EXTRACTION OF THE ESSENTIAL OIL OF *AQUILARIA MALACCENSIS* (GAHARU) USING HYDRO-DISTILLATION AND SOLVENT EXTRACTION METHODS

ASHWIN CHARLES BENEDICT

Thesis submitted to the Faculty of Chemical and Natural Resources Engineering in Partial Fulfillment of the Requirement for the Degree of Bachelor Engineering in Chemical Engineering

Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

APRIL, 2009

I declare that this thesis entitled "Extraction of the Essential Oil of *Aquilaria Malaccensis* (Gaharu) Using Hydro-distillation and Solvent Extraction Methods" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any degree.

Signature :

Name : ASHWIN CHARLES BENEDICT

Date :

I dedicate this thesis to my family, without whom none of this would have been worth the challenge…

Supportive parents;
Benedict Charles and Hazel Mirandah

Not-so-little sisters;
Evelyn Charles and Jocelyn Charles

This is for the four of you.

ACKNOWLEDGEMENT

This final year project has been both a challenge and an experience to cherish for a life time. Although a lot of hard work and sacrifice did come from my part, there are many without whom this project would not have even lifted off the ground, let alone come to completion.

First and foremost, I would like to extend my deepest gratitude to my final year project supervisor, Mr Saiful Nizam for his endless support and guidance during the infancy of this project and throughout its implementation. I will be forever grateful for his professionalism and willingness to listen and consider my many suggestions and amendments to the proposed project as well as for his brilliant advice and ideas.

Secondly, I would like to extend my sincere thanks to the lab assistant who has been there for me providing a light in the darkness, Pn Rosmaria Mohamad Mokhtar. Without her aid and support this project would not have made it far.

Finally, I would like to thank my fellow research group mates, Siti Faridah, and Norsuzieana who were right there by my side throughout the duration of PSM 1 and 2. I would like to thank them for all their help and support. It has been a pleasure, working with them.

ABSTRACT

Agarwood oil is regarded as one of the most expensive natural products in the world due to the fragrance inducing compounds it contains. However, current studies on the chemical composition of agarwood essential oil are woefully lacking and this poses a threat to the agarwood industry. This research aims to identify the best extraction method for isolating gaharu essential oil and to create a list of compounds contained in a sample of grade C agarwood. In the present work, the composition of agarwood essential oil obtained through hydro-distillation and solvent extraction with acetone, dichloromethane and hexane as the solvents were analyzed for marker compound identification using gas chromatography-mass spectrometry (GC-MS). Studying another parameter of this experiment, the sample hydro-distillated in the lab was compared with industrial grade hydro-distillation to determine the difference in quality between industrial and lab scale hydro-distillation. Of the three solvents used, acetone eluted the highest number of compounds. The lab scale hydro-distillated sample eluted 34 compounds at a quality of 50% and above whereas the solvent extraction sample eluted 25 compounds. There was no significant difference found between lab scale and industrial scale hydro-distillation.

ABSTRAK

Minyak gaharu dianggap antara produk asli yang paling mahal di dunia kerana komposisi kimianya yang menghasilkan bau yang sangat harum. Namun demikian, kajian-kajian yang dijalankan sebelum ini tidak menyeluruh dan informasi yang tidak mencukupi mengenai komposisi kimia minyak gaharu menjadi punca ancaman terhadap industry gaharu. Kajian ini bertujuan untuk memutuskan kaedah penyulingan minyak gaharu yang paling efektif dan juga untuk menghasilkan satu senarai kompaun dalam sampel gaharu gred C. Komposisi kimia minyak gaharu yang diperolehi dari kaedah 'hydro-distillation' dan 'solvent extraction' dianalisa menggunakan GC-MS. Satu lagi aspek yang dikaji dalam experimen ini adalah perbandingan antara 'hydro-distillation' pada skala berbeza yakni skala makmal dan skala industri. Perbandingan antara tiga pelarut yang digunakan menunjukkan acetone sebagai pelarut yang terbaik. 34 kompaun dikenal pasti dari sampel 'hydrodistillation' manakala 25 didapati dari sampel 'solvent extraction' menggunakan acetone apabila dianalisa pada kualiti 50%. Perbandingan antara sampel-sampel 'hydro-distillation' menunjukkan bahawa tiada perbezaan ketara dari segi kualiti antara prosedur makmal dan prosedur pada skala industri.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CAS	Chemical Abstracts Service
CITES	Convention on International Trade in Endangered Species and Wild Fauna and Flora
D	Debye
Dbh	Diameter Breast Height (for bark of trees)
D, d	Diameter
GCMS	Gas Chromatography Mass Spectrometry
Ha	Hectare
ID	Identification
MAHA	Malaysian Agriculture and Horticulture Agrotourism
MF	Molecular Formula
RT	Retention Time
VDW	Van der Waal’s

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Aquilaria Malaccensis is a species of plant in the Thymelaeaceae family found primarily in Bangladesh, Bhutan, India, Indonesia, Iran, Laos, Malaysia, Myanmar, the Philippines, Singapore, and Thailand. The Aquilaria genus is more broadly recognized as Agar wood, Jin Koh, Aloes wood, Gaharu, Eagle wood, Jinkoh and Oud. Among the other species of agarwood besides the Malaccensis include agallocha, gradiflora, ophispermum, sinesis, crassna, pentandra and yunnanensis. The term agarwood, although widely used to refer to the members of the Aquilaria genus, more specifically refers to the resinous heartwood from the Aquilaria trees.

Occasionally the heartwood gets infected by a parasitc ascomycetous mold, *Phaeoacremonium parasitica*. As a response, the tree produces a resin high in volatile organic compounds that aids in suppressing or retarding the fungal growth (Wikipedia, 2008). This resin and its oil are valuable for their use in medicine, perfumery and other aromatic products.

There are many grades of agarwood, and the highest quality wood is extremely expensive. In fact, the first-grade wood is one of the most expensive natural products in the world, with prices of up to $13,000 per pound (Eden Botanicals, 2007). However the finest grade of agarwood is produced from naturally occurring fungal infection which happens slowly and very infrequently.

Because of its immense value and rarity, indiscriminate cutting of trees and over harvesting in hope of finding the treasured resin has lead to depletion of wild trees. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) has listed Aquilaria Malaccensis as an endangered species. Efforts to protect the species involve inoculation of grown agarwood with the fungus as well as intentional injuring of the tree to encourage fungal infection to produce the required resin. However resin produced in this manner is deemed to be of secondary quality and has inferior market value compared to naturally harvested agarwood resin.

Essential oils from agarwood can be extracted by several methods which include distillation (typically using water or steam), solvent extraction, carbon dioxide extraction, cold pressing as well as florasol/phytol extraction. These extraction methods will be further discussed in the literature review.

1.2 Objectives

The main objectives of this preliminary study are:

1. To extract the Gaharu essential oil using the hydro-distillation and solvent extraction methods.
2. To analyze the chemical compounds present in the essential oil using Gas Chromatography – Mass Spectrometer (GCMS)
3. To compare the results of analysis between different extraction methods.
4. To compare the results of analysis between different sources of essential oil for the hydro-distillation method.
5. To compare the results of analysis between different solvents used for the solvent extraction method.

1.3 Scope of Study

The scope of this study is essentially to compare between the methods of hydro-distillation and solvent extraction. For hydrodistillation, the lab grade sample of agarwood distilled to obtain essential oil will be compared with previously procured samples from MAHA and Kelantan to examine differences in composition. In the solvent extraction procedure, three solvents used – hexane, dichloromethyl and acetone to extract three samples of essential oil. These three samples are then compared to the sample procured from hydro-distillation of the same lab grade agarwood to determine significant differences and similarities. The GCMS is used to perform analysis to determine the composition of each of the six samples.

1.4 Problem Statement

The main problem which needs to be addressed revolves around the issue of creating a standard by which to evaluate and assess the quality of essential oil produced from agarwood. Currently no applicable standard exists and prices as well as the quality of oil are arbitrarily determined by traders and clients.

Another problem which is highly correlated to the problem of quality is the reproduction of a successful formula. Current methods of producing perfumes and aromatic products from agarwood essential oil depend greatly on experience and lack scientific backing and suitably rigid procedures to ensure that a successful formula can be replicated.

This also brings to light the problems faced by law enforcement agencies and nature preservation groups that are trying to stop indiscriminate felling of Aquilaria trees. The woeful lack of information on agarwood and its essential oil has lead to exports being approved with little information on the species and not knowing whether exploitation is within sustainable levels.

1.5 Rationale and significance

The issue of finding a solvent or an extraction method that can be used to conveniently determine the quality of a sample, a majority of its components as well as the species from which the essential oil was obtained is a crucial part of this study. The discovery of such a solvent or any research leading to that discovery would pave the first steps towards solving the problems stated above.

CHAPTER 2

LITERATURE REVIEW

2.1 Background of Gaharu

Valued mainly for its aromatic, fumigatory, and medicinal properties, *gaharu* is the fragrant, resin-impregnated wood found in approximately 17 species of sub-canopy trees of the genus *Aquilaria* (Thymelaeaceae) commonly found in mixed hardwood hill forests across tropical Southeast Asia (Chung and Purwaningsih, 1999). Generally agreed to be the result of a pathological condition, this aromatic resin is produced as the tree sap thickens in response to injury and fungal infection. The degree to which the resin saturates the heartwood determines the market value of this product. In lesser quality specimens, the resin creates a mottled or speckled appearance in the naturally pale wood, but higher quality specimens are nearly solid in color—glossy and black. Through distillation, the most valuable specimens can yield an essential oil that is a key perfume ingredient; distillation residues and lesser quality material are commonly processed for incense. The species that produce high quality resin include *A. agallocha*, *A. crassna*, *A. bailloni*, and *A. grandiflora* (Burkill 1966, Soehartono 1997).

A member of the family Thymelaeaceae, *Aquilaria* is a relatively slow-growing, medium-sized tree, on average 15–25 m tall. Having a moderately straight stem, it can achieve a diameter (dbh) of up to 250 cm. Most *Aquilaria* species have smooth, thin, pale gray bark with dense, dark foliage of shiny elliptical to oblong leaves (7.5–12 cm long by 2.5–5.5 cm wide) (Ding Hou 1960). The small, pale blooms flowering in clusters on the short stalks of the leaf stems produce 3–5 cm long, bi-valved fruit capsules in August.

La Frankie (1994) found *A. malaccensis* widely distributed but relatively uncommon (2.5 stems per ha, >1 cm dbh) in the Pasoh Forest Reserve of peninsular Malaysia. Despite the fact that *Aquilaria* regenerates freely under natural conditions as seedlings around the mother tree or sprouts from the stumps of harvested trees, mother trees are becoming scarce in many areas because of over-exploitation (Beniwal 1989, Paoli et al. 1994, Hasnida et al. 2001, Soehartono and Newton 2002, Quan et al. 2003). Although this condition may not lead to local extinction of the species, it may severely affect the availability of the product and, thus, the local *gaharu* economy.

The occurrence of the tree itself does not guarantee the presence of the resin. Scientists estimate that only 10% of the *Aquilaria* trees in the forest may contain *gaharu* (Gibson 1977). The resin forms in response to wounding and subsequent fungal infection, and is found in many parts of the tree, according to some sources in the bark and the roots as well as the heartwood (Jalaluddin 1977). Under natural conditions, the resin is more commonly found in trees of about 20 years or older, with trees more than 50 years old reportedly having the highest concentration (Sadgopol 1959).

2.2 Properties of Essential Oil

An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. They are also known as volatile or ethereal oils, or simply as the "oil of" the plant material from which they were extracted, such as *oil of clove.* An oil is essential in the sense that it carries a distinctive scent or essence of the plant. Essential oils do not as a group need to have any specific chemical properties in common, beyond conveying characteristic fragrances. They are not to be confused with essential fatty acids. Essential oils are multi-component chemicals. The mixture of oil compounds that constitute the essential oil comprises polar and non-polar compounds (Fliesher and Fliesher; 1991, Bohra *et al.*, 1994; Masango, 2004).

2.2.1 Physical Properties

A vast majority of essential oils are colourless, particularly when fresh. *Gaharu* oil however can be distinguished by colours, specifically ‘reddish brown’ and ‘greenish brown’ (Fatmawati Adam *et al* 2005). Essential oils also known as volatile oils because are easily to evaporate. Unlike vegetable oils expressed from nuts and seeds, essential oils are not actually oily. Some essential oils are viscous; others are fairly solid and most are somewhat watery.

2.2.2 Chemical Properties

Essential oils, like all organic compounds, are made up of hydrocarbon molecules and can further be classified as terpenes, alcohols, esters, aldehydes, ketones and phenols etc (Nor Azah Mohd Ali, 2002). The terpenes in *Gaharu* oil can be further divided into monoterpenes and sesquiterpenes. Most monoterpenes in *Gaharu* oil have a structure consisting 10 carbon atoms and at least one double bond. Terpenes react readily with air in the presence of even the smallest heat source. This is the reason citrus oils degrade quickly unless properly stored. Sesquiterpenes on the other hand consist of 15 carbon atoms and have complex pharmacological actions which include anti-inflammatory and anti-allergy properties. Professor Otto Wallach attributes the fragrance of *Gaharu* oil mostly to the presence of terpenes and cites the terpenes as having greatly influenced the oil industry.

In addition, for oxygenated compounds, they are contains phenols and alcohols such as monoterpene and sesquiterpene alcohol. The phenols found in essential oils normally have a carbon side chain and here we can look at compounds such as thymol, eugenol and carvacrol. These components have great antiseptic, anti-bacterial and disinfectant qualities and also have greatly stimulating therapeutic properties.

Specifically the chemical compounds of interest discovered in *Aquilaria Malaccensis* Benth are α-agarofuran, (-)-10-epi-γ-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% (Yoneda *et al*, 1984, Nakanishi *et al*, 1984). A more comprehensive and more recent list of compounds found in the Aquilaria species is shown in Table 2.1:

Table 2.1: List of compounds in different species of Aquilaria referred to from different sources

Compound	Species	Source
β-agarofuran	*Aquilaria agallocha*	Manfred Meier, Birgit Kohlenberg and Norbert A. Braun - Isolation of Anisyl Acetone from Agarwood Oil (February 2003)
Epi-γ-eudesmol		
Agarospirol		
Jinkoh-eremol		
Valerianol		
6,10,10-trimethyl-11-oxatricyclo-[7.2.10]dedecane-2-carbaldehyde		
2-isopropylidene-10-methyl-spiro[4.5]-dec-6-ene-6-carbaldehyde		
2-(1,2,6,7,8,8a-hexahydro-8,8a-dimethyl-2-naphthyl)-propan-2-ol		
Dihydrokaranone		
α-guaiene	*Aquilaria agallocha*	Masakazu Ishihara and Tomoyuki Tsuneya – Components of the Volatile Concentrate of Agarwood (June 1993)
α-bulnesene		
Nor-ketoargarofuran		
1,10-epoxybulnesene		
1,5-epoxy-nor ketoguaiene		
Kusunol		
Dehydrojinkoh-eremol		
Selina-3,11-dien-9-one		
Routundone		
Selina-3,11-dien-9-ol		

Selina-3,11-dien-14-al		
Neopetasane		
Selina-3,11-dien-14-ol		
Guaia-1(10),11-dien-9-one		
Selina-4,11-dien-14-1l		
Guaia-1,(10),11-dien-15-ol		
Sinenofuranol		
Guaia-1,(10),11-dien-15-al		
Karanone		
Oxo-agarospirol		
Guaia-1,(10),11-dien-15,2-olide		
Guaia-1,(10),11-dien-15-oic acid		
2-hydroxyguaia-1,(10),11-dien-15-oic acid		
Selina-4,11-dien-14-oic acid		
Selina-3,11-dien-14-oic acid		
9-hydroxyselina-4,11-dien-14-oic acid		
(s)-4a-methyl-2-(1-methylethyl)-3,4,4a,5,6,7-hexahydronaphthalene	*Aquilaria agallocha*	R. Naf, A. Velluz, R. Brauchli and w. Thommen – Agarwood Oil (Aquilaria agallocha Roxb.). Its Composition and Eight New Valencane-, Eremophilane- and Vetispirane-Derivatives (1995)
β-vetispirene		
4-phenyl-butan-2-one		
α-vetispirene		
Rel-(1R,2R)-9-(isopropyl-2-methyl-8-oxatricyclo[7.2.1.0]dodec-5-ene		
Rel-(1R,2R)-9-(isopropyl-2-methyl-8-oxatricyclo[7.2.1.0]dodeca-4,6-diene		
(2R,4aS)-2-(4a-methyl-1,2,3,4,4a,5,6,7-octahydro-2-naphthyl)-propan-2-ol		
2-(1,2,3,5,6,7,8,8a-octahydro-8,8a-dimethyl-2-naphthyl)-propanal		
Valerianol		
(1S,2S,6S,9R)-6,10,10-trimethyl-11-oxatricyclo[7.2.1.0]dodecane-2-carbaldehyde		
4-(4-methoxyphenyl)-butan-2-one		

rel-(5R,10R)-2-isopropylidene-10-methyl-spiro[4.5]dec-6-ene-6-carbaldehyde		
rel-(2R,8S,8aS)-2-(1,2,6,7,8,8a-hexahydro-dimethyl-2-naphthyl)-propan-2-ol		
rel-(5R,7S,10R)-2-isopropylidene-10-methyl-6-methylene-spiro[4.5]decan-7-ol		
rel-(2R,8R,8aS)-2-(1,2,3,5,6,7,8,8a-octahydro-8,8a-dimethyl-2-naphthyl-prop-2-en-1-ol		

The list of compounds above omits recurring compounds and thus any one list may be an incomplete reproduction of the original. However, the compounds that consistently appear in almost every list besides the ones listed by Yoneda *et al* are: β-agarofuran, valerianol, dihydrokaranone, α-bulnesene, α-guaiene, β-vetispirene, α-vetispirene and 4-phenyl-butan-2-one.

2.3 Extraction of essential oils

The demand for *gaharu* oil has created the necessity to extract this oil from the resin using one of a vast number of methods available. Extraction of *gaharu* oil falls under the category of fragrance extraction which means the extraction of aromatic compounds from raw material. Among the methods commonly employed for fragrance extraction are water/steam distillation, solvent extraction, enfleurage, expression, carbon dioxide extraction, hydrodiffusion, percolation and maceration. The results of the extraction are either essential oils, absolutes, concretes or butters depending on the amount of waxes in the extracted product. Despite the vast variety of methods available the most frequently used methods of *gaharu* extraction are hydro-distillation and solvent extraction. The extraction method employed is of central interest because it determines the quality of the oil produced. An incorrect or wrongly carried out extraction procedure would produce inferior quality oil as it would change the chemical signature of the original *gaharu* oil.

2.3.1 Hydro-Distillation

Hydro distillation is used in the manufacture and extraction of essential oil. This is the simplest and usually the cheapest process of distillation. Hydro distillation seems to work best for powders and very tough materials like roots, wood, or nuts. The main advantages of this method are that less steam is used, shorter processing time and a higher oil yield.

In distillation, the plant material is heated, either by placing it in water which is brought to the boil or by passing steam through it. The heat and steam cause the cell structure of the plant material to burst and break down, thus freeing the essential oils. The essential oil molecules and steam are carried along a pipe and channelled through a cooling tank, where they return to the liquid form and are collected in a vat. The emerging liquid is a mixture of oil and water, and since essential oils are not water soluble they can be easily separated from the water and siphoned off. Essential oils which are lighter than water will float on the surface.

2.3.2 Solvent Extraction

Some plant material cannot tolerate the heated forms of extraction such as steam distillation. High pressure damages these plants and once damaged, their essential oils too are damaged and are no longer able to be extracted. For these plants solvents such as ether, ethanol, methanol, hexane, alcohol and petroleum are used instead. The problem with using solvents to extract essential oils is that most of the time, residual solvents or impurities remain in the product.

Plant material is first washed in a bath of hydrocarbon solvents. This process dissolves the necessary plant materials including the aromatic molecules, waxy matter and pigment and the dissolved matter mixes in with the solvent. The solvent mixture is then filtered and distilled using low pressure. After distillation and further processing, either a resin or a concentrated concrete remain. Additional processing using alcohol does in fact help in the process of extracting the essential oils.

2.4 Analysis using GC/MS

GC/MS is the most frequently used technique for analyzing essential oil composition. This method of testing requires an analytical component, a gas chromatograph, coupled with a detection component, a mass spectrometer.

A small sample of an essential oil is introduced into the GC, where it is heated to vapor and then carried along a column by an inert gas, such as helium. As the vaporized oil passes through the column, it separates into individual molecular constituents as it interacts with the stationary phase of the column. The separated constituents then pass into the MS. In the MS module, the constituents become charged, or ionized. The ionized constituents are then amplified and detected as current by the MS.

Each constituent is represented by a peak in a chromatograph, and the peaks can be compared to a library of molecules to identify the substance. Even though a GC/MS can produce a “fingerprint” of an oil, it cannot detect some synthetic and natural diluents. It can, however, detect a mixture of two or more similar oils, an oil that has had the terpenes removed, an oil that has been rectified, and traces of solvents or mineral oils.