Anatomical observation and characterization on basic properties of Agarwood (Gaharu) as an Appendix II CITES

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Abstract. High demand for Agarwood (Gaharu) from natural forest causes the decreasing of their population. In 2004, Indonesia proposed that all natural Agarwood from Gyrinops and Aquilaria generas should be included in the Appendix II CITES (Convention on International Trade in Endangered Species) list, which could be internationally traded but in certain quota. This paper deals with anatomical observation and characterization on basic properties to find out the difference between Gyrinops and Aquilaria for classification and standardization. Explorations have been conducted at community forests in Flores, East Nusa Tenggara for Gyrinops versteegii (Gilg) Domke; while A. malaccensis Benth., A. microcarpa Baill., A. beccariana Tiegh., and Aquilaria sp. were extracted from Sangau and Ketapang Regencies, West Kalimantan. Wood samples were taken by drilling the trees stem by using a Drill tool to get a pencil-shaped sample of about 20 cm in length and 0.5 cm in diameter. Another sample in form of chips contain bark and wood were also extracted. The results showed that the average specific gravity of Gyrinops and Aquilaria was between 0.31 – 0.35, therefore it was included in the Strength Class IV. Chemical components of injured G. versteegii contained 50.74% holocellulose, 39.50% α-cellulose, 31.98% lignin, and 16.69% extractives. The composition of ray cells in G. versteegii was more complex than Aquilaria generas.

1. Introduction

Agarwood is a blackish wood and contains a typical resin produced by a number of tree species from the genus Aquilaria, especially A. malaccensis. This resin is used in perfume industry because it smells good. Since the beginning of the modern era (2000 years ago), agarwood has become a trading commodity from the Archipelago to India, Persia, the Arabian Peninsula, and East Africa [1].

Plants produce agarwood in response to microbes entering the injured tissue. The wounds on woody plants can be caused naturally by broken branches or peeled skin, or intentionally with drilling and sawing [2]. The entry of microbes into plant tissues is considered as a foreign object so that the plant cells will produce a phytoalexin compound which functions as a defense against diseases or pathogens. Phytoalexin compounds can be in the form of brown and fragrant resin, and accumulate in the xylem and phloem vessels to prevent wounds from spreading to other tissues [3]. However, if the microbes that infect the plant can defeat the plant's defense system, then the Agarwood is not formed, and the injured
plant parts can rot. The characteristics of the part of the plant that has produced gaharu are that the bark becomes soft, the crown of the plant turns yellow and falls out, and the swelling, bending, or thickening of the stems and branches of the plant occur [4]. Gaharu compounds can produce a fragrant aroma because they contain known Guia compounds, selina-dienone, and selina dienol. For commercial purposes, the community drills the stem of the gaharu-producing plant and inserts the fungus inoculum into it. Every gaharu-producing tree species has specific microbes to induce large amounts of Agarwood income. Some examples of fungi that can be used as inoculums are Acremonium sp., Cylindrocarpon sp., Fusarium nivale, Fusarium solani [5], Fusarium fusariodes, Fusarium roseum, Fusarium lateritium, and Chepalosporium sp.

Agarwood is widely traded with a very high selling price, especially for Agarwood from the family Themelaceae plant with Aquilaria spp., which in the world of trade is called the gaharu beringin. For this type of Agarwood with a relatively low selling value, it is usually referred to as gaharu buaya. In addition to being determined by the species of plant it produce, the quality of the Agarwood is also determined by the amount of resin content in the wood tissue. The higher the content of the resin in it, the higher/more expensive the price of the Agarwood will be [6]. In general, the trade in Agarwood is classified into three major classes, namely sapwood, kemedangan, and ash. Sapwood is a black or brownish-black wood and obtained from the part of a gaharu-producing tree that contains strong-scented resin. Kemedangan is a type of gaharu with mastic content and a weak aroma. The wood is soft with brown to gray physical appearance and coarse fiber. The last class is the ashes of gaharu which is the sawdust from the grinding or remaining destruction of Agarwood [7].

The high demand for Agarwood from natural forest causes the decreasing of their population in nature. On the other hand, currently, the Agarwood cultivation has not been able to meet the market demand. In 1994, the CITES (Convention on International Trade in Endangered Species) convention in the United States determined that the A. malaccensis species of Agarwood were included in Appendix II, which is a restricted plant for trade [8]. The determination is due to the fact that the gaharu-producing plant population is shrinking in nature because the Agarwood traders cannot recognize precisely which plants already contain gaharu and are ready to be harvested. To look for gaharu-producing trees, the businessmen cut down the wrong trees (did not produce gaharu), so the number of trees was greatly reduced [9]. In 2004, Indonesia proposed that all natural Agarwood from the genera of Gyrinops and Aquilaria should be included in the Appendix II list to restrict their trade so that the Agarwood trade must have permission from CITES with certain quota [10]. It is done to ensure that natural Agarwood tree species can develop and spread well.

This paper deals with anatomical observation and characterization on basic properties of Agarwood to find out the difference between Gyrinops and Aquilaria genera for classification and standardization. The characterizations are expected to contribute in providing data on its anatomical, physical, mechanical, and chemical properties from exploration results in Flores and West Kalimantan.

2. Materials and methods

Explorations and field studies were conducted at community forests of Rua Village in Flores, East Nusa Tenggara from April 8 to 13, 2018; and at Sangau and Ketapang Regencies, West Kalimantan from July 22 to 27, 2018 (figure 1).

Wood samples were taken by drilling a tree using a Riap Drill tool at breast height to get a pencil-shaped sample of about 20 cm in length and 0.5 cm in diameter (figure 2, left). Another sample was in chips of bark and xylem (figure 2, right). Both samples were used to identify and determine the anatomical structure, as well as the basic properties of the wood. In addition to the sampling, the data recorded were the tree diameter, tree height, soil pH, GPS location, altitude, coordinates, and ambient temperature.
Figure 1. Explorations in Flores, East Nusa Tenggara (left) and West Kalimantan (right).

Figure 2. Wood samples: pencil-shaped (left); bark and xylem in chips (right).

2.1. Anatomical observation
Microstructure of the wood was observed in axial, radial, and tangential sections at 40 µ thickness by using sliding microtome. Dehydration was carried out using 30%, 50%, 70%, 96%, and absolute alcohol. Replenishment was carried out by soaking it for a while in carboxylol and toluene, then gluing it tightly on top of the object glass. Characteristics of wood anatomy were observed according to the International Association of Wood Anatomist [11].

2.2. Physical properties
The pencil-shaped wood sample of about 20 cm in length and 0.5 cm in diameter was cut into 1 cm length, weighed, and volumetric measured, before drying in the oven at 103 ± 2°C for 24 hours until the weight was constant so that the oven dry weight was obtained. Specific gravity was calculated by oven-dried weight/green volume.

2.3. Mechanical properties
Mechanical properties were carried out to determine the absolute bending and compression strengths. These values could be obtained through the values of specific gravity based on table 1. Furthermore, strength class could be predicted, as well.
Table 1. Strength class of wood [12].

| Strength classes | Specific gravity | Absolute bending strength (kg/cm²) | Absolute compression strength (kg/cm²) |
|------------------|------------------|-----------------------------------|---------------------------------------|
| I                | over 0.90        | over 1100                          | over 650                              |
| II               | 0.60 to 0.90     | 725 to 1100                        | 425 to 650                            |
| III              | 0.40 to 0.60     | 500 to 725                         | 300 to 425                            |
| IV               | 0.30 to 0.40     | 360 to 500                         | 215 to 300                            |
| V                | under 0.30       | under 360                          | under 215                             |

2.4. Chemical properties

The wood sample was chopped, milled, and sieved to obtain 40 – 60 mesh wood powder. The samples were air dried, then stored in plastic before conducting chemical component analysis. The wood chemical components were carried out by analyzing moisture content [13], extractive content (NREL/TP-510-42619), acid-insoluble lignin content (LAP) – 003, holocellulose content by using sodium chlorite delignification [14], and α-cellulose content [15]. Hemicellulose content was calculated by subtracting holocellulose content with the α-cellulose content.

3. Results and discussions

Turjaman and Hidayat [16] reported that Aquilaria malaccensis, A. microcarpa, A. beccariana, and A. hirta are distributed in Sumatera and Kalimantan Islands. On the other hand, A. cumingiana, A. filaria, A. tomentosa, Gyrinops versteegii, G. decipiens, G. landermanii, G. salicifolia, G. audate, and G. podocarpus are distributed in Sulawesi, Maluku, West and East Nusa Tenggara, and Papua Islands. Therefore, the explorations were conducted in East Nusa Tenggara to acquire the Gyrinops genus, and in West Kalimantan to obtain the Aquilaria.

3.1. Exploration at Flores, East Nusa Tenggara

From the exploration, it was found that there were five trees of cultivated Gyrinops versteegii, which were planted in 1982/1983 (36 years-old) and seventeen trees of natural G. versteegii. The samples were taken from 6.0 – 7.0 soil pH, 630 – 707 m sea-level altitude (highland), and 68 – 86°F ambient temperature.

The average diameter, tree height and specific gravity of cultivated G. versteegii were 19.57 cm, 8 m, and 0.31, respectively. On the other hand, those of natural G. versteegii were 10.04 cm (4.00 – 23.25 cm), 5.48 m (3.00 – 10.00 m), and 0.32 (0.29 – 0.35), respectively. There was no correlation between diameter and tree height, but cultivated G. versteegii was grown faster than natural G. versteegii. However, G. versteegii could be classified into slow-growing species because the average diameter and tree height were 19.57 cm and 8 m, even the age of trees was 36 years-old (diameter increment 0.5 cm/year).

The results showed that the average specific gravity of cultivated and natural G. versteegii was between 0.31 – 0.32. Therefore, it was classified into the strength class IV with bending strength between 360 to 500 kg/cm² (table 1).
3.2. Exploration at Sangau and Ketapang Regencies, West Kalimantan

Table 2. Exploration data of cultivated Aquilaria from Sangau and Ketapang Regencies, West Kalimantan.

| Species               | Planted year | Diameter (cm) | Tree height (m) | Soil pH | Altitude (m) | Specific gravity |
|-----------------------|--------------|---------------|-----------------|---------|--------------|------------------|
| Aquilaria malaccensis | 2003         | 14.0          | 15.0            | 6.7     | 57           | 0.27             |
| Aquilaria malaccensis | 2003         | 28.7          | 15.0            | 6.7     | 57           | 0.36             |
| Aquilaria microcarpa  | 2003         | 33.1          | 25.0            | 6.8     | 55           | 0.42             |
| Aquilaria microcarpa  | 2004         | 18.2          | 12.0            | 6.7     | 64           | 0.30             |
| Aquilaria microcarpa  | 2005         | 22.3          | 12.0            | 6.7     | 64           | 0.30             |
| Aquilaria microcarpa  | 2013         | 12.7          | 7.0             | 6.4     | 58           | 0.27             |
| Aquilaria microcarpa  | 2013         | 12.4          | 5.0             | 6.5     | 51           | 0.32             |
| Aquilaria microcarpa  | 2013         | 10.2          | 4.0             | 6.7     | 57           |                  |
| Aquilaria beccariana  | 2003         | 21.7          | 8.0             | 6.6     | 66           | 0.38             |
| Aquilaria beccariana  | 2006         | 28.7          | 8.0             | 6.7     | 63           | 0.30             |
| Aquilaria beccariana  | 2013         | 9.6           | 8.0             | 6.8     | 54           | 0.31             |
| Aquilaria sp.         |              | 14.3          | 11.0            | 6.8     | 59           | 0.38             |

From the exploration, it was found that there were two trees of Aquilaria malaccensis, six trees of A. microcarpa, two trees of A. beccariana, and two trees of Aquilaria sp. cultivation, as shown in table 2. The wood samples were taken from 6.4 – 6.8 soil pH, and 51 – 66 m sea-level altitude (lowland). A. malaccensis trees were planted in 2003 (15 years-old); Aquilaria microcarpa trees were between 2003 – 2013; and A. beccariana trees were in 2003 and 2006. The diameter and tree height increased with the increasing age for A. microcarpa, however, that for A. malaccensis and A. beccariana were no correlation. Aquilaria was grown faster (around 1 – 2 cm/year) than the genus of Gyrinops. It was probably due to the differences in growing conditions.

The results showed that the average specific gravity of A. malaccensis, A. microcarpa A. beccariana, and Aquilaria sp. was 0.32, 0.32, 0.34, 0.35, respectively. Therefore, it was classified into the strength class IV with bending strength between 360 to 500 kg/cm² (Tabel 1), as well.

3.3. Anatomical observations

Figure 3 shows microscopic structure of natural Gyrinops verstegii at axial direction. Observation showed there were no differences on wood anatomical structures between injured and non-injured trees, namely growth ring boundaries indistinct, diffuse porous, vessel in radial multiples of four common, very thin-walled fibres, and diffuse included phloem were observed. Fibre cell dimensions of injured G. verstegii were 864.43 µm in length, 35.87 µm in diameter, and 4.38 µm in cell wall thickness; while vessel length and diameter were 367.08 µm and 98.97 µm, respectively. Non-injured G. verstegii had longer fibre length that was 939.12 µm, with almost similar fibre diameter and cell wall thickness namely 35.58 µm and 4.51 µm (compared to the injured one). Furthermore, compared to the injured G. verstegii, vessel length of non-injured one was also longer, 412.72 µm, but the vessel diameter that is 96.50 µm, was nearly similar. Until this point, injuring G. verstegii might reduce the rate of cell length developing.

In general, the wood anatomical structure of the three species of Aquilaria planted in Sanggau and Ketapang Regencies, West Kalimantan, showed indistinct growth ring boundaries, diffuse porous, vessel in radial multiples of 2 – 3 or more were commons, and at A. macrocarpa, a structure of vessel...
cluster was observed. The wood had simple perforation plates, opposite inter-vessel pits, distinctly bordered pits fibres, very thin walled fibres, ray width 1 – 3 cells, and the ray cells composition were all upright.

Similar to *G. verstegii*, Aquilaria also had diffuse included phloem. Another similar structure was the simple perforation plates, inter-vessel pits alternate, vessel-ray pitting with distinct borders; similar to inter-vessel pits in size and shape throughout the ray cells. Non septate fibres were present, and both genera had fibres with distinctly bordered pits. Ray has width of 1 – 3 cells, but more exclusively uniseriate. The difference between Gyrinops and Aquilaria genera was the composition of ray cells in Gyrinops which was more complex. Gyrinops had body rays cell procumbent with one to more of four rows of upright/square marginal cells. The characteristics of cultivated *Aquilaria* spp. wood from West Kalimantan were similar to the wood anatomical structure described in Inside-Wood.

![Figure 3](image-url)

Figure 3. Wood anatomical structures of injured (left) and non-injured natural *Gyrinops verstegii* grown in Flores, East Nusa Tenggara.
3.4. Chemical component analysis

Chemical components of injured (cultivated) *G. versteegii* have an average of 50.74% holocellulose, 30.50% α-cellulose; 31.98% lignin; and 16.69% extractives, as shown in table 3.

**Table 3.** Chemical component analysis of injured and induced Agarwood.

| Chemical component composition (%) | This study (%) | Control (1) | Sap-wood1 | Sap-wood1 | Gonystillus sp. |
|-----------------------------------|---------------|-------------|-----------|-----------|----------------|
| Moisture content                  | 3.11 ± 0.06   | 5.00        |           |           |                |
| Ethanol-benzene extractives       | 16.69 ± 1.88  | 30.83       | 35.00     | 40.00     | 1.2 61.6       |
| Acid insoluble lignin, AIL        | 31.98 ± 0.35  | 22.30       | 24.10     | 30.80     | 28.0 28.8      |
| Holocellulose                     | 50.74 ± 2.83  |             |           |           |                |
| α-cellulose                       | 30.50 ± 1.39  | 35.00       | 27.50     | 26.66     | 62.0 47.6      |
| Hemicellulose                     | 20.24 ± 1.45  | 35.50       | 22.83     | 19.33     | 19.0 9.0       |

1*Aquilaria malaccensis* Lamk [17], 2[18]. 3[19]

Table 3 shows the chemical composition of injured Agarwood in this study compared to some studies of induced Agarwood that have been reported before. Ethanol-benzene extractives in this study tended to be lower than that of induced Agarwood (*Aquilaria malaccensis* Lamk) [17] and *Gonystillus* sp. [19]. However, this extractives was higher than that of *Gonystillus* sp. wood. Extractives secondary metabolite has an important function as a defense system of the tree against attack of disease-causing microorganism [17]. Extractives component that was soluble in ethanol-benzene consisted of oil, fat, less tannin and resin [20]. The extractive content of Agarwood in this study is classified as high content compared to the classification of Indonesian hardwood based on the chemical component (>4%) [12]. It means that this wood has been infected by a microorganism, and then the defense process occurred as showed by high extractive content. Syahri [19] also stated that the degradation of cellulose and pentosan (hemicellulose) by the enzyme of fungus then was converted into extractive component caused the increase of extractive content. It might be derived from the degradation of all wood chemical components (hemicellulose and cellulose), except lignin as explained in the next paragraph.
Lignin of this study included in a high content and was equal to Agarwood (*A. malaccensis* Lamk) induced for twelve months. This content has increased slightly compared to lignin of *Gonystillus* sp. wood [18]. Herawati *et al.* [17] reported that lignin content tended to increase along with induced period. It indicates that the induced process in the Agarwood samples has occurred. This content was compared to the lignin content based on the classification of Indonesian hardwood (18 – 33%) [12], and the result showed the lignin content of Agarwood is in moderate class.

Alpha-cellulose content of induced Agarwood was lower than that of *Gonystillus* sp. as reported by Martawijaya *et al.* [18], Syahri [19], and also the control of *A. malaccensis*. This content is classified into low class (< 40%) based on the classification of Indonesian hardwood α-cellulose content [12]. Cellulose of induced wood tissue would eventually be degraded by stimulant as result of pathogen work in Agarwood sapwood tissue, while cellulose would be replaced by resin. This condition was required to observe the success of Agarwood formation in the sapwood. The cellulose structure has a strong chain, therefore the degradation occurs slow [17]. The hemicellulose of Agarwood was lower than that of control of *A. malaccensis Lamk*; however, it was equal to *Gonystillus* sp. hemicellulose and sapwood of *A. malaccensis* Lamk induced for twelve months.

Hemicellulose is polymer having branch structure and lower polymerization degree than that of cellulose. Therefore, it is easier to be degraded by pathogen induced on sapwood tissue of agarwood. Therefore, this sapwood of agarwood provides a response to protect itself by removing fragrant resin which degrades the existing chemical component [17]. The hemicellulose content obtained in this study was in low class based on the classification of Indonesia hardwood and based on the chemical component especially hemicellulose (<21%) [12].

4. Conclusions
The average specific gravity of Gyrinops and Aquilaria genera were between 0.31 – 0.35, therefore they were included in the Strength Class IV. Chemical components of injured *G. versteegii* wood contained 50.74% holocellulose, 30.50% α-cellulose; 31.98% lignin; and 16.69% extractives.

Non-injured *G. versteegii* had longer fibre length, with almost similar fibre diameter and cell wall thickness compared to the injured one. The vessel length of non-injured *G. versteegii* was also longer, while the vessel diameter was nearly similar to the injured one. Injured *G. versteegii* might reduce the rate of cell length developing. The composition of ray cells in *G. versteegii* was more complex than *Aquilaria*.

5. References
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