

STUDY ON PRETREATMENT OF *GAHARU* OIL EXTRACTION PROCESS

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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”

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Special dedication to my beloved family, lectures and friends.....

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ABSTRACT

Gaharu, the names for the resinous, fragrant and highly valuable heartwood produced by *Aquilaria Malaccensis*. *Gaharu* is classified as one of the most valuable forest products traded internationally. *Gaharu* oil is widely use in religious ceremony, meditation, incense and perfume. Currently, local entrepreneur has adopted hydro distillation technique to complete the extraction process because the advantages of the hydro distillation are less steam usage, the cheapest and simplest process and this is the best method for the powder. However, the percentage of yield is very low and longer time required extracting *gaharu* oil. To overcome this problem, a study on pretreatment methods will be performed to increase the effectiveness of *gaharu* oil extracted using hydrodistillation. There were three pretreatment methods studied which are soaking, ultrasonic and ultrasonic with enzymatic. From three of pretreatment methods, hydro distillation process with the assistant of ultrasonic with enzymatic pretreatment contribute the highest efficiency and capacity of *gaharu* oil extraction which gave 0.12% percentage yield of *gaharu* oil. However, the further method of pretreatment the *gaharu* oil in this study was ultrasonic because the increasing percentage yields of ultrasonic was 0.023% compared to the increasing of ultrasonic with enzymatic only 0.009% while the lowest was soaking 0.016%. The percentage of yield with the ultrasonic pretreatment was increased when the duration was increased. The percentage of yield was proportional with time.9 hours of pretreatment time showed the highest percentage of yield rather than other durations (1, 3 and 6 hours). As the conclusion, the ultrasonic pretreatment method is very crucial to improve the extraction process of *gaharu* essential oil.

ABSTRAK

Gaharu, nama untuk resin, haruman dan mempunyai nilai yang sangat tinggi adalah sejenis kayu dari Aquilaria Malaccensis. Gaharu diklasifikasikan sebagai salah satu hasil dari pokok yang mempunyai nilai yang tinggi di peringkat antarabangsa. Gaharu banyak digunakan dalam upacara keagamaan, perubatan, haruman dan wangian. pengusaha gaharu telah menggunakan teknik penyulingan hidro untuk proses pengekstrakan dan kelebihan menggunakan kaedah penyulingan hidro adalah kerana kurang stem yang digunakan, proses yang paling mudah dan murah serta paling bagus untuk serbuk. Walaubagaimanapun, peratusan pati minyak adalah sangat sedikit dan memerlukan masa yang panjang. Untuk mengatasi masalah ini, kajian dijalankan menggunakan rawatan awal melalui kaedah pengekstrakan penyulingan hidro. Terdapat tiga jenis rawatan awal yang dikaji iaitu perendaman, ultrasonic dan ultrasonic berenzim. Daripada ketiga-tiga rawatan awal, penyulingan hidro dengan dibantu oleh ultrasonik berenzim boleh meningkatkan kecekapan pengekstrakan minyak gaharu iaitu sebanyak 0.12% pati minyak. Walau bagaimanapun, peningkatan peratusan pati minyak menggunakan ultrasonik berenzim adalah paling rendah iaitu 0.009% berbanding peningkatan peratusan pati minyak oleh ultrasonik iaitu sebanyak 0.023% dan peningkatan peratusan pati minyak menggunakan perendaman sebanyak 0.016%. Peratusan pati minyak berkadar terus dengan masa untuk rawatan awal ultrasonik di mana 9 jam peratusan paling tinggi. Sebagai kesimpulan, rawatan ultrasonik sangat bagus untuk meningkatkan proses pengekstrakan minyak gaharu.

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LIST OF SYMBOLS

SYMBOLS	TITLE
w/v	Weight/ Volume
kg	Kilogram
rpm	Rotation per Minute
$^{\circ}\text{C}$	Degree Celsius
g	Gram
L	Liter
mL	Mililiter
T_{C}	Critical Temperature
P_{C}	Critical Pressure
Atm	Atmospheric

CHAPTER 1

INTRODUCTION

1.1 Research Background

Gaharu or agarwood is the resin impregnated heartwood or *Aquilaria* species of the family *Thymelaeaceae* (Chakrabarty, 1994; Soehartono & Newton, 2000). It gives off a unique aromatic scent when the wood is burnt. There are no less than twenty names associated with it, and this reflects its long history and widespread usage. Some of these names include agaru, aloes wood, eagle wood, oud, chen-xiang, jinkoh and so on depending on the region (Chakrabarty, 1994; Soehartono & Newton, 2000).

Gaharu is divided into several grades in the market. The best and darkest are used in incense mixtures while the lower grades are extracted by hydrodistillation to produce *gaharu* oil used in perfumery (Gibson 1977). As noted in Barden *et al.*, 2000, grading *gaharu* is a complicated process. This includes evaluating the size, colour, odour, weight (on scales and in water) and flammability of the wood but application of grade codes (Super A, A, B, C, D, E) varies between buyer.



Figure 1.1 Pieces of *Gaharu* from Forest Trees

(Source: forestpathology.agarwood.nd)

Five species of *Aquilaria* are recorded for Peninsular Malaysia and all are believed to be able to produce oleoresins (Chang *et al.*, 2001). There is very little information on the quality of the different *gaharu* produced. The most popular species generally associated with *gaharu* is *A.malaccensis*. This species is synonymous with *A.agallocha* from India (Hou 1960). In the market, *gaharu* is the trade name generally refers to the resinous wood from any of the *Aquilaria* species despite the difference in or absence of local names. It is very difficult to identify the species from the resinous wood alone.

Table 1.1: Gaharu Producing Species of *Aquilaria* in Peninsular Malaysia

SPECIES	LOCAL NAME FOR RESINOUS WOOD	GRADE
<i>A.malaccensis</i>	Gaharu	Medium
<i>A.microcarpa</i>	Garu	-
<i>A.hirta</i>	Chandan	Medium
<i>A.rostrata</i>	-	-
<i>A.beccariana</i>	Gaharu, tanduk	-

(Source: Chang *et al.*, .2000)

Aquilaria malaccensis is distributed throughout Malaysia, except in the states of Perlis and Kedah (Whitmore 1972), and is known to produce medium quality grade *gaharu* (Burkill 1966). There are also unconfirmed reports of *A. malaccensis* in Bangladesh, although recent reports indicate that this species no longer exists in the wild (Khan 1993 cited in Chakrabarty *et al.*, 1994). In India *A. malaccensis*, formerly known as *A. agallocha*, is native to Arunachal Pradesh, Assam, West Bengal, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, and Tripura (Chakrabarty *et al.*, 1994). In natural habitats, this species can be found growing at an elevation of 1000 m, but is localized mainly in the foothills and undulating slopes of evergreen and semi evergreen forests.

Gaharu is one of the most expensive and highly prized commodities (Barden *et al.*, 2000). Several raw forms are traded. They range from large sections of trunk to chips, flakes, oil and even the spent powder wastes after oil extraction. Large pieces of *gaharu* are hard to come by nowadays. In Malaysia, chips, flakes, oil and powder waste after extraction are the most common forms traded. Depending on the grade and quality, prices could range from 60 sen per kg for the low and mixed grades to more than RM 2000 per kg for the high grade (Chang *et al.*, 2000)

The *gaharu* wood is hard and light with rough texture, white or brownish yellow. It has a highly appreciated and priced fragrant wood caused by accumulation of scented resin. Production of *gaharu* may be influenced both by genetic and environmental factors but the general understanding is that the fragrant oleoresin that permeates the heartwood of some trees is produced as a response to wounding and/or fungal infection. They have a wide use in medicine (general pain reducer, dental pain, kidney and rheumatism medicine), as venom repellent, in perfume and as incense raw material. Wood without or with low content of resin can be used for boxes, interior or veneer. The inner fibrous bark has occasionally been used locally as raw material for clothing and ropes. *Gaharu* contains more than 12 chemical components that can be extracted.

Since *gaharu* is valuable, local entrepreneur has adopted hydrodistillation technique that much practice traditionally especially in rural areas of Cambodia and

India (Chang *et al.*, 2002). But now, local entrepreneur more prefer effective technique that produce higher yield of oil using other extraction techniques (Reverchon *et al.*, 1992).

1.2 Problem Statement

Gaharu wood has been driven to endangered status due to human harvest of the wood to extract valuable resins used in perfume making (Lagenheim *et al.*, 2003). High quality *gaharu* is still used in the manufacture of high quality joss-sticks in China, Japan and India. Currently, *gaharu* is also used in products such as *gaharu* essence, soap and shampoo. These products are marketed at prices about ten times more expensive than the common brands of toilet soaps and shampoos. With advancing technology, it is expected that in future more new products derived from *gaharu* will appear in the market.

The drawbacks associated with hydro distillation method include long hours required to complete the extraction process and produce very low yield. In this study, ultrasonic pretreatment step is introduced in order to speed up the breakage *gaharu* cell's wall, hence reduce the mass transfer resistances and releases the oil into extraction medium during the distillation process. Hydro distillation process with the assistant of ultrasonic pretreatment may contribute to improve the efficiency and capacity of *gaharu* oil extraction.

1.3 Objective of the Research

The objective of this study is to examine the feasibility of pretreatment method in hydro distillation *gaharu* oil extraction process.

1.4 Scopes of the Research

In order to achieve the objective, the following scopes have been identified:

- i) *Gaharu* oil extraction without pretreatment.
- ii) *Gaharu* oil extraction with soaking as a pretreatment method.
- iii) *Gaharu* oil extraction with ultrasonic pretreatment.
- iv) *Gaharu* oil extraction with enzymatic and ultrasonic pretreatment

CHAPTER 2

LITERATURE REVIEW

2.1 Gaharu

2.1.1 Gaharu

Aquilaria malaccensis is one of 15 tree species in the Indomalaysian genus *Aquilaria*, family *Thymelaeaceae* (Mabberley, 1997). It is a large evergreen tree growing over 15-30 m tall and 1.5- 2.5 m in diameter, and has white flowers (Chakrabarty *et al.*, 1994). It bears sweetly-scented, snow-white flowers. *A. malaccensis* and other species in the genus *Aquilaria* sometimes produce resin-impregnated heartwood that is fragrant and highly valuable. There are many names for this resinous wood, including agar, agarwood, aloeswood, eaglewood, *gaharu* and *kalamabak*, this wood being in high demand for medicine, incense and perfumes across Asia and the Middle East. The trees frequently become infected with a parasite fungus or mold *Phialophora parasitica*, and begin to produce an aromatic resin, in response to this attack. For convenience, agar or *gaharu* will be used from hereon.

In Malaysia, the tree of *Aquilaria* is called *karas* and its fragrant wood is known as *gaharu*. The *gaharu* is traditionally used to produce incense in the Far East. The grade of *gaharu* is divided by 5 types, which are Grade Super A, A, B, C and D. Grade Super A is most expensive compared to the others. The grades are depends on the essential oil that can be extracted. The main extract of the *Aquilaria* wood contained sesquiterpene namely alfa-agarofuran, (-)-10 epi-gama-eudesmol and oxo-agorospinol that produce the aromatic smell of the *Aquilaria* incense wood (Cheksum *et al.*, 2002).

Gaharu is frequently found as irregular patches or streaks in the wood of about 20 years old trees. Very often, the quantity and quality of *gaharu* produced increase with age, with the best yields occurring in trees aged 50 and above (Sadgopal 1960). It was reported that only about 10% of wild *Aquilaria* spp. Produce oleoresins (Gibson, 1977). The oleoresin-deposited trees are often identified by the appearance of decay and other signs which indicate disease. The affected wood containing *gaharu* appears as light brown to black regions against the background normal white wood. Traces of aromatic oils can be found in the peripheral white wood as well as in the colored portions.

2.1.2 Geographical Distribution and Habitat.

The genus *Aquilaria* of the family *Thymelaeaceae* comprises eight tree species which are distributed throughout India, China, Southeast Asia and the East Indies (Willis, 1955). Several species are known to produce *gaharu*. Of the 15 species in the genus *Aquilaria*, only *Aquilaria malaccensis* and *Aquilaria agallocha* have received significant attention in the last few decades.

Aquilaria malaccensis is widely distributed in south and Southeast Asia. There are differing accounts of the countries in which it occurs. According to Oldfield *et al.*, (1998), *A. malaccensis* is found in 10 countries which are in Bangladesh, Bhutan, India, Indonesia, Malaysia, Myanmar, Philippines, Singapore and Thailand. *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 20-22⁰C (Afifi *et al.*, 1995).

2.1.2.1 Malaysia Distribution

A.malaccensis is distributed throughout Peninsular Malaysia, except for the States of Kedah and Perlis (Barden *et al.*, 2000), but although the species has good geographical coverage, its occurrence is rather rare, with trees often locally scattered. La Frankie (1994) studied the population dynamics of *A.malaccensis* in Pasoh Forest Reserve and suggested a typical lowland Malaysia forest density of 2.5/ha and found that the growth rate varied between 0-1.95 cm/year.

Malaysia has a long history in the trade in *gaharu* wood, which has long been collected by the indigenous peoples of the interior of Peninsular Malaysia, Sabah and Sarawak to supplement their income. In Peninsular Malaysia, the *gaharu* products in domestic trade are woodchips and powder or sawdust (L.Chua, Forest Research Institute Malaysia, *in litt.* to TRAFFIC Southeast Asia, 2003). Some use has been recorded locally for medicinal purposes, but it appears that the majority of *A.malaccensis* harvested is exported (Barden *et al.*, 2000). The wood is also used for making small boxes in Sabah (Sabah Forest Department, *in litt.* to TRAFFIC Southeast Asia, 2003).

2.1.3 Conservation Status

Aquilaria trees are now protected in most countries and the collection of *gaharu* wood is illegal from natural forests. International agreements, such as CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora), accepted by 169 countries, is designed to ensure trade in *gaharu* products from wild trees does not threaten the survival of *Aquilaria*. Despite these efforts *gaharu* products from illegally cut trees continues to be sold and unknowing consumers create a demand that helps to destroy the last old growth *Aquilaria* trees in existence.

Aquilaria malaccensis is considered 'Vulnerable' according to the current IUCN Red List Categories, and has been included in *The World List of Threatened Trees* (Oldfield *et al.*, 1998). Considerable harvest pressure was noted in range States such as Indonesia, Malaysia and Thailand at the time when the species was proposed. Very little recently published information is available regarding the status of this species. Information collected during this study indicates that overexploitation remains a significant concern. A report by Chakrabarty *et al.*, (1994) documenting India's trade in *gaharu* wood concluded that *A. malaccensis* is highly threatened in that country due to exploitation of the species for commercial purposes.

The government of each of Malaysia's three administrative jurisdictions requires that permits be obtained from the relevant State Forestry Department to harvest and trade all *gaharu* wood -producing species. Harvesting from national parks or wildlife sanctuaries is prohibited in all states.

2.1.4 Hypotheses of Gaharu Formation

The investigation of *gaharu* wood formation was first initiated in 1962 by Bose. As yet, however, neither the casual agent nor the exact mechanism for *gaharu* wood formation has been determined. It is uncertain whether the fragrant wood results from fungal infection which brings about pathological conditions, certain chemical changes in the tree, or the environmental factors. Three hypotheses exist regarding *gaharu* formation, namely that it is the result of pathological, wounding/pathological and/or nonpathological processes (Ng *et al.*, 1997). According to Ng *et al.*, (1997), studies have not provided conclusive evidence for any of these hypotheses. Oldfield *et al.*, (1998) states that resin production is in response to fungal infection, and Heuveling van Beek (TRP, *in litt.* to TRAFFIC International, 2 May 2000) that it is in response to wounding. He adds that fungal infection can increase resin production as a host response to increased damage due to fungal growth. *Aquilaria* trees are naturally infected by a variety of fungi including: *Aspergillus* spp., *Botryodyplodia* spp., *Diplodia* spp., *Fusarium bulbiferum*, *F. laterium*, *F. oxysporum*, *F. solani*, *Penicillium* spp., and *Pythium* spp. (Soehartono *et al.*, 1997). The ecological interaction between the host tree and the wound and/or the fungi in order to produce *gaharu* is poorly understood.

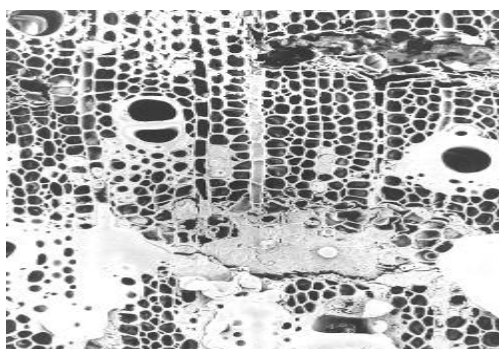


Figure 2.1 Cell Structure within an *Aquilaria* tree; White Areas Indicate Resin Deposits

(Source: Blanchette *et al.*, 2004)