

The investigation of different extraction techniques to extract gaharu oil

by

Abdul Mudzil bin Mahamod

**Dissertation submitted in partial fulfilment of
the requirements for the
Bachelor of Engineering (Hons)
(Chemical Engineering)**

JANUARY 2009

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Bandar Seri Iskandar
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CERTIFICATION OF APPROVAL

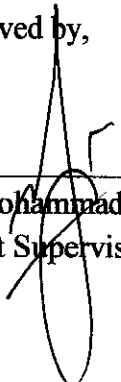
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A project dissertation submitted to the
Chemical Engineering Programme
Universiti Teknologi PETRONAS
in partial fulfilment of the requirement for the
BACHELOR OF ENGINEERING (Hons)
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Approved by,



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ABSTRACT

Gaharu or agarwood, scientifically known as *aquilaria malaccensis*, is one of the most expensive woods in the world. Analysts have differentiated the quality of gaharu based on its commercial grade, at which it ranges from A Super, A, B and C. Due to its rarity and high demand, agarwood extract (gaharu oil) brings high prices. The price is around RM 30 000 per litre for lower grade and superior grades could be priced up to RM 60 000 per litre. It is anticipated that the prices of gaharu will remain high in the future because of the high demand for gaharu material in Arabic countries, introduction of new applications for gaharu materials in the cosmetic industry and the traditional users of gaharu in China, Japan and India for manufacturing joss-sticks and other.

Traditionally, gaharu oil is extracted by distilling the grinded gaharu sample in a copper still. However, the process is less effective as the yield of oil is relatively small and it acquires high temperature. This project is mainly about investigating the alternatives to traditional hydro distillation to extract gaharu oil. Experiments are to be conducted to study the effectiveness of ultrasonic technology, microwave technology, solvent extraction and supercritical fluid extraction (SFE) in terms of oil yield and other related parameters. Proposed methodology is carried out to accomplish the aims of the project.

Three extraction methods were selected, namely as solvent extraction and ultrasonic extraction, and steam distillation as control. The gaharu chips were initially grinded into small particles and mixed with different type of solvent for extraction. For solvent extraction, petroleum ether is not suitable used as solvent at specified temperature (35°C) due to its dissimilarity in polarity as the chemical compounds for the raw material obtained were mainly sesquiterpenes. For ultrasonic extraction, the highest oil yield recovery is 0.1% at 100% power output of ultrasonic bath with temperature of 50±2°C. Several modifications on the parameters experimented need to be done to produce higher oil yield recovery.

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CHAPTER 1: INTRODUCTION

1.1 PROBLEM STATEMENT

Zakaria (2006) reported that the extraction of gaharu essential oil using spinning band distillation (batch distillation) and water as solvent at heating rate and temperature cut ranging from 20 – 40% and 25 - 100°C respectively was studied. The size of gaharu particle that will be used is < 1.00mm. Result obtained after the experiment is 0% of oil yield in parameter stated. This extraction technique is not suitable to extract the gaharu essential oil at specified parameter.

Hence, this project will study the effectiveness of other gaharu oil extraction methods inclusive of ultrasonic technology, microwave technology, solvent extraction and supercritical fluid extraction (SFE) as alternatives to replace traditional steam or hydro distillation. As the commercialization of gaharu product is growing, the project would assist in providing the best possible extraction methods for higher economic profit.

1.2 BACKGROUND OF STUDY

The data results from National Forestry Inventory 4 (NFI-4) conducted in 2002 showed that the *Aquilaria spp.* stock population is about 3.06 million trees with volume estimation of 1.83 million m³ ranging from 15 cm diameter in size and above. The data also indicated high percentage of trees with diameter range of 15 – 45 cm which is 95% from total number of trees surveyed, which also can be estimated to be about 66.8% from total volume of agarwood in Malaysian forestry. Kelantan shows the highest number of trees population while Pahang shows the highest estimated volume of agarwood.

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 its rarity and high demand, agarwood extract (gaharu oil) brings high prices. The price is around

RM 30 000 per litre for lower grade and superior grades could be priced up to RM 60 000 per litre. 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 cosmetic industry and the traditional users of gaharu in China, Japan and India for manufacturing 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 (*Mazni, 2007*).

1.3 OBJECTIVES AND SCOPE OF WORK

The objectives of this study are:

- To investigate the oil yield of each extraction technique, inclusive of
 - Conventional method; steam distillation
 - ultrasonic technology
 - microwave technology
 - solvent extraction
 - supercritical fluid extraction (SFE)
 - soxhlet extraction
- To investigate the most economical extraction technique of gaharu oil by tabulating the comparison between extraction techniques as listed above.

This one-year project includes literature review of the researches, articles and information related to the topic. Apart from that, experimental work would be carried out to analyze the oil yield and other related parameters of each extraction method for comparison.

CHAPTER 2: LITERATURE REVIEW

2.1 GENERAL OVERVIEW OF GAHARU

2.1.1 History of Gaharu

Agarwood or just Agar (from the Malay gaharu) is the resinous heartwood from *Aquilaria* trees, large evergreens native to Southeast Asia. The trees occasionally become infected with mould and begin to produce an aromatic resin in response to this attack. As the infection grows, it results in a very rich, dark resin within the heartwood. It is this precious resinous wood that is treasured around the world. The resin is commonly called Gaharu, Jinko, Aloeswood, Agarwood or Oud and is valued in many cultures for its distinctive fragrance, thus it is used for incense and perfumes. One of the reasons for the relative rarity and high cost of agarwood is the depletion of the wild resource.

2.1.2 Molecular Structure of Gaharu

Jinkohol (2b-hydroxy-(+)-prezizane) was characterized by Nakanishi *et al.* (1980) following work on a benzene extract of Indonesian agarwood imported via Singapore. In 1983, two new sesquiterpenes: jinkoh-eremol and jinkohol II were characterized by the same author from "*Aquilaria* sp; (not *A. agallocha*) probably *A. malaccensis* Benth." from agarwood "collected in Indonesia and imported via Singapore", which they called "type" B agarwood, reserving the description "type A" for agarwood from *A. agallocha*.

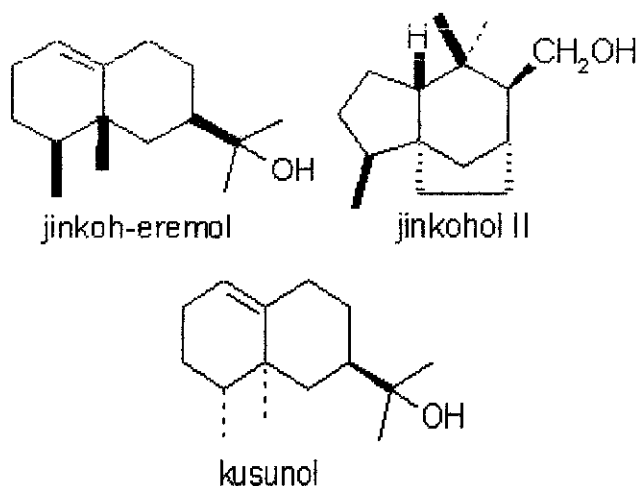


Figure 1: Molecular structure of *aquilaria malaccensis*

Nakanishi *et al.* (1980) reasoned that “type B” agarwood contained nootkatane (e.g. kusunol, jinkoh-eremol), spirovetivane (e.g. agarospirol) and tricyclic (+)-prezizane (e.g. jinkohol, jinkohol II) type sesquiterpenes, and that these structural types also occur in vetiver oil, and may evolve from a common precursor. The authors also indicated that four compounds possessed intense odors (agarospirol, kusunol, jinkohol & jinkohol II) and **“appear to be the source of the fragrance of agarwood”**.

Subsequently Nakanishi *et al.* (1984) isolated alpha-agarofuran, (-)-10-epi-gamma-eudesmol and oxo-agarospirol as major constituents from “type B” agarwood. Yoneda *et al.* (1984) were able to list the major sesquiterpenes of agarwood from type A and type B agarwoods imported from Indonesia and Vietnam through Singapore. *A. agallocha* (agarwood type A) was found to contain b-agarofuran 0.6%, nor-ketoagarofuran 0.6%, agarospirol 4.7%, jinko-eremol 4.0%, kusunol 2.9%, dihydrokaranone 2.4%, and oxo-agarospirol 5.8%. In type B agarwood the following compounds were identified a-agarofuran, (-)-10-epi-g-eudesmol 6.2%, agarospirol 7.2%, jinkohol 5.2%, jinko-eremol 3.7%, kusunol 3.4%, jinkohol II 5.6%, and oxo-agarospirol 3.1%. From their findings the authors concluded that type A wood exclusively contains nor-ketoagarofuran and dihydrokaranone, but does not contain (-)-10-epi-gamma-eudesmol, jinkohol and jinkohol II, findings which might be used to distinguish the products.

2.1.3 Gaharu Cultural Value

Agarwood gained great cultural and religious significance in ancient civilizations around the world. Agarwood is usually classified as Black Agar Wood (Grade-1), Brown Agar Wood (Grade-2), Brownish Yellow Agar Wood (Grade-3) and Yellow Agar Wood (Grade-4). Often, agarwood is used as a raw material in perfume and incense making. Natural carvings can be made from it by cutting out the wood portion into special artistic shapes. Agarwood bark was used as sachpat, a writing material immune to insect attack used in writing religious scriptures. Wood with or without resin content has been used for boxes, musical instruments and interior or veneer. The inner fibrous bark is used as a raw material for

clothing, belts and ropes. This can also be used as an anti-biotic preservative. It is the best preservative in making high quality perfumes.

It was also one of the most important ingredients used in Egyptian mummifications. It can be dusted on clothes and skin as a repellent against fleas and lice. It has been used in painting formal attire of palaces in China and Korea. Agar is also said to relieve general pain, dental pain, to check vomiting, as a venom repellent, and also as a medicine for kidney disorders and rheumatism. Agar wood has been used to enhance cerebral function, balancing of mind - body coordination through nervous system (*gardenpoint.org*, 2008).

2.1.4 The Production of Gaharu in Malaysia

Latest move by Peninsular Malaysia Forestry Development to develop a uniform national grading system for the fragrant resin has marked another milestone for Malaysia to expedite the commercialization of agarwood. They highlight that the absence of a standardized grade of agarwood has hampered administration and regulation of the non-timber forest product. Currently, the 10% royalty payment for revenue from gaharu is based on weight, not on the quality, hence resulting in the government to lose out on revenue collection. They are recommending four grades: A Super, A, B and C which would be presented at the next state forestry directors' conference for consideration and adoption (Chiew, 2007).

In 2007, the government has capped export of gaharu at 200 tonnes per year. In 2004, all eight *aquilaria* species and a species of *Gyrinops* that also produces aromatic resins were included in Appendix II of the Convention on International Trade in Endangered Species (CITES) to ensure survival of the species in the wild. A listing in Appendix II subjects trade in the species to the CITES permit system that covers export, import and re-export. Since it began issuing CITES export permits for the substance in 2002, the Malaysian Timber Industry Board (MTIB, the CITES management authority for timber and timber-related products) has registered exports of 1.3 million tonnes of woodchips and sawdust. The highest volume was 357 tonnes in 2003. Initially doubted by the CITES Secretariat which demanded to know the justification for the quota as it was felt to

be too high, Malaysia escaped trade suspension when it convinced the Secretariat that the precautionary principle was applied in determining the harvest quota and that it would not diminish wild populations. The argument was based on data from the 4th National Forestry Inventory which showed that there to be at least three million stands of *Aquilaria*, mostly *Aquilaria malaccensis*, in peninsula forests. The quota was derived from a conservative estimation that only 10% of the matured trees would be impregnated with the resin.

Malaysia is working towards a more comprehensive management plan with new information on the biology and trade level and aims to submit the information to CITES by year-end. However, oil products from gaharu still escape CITES scrutiny due to its exclusion from the Customs export prohibition order. However, it is revealed that the Customs and Excise Department is in the final stage of amending the order to control the export of processed gaharu in oil form, which indicates that gaharu derivatives like oil and the high-grade, resin-embedded wood would be subjected to declaration under the export prohibition order.

2.1.5 **Commercialization of Gaharu in Malaysia**

Being initially deemed as worthless, the commercialization of gaharu oil products in Malaysia is expected to increase as numerous researches on it are being currently carried out, due to its high market value in worldwide. The value of first-grade agarwood is extremely high. A whole range of qualities and products is on the market varying with geographical location and cultural deposition. Prices range from a few dollars per kilo for the lowest quality to over thirty thousand US dollars for top quality oil and resinous wood. *Aquilaria crassna* is listed as an endangered species in Viet Nam, and *A. malaccensis* is listed as endangered by the World Conservation Union, IUCN.

Based on the research conducted by CITES, 20 countries are actively involved in gaharu trade and business, at which Malaysia and Indonesia are the largest producers currently. From 1995 to 1997, Malaysia has produced approximately 340 tonnes of gaharu from peninsula, and 530 tonnes from Sarawak, at which the derivatives of gaharu are largely exported to Singapore, Taiwan and Middle East

countries. However, the statistics may be largely less from the actual number of production of gaharu, based on the information from Malaysian Nuclear Agency (MINT) analysts. Besides, statistical data (CITES, 1997) shows that Southeast Asians countries have exported more than RM48 million to Saudi Arabia, at which 26% of it may come from Malaysia. Globally, gaharu trading officially amounts more than RM200 million worldwide.

Chakrabarty et al (1994) stated that the lowest grade of Malaysian agarwood could be obtained for USD19/kg in the Middle East. The high grades, normally reserved for exclusive buyers, can cost up to USD 9,589/kg. More expensive grades are also available and can sell for as much as USD 27,400/kg. The *A. malaccensis* tree is comparative to gold and is becoming very rare in the wild due to illegal extraction. Thus, the species has been CITES listed in Appendix II. The listing subjects the species to limited commercial use and stringent monitoring through a permit system. Under CITES rules, the Management Authority will issue CITES Export Permit to exporters of gaharu which originate from Malaysia, CITES Import Permit to importers of gaharu, and CITES Re-Export Certificate to traders of this species which originate from other countries. The Importers are requested to furnish the CITES Export Permit from the exporting country before the Management Authority can issue the CITES Import Permit.

In Malaysia, two states, namely Kelantan and Terengganu have already put a step ahead from other states in taking serious efforts to commercialize gaharu production. Kelantan through its State Economic Planning committee has initiated incentives for researchers and investors by setting aside 404ha state-owned integrated agarwood plantation in Gua Musang. The offers, remarked by the state inventory of about 1.6 million agarwood trees of various species of which many are growing in the wild at forest reserves and plantation, would boost up the state economy due to the high demand of gaharu itself, especially from Middle East Countries, such as Saudi Arabia, Qatar and Yaman (Mcintyre, 2008).



Figure 2: Gaharu Distillation Plant in Gua Musang, Kelantan

In general, Malaysia, located in Southeast Asia, experiences tropical weather which is suitable for agarwood plantation in a bigger scale. Gaharu derivatives from Malaysia are famously known due to its high aromatic oil and resin quality and its abundant sources of agarwood available in forestry. At this point moment, the Malaysian analysts are working hard to conduct researches in resin production via artificial inoculation.

2.1.6 Forestry Law

Following on from the resolutions of the 13th CITES meeting in Bangkok in Oct 2004, the EU Commission has a new regulation (Commission Regulation (EC) No 1332/2005 of 9 August 2005) amending Council Regulation (EC) No 338/97 on the protection of species of wild fauna and flora, by regulating trade therein (this follows on from the resolutions of the 13th CITES meeting in Bangkok in Oct 2004). For our purposes, and considering only the aromatic species affected, it is a reclassification whereby *Aquilaria* spp. (except for *A. malaccensis*, which was already listed in Appendix II), *Gyrinops* spp. and *Gonystylus* spp. (previously listed in Appendix III) were included in Appendix II* to the Convention.

The regulation affects all parts and derivatives of the above species, except: (a) seeds, spores and pollen (including pollinia); (b) seedling or tissue cultures

obtained in vitro, in solid or liquid media, transported in sterile containers, and (c) cut flowers of artificially propagated plants.

[*Appendix II includes species not considered to be under the same threat as those in Appendix I, but which may become so if trade is not regulated. International trade in these species is monitored through a licensing system to ensure that trade can be sustained without detriment to wild populations. Appendix III contains species that are not necessarily threatened on a global level, but that are protected within individual states where that state has sought the help of other CITES Parties to control international trade in that species].

This outcome may give a little more muscle in the fight to combat the "eco-mafia" who makes a trade out of smuggling protected species. However as is stated elsewhere in this data-base, licenses are easily fabricated at point of export by the "eco-mafia" and dispatched to the receiving customers of these illicit goods.

States in Peninsular Malaysia have been told to keep a close eye on extraction of the heartwood by emphasizing on enforcement of Section 15 of the National Forestry Act 1984, which requires any removal of the valuable product to be accompanied with a removal pass. All states are encouraged to pay attention to the forest product that is coveted by both local and foreign poachers.

Former Kelantan forestry director, Datuk Dahlan Taha claimed that greater awareness upon all forestry activities has led to better protection of the heartwood against illegal collection, as indicated by zero arrest in the last two years. This includes routine enforcement efforts at checking stations within logging areas, at which special attention are also being paid to gaharu. The last arrest was made in 2004, when one poacher was found with 2.5kg of gaharu. The biggest haul was in 2003 where five persons were nabbed in three separate cases that led to the recovery of 100kg of gaharu (*Chiew, 2007*).

2.2 EXTRACTION TECHNIQUE AND RECENT TECHNOLOGY

2.2.1 Steam Distillation

2.2.1.1 Introduction

Steam distillation, or also known as hydro-distillation, is a special type of distillation for *temperature sensitive* materials like natural aromatic compounds. Many organic compounds tend to decompose at high sustained temperatures. Separation by normal distillation would then not be an option, so water or steam is introduced into the distillation apparatus. By adding water or steam, the boiling points of the compounds are depressed, allowing them to evaporate at lower temperatures, preferably below the temperatures at which the deterioration of the material becomes appreciable. If the substances to be distilled are very sensitive to heat, steam distillation can also be combined with vacuum distillation. After distillation the vapors are condensed as usual, usually yielding a two-phase system of water and the organic compounds, allowing for simple separation (*Wikipedia*, 2008).

When a mixture of two practically immiscible liquids is heated while being agitated to expose the surfaces of both the liquids to the vapour phase, each constituent independently exerts its own vapour pressure as a function of temperature as if the other constituent were not present. Consequently, the vapour pressure of the whole system increases. Boiling begins when the sum of the partial pressures of the two immiscible liquids just exceeds the atmospheric pressure. In this way, many organic compounds insoluble in water can be purified at a temperature well below the point at which decomposition occurs. For example, the boiling point of bromobenzene is 156 °C and the boiling point of water is 100 °C, but a mixture of the two boils at 95 °C. Thus, bromobenzene can be easily distilled at a temperature 61 C° below its normal boiling point (*Wikipedia*, 2008). Inevitably, steam distillation process is the simplest extraction technique. Yet, the process of

extracting gaharu oil using steam distillation takes several hours with low yield, thus making the process less effective (*Mazni, 2007*).

Steam distillation, hydro distillation and simultaneous distillation–extraction methods are known to be the most common methods for the extraction of essential oils. It is known that conventional methods used for the extraction of essential oils from plant materials have some disadvantages mainly concerned with the quality of the final product. Losses of some volatile compounds, low extraction efficiency, degradation of unsaturated or ester compounds through thermal or hydrolytic effects and toxic solvent residue in the extract may be encountered using these extraction methods (*Ferhat et al., 2007*). Moreover, these extraction methods are time-consuming. Therefore, new technologies have been developed for obtaining essential oils. [*Bayramoglu, 2007*]

2.2.1.2 Apparatus and Procedure

Regarded as the simplest extraction method, agarwood and water can be simultaneously boiled to extract its oil. Due to its simplicity, it is often used as a controller method when experiments on other type of extractions are being conducted.

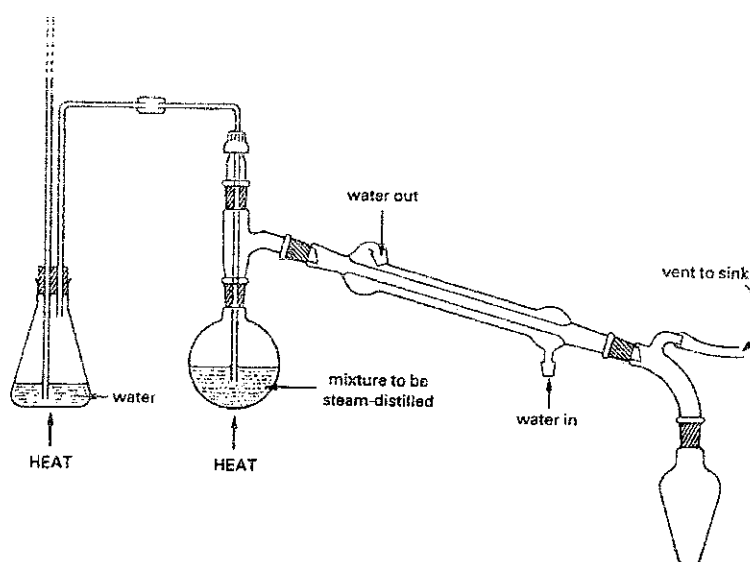


Figure 3: Typical Apparatus of Steam Distillation

Many researchers were using conventional methods of extraction, commonly steam distillation as reference method and comparison to another technique of extraction. Among of them are *Zhang et al* (2007), *Veličković et al* (2007), *Lucchesi et al* (2004) and *Bayramoglu* (2007) on different types of reagents and materials.

In extracting oil from flaxseed as researched by *Zhang et al* (2007), the crushed flaxseed powder (10 g) was mixed with *n*-hexane in a flask. The flask was put into the water bath with a controlled temperature. After each extraction experiment, the extracts were filtered through the Whatman No. 1 filter paper under vacuum, and then the solution was collected and concentrated with a rotary evaporator (RE-2S, Beijing Jing Zhi Jie Laboratory Apparatus Co. Ltd., China) to acquire the flaxseed oil. The acquired flaxseed oil was further dried in a vacuum dryer to remove the residual *n*-hexane.

In comparison to the solvent-free microwave extraction (SFME) method being studied by *Lucchesi et al* (2004), 500 grams of each aromatic herb were submitted to hydro-distillation with a Clevenger-type apparatus according to the European Pharmacopoeia and extracted with 6 L of water for 4.5 h (until no more essential oil was obtained). The essential oil was collected, dried under anhydrous sodium sulphate and stored at 0°C until used.

Veličković et al (2007) in his study on the ultrasonic extraction of waste solid residues from the *Salvia* sp. essential oil hydro distillation also reported in their study that the ground herb material (400 g plus 5 L of distilled water) was submitted to hydro distillation in a Clevenger-type apparatus equipped with a 10 L distillation flask for 2 h. The essential oil was separated from the aromatic water, dried over anhydrous Na₂SO₄, diluted by ethanol (1 mL of the oil and 99 mL of ethanol) and immediately analyzed. The hydrosol was separated by

vacuum filtration and the remaining SPM was dried in a well-aired place in a thin layer for 5 days. The moisture content, determined by drying at 105 °C to constant mass, was about 12% for the SPM of both plant species.

2.2.2 Ultrasonic Extraction

2.2.2.1 Introduction

Ultrasound, the term used to describe sounds ranging from 20 kHz to 1 GHz, is usually generated by a transducer which converts mechanical or electrical energy into high frequency vibrations. *Vinatoru* (2001) and *Romdhane and Gourdon* (2002) reported that the enhancement of extraction efficiency of organic compounds using ultrasound is attributed to a phenomenon called cavitation produced in the solvent by the passage of an ultrasonic wave. They found that cavitation bubbles are produced and compressed during the application of ultrasound, allowing higher penetration of the solvent into the raw plant materials and intracellular products released by disrupting the cell walls. *Albu et al.* (2004) and *Rostagno et al.* (2003) reported that ultrasound has been shown to aid extraction in a number of plant materials by significantly reducing extraction time and increasing maximum extraction yield, respectively (*Sun and Wang*, 2008).

Recent studies have shown that the ultrasound-assisted extraction can enhance the extraction efficiency through acoustic cavitation and some mechanical effects. Acoustic cavitation can disrupt cell walls facilitating solvent to penetrate into the plant material and allowing the intracellular product release. Another mechanical effect caused by ultrasound may also be the agitation of the solvent used for extraction, thus increasing the contact surface area between the solvent and targeted compounds by permitting greater penetration of solvent into the sample matrix. Therefore the main advantages of ultrasound-assisted extraction include the reduced extraction time and reduced solvent consumption. In addition, ultrasound-assisted extraction can be

carried out at a lower temperature which can avoid thermal damage to the extracts and minimize the loss of bioactive compounds (Zhang *et.al*, 2007).

Currently, there is no extensive research on ultrasonic-assisted extraction (UAE) to extract gaharu oil, while several researches including ultrasonic extraction of ferulic acid from *Ligusticum chuanxiong* that was conducted by Sun and Wang (2008), ultrasound-assisted extraction of oil from flaxseed by Zhang *et.al.* (2007) and ultrasonic extraction and separation of anthraquinones from *Rheum palmatum* L. conducted by Wang *et.al.* (2007) would provide thorough experimental procedures for ultrasonic extraction on gaharu oil.

2.2.2.2 Apparatus and Procedures

Current literature review on experimental procedures of extracting oil from flaxseed reveals that For the UAE experiments, a 250 W, 20 kHz ultrasonic emulsifier (88-1, Institute of Acoustics, Chinese Academy of Sciences, China) with a 2.00 cm flat tip probe was used (Figure 3).

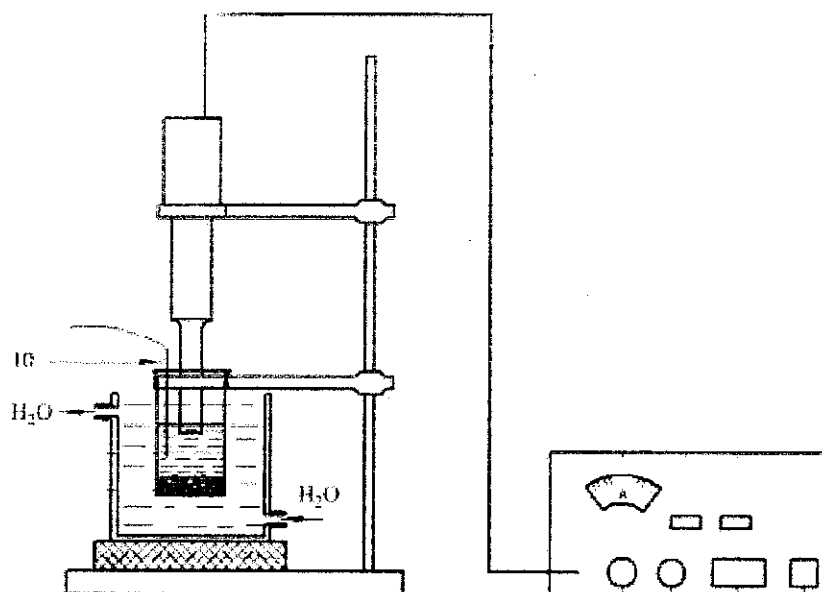


Figure 4: Flat tip probe for ultrasonic-assisted extraction (UAE)

The ultrasonic output power could be set to a desired level ranging from 0 to 100% of the nominal power by the amplitude controller. Ultrasonic output powers were determined calorimetrically and ranged from 10 to 50 W according to the method described by Li et al. (2007). The ultrasound-assisted extraction used in this study was similar to that described by Zhao et al. (2007), with a little modification. The flaxseed powder was mixed in 100 mL *n*-hexane contained in a 200 mL plastic beaker. The ultrasonic probe was inserted into the mixture directly. The samples were extracted under continuous ultrasonic waves at 20 kHz at different levels of power output. During extraction, the temperature was controlled at a desired level within ± 1 °C. The post-treatment of the extracts was the same as that mentioned in the conventional extraction. In this study, all the experiments were performed in triplicates, and the results reported here are the means of the three trials.

2.2.3 Microwave Extraction

2.2.3.1 Introduction

Microwaves are high frequency electromagnetic radiation with a typical wavelength of 1mm to 1 m. Many microwaves, both industrial and domestic, operate at a wavelength of around 12.2 cm (or a frequency of 2.45 GHz) to prevent interference with radio transmissions (Zlotorzynski, 1995). Microwaves are split into two parts, the electric field and the magnetic field component. These are perpendicular to each other and the direction of propagation (travel) and vary sinusoidally. Microwaves are comparable to light in their characteristics. They are said to have particulate character as well as acting like waves. The ‘particles’ of microwave energy are known as photons. These photons are absorbed by the molecule in the lower energy state (E_0) and the energy raises and electron to a higher energy level (E_1). Since electrons occupy definite energy levels, changes in these levels are discrete and therefore do not occur continuously. The energy is said to be quantized. Only charged particles are affected by

the electric field component of the microwave. The Debye equation for the dielectric constant of a material determines the polarisability of the molecule. If the charged particles or polar molecules are free to move, this causes a current in the material. However, if they are bound strongly within the compound and, consequently, are not mobile within the material, a different effect occurs. The particles re-orientate themselves so that they are in-phase with the electric field. This is known as dielectric polarization (*Jacob and Boey, 1995*). [*Dean et al., 1999*]

Microwave heating has been recently used for the isolation and analysis of essential oils ([*Lucchesi et al., 2004a*], [*Lucchesi et al., 2004b*] and [*Lucchesi et al., 2007*]). Solvent-free microwave extraction (SFME) is a new technique which combines microwave heating with dry distillation at atmospheric pressure for the isolation and concentration of the essential oils in fresh plant materials. SFME has been used to obtain essential oils from three different spices (ajowan, cumin, and star anise) (*Lucchesi et al., 2004a*), three different aromatic herbs (basil, garden mint, and thyme) (*Lucchesi et al., 2004b*), and cardamom seed (*Lucchesi et al., 2007*). However, there are no reports on the solvent-free microwave extraction of essential oils from gaharu (agarwood). In SFME method, there is no need to add any solvent or water if fresh plant material is used. If dry plant material is used, the sample is rehydrated by soaking in water for some time and then draining off the excess water. In the present study, the applicability of SFME in the extraction of essential oil from *aquilaria malaccensis* would be examined. The effects of microwave power and extraction time on the yield and composition of the product are investigated. The results would be compared with those obtained with the hydro distillation method, which would be performed as control. The essential oils obtained at different conditions were analyzed both qualitatively and quantitatively (*Bayramoglu et al., 2007*).

2.2.3.2 SFME Apparatus and Procedure

Solvent free microwave extraction has been performed in a Milestone “DryDist” microwave laboratory oven. This is a multimode microwave reactor 2455MHz with a maximum delivered power of 1000W variable in 10W increments. The dimensions of the PTFE-coated cavity are 35 cm × 35 cm × 35 cm. During experiments, time, temperature, pressure, and power can be controlled with the “easy-WAVE” software package. Temperature was monitored by a shielded thermocouple (ATC-300) inserted directly into the sample container and by an external infrared (IR) sensor. Temperature was controlled by a feedback to the microwave power regulator. The SFME apparatus is illustrated in Figure 4. The experimental SFME variables have been optimized by the univariate method in order to maximize the yield of essential oil. In a typical SFME procedure performed at atmospheric pressure, 250 g of fresh plant material was heated using a fixed power of 500W for 30 min without added any solvent or water. A cooling system outside the microwave cavity condensed the distillate continuously. Condensed water was refluxed to the extraction vessel in order to provide uniform conditions of temperature and humidity for extraction. The extraction was continued at 100°C until no more essential oil was obtained. The essential oil was collected, dried under anhydrous sodium sulphate and stored at 0°C until used (*Luccessi et al.*, 2004).

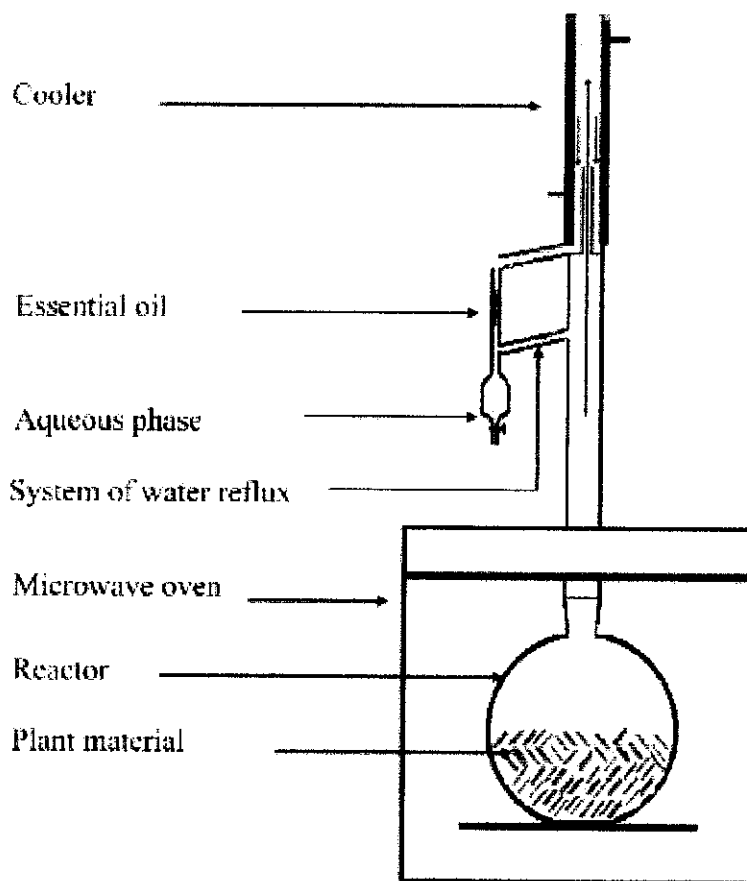


Figure 5: Apparatus for Solvent-free Microwave Extraction (SFME)

2.2.4 Solvent Extraction

2.2.4.1 Introduction

Solvent extraction is the process of separating one constituent from a mixture by dissolving it into a solvent in which it is soluble but in which the other constituents of the mixture are not (Isaacs *et al.*, 1989), or at least less soluble. The separation process is performed by the process of percolation, where a solvent is passed through the mixed solids and, thus, the analyte which is soluble in the solvent is extracted into that solvent. If the solvent used is water, then this process can be termed leaching (Thorpe and Whitely, 1940). When the term solvent extraction is used, it is usually taken as meaning extraction involving two or more liquids, i.e. liquid-liquid extraction. In liquid-liquid extraction, the solution containing the desired constituent must be immiscible with the liquid used to extract the desired constituent

(Isaacs *et al.*, 1989). When the extraction process has occurred, the phase which contains the extracted analyte is known as the extract phase; while the sample from which the analyte has been removed is named the refined phase (*Ulicky and Kemp*, 1992).

The extraction of an analyte from one phase into a second phase is dependent upon two main factors: solubility and equilibrium. The principle by which solvent extraction is successful is that 'like dissolves like'. That is, to remove a polar solute from a solution a polar solvent should be used. Qualitative predictions can be made on the likely success of an extraction by considering the polarity of the analyte of interest and of the two solvents used. Uncharged solutes are more easily extracted into nonpolar organic solvents and the less polar the solute the more efficient the extraction process. If the solute is charged, then it is usually best to form an ion-pair with a counter ion and extract the newly formed neutral complex into a nonpolar solvent. (*Holden*, 1999).

2.2.4.2 **Apparatus and Procedures**

Holden (1997) described that one of the most important decisions when using any solvent extraction system is the selection of the solvent to be used. The properties which should be considered when choosing the appropriate solvent are: selectivity; distribution coefficients; insolubility; recoverability; density; interfacial tension; chemical reactivity; viscosity; vapour pressure; freezing point; safety and cost (*Price*, 2007). Table 1 presents an example of the typical processes which are involved in a solvent extraction process.

Table 1: Example of the typical processes of Solvent Extraction

Steps	Example
1. Prepare a solution of the solutes to be separated. If the solute is in a solid matrix, then a solution may be prepared.	Put air filter into a solution of cyclohexane and place in an ultrasonic bath. The analyte, PAHs, will extract into the cyclohexane solution along with any polar compound.
2. Adjust solution to ensure most efficient extraction conditions. This may involve preparing derivatives of the solute or adjusting the chemistry of the solution (e.g. pH) to maximise the difference in solubility of the solute between the two solvents.	
3. Add a second solvent to the system which is immiscible with the initial solvent that the solute is dissolved in.	Add DMF containing 3% water, which will extract the polar compound but not the PAHs.
4. Shake the mixture in a sealed, stoppered container.	Shake
5. Allow the mixture to stand and the two phases to separate into two distinctive layers.	
6. Collect each layer separately and analyse as required.	Collect the cyclohexane layer and dry with anhydrous sodium sulphate. This fraction will contain the PAHs.

2.2.5 Supercritical Fluid Extraction (SFE)

2.2.5.1 Introduction

A supercritical fluid is any substance at a temperature and pressure above its thermodynamic critical point. It can diffuse through solids like a gas, and dissolve materials like a liquid. Additionally, close to the critical point, small changes in pressure or temperature result in large changes in density, allowing many properties to be “tuned”. Supercritical fluids are suitable as a substitute for organic solvents in a range of industrial and laboratory processes. Carbon dioxide and water are the most commonly used supercritical fluids, being used for decaffeination and power generation respectively (*Wikipedia*, 2008).

Supercritical fluids extraction (SFE) is an efficient alternative for the extraction of natural substances from foods ([*Mendes et al.*, 2003] and [*Sun and Temelli*, 2006]). Supercritical fluids possess excellent extractive properties such as high compressibility, liquid-like density, low viscosity, high diffusivity (*Lim et al.*, 2002). They have been widely used in many industrial applications, i.e. the decaffeination of coffee, the extraction of hops, the synthesis of polymers, the purification and the formation of nano particles ([*Lim et al.*, 2002], [*Kopcak and Mohamed*, 2005] and [*Machmudah et al.*, 2006]). For extraction of natural substances, supercritical carbon dioxide (SC-CO₂) is generally used. It has a greater ability to diffuse through the ultra fine complex matrix than conventional organic solvents and can be easily separated from the products by depressurizing process. Furthermore, low critical temperature of carbon dioxide means that the SC-CO₂ system could be operated at moderate temperature, preventing the degradation of the substance due to heat induction ([*López et al.*, 2004], [*Vasapollo et al.*, 2004] and [*Machmudah et al.*, 2006]). As a result, the obtained product is pure and of great quality, and thus safe for use as nutritional additives and for pharmaceutical applications (*Mendes et al.*, 2003).

2.2.5.2 Supercritical Fluids

Table 2: Substance useful as supercritical fluids, parameters from Reid *et al.* (1987)

	Critical Temperature, T_c (K)	Critical Pressure, p_c (bar)	Critical compression factor, Z_c	Acentric factor, ω
Carbon Dioxide	304	74	0.274	0.225
Water	647	221	0.235	0.344
Ethane	305	49	0.285	0.099
Ethene	282	50	0.280	0.089
Propane	370	43	0.281	0.153
Xenon	290	58	0.287	0
Ammonia	406	114	0.244	0.250
Nitrous oxide	310	72	0.274	0.165
Fluoroform	299	49	0.259	0.260

Table 2 shows the critical parameters of some of the important compounds useful as supercritical fluids. One compound, carbon dioxide, has so far been the most widely used, because of its convenient critical temperature, cheapness, chemical stability, non-flammability, stability in radio-active application and non-toxicity. Large amount CO₂ released accidentally could constitute a working hazard, given its tendency to blanket the ground, but hazard detectors are available. It is an environmentally friendly substitute to other organic solvents. The CO₂ that is used is obtained in large quantities as a by-product of fermentation, combustion and ammonia synthesis and would be released into the atmosphere sooner rather than later, if it were not used as a supercritical fluid (Clifford, 1999).

Its polar character as a solvent is intermediate between a truly non-polar solvent such as hexane and weakly polar solvents. Because the molecule is non-polar, it is often classified as a non-polar solvent, but it has some limited affinity with polar solutes because of its large

molecular quadrupole. To improve its affinity with polar molecular further, CO₂ is sometimes modified with polar entrainers as is discussed below. However, pure CO₂ can be used for many organic solute molecules even if they have some polar character. It has a particular affinity for fluorinated compounds and is useful for working with fluorinated metal complexed and fluoropolymers (Clifford, 1999).

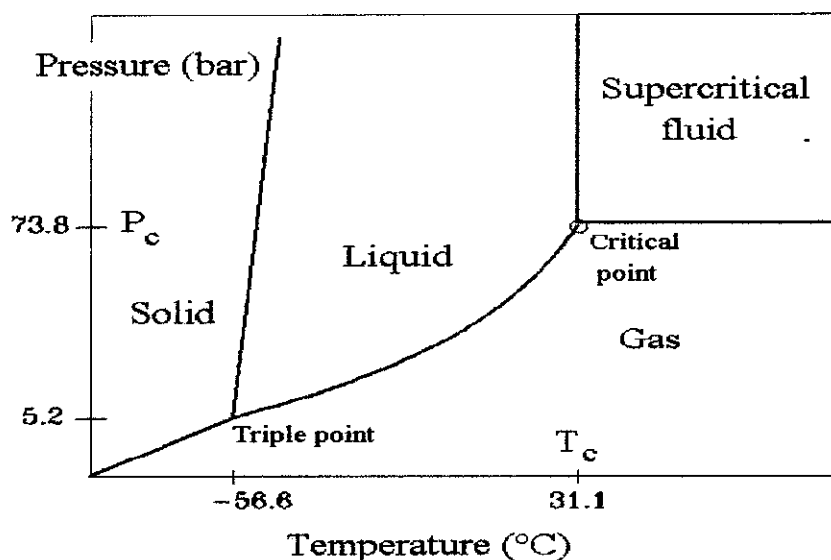


Figure 6: The phase diagram of Carbon Dioxide

CO₂ is not such a good solvent for hydrocarbon polymers and other hydrocarbons of high molar mass. Ethane, ethene and propane become alternatives for these compounds, although they have the disadvantages of being hazardous because of flammability and of being somewhat less environmentally friendly. However, small residues of lower hydrocarbons in foodstuffs and pharmaceuticals are not generally considered a problem. Water has good environmental and other advantages, although its critical parameters are much less convenient and it gives rise to corrosion problems. Supercritical water is being used, at a research level, as a medium for the oxidative destruction of toxic waste. There is a particular interest in both supercritical and near-critical water because of the behaviour of its polarity. Ammonia has similar behaviour, is often considered and discussed, by not often used. Many halocarbons have the disadvantage

of cost or of being environmentally unfriendly. Xenon is expensive, but is useful for small-scale experiments involving spectroscopy because of its transparency in the infrared, for example (Clifford, 1999).

2.2.5.3 Apparatus and Procedures

Machmudah et al (2007) in studying the pressure effect in supercritical CO₂ extraction of plant seeds used the SC-CO₂ extraction apparatus as shown below.

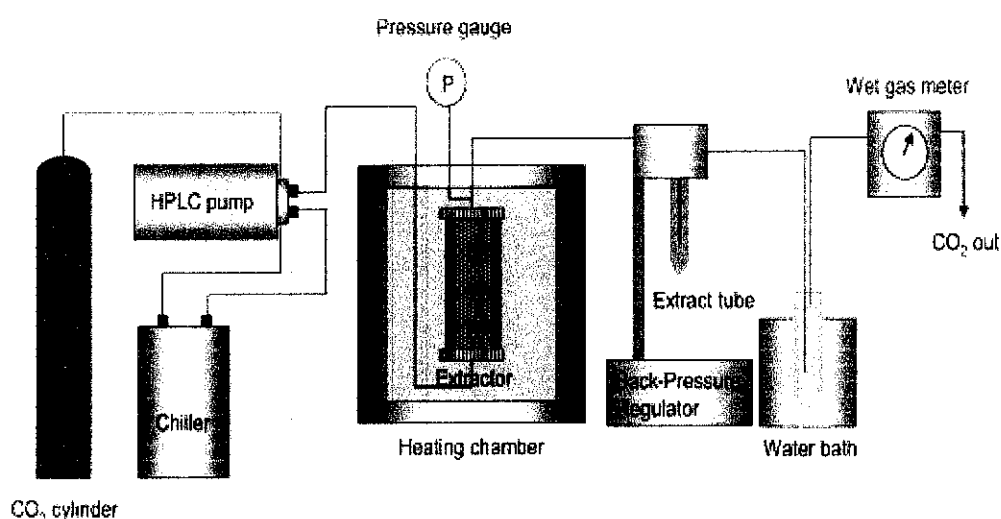


Figure 7: Schematic Diagram of Supercritical CO₂ Extraction System

The apparatus includes a chiller (Cooling Unit CLU-33, Iwaki Asahi Techno Glass, Japan), an Intelligent HPLC Pump (PU-2080-100 MPa, Jasco, Japan) for rosehip and loquat seeds extractions or a Syringe Pump (Model 260D, ISCO, USA) for physic nut extraction, a heating chamber (ST-110, ESPEC Corp., Japan), an extractor (Thar Tech, Inc., USA) with 10 ml in volume for rosehip and loquat seeds extractions or an extractor (AKICO, Japan) with 15 ml in volume for physic nut extraction, a back-pressure regulator (SCF-Bpg, Jasco, Japan) for rosehip and loquat seeds extractions or a back-pressure regulator (880-81 Back-Pressure Regulator, Jasco, Japan) for physic nut extraction, collection vials, and a wet gas meter (Sinagawa Co., Japan). Extraction was conducted under pressures of 15–49 MPa, temperatures of 40, 60

and 80 °C and CO₂ flow rate of 3 ml/min (based on the inlet pump flow rate at room temperature and 0.1 MPa).

In each experiment, approximately 3.5, 4 and 3 g of rosehip, loquat and physic nut samples, respectively, were loaded into a 10 ml extraction vessel and the remaining volume was filled with glass beads in the bottom and upper of the cell. The cell was placed in the heating chamber to maintain the operating temperature. The extract was collected in the vial at every 10, 20, 30 and 40 min for 180 and 300 min, and weighed immediately after the collection. The yield was calculated based on the cumulative mass of extract. To compare the recovery efficiency of SC-CO₂ and conventional extraction process, 8 g of rosehip seeds, 11 g of loquat and 5.5 g of physic nut seeds were extracted with 150 ml of hexane for rosehip and physic nut, and 150 ml of methanol for loquat seeds using a soxhlet apparatus at 80 °C for 8 h then evaporated using Buchi Rotavapor R-205 (*Machmudah, 2007*). Above all, the experiments indicate that the extractions of gaharu using methods described above are possible to be conducted.

2.2.6 Soxhlet Extraction

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. However, a Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only required where the desired compound has a *limited* solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance (*Wikipedia, 2008*).

Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask.

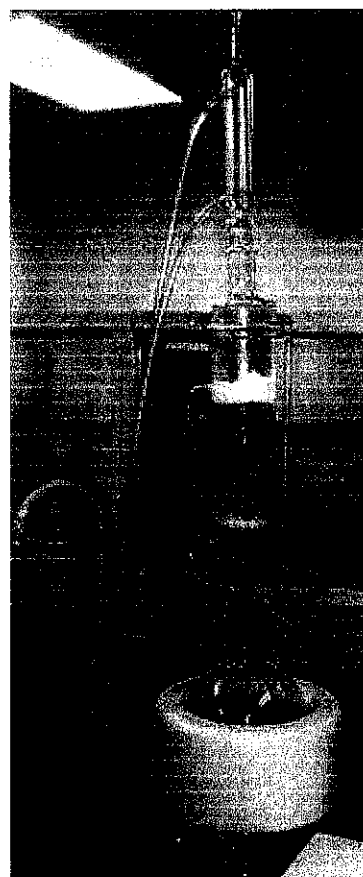


Figure 8: Fruit Extraction in Progress

This cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

2.2.7 Gas Chromatography-Mass Spectrometry

Gas chromatography-mass spectrometry (GC-MS) is laboratory equipment composed of two major parts. The parts are gas chromatography and mass spectrometry. Its main function is to identify different substances during sample test. GC-MS is used widely in crime and forensic investigation, fire investigation,

environmental and pollution analysis, and identification of unknown samples. Similar concept of GC-MS is applied in airport to conduct check on specific substance in luggage or on human beings.

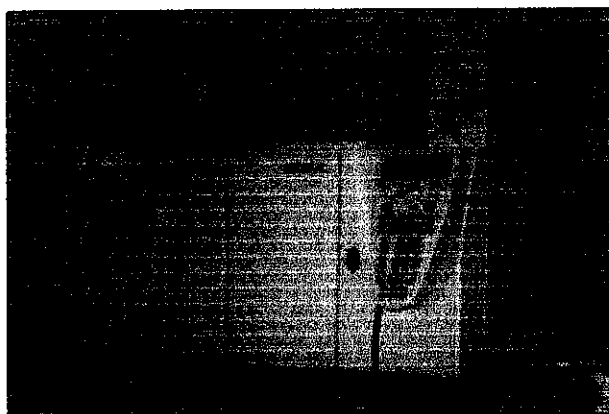


Figure 9: Gas chromatography-mass spectrometry

Nor Azah *et al.* (2008) in their analysis of the comparison of chemical profiles of selected gaharu in Malaysia had used GC-MS analysis was performed using a Hewlett-Packard GCMSD 5890 series II /5971A equipment, on fused silica capillary column DB1 (J & W Scientific 30 m x 0.25 mm, 0.25 μm film thickness). The column oven temperature was programmed from 60°C to 230°C with an increase of 3°C/min. The injector and GC/MSD interface temperatures were maintained at 250°C and 300 °C respectively with helium as carrier gas and electron energy (70eV).

2.2.8 Supercritical Fluid Chromatography

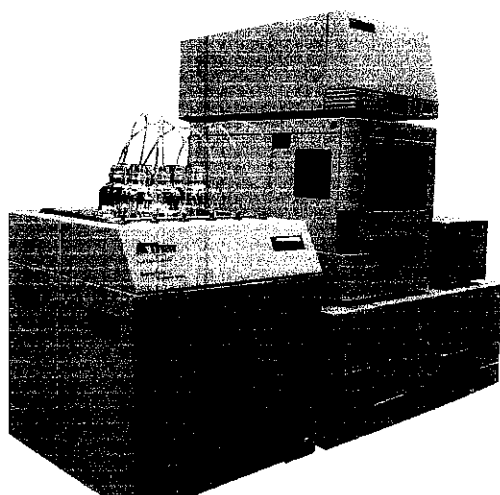


Figure 10: Standard SFC System for Analytical Use

Supercritical Fluid Chromatography is a form of normal phase chromatography that is used for the analysis and purification of low to moderate molecular weight, thermally labile molecules. It can also be used for the separation of chiral compounds. Principles are similar to those of high performance liquid chromatography (HPLC), however SFC typically utilizes carbon dioxide as the mobile phase therefore the entire chromatographic flow path must be pressurized. SFC finds use in industry primarily for separation of chiral molecules, and uses the same columns as standard HPLC systems. SFC is now commonly used for achiral separations and purifications in the pharmaceutical industry (*Wikipedia*, 2008).

SFC utilizes carbon dioxide pumps that require that the incoming CO₂ and pump heads be kept cold in order to maintain the carbon dioxide in a liquid state where it can be effectively metered at some specified flow rate. The chemist uses software to set mobile phase flow rate, co-solvent composition, and column temperature. In addition, SFC provides an additional control parameter, pressure, by using an automated back pressure regulator. From an operational standpoint, SFC is as simple and robust as HPLC but fraction collection is more convenient because the primary mobile phase evaporates leaving only the analyte and a small volume of polar co-solvent. Similar to an HPLC system, SFC can use a variety of detection methods including UV/VIS, mass spectrometry and evaporative light scattering (*Wikipedia*, 2008).

2.2.9 Comparison between All Extraction Techniques

In general, a comparison between all extractions techniques discussed previously is tabled to provide clearer picture on extraction technique available for extraction of gaharu oil. All techniques are compared with hydro distillation, based on numerous researches on each type of extraction. The advantages and disadvantages of each technique are described as below, in Table 3.

Table 3: Table of comparison between all extraction techniques

Technique	Advantage(s)	Disadvantage(s)
Steam Distillation	<ul style="list-style-type: none"> ▪ The simplest method ▪ Only requires cheap and simple apparatus setup with only one or two solvents to be used, depending on the parameters of experiments. ▪ Usually performed as control in any extraction experiment. 	<ul style="list-style-type: none"> ▪ Low oil yield (<i>Mazni,2007</i>) ▪ Time consuming ▪ Exposure to heat may cause degradation of thermally labile compounds
Ultrasonic Extraction	<ul style="list-style-type: none"> ▪ Higher oil yield and lower solvent consumption, compared to conventional method of extraction ▪ Extraction time is significantly shortened compared to hydro distillation ▪ Effective and indeed feasible method for the production of the plant oil ▪ Does not affect the oil composition too much, based on the extraction of oil from flaxseed (<i>Zhang et al, 2007</i>) 	<ul style="list-style-type: none"> ▪ Expensive equipment
Microwave Extraction	<ul style="list-style-type: none"> ▪ Shorter extraction times (30 min for SFME method against 4.5 hours for hydro-distillation) [<i>Lucchesi, 2004</i>] ▪ Substantial saving of energy, compared to hydro distillation method [<i>Lucchesi, 2004</i>] ▪ Reduced environmental burden (less CO₂ rejected in the atmosphere) 	<ul style="list-style-type: none"> ▪ Safety concern, in case of any organic solvent leakage, which may cause fire

Technique	Advantage(s)	Disadvantage(s)
Solvent Extraction	<ul style="list-style-type: none"> ▪ Simple extraction method ▪ Easy to setup the apparatus 	<ul style="list-style-type: none"> ▪ Longer extraction time ▪ Suitable solvent need to be identified first before proceeding experiment
Supercritical Fluid Extraction (SFE)	<ul style="list-style-type: none"> ▪ Unique properties of supercritical fluids, making it suitable for aromatic natural compounds ▪ excellent extractive properties such as high compressibility, liquid-like density, low viscosity, high diffusivity ▪ low critical temperature of carbon dioxide means that the SC-CO₂ system could be operated at moderate temperature, preventing the degradation of the substance due to heat induction ▪ high product purity and quality 	<ul style="list-style-type: none"> ▪ Expensive equipment ▪ Complicated process of method development in SFE
Soxhlet Extraction	<ul style="list-style-type: none"> ▪ Simple extraction method ▪ Easy to setup the apparatus ▪ Only one batch of solvent is recycled, thus saving the solvent consumption and increase the efficiency 	<ul style="list-style-type: none"> ▪ Longer extraction time ▪ Suitable solvent need to be identified first before proceeding experiment

2.3 CHEMICAL PROPERTIES

2.3.1 Description of Agarwood

Agarwood is a resinous wood that sometimes occurs in trees belonging to the *Aquilaria* genus, *Thymelaeaceae* family. *Aquilaria* is a fast-growing, archaic tropical forest tree, which occurs in South and Southeast Asia, from the foothills of the Himalayas to the rainforests of Papua New Guinea. The tree grows in natural forests at an altitude of a few meters above sea level to about 1000 meters, and it grows best around 500 meters. It can grow on a wide range of soils, including poor sandy soil. Seedlings need a lot of shade and water. Trees grow very fast, and start producing flowers and seeds as early as four years old. At least fifteen species of *Aquilaria* trees are known to produce the much sought-after Agarwood. In South Asia *Aquilaria achalloga* is found, particularly in India, *Aquilaria malaccensis* is mostly known from Malaysia and Indonesia, and *Aquilaria crassna* principally grows in Indochina. A number of other species are known such as *Aquilaria grandfolia*, *Aquilaria chinensis* etc (*The RainForest Project*, 2002).

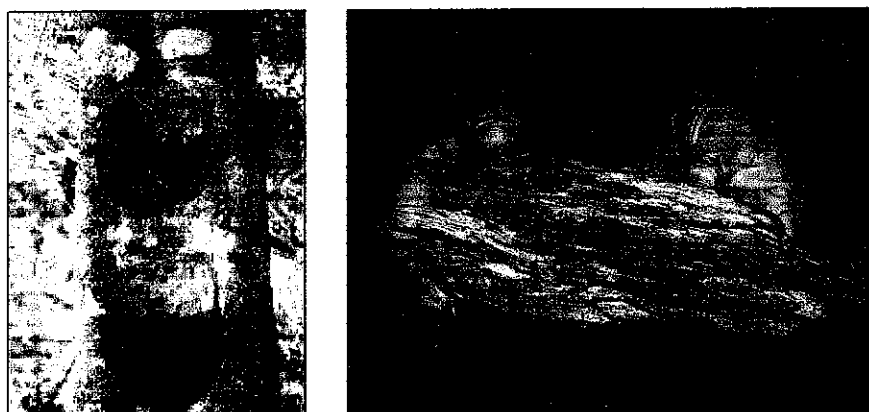


Figure 11: *aquilaria malaccensis*

2.3.2 Uses of Agarwood

Agar wood is the rare and famous, resin containing heartwood produced from old and diseased trees of several *Aquilaria* species of which *A. malaccensis*, *A. crassna* and *A. sinensis* are most important. The fragrance produced by the burning agar wood has been highly valued for thousands of years, and its use as incense for ceremonial purposes in Buddhism, Confucianism and Hinduism is

widespread throughout eastern and southern Asia. The wood is only partly saturated with resin but still fragrant, and occasionally, the wood remaining after distillation, is made into sticks called 'joss sticks' or 'agarbattis' which are burnt as incense. Agar wood oil is an essential oil obtained by water and steam distillation of agar wood, which is used in luxury perfumery. The incense is also used as an insect repellent and for medicines. The timber of undiseased trees, known as 'karas', is very light and is only suitable for making boxes, light indoor construction and veneer (*Angela Barden et al, 2000*).

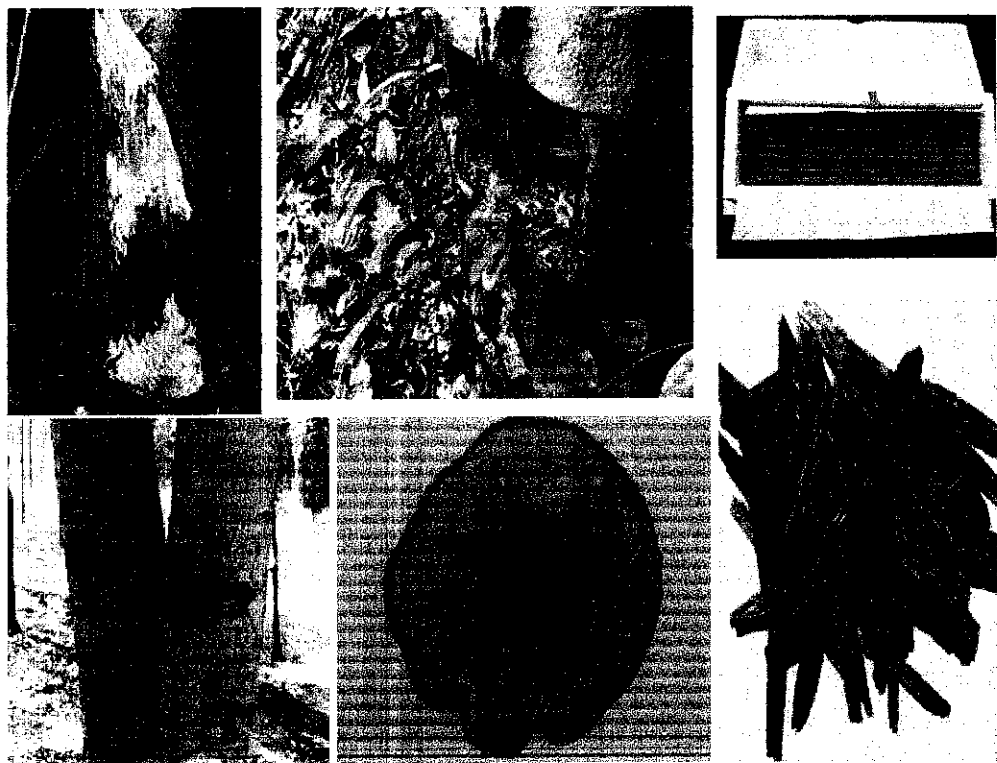


Figure 12: Pictures of agarwood

2.3.3 Formation of Agarwood

Gaharu or agar wood formation is a pathological process taking place in the stem or main branches where an injury has occurred. Fungi are involved in the process, but the process itself is not yet fully understood. Damage by boring insects is often associated with the infection. It is believed that the tree is first attacked by a pathogenic fungus, which causes it to weaken. Infection by a second fungus causes the formation of agar wood, but it is unclear whether it is a product of the fungus or the tree. The fungus implicated in the formation of agarwood in *A.*

malaccensis is *Cytosphaera mangiferae*, while *Melantos flavolives* is assumed to play a similar role in *A. sinensis*. *A. malaccensis* forms an association with *endotrophic mycorrhizal* fungi. In natural forests, only 7-10% of the trees are infected by the fungus (Ng. *et al*, 1997). A common method in artificial forestry is to inoculate the trees with the fungus.

2.3.4 Species of Agarwood

Table 4: The Characteristics of *Aquilaria* species

SCIENTIFIC NAME	NATURAL POPULATION AREA	CHARACTERISTICS
<i>Aquilaria Hirta</i>	Terengganu, Pahang and Johor	Small trees in 15m high, small hair at bottom side of leaves, leaves' shape: <i>ovate oblong</i>
<i>Aquilaria Beccariana</i>	Growing in south peninsula (Johor)	Moderate trees in 20m tall, leaves' colour is grey, clearer leaves' secondary capillary
<i>Aquilaria Rostrata</i>	Pahang (Gunung Tahan), mountains	Secondary capillary is unclear
<i>Aquilaria Malaccensis</i>	Available in the whole peninsula of Malaysia, Sabah & Sarawak, except of Kedah and Perlis	Shining leaves' surface and elliptic
<i>Aquilaria Microcarpa</i>	Johor	Large trees in 36m tall, small fruits in heart shape

(JASBEN PLANTATION, 2008)

2.3.5 Chemical Components

The table below listed main chemical components found in agarwood based on numerous researches worldwide.

Table 5: Sesquiterpenoids in agarwood

Compound	Plant Species	Reference
Kusunol	<i>A. agallocha</i>	Nakanishi et al. (1983b)
	<i>A. malaccensis</i>	Yoneda et al. (1986)
	<i>A. sinensis</i>	Yoneda et al. (1986)
Jinkoh-eremol	<i>A. agallocha</i>	Yoneda et al. (1986)
	<i>A. malaccensis</i>	Yoneda et al. (1986)
	<i>A. sinensis</i>	Yoneda et al. (1986)
(+) -Dihydrokaranone	<i>A. agallocha</i>	Nagashima et al. (1983a)
	<i>A. malaccensis</i>	Nagashima et al. (1983a)
	<i>A. sinensis</i>	Nagashima et al. (1983a)
(-)-10-Epieudesmol	<i>A. agallocha</i>	Nakanishi et al. (1984)
	<i>A. malaccensis</i>	Nakanishi et al. (1984)
Agarol	<i>A. agallocha</i>	Nakanishi et al. (1984)
	<i>A. malaccensis</i>	Nakanishi et al. (1984)
α -Agarofuran	<i>A. agallocha</i>	Maheshwari et al. (1963a)
	<i>A. malaccensis</i>	Maheshwari et al. (1963a)
β -Agarofuran	<i>A. agallocha</i>	Maheshwari et al. (1963a)
	<i>A. sinensis</i>	Maheshwari et al. (1963a)
nor-keto-agarofuran	<i>A. agallocha</i>	Maheshwari et al. (1963a)
3,4-Dihydroxydihydroagarofuran	<i>A. agallocha</i>	Maheshwari et al. (1963a)
8H-Dihydrogmelofuran	<i>A. agallocha</i>	Nakanishi et al. (1984), Pant and Rastogi (1980)
Gmelofunran	<i>A. agallocha</i>	Maheshwari et al. (1963a)
Baimuxinol	<i>A. sinensis</i>	Yang and Chen (1986)
Dehydrobaimuxinol	<i>A. sinensis</i>	Yang and Chen (1986)
Dihydroagarofuran	<i>A. agallocha</i>	Maheshwari et al. (1963a)
4- Dihydroxydihydroagarofuran	<i>A. agallocha</i>	Maheshwari et al. (1963a)
Agarospirol	<i>A. agallocha</i>	Yang and Chen (1986)
	<i>A. sinensis</i>	Yang and Chen (1986)
	<i>A. malaccensis</i>	Nakanishi et al. (1984)
Oxo-agarospirol	<i>A. agallocha</i>	Nakanishi et al. (1984)
	<i>A. sinensis</i>	Nakanishi et al. (1984)
	<i>A. malaccensis</i>	Nakanishi et al. (1984)
Baimuxinic acid	<i>A. sinensis</i>	Yang and Chen (1986)
Jinkohol	<i>A. malaccensis</i>	Nakanishi et al. (1981)
Jinkohol II	<i>A. malaccensis</i>	Nakanishi et al. (1983b)
(+) -Guaia-1(10),11-dien-9-one	<i>A. agallocha</i>	Ishihara et al. (1991b)
(-) -1, 10-Epoxyguai-11-ene	<i>A. agallocha</i>	Ishihara et al. (1991b)

Compound	Plant Species	Reference
(-)-Guaia-1(10),11-dien-15,2-olide	<i>A. agallocha</i>	Ishihara et al. (1991b)
(-)-Rotundone	<i>A. agallocha</i>	Ishihara et al. (1991b)
(-)-Guaia-1(10),11-dien-15-ol	<i>A. agallocha</i>	Ishihara et al. (1991b)
(-)-Guaia-1(10),11-deine-15-carboxylic acid	<i>A. agallocha</i>	Ishihara et al. (1991b)
Methyl guaia-1(10),11-deine-15-carboxylate	<i>A. agallocha</i>	Ishihara et al. (1991b)
(-)-Guaia-1(10),11-dien-15 al	<i>A. agallocha</i>	Ishihara et al. (1991a)
Sinenofuranal	<i>A. sinensis</i>	Xu et al. (1988)
Sinenofuranol	<i>A. sinensis</i>	Xu et al. (1988)

2.3.6 Concluding Remarks

Throughout this project, the species used would be from *aquilaria malaccensis*, which is mostly found in Malaysia and Indonesia for its easier sources accessibility. In Malaysia, the agarwood species from *aquilaria malaccensis* is found mostly in Kelantan. In line with Kelantan initiative to consolidate its position as a hub for the agarwood industry, the source of agarwood trees would be available for researchers and investors, thus providing the source of this project. In fact, several gaharu distilling plants are operating in small-scale in Kelantan, at which the extra information of processing gaharu would be available via survey and site visit.

CHAPTER 3: METHODOLOGY & PLANNING

3.1 KEY MILESTONE

3.1.1 Semester 1

Research works on previous semester were focused on the literature review on each extraction technique, including:

- Supercritical Fluid Extraction
- Solvent Extraction
- Steam Distillation
- Ultrasonic Extraction
- Microwave Extraction

The whole work planning is illustrated in the Gantt chart. (Refer Appendix 1)

While the literature review of each extraction technique was successfully understood based on numerous journals and paper works available local and worldwide, the step-by-step experimental methods were not included. Therefore, the second semester would provide the proposed step-by-step experimental methods based on the literature studied.

3.1.2 Semester 2

This semester's works would be emphasized on three extraction techniques, namely solvent extraction, ultrasonic extraction and steam distillation due to availability of facilities in laboratory. The experimental procedures for each selected technique would be detailed in next section, which is set up based on literature studied on previous reports of extracting essential oil including journals, books and reports from previous students. The related parameters that would be analyzed would also be explained in later section. Due to several limitations on raw material supply, the type of gaharu used would be from grade 'D', which was obtained from Pahang. It is expected to have lesser oil yield and darker oil, if any, due to the lower grade used for experimental works later.

3.2 Planned Methodology

Below is listed the planned methodologies for each extraction technique with reference to the specific journal and research paper works on experimental setup of extracting essential oils from natural plant. Prior to conducting the experimental works, the gaharu chips were ground and granulated into small particles to provide larger surface area. The ground gaharu woods were shown below:

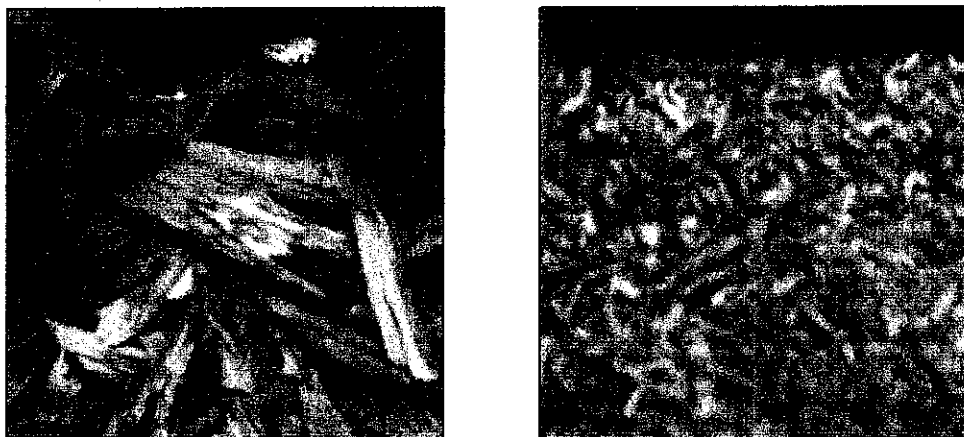


Figure 13: Gaharu chip before and after grinding process

The experimental works were carried on based on the procedures below:

3.2.1 Solvent Extraction

3.2.1.1 10g of gaharu powder is weighed and prepared.

3.2.1.2 100mL of petroleum ether, as solvent, is measured. The ratio of solvent to gaharu is set at 1:10.

3.2.1.3 The gaharu powder is then mixed into the petroleum ether.

3.2.1.4 The solution is put into water bath with controlled temperature at 40°C for two hours.

3.2.1.5 After two hours, the essential oil is collected using rotary evaporator to remove petroleum ether from the impure oil.

3.2.1.6 The volume of essential oil collected is measured.

3.2.1.7 The oil yield is recorded.

3.2.1.8 The experiment is repeated using different solvents; water and ethyl acetate.

3.2.2 Ultrasonic Extraction

3.2.2.1 10g of gaharu powder is weighed and prepared.

3.2.2.2 100mL of ethanol, as solvent, is measured. The ratio of solvent to gaharu is set at 1:10.

3.2.2.3 The gaharu powder is then mixed into the petroleum ether.

3.2.2.4 The solution is put into ultrasonic water bath with controlled temperature at 50°C for four hours.

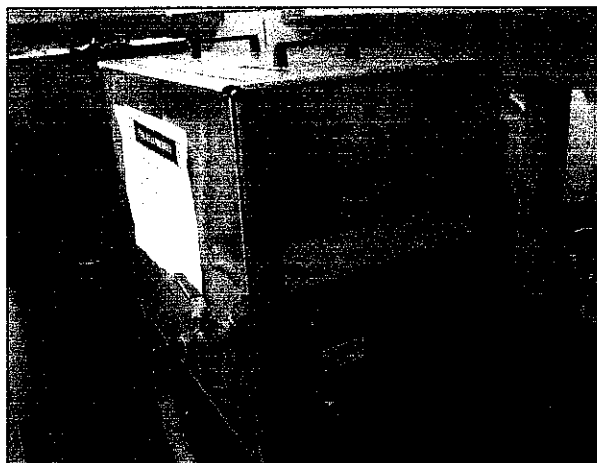


Figure 14: Ultrasonic Water Bath

3.2.2.5 The power output is set at 60%

3.2.2.6 After four hours, the essential oil is collected using rotary evaporator to remove ethanol from the impure oil.

3.2.2.7 The volume of essential oil collected is measured.

3.2.2.8 The oil yield is recorded.

3.2.2.9 The experiment is repeated using different solvents; water and ethyl acetate and different power output; 80 and 100.

3.2.3 Steam Distillation

3.2.3.1 The apparatus was set up as shown in figure below.

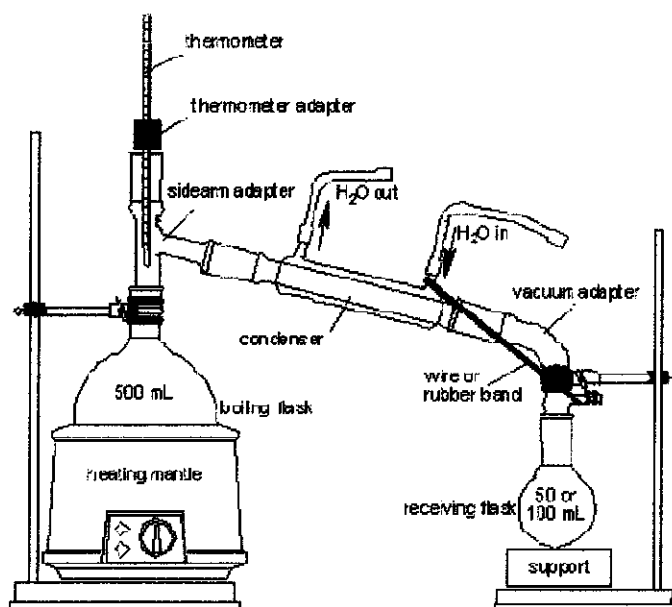


Figure 15: Steam Distillation apparatus

3.2.3.2 10g of gaharu powder is weighed and prepared.

3.2.3.3 The gaharu powder is then mixed into the 500 mL of distilled water in a boiling flask.

3.2.3.4 The water-gaharu solution is allowed to undergo steam distillation for six hours.

3.2.3.5 After six hours, 1g of salt per 10mL of extract is added and dissolved to increase the separation of the essential oil from the water.

3.2.3.6 A separatory funnel is used to extract the oil from the water.

3.2.3.7 Volume of essential oil collected is measured and oil yield is calculated.

3.2.4 Soxhlet Extraction

3.2.4.1 10g of gaharu powder is weighed and prepared.

3.2.4.2 100mL of petroleum ether, as solvent, is measured. The ratio of solvent to gaharu is set at 1:10.

3.2.4.3 The gaharu powder is then mixed into the petroleum ether.

3.2.4.4 The solution is put into round bottomed flask.

3.2.4.5 The water-solvent mixture is then allowed to undergo soxhlet extraction for six hours.

3.2.4.6 After six hours, the essential oil is collected using rotary evaporator to remove petroleum ether from the impure oil.

3.2.4.7 The volume of essential oil collected is measured.

3.2.4.8 The oil yield is recorded.

3.2.4.9 The experiment is repeated using different solvents; water and ethyl acetate.

3.3 **Parameter Analysis**

3.3.1 **Essential Oil Yield**

According to Bayramoglu (2008), essential oil yield can be expressed in terms of the volume of the oil collected in mL per gram of dry plant material.

$$\text{oil yield} = \frac{\text{essential oil collected, mL}}{\text{dry plant material, g}}$$

3.3.2 **Composition of Essential Oil**

Based on the chemical oil analysis conducted by (Azah M.A., et al., 2008), the samples would be injected in the split mode, using pressured controlled helium as the carrier gas at flow rate of 1 mL/min. The gas chromatograph would be programmed initially at 60°C for 10 minutes, then to 230°C at increment of 3°C / min. The relative amounts of individual components are based on peak areas obtained. Retention indices would be determined from the gas chromatogram by logarithmic interpolation between bracketing alkanes using a homologous series of *n*-alkanes as standards and in accordance with established method (Kovats, 1965).

The column oven temperature of GC equipment (whichever available in laboratory) would be programmed from 60°C to 230°C with an increase of 3°C / min. The injector and GC/MSD interface temperature would be maintained at 250°C and 300°C respectively with helium as carrier gas and electron energy (70 eV).

3.3.3 Physical Properties

The physical properties of essential oils collected would also be examined in terms of its colour, odour and aroma.

3.4 Manipulated Variables

The aim is to manipulate these variables to obtain the most optimum essential oil yield, at which different extraction techniques would require certain set of specified manipulated parameters wherever suitable with the extraction method. Throughout this study, the manipulated variables would be specified on steam distillation and solvent extraction techniques.

3.4.1 Extraction Time

The effect of extraction time is essential as one of the main project objectives is to find out the optimal time taken for certain selected extraction method upon experiments in deciding which extraction method is the most economical.

3.4.2 Extraction Temperature

The effect of temperature would only be applicable for other than steam distillation method. This would include the ultrasonic extraction, soxhlet and solvent extraction.

3.4.3 Solvent/Gaharu Ratio

The effect of solvent / gaharu ratio would be studied to determine the efficiency of solvent used with respect to the amount of gaharu wood used. It can be expressed in term of volume / mass.

CHAPTER 4: RESULTS & DISCUSSION

The aim of this work was to find the condition providing the highest oil yield recovery of *aquilaria malaccensis* inside the experimental domain explored, and the results were compared with essential oil composition obtained through steam distillation. Below is the comparison table that showed relevant information pertaining to the experiments that had been conducted so far.

Table 6: Table of Comparison between experimented extraction methods

Methods/ Parameter	Ultrasonic Extraction	Solvent Extraction	Steam Distillation
Oil yield (%)	0.1	0.0	0.0
Time taken	4 hours at 100% power output	-	16 hours (literature)
Solvent (s) tested	Ethanol, Water	Petroleum Ether, Water	Steam (Water)
Solid: Solvent Ratio	1:10	1:10	1:10

Based on the table of data shown above, the ultrasonic extraction had produced the highest oil yield, so far, at 0.1% of oil yield recovery in 4 hours of 100% ultrasonic power output using ethanol as the solvent. The solid to solvent ratio was set constant at 1:10 for all types of experiments. Below is the analysis on each extraction method tested to explain the result of each column listed above in Table 6.

4.1 Solvent Extraction

The following tabulated data showed the results of solvent extraction on obtaining the gaharu essential oil. The time interval was set at 2, 4 and 6 hours for each solvent used at temperature range of $35\pm 1^{\circ}\text{C}$. The solid to solvent ratio used was kept constant at 1:10, at which for all samples, 10g of gaharu powders were used and mixed with 100mL of solvents.

Table 7: Experimental Data on Solvent Extraction

Time interval	Type of solvent	Solvent volume (mL)	Gaharu mass (g)	Volume oil collected (mL)	Oil yield, ($\frac{mL}{g}$)
2 hrs	Petroleum Ether	100	10	0.0	0.0
	Water	100	10	0.0	0.0
4 hrs	Petroleum Ether	100	10	0.0	0.0
	Water	100	10	0.0	0.0
6 hrs	Petroleum Ether	100	10	0.0	0.0
	Water	100	10	0.0	0.0

The results provided that petroleum ether and water produced 0% oil yield recovery. This was determined both by observation made on the samples and the rotary evaporation conducted on each sample where no presence of dark brown oil, as expected from grade D gaharu woods.

Gaharu powder under listed condition above was observed not dissolving into the solvent used. This predicted outcome was explained by the molecular structure of both solvents used, comparing to the chemical components inside gaharu as studied before in literature. **Table 8** below shows the related chemical compounds found in gaharu from different states in Malaysia, as per reported by Azah *et. al.* (2008).

Table 8: Temperature programmed linear retention indices on CBP5, relative to alkanes

Chemical compounds	RI	Selangor (%)	Kelantan (%)	Pahang (%)	Terengganu (%)
3-phenyl-2-butanone	1249	1.50	5.77	7.80	0.79
α -guaiene	1448	-	0.67	-	-
β -agarofuran	1477	1.69	1.98	0.69	0.50
α -agarofuran	1553	4.83	2.96	1.48	1.57
Nor-ketoagarofuran	1557	2.09	-	-	-
10-epi- γ -eudesmol	1618	11.54	9.03	8.10	3.32
Agarospinol	1631	14.86	5.49	7.11	18.86
β -eudesmol	1649	-	-	-	5.74
Jinkoh-eremol	1650	10.62	7.70	6.31	-
Kusunol	1659	18.94	-	-	-
Jinkohol II	1751	4.71			

As the raw material was obtained from Pahang, most chemical compounds were 3-phenyl-2-butanone, 10-epi- γ -eudesmol, agarospirol and Jinkoh-eremol. These chemical compounds are categorized under sesquiterpene group, a complex chemical compound usually found in natural plant. Above all, the principle of solvent extraction requires similar polarity between solvate and solvent, as indicated by the concept of polar-like-polar. Thus, the use of petroleum ether is proved to be not suitable to extract gaharu as it is considered non-polar compounds. Further experiments on different solvents, such as ethanone and ethyl acetate are preferred to enhance the results quality and accuracy.

4.2 Ultrasonic Extraction

The following tabulated data (Table 9) showed the results of ultrasonic extraction on obtaining the gaharu essential oil. The duration was set at 4 hours for each sample, using different type of solvent at constant temperature of $50 \pm 2^\circ\text{C}$.

Table 9: Experimental Data for Ultrasonic Extraction

Power Output (%)	Solvent type	Solvent volume (mL)	Gaharu mass (g)	Volume oil collected (mL)	Oil yield, ($\frac{\text{mL}}{\text{g}}$)
60	Water	100	10	0.0	0.0
	Ethanol	100	10	<i>Wax</i>	-
80	Water	100	10	0.0	0.0
	Ethanol	100	10	<i>Wax</i>	-
100	Water	100	10	0.0	0.0
	Ethanol	100	10	1.0	0.1

It was found that almost all samples produced oil wax when undergoing separation using rotary evaporator. This experiment had highlighted several important issues upon conducting experimental works on gaharu:

4.2.1 Type of solvent

The basis of this experiment, which used water as one type of solvent, had provided insightful knowledge on the suitable parameters that should be applied upon using water as the solvent. The parameters need to be modified included the duration of experimental works and the solid to solvent ratio, which indeed should be implemented to all samples and solvents as well. The result of using water at

50±2°C had produced 0% oil yield, due to the observation made, such as the water and gaharu powders were not fully dissolved and no aroma smell was detected.

On the other hand, the usage of ethanol provided quite an interesting result on the essential oil recovery, at which the three different power output set up (60, 80, 100) produced oil wax at the end of experiments, except for 100% power output where the collected essential oil is about 1 mL. This is in high relation to the further procedures that need to be taken care of when handling the samples after the completion of each experiment, at which many journals states to store the sample of essential oil recovered over anhydrous sodium sulphate in 0°C (incubator). It was believed the step would eliminate, or reduce the possibility of collecting oil wax inside the flask.

4.2.2 Gaharu to solvent ratio

The gaharu to solvent ratio used, which is 1:10, was considered constraining the efficiency of getting good recovery of essential oil. According to Zubair (2008), who had manipulated the solid to solvent ratio which are from 1:8, 1:12, 1:16 and 1:20, the results showed that the gaharu oil yield increased along with the increment of extraction time and solid to solvent ratio. The highest oil yield percentage for solid to solvent ratio is for 1:20 which is 0.139% while 9-hour extraction time produced highest oil yield at 0.169%. While this experiment produced 0.1% of oil yield recovery at 100% power output, the modification on parameter time and solid to solvent ratio can be made to produce higher oil yield.

4.3 **Steam Distillation**

Steam distillation, as set up in figure above was used as control. The mixture of gaharu powders and water were steam-distilled for 6 hours. The distillate collected is about 32 mL, however the presence of essential oil was undetected. This shows the original time taken (6 hours) is not sufficient enough to extract gaharu essential oil. According to Azah *et.al* (2008), the hydro distillation of gaharu was run for 16 hours using Clavenger-type apparatus. Thus, comparing the results, the extraction of gaharu oil using steam distillation at 6-hour operation is not feasible.

CHAPTER 5: RECENT RESEARCH

5.1 RESEARCH IN UTP

Currently, there is no specific research in any level of doctorate that is specifically related to the study on the extraction techniques of gaharu, or agarwood. Also, research on extraction technique on any natural compound seems unavailable to be accessed via information centre. There is one, entitled “*Chlorophyll Extraction from Pan Danusaceae Amaryllifolius Roxb (Pandan Leaves), Pipier Betel Linn (Sirih Leaves) and Codiaeum Variegatum (Red and Yellow Croton Leaves) and Detetermination of Their Metal Composition*” at which the study was conducted by Mohd Nirman Mohd Halil (2001). However, it was inaccessible through information research centre, leaving the author with limited option to enhance research on agarwood extraction techniques. Hence, this study would be the pioneer of other research that is related to agarwood in the future.

5.2 RESEARCH FROM OTHERS

Given the huge potential of gaharu in term of its market profitability and opportunity for further studies especially on its numerous chemical compounds, many local parties including government staffs, lecturers and students take part actively in conducting researches on gaharu in large area of interest. Below are some research conducted within local:

5.2.1 Study on Comparison of Chemical Profiles of Selected Gaharu Oils from Peninsular Malaysia by (Azah M.A., et al., 2008)

This research mainly focuses on the different chemical profiles of selected gaharu oils, where gas chromatographic analysis was conducted and the results were compared to each other, with reference to the literature review. This research is very pivotal in providing insightful knowledge to this project in term of the procedures to conduct GC analysis on gaharu essential oil.

5.2.2 Extraction of Gaharu Essential Oil Using Ultrasonic Assisted Hydrodistillation by (Zubair, 2008)

CHAPTER 9: CONCLUSION

The results and interpretation made represent a huge need in further investigating the optimized parameters to produce higher essential oil yield recovery. These three methods used were proven to be feasible to extract essential oil from gaharu, with several modifications on parameters are needed. The experimental data obtained was proved to be similar with literature data studied in earlier chapters. Based on the experiments, ultrasonic extraction provided the highest oil yield recovery at 0.1% using ethanol as solvent, compared to solvent extraction and steam distillation method.

Upon the completion of the project, the aim on providing the alternative for the current extraction technique is proved fruitful with several modifications to be made to enhance the results accuracy and reliability. Thus, several limitations should be eliminated as to progress for further study on this field. This includes the necessity on using higher grade of gaharu, as the grade D used throughout this project does not deliver the expected outcomes. Therefore, more financial aid would be a great assistance as the gaharu cost, as mentioned in earlier part, could possibly reach RM500 – RM1000 per kg for grade C gaharu woods.

Site visit to the distillation plant is also recommended for any researcher who pursues study in this field. The opportunity to witness the real-life industrial practice would provide deeper understanding on the importance of seeking the alternative for better extraction of gaharu, as mentioned in the project objectives. Consultation on experts is also recommended to know further on the nature of gaharu and its important facts upon undergoing extraction process. This is to correspond to the several limitations on gaharu data, such as its boiling point temperature, the criteria used for categorizing its quality into different grades and others, as found throughout this project.

It is recommended this project to be pursued further by improvising on analytical aspects, including the gas chromatographic analysis, the effect on different type of solvents, and the analysis on economical aspect, including the economic potential and the feasibility of the extraction techniques to be expanded into industrial scale.

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