



Faculty of Resource Science and Technology

SYSTEMATIC STUDY OF *AQUILARIA* SPP. IN SARAWAK

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I declare that no portion of this research work has been submitted to support the application of other degree or qualification at any other universities or institutions of higher learning.

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LIST OF ABBREVIATIONS

CITES: Convention on International Trade in Endangered Species

LM: Light microscopy

PBUH: Peace Be Upon Him

RLS: Radial Longitudinal Section

SAR: Sarawak Herbarium

SEM: Scanning Electron Microscopy

Sp.: Species (Singular)

Spp.: Species (Plural)

TCM: Traditional Chinese Medicine

TEM: Transmission Electron Microscopy

TLS: Tangential Longitudinal Section

TS: Tangential Section

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Systematic Study of *Aquilaria* spp. in Sarawak

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ABSTRACT

There are three species of *Aquilaria* found in Sarawak which are *A. microcarpa* Bail., *A. malaccensis* Lam., and *A. beccariana* Teigh. They are trees which have the commercial values which can be used as medicinal purposes and essential oil. This study compared the differences among those three species in morphological and anatomical characteristics of the leaves and wood. The leaves were observed by using herbarium samples and fresh samples. The morphological observations were made by using stereo microscope and the anatomical observations done under light microscopy and Scanning Electron Microscopy (SEM). *Aquilaria beccariana* had the largest leaves with size of 14.0 – 17.0 cm x 6.0 – 7.5 cm. The three species were different in the morphology of leaves epidermal cell where *A. microcarpa* had polygonal isodiametric arrangements of cells, *A. malaccensis* had the tetragonal rectangular arrangements of cells and *A. beccariana* had the random isodiametric arrangements of cells. In anatomical characteristics observed, *A. microcarpa* had the largest stomata with 20.3 μm x 17.2 μm of size. The diameter of the vessels identified *A. microcarpa* had the largest diameter with 80 μm – 117 μm . Thus, the three species can be differentiated through the observations of the leaves morphological and anatomical characteristics.

Key words: *A. beccariana*, *A. malaccensis*, *A. microcarpa*, anatomy, morphology, stomata, wood

Terdapat tiga spesies Aquilaria yang terdapat di Sarawak iaitu A. microcarpa Bail., A. malaccensis Lam., dan A. beccariana Teigh. Spesies tersebut merupakan pokok-pokok yang mempunyai nilai-nilai komersial yang boleh digunakan sebagai tujuan perubatan dan produk minyak wangi. Kajian ini membandingkan perbezaan antara ketiga-tiga spesies dalam ciri-ciri morfologi dan anatomi daun dan kayu. Daun diperhatikan dengan menggunakan sampel herbarium dan sampel segar. Pemerhatian morfologi telah dibuat dengan menggunakan mikroskop stereo dan pemerhatian anatomi dilakukan di bawah mikroskop cahaya dan Mikroskop Pengimbas Elektron (SEM). Aquilaria beccariana mempunyai daun terbesar dengan saiz 14,0-17,0 cm x 6.0 - 7.5 cm. Tiga spesies mempunyai berbeza dalam morfologi sel epidermis daun di mana A. microcarpa mempunyai susunan sel isodiametrik poligonal, A. malaccensis mempunyai susunan sel segi empat tepat tetragonal dan A. beccariana mempunyai susunan sel isodiametrik rawak. Dalam ciri-ciri anatomi diperhatikan, A. microcarpa mempunyai stomata terbesar dengan 20.3 μm x 17.2 μm saiz. Diameter pembuluh dikenalpasti A. microcarpa mempunyai garis pusat terbesar dengan 80 μm - 117 μm . Oleh itu, ketiga-tiga spesies dapat dibezakan melalui pemerhatian daripada daun morfologi dan ciri-ciri anatomi.

Kata kunci: *A. beccariana*, *A. malaccensis*, *A. microcarpa*, anatomi, morfologi, stomata, kayu

CHAPTER 1

INTRODUCTION

Aquilaria spp. is categorized into Magnoliopsida class under Myrtales order in the family of Thymelaeaceae. *Aquilaria* species are types of trees where the species are very well-known due to distributions are widely spread mostly within the Asia. *Aquilaria* or the common name called aloeswood, eaglewood, agarwood, and sandalwood have economical values (Donovan & Puri, 2004). This tree is also known as ‘Gaharu’ in Malaysia. The most common species in Sarawak are *A. beccariana* Teigh., *A. malaccensis* Lam. and *A. microcarpa* Bail. Generally, the *Aquilaria* spp. are described as it have height of 40 m tall with 1.2 m – 2.5 m of diameter with dense bark which is smooth, thin and pale; leaves are elliptical to oblong with shiny foliage about 7.5 cm – 12 cm long by 2.3 cm – 5.5 cm wide (CITES, 2003; Donovan & Puri, 2004). The special characteristic is the wood can produce aromatic smell which produces resin-impregnated heartwood that is fragrant where it can be process into various types of products such as perfumes (Donovan & Puri, 2004). Products that commonly use from these species are for medicinal, religious and cosmetics purposes.

Besides that, the usage of *Aquilaria* in medicinal purposes have been practiced from a long time ago which can be known as traditional medicine. Ayurveda is the therapy which mainly used by the Hindus which use the agarwood by burning the agarwoods to promotes meditational state as it gives warming and centering effect (Burfield & Kirkham, 2005). In addition, in Muslims’ believes there is a ‘hadith of Sahih Muslim’ where the Prophet Muhammad PBUH has introduced the wood of *Aquilaria* for medicinal purposes (Barden,

Awang-Anak, Mulliken & Song, 2000). In religious purposes, it also used by the Hindus, Muslims, Christians and Buddhist (Lim, Mamat, & Chang, 2007).

In addition, the ecology of *Aquilaria* spp. can be determined through the seed production, pollination and seed germination as to manage the species which is highly being harvested and need to sustain the necessity (Soehartono & Newton, 2000). The distribution of the species is different for each species and some of species become threatened due to highly demand and need to manage it by the dispersal activity of the species and control the harvest.

In anatomical research, through the extraction process, the chemical properties of *Aquilaria* spp. resins are found to have special characteristics which are difference to other tree especially family of Dipterocarpaceae which also produced resins. Furthermore, although with the same family, Thymelaeaceae, *Aquilaria* spp. are the species with this special characteristic of the wood. Nevertheless, the research on the morphological and anatomical of *Aquilaria* spp. needed are to differentiate among the species of *Aquilaria*. The morphological study is basically referring to the physical appearance of the tree. For anatomical study, it is more specific where the microstructure of the tree is observed. Therefore, this study focused on the morphological and anatomical characteristics of the three different species of *Aquilaria* in Sarawak which could be used as evidence to facilitate identification in the absence of the reproductive parts.

1.1 Problem statement

The morphological and anatomical characteristics of these three species are not well described and lacked of information, apart from that these three species is hard to compare due to their similarities.

1.2 Objectives

- To produce details of morphological description characteristic for these three species found in Sarawak.
- To produce anatomical characteristics of the microstructures of these *Aquilaria* spp.
- To differentiate the species on morphological and anatomical characteristics.

CHAPTER 2

LITERATURE REVIEW

Sarawak Forestry (2006), the species of *Aquilaria* that are commonly found is *A. beccariana*, *A. malaccensis* and *A. microcarpa*. The genus of *Aquilaria* is from the class of Magnoliopsida, order of Myrtales and family of Thymelaeaceae.

2.0.1 Class: Magnoliopsida

Under Magnoliopsida class characteristics, it is identified the plants are dicotyledons which have two seed leaves and stems have cambium tissue (Columbia University Press, 2012). This class has been divided into 12 subclasses, 35 superorders, 87 orders, 40 suborders, 472 families, and 400 subfamilies (Thorne & Reveal, 2007). Plants in this class are flowering plants (Thorne & Reveal, 2007).

2.0.2 Order: Myrtales

Order of Myrtales have special characteristic where in one order it divides into two difference of wood anatomy and not usually found in other flowering plants groups which are bicollateral vascular bundles in the primary stem and bordered pits of the secondary xylem consist of vestures (Conti, Litt, & Sytsma, 1996).

2.0.3 Family: Thymelaeaceae

Herber (2011) have distinguished the four subfamily of Thymelaeaceae on observing pollen morphology using LM, SEM and TEM where the distribution of pollen types shows the relations with the existence of characters from wood anatomy and flower morphology.

2.1 Ecology and distributions

The species of *A. malaccensis* is the most dominant compare to *A. beccariana* and *A. microcarpa* (Saikia & Khan, 2014). In India, quadrat method is done to identify the distribution of *A. malaccensis* ecology where the estimation of population, analysis of ecology and appraisal of *A. malaccensis* were done in twenty-seven selected villages located in Jorhat and Golaghat districts of upper Assam in northeast India (Saikia & Khan, 2014). In Indonesia, the studies of distribution of the ecology of *Aquilaria* spp. through the seed production, pollination and seed germination as to manage the species which is highly being harvested and need to sustain the necessity were carried out (Soehartono & Newton, 2000). The research was conducted on several species of *Aquilaria* such as *A. beccariana*, *A. malaccensis*, *A. microcarpa*, *A. hirta*, *A. crasna* and *A. filaria*. The distributions of the species were different for each species and some of species were identified as highly demanding activities. The *A. malaccensis* need to manage and monitored and control the harvest in *ex situ* (Saikia & Khan, 2014).

2.2 Morphological and Anatomical research

2.2.1 Morphology

Plant morphology is the study of plant physical structure such as leaf, wood, seed, fruit, and flower on shape, size, texture, and colour. Moreover, the morphological study on plant can act on the identification of nutrient deficiency such as boron deficiency. Throughout the morphology characteristics, *Aquilaria* spp. have been compared with *Gyrinops* spp. where two of the species was said to be resemble of each other, however it differentiated by the grade of plants (CITES, 2003). Besides that, *Aquilaria* spp. are described as it have height of 40 m tall with 1.2 m – 2.5 m of diameter with dense bark which is smooth, thin

and pale; leaves are elliptical to oblong with shiny foliage about 7.5 cm – 12 cm long by 2.3 cm – 5.5 cm wide (Donovan & Puri, 2004).

2.2.2 Anatomy

Anatomical structure highlight on the production of agarwood by the *Aquilaria* spp. which have the special chemical compounds. Resins of *Aquilaria* are produced from the wood and highly on demand (Persoon, 2008). The chemical compound in *Aquilaria* is identified through the extraction. The identified compound were “3-phenyl-2-butanone, alpha-cubebene, and globulol” (Yumi, Hashim, Ismail, & Abbas, 2014). Apart from that the secretion and accumulation of resin in the cell can be observed through light microscope (Chong, Abdul-Rahim, & Awang, 2014). There were differences between the microstructure of juvenile and mature trees are observed under light microscope (Mohamed, Wong, & Halis, 2013). The resin-filled tracheids and the vessels of separated cells filled with resin can see under light microscope (Chong, Abdul-Rahim, & Awang, 2014). Mohamed, Wong, & Halis (2013) stated that the juvenile tree of the Gaharu produces the resin as the same as the mature tree through the observations on the anatomical microstructures of the wood of *Aquilaria malaccensis*.

2.3 Uses of *Aquilaria*

2.3.1 Medicine

Ayurveda is the therapy which mainly used by the Hindus which use the agarwood by burning the agarwoods to promotes meditational state as it gives warming and centering effect (Burfield & Kirkham, 2005). Nowadays, the medicinal uses of agarwood have been

simplified and one of the examples is medicinal soap which can treat skin diseases. In addition, TCM herbs are acts as it reorganise the body constituents to balance state called Qi (Burfield & Kirkham, 2005).

2.3.2 Religious

Wood of *Aquilaria* is mainly used by the Buddhist and Hindus for serves instead of medical usage and even the tree is called “the wood of the gods” (Persoon, 2008). Buddhist and Hindus will burn the incense which are made from the agarwood before they serves at the temple.

2.3.3 Cosmetics and perfumes

The aromatic wood characteristics make it possible for the agarwood be made into fragrance or mostly become perfumes which have been commercialized and used by the Muslims, or Malays called “minyak attar”. In addition, Hindus also practice the same as the Muslims but it encouraged by the therapy of Ayurveda and used variety of products such as body soap itself.

2.4 Agronomy and ecosystem sustainable

Agarwood are said to be demanded nowadays as it have been overexploited and this lead to the threatened of *Aquilaria* spp. (Barden, Awang-Anak, Mulliken & Song, 2000; AB-Rahman, 2009). *Aquilaria* spp. are category under threatened also have been listed in The IUCN Red List of Threatened Species due to the uses of the product from the tree on human necessity (The IUCN Red List of Threatened Species, 2014). However, recently from the study it revealed that supply rates of agarwood is about 40% of the demand only and agarwood oil per litre can consumed at around \$US10,000 - 14,000 on the market (Gratzfeld & Tan , 2008). Due to human needs the wood of *Aquilaria* has become one of

the expensive wood and even the specialized consumer which used the products as necessity can pay more (Gratzfeld & Tan, 2008). Agarwood from *Aquilaria* spp. will have a cycle of harvest after planting for 7 years. Sixty percent of resin are estimate to produce on 1 000 hectare where each hectare is planted with 833 trees where at the end of cycle, only about 500 trees are produced agarwood resin and 500 kg of agarwood will produced if each of trees produces 1000g of resin. (Mamat, Yacob, Lim & Rdam, 2010).

The Penan also believes that there are special relationships between the insects and the tree (Donovan & Puri, 2004). However, the Ethnobiologist have found the Penan have lack of knowledge on the importance of *Aquilaria* product to the ecosystem (Donovan & Puri, 2004). The resin of agarwood can act as fumigator which reduce the usage of chemicals which are full of toxicity and hazard that can cause water pollution when the soil leaching and affected to the water organisms. Thus, agarwood resin become a biological control and reduces the pollution of environments.

CHAPTER 3

MATERIALS AND METHODS

3.1 Herbarium specimen and fresh sample collection

Sample of *Aquilaria* spp. obtained from the Herbarium Universiti Malaysia Sarawak, Herbarium of The Forest Department Sandakan and Sarawak Forest Department. The fresh samples are collected from the green house and Arboretum at the external lab in Unimas.

Herbarium Storage	Plant Species	Herbarium number
Herbarium Universiti Malaysia Sarawak	<i>Aquilaria microcarpa</i>	MA 002
	<i>Aquilaria microcarpa</i>	MA 001
	<i>Aquilaria microcarpa</i>	MA 003
	<i>Aquilaria microcarpa</i>	SD 42
	<i>Aquilaria microcarpa</i>	SD 41
	<i>Aquilaria microcarpa</i>	SD 41
	<i>Aquilaria microcarpa</i>	SD 41
	<i>Aquilaria microcarpa</i>	SD 41
	<i>Aquilaria microcarpa</i>	SD 42
	<i>Aquilaria microcarpa</i>	SD 42
	<i>Aquilaria microcarpa</i>	SD 42
	<i>Aquilaria microcarpa</i>	SD 42
	<i>Aquilaria microcarpa</i>	SD 40
	<i>Aquilaria microcarpa</i>	SD 37
	<i>Aquilaria microcarpa</i>	SD 37
Sandakan Herbarium	<i>Aquilaria beccariana</i>	SAN 101710
	<i>Aquilaria beccariana</i>	SAN 93196
	<i>Aquilaria malaccensis</i>	44685
	<i>Aquilaria malaccensis</i>	128153
	<i>Aquilaria malaccensis</i>	63887
Sarawak Herbarium	<i>Aquilaria beccariana</i>	S 34204
	<i>Aquilaria microcarpa</i>	S 15838

Table 1: List of herbarium specimens observed

3.2 Morphological observations

The morphological characteristics were observed on the sample of *Aquilaria* spp. provided such as shape of leaves, length, apex and base of leaves. For more specific, observes the texture and the ornamental of the leaves by using a stereo microscope.

3.3 Anatomical observations

3.3.1 Leaves Anatomy

Leaves observation on abaxial and adaxial part of leaf surface were observed. The characteristics observed were the epidermis cells and the subsidiary cells of stomata.

Firstly, the middle portion of the lamina 1 x 1 cm was cut and cleaned it thoroughly in distilled water. Fifteen percent of nitric acid was poured into 250 ml beaker and the portion of the leaf is put into the beaker contain 15% of nitric acids. The leaf was then be heated using the hotplate. Turn off the hotplate and take the tissue using paint brush and place it on watch glass contain of 5% of acetic acids in 30 seconds. The tissue was then transferred to other watch glass contains distilled water for rinse. The tissue was then transferred to the other watch glass contains Sodium Hypochlorite (NaOCl) to act as bleach which decolourised the brown colour of the mesophyll on the tissue. The tissue was then transferred back into the watch glass contains distilled water and transferred into the watch glass contains 50% of ethanol for 2 minutes. Then, the tissue was transferred into the watch glass contains Safranin in 3 minutes. The tissue was then undergoes the series of alcohol for dehydration by using 50%, 70%, 80%, and 90% of ethanol. The slide preparation was done by putting the tissue on the slide and the tissue layer was spread out to avoid the overlap, then put a drop of Eupharal on top of the tissue and put the cover slit.

Lastly, the slides prepared were left on the slide dry bench in 24 hours to ensure the slide is dry when observing under the light microscope. Apart from that, observation of the leaf microstructure was made using SEM but the tissue was not soaked into the Safranin as the image in SEM is black and white. The microstructures of the leaves were analysed in full and compared among species.

3.3.2 Wood Anatomy

Through SEM, the anatomical observations towards the wood of *Aquilaria* spp. where the sample need to be sliced into very thin pieces at three different sides which in tangential longitudinal section (TLS), radial longitudinal section (RLS) and transverse section (TS) using sliding microtome.

Firstly, wood preparation was done by preparing the cube of 1 x 1 cm and boiled 1-2 hours until the wood cube sinks. The boiling was for the wood to be softened to easily cut the wood. The wood was then soaked into the water to avoid the drying and hardened. The wood cube was then sliced using the microtome with care handling to avoid injuries. Therefore the TS, TLS, and RLS were identified during the cuttings to make the wood cubes and cut the section and differentiate by putting on different petri dish containing water. The slides was prepared by putting the wood sliced onto the Safranin and put the slices onto the clean slides and put the Eupharal glue and cover with cover slit, then the slides were dried on the slide dry bench in 24 hours to ensure the slides dry when observing under the light microscope. Apart from that, observation of the microstructure was made using SEM but the wood sliced was not soaked into the Safranin as the image in SEM is black and white. To ensure the dryness of the sample before observing under the