EXTRACTION OF GAHARU ESSENTIAL OIL USING ENZYMATIC HYDRODISTILLATION

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“ I declare that this thesis is the result of my own research except as cited references. The thesis has not been accepted for any degree and is concurrently submitted in candidature of any degree.”

Signature : ……

Name of Candidate : ……

Date : ……...

DEDICATION

Dedicated to my beloved father, mother and brothers…

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ABSTRACT

Gaharu is one of the most precious trees in the world and its essential oil is one of the most expensive natural products existing today. The main uses of gaharu essential oil are in medicinal, perfumery and incense. Grade C gaharu from peninsular Malaysia was used in this study. Previous method of hydrodistillation is not effective, yield of gaharu essential oil is relatively small and consumes a lot of time. This study of extraction of gaharu essential oil using enzymatic hydrodistillation was developed to improve the previous method. The parameters that are manipulated in this study are solid to solvent ratio and pretreatment time to achieve the optimum yield of essential oil. From the study, gaharu essential oil that extracted using enzymatic hydrodistillation produce highest yield at lowest solid to solvent ratio and highest pretreatment time. Based on the results, it was found that the ratio 1:8 give 0.1092% yield which is the highest yield compare to yield at 1:12, 1:16 and 1:20 solid to solvent ratio. For pretreatment time, the highest yield of extracted oil occurs at nine hours of pretreatment time which is 0.1375%. This study had proved that the parameter, solid to solvent ratio and pretreatment time are suitable in optimizing the yield of essential oil.

ABSTRAK

Gaharu adalah di antara salah satu pokok paling berharga dalam dunia dan minyak patinya adalah diantara hasil semulajadi yang termahal pada hari ini. Kegunaan utama pati minyak gaharu adalah untuk tujuan perubatan, haruman dan setanggi. Gaharu gred C dari semenanjung Malaysia telah digunakan dalam kajian ini. Kaedah penyulingan hidro sebelum ini adalah tidak efektif, menghasilkan pati minyak gaharu yang sedikit dan memakan masa yang panjang. Kajian mengenai pengekstrakan pati minyak gaharu menggunakan penyulingan hidro berenzim telah dijalankan untuk memperbaiki kaedah yang lepas. Parameter yang dimanipulasi dalam kajian ini adalah nisbah pepejal-larutan dan masa pra-rawatan untuk mencapai penghasilan minyak pati yang optimum. Berdasarkan kajian, minyak gaharu yang diekstrak menggunakan penyulingan hidro berenzim mengeluarkan hasil yang tertinggi pada nisbah pepejal-larutan yang terendah dan masa pra-rawatan yang tertinggi. Merujuk kepada keputusan, didapati nisbah 1:8 memberikan hasil 0.1375% dimana ia merupakan hasil yang tertinggi berbanding hasil pada nisbah pepejal-pelarut 1:12, 1:16, 1:20. Bagi masa pra-rawatan, hasil yang tertinggi adalah pada masa 9 jam iaitu 0.1375%. Kajian ini telah membuktikan, parameter nisbah pepejal-pelarut dam masa pra-rawatan adalah sesuai untuk mengoptimumkan penghasilan pati minyak gaharu.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Introduction

Agarwood is the resinous heartwood from Aquilaria trees, large evergreens native to Southeast Asia. It is commonly referred to as Gaharu in Malaysia but is also known as Eaglewood, Aloes Wood and Agalocha. The common names of Gaharu for certain countries are stated in Table 1.1

Table 1.1 : Common Names of Gaharu

ENGLISH	Agarwood, Aloeswood, Eaglewood
FRENCH	Bois d'aigle, Bois d'aloes
VIETNAMESE	Tram Huong
CHINESE	Chen-xiang
JAPANESE	Jinkoh
ARABIC	Oud
INDONESIAN	Gaharu
MALAY	Gaharu, Oudh
THAI	Kritsana noi

(Source: www.360.yahoo.com)

Gaharu is a fast growing, evergreen tree, that normally grows to 18 - 21 m but sometimes up to 40 m in height. The trees occasionally become infected with a parasite mould and begin to produce an aromatic resin in response to this attack. As

the fungus grows, the tree produces a very rich, dark resin within the heartwood. The resin is commonly called Jinko, Aloeswood, Agarwood or Oud and is valued in many cultures for its distinctive fragrance, thus it is used for incense and perfumes. Normally harvesters would cut only the infected parts in the hope that the tree would produce more of this resinous wood. The oldest of the cultivated trees containing the oleoresin are 12 years of age whereas the resin found in wild harvested trees has been developing for 30 years or more. So the age of the raw material has a lot to do with the richness and complexity of the resin produced therein. Still there are many merits in the oil from the cultivated trees.

However, fungal introduction (or inoculation) still poses a major problem, making it difficult to produce Gaharu on a significant scale. In fact in the years to come it is going to be the only source of agarwood oil as the wild harvested material will eventually run out. Information on the taxonomy and morphology of Aquilaria spp. is available in Table 1.2

Table 1.2 : Taxanomic Position of Gaharu Trees:

KINGDOM	*Plantae*
PHYLUM	*Spermatophyta*
SUBPHYLUM	*Angiospermae*
CLASS	*Dicotyledonae*
ORDER	*Thymelaealeales*
FAMILY	*Thymelaeaceae*
SPECIES	*Aquilaria Malaccensis*

Gaharu has been used to make high quality incense. The Chinese continue to use it in religious and festive celebrations, as do Arabians, Indian and Japanese people. It is also part of many traditional pharmacopoeias, dating back to medieval times and Chinese doctors still prescribe it for colds and digestion problem. Oil extracted from Gaharu is used in Arabic countries as a perfume as well. Unlike many industrial perfumes, it is suitable for hot climates. In spite of its unique qualities though, Gaharu is rarely used in European perfumeries because of its cost, and good quality synthetic substitutes are yet to be created.

1.2 Problem Statement

Current method of extracting gaharu essential oil is using traditional water distillation method (Chang et al, 2002) or hydrodistillation. The efficiency of this method is relatively low and it is too time consuming. All of this will result in higher operating cost because of the process is slow and the distillation time is much longer. Furthermore, prolong action of hot water can cause hydrolysis of some constituents of the essential oils such as ester.

Figure 1.0: Traditional Hydrodistillation

1.3 Objective

- To examine the feasibility of enzymatic Hydrodistillation as an improved method for gaharu oil extraction process.

1.4 Scope of Study

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

- The effect of treatment time on the yield of gaharu essential oil.
- The influence on solid to solvent ratio on the yield of gaharu essential oil.

CHAPTER 2

LITERATURE REVIEW

2.1 Gaharu

Gaharu is a large evergreen trees that growing over 15-30 m tall and 1.5-2.5 m in diameter, and has white flowers (Chakrabarty et al., 1994). The trees occasionally become infected with a parasite mould and begin to produce an aromatic resin in response to this attack. As the fungus grows, the tree produces a very rich, dark resin within the heartwood. Normally harvesters would cut only the infected parts in the hope that the tree would produce more of this resinous wood. The oldest of the cultivated trees containing the oleoresin are 12 years of age whereas the resin found in wild harvested trees has been developed for 30 years or more. So the age of the raw material has a lot to do with the richness and complexity of the resin produced therein. Still there are many merits in the oil from the cultivated trees.

2.2 Distribution and Habitat

Aquilaria species have adapted to live in various habitats, including those that are rocky, sandy or calcareous, well-drained slopes and ridges and land near swamps. They typically grow between altitudes of 0-850 m, and up to 1000 m in locations with average daily temperatures of 20-22°C (Ding Hou, 1960; Afifi, 1995; Keller and Sidiyasa, 1994; Wiriadinata, 1995).

Three species of *Aquilaria* are found in Malaysia: *A. hirta*, *A. malaccensis* and *A. rostrata*. *Aquilaria malaccensis* is well distributed throughout Peninsular Malaysia, except for the States of Kedah and Perlis. It is confined mainly to plains, hill slopes and ridges up to 750 m in both primary and secondary Malaysian lowland and hill dipterocarp forests (Jantan, 1990). The average diameter growth rate of *A. malaccensis* in native forests in Malaysia is rather low, e.g. a mean of 0.33 cm/ year, but the fastest-growing larger specimens are reported to grow at 0.8-1 cm/year (La Frankie, 1994). Although *A. malaccensis* enjoys good geographical coverage, its occurrence is rather rare.

2.3 Conservation Status

Aquilaria trees are now protected in most countries and the collection of agarwood is illegal from natural forest. International agreements, such as CITES (the Convention on International Trade in Endangered Species of Wild fauna and Flora), accepted by169 countries, is design to ensure trade in agarwood products from wild trees does not threatened the survival of *Aquilaria*.

Aquilaria malaccensis is considered threatened species due to its high value in today's market and has been included in The World List of Threatened Trees (Oldfield et al, 1998). The government of each of Malaysia's States requires that special permits be obtained from the relevant State Forestry Department to harvest and trade agarwood. Harvesting from national parks or wildlife sanctuaries is

prohibited in all States. In Peninsular Malaysia, the Department of Wildlife and National Parks (PERHILITAN) is responsible for reporting to national and international agencies. PERHILITAN is also accountable for monitoring trade via an enforcement unit that inspects shipments, and which occasionally acts on information received from the public.

2.4 Production of Agarwood

Formation of agarwood occurs in the trunk and roots of trees that have been infected by a fungus. As a response, the tree produces a resin high in volatile organic compounds that aids in suppressing or retarding fungal growth. While the unaffected wood of the tree is relatively light in color, the resin dramatically increases the mass and density of the affected wood, changing its colour from pale beige to dark brown or black.

Not all of Aquilaria trees produce the resin, (Gianno, 1986) suggested that only 10% of mature *Aquilaria* trees above 20 cm diameter at breast height (dbh) produce agarwood. (Chakrabarty *et al*, 1994) stated that infected trees produce resin from the age of 20 years onwards, while (Hooper, 1904) had noted that trees that were at least 50 years old yielded the largest amount of resin.

2.5 Grading of Gaharu

There are various systems adopted for agarwood grading. Many traders claim that each country (of origin or import) has its own agarwood grading system, but there is no record of a systematic explanation of these systems. Based on the information supplied by the traders surveyed, the following characteristics are the major indicators for distinguishing agarwood grades:

2.5.1 The amount of resin content and distribution

The higher resin content an agarwood piece has, the higher the price. High resin content allows wood pieces to produce a purer or higher-level scent as well as provide greater therapeutic effect. The most common method of grading is to place agarwood pieces into water, and then the pieces are classified into three basic grades: sinking, half-sinking (or half-floating) and floating. Sinking pieces are top grade and the rest are divided into different grades based on diverse standards, including the pattern of resin distribution in each piece. Higher resin content also gives an agarwood piece more weight than others in similar size. Many traders also stated that agarwood grading is very subjective, meaning that it takes years of experience to learn to distinguish accurately between different types and grades of agarwood.

2.5.2 Colour and scent

A number of traders who specialize in high-grade agarwood indicate that agarwood from different countries/islands of origin contains distinctive resin coloration. It is said that the colour of resin that an agarwood piece holds is the main factor determining its scent when it is burnt. The colours mentioned include: green, dark green, yellow, golden, red (purple), black, brown, and white. The darker an agarwood piece, the higher resin content and therefore the higher grade (*i.e.* sinking in water plus dark colour) (Barden *et al*, 2000; Song, 2002). In the general retail market, most traders (retailers) explained that scent is the major factor influencing a consumer's decision. In general, agarwood materials and products producing a softer scent are considered as higher grade, are more popular and are sold at higher prices than those producing a more intense scent. There are no systematic indicators that demonstrate a uniform relation between colour, scent, grading and pricing.

2.5.3 Causes of formation, age and location in a tree

Agarwood raw materials extracted from dead trees buried in the ground or from a swamp are generally considered more ‘mature’ material, which can contribute to higher grading and higher prices than agarwood extracted from a standing tree. When comparing agarwood taken from different parts of the same tree, agarwood from the roots is considered higher grade than agarwood from higher parts of a tree.

2.5.4 Size and form

For agarwood pieces, when two pieces are at a similar level of grade according to other characteristics, the value of the larger piece could be many times more than the ratio between the pieces’ respective weights. Agarwood pieces that have natural shapes of aesthetic value are usually picked out by traders to be sold at higher prices to agarwood (‘art’) collectors.

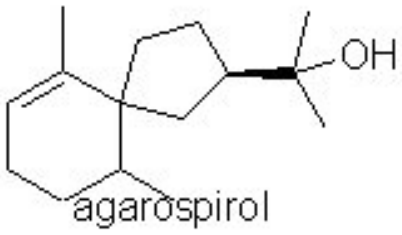
2.5.5 Source and scarceness

Agarwood items from sources known to have increasingly scarce supplies, such as those from Vietnam, are sold at much higher price than other items of similar grade.

2.6 Chemical Component of Gaharu

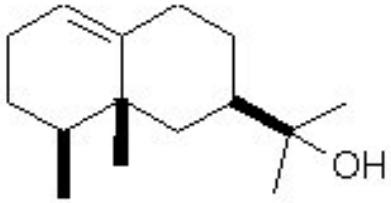
Agarwood contains more than 12 chemical components that can be extracted. 3,4-dihydroxy-dihydroagarofuran, 4-hydroxydihydroagarofuran, agarol, agarospirol, agarotetrol, alpha- agarofuran, aquillochin, benzylacetone, beta-agarofuran, dihydroagarofuran, dihydroxyagarofuran, gmelofuran, liriodenine, norketoagarofuran, noroxoagarofuran,oxo-nor-agarofuran, p-methoxybenzylacetone, p-methoxycinnamic-acid, and oleoresin.

Based on the study by FRIM on several grades of gaharu obtained from the wild, samples from companies and through inoculation trials, chemical profiles of each grade such as grade A, B and C were different. Further comparison of gas chromatograph of gaharu oils obtained from different sources in Peninsular Malaysia demonstrated that they were mostly of grade C quality. The gas chromatograms showed similar GC profiles suggesting a region of peaks with retention times ranging from 28.0 to 42.0 minutes to be indicative of gaharu presence. Several chemical compounds such as agarospirol, guaiene, jinkohol and jinkohol II have been detected in Malaysian gaharu oil.



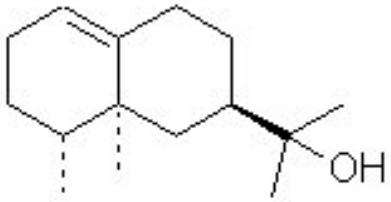
agarospirol

IUPAC: 2-(6,10-dimethyl-2-spiro[4.5] dec-9-enyl)propan-2-ol



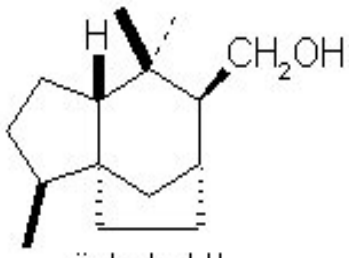
jinkoh-eremol

IUPAC: 2-(8,8a-dimethyl 2,3,4,6, 7,8-hexahydro-1H- elicoids -e-2-yl)propan-2-ol



kusunol

IUPAC: 2-(2,4-dihydroxyphenyl)-3-7-dihydroxy-8-(5-elicoid-5-methyl -2-prop-1-en-2-yl-hexyl)-5-methoxy-chroman-4-one



jinkohol II

MF: $C_{15}H_{26}O$

Figure 2.0 Chemical Component of Gaharu

(Source: www.equitech.biz)

2.7 USES OF GAHARU

The use of agarwood for perfumery extends back several thousands of years, and is referenced, for example, in the Old Testament several times using the term ‘aloes’. Both agarwood smoke and oil are customarily used as perfume in the Middle East (Chakrabarty et al, 1994). In India, various grades of agarwood are distilled separately before blending to produce final ‘attar’. Minyak attar is a water-based perfume containing agarwood oil, which is traditionally used by Muslims to lace prayer clothes (Yaacob, 1999).

The stem of *Aquilaria agallocha* Roxb. (Thymelaeaceae), agarwood has been used in the treatment of cough, acroparalysis courap, asthma, and it also has been used as a stomachic, tonic, sedative, and expectorant in Korea (Yuk et al, 1981; Takagi et al, 1982). The benzene extract of this plant has shown the central nervous system depressant activity (Okugawa et al, 1993), and three cytoxic compounds have been isolated (Gunasekera et al, 1981). Based on the use of this plant in Korean folk it has been expected that the stem of *Aquilaria agallocha* has an antialergic activity and it is also proclaimed as an aid to spiritual meditation such as aphrodisiac.

Agarwood incense is burned to produce a pleasant aroma, its use ranging from a general perfume to an element of important religious occasions. Irregular chunks of agarwood, usually a few centimeters long and weighing 10-200 g, may be cut or broken into smaller pieces and then burned, usually in a specially made incense burner. Agarwood powder and dust cannot be burned directly in incense holders, but can be used to make incense sticks or coils for indoor fragrance, and are used for religious purposes by Muslims, Buddhists and Hindus (Yaacob, 1999).

2.8 Essential Oil

Essential oils are products of the secondary metabolism of plants, and generally are fragrant volatile materials consisting of complex mixtures of mono- and sesqui-terpene hydrocarbons, and oxygenated materials biogenically derived from them. Other common constituents include phenyl propanoids from the Shikimic acid pathway, and their biotransformation products, and other compounds from the metabolism of fatty acids and amino acids. As well as these major groups of compounds, a large number of other types of chemical components are also found, including nitrogen & sulphur compounds.

Essential oils may be present in many different types of plant materials (wood, bark, leaves, stems, flowers, stigmas, reproductive parts etc.) at concentrations ranging from thousandths of a percent to one or several percent. Oil is often contained in specialised secretory structures which include secretory cells, ducts, cavities, glandular trichomes etc. The yield of essential oils from seeds can often be high - in the several tens of percentage - but for the majority of other materials, the main range is 0.1% to 1%.

The fact that essential oils are, traditionally, perfumery materials, and that perfumers do not necessarily require oils to be pure (just consistent in quality batch to batch, and to be able to achieve the desired effect in product use) has lead to some problems for Aromatherapists who require pure essential oils, 100% derived from the named source. Aromatherapists also use certain oils which they believe have efficacious properties and bring benefits to clients in application, these oils are not necessarily always those in the mainstream of popular use for example rosemary oil verbenone type, or *Helichrysum italicum* ssp. *serotinum*. This has lead to the establishment of a small industry of essential oil distillers supplying the Aromatherapy market.