COMPARISON OF GAHARU (AQUILARIA MALACCENSIS) ESSENTIAL OIL COMPOSITION BETWEEN EACH COUNTRY

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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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DEDICATION

Special dedication to my beloved father, mother, brothers and sisters.....

ACKNOWLEGMENT

In preparing this thesis, I was in contact with many people, researchers, academicians and practitioners. They have contributed towards my understanding and thoughts. In particular, I wish to express my sincere appreciation to my beloved supervisor, Mr. Saifulnizam Tajuddin for encouragement, guidance, critics and friendship. I also indebted to FKKSA lecture for their guidance to complete this thesis. Without their continued support and interest, this thesis would not have been the same as presented here.

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ABSTRACT

Aquilaria is an evegreen tree growing up to 40 meters high and 60 centimetres in diameter. These trees frequently become infected with a fungus and begin to produce an aromatic resin commonly called Aloeswood, Agarwood and Oud. The resin is created in response to an attack from Phialophora parasitica, which is a parasite fungus or mold. The purpose of this project were to know the compounds in essential oil from gaharu that been produced by hydrodistillation, to analyse of the compound in the gaharu essential oil and to determine the quality, chemical compound and method use to extract the essential oil of gaharu between different origins. Chemical compound of an agarwood originating from agarwood (Aquilaria sp. probably A. malaccensis) were investigated by GC-MS. The differences in chemical composition between the agarwood in four difference countries are discussed. The samples are taken from gaharu production industry at Kelantan, China, India and Thailand. The extraction of gaharu essential oil also been done by using hydrodistillation. Firstly preparations of the sample were done by make the sample to the sawdust and after that soak it with water. Then setting the hydrodistillation set and heated up the sample that was soaked before. The temperature of the sample was maintained at 98°C to 99°C for three days. Collect the sample that diluted in solvent of ethyl acetate (EtOAc) and put it in sample bottle. Cleaned the sample from the water existed, before analyze it with GC-MS. The data from GC-MS were recorded in the understandable way. The compound between the four countries have large dissimilar, but it still some component is comparable between each country. The essential oil had been produced and been analyzed. If look from analysis result, component from oil sample of industry and laboratory were not identical

ABSTRAK

Aquilaria adalah pokok malar hijau yang hidup sepanjang 40 meter dan berdiameter 60 sentimeter. Pokok ini selalunya telah dijangkiti oleh fungus dan mula menghasilkan resin yang wangi dipanggil Aloeswood, Agarwood dan Oud. Resin ini dihasilkan untuk bertindakbalas dari serangan Phialophora parasitica, iaitu fungus parasit ataupun kulat. Tujuan projek ini adalah untuk mengetahui campuran minyak asal gaharu yang dihasilkan dengan menggunakan penyulingan hidro, untuk menganalisis sebatian minyak asal gaharu dan untuk menentukan kualiti, sebatian kimia dan cara yang digunakan untuk memerah minyak asal gaharu dari tempat yang berbeza. Sebatian kimia gaharu berasal dari agarwood (Aquilaria barangkali A. Malaccensis) telah disiasat dengan GC-MS. Perbezaan dalam komposisi kimia diantara gaharu dalam empat negara yang berbeza telah dibincangkan. Sampel telah diambil dari industri yang menghasilkan gaharu di Kelantan, China, India dan Thailand. Pemerahan minyak asal gaharu telah dibuat dengan menggunakan penyulingan hidro. Pertamanya, penyediaan sampel telah dibuat dengan menjadikan sampel kepada habuk kayu dan selepas itu proses merendam habuk kayu itu ke dalam air. Kemudian set penyulingan hidro disediakan dan panaskan sampel yang telah direndam sebelum ini. Suhu sampel telah dikekalkan pada suhu 98°C hingga 99°C selama 3hari. Sampel yang telah dilarutkan dalam pelarut dari ethyl acetate (EtOAc) dikutip dan dimasukkan ke dalam botol sampel. Sampel dibersihkan daripada sebarang kehadiran air, sebelum dianalisa dengan GC-MS. Maklumat dari GC-MS telah dicatatkan dalam cara yang difahami. Dari keputusan analisa, terdapat perbezaan yang besar diantara sebatian empat negara ini, tetapi masih ada sebahagian komponen yang sama diantara setiap negara. Minyak asal telah dihasilkan dan dianalisa. Jika dilihat dari keputusan analisa, komponen daripada sampel minyak dari industri dan makmal tidak serupa.

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CHAPTER 1

INTRODUCTION

1.0 Essential Oil

An essential oil is a concentrated hydrophobic liquid containing volatile aromatic compounds extracted from plants. It may be produced by distillation, expression or solvent extraction. Essential oil is used in perfumery, aromatherapy, cosmetics, incense, medicine, household cleaning products and for flavouring food and drink. They are valuable commodities in the fragrance and food industries. Essential oil is also known as volatile oil and ethereal oil. It may also be referred to as "oil of" the raw plant material from which it was extracted, such as oil from clove. The term essential is intended to convey that the oil is an essence of the plant it is extracted from, and not in the more common sense of being indispensable, do not confuse them with essential fatty acids. Essential oil contains the true essence of the plant it was derive from. Essential oils are not the same as perfume oils or fragrance oils. Where essential oils are derived from the true plants, perfume and fragrance oils are artificially created fragrances, contain artificial substances or are diluted with carrier oils and do not offer the calibre of therapeutic benefits that essential oils offer.

Agar oil is distilled from the resinous portions of the wood of Aquilaria agallocha. This resinous wood is traded under the names "agar", "aloe wood" or "eagle wood". The tree occurs in patches in Bhutan's southern hills, in Assam in India and in parts of West Bengal. Very little is known regarding why irregular portions of dark wood, highly charged with oleo-resin, appear in some trees but not in others, especially in and around old wounds and hollows. It is known that resinous infiltration occurs because of fungal attack, but the specific fungus responsible for the formation of agarwood has not yet been identified. Attempts to impregnate trees by driving pegs from trees already containing agar wood into trees not infected have not been successful. The distillation processes for agar oil consist of soaking agarwood in water for 60-70 hours. The wood is then disintegrated into powder in a chopper. The powdered wood, suspended in water to which 5 percent by weight of common salt has been added, is placed in a retort and heated over a furnace. The retort has a swan neck with a device for replenishing the water, without removing the lid during distillation. A Florentine flask made of glass or copper constitutes the receiver for the distillate. Distillation takes 30-32 hours. Because distillation takes place at atmospheric pressure, the process of total exhaustion of the wood is lengthy. The oil yield ranges from 0.75-2.5 percent of the wood.

Agarwood is a scented product obtained from a pathological condition of the wood of standing trees of certain Aquilaria species. Aquilaria is an evergreen tree growing up to 40 meters high and 60 centimetres in diameter. Aquilaria is native to Northern India, Laos, Cambodia, Malaysia, Indonesia, China and Vietnam as shown in (Figure 1.1). These trees frequently become infected with a fungus and begin to produce an aromatic resin commonly called Aloeswood, Agarwood and Oud. This resin is used by Traditional Chinese, Unanai, Ayuravedic and Tibetan physicians. This resin is created in response to an attack from Phialophora parasitica, which is a parasite fungus or mold. The fungus and decomposition process continues to generate a very rich and dark resin to form within its heartwood. The resin created as an immune response makes the most sacred oil on the planet.



Figure 1.1Distribution of Gaharu Across Asia Country

(Source: <u>www.unep.org</u>)

Aquilaria malaccensis is a species of plant in the Thymelaeaceae family. It is found in <u>Bangladesh</u>, <u>Bhutan</u>, <u>India</u>, <u>Indonesia</u>, <u>Iran</u>, <u>Malaysia</u>, <u>Myanmar</u>, <u>Philippines</u>, <u>Singapore</u> and <u>Thailand</u>. It is threatened by habitat loss (Barden, Angela, 2000). Aquilaria malaccensis is the major source of agarwood, resinous heartwood, used for perfume and incense (Broad, S., 1995). The resin is produced by the tree in response to infection by a parasitic <u>ascomycetous</u> mould, *Phaeoacremonium parasitica* (P. W. *et al.* 1996) and a dematiaceous (dark-walled) fungus.

The grade of agarwood essential oil is divided by 5 types, which are Grade Super A, A, B, C, and D. The Grade Super A is the most expensive compared to the others grade. The grade (and hence value) of agarwood and agarwood derivatives such as oil is determined by a complex set of factors including: country of origin; fragrance strength and longevity; wood density; product purity; resin content; colour; and size of the form traded. According to Heuveling van Beek and Phillips (1999), agarwood oil is graded based on the quality of raw materials, the method of distillation and the skill used in processing. It is said to be now virtually impossible to find pure agarwood oil (although a supposedly pure sample was received by TRP from a large international agarwood-trading group in Dubai). The grades are depends on the essential oil that can be extracted. Plant extracts as seen as a way of meeting the demanding requirement of the modern industry for the past two decades (Simandi *et al.*, 1996). Five basic odour classifications of agarwood incense aromas in Japan which is sweet, sour, hot, salty, and bitter (Morita, 1992).

Traders have quoted prices for pure agarwood oil as high as USD30 000/kg, such oil only being made to order. Grade-two oil costs approximately USD15 000/kg, but generally oil prices are between USD5000/kg and USD10 000/kg. However, cheaper oils, adulterated with perhaps a mixture of sandalwood and sesame seed oil, can be bought for a few hundred dollars per kilograms. Few traders nowadays, if any, can assess oil quality or purity and it is unlikely that there is much consistency between oil batches. Where agarwood prices have been obtained in currencies other than US dollars, they have been converted to that currency using the average interbank exchange rate for the period 1 January to 31 March 1999, based on rates provided by an on-line currency converter (OANDA, 2000). The following conversion rates were used: INR1 (Indian Rupee) = USD0.02353; IDR1 (Indonesian Rupiah) = USD0.00011; MYR1 (Malaysian Ringgit) = USD0.26311; THB1 (Thai Bhat) = USD0.02695. Agarwood powder is generally much less expensive than chips or flakes, with prices varying from around USD20-60/kg.

The first investigation on the chemical components of agarwood was reported by Kafuku and Ichikawa 1935 cited in (Shimada *et al., 1982*). Agarwood contains a sesquiterpene alcohol which produces its characteristic aroma. It was reported that 2-[2-(4'-methoxyphenyl) ethyl]chromone and 2(2-phenylethyl) chromone (Figure 1.2) (or flidersiachromone), through pyrolysis at 150°C produces 4-methoxybenzaldehyde and benzaldehyde respectively (Hashimoto *et al.,* 1985). These molecules are odourless at room temperature but produce a long lasting fragrance upon burning.



Figure 1.2 2(2-phenylethyl) Chromone

Form the chloroform-soluble fraction of the alcoholic extract of the alcoholic extract of A. agallocha, (Bhandari *et al.*, 1982) identified the molecules called aquillochin (a coumarinolignan), gmelofuran and agarol. Two chromones, known as chromone 1 (2-[2-(4'-methoxyphenyl)ethyl]chromone) and chromone 2(6-methoxy-2-[2-(4'-methoxyphenyl) ethyl]chromone), are components characteristic of "kanankoh" (in Japanese for the best quality agarwood from A. *agallocha*) and are either absent entirely or present only in small amounts in "jinkoh" (in Japanese for the lesser quality agarwood from other species of *Aquilaria*) (Hashimoto *et al.*, 1985, Nakanishi *et al.*, 1984,1986, Ishihara *et al.*, 1991). Differences in other chemical components were also noted between the best and lesser quality agarwood (Table 1.1). According to the latest study of (Ishihara *et al.*, 1993a, 1993b), there are two types of kanankoh; one of them is rich in oxygenated guaiane and eudesmane derivatives, while the other contains oxoagarospirol as a major sesquiterpene component. Similar chemical studies were conducted on agarwood from A. *agallocha* and other species of *Aquilaria* (Jain & Bhattacharyya 1959, Varma *et al.*, 1965, Maheshwari *et al.*, 1963a, 1963b, Barrett &

Buchi 1967, Yoneda *et al.*, 1984). The results from a study by Yoneda and co-workers (1984) suggest that agarwoods of different origins may be distinguished chemically (Table 1.2).

 Table 1.1: Differences in Chemical Component between Best and Lesser Quality

 Agarwood

Grade of agarwood	Compounds identified	Remark
Best	Sesquiterpenes:	Absent from lesser quality
	(-)-guaia-1(10),11-dien-15-al	agarwood
	(-)-selina-3,11-dien-9-one	
	(+)-selina-3,11-dien-9-ol	
Lesser	Kusunol	Present in considerable
	Dihydrokaranone	amounts
	Karanone	
	Oxo-agarospirol	

Table 1.2: Chemical Comparisons between Agarwood of Different Origins

Agarwood	Chemical components	Remark
Type A (A. agallocha)	Agarospirol	Abundant
	Jinkoh-eromol	
	Oxo-agarospirol	
	α - and β -agarofuran	
	Dihydroagarofuran	
	Kesunol	
	Nor-ketoagarofuran	Not present in type B
	Dihydrokaranone	
Type B (Aquilaria spp.)	Agarospirol	

Kusunol	
Jinkoh-eremol	
Oxo-agarospirol	
α-agarofuran	
(-)-10-epi-γ-eudesmol	
Jinkohol	In large amounts, absent
Jinkohol II	from type A

1.1 Problem Statement

Since *gaharu* is valuable, local entrepreneur has adopted water distillation technique that very 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 hydrodistillation. Although several method of extracting essential oil is developed such as solvent extraction, expression and critical fluid extraction most are produce by hydrodistillation (Reverchon *et al., 1992*). Researchers are looking at various inducement techniques to produce aromatic *gaharu* on a commercial scale. The Forest Research Institute of Malaysia (FRIM) began researching in the late 1990s following a surge in market demand for *gaharu* and is still refining its inoculation technique. Based on anecdotes from Orang Asli collectors, researchers deliberately wound the tree trunk and indeed, *gaharu* was produced in varying degrees of formation, suggesting that it can be induced in standing Aquilaria trees by artificial means. But the grade obtained was inconsistent.

In *gaharu* producing species like Aquilaria, the tree will produce the resin to contain the infection from spreading, covering the wound and blackening the whitish heartwood. That's how *gaharu* is produce. "The challenge is to come out with high

quality or the desire grade and predictable volume to make planting a viable solution to over-harvesting of wild species," say Chang. But in Malaysia there is no instrument that can grade the *gaharu* essential oil base on the scientific ways. In Malaysia, most of the *gaharu* essential oil are been grading base on the experience, and it just from the physical look and its sense. So now there is still in the research about to grading the *gaharu* essential oil by using the technological methods.

1.1 Objective

- To know the compounds in essential oil from *gaharu* that been produced by hydrodistillation.
- To analyse of the compound in the *gaharu* essential oil.
- To determine the quality, chemical compound and method use to extract the essential oil from *gaharu* between different origins.

1.3 Scope of Study

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

- Study how to distil Malaysia gaharu by using hydrodistillation.
- Study the grade of *gaharu* essential oil that been produced in Malaysia and others country.
- Study the comparison *gaharu* compound among Thailand, China, India and Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.0 Gaharu

Agarwood or eaglewood is the most expensive wood in the world. It is valued in many cultures for its distinctive fragrance, and used extensively in incense and perfumes. Agarwood is the occasional product of two to four genera in the family *Thymelaeaceae*, with *Aquilaria agallocha*, *Aquilaria crassna* and *Aquilaria malaccensis* the best known species. The wood is formed as a result of the tree's immune response to fungal infection. The odor of agarwood is complex and pleasing, with few or no similar natural analogues. As a result, agarwood and its essential oil gained great cultural and religious significance in ancient civilization around the world. Agarwood , eaglewood , gaharu, aloeswood are just a few of the name for the resinous, fragrant and highly valuable heartwood produced by *Aquilaria malaccensis* and other species of the *Indomalesian* tree genus *Aquilaria*. The wealth of names for this dark and heavy wood (its Chinese name literally means 'wood that sinks') reflects its widespread and varied use over thousands of years.

2.0.1 Application of Agarwood

Agarwood has three principal uses, such as in medicine, perfume and incense. Smaller quantities are used for other purposes, such as carvings. These uses are described in more detail below.

2.0.1.1 Medicine

Agarwood has been used for medicinal purposes for thousands of years, and continues to be used in Ayurvedic, Tibetan and traditional East Asian medicine, for example (Chakrabarty *et al.*, 1994; Fratkin, 1994). The Sahih Muslim, which dates back to approximately the eighth century, refers to the use of agarwood for the treatment of pleurisy and its use is referenced in the Ayurvedic medicinal text the Susruta Samhita. Agarwood is prescribed in traditional East Asian medicine to promote the flow of qi, relieve pain, arrest vomiting by warming the stomach, and to relieve asthma (Anon., 1995a). High-grade agarwood powder is prescribed in Chinese medicine (Yaacob, 1999) and is also used in the production of pharmaceutical tinctures (Heuveling van Beek and Phillips, 1999). (Burkill 1966) reported that Malaysians used agarwood mixed with coconut oil as a liniment, and also in a boiled concoction to treat rheumatism and other body pain. (Chakrabarty *et al.*, 1994) report that the often-discarded uninfected wood is used as *Kayu gaharu lemppong* by Malaysians to treat jaundice and body pains.

Bull 1930, cited in (Chakrabarty *et al.*, 1994) notes agarwood's use as a complex ointment for smallpox and for various abdominal complaints. Agarwood is also prescribed for dropsy, as a carminative, a stimulant, for heart palpitations, and as a tonic taken particularly during pregnancy, after childbirth and for diseases of female genital organs (Chakrabarty *et al.*, 1994).

2.0.1.2 Perfume

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). Agarwood perfumes are seldom pure agarwood oil, but instead use an alcoholic or non-alcoholic carrier, such as sandalwood oil. The cheapest agarwood perfumes are either synthetic or a blend of oils, each with different qualities and fragrances. Although there are several commercially available synthetic agarwood fragrance compounds, they can produce only low-quality agarwood fragrances, owing to the chemical structure of natural oil (Heuveling van Beek and Phillips, 1999). Agarwood essences have recently been used as a fragrance in soaps and shampoos (Kadir *et al.*, 1997), cited in Schippmann, 1999. Agarwood is said to have been highly prized by European perfumers in the mid-1990s (cited in Chakrabarty *et al.*, 1994).

2.0.1.3 Incense

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 centimetres long and weighing 10-200 g, may be cut or broken into smaller pieces and then burned, usually in a specially made incense burner (Heuveling van Beek and Phillips, 1999). 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).

Taiwanese consumers purchase agarwood for the manufacture of incense sticks, which are used in Agarwood perfume, chips and powder in Malaysia, 1999 Agarwood incense sticks on display in Taiwan, 1998 prayers during many traditional festivals and ceremonies to bring safety and good luck (TRAFFIC East Asia-Taipei, in litt. to TRAFFIC International, 2 May 2000). Both Indians and Chinese have used agarwood

as an essential ingredient of incense sticks in the past, but in the present day incense sticks generally do not contain agarwood, although Indian traders report that highquality Indian incense sticks destined for export may have a drop of agarwood oil added to them (Chakrabarty *et al.*, 1994). Agarbattis are incense cones, which also originally contained agarwood powder but seldom do so now because of the high price of agarwood. Instead, the light cream/brown powdery waste material obtained from oil distillation (with little or no resin content) is used to provide a basic carrier for other, cheaper, fragrant ingredients. This waste agarwood powder sells for around USD5/kg.

Japanese incense products are very different, with most of the highest-grade products made using natural raw materials which include ground agarwood extracts combined with other ingredients such as sandalwood and benzoin and then carefully molded and baked. Pure agarwood is also burned as incense in Japan. The user breaks pieces off and burns small pieces as required, hence large sections of wood will last several years (Heuveling van Beek and Phillips, 1999). In Japan, a revival in the ancient art of Koh doh, the incense ceremony, has revitalised interest in agarwood (Katz, 1996).

In Malaysia, Muslims burn agarwood splinters or chips to produce incense during special religious occasions, particularly at gatherings, and agarwood incense has been recorded in use there during Ramadan prayers (Chakrabarty *et al.*, 1994). Some Malay tribes fumigate paddy fields with agarwood smoke to appease local spirits (Chakrabarty *et al.*, 1994). Agarwood incense is used for various purposes in the Middle East, especially during prayers (Yaacob, 1999). Agarwood chips and splinters are also burned in bathrooms and incense is used as a customary perfume. Party hosts place agarwood chips over hot charcoals, the aroma signifying the end of a party.

2.0.1.4 Other Application

Burkill (1966) reported that grated agarwood has been used in Malaysia for cosmetic purposes, particularly during sickness and after childbirth. The use of agarwood bark as a writing material has also been documented extensively and agarwood is used for chronicles of important and sacred religious books. Use as a substitute for paper is also known from the mountaineers of Annam (Vietnam) and from China (Chakrabarty *et al.*, 1994). Twine is reported to be made from Aquilaria in Malacca (a province of Malaysia) (Chakrabarty *et al.*, 1994).

Although it may be possible to use healthy Aquilariawood to make simple ornamental boxes, this wood is typically too light and fibrous (rather like balsa wood) to be suitable for furniture, construction or even carving. Some foresters in India have suggested using Aquilaria wood for constructing tea-boxes (Chakrabarty *et al.*, 1994). Aquilaria bark was reportedly used for this purpose during the nineteenth century (Heuveling van Beek and Phillips, 1999). There are a considerable number of craft shops offering religious 'agarwood' sculptures, usually Bhuddhist figures. Although a proportion of immature agarwood is used in this trade, most statues are not made with agarwood, owing to its soft and flaky properties, which make it unsuitable for carving. Instead, tropical hardwoods are treated to resemble agarwood. The wood is blackened by injecting oil or tar into tree trunks and may also be impregnated with agarwood perfume (Heuveling van Beek and Phillips, 1999).

Agarwood is used to produce statues and religious objects (e.g. statues of Buddha) in Taiwan (TRAFFIC East Asia-Taipei, in litt. to TRAFFIC International, August 1999). As with carvings, most agarwood rosary and 'worry beads' offered for sale are fake, owing to the cost of shaping and drilling perfectly round beads of authentic agarwood. Instead, other dark woods may be submerged in agarwood oil for several weeks until the fragrance of agarwood has been absorbed and these are hen used in place of agarwood. Authentic agarwood bead necklaces cost approximately USD1500/kg (Heuveling van Beek and Phillips, 1999). Agarwood is used as an aromatic ingredient of Chu-Yeh Ching and Vo Ka Py wine in Taiwan (TRAFFIC East Asia-Taipei, in list. to TRAFFIC International, May 1999). Agarwood powder is known to be sprinkled on clothes and skin as an insect repellant effective against fleas and lice (Heuveling van Beek and Phillips, 1999). The *Sahih Muslim* refers to the use of agarwood for fumigation purposes. Agarwood may also be added to funeral pyres (Chakrabarty *et al.*, 1994) and is used in the preparation of bodies for burial (Yaacob, 1999). In Malaysia, it is used as a libation ingredient poured at gravesides (Chakrabarty *et al.*, 1994).

2.0.2 Grading and Prizing of Gaharu

The grading given an individual is different from others because they totally depend on their senses and sense between human are different. Different people give different grade values. This is always the problem because there is no standard on standardizing the grade of *gaharu*.

According to (Zich *et al.*, 2001) price per kilogram in May 2001 averaged as follows:

A grade = USD 341 B grade = USD 237 C grade = USD 172 D grade = USD 111

Gaharu is divided into several grades in market. The best and darkest are used in increase mixtures while the lower grades are extracted by steam distillation to produce *gaharu* oil used in perfumery (Gibson 1977).In 1991, Saudi Arabia imported about RM48.3 million of *gaharu* from Southeast Asia (Central Department of Statistics, Riyadh, Saudi Arabia) in which Malaysia was the leading supplier, clinching 26.3% of the market with an import value of RM12.6 million (Anonymous 1994). Singapore as a second-hand trader was in second place, with an import value of RM11.7 million, while Indonesia, Thailand and India trailed close behind. The annual agarwood trade in the

Middle East was reported to be about US\$40 million or more (Anonymous 1994). The retail prices of *gaharu* vary between countries depending on the quality (Table 2.1). In Dubai, the lowest grade *gaharu* was traded at Dhs100 (about US\$27) kg⁻¹ in 1993, while the most expensive grade was reported to be priced at at Dhs35 000 (US\$9589) kg^{-1.} These two products differed little in their visual appearance, but users can still discern the product's quality. In general, the most expensive grade *gaharu* are not displayed for the average buyer. Due to recent shortages of good quality *gaharu* on the market, the price of top quality *gaharu* has been reported to soar to an astronomical rate of Dhs100 000 (US\$27 400) kg⁻¹ (Chakrabarty *et al.* 1994).

 Table 2.1: Prices of Various Grades of Gaharu

Country	Grade	US\$kg ⁻¹	Source
India	Various	Up to 1250	Chakrabarty et al. 1994
Malaysia	Medium	50-250	Unverified
Vietnam	Unknown	100-1200	Chakrabarty et al. 1994

Loas	Unknown	2000-2400	Chakrabarty et al. 1994
Thailand	Various	1.2-1000	Chaiwong- Kiet 1994
	Finest	2800	
Dubai	Low	275-1250	Chakrabarty et al. 1994
	High	Up to 9589	
	Best	27400	

In terms of *gaharu* oil, it is reported to be as expensive as gold. Its units of trading in the Middle East market is in tola (one tola is equal 11.62 g) which is also used to weigh gold biscuits (of 99.99 purity) in Switzerland. The price of *gaharu* oil ranges between Dhs50 and 800 per tola (Chakrabarty *et al.*, 1994). The popular price is around Dhs600 tola⁻¹ which is equal to US\$14g⁻¹. Superior grades could be priced up to Dhs3000 (US\$825) tola⁻¹ .High quality of *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 then 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.

2.0.2.1 Malaysia

(Chakrabarty *et al*, 1994) stated that the lowest grade of Malaysian agarwood (not necessarily *Aquilaria malaccensis*) could be obtained for USD19/kg in the Middle East. The high grades, normally reserved for exclusive buyers, are said to cost up to USD9589/kg. More expensive grades are also available and can sell for as much as USD27 400/kg. (Ng *et al*, 1997) reported that, in 1991, south-east Asian countries exported approximately MYR48.3million (approximately USD17.6 million) worth of agarwood to Saudi Arabia, of which approximately 26% was sourced from Malaysia.

Trade data compiled by Ng and Azmi (1997) show that the value of agarwood chips alone (therefore representing only a small part of the actual trade in agarwood and its products) exported from Malaysia was MYR393 065 in 1995 (USD156 746, or USD164 787 adjusted for inflation to 1998 values) and MYR546 289 in 1996 (USD217 144, or USD223 897 adjusted for inflation to 1998 values). The data of *gaharu* price in Malaysia is shown in (table 2.2) and (table 2.3)

Gaharu Grade	Market price
Grade A	RM3,200 to RM4,000 per kg
Grade B	RM1,800 to RM2,500 per kg
Grade C1	RM 400 to RM 800 per kg
Grade C2	RM 40 to RM 80 per kg
Grade D	RM 8 to RM 30 per kg

Table 2.2: Prices of Grades of Gaharu at Terengganu

Table 2.3: Prices of Grades of Gaharu at Kelantan

Gaharu Grade	Market price
Grade Double Super	RM10,000 to RM12,000 per kg

Grade Super	RM 8,000 to RM10,000 per kg
Grade A	RM 4,000 to RM 8,000 per kg
Grade B	RM 3,000 to RM 4,000 per kg
Grade C	RM 1,000 to RM 2000 per kg
Mixing Grade	RM 60 to RM 250 per kg
Essential oil	RM19,000 to RM38,000 per kg

2.0.2.2 India

Agarwood chips in Mumbai are generally available for INR2000-5000/kg (USD47-118/kg). Traders said that chips can actually be obtained for anything between INR200-100 000/kg (USD5-2353/kg). It is very likely that those priced at INR200 are almost certainly fake, heavily adulterated and contains virtually no resin. Chips of the highest grade ('double super') are reportedly available at the border with Myanmar for up to INR60 000/kg (USD1412/kg), rising to INR100, 000/kg (USD2353/kg) in the Mumbai market.

Agarwood chips available in a Mumbai medicinal plant market rangedfrom INR5000-6000/kg (USD118-141/kg). Their authenticity could not be verified. Agarwood oil of varying grades and content was offered for sale at INR20-3000/tola (1 tola=10 g, equating to USD0.5-71/tola or USD47-7059/kg). North-eastern prices are typically higher than those in Mumbai. Agarwood chips cost INR100, 00-50000/kg (USD235-1177/kg) and dust can be obtained for INR7500-35000/kg (USD176-824/kg). Manufactured products were also available for purchase. Perfume prices ranged between INR2000- 4500/tola (USD47-106/tola or USD4706-10 589/kg), and incense was in the range of INR45000-55000/kg (USD1059-1294/kg). Wholesale agarwood

prices are fixed by certain forest divisions in the north-east. The Forest Department collects and reviews existing market prices for the various grades of agarwood from different Divisional Forest Officers to decide the price. The Principal Chief Conservator of Forests then approves these. Table 2.4 shows the price ranges allocated by the Assam Forest Department to various grades between 1993 and 1998.

Table 2.4: Wholesale Prices for Aquilaria malaccensis Fixed by Assam Forest Department

Source: Gupta, 1999

Year	Grade	Price(INR/kg)	Price(USD/kg)	Price adjusted for inflation to 1998 dollars(USD/kg)
1993	1 st class Black Agar	30 000	956	1054
	2 nd class Bantang	20 000	638	703
	3 rd class Plutas, Kalaguchi	7 500	239	264
	4 th class Dlum	100-250	3-8	3-9
1994	1 st class Black Agar	65 000	2 072	2 231
	2 nd class Bantang	15 000	478	515
	3 rd class Plutas, Kalaguchi	7 000	223	240
	4 th class Dlum	350	11	12
1998	1 st class Black Agar	50 000	1 213	1 213

2 nd class Bantang	30 000	728	728
3 rd class Plutas, Kalaguchi	10 000	243	243
4 th class Dlum	450	11	11

Based directly on the agarwood prices in Indian rupees shown above (therefore not accounting for inflation), between 1993 and 1994 the prices of 2nd class and 3rd class agarwood decreased by 25% and 7%, respectively. However, both the lowest class (Dhum Agarwood) and the highest class (Black Agar) increased in price by between 40-250% and by 117%, respectively, in a single year. The timing of these changes in price corresponded to the submission of the CITES listing proposal for *Aquilaria Malaccensis* by India. Interviews conducted with traders, exporters and suppliers within India indicated that agarwood was available (and therefore available for export) in raw, partially processed and processed forms. The latter include medicine, incense and perfumes. The vast majority of agarwood is exported in the form of oil. Destinations reported by traders included Bahrain, Kenya, Kuwait, Oman, Qatar, Saudi Arabia and UK.

2.0.2.3 Thailand

Agarwood can be used in different ways. Low grade agarwood is distilled to produce agar attar, which is used in the perfume and tobacco industries. High grade agarwood is exported to Arab countries, where it is used as incense and in the manufacture of joss sticks. The wood has been variously described as a stimulant, a tonic and a carminative, and is an ingredient of several medical preparations for rheumatism, body pains, and heart palpitation. Agarwood sells for US\$ 15-692 per kilogram, depending on quality, while oil distilled from the wood sells for US\$ 154-192 per 10 millimeter bottles.

2.0.3 Chemical properties



Figure 2.1 Agarotetrol



Figure 2.2 Isoagarotetrol

In a structural investigation of the chemical extracts of agarwood (A. Malaccensis) from Kalimantan, (Shimada *et al.*, 1982, 1986a) initially identified six molecules, namely (Figure 2.1 and 2.2). The 2-phenylethyl chromones have not been detected in normal tissues, except in the resinous parts of A. *Malaccensis*. It was therefore assumed that the presence of such constituents is specific to the process of resinification in *Aquilaria* plants.

2.0.3.1 India

The presence of eight sesquiterpenes including agarol and agarospirol together with α - and β - agofuran, were characterised by (Battacharrya *et al.* in 1959 & 1965) [Jain & Battacharrya (1959), Varma, Maheshwari & Battacharrya (1965)]. Maheshwari *et al.* (1963, 1963a) characterised alpha- and beta-agaofuran, dihydroagarofuran, norketoaragofuran, 4-hydroxydihydroagarofuran and 3, 4 dihydroxydihydroagarofuran by solvent extraction of infected agarwood of Indian origin. Subsequent total synthesis of nor-ketoagarofuran by Heathcock & Kelly (1968) showed the formula presented by Maheshwari *et al.* was incorrect. Pant & Ragosti (1980) used an instrumental technique to show that dihyroagarofuran was dihydro-beta-agarofuran and isodihydroagarofuran was isodihydro-alpha-agarofuran.



Pant & Rastogi (1980) also confirmed the sesquiterpenes: agarol & gmelofuran in the fragrant wood. (Ishihara *et al*, 1991a) described seven newly identified compounds in agarwood oil form *Aquilaria Agallocha* based on the guaiane skeleton, including guaia-1(10),11-dien-15-ol, guaia-1(10)-11-dien-15- carboxylic acid, methylguaia-1(10),11-dien-15-carboxylate, guaia-1(10),11-dien-9-one, 1,1'0epoxyguaia-11-ene, guaia-1(10),11-dien-15,2-olide and guaia-1(5),11-dien-2-one. In a subsequent paper Ishihara (1991b) describe a further 3 sesquiterpenes and ascribed odour descriptions to some characterised components.

(NДf *et al.*, 1992, 1995) described components in fresh agarwood oil distilled from some freshly purchased Indian wood (identified as A. *agallocha*), the authors identified beta-agarofuran, vetispira-2(11), 6(14)-dien-7-ol, dihydrokaranone and valerianol as major constituents of Indian agarwood oil. Eight new valencane-, eremophilane- and vetispirane- derivatives were described, including (3R, 7R, 9R, 10S)-9,10-dimethyl-6-methylene-4-oxatricyclo[7.4.0.0^{3,7}]1-tridecene, and compounds

synonymous with 2,14-epoxyvetaspira-6-ene and 2,14-epoxyvetaspira-6(14),7-diene were also described (1995), odour descriptions being ascribed to three of the characterised compounds (table 2.6):

Table 2.5: Odour descriptions of compounds from India agarwood

Compound	Odour	
8,12-epoxy-eremophila-9,11(13)-	Woody with vetiver subtonality	
diene		
2,14-epoxy-vetispir-6-ene	Woody with typically agarwood-like, sweet,	
	woody, smoky, phenolic like oak-moss but	
	weak.	
2,14-epoxy-vetaspira-6,(14),7-diene	Woody but nearly odourless	





(3R, 7R, 9R, 10S)-9,10-dimethyl-6-methylene -4-oxatricyclo[7.4.0.0]1-tridecene

2,14-epoxy-vetaspira-6-ene



2,14-epoxy-vetaspira-6(14),7-diene

2.0.3.2 China

(Yoshi *et al.*, 1983) elicidated the structure of the highly oxygenated chromone, agarotetrol from Jinkoh(A. sinesis). In a series of published studies, Yang and Cheng(1983,1986)(Yang *et al.*, 1989a, 1989b, 1990) identified the presence of benzylacetone, p-methoxybenzylacetone, anisic acid and beta-agarofuran as well as the sesquiterpenese baimuxinic acid, baimuxinol, dehycrobaimuxinol and isobaimuxinol.



Figure 2.3 Characterised Six Compounds in Chinese Agarwood (A. sinensis)(Xu *et al.*, 1988)
(Yang et al., 1989) from an ether fraction of an alcohol soluble extract of A. sinesis distinguished a new chromone, 6-hydroxy-2-[2-(4'-methoxylphenyl) ethyl] chromone and the other five known chromones: 2-(2-phenylethyl) chromone, 6methoxy-2-(2-phenylethyl)chromone, 6.7-dimethoxy-2-(2-phenylethyl) chromone, 6methoxy-2[2-(3'-methoxyphenyl) ethyl] chromone and 6-hydroxy-2-(2-phenylethyl) chromone. Later Yang & Wang (1990) elucidated the structure of two 2-(2phenylethyl)chromone derivatives isolated from the ethyl acetate soluble fraction of the alcoholic extract of Aquilaria sinensis (Lous. Gilg) which is 5,8-dihydroxy-2-(2-pmethoxyphenylethyl)chromone 6,7-dimethoxy-2-(2-p-methoxyphenylethyl) and chromone. This was followed by Yagura (2003) who identified 5-hydroxy-6-methoxy-2-(2-phenylethyl) chromone, 6-hydroxy-2-(2-hydroxy-2-phenylethyl)chromone, 8chloro-2-(2-phenylethyl)-5,6,7-trihydroxy-5,6,7,8-tetrahydrochromone,6,7-dihydroxy-2-(2-phenylethyl)-5,6,7,8-tetrahydrochromone were isolated from a methanol extract of withered wood of Aquilaria sinensis.

Hsu (1996) notes the presence of agarospirol, agarol, agarofuran, dihydroagarofuran, 4-hydroxyagarofuran, 3,4-hydroxydihydroagarofuran, and norketoagarofuran in infected extracted wood, and the presence of benzylacetone, pmethoxybenzylacetone, hydrocinnamic acid, p-methoxyhydrocinnamic acid, agarospirol, agarol, agarofurans and agarotetrol in the essential oil, the inference being this is from non-infected wood.

benzylacetone





p-methoxybenzylacetone





2.1 Method

2.1.1 Extraction

The vast majority of true essential oils are produced by distillation. There are different processes used. In all of them, water is heated to produce steam, which carries the most volatile chemicals of the aromatic material with it. The steam is then chilled (in a condenser) and the resulting distillate is collected. The Essential Oil will normally float on top of the Hydrosol (the distilled water component) and may be separated off.

The temperature for the heating is between 99-100 0 C, with that the oil that produce will called as essential oil. There is a wide variety of analytical methods is used to extract the volatile compounds from plant material. Techniques commonly used to extract the essential oils include steam distillation, hydrodistillation, dynamic and static headspace, supercritical-fluid extraction and solvent extraction (N.S. Kim *et al.*, 2002). For extraction of gaharu essential oil the type of distillation that need be used is hydrodistillation.

The proportion of different essential oil extracted by hydrodistillation is 93% and the remaining 7% is extracted by other method (Masango P. 2001). Usually hydrodistillation method produces pure quality of essential oil because it only use water rather then other method that use solvent. Using of solvent in extraction method may lead to produce essential oil and its compound of the composite oil in the wastewater (Bohra *et al., 1994*). This will be a problem in separating the essential oil but majority cases of steam distillation extraction produce the oil that less density than the water and so form the top layer of distillate. The partition between the water and oil phases of distillate make the separation of the oil is easy and more economically (Masango P. 2000).

2.1.2 GC-MS

The use of a mass spectrometer as the detector in gas chromatography was developed during the 1950s by Roland Gohlke and Fred McLafferty (Gohlke, *et al.*, 1993).These sensitive devices were bulky, fragile, and originally limited to laboratory settings. The development of affordable and miniaturized computers has helped in the simplification of the use of this instrument, as well as allowed great improvements in the amount of time it takes to analyze a sample. In 1996 the top-of-the-line high-speed

GC-MS units completed analysis of fire accelerants in less than 90 seconds, whereas first-generation GC-MS would have required at least 16 minutes. This has led to their widespread adoption in a number of fields.

Gas chromatography-mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. GC-MS can also be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification.

The GC-MS has been widely heralded as a "gold standard" for forensic substance identification because it is used to perform a *specific test*. A specific test positively identifies the actual presence of a particular substance in a given sample. A *non-specific test*, however, merely indicates that a substance falls into a category of substances. Although a non-specific test could statistically suggest the identity of the substance, this could lead to false positive identification.

CHAPTER 3

METHODOLOGY

3.0 Sample preparation

Based on the study by FRIM, *gaharu* wood actually can be devided into several main grades that are grade A, B and C (Chang *et al.*, 2002). This grade is given based on the quality and yield of essential oil that can be extracted from the wood. Grade A *gaharu* can produce high yield of essential oil but it price is very high and this increase the overall cost. For this experiment, *gaharu* grade C was use to extract as much as possible of essential oil with low cost of operating process. Grade C *gaharu* wood can be bought through local entrepreneur *gaharu* trade by middle man at the cost of RM100 per kilogram.

Gaharu wood firstly has been cut into small pieces to increase surface area of *gaharu* chips for easier drying process. The purpose of this drying process is to avoid any blockade in grinding.

Second steps of sample preparation are grinding process. The pieces of dry *gaharu* wood are ground into sawdust with the size of 1mm. In extraction process, the

rate of extraction is increase when the area of contact between the solvent and solid is high. So, the higher surface area of *gaharu* sawdust, more *gaharu* essential oil can be extracted.

Before the extraction process, grinded *gaharu* must be soaked in water. The ratio of *gaharu* to water is 1:8 (Dong-ping *et al.*, 1999) for period of three to seven days in order to break down the parenchymatous and oil glands (Chang *et al.*, 2002). For this experiment five days was chosen in order to maximize the soaking effect. The amount of *gaharu* sawdust used is 500 gram and water equal to 4000 mL.

3.1 Hydrodistillation

The equipment of hydrodistillation process was set up. Then, the mixture of 500 gram sawdust of *gaharu* wood and 4000 mL of water is put into the flask of distillation unit. After that, the heating mantel and re-circulating cooler are switched on. The temperature is set at boiling point of water should be below of 100° C for the whole experiment. Aluminium foil needs to be wrapped all over the apparatus to make sure there is no heat loss occurs. The extraction process is run for three days. The essential oil present in the flask is vaporizes. Steam and essential oil vapors are passed through a condenser. The condensate, which has a mixture of water and essential oil, is collected in a receiving flask. At the receiving flask the layer of essential oil is decanted and collected to sample bottle. The laboratory apparatus for the experiment are shown in (Figure 3.1) below.



Samples





Grind & soak (3-7 days)



(6-8 h)



Put the gaharu essential oil in the sample bottle

Figure 3.1 Laboratory Set Up Equipment

3.2 GC-MS

The GC-MS is composed of two major building blocks: the <u>gas chromatograph</u> and the <u>mass spectrometer</u>. The gas chromatograph utilizes a capillary column and depending on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. (5% (phenyl)polysiloxane) the difference in the chemical properties between different <u>molecules</u> in a <u>mixture</u> will separate the molecules as the

sample travels the length of the column. The molecules take different amounts of time (called the retention time) to come out of (elute from) the gas chromatograph and this allows the mass spectrometer downstream to capture, ionize, and detect the molecules separately. The mass spectrometer does this by breaking each molecule into <u>ionized</u> fragments and detecting these fragments using their mass to charge ratio.

These two components, used together, allow a much finer degree of substance identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. Flame Ionization Detector) detect multiple molecular that happen to take the same amount of time to travel through the column (*i.e.* have the same retention time) which results in two or more molecules to co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes makes it extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically lends to increased certainty of the analyzing of interest is in the sample.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Result

4.1.1 Thailand



Figure 4.1 Spectrum of Thailand Agarwood Compound



2-butanone, 4-phenyl



-cyclopropa[a] naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.al pha.)]-





1-oxaspirol[2.5] octane, 5,5-

dimethyl-4-(3-methyl-1,3-

butadienyl)-







Bicyclo[2.2.2]oct-2-ene, 1,2,3,6-tetramethyl-



2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-, (2R-cis)-



Aristolene



1,3-hexadiene, 2,3,5-trimethyl-



2(3H)-naphthalenone, 4,4a,5,6,7,8-hexahydro-4a,5dimethyl-3-(1methylethylidene)-, (4ar-cis)-



trimethyl-, methyl ester, [1R-(1.alpha.,4a.beta.,4b.alpha.,7.alph

a.,10a.alpha.)]-, dihydro deriv.



4-ylideno]

[6,8-dioxa-2-(3-nitro-1,2,4-

triazol-1yl) bicyclo[3.2.1]octan-

Benzenemethanol, 3,5dimethyl-



3-methylbenzyl alcohol



1,2,3,4,4a,4b,5,6,7,9,10,10adodecahydro-1,4a,7-trimethyl-, methyl ester, [1R-(1.alpha.,4a.beta.,4b.alpha.,7.beta .,7a.alpha.)]-



diphenyl-

NH₂ N



1-phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,10,10adodecahydro-1,4a,7-



4(1H)-pteridinone, 2-amino-

Benzhydrazide, 3-hydroxy-N2-



1-phenanthrenecarboxylic acid, 1,2,3,4,4a,5,6,7,8,9,10,10adodecahydro-1,4a-dimethyl-7-(1-methylethyl)-, methyl ester, [1R-

(1.alpha.,4a.beta.,7.beta.,10a.alp ha.)]-



,4,6-octatriene, 2,6-dimethyl-



Tetrahydroabietic acid



1-phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a octahydro-1,4a-dimethyl-7-(1-methylethyl)-, methyl ester, [1R(1.alpha.,4a.beta., 10a.alpha.)]-





1-phenanthrenecarboxylic acid,

1,2,3,4,4a,4b,5,6,7,8,10,10adodecahydro-1,4a-dimethyl-7-(1-methylethyl)-, methyl ester, [1R-(1.alpha.,4a.beta.,4b.alpha.,7.

alpha.,10a.alpha.)]-

2-methylenebornane





1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) este



Figure 4.2 Spectrum of Malaysia Agarwood Compound (Industry Scale)



2-butanone,4-phenyl

azulene, 1,2,3,4,5,6,7,8octahydro-1,4-dimethyl-7-(1-

methylethenyl)-, [1s-

(1.alpha.,4.alpha.,7.alpha.)]



Thujopsene

1-oxaspirol[2.5] octane, 5,5dimethyl-4-(3-methyl-1,3butadienyl)-



eudesma-4(14), 11-diene



(-)- aristolene



Naphthalene, 1, 2, 4a, 5, 6, 8ahexahydro-4, 7-dimethyl-1-(1-

methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)



azulene, 1,2,3,5,6,7,8,8aoctahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1s-(1.alpha.,7.alpha.,8a.beta.)]-



Cyclohexanemethanol, 4ethenyl-.alpha.,.alpha.,4trimethyl-3-(1methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]-



1-formyl-2,2-dimethyl-3-trans-(3-methyl-but-2enyl)-6methylidene-cyclohexane



Cyclohexene, 6-ethenyl-6methyl-1-(1-methylethyl)-3-(1methylethylidene)-, (s)-



Aristolene



1H-indene, 1ethylideneoctahydro-7amethyl-, (1Z,3a.alpha.,7a.beta.)-



Ocimene

1H-cyclopropa[a] naphthalene, 1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl-, [1aR-(1a.alpha.,3a.alpha.,7b.alpha.)]-



Copaene

Naphthalene, 1,2,3,4,4a,5,6,8aoctahydro-7-methyl-4methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)]-



2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro-.alpha.,.alpha.,4a,8tetramethyl-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-



4a, trans-8a-perhydro-cis-2-(2hydroxy-2-propyl)-4a,cis-8dimethylnaphthalene



2-Naphthalenecarboxylic acid, 8-ethenyl-3,4,4a,5,6,7,8,8aoctahydro-5-methylene



1,3-cyclopentadiene, 5,5dimethyl-1-ethyl-

n-hexadecanoic acid



1-[1-methyl-1-(4 -methylcylohex-3-enyl) – ethyl] -1Hpyrrole



(4-methyl-cylohex-3-enyl)methanol



OH OH

1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) este



Trans-2-isopropylbicylclo [4.3.0] non-3-ene-8-one

Phenol, 3,5-dimethyl-

4.1.3 China



Figure 4.3 Spectrum of China Agarwood Compound



3,7,11-trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate





(-)- aristolene

Cyclopropanecarboxylic acid, 2,2-dimethyl-3-(1propenyl)-, methyl ester, [1.alpha.,3.beta(z)]-





1H-cyclopropa[a] naphthalene, 1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl-, [1aR-(1a.alpha.,3a.alpha.,7b.alpha.)]-

1H-cyclopropa[a] naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b .alpha.)]-



2,4-pentanedione, 3-

(phenylmethyl)-



2,6-octadiene, 2,6dimethyl-

2(3H)-naphthalenone, 4,4a,5,6,7,8-hexahydro-4a,5dimethyl-3-(1methylethylidene)-, (4ar-cis)-



Figure 4.4 Spectrum of India Agarwood Compound



- Heptanoic acid
- Diethyl phthalate

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Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-

methylethenyl)-,[1S-(1.alpha.,7.alpha.,8a.alpha.)]





Guaiol



(-)-aristolene

1H-cycloprop[e] azulene, decahydro-1,1,7-trimethyl-4methylene-, [1aR-

(1a.alpha.,4a.beta.,7.alpha.,7 a.beta.,7b.alpha.)]-

Dodecanoic acid



1,3-dimethyl-5-(propen-1yl) adamantine



2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7octahydro-.alpha.,.alpha.,4a,8tetramethyl-, (2R-cis)-



59

este

1,2-benzenedicarboxylic



Figure 4.5 Spectrum of Malaysia Agarwood Compound (Laboratory Scale)







acetic acid, 2-methylpropyl ester

1,3-cyclohexadiene, 1,3,5,5-tetramethyl-



ethyl acetate





HO

1-butanol, 3-methyl



0 octanal



butanal, 2-methyl-



furfural



n-propyl acetate



cyclopentanone, 3-methyl-



benzaldehyde, 2-hydroxy-



Copaene



4,7-methanoazulene, 1,2,3,4,5,6,7,8 -octahydro -1,4,9,9-tetramethyl-, [1S-(1.alpha.,4.alpha.,7.alpha.)]-



4-methylene-1-methyl-2- (2-methyl-1 -propen-1-yl) -1-vinyl-cycloheptane,





1H-3a, 7-methanoazulene, 2,3,4,7,8,8ahexahydro - 3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]

Ĥ



azulene, 1,2,3,5,6,7,8,8a-octahydro - 1,4-dimethyl -7-(1-methylethenyl)-, [1S- (1.alpha,7.alpha,8a.beta.)]-





1- (3-methyl-cyclopent-2-enyl)- cyclohexene



azulene, 1,2,3,4,5,6,7,8-octahydro - 1,4-dimethyl -7-(1-methylethenyl)-, [1S- (1.alpha.,4.alpha.,7.alpha.)]-





naphthalene, 1,2,3,4-tetrahydro-1,6dimethyl- 4-(1-methylethyl)-, (1S-cis)-



2,4-quinolinediol



1-methoxy- 1,3-cyclohexadiene



4,6,6-trimethyl-2-(3-methylbuta-1,3-dienyl) -3-oxatricyclo [5.1.0.0(2,4)] octane





Countries	Malaysia	India	Thailand	China
(-)-aristolene	\checkmark	\checkmark		\checkmark
1,2-benzenedicarboxylic acid, mono (2-ethylhexyl)				
este	\checkmark	\checkmark	\checkmark	
1-formyl-2,2-dimethyl-3-trans-(3-methyl-but-2-enyl)-				
6-methylidene-cyclohexane	\checkmark		\checkmark	
1H-cyclopropa[a] naphthalene, 1a,2,3,3a,4,5,6,7b-				
octahydro-1,1,3a,7-tetramethyl-, [1aR-				,
(1a.alpha.,3a.alpha.,7b.alpha.)]-	\checkmark			V
1H-cyclopropa[a] naphthalene, 1a,2,3,5,6,7,7a,7b-				
octahydro-1,1,7,7a-tetramethyl-, [1aR-				
[la.alpha.,/.alpha.,/a.alpha.,/b.alpha.)]-			\checkmark	V
1-oxaspirol[2.5] octane, 5,5-dimethyl-4-(3-methyl-				
1,3-butadienyl)-	\checkmark		\checkmark	
2(3H)-naphthalenone, 4,4a,5,6,7,8-hexahydro-4a,5-				
dimethyl-3-(1-methylethylidene)-, (4ar-cis)-			\checkmark	\checkmark
2-butanone, 4-phenyl	\checkmark		\checkmark	
2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydro-				
.alpha.,.alpha.,4a,8-tetramethyl-, (2R-cis)-		\checkmark	\checkmark	
3-pentanone, 1,5-diphenyl-		\checkmark	\checkmark	
Aristolene	\checkmark		\checkmark	
Copaene	\checkmark		\checkmark	
Diethyl phthalate		\checkmark	\checkmark	
Heptanoic acid		\checkmark		\checkmark
n-hexadecanoic acid	\checkmark	\checkmark		\checkmark

Table 4.1: Similarity of Compound between Four Countries

4.2 **DISCUSSION**

From the result of four different countries, there are several compounds that are including in 2 or more countries. The compounds including (-)-aristolene, 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) este, 1-formyl-2,2-dimethyl-3-trans-(3-methyl-but-2-enyl)-6-methylidene-cyclohexane, 1H-cyclopropa[a] naphthalene, 1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl-, [1aR-(1a.alpha., 3a.alpha., 7b.alpha.)]- and others. The summary of the result can be shown in table 4.1. Almost of the compound is same between Malaysia and Thailand. Compounds from Thailand have more similar to other countries compare to China, India and Malaysia. From the analysis result, the amount of compounds that differ between each country is large. This is because the trees were plant is not in the same origin. The factor of where the wood was plant gives the effect of the compounds in those countries.

Base on overall review, there are component that classified in monoterpene and sesquiterpene. Example for monoterpene component such as 1-formyl-2,2-dimethyl-3trans-(3-methyl-but-2-enyl)-6-methylidene-cyclohexane, 3-methyl benzyl alcohol, 2-methyl-5-(1-methylethenyl)-, 3-Cyclohexanone, trans-, 2,4-pentanedione, (phenylmethyl)- and others. For sesquiterpene component can be shows as eudesma-4(14), 11-diene, Copaene, 2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-, [2R-(2.alpha., 4a.alpha., 8a.beta.)]-, 1-[1-methyl-1-(4 methyl-cylohex-3-enyl) – ethyl] -1H-pyrrole and others.

From the first appearance, the color of agarwood essential oil is green, after a few weeks later the color of agarwood essential oil was change to light yellow. The green color shows that pigment of chloroform was inside the oil. The essential oil were collected is in small amount, in range 2mL to 3mL. This is because the method that

used is the general method, so that the essential oil that produces by this method is less if compared to other method that added some steps of pretreatment. Furthermore the samples that need to be analyzed are in the small amount. For the oil sample from industrial production, it is more viscous compare to oil sample from laboratory extraction. This is happened might be because of the amount of the oil and the solvent. In the sample from industry the content of the solvent is almost zero. But in the sample from laboratory the content of solvent is almost 80 percent. The color of *gaharu* essential oil from industry is dark yellow or brown.

In this experiment there are certain preventative measures that need to be stress up. It is include the preparation step of the sample. The sample need to prepared in the cleaned place, make sure there is no contamination. If there is slightly an impurity it will give effect on the result when the analysis method was run. The boiling point also will not stable if there is impurity in the sample. When soaking and heating process were run, there is several thing that need to give an extremely attention. The soaking and heating process time requirement must be done properly. Avoid running or shutting down the process in the weekend. This matter need have a supervision of the lab owner or other lecturer, so it is give a difficulty to others. During the soaking process, make sure closed the tunnel with the rubber stopper. This is done to keep away from the insect that will affect the sample. While the heating sample were run, stay away from touch the equipment, this is because the equipment will became hot. When the experiment was done, evade sorting out the equipment when it is still hot and in rushing situation. This action will make the equipment broken and give an injury to the body. In this hydrodistillation method, the water supplied for the condenser must be in consistent state. If the water supplied in condenser were disconnect, the equipment will become heat from this experiment cannot been cooled and can make it burn or exploded. The most importance that needs to be emphasized is GC-MS during analysis procedure. This equipment need to be handling gentle, because it is very expensive equipment. This equipment just can be run by lab owner, lecturer and science assistance.

This hydrodistillation method was commonly applied by other industries. It is the suitable way to extract *gaharu* essential oil because of the low costing, high purity and easy to run. *Gaharu* essential oil was hardly to extract and have high market demand even thought it was sold in extremely high price. The *gaharu* essential oil also been applied in many industries including in medical, agriculture, and construction. **CHAPTER 5**

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The purpose of this experiment had been achieved. First, to know the type of oil from *gaharu* that been produced by hydrodistillation, which is can be determine as agarwood oil or essential oil. So that in this experiment essential oil were been collected. After run the experiment and analyze it the chemical compound in *gaharu* essential oil can be determine. The list of chemical compound was compiling at the table 5.1 below. From the samples that take from several countries, the comparison between the quality, method and chemical compound in different species can be analyzed. The details are same with the literature review that had been done.

compound	malaysia	india	thailand	china
(-)alphapanasinsen		\checkmark		
(-)-aristolene	\checkmark	\checkmark		V
(4-methyl-cylohex-3-enyl)-methanol	\checkmark			
.alphasantalol				V
1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) este	\checkmark	\checkmark	\checkmark	
1,3-cyclopentadiene, 5,5-dimethyl-1-ethyl-	\checkmark			
1,3-dimethyl-5-(propen-1-yl) adamantine		V		
1,3-hexadiene, 2,3,5-trimethyl-			V	
1,5,7-octatrien-3-ol, 3,7-dimethyl-				V
1-[1-methyl-1-(4 -methyl-cylohex-3-enyl) – ethyl] -1H- pyrrole	\checkmark			
1-formyl-2,2-dimethyl-3-trans-(3-methyl-but-2-enyl)-6- methylidene-cyclohexane	\checkmark		\checkmark	
1H-cycloprop[e] azulene, decahydro-1,1,7-trimethyl-4- methylene-, [1aR- (1a.alpha.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-		\checkmark		
1H-cycloprop[e]azulene, decahydro-1,1,4,7- tetramethyl-, [1aR- (1a.alpha.,4.beta.,4a.beta.,7.beta.,7a.beta.,7b.alpha.)]-				\checkmark
1H-cyclopropa[a] naphthalene, 1a,2,3,3a,4,5,6,7b- octahydro-1,1,3a,7-tetramethyl-, [1aR- (1a.alpha.,3a.alpha.,7b.alpha.)]-	\checkmark			\checkmark
1H-cyclopropa[a] naphthalene, 1a,2,3,5,6,7,7a,7b- octahydro-1,1,7,7a-tetramethyl-, [1aR- (1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]-			\checkmark	\checkmark
1H-indene, 1-ethylideneoctahydro-7a-methyl-, (1Z,3a.alpha.,7a.beta.)-	\checkmark			
1-oxaspirol[2.5] octane, 5,5-dimethyl-4-(3-methyl-1,3- butadienyl)-	\checkmark		\checkmark	
1-phenanthrenecarboxylic acid, 1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-1,4a- dimethyl-7-(1-methylethyl)-, methyl ester, [1R-			\checkmark	

Table 5.1: List of Chemical Compound in Four Countries

(1.alpha.,4a.beta.,4b.alpha.,7.alpha.,10a.alpha.)]-				
1-phenanthrenecarboxylic acid, 1,2,3,4,4a,5,6,7,8,9,10,10a-dodecahydro-1,4a- dimethyl-7-(1-methylethyl)-, methyl ester, [1R- (1.alpha.,4a.beta.,7.beta.,10a.alpha.)]-			\checkmark	
1-phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a- octahydro-1,4a-dimethyl-7-(1-methylethyl)-, methyl ester, [1R-(1.alpha.,4a.beta.,10a.alpha.)]-			\checkmark	
1-phenanthrenecarboxylic acid, 7-ethenyl- 1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-1,4a,7- trimethyl-, methyl ester, [1R- (1.alpha.,4a.beta.,4b.alpha.,7.alpha.,10a.alpha.)]-, dihydro deriv			\checkmark	
1-phenanthrenecarboxylic acid, 7-ethyl- 1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,4a,7- trimethyl-, methyl ester, [1R- (1.alpha.,4a.beta.,4b.alpha.,7.beta.,7a.alpha.)]-			\checkmark	
2(3H)-naphthalenone, 4,4a,5,6,7,8-hexahydro-4a,5- dimethyl-3-(1-methylethylidene)-, (4ar-cis)-			\checkmark	\checkmark
2,4,6-octatriene, 2,6-dimethyl-			V	
2,4-pentanedione, 3-(phenylmethyl)-				
2,6-octadiene, 2,6-dimethyl-				\checkmark
2-butanone, 4-phenyl	\checkmark		V	
2-cyano-3-fluorophenylhydrazine				\checkmark
2-methylenebornane			V	
2-Naphthalenecarboxylic acid, 8-ethenyl- 3,4,4a,5,6,7,8,8a-octahydro-5-methylene-				
2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydro- .alpha.,.alpha.,4a,8-tetramethyl-, (2R-cis)-		\checkmark	\checkmark	
2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro- .alpha.,.alpha.,4a,8-tetramethyl-, [2R- (2.alpha.,4a.alpha.,8a.beta.)]-	\checkmark			
2-penten-1-ol, 2-methyl-5-(2-methyl-3- methylenebicyclo[2.2.1] hept-2-yl)-, [1S- [1.alpha.,2.alpha.(z),4.alpha.]]-				
3,7,11-trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate				
3-heptyne, 5-ethyl-5-methyl-			\checkmark	

3-methylbenzyl alcohol			V	
3-pentanone, 1,5-diphenyl-		\checkmark	V	
4(1H)-pteridinone, 2-amino-			V	
4a, trans-8a-perhydro-cis-2-(2-hydroxy-2-propyl)- 4a,cis-8-dimethylnaphthalene	\checkmark			
5-azulenemethanol, 1,2,3,3a,4,5,6,7-octahydro- .alpha.,alpha.,3,8-tetramethyl-, [3S- (3.alpha.,3a.beta.,5.alpha.)]-		\checkmark		
8H-pyrano [2,3-e] benzothiophen-8-one, 4-formamido- 6-methyl-		\checkmark		
9-hexadecenoic acid		V		
Aristolene	\checkmark		V	
azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1- methylethenyl)-, [1s-(1.alpha.,4.alpha.,7.alpha.)]	\checkmark			
azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1- methylethenyl)-, [1s-(1.alpha.,7.alpha.,8a.beta.)]-	\checkmark			
Benzene, (1-methylbutyl)-				\checkmark
Benzenemethanol, 3,5-dimethyl-				
Benzhydrazide, 3-hydroxy-N2-[6,8-dioxa-2-(3-nitro- 1,2,4-triazol-1yl) bicyclo[3.2.1]octan-4-ylideno]			\checkmark	
Bicyclo[2.2.2]oct-2-ene, 1,2,3,6-tetramethyl-				
Camphene				
Copaene	\checkmark			
Cyclohexanemethanol, 4-ethenylalpha.,.alpha.,4- trimethyl-3-(1-methylethenyl)-, [1R- (1.alpha.,3.alpha.,4.beta.)]-	\checkmark			
Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans-				\checkmark
Cyclohexene, 6-ethenyl-6-methyl-1-(1-methylethyl)-3- (1-methylethylidene)-, (s)-	\checkmark			
Cyclopropanecarboxylic acid, 2,2-dimethyl-3-(1- propenyl)-, methyl ester, [1.alpha.,3.beta(z)]-				\checkmark
Diethyl phthalate		V	\checkmark	
D-limonene				\checkmark
Dodecanoic acid		V		
eudesma-4(14), 11-diene	\checkmark			
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Guaiol		V		
Heptanoic acid		V		\checkmark
Hexadecanoic acid, methyl ester		V		
Hexanoic acid		V		
Methyl abietate			\checkmark	
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4- methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)]-	\checkmark			
Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a- dimethyl-7-(1-methylethenyl)-,[1S- (1.alpha.,7.alpha.,8a.alpha.)]-		\checkmark		
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1- methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-			\checkmark	
Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1- (1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)	\checkmark			
n-hexadecanoic acid	\checkmark	\checkmark		V
ocimene	\checkmark			
Octadecanoic acid		\checkmark		
Octanoic acid				\checkmark
Pentadecanoic acid		V		
Phenol, 3,5-dimethyl-	\checkmark			
p-menth-1-en-8-ol			\checkmark	
Tetradecanoic acid		V		
Tetrahydroabietic acid			V	
thujopsene	\checkmark			
Trans-2-isopropylbicylclo [4.3.0] non-3-ene-8-one	V			
Tridecanoic acid		V		
Undecanoic acid		\checkmark		

5.2 **RECOMMENDATION**

Gaharu essential oil extraction using hydrodistillation study is an important research that must be continued due to the value and demand of gaharu oil nowadays. More importantly it is not only can improved the research study but also can help local entrepreneur who involved in gaharu oil extraction industry. In order to achieve the objective of the project there are some recommendations that need to be applied to improve the yield and quality of gaharu essential oil extracted. Below are few of the achievable way that can increase the precision of the result:

- i. It is hard to collect the product because of the amount of gaharu essential oil extraction is too small. The essential oil is stuck at the wall of the equipment apparatus. The best solution that was suggested is clean the essential oil with the solvent ethyl acetate (EtOAc). The oil will dissolve in solvent liquid.
- Product of gaharu essential oil should be analyzed using GC before run it at GC-MS to ensure the amount of water in essential oil. This is because GC-MS very sensitive to water, if just a little quantity of water in GC-MS, it can cause damage to that equipment.
- iii. There is some technical skill that is student did not know to apply. There also some equipment those students do not have the specialty to run it, so they cannot apply it at industry. The suggestion was been recommended is expose more technical skill to student, it can help them to applied it at work field.

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APPENDIX A



Flow Diagram of the Extraction Process