THE YIELD AND QUALITY OF GAHARU OIL (AQUILARIA MALACCENSIS) 
EXTRACTED BY THREE METHODS

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THE YIELD AND QUALITY OF GAHARU OIL (AQUILARIA MALACCENSIS) EXTRACTED BY THREE METHODS

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A thesis submitted in fulfilment of the requirements for the award of the degree of Master of Engineering (Bioprocess)

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To my beloved families
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ABSTRACT

Gaharu (*Aquilaria malaccensis*) oil and resin are among the most valuable products from forest. The essential oil and resinous wood of gaharu are widely used for their aromatic, fumigatory and medicinal properties. Currently, the main problems in the production of gaharu oil were the issues of the various extraction methods which were not uniform and the lack of establishment of gaharu standard to evaluate its quality. The prices as well as the quality of gaharu oil are arbitrarily determined by traders and clients due to no established standard that can be referred. Therefore, the aim of this research was to study the appropriate extraction methods for the production of gaharu oil. The work focused on the performance of three different extraction methods i.e. Soxhlet extraction, hydro distillation and Accelerated Solvent Extraction (ASE). The results showed that the yield of gaharu oil increased with the increasing of extraction temperature, duration and solvent volume. The ASE method yielded higher percentage (2.28% ± 0.02) of gaharu oil than Soxhlet extraction (1.67% ± 0.01) and hydro distillation (0.18% ± 0.01). Moreover, the colour of ASE oil at elevated temperature was dark brown in comparison to oil from Soxhlet (brownish) and hydro distillation (dark green). Analysis of chemical compounds of the oils extracted via different methods showed a similar pattern of chemical profile but significant difference in the percentage of specific chemical compounds. ASE method was selected for further study, hence the gaharu oil extracted via Soxhlet was used as benchmark in relation to the presence of all expected chemical compound detectable in the gaharu oil at appreciated percentage. ASE method was optimized at the extraction temperature of below 150 °C because increased extraction temperature promoted the degradation of chemical components in the gaharu oil. The optimal parameters of ASE (ASE OPT) were found to be at temperature of 141 °C, duration of 90 min, and solvent volume of 90%. The yield from ASE OPT was 1.74%. Gas chromatopgraphy-mass spectrometer (GC-MS) was used to identify the specific chemical compounds of ASE OPT gaharu oil. The data was highly comparable with Soxhlet extraction result in which the percentage of most of the chemical compounds were significantly higher in the ASE OPT oil i.e. 3-phenyl-2-butanone (1.59%), α-agarofuran (0.97%), 10-epi-γ-eudesmol (10.20%) and agarospirrol (6.72%) than Soxhlet extraction (0.25, 0.22, 0.72 and 5.49% respectively). However α-guaiene (2.64%) were found slightly lower in the ASE OPT gaharu oil compared to gaharu oil of Soxhlet extraction (2.83%). The ASE OPT gaharu oil was categorized under viscous essential oil and it also tends to have heavier aromatic compounds.
ABSTRAK

Minyak dan resin gaharu (Aquilaria malaccensis) adalah di antara produk yang sangat berharga daripada hutan. Minyak pati dan kayu gaharu digunakan secara meluas untuk aromatik, setanggi dan kegunaan perubatan. Pada masa ini, masalah utama dalam pengeluaran minyak gaharu adalah isu-isu pelbagai kaedah pengekstrakan yang tidak seragam dan penghasilan minyak gaharu piawai untuk menilai kualitinya. Harga serta kualiti minyak gaharu ditentukan dengan sewenang-wenangnya oleh peniaga-peniaga dan pelanggan kerana tiada standard yang boleh dirujuk. Oleh sebab itu, tujuan penyelidikan ini adalah untuk mengkaji kaedah pengekstrakan yang sesuai untuk pengeluaran minyak gaharu. Penyelidikan ini memberi tumpuan kepada prestasi tiga kaedah yang berbeza iaitu penyulingan hidro, pengekstrakan soxhlet dan pengekstrakan pelarut dipercepatkan (ASE). Hasil kajian menunjukkan bahawa hasil minyak gaharu meningkat dengan peningkatan suhu pengekstrakan, tempoh dan jumlah pelarut. Kaedah ASE menghasilkan peratusan minyak gaharu yang lebih tinggi (2.28% ± 0.02) daripada pengekstrakan Soxhlet (1.67% ± 0.01) dan hidro penyulingan (0.18% ± 0.01). Selain itu, warna minyak ASE pada suhu tinggi adalah coklat gelap berbanding dengan minyak dari Soxhlet (perang) dan hidro penyulingan (hijau gelap). Analisis bahan kimia minyak yang diekstrak dengan menggunakan kaedah yang berbeza menunjukkan persamaan dalam corak profil kimia tetapi perbezaan yang ketara di dalam peratusan bahan kimia yang tertentu. Kaedah ASE telah dipilih untuk kajian lebih lanjut dan minyak gaharu yang diekstrak melalui kaedah Soxhlet digunakan sebagai penanda aras berhubung dengan kehadiran semua sebatian kimia yang dijangka dikesan dalam minyak gaharu di dalam peratusan yang dihargai. Kaedah ASE dioptimumkan pada suhu pengekstrakan dibawah 150 °C kerana peningkatan suhu mengalakkan kemusnahkan komponen kimia minyak gaharu. Parameter ASE yang optimum (ASE OPT) adalah pada 141 °C, tempoh 90 minit, menggunakan 90% isipadu pelarut. Hasil pengekstrakan minyak daripada ASE OPT adalah 1.74%. Gas chromatopgraphy-mass spectrometer (GC-MS) telah digunakan untuk mengenal pasti bahan kimia didalam minyak gaharu dari ASE OPT. Data yang diperolehi adalah sangat setanding dengan hasil pengekstrakan Soxhlet dimana peratusan kesemua bahan kimia adalah lebih tinggi dalam minyak ASE OPT iaitu 3phenyl-2-butanone (1.59%), α-agarofuran (0.97%), 10-epi- γ-eudesmol (10.20%) dan agarospirol (6.72%) daripada pengekstrakan Soxhlet (0.25, 0.22, 0.72 dan 5.49% masing – masing ). Walau bagaimanapun α-guaiene (2.64%) didapati lebih rendah dalam minyak gaharu ASE OPT berbanding dengan minyak gaharu daripada pengekstrakan Soxhlet (2.83%). Minyak gaharu daripada ASE OPT dikategorikan dalam minyak pati likat dan ia juga cenderung untuk mempunyai sebatian aromatik yang lebih berat.
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LIST OF SYMBOLS

µg - microgram
ANOVA - analysis of variance
ASE - accelerated solvent extraction
CITES - convention on international trade in endangered species
cm - centimeter
g - gram
GC - gas chromatography
GC-FID - gas chromatography-flame ionization detector
GC-MS - gas chromatography-mass spectrometer
hr - hour
kg - kilogram
m - meter
min - minutes
ml - milliliter
mm - millimeter
°C - degree celcius
PLE - pressurized liquid extraction
PNGFA - Papua New Guinea Forestry Authority
RSM - response surface methodology
s - second
SFE - supercritical fluid extraction
US EPA - United States Environmental Protection Agency
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CHAPTER 1

INTRODUCTION

1.1 Research Background

Gaharu is a natural plant resinous which accumulated in the plants species of within four genera; Gyrinops, Aetoxylon, Gongystylis and more commonly from Aquilaria within the family Thymelaeaceae. These plants are natively grown widely in South and South East Asia. There are 15 species reported to produce gaharu in Asia (Nor Azah et al., 2008). This resinous wood also known as agarwood, otherwise known as eaglewood, aloeswood depending on the ethnic and country (Gunn et al., 2004); which in Malaysia, this tree is known as karas and its resinous wood is called gaharu. The species of Aquilaria and Gyrinops are the major types of gaharu sources from Malaysia and Indonesia.

Gaharu may be classified into various grades; Grade A, B, C and D and the grading of the gaharu usually based on the physical properties, gaharu formation and its unique scent. The lower grades such as C and D can be used as a raw material for extraction of gaharu oil using hydro distillation method. Currently, hydro-distillation and solvent extraction are the methods that practiced by Malaysian producers (Nor Azah et al., 2008).
The Gaharu and its essential oil are the most valuable products due to its specialty and peculiarity properties such as strong odor, sweet and its medicinal benefit. Due to its unique characteristics, it has been required during Buddhist and Islamic ceremony. The uses of gaharu are infinite as it can be used in wooden sculptures, perfumery, culinary, medicine and aromatherapy (Beevi and Seema, 2009). The gaharu oil is also described as a stimulant, car diatonic and carminative. It is also used in the cosmetic and pharmaceutical industries.

The resinous wood and oil of gaharu are extremely expensive due to low in oil extraction yield and the resinous wood formation rarely occurs in natural wild trees and relatively young trees. The price for a good quality of gaharu can reach up to RM10,000 per kg depending on the grade of the resinous wood. A 12g of gaharu oil is sold in the range of RM50 and RM200 (Chiew, 2005).

However not all of these trees will produce the resinous gaharu by itself. The formation and accumulation of resinous in plant have been reviewed by Blanchette (2006). The formation of gaharu is considered to be a pathological product produced by fungal invasion of the host (Qi et al., 1992). The tree is unable to produce resinous without injuries then followed by the infection. The formation of resinous usually occurs in the trunk, brunch and root that have been infected by fungus due to the injuries.

The global demand for gaharu has increased and gaharu trees are becoming rare and difficult to find in natural forest. The huge amount of non-infected gaharu trees are increasingly being cut due to low estimation of profit from the harvest of just a few kilos of gaharu wood even in the protected area.

The non-infected gaharu wood is nearly odorless until a fungus invades the wood. Moreover the chemical composition of gaharu not only depends on the Aquilaria species, but also the soil and climate of growth. According to Kaiser
(2006) many investigations for variety of sesquiterpenes compounds due to the importance of this compound in the perfumery industry.

Gaharu oil can be extracted by several methods including hydro-distillation, solvent extraction, carbon dioxide extraction and phytosol extraction. The present of chemical components can be analyzed by GC and GC-MS. The increasing consumer demand on gaharu products leads to further development of extraction methods for better yield and quality. The relevant extraction methods will be discussed further in Chapter 2.

1.2 Problem statement

Gaharu is a resin product which produced in the plants commonly from the species of Aquilaria and it has a certain high commercial value for perfume and cosmetics products. There are many grades of gaharu wood and the highest quality of the wood is extremely expensive. The first-grade wood is become one of the most expensive natural products in the world, with prices as high as USD30000/kg (Gunn et al., 2004).

Nowadays, the common method to extract gaharu oil is using traditional hydro distillation method. This method involves submerging the raw material (gaharu chips) in water in the still and brought to boil, and the steam produced is collected and condensed to get the gaharu oil. This extraction method acquires long extraction times and consumes a lot of fuel for heating purposes. The extraction process did not produce the maximum yield of oil because the efficiency of the method itself is relatively low. Moreover, there is no established standard that can be referred to determine the grade of gaharu oil, therefore prices as well as the grading of gaharu oil are arbitrarily determined by traders and clients.
However, throughout the year, there were still problems in extracting gaharu oil. Therefore, the target of the research is to obtain high yield and quality of gaharu essential oil with reasonably low extraction time and less solvent consumed. This project will study the extraction yields and quality of gaharu oil via extraction methods inclusive of hydro distillation, Soxhlet extraction and ASE method. As the commercialization of gaharu product is growing, the project aims to contribute to the appropriate extraction method for the production of high yield and standardized gaharu oil.

1.3 Research Objective

The objective of current research is to determine the yield of gaharu essential oil by using different extraction methods. The extraction methods applied are hydro distillation, Soxhlet extraction and Accelerated Solvent Extraction (ASE). The extraction yields obtained will be compared to determine the most appropriate extraction method and factors that influence the extraction processes. In addition, the compositions of some chemical compounds in gaharu oils are evaluated to determine the oil quality.

1.4 Research Scope

The important scopes have been identified for this research in achieving the objectives are divided into four main parts:

1) To extract gaharu oil using three different methods and determine the maximum yield of the essential oil produced. The methods involved are hydrodistillation, Soxhlet extraction and ASE method. The raw material of grade C gaharu wood will be used for the extraction of the oil.
2) To carry out analysis of gaharu oil using GC-FID. The analysis is performed to identify the variation in percentages of some chemical components in extracted gaharu oils. The finding will be the basis / reference point for the next study.

3) To optimize the extraction of gaharu oil using the appropriate method and optimized parameters that have been chosen from parts (1) and part (2).

4) To carry out GC-MS analysis of optimized gaharu oil from part (3), in order to verify the yield and quality.

1.5 Contribution of Study

Nowadays, the natural product related market includes herbal and phytochemical based industries are estimated to be worth US$200 billion in 2008 and US$5 trillion in 2050 (Aljadi and Kamaruddin, 2002). The Malaysian market for natural products has been estimated to be worth RM4.55 billion with the growth rate of 15 to 20 percent (Ramlan, 2003). The demand of natural products has been increasing due to the awareness of consumers regarding the toxicity and side effects of synthetic or chemical based product. The statistics show ample opportunities for local companies to embark in the sector of phytochemical and its commercialization.

The essential oil from aromatic plants is the most volatile part and there are several types of extractions method have been used to produce the essential oil such as hydro-distillation, steam-distillation and solvent extraction. The gaharu raw material of grade C can be distilled to obtain the gaharu oil (Nor Azah et al., 2008), which further being used in this study. The most practiced of conventional hydro-distillation or steam-distillation is deployed in the production of gaharu oil. As a result, various grade of gaharu oils appeared in the market due to different extraction methods which are employed with different set of operation parameters.
At present, there is no established standard that can be referred to determine the quality of gaharu oil except by using traditional or individual preferences by the customer or traders. Therefore, these scenarios caused the fluctuation in price of gaharu as such trigger the traders to manipulate the price to manufacturers. Besides, the dependency on human to identify the quality of gaharu especially by using visual approach or naked eyes leads to several problems such as inconsistent grading result, hence only experienced workers are able to classify the gaharu. Furthermore, the grading process also depends much on the expert opinion.

The need for establishment of gaharu oil extraction standard is crucial especially in fulfilling the requirements of cosmeceutical and perfumery industries. The understanding of the effects of processing methods on the yield and quality of gaharu oil are useful in designing a better processing technology to achieve optimum extraction in its production. The current study aims to contribute to the appropriate extraction method for the production of high quality gaharu oil.
REFERENCES


