential of this product is substantial.

It is expected that through understanding of the mechanism of gaharu oil extraction, there will be sufficient supply to meet market demands. Therefore, there is strong incentive to optimize gaharu oil extraction yield.

One of the challenges in commercializing Gaharu in Peninsular Malaysia is to produce high quality Gaharu essential oil (Mohd Paiz, 2006). The Malaysian Timber Industry Board (MTIB) projected that Malaysia can be a leader in Gaharu trading if Gaharu essential oil is produced using high technology (Azmi, 2006).

Currently, the gaharu oil is extracted from the agarwood chips by heating it through hydrodistillation process. The drawbacks associated with the hydrodistillation method include long hours required to complete the extraction process and produce very low yield. A few researches have been done to improve the efficiency of gaharu oil extraction. In this research, an enzymatic pretreatment step is introduced in order to speed up the breakage agarwood cell's wall, hence reduce the mass transfer resistances and releases the cell's contents into the extraction medium during the distillation process. Thereby; the oil is released easier in the subsequent separation process. Hydro distillation process with the assistant of enzymatic pretreatment may contribute to improve the efficiency and capacity of gaharu oil extraction.

## 1.3 Objective of the Research

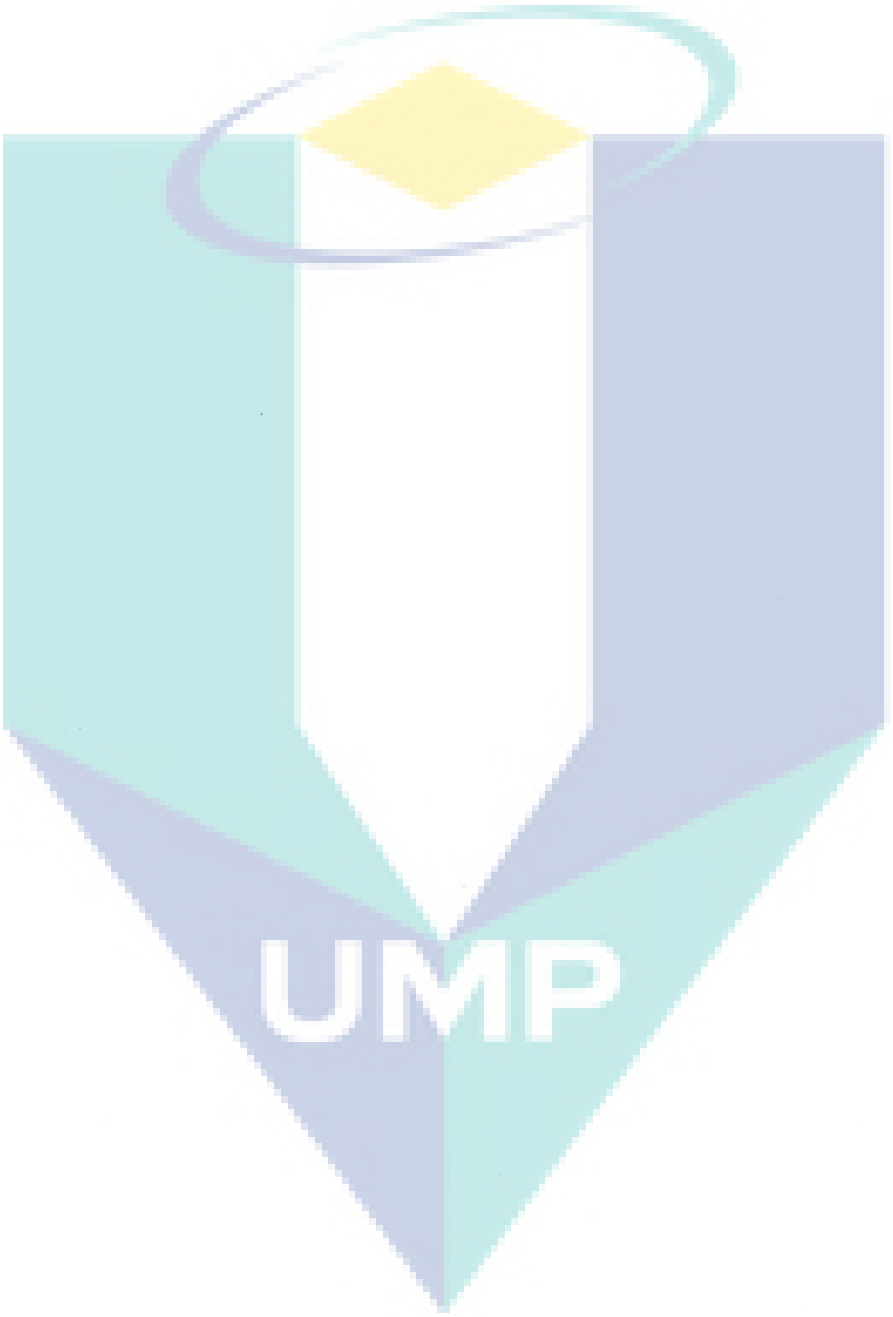
The main objectives of this research are:

i) To examine the feasibility of the enzymatic pre-treatment in hydro distillation process

ii) To identify the most influential operational parameters that affect the percentage of oil collected in the process.

iii) To extract gaharu oil using appropriate new technology for better extraction process.

iv) To identify the composition and profile of the essential oil from the different grades.



# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Gaharu

Gaharu or agarwood is the resinous wood from the aquilaria tree, an evergreen tree native to northern India, Laos, Cambodia, Malaysia, Indonesia and Vietnam. Its scientific name is *Aquilaria Malaccensis*. Others common name for gaharu is Aloeswood, Agarwood, Jin koh, Jinko, Eagle wood, Oud and Ood ud.

Gaharu tree (Figure 2.1) growing up to the 40 meters high and 60 centimeter in diameter (Chang *et al.* 2002). The resin of the tree from a natural fungal attack and immune response is commonly known as an agarwood. An inferior resin is created by the deliberate wounding of Gaharu tree, leaving it more susceptible to a fungus attack by using a force method. The fungus and decomposition process continue to generate a very rich and dark resin forming within the heartwood. Figure 2.2 shows the cross section of gaharu wood.

Figure 2.1: Gaharu Tree

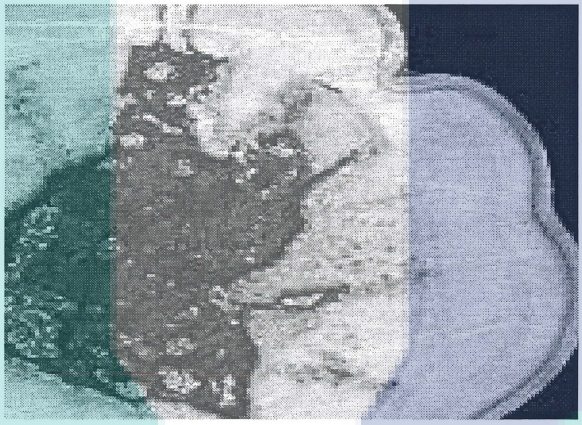


Figure 2.2: Cross Section Of Gaharu Wood

In Malaysia, gaharu in the form of chips (Figure 2.3), flakes (Figure 2.4), oil and powder waste after extraction are the most commons forms traded.

Figure 2.3: Gaharu Chips

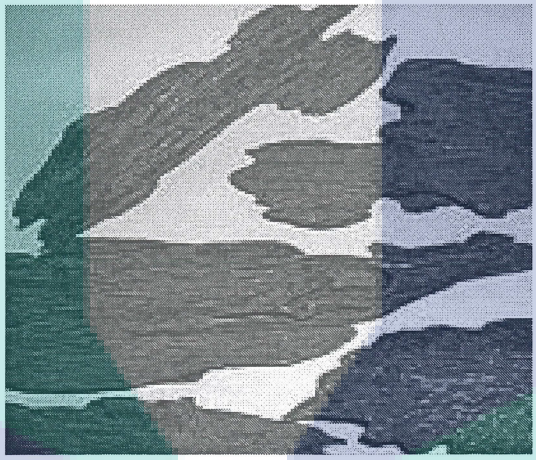


Figure 2.4: High quality Gaharu flakes

In Malaysia there are records of the use of Gaharu in various folk remedies for the treatment of weakness, stomach pains, in pregnancy, after delivery, fever, chest pains, body pains, rheumatism, women diseases and dropsy (Chang, *et al.* 2002).

A decoction of the wood is used for abdominal pain, asthma, cancer, colic chest, congestion, diarrhea, hiccups, nausea, nerves and also regurgitation. Other than that, the resinous wood and the resin is considered tonic for the kidneys. It is used to expel gas, treat male disorders and shortness of breath. In the Islamic

societies of the Middle East, oud (Arabic for wood) is a symbol of status, wealth and hospitality. Gaharu incense is used in religious ceremonies by Buddhists, Hindus and Muslims, while a revival of the 'Koh doh' incense ceremony in Japan has rekindled interest in Gaharu in that country.

## 2.2 Chemical Components of Gaharu

Generally, gaharu essential oils are mixture of sesquiterpenes, sesquiterpene alcohols, oxygenated compounds, chromone derivatives and resin. The most important compounds in the gaharu essential oils are agarospirol, jinkohol-eremol, jinkohol and kusenol that may contribute to the characteristic aroma of gaharu. Other compounds such as 2-(2-4'-methoxyphenylethyl)chromone produce a long lasting fragrance upon burning.

### a) Agarospirol

Agarospirol is a one of important compounds that contribute the special aroma for gaharu essential oil. The IUPAC name for Agarospirol is 2-(6,10-dimethyl-2-spiro[4.5]dec-9-enyl)propan-2-ol. The formula molecule for Agarospirol is $C_{15}H_{26}O$. This compound has a molecular weight 222.366 g/mol. The functional groups in agarospirol are hydroxyl and alkenes. The structure of Agarospirol is aromatic.

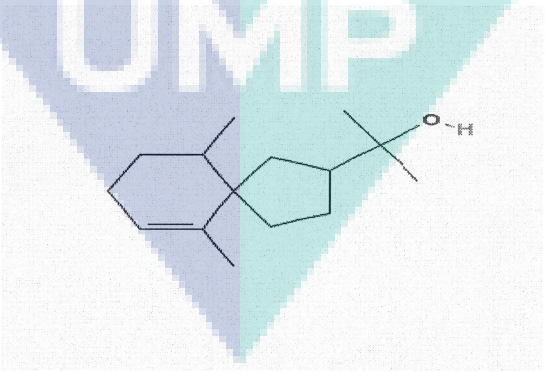



Figure 2.5: The structure of agarospirol

**b) Jinkoh-eremol.**

The IUPAC name for jinkoh-eremol is 2-(8,8a-dimethyl-2, 3,4,6,7,8-hexahydro-1H-naphthalen-2-yl)propan-2-ol. The formula molecule for Jinkoh-eremol is $C_{15}H_{26}O$. This compound has a molecular weight 222.366 g/mol. The functional groups in Jinkoh-eremol is hydroxyl and alkene. The structure of Jinkoh-eremol is aromatic.

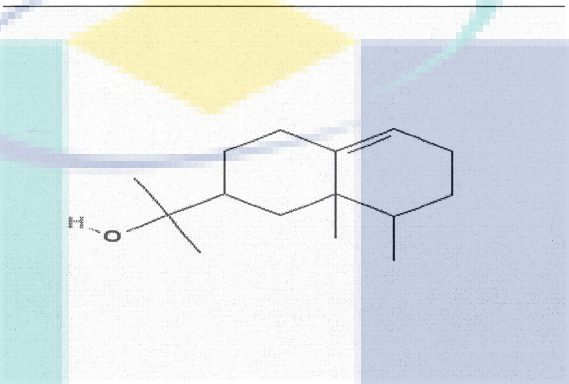


Figure 2.6: The structure of Jinkoh-eremol

**c) Khusenol**

The IUPAC name for Khusenol is 2-(2,4-dihydroxyphenyl)-3,7-dihydroxy-8-(5-hydroxy-5-methyl-2-prop-1-en-2-yl-hexyl)-5-methoxy-chroman-4-one. The formula molecule for Khusenol is $C_{26}H_{32}O_8$. This compound has a molecular weight 472.527 g/mol. The functional groups in Jinkoh-eremol are hydroxyl, alkene, ether, and ester. The structure of Khusenol is aromatic.

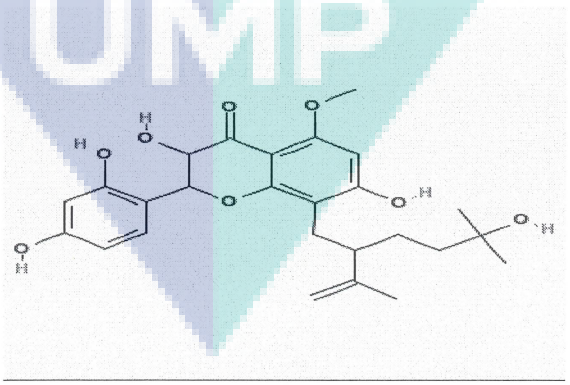


Figure 2.7: The structure of Khusenol

## 2.3 The Principles of Solid-Liquid Extraction

Solid-liquid extraction is among the most commonly employed methods of separation, which appears in many industrial processes for example pharmaceutical industry, perfumes or pesticides manufacturing industries to recover active principles from plants (Luque de Castro and Garcia-Ayuso, 1998; Romdhane and Gourdon, 2002).

Extraction is a separation process to separate the desired solute or removed an undesirable solute component from the solid phase where the solid is contacted with a liquid phase. The two phases are in intimate contact and the solutes can diffuse from the solid to the liquid phase, which causes a separation of the components originally in the solid. Solid-liquid extraction also known by a variety of other names, such as leaching, washing, percolation, digestion, steeping, lixiviation and infusion but of this only one term, leaching has widespread use (Geankoplis, 1993; Ruthven, 1997; Luque de Castro and Garcia-Ayuso, 1998).

Leaching is widely used as a separation process for the following (Phipps and Eardley, 1982; Mizubuti *et al.*, 2000; Dickey *et al.*, 2002; Xuejun Pan *et al.*, 2003):

1) Extraction of edible oils from seeds, beans, nuts, rice bran, wheat germ, coconut and other sources.
2) Extraction of essential oils from flowers, leaves and seeds.
3) Extraction of oleoresins from spices.
4) Extraction of sugar from sugar beet and sugarcane.
5) Extraction of coffee from coffee beans.
6) Extraction of fish oil from fish meal.
7) Extraction of active ingredients from leaves, pods, seeds, flowers and barks e.g., extraction of tocopherols.
8) Extraction of copper sulphate from copper ore.

The simplest extraction system comprises three components:

i) Solute, or the material to be extracted

ii) Solvent, which may be a liquid or a supercritical fluid at process conditions

iii) Carrier, or nonsolute portion of the feed mixture to be separated.

For the case of countercurrent extraction and a light solvent, the flow of these materials are as shown in figure 2.8.

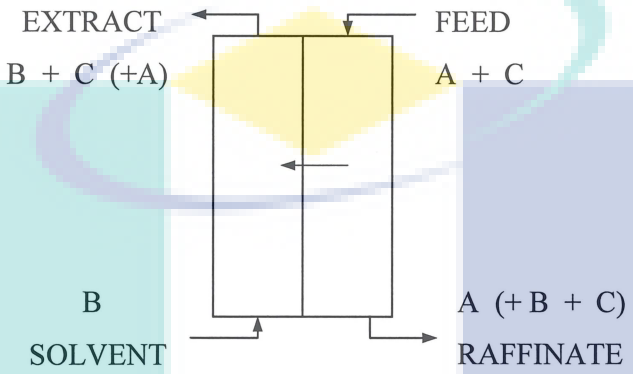



Figure 2.8: Extraction notation

Where,

Raffinate phase: feed stream minus extracted material

Extract phase: solvent stream plus extracted material

A = Carrier, B = Solvent, C = Solute

For such a system the carrier and the solvent are essentially immiscible, while the carrier-solute and solvent-solute pairs are miscible.

The general operation scheme for solvent extraction is presented in figure 2.9:

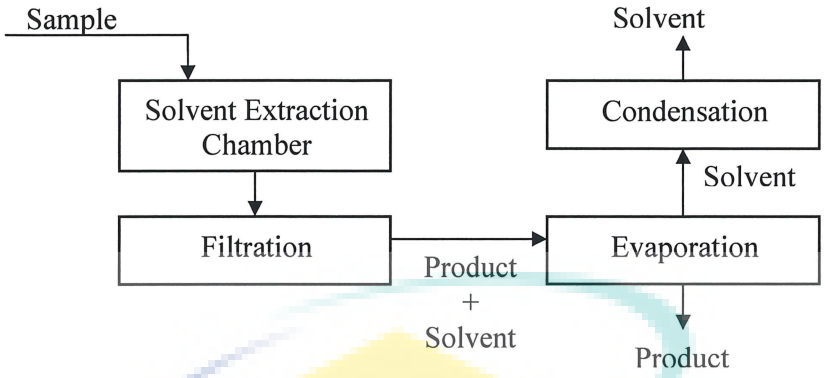



Figure 2.9: General scheme for a solvent extration unit (Vinatoru *et al.*, 1997)

### 2.3.1 Mass Transfer in Extraction Process

In general, the following steps can occur in an overall solid-liquid extraction process (Geankoplis, 1993; Holland, 1975):

i) Solvent transfer from the bulk of the solution to the surface of solid
ii) Penetration or diffusion of the solvent into the pores of the solid
iii) Dissolution of the solvent into the solute
iv) Solute diffusion to the surface of the particle
v) Solute transfer to the bulk of the solution

Any one of the five basic steps may be responsible for limiting the extraction rate. The rate of transfer of solvent from the bulk solution to the solid surface and the rate into the solid are usually rapid and are not rate-limiting steps and the dissolution is usually so rapid that it has only small effect on the overall rate (Geankoplis, 1993; Ruthven, 1997).

The overall extraction process is sometime subdivided into two general categories according to the main mechanism responsible for the dissolution stage:

1) Operations that occur because of the solubility of the solute or its miscibility with the solvent, e.g., oilseed extraction

2) Extractions where the solvent must react with a constituent of the solid material in order to produce a compound soluble in the solvent, e.g., the extraction of metal from metalliferous ores

In the former case the rate of extraction is most likely to be controlled by diffusion phenomena, but in the latter the kinetics of the reaction producing the solute may play a dominant role.

As state before, the process of solid-liquid extraction involves the transfer of material from one phase to another, therefore it is categorized as a mass transfer process. Mass transfer mean that the redistribution of molecules under the influence of a potential or driving force. The potential used to bring about this change may be a chemical potential (created by concentration differences) or other forms of potential e.g. electrical, gravitational etc (Phipps & Eardley, 1982; Gekas, 2001).

When mass is being transferred from one distinct phase to another or through a single phase, the basic mechanism are the same whether the phase is a gas, liquid or solid. The equation for molecular diffusion of mass is Fick's law. It is written as follows for constant total concentration in a fluid (Geankoplis, 1993; Lydersen, 1983; Seader and Henley, 1998).

$$J^*_{AZ} = -D_{AB}\frac{dc_A}{dz} \qquad 2.1$$

Where $J^*_{AZ}$ is the molar flux of component $A$ in the z direction due to molecular diffusion in kg mol A/s.m$^2$, $D_{AB}$ is the molecular diffusivity of the molecule $A$ in $B$ in m$^2$/s, $c_A$ the concentration of $A$ in kg mol/m$^3$, and $z$ the distance of diffusion in m.

This equation is more commonly used in many molecular diffusion processes. If $c_A$ varies to some extent, an average value is often used with equation 2.1. Rearranging equation 2.1 and integrating,

$$J^*_{AZ} = \frac{D_{AB}(c_{A1} - c_{A2})}{z_2 - z_1} \qquad 2.2$$

The general Fick's law equation can be written as follows for a binary mixture of $A$ and $B$:

$$J^{*}_{AZ} = -cD_{AB}\frac{dx_A}{dz} \qquad 2.3$$

Where $c$ is total concentration of $A$ and $B$ in kg mol $(A + B)/m^3$, and $x_A$ is the mole fraction of $A$ in the mixture of $A$ and $B$. If $c$ is constant, then $c_A = cx_A$,

$$c\,dx_A = d(cx_A) = dc_A \qquad 2.4$$

### 2.3.1.1 Convective Mass Transfer Coefficient

When a fluid is flowing outside a solid surface in forced convective motion, the rate of convective mass transfer, $N_A$ from the surface to the fluid, or vice versa can be expressed by the following equation:

$$N_A = k_c\,(c_{L1} - c_{Li}) \qquad 2.5$$

Where $k_c$ is a mass transfer coefficient in m/s. This is an indirect measure of the resistance to transfer. The effectiveness or efficiency with large $k_c$ values indicate an effective transfer for the reason that a low transfer resistance exists. Similarly the larger mass transfer coefficient will produce the more rapid rate of extraction. $c_{L1}$ is the bulk fluid concentration in kg mol $A/m^3$, and $c_{Li}$ the concentration in the fluid next to the surface of the solid (Sherwood and Pigford, 1952; Schweitzer, 1979).

### 2.3.1.2 Molecular Diffusion in Solid

Transport in solids can be classified into two types of diffusion(Geankoplis, 1993);

i) Diffusion that can be considered to follow Fick's law and does not depend primarily on the actual structure of the solid

ii) Diffusion in porous solids where the actual structure and void channels are important

Diffusion in solids following Fick's Law does not depend on the actual structure of the solid. The diffusion occurs when the fluid or solute diffusing is actually dissolved in the solid to form a more or less homogeneous solution for example in leaching, where the solid contains a large amount of water and a solute is diffusing through this solution (Geankoplis, 1993; Treybal, 1980).
Generally, simplified equation is used.

$$N_A = \frac{-D_{AB} dc_A}{dz} \qquad 2.6$$

where $N_A$ is flux in kg mol $A$/s.m$^2$, $D_{AB}$ is diffusivity in m$^2$/s of $A$ through $B$ and usually is assumed constant and independent of pressure for solids.
Integration of equation 2.6 gives

$$N_A = -\frac{D_{AB}(c_{A1} - c_{A2})}{z_2 - z_1} \qquad 2.7$$

### 2.3.1.3 Turbulent Diffusion Equations for Mass Transfer

If the fluid is agitated or mixed, transfer takes place by the relatively fast process of eddy diffusion. Conditions favorable to eddy diffusion may be maintained

by special mixing devices or agitators, or by the establishment of turbulent flow (Sherwood and Pigford, 1952; Treybal, 1980; Geankoplis, 1993).

For turbulent mass transfer for constant *c*,

$$J_A^* = -(D_{AB} + \varepsilon_M)\frac{dc_A}{dz} \qquad 2.8$$

where $D_{AB}$ is the molecular diffusivity in $m^2/s$ and $\varepsilon_M$ is the turbulent or mass eddy diffusivity in $m^2/s$. Integrating equation 2.8,

$$J_A^* = \frac{D_{AB} + \varepsilon_M}{z_2 - z_1}(c_{A1} - c_{A2}) \qquad 2.9$$

The simplified equation is written using convective mass transfer coefficient

$$J_A^* = k_c^{'}(c_{A1} - c_{A2}) \qquad 2.10$$

Where $k_c^{'}$ is $(D_{AB} + \varepsilon_M)/(z_2 - z_1)$

### 2.3.2 Factors Influencing Leaching Rate

There are four important factors influencing the rate of extraction which are type of solvent used, physical characteristic of solids, temperature of the process and agitation of the fluid. These factors are discussed in the following sections.

#### 2.3.2.1 Type of Solvent

By definition, solid-liquid extraction is the use of a liquid (solvent) to extract selective component from a solid phase, by dissolving that component in the liquid. The characteristics of the solvent, which must interlock with the selected characteristics of the solute being separated, need to be identified. Solvents can be

divided into two broad chemical classes, polar solvents such as water and the alcohols, and non-polar solvents such as paraffins and benzene. Substances which dissolve in polar solvents are themselves generally polar; likewise, substrates which dissolve in non-polar solvents are non-polar in nature. There are, however, many exceptions. For example, as would be predicted, polystyrene will dissolve in several non-polar solvents; yet it is also soluble in the polar diethyl ketone although insoluble in the non-polar hexane (Phipps and Eardley, 1982).

Choice of solvent is determined by a number of factors (Phipps and Eardley, 1982; Ruthven, 1997):

a) Selectivity:

Selectivity is the ability of the solvent to extract only the desired component whilst leaving all other material in the solid phase. Solvent selectivity intimately linked to the purity of the recovered extract, and obtaining a purer extract can reduce the number and cost of subsequent separation and purification operations.

b) Physical Properties:

Low surface tension facilitates wetting of the solids in the first extraction stage and low viscosity assists diffusion rates in the solvent phase. A low solvent density is desirable to reduce the mass of solvent held up in the solid being extracted. A high boiling solvent with a high latent heat of evaporation requires recovery conditions that may be adverse for thermally sensitive extracts and increases the cost of solvent recovery.

c) Thermal stability:

At processing temperatures in both the extraction and recovery plants, the solvent should be completely stable to avoid expensive solvent losses; contamination of the solvent by any solvent breakdown must be avoided.

d) Hazards:

The solvent should be nontoxic and nonhazardous. Adequate design must take into account flammability and explosivity characteristics of the solvent.

e) Cost:

The cost of fresh solvent is reflected in the operating costs in the form of solvent make-up charges. Avoidance of solvent losses, and hence a reduction of operating costs, may be obtainable through better plant design which is usually associated with increased capital costs.

Very often, a compromise in the selection of the solvent is required.

### 2.3.2.2 Physical Characteristics of Solids

Knowledge of the physical characteristics of the carrier solid is very important to determine whether it needs prior treatment to make the solute more accessible to the solvent. Prior treatment may involve crushing, grinding, cutting into pieces, or re-forming into special shapes such as flakes.

Solute particles may exist in the inert solid in variety of ways. It may exist on the surface of the solid, may be surrounded by a matrix of inert material, may be chemically combined, or may exist inside cells as in the case of many vegetable and animal bodies.

Solute adhering to the solid surface is readily removable by the solvent. When the solute exists in pores surrounded by a matrix of inert material, the solvent has to diffuse to the interior of the solid to capture solute and then diffuse out before a separation can result. In such cases, subdivision of the solid by crushing, grinding, or cutting increases the exposed to the solvent. However, reduction of solids to finer particle size has its limitations. In some instances, the amount of solute to be recovered is small in relation to the amount of material to be treated, in which case grinding becomes uneconomical (Schweitzer, 1979).

Too-fine division may result in packing of solids during extraction, preventing free flow of the solvent through the solid bed. In such a case, the extraction is much more difficult, especially when finely divided solids are treated in an unagitated state (Schweitzer, 1979).

### 2.3.2.3 Solvent Agitation

Agitation of the solvent increases local turbulence and the rate of transfer of material from the surface of the particles to the bulk of the solution. Agitation should prevent settling of the solids, to enable most effective use of the interfacial area (Ruthven, 1997).

### 2.3.2.4 Temperature

Temperature plays an important role in solid extraction. Both the solubility of the material being extracted and its diffusivity usually increase with temperature, permitting higher rates of extraction. However, higher temperatures may also mean high solvent losses, extraction of some undesirable constituents and damage to some sensitive components in the solid material. A compromise is necessary in the selection of operating temperature (Ruthven, 1997).

### 2.3.3 Extraction Techniques

This section briefly describes fundamental of conventional and non conventional extraction methods.

#### 2.3.3.1 Conventional Extraction Techniques

The classical techniques for the solid-liquid extraction are based upon the correct choice of solvent coupled with the use of heat and/or agitation. Almost all classical methods (shake-flask, direct distillation, water steam distillation, maceration and enfleurage) are time and solvent-consuming. Due to the long extraction process, degradation of the heat sensitive material can occur when it involve heat sensitive substances (Vinatoru *et al.*, 1997; Skerget and Knez, 2001; Toma *et al.*, 2001).

The conventional Soxhlet extraction method is the most commonly used example of a semi-continuous method. In this method, solid materials are extracted by repeated washing (percolation) with an organic solvent, under reflux in special glassware. Although the conventional Soxhlet extraction can be used to extract compounds of interest with high efficiency, they still require long extraction time and large volumes of extraction solvents, which is not only expensive to dispose off but which can itself cause additional environmental problems (Babic *et al.*, 1998; Saim *et al.*, 1997).

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 heat sensitive 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 or concentration step after the extraction is mandatory (Luque de Castro and Garcia-Ayuso, 1998).

### 2.3.3.2 Non conventional Extraction Techniques

Although conventional methods offer simplicity, it is time-consuming and imprecise methods of extraction. In the last few years, a number of new extraction techniques have been developed to reduce the volume of solvents required for extraction, improve the amount of analyte recoveries, and reduce extraction time. Such techniques include Microwave Extraction, Supercritical Fluid Extraction and assisted solvent extraction using electrical, magnetic or sonic field as potential alternatives to conventional extraction (Babic *et al.*, 1998; Kim *et al.*, 2003; Li *et al.*, 2003).

a) Supercritical Fluid Extraction:

In particular, Supercritical Fluid Extraction is a separation process based on the contact of a substance containing the extractable compound with a solvent in supercritical conditions. Fluids under supercritical conditions exhibit enhanced dissolving power and have transport properties that favor high extraction capabilities. In particular, supercritical fluids have a relative lower viscosity and higher diffusivity than liquid solvents. Therefore, they can penetrate into porous solid materials more effectively than liquid solvents (Riera *et al.*, 2004).

The most employed supercritical solvent is carbon dioxide. Some of the motivations for its employment are that the solvent is non-toxic, recyclable, cheap, relatively inert, non-flammable and the process improves product quality and product recovery. Its low critical temperature and pressure ($T_c$ = 31.18C, $P_c$ =78.0 atm) also make it a preferred solvent. In the Supercritical Fluid Extraction process, the recovery of solvent and the isolation of extract can be accomplished by a simple mechanical pressure reduction. Nevertheless, the use of the supercritical extraction as an industrial separation has the drawback of the capital (complicated equipment is required) and operation costs at high pressure (Montero-Vazquez *et al.*, 2003).

Supercritical Fluid Extraction of oil from a solid matrix has a slow dynamics even when solute free solvent is recirculated and therefore improvements in mass

transfer are required for oil extraction using Supercritical Fluid Extraction (Riera *et al.*, 2004).

b) Microwave Extraction:

Extraction utilizing microwave energy is a sample extraction technique patented by Environment Canada under the name MAPTM. This novel technique can also reduce solvent usage and shorten extraction time. MAPTM extractions can be performed under closed or open vessel conditions. In this form of extraction, the solid sample is heated rapidly in a polar solvent by microwave energy. Microwave energy causes molecular motion by migration of ions and rotation of dipoles. Therefore microwave heating depends on the presence of polar molecules or ionic species (Li *et al.*, 2003; Pastor *et al.*, 1997).

Instrumentation is relatively simple and method development time is greatly reduced. It is, however, subjected to potential interferences from the presence of microwave energy absorbing materials in the sample matrix such as ferrous materials and charcoal, in which these can cause localised 'hot spots' and can be a safety issue (Li *et al.*, 2003). The application of microwave energy to flammable organic compounds, such as solvents, can become hazardous in inexperienced hands (Llompart *et al.*, 1997). At the end of the extraction, extraction vessels must be cooled to room temperature before they can be opened, thus increasing the overall extraction time (Li *et al.*, 2003).

c) Accelerated solvent extraction.

Accelerated solvent extraction (ASE) constitutes a leaching technique which is based on the principles similar to those of microwave assisted extraction, but microwave energy is replaced in ASE by conventional heating in an oven (Li *et al.*, 2003; Luque de Castro and Garcia-Ayuso, 1998).

Accelerated solvent extraction process performed at elevated temperature, usually between 50-200 °C and pressure of 1500-2000 psi. Samples are placed in a cartridge and the hot pressurized liquid solvent is passed through the sample, cooled

and then collected. This technique is applicable to polymer, plant and animal tissue and food (Luque de Castro and Garcia-Ayuso, 1998).

d) The Vortical (Turbo) Extraction:

The vortical (turbo) extraction procedure uses a high speed stirrer that induces hydrodynamic cavitation, enhancing thereby the extraction yield. It is obvious that using a high speed stirrer, the contact between solid material and solvent is improved and therefore the diffusion process is increased. Moreover, during vertical extraction hydrodynamic cavitation bubbles are produced and their collapse acts in a similar way to the effect of ultrasonic devices (Vinatoru, 2001). However, due to the high speed rotation employed in the extraction, i.e. involves lots of mechanical movement, the wear and tear of the system is rather high. In addition, it frequent alignment of the rotor is required to reduce vibration of the system.

e) Electrical Extraction:

Electrical discharges within the extraction mixture were also claimed to increase the extraction yield. During electrical discharges, cavitation bubbles are produced and that this technique therefore has similarities to ultrasonic extraction (Vinatoru, 2001). However, this technique is only effective when the liquid medium is polar in nature. The bubbles produced are also weak and incompetent as compared to the vigorous cavitation bubbles produced from ultrasound wave fields.

## 2.4 Gas Chromatography- Mass Spectrometry (GC-MS)

Gas chromatography (GC) and mass spectrometry (MS) make an effective combination for chemical analysis. The GC instrument is effective is separating compounds into their various components. MS identifies substances by electrically charging the specimen molecules, accelerating them through a magnetic field, breaking the molecules into charged fragments and detecting the different charges.

GCMS is able to detect the presence of significant chemical components in the gaharu oil. The identification of the gaharu oil chemical components is based on the existing Wiley library spectral data and literature references.

### 2.4.1 Gas Chromatography (GC)

The function of GC is to separate all chemical components in a sample and provide a representative spectral output. The chemicals sample are injects into injection port of the GC device. The sample will vaporized and then separates and analyzes the various components.

Each chemical components will produces a specific spectral peak and it will be record on electronically or paper chart. Retention time (RI) is duration needed between injection and elution. The retention time can help to differentiate between some compounds. The size of the peak is proportional to the quantity of the corresponding substances in the specimen analyzed. The peak is measured from the baseline to the tip of the peak. The different duration time for each component take to travel to the top depend on the characteristics of each components. The lighter component travel more quickly and some components take longer time due to their shape. The different components interact with each other may hinder or acceleration the component's travel as the components strike each other.

GC analysis depends on similar phenomena to separate chemical substances. A mixture of chemicals presents in a specimen can be separated in the GC column. Some chemicals and physical characteristics of the molecules cause them to travel through the column at different speeds. If the molecules has low mass it may travel more swiftly. Also, the molecule's shape also may cause the time needed to exit the column. How the different substances relate to each other may cause the time needed to travel the column to increase or decrease.

### 2.4.1.1 Description of GC Process

The equipment used for Gas Chromatography (GC) generally consists of an injection port at one of a metal column packed with substance material and a detector at the other end of the column. A carrier gas propels the sample down the column. Carrier gases are usually argon, helium, nitrogen, or hydrogen. The gas that does not react with the sample or column is essential for reliable results.

Normally the sample is injected into the injection port with a hypodermic needle and syringe or capable of measuring the specimen amount. The needle is stuck into a replaceable neoprene or silicon rubber septum that covers the injection port. The injection port is maintain at a temperature at which the sample vaporizes immediately.

The column is a metal tube, often packed with a sand-like material to promote maximum separation. As the sample moves thought the column, the different molecular characteristics determine how each substance in the sample interacts with the column surface and packing. The column allows the various substances to partition themselves.

Substance that do not like to stick to the column or packing move through the column rapidly. Substances that do not like to stick the column or packing are impeded but eventually elute from the column. Ideally, the various components in the sample separate before eluting from the column end.

The GC instrument uses a detector to measure the different as they emerge from the column. Among the available detectors are the argon ionization detector, flame emission detector, cross section detector, thermal conductivity detector and the electron capture detector.

#### 2.4.1.2 Injection Port Temperature

The temperature of the GC injection port must be high enough to vaporize a liquid specimen instantaneously. If the temperature is too low, separation is poor and broad spectral peaks should result or no peak develops at all. If the injection temperature is too high, the specimen may decompose or change its structure. If this occurs, the GC result will indicate the presence of compounds that were not in the original specimen.

### 2.4.2 Mass Spectrometry (MS)

MS identifies substance by electrically charging the specimen molecules, accelerating them through a magnetic field, breaking the molecules into charged fragments and detecting the different changes.

MS analysis required a pure gaseous sample. The sample inlet is maintain at an high temperature, up to 400 $^{o}$C, to ensure that the sample stays a gas [3]. Next the specimen enters the ionization chamber. A beam of electrons is accelerated with a high voltage. The specimen molecules are shattered into well-defined fragments upon collision with the high voltage electrons. Each fragment is charged and travels to the accelerator as an individual particle. In the acceleration chamber the charged particle's velocity increase due to the influence of an accelerating voltage. For one value of voltage only one mass accelerates sufficiently to reach the detector. The accelerating varies to cover a range of masses to that all fragment reach the detector.

The charged particles travel in a curved path towards the detector. When as individual charged particle collides with the detector surface, several electrons (also charged particles) emit from the detector surface. Next, these electrons accelerate towards a second surface, generating more electrons, which bombard another surface. Each electron carries a charge. Eventually, multiple collisions with multiple surfaces generate thousand of electrons that emit from the last surface. The result is amplification or the original charge through a cascade of electrons arriving at the

collector. At this point the instrument measures the charge and record the fragment mass as the mass is proportional to the detected charge.

The MS instrument produces the output by drawing a array of peaks on a chart, the 'mass spectrum'. Each peak represents a value for a fragment mass. A peak's height increase with the number of fragments detected with one particular mass. As in the case of the detectors, a peak may differ in height with the sensitivity of the detector used.

### 2.4.2 Kovat Index (KI)

Kovet index is used to calculate or to determine the number of chemical components exist in the sample of Gaharu essential oil. To determine the unknown substances, the value of kovet index (KI) will be compared with journal. The kovet index (KI) value from journal with a range of +/- 5 will be acceptable.

$$KI = \frac{100\,[\,\mathrm{Log}\,(Tx - Tm) - \mathrm{Log}\,(Tn - Tm)\,] + 100\,(N)}{[\,\mathrm{Log}\,(Tn + 1\text{-}\,Tm) - \mathrm{Log}\,(Tn - Tm)\,]}$$

Where,

Tm = Mobile Phase retention time

Tx = Sample compounded retention time

Tn = Standard hydrocarbon containing carbon retention time

Tn+1 = The next standard hydrocarbon containing carbon retention time

N = Lowest carbon value

# CHAPTER 3

# METHODOLOGY

## 3.1 Chemical Component of Gaharu Oil Analysis

Two gaharu oil samples; distinguished by their colours; 'reddish brown' and 'greenish brown' were obtained by hydrodistillation process from a cottage industry in Pahang. The oils were analysed using gas chromatography (GC) and a combination of gas chromatography- mass spectrometer (GC-MS). Both gaharu oil samples were found to be made up of complex mixtures of sesquiterpene hydrocarbons and their oxygenated derivatives.

Gas chromatography – mass spectrometer (GC-MS) is the most suitable analysis equipment to detect the chemical composition of gaharu essential oil. Accurate mass (high resolution) mass spectrometer is a valuable tool for confirmation of the molecular formula of a detected component. Figure 3.1 shows Gas Chromatography – Mass Spectrometer (GC-MS) used to analyze chemical composition of gaharu oil.

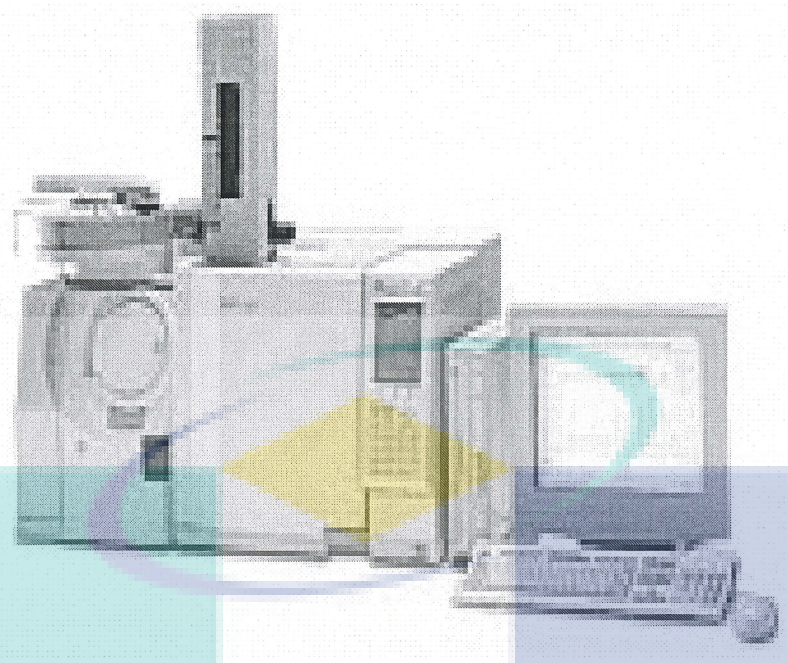


Figure 3.1: Gas Chromatography – Mass Spectrometer (GC-MS)

## 3.2 Gaharu Oil Extraction

Gaharu oil was extracted using enzymatic pre-treatment hydro distillation process and Supercritical Fluid Extraction. In hydro distillation process, 375 gram of gaharu was extracted with 2625 ml water for every batch of experiment (1:7 solid to solvent ratio).

### 3.2.1 Enzymatic Pre-treatment Hydro Distillation Process

Extraction time and temperature were varied to study the performance of Enzymatic Pre-treatment Hydro Distillation process. Three durations (6, 9, and 12 hours) and five temperatures (80, 100, 120, 140 and 160°C) were investigated. The process is outlined below.

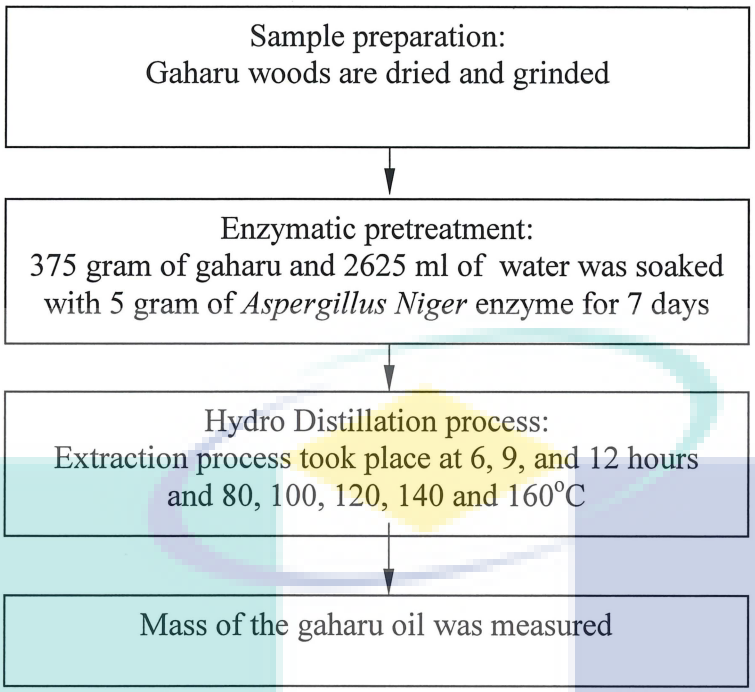



Figure 3.1: Experimental procedure for enzymatic pre-treatment hydro distillation process

### 3.2.1.1 Wood Samples Selection (Grade C)

Grade C of gaharu wood is selected in the process due to the maximum profitability compares to other grades. Also the availability of this type of grade C sample is easier found than other grades.

### 3.2.1.2 Drying

The samples need to be dried first at certain moisture before grinding. In this process, tray dryer is used to dry the samples. The tray dryer able to shorten the processing time compare to natural air drying method.

### 3.2.1.3 Grinding

The samples are ground to small particle size before the extraction process. Small particle size is able to increase the extraction rate due to the increase the interfacial area for mass transfer occurs.

## 3.2.2 Supercritical Fluid Extraction Experimental Work

Extraction time was varied to study the performance of Supercritical Fluid Extraction as a comparison method to Enzymatic Pre-treatment Hydro Distillation process. Two durations (15 and 30 minutes) were investigated using Supercritical Fluid Extraction. The temperature and pressure were set at 40°C and 100 bar respectively. The process is outlined below:

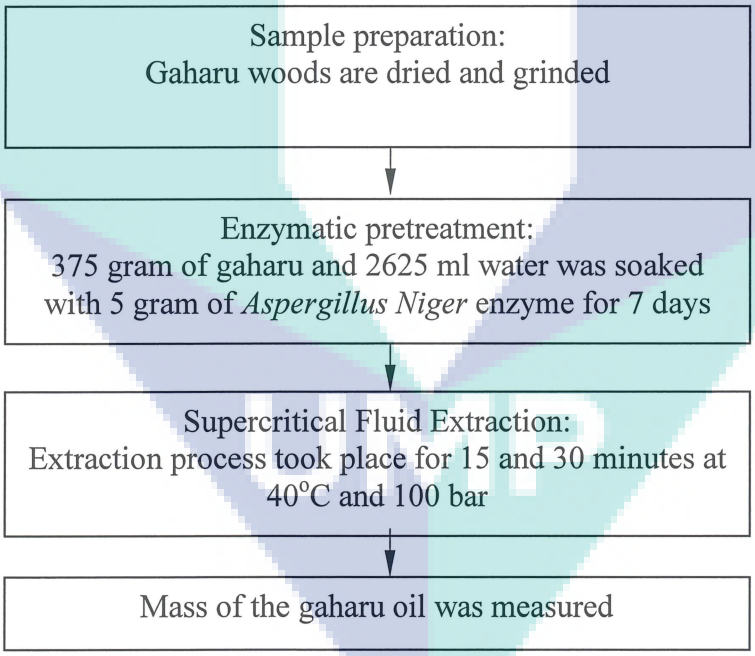



Figure 3.2: Experimental procedure for Supercritical Fluid Extraction

# CHAPTER 4

# RESULTS AND DISCUSSIONS

## 4.1 Chemical Component of Gaharu Oil Analysis

In this preliminary study, two different gaharu oil samples were collected from a cottage industry with the objective to analyze their chemical components. The samples were the gaharu from C grade. Both of their chemical components were different in color, odor and the durability even though they have been processed by the same hydro distillation process method and batch sample. Consumers claimed that the 'Reddish brown' colour sample is of a lower quality of agarwood oil, while the 'Greenish brown' has a higher quality of oil. A preliminary study of organic constituents for the samples have been tested using the GC and GC/MS in order a further investigation can be made to study the process parameter effect on the quality and quantity of the oil. In most essential oil, the distinctive aromas comes from the complex mixture of organic constituents may undergo chemical alteration and less stable when subjected to high temperature.

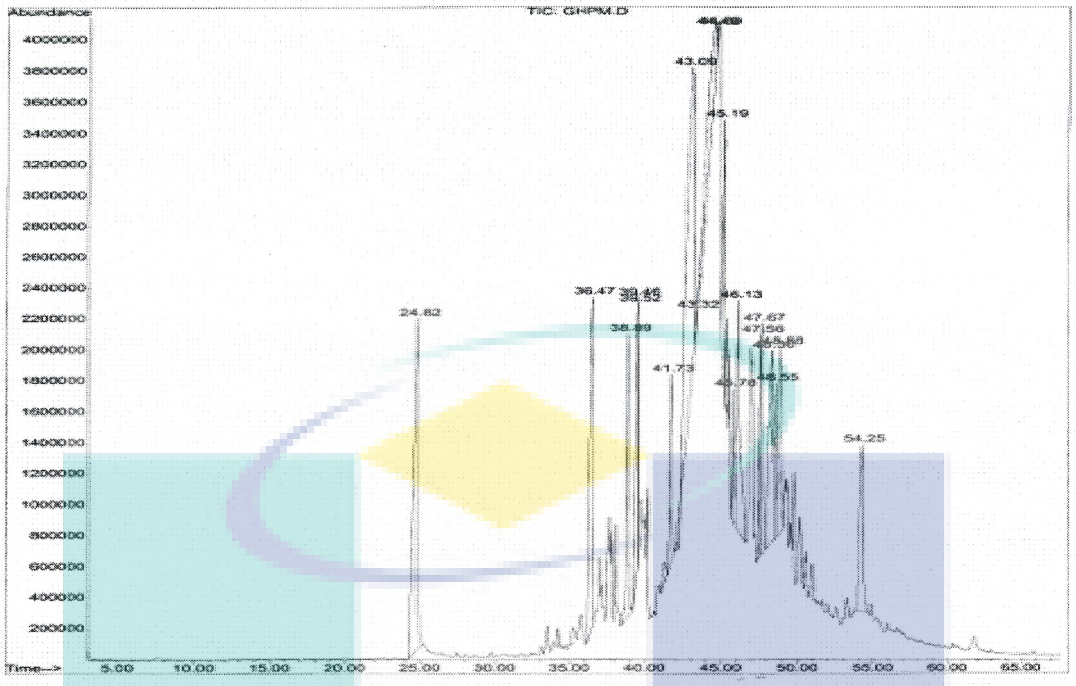



Figure 4.1: GCMS of Gaharu Reddish brown

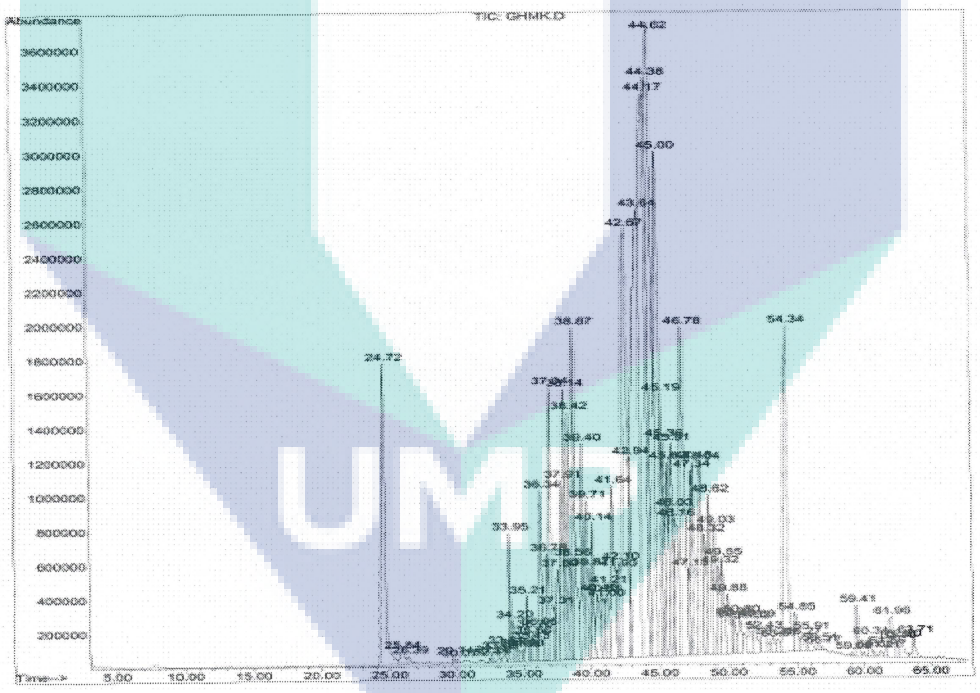



Figure 4.2: GCMS of Gaharu Greenish brown

From the GC and GC/MS analysis result (Table 4.1), agaruspirol (13.49%) and selin-4,7(11)-diene (13.11%), were identified as major components of agarwood oil (Reddish brown), meanwhile 'Greenish brown' sample consists of delta-selinene (11.11%) and rotundone (8.37%) as the major component. Other constituents traced that contribute to the mixture complexity are as in Table 4.1. Chang et al. (2002) reported that several chemical compounds such as agarospirol, guaiene, jinkohol and jinkohol II have been detected in Malaysian gaharu oil. This preliminary study shows that Reddish brown Sample consists of 15.4% of agarospirol and 0.88% guaiene. Meanwhile Greenish brown sample consists of 7.76% agarospirol and 1.71% guaiene. Neither jinkohol nor jinkohol II was detected from both samples. A research done by Meier et al. (2003) stated that the main constituents of Aquilaria agallocha were agarospirol (12.1%) and jinkoh-eremol (10.0%). According to Jantan and Abdul Razak (1990), the distillation of leaves, wood and barks, the factors such as moisture content, size of plant material, steam pressure and duration of distillation may have some influence on the yield. During the distillation, the direct contact of plant material with the hot furnace able to make the plant material getting charred and causing hydrolysis of some constituents of the oil. Ng et al. (1996), cited that the quantity and quality of gaharu produced increase with age, with the best yields occurring in trees aged 50 and above (Sadgopal 1960).

Table 4.1: Main Chemical Constituents Traced in The GC/MS Peak of Both Samples.

| **Reddish brown Sample** | **Area %** | **KI** | **Greenish brown Sample** | **Area %** | **KI** |
|---|---|---|---|---|---|
| 1. 2-Butanone, 4-phenyl | 3.92 | 1252.5 | 1. 2-Butanone, 4-phenyl | 4.52 | 1250.1 |
| 2. β-bulnesene | 2.29 | 1505.6 | 2.α-guaiene | 0.13 | 1441.3 |
| 3. nor-ketoagarofuran | 1.69 | 1558.3 | 3.α-muurolene | 2.94 | 1541.1 |
| 4. epoxybulnesene | 1.84 | 1573.9 | 4.benzyl n-hexanoate | 2.31 | 1519.6 |
| 5. elemol | 1.50 | 1581.1 | 5.delta-guaiene | 1.58 | 1537.5 |
| 6. agaruspirol | 15.4 | 1695.6 | 6.nor-ketoagarofuran | 2.43 | 1557.5 |
| 7. rotundone | 7.86 | 1701.9 | 7.elemol | 1.43 | 1580.6 |

| | | | | | |
|---|---|---|---|---|---|
| 8. selina-3, 11-dien-9-ol | 4.95 | 1710.0 | 8.agarospirol | 7.76 | 1693.5 |
| 9. selina-4, 11-dien-14-oic acid | 2.96 | 1728.0 | 9.kusunol | 7.61 | 1653.5 |
| 10. selina-3, 11-dien-14-al | 2.71 | 1735.1 | 10.selina-3,11-dien-9-one | 6.14 | 1689.0 |
| 11. β-selinene | 4.23 | 1744.0 | 11.eudesmol | 4.28 | 1699.0 |
| 12. guaia-1 (10), 11-dien-9-one | 2.33 | 1752.4 | 12.selina-3,11-dien-14-al | 2.91 | 1738.4 |
| 13.sinenofuranol | 2.02 | 1775.4 | 13.selina-3,11-dien-14-ol | 3.61 | 1747.6 |
| 14.dihydrokaranone | 2.03 | 1796.4 | 14.guaia-1(10),11-dien-15-al | 1.09 | 1806.2 |
| 15.guaia-1(10),11-dien-15-al | 1.57 | 1804.3 | 15.n-butyl n-tetradecanoate | 5.14 | 1976.5 |
| 16.karanone | 2.69 | 1814.4 | | | |

## 4.2 Gaharu Oil Extraction Using Enzymatic Pre-treatment Hydro Distillation

Extraction process with the enzymatic pre-treatment may contribute to improve the efficiency and capacity of extraction for recovery oil product from plant material. Enzyme will break the cell wall which is responsible in accelerating the extraction process. The phenomena facilitate the diffusion of solute from samples into the solvent.

In order to demonstrate the utility of enzymatic as improved method, the approach has been applied in this research using water as extractant to extract oil from gaharu.

Effective application of enzymatic pre-treatment hydro distillation requires consideration of a number of variables. While time remains important in extraction process, temperature also must be considered to maximize the effectiveness of the process. These variables have been studied in this research to investigate the effectiveness of the proposed method.

In the first set of experiment, the extraction efficiencies of extraction time were compared. In the second set of experiment, the optimum temperature was determined.

The amount of extracted gaharu oil was determined in each experiment, and percentage of gaharu oil extracted was calculated.

Percentage of gaharu oil extracted is defined as follow:

$$\text{Percentage of gaharu oil extracted (\%)} = \frac{\text{mass of gaharu oil extracted}}{\text{mass of gaharu oil in samples}} \times 100\%$$

Extractions were performed at durations of 6, 9 and 12 hours , and temperature of 80, 100, 120, 140 and 160 $^{o}$C respectively.

### 4.2.1 Influence of Extraction Time on Hydro Distillation With and Without Enzymatic Pre-treatment

Investigations were carried out to examine the effect of extraction time on gaharu oil extracted for hydro distillation with and without pre-treatment process. Three durations were tested in both processes.

The findings were shown in figure 4.3 which shows the percentage of gaharu oil extracted for various durations. It was clear from the figure that the amount of gaharu oil extracted using hydro distillation showed an increasing trend with the increase of extraction time. Both methods show similar trend.

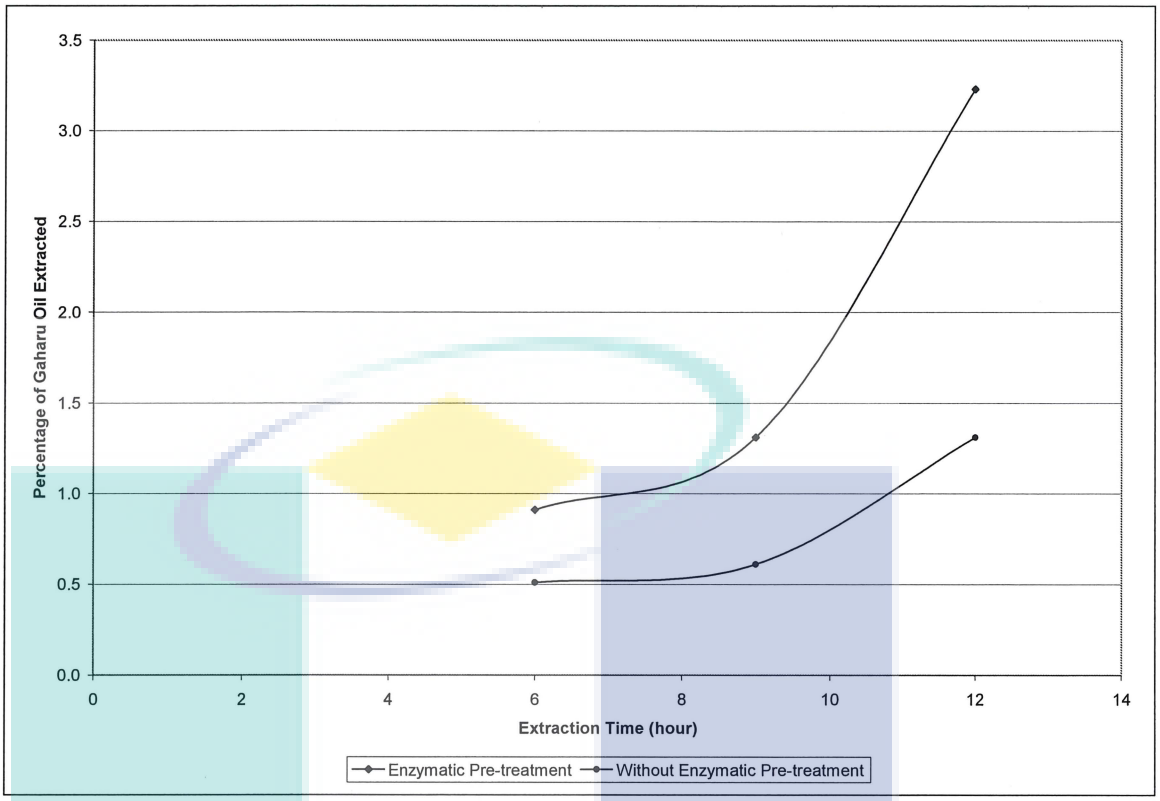



Figure 4.3: The effect of extraction time on gaharu oil extracted using hydro distillation with and without pre-treatment process

The increment of extraction time was intended to increase the amount of mineral oil extracted due to the longer duration allowed for the process to take place. In the range studied, maximum percentage of mineral oil extracted (3.23%) was obtained at 12 hours using enzymatic pre-treatment process. Meanwhile, at the lowest extraction time (3 hours) examined, the percentage of mineral oil extracted was 0.51% (without enzymatic pre-treatment).

The optimum condition has not being established for this set of experiment. Therefore, higher duration is needed for the extraction to reach the optimum stage during extraction process. The percentage of gaharu oil is expected to increase with further increase of duration until it reaches plateau condition. After plateau condition was achieved, this variable has no significant effect on the gaharu oil extracted.

Figure 4.3 indicates that both methods are applicable to extract gaharu oil. However, gaharu oil extracted was higher when enzymatic pre-treatment was employed. The percentage of improvement in recovering gaharu oil using enzymatic pre-treatment for all the investigated duration is shown in Tables 4.2

Table 4.2: Percentage of improvement in recovering gaharu oil using enzymatic pre-treatment hydro distillation compared to without enzymatic pre-treatment at various extraction time

| Extraction time (hour) | Percent Improvement (%) |
|---|---|
| 6 | 44 |
| 9 | 53 |
| 12 | 59 |

Enzymatic pre-treatment that breaks the cell wall managed to improve the percentage of gaharu oil extracted within the range of extraction time investigated in this study. This phenomenon increases the mass transfer process where the solid particles become more exposed to the water. Furthermore, disruptions of cell wall released the essential oil easily.

### 4.2.2 Influence of Temperature on Enzymatic Pre-treatment Hydro Distillation

Temperature is an important factor to enhance the extraction process. Therefore, the variation of temperature range from 80 to 160$^{o}$C was tested to study the effect of the temperature on gaharu oil extracted using enzymatic pre-treatment hydro distillation. Figure 4.4 shows the results of varying the temperature on gaharu oil extracted.

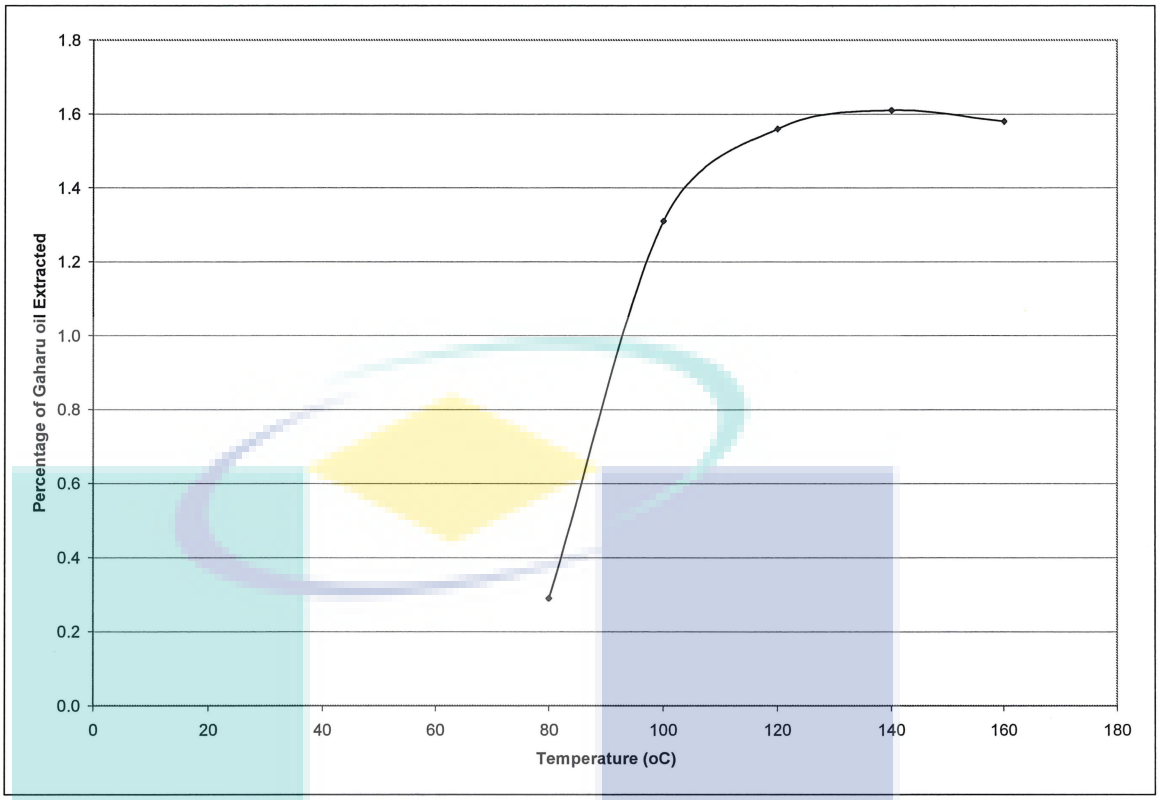



Figure 4.4: The effect of temperature on gaharu oil extracted using enzymatic pre-treatment hydro distillation process

As can be seen, the amount of gaharu oil extracted gradually increased as the temperature time increased up to 120°C. The result indicates that the extraction gained the highest extraction of gaharu oil (1.61%) for temperature of 140°C. However, as the temperature was increased, the percentage of gaharu oil extracted decreased.

High liquid temperature caused the water molecules to have high kinetic energy and hence the molecules diffuse faster into solid samples, thus increased the rate of extraction.

In the event of increasing the temperature of the water above 140°C contributes to high solvent losses through evaporation of water. As a result, the amount of water left in the flask was less compared to its amount at the beginning of the extraction process. Besides that, the evaporated solvent carried along some extracted gaharu oil to the surrounding.

As a conclusion, the optimum temperature with the highest percentage of extraction was 140°C.

## 4.3 Influence of Extraction Time on Supercritical Fluid Extraction

Experiment on Supercritical Fluid Extraction also conducted to study the reliability of this new technique. Supercritical extraction is a method that use the solvent at supercritical condition. $CO_2$ is used as solvent in this extraction technique. The $CO_2$ is pressurized to be converted into an effective solvent. Supercritical Fluid Extraction was able to extract 11.41% gaharu oil within 15 minutes. It is faster method compares hydro distillation method. However, the high capital cost and highly-skilled labor skill requirements of the supercritical carbon dioxide extraction limits the widespread application of this extraction process.

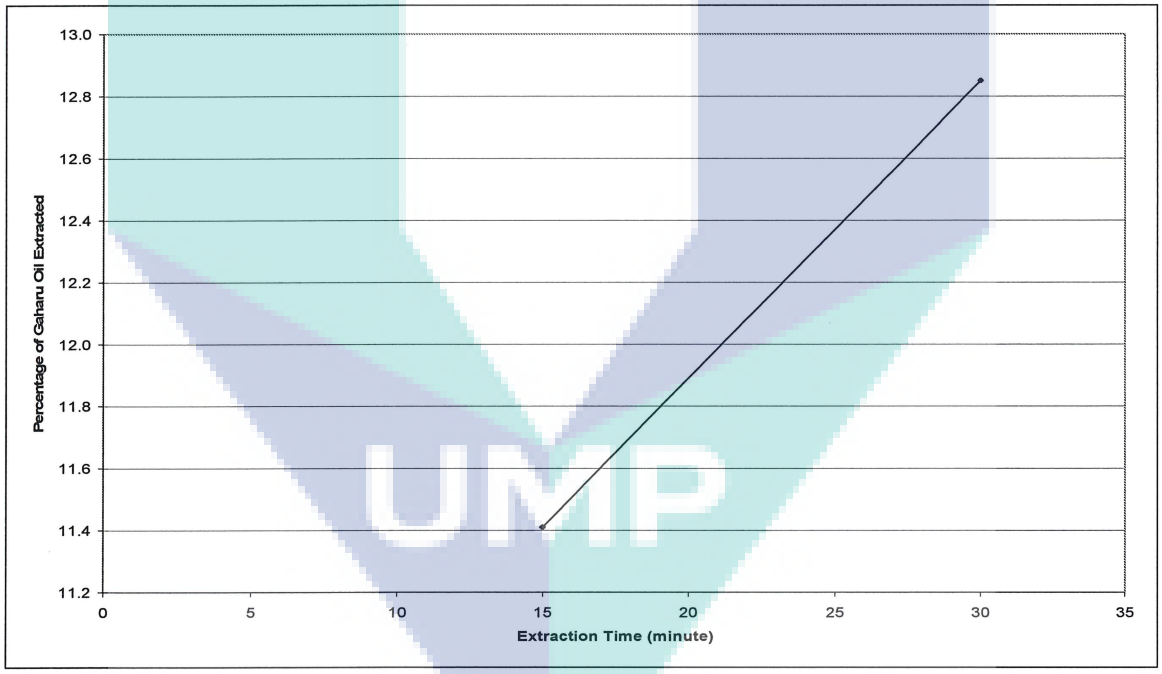



Figure 4.5: The effect of extraction time on gaharu oil extracted using Supercritical Fluid Extraction

# CHAPTER 5

# CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Conclusions

Generally, the study was carried out to determine the feasibility of enzymatic pre-treatment hydro distillation to enhance extraction of gaharu oil. The effects of the extraction time and temperature on the percentage of gaharu oil extracted were examined.

The main conclusions than can be drawn from this study are summarized as followed:

1) Enzymatic pre-treatment hydro distillation provides high extraction yield (an increase of up to 59%) compared to without enzymatic pre-treatment hydro distillation. The enhancement was achieved by the breakage of gaharu cell's wall, hence reduce the mass transfer resistances and releases the cell's contents into the extraction medium during the distillation process. Thereby; the oil is released easier in the subsequent separation process.

2) Supercritical Fluid Extraction was able to extract gaharu oil faster compared to hydro distillation method. However, the high capital cost and highly-skilled labor skill requirements of the supercritical carbon dioxide extraction limits the widespread application of this extraction process.

## 5.2 Recommendations

Recommendations are made to suggest future work which can be performed to give a better understanding and improvement of the gaharu oil extraction system. Below are some recommendations for future work:

1. The gaharu samples must be kept agitated otherwise it may settle in the bottom of the still and become damaged by the heating.
2. In order to produce superior quality of gaharu oil, oxygen may inject into decanter to enrich the main chemical constituents with oxygen compound.
3. Ultrasonic is the most simple and versatile method for the disruption of cells and the production of extracts. Ultrasonic cavitations create shear forces that break cell walls mechanically and improve mass transfer. Ultrasonic can be use to enhance the gaharu oil extraction process.
4. Agaruspirol (13.49%) and selin-4,7(11)-diene (13.11%), were identified as major components of agarwood oil (Reddish brown), meanwhile 'Greenish brown' sample consists of delta-selinene (11.11%) and rotundone (8.37%) as the major components. The number of chemical constituents between the two samples is quite different even though the two samples were extracted from the same process and raw materials. Therefore, universal standard for profiling of essential oils from *Aquilaria malaccensis* (Gaharu) using known marker compounds should be develop so that qualities of essential oils can be made quantitatively.

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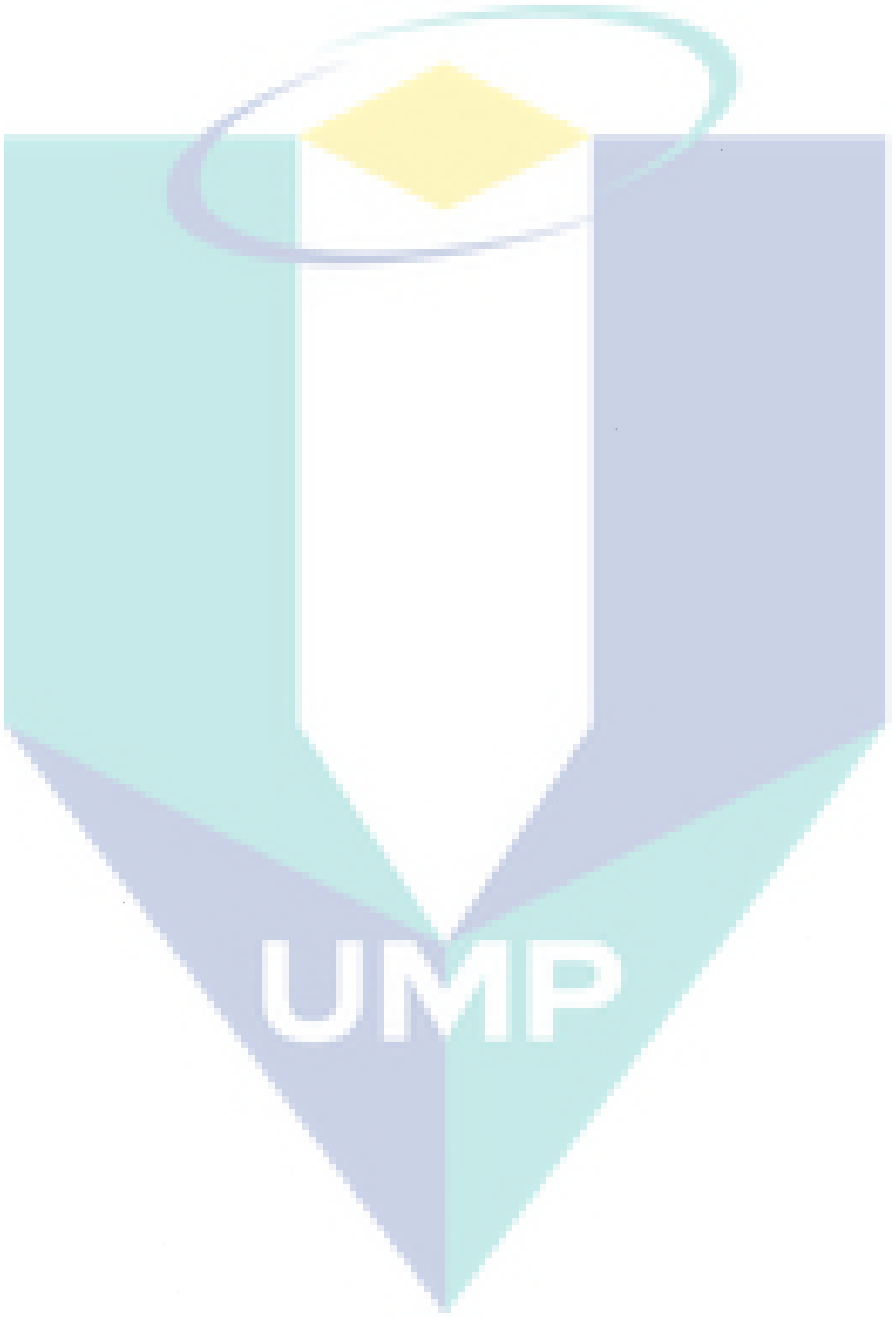
# APPENDIX

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