YOUNG STEM AND PETIOLE ANATOMY OF AQUILARIA BECCARIANA VAN TEIGH AND AQUILARIA MICROCARPA BAILL (GAHARU ENGKARAS) IN SARAWAK

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YOUNG STEM AND PETIOLE ANATOMY OF \textit{AQUILARIA BECCARIANA} VAN TEIGH AND \textit{AQUILARIA MICROCARPA BAILL} (GAHARU ENGKARAS) IN SARAWAK.

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<table>
<thead>
<tr>
<th>TABLE OF CONTENT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Title Page</td>
<td>i</td>
</tr>
<tr>
<td>Acknowledgement</td>
<td>ii</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>iii-iv</td>
</tr>
<tr>
<td>List of Plates</td>
<td>v</td>
</tr>
<tr>
<td>List of Tables</td>
<td>vi</td>
</tr>
<tr>
<td>Abstract/ Abstrak</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER 1.0: INTRODUCTION</td>
<td>2-3</td>
</tr>
<tr>
<td>CHAPTER 2.0: LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>2.1 Characteristics of <em>A. beccariana</em> Van Teigh and <em>A. microcarpa</em> Baill</td>
<td>3</td>
</tr>
<tr>
<td>2.1.1 Characteristics of <em>A. beccariana</em> Van Teigh</td>
<td>4-5</td>
</tr>
<tr>
<td>2.1.2 Characteristics of <em>A. microcarpa</em> Baill</td>
<td>5</td>
</tr>
<tr>
<td>2.2 Anatomical Characteristics of <em>Aquilaria</em> species</td>
<td>5-6</td>
</tr>
<tr>
<td>2.3 The Importance of Microscopic Anatomical studies</td>
<td>6</td>
</tr>
<tr>
<td>2.3.1 Vessels</td>
<td>6-7</td>
</tr>
<tr>
<td>2.3.2 Rays</td>
<td>7-8</td>
</tr>
<tr>
<td>2.3.3 Fibres</td>
<td>8-9</td>
</tr>
<tr>
<td>2.3.4 Included Phloem</td>
<td>9</td>
</tr>
<tr>
<td>2.3.5 Vascular Bundle Arrangements</td>
<td>10</td>
</tr>
<tr>
<td>CHAPTER 3.0: MATERIALS AND METHODS</td>
<td>11</td>
</tr>
<tr>
<td>3.1 Samples Collection</td>
<td>11</td>
</tr>
</tbody>
</table>
3.2 Samples Preparations

3.2.1 Preparations of Slides

3.2.2 Microscopical Observations

3.2.2.1 Pore and Included Phloem Measurements

3.2.2.2 Vascular Bundle Arrangement

CHAPTER 4.0: RESULTS AND DISCUSSION

4.1 Morphological Characteristics of A. beccariana and Aquilaria microcarpa

4.2 Microscopic Anatomical Characteristics of A. beccariana and A. microcarpa

4.2.1 Vessels

4.2.2 Included phloem

4.2.3 Vascular Bundle Arrangement

CHAPTER 5.0: CONCLUSION

REFERENCES

Appendix I: Flow Chart of the Preparations of Microscopic Slide

Appendix II: Measurements for vessels of Aquilaria beccariana

Appendix III: Measurements for vessels of Aquilaria microcarpa

Appendix IV: Measurements for included phloem of Aquilaria beccariana.

Appendix V: Measurements for included phloem of Aquilaria microcarpa.
List of Plates

Plate 1: Transverse Section of the young twig *A. beccariana* wood showing A & B: solitary vessel; C: solitary vessel (small); D: solitary vessel (multi solitary vessel); E: included phloem. Scale bar = 100μm. 16

Plate 2: Transverse Section of the young twig *A. microcarpa* wood showing A: solitary vessel; B: solitary vessel (small); C: solitary vessel (large); D: multi solitary vessel; E: included phloem. Scale bar = 100μm. 16

Plate 3: Vascular bundle of the *A. beccariana* petiole; A: Cortex; B: xylem; C: phloem; D: crystal inclusion. Scale bar = 100μm. 17

Plate 4: Vascular bundle of the *A. microcarpa* petiole; A: Cortex; B: phloem; C: Xylem. Scale bar = 100μm. 18
List of Tables

Table 1: One-way ANOVA analyses on the vessels of Aquilaria beccariana and Aquilaria microcarpa. 19

Table 2: One-way ANOVA analyses on the included phloem of Aquilaria beccariana and Aquilaria microcarpa. 20
Young stem and petiole anatomy of *Aquilaria beccariana* Van Teigh and *Aquilaria microcarpa* Baill (Gaharu engkaras) in Sarawak.

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ABSTRACT

*Aquilaria beccariana* Van Teigh and *Aquilaria microcarpa* Baill are both protected species found in Sarawak and indiscriminately harvested for gaharu. Anatomical studies of their young stems and petioles were conducted to find out their differences of their vessels, included phloem and vascular arrangement. This anatomy studies aimed to uncover the differences between the *A. beccariana* and *A. microcarpa*. Young saplings and leaves from each species were obtained from the UNIMAS green house. Part of twigs was taken from the young saplings and petioles of mature leaves were selected. The results showed that there are differences between *A. beccariana* and *A. microcarpa* in their anatomical characteristics of their vessels, included phloem and the arrangement of the vascular bundles in the petioles. However, with the anatomical characteristics that have been obtained can be done for further research is required to be conducted to support these new findings.

Key words: *Aquilaria beccariana*, *Aquilaria microcarpa*, anatomy, vessel and included phloem

ABSTRAK


Kata kunci: *Aquilaria beccariana*, *Aquilaria microcarpa*, anatomi, vesel dan floem terkandung
1.0 INTRODUCTION

Aquilaria beccariana Van Teigh and Aquilaria microcarpa Baill are treelets to large trees classified under the family Thymelaeaceae. Thymelaeaceae is important because it contains the curious Eaglewood Tree (Aquilaria) fragrance (Corner, 1988). The important source for producing the value oil extract and 1 kg of the oil fetched up to RM35,000 exported to Arab countries. The distribution of the species could be found around India, Burma, Indo-China, South China, Thailand, Indonesia and Philippines and in Malaysia only 5 spp. namely A. beccariana, A. microcarpa, A. malaccensis, A. hirta and A. filaria (Whitmore, 1973).

In Sarawak, A. beccariana and A. microcarpa are listed under protected plants under The Wildlife Ordinance 1982. The occurrence of this species are getting scare in their natural habitat due uncontrol and over exploitation for the gaharu.

The standard Malaysian name for the timber of Aquilaria spp. (Thymelaeaceae) and the timber is soft and light with density ranging from 335 – 400 kg/m (Wong, 1982). The general characteristics are the one noteworthy feature which is presence of included phloem and the density is about 23 lb/cu.ft. (Burgess, 1966).
According to Cutler et. al. (1987), the bark has solitary crystals abundant in the cortex and cluster crystals rare in the ray cells. Certain numbers of the closely related Aetoxylon and even Gonystylus (Ramin), have been recorded as producing a wood similar to Gaharu.

Both of these species, *A. beccariana* and *A. microcarpa* can be considered as one of the important hardwood in Sarawak. As not many study have been done on this species, hence the objectives is to obtain detail information on it is anatomical structures and the variation of the two species based on its microscopic anatomical characteristics and the arrangement of the vascular bundle in the petiole.
2.0 LITERATURE REVIEW

*A. beccariana* Van Teigh and *A. microcarpa* Baill are both protected species and are classified under the Thymelaeaceae family which these species is important because of they produces valuable source of gaharu and also as a source of oriented perfume (Corner, 1988). According to Whitmore (1973), in Malaysia only have 5 spp. namely *A. malaccensis, A. beccariana, A. microcarpa, A. hirta* and *A. filaria*. *Aquilaria* wood has less potential as timber because the wood is light and soft.

2.1 Characteristics of *A. beccariana* Van Teigh and *A. microcarpa* Baill.

2.1.1 Characteristics of *A. beccariana* Van Teigh

The habitat of this species is in Mixed Dipterocarp forest and sometimes in Kerangas forest from lowland to 1200 meter above sea level. According to Whitmore (1973), this species can be found in the secondary forest which is near to the streams. This species could be found throughout Sarawak. *A. beccariana* is in the category as a medium-sized tree which could be reached up to 26 meter tall and 50 cm diameter at breast height (dbh).

According to Cockburn (1976), *A. beccariana* often has large blades leaves to 11 x 6 – 27 x 8.5 cm. The inflorescence of this species are terminal, axillary or
above the axils. Flowers in stalked clusters, small and it is usually in white, yellow and green in colour (Whitmore, 1973).

2.1.2 Characteristics of \textit{A. microcarpa} Baill

This species is a big tree and could be reached up to 36 meter tall. The habitat is in lowland forest up to 200 meter above sea level. This species is also found in throughout Sarawak. The vegetation \textit{A. microcarpa} is almost exactly as \textit{A. malaccensis} (Cockburn, 1976). The vegetation is distributed throughout the lowlands in Sabah (Burgess, 1966).

According to Kochummen (1997), the fruits of \textit{A. microcarpa} are in pear-shaped and capsule. \textit{A. microcarpa} Van Teigh is almost have the similar characteristic as \textit{A. malaccensis} but the fruit is half the size and the stamens equal or shorter than the petal (Cockburn, 1976).

2.2 Anatomical Characteristics of \textit{Aquilaria} species

The normal wood is usually the unscented wood. The vessels are very small to medium sized, not visibly or barely visible to the naked eye and solitary. The rays are numerous and mostly uniseriate. Included phloem is always present in the form of vertical strands and usually oval or round in shape and wider in tangential section (Ramesh & Dayal 1992). The scented wood has different appearance and general characteristics from normal wood. This is
because the wood has an aromatic resinous deposit. The resin is concentrated mostly in the strands of included phloem, other elements such as vessels and fibres may be plugged to lesser extent.

2.3 The Importance of Microscopic Anatomical Studies

The anatomical features of angiosperms are considerably more complex than that of the gymnosperms. It is not surprising that angiosperms are distinctive enough to be recognized with an eye or at most with a hand lens, so microscopes are often needed for certain identification of angiosperms particularly within genera or species level.

2.3.1 Vessels

According to Hoadley (1980), vessels elements are extremely large in diameter but have relatively thin walls. Vessels vary in sizes among and within species. Some can be seen clearly and easily with the naked eye. Some require hand-lens magnification but are distinguishable from other cell types because they are slightly larger. When vessels are cut transversely, the exposed open end is referred to as a pore and sometimes is called porous wood. Vessels are composed of single cells, the vessels elements. These joining end to end in wood, from longitudinal tubes, which can be several meters long (Anon, 1995).
In cross section, most vessels are oval in shape, but some vessels tend to be angular. The length of vessels, which varies greatly among species and different parts of the same tree, is positively correlated with vessel diameter (Kozlowski et.al., 1997).

2.3.2 Rays

Ray cells or also known as ray parenchyma is another important feature in the identification of a particular species. A few hardwoods usually have only uniseriate rays and some hardwoods also have rays of two distinct sizes- short uniseriates, and broad, high multiseriates. Generally rays can be classified into various types (Butterfield & Meylan, 1980):

A. Homogeneous Rays

i. **Uniseriate Homogeneous Rays** – These types of ray cells are one cell thick and made up of cells of equal size. All cells are composed of procumbent cells only.

ii. **Biseriate Homogeneous Rays** – These types of ray cells are two cells thick and made up of cells of equal size. All cells are composed of procumbent cells only.

iii. **Multiseriate Homogeneous Rays** – These types of ray cells are more than two cells thick and made up of cells of equal size. All cells are composed of procumbent cells only.
B. Heterogeneous Rays

i. Rays entirely uniseriate - These types of ray cells are one cell thick consisting of both the procumbent and upright cells.

ii. Heterogeneous Rays Type I – These types of ray cells are two or more cells thick. It is a mixture of uniseriate ray cells and multiseriate cells or biseriate ray cells. Uniseriate ray cells composed of upright cells and are longer than the broad multiseriate or biseriate, which composed of the procumbent cells.

iii. Heterogeneous Rays Type II – These types of ray cells are two or more cells thick. It is a mixture of uniseriate ray cells or multiseriate or biseriate ray cells. Uniseriate ray cells composed of upright cells and are shorter than the broad multiseriate or biseriate, which composed of the procumbent cells.

iv. Heterogeneous Rays Type III – these types of ray cells are two or more cells thick. It is a mixture of uniseriate ray cells and multiseriate or biseriate ray cells. Uniseriate ray cells composed of upright cells or procumbent cells and occur as single cell forming the tail of multiseriate or biseriate which composed of the procumbent.

2.3.3 Fibres

There are five categories commonly used to classify the average fibre wall thickness based on their appearance in a transverse section. There are (Butterfield & Meylan, 1980):
i. Very thin walled – wall thickness much less than fibre lumen diameter.

ii. Thin walled – wall thickness slightly less than fibre lumen diameter.

iii. Moderately thick walled – wall thickness about the same as the fibre lumen diameter.

iv. Thick walled – wall thickness greater than the fibre lumen diameter.

v. Very thick walled – wall so thick that the fibre lumen is almost closed up.

According to Hoadley (1980), fibres are smallest in diameter with closed ends and thick walls.

2.3.4 Included phloem

According to Butterfield & Meylan (1980), in some hardwoods, strands of phloem can be found embedded within the wood. These strands develop in a number of different ways as the results of unusual forms of cambial activity.

Included phloem is generally subdivided into two types depending on the distribution of the strands when can be viewed in the transverse section. Foraminate included phloem strands are scattered more or less randomly throughout the wood while concentric included phloem strands lie in the definite rings.
2.3.5 Vascular bundle arrangement

According to Rudall (1994), vascular bundle can be in form of collateral with xylem and phloem adjacent to each other, bicollateral with phloem on both sides of the xylem or amphivasal with xylem surrounding the phloem. The collateral forms are arranged either in a continuous cylinder or in a cylinder of separate or fused collateral bundles with phloem external to the xylem. For bicollateral arrangement, phloem is present in the external phloem. And amphivasal arrangement, the bundles are relatively unusual where the vascular cambium produces secondary vascular tissue, is situated between the xylem and phloem and eventually forming a complete cylinder.
3.0 MATERIALS AND METHODS

3.1 Samples collection

The samples were obtained from the sapling grown at University Malaysia Sarawak green house. The samples are young saplings of *A. beccariana* and *A. microcarpa*. The part of the twigs of the young saplings and petioles of mature leaves were selected for microscopic observations. The young twig samples are sliced into three sections which consist of transverse section, tangential section and radial section. Microscopic observations are done based on vessels structure and length, fibre length, parenchyma ray, included phloem and crystal inclusion and vascular bundle arrangement and structure of the petiole.

3.2 Samples Preparations

Each young twig was taken from the two species. The twigs are cut 3 – 4 cm in length. Fresh cut twigs are then boiled for \( \frac{1}{2} \) to 1 hour to soften the wood, so that it is easier to slice. Leaves are taken randomly from the two species. Middle parts of the petiole selected for the observation of the vascular bundle arrangement and the structure of the petiole.
3.2.1 Preparation of Slides

The boiled young twigs are sliced by using the Sliding Microtome with the thickness of 16 and 10 micrometer. One portion must be slice – Transverse section.

The sliced sections were then stained with 5% aqueous safranin for at least 10 minutes. The purpose of using safranin is to get anatomy structure from the sliced so that could be seen clearly under the microscope. The stained slices sections were then rinsed with in a series of dehydration 50%, 70%, 80%, 90% and 100% concentration ethanol. Then the dehydrated sliced sections were treated with Clove Oil and followed by xylene for about 10 minutes to harden the tissues. Finally, the sliced section was mounted on clean slides, glued with Canada balsam and then cover with cover slips. All the slides were then label accordingly. The ready made microscopic slides was then kept and dry for 10 days. The slides were prepared for permanent slides. Flow chart of the preparation can be seen in APPENDIX I. Photographs were also taken using Leica MPS 32.

Slices of petiole of both two species are cut freshly of 5mm long. The samples were cut by using razor blade of uniform thickness. Then the selected sections were soaked in distilled water for 10 minutes to expand the tissues. Lastly, observations were made immediately. The arrangements of the vascular
bundle of the two species were observed. Photographs of the structure of the petiole were taken using Leica MPS 32.

3.2.2 Microscopical Observations

3.2.2.1 Pore and included phloem Measurement

The pore or vessels and included phloem measurement were based on the dimension of the length and width. Observation is made on the transverse section of the microscopic slide. Thirty measurements were taken randomly for every sample of each species. Photographs of these cells were taken using Leica microscope.

3.2.2.2 Vascular bundle arrangement

The vascular bundle arrangements of the two species are observed. Several slices of the petiole are cut and observed under the microscope to obtain good section. Observation were made directly under the microscope and photograph were taking using Leica MPS 32.
4.0 RESULTS AND DISCUSSION

4.1 Morphological Characteristics of *A. beccariana* and *A. microcarpa*.

*Aquilaria* species can be found in peat swamp forest. The distribution of these two species are scattered around Sarawak. *A. beccariana* can be found throughout Sarawak and *A. microcarpa* can be found in Kuching and Samarahan Division. The morphological characteristics of these two species are from the sizes of the leaves, flowers and fruit. *A. beccariana* has big leaves and bear flowers. However, *A. microcarpa* has small – medium sized leaves and bears fruits.

4.2 Anatomical Characteristics of *A. beccariana* and *A. microcarpa* young stem

The microscopic anatomical characteristics study of *A. beccariana* and *A. microcarpa* wood only covered two main important features that are the vessels, included phloem and vascular bundle arrangement and structure of the petiole.
4.2.1 Vessels

Vessels or pore is one of the important features in wood identification. In this study, there are one main characteristic of the vessels were studied which is vessels diameter measurements.

The microscopic anatomical features showed that the vessels in both species are mostly solitary vessels. There are two types of solitary vessels can be found in both species which is large solitary vessel and small solitary vessel. Large solitary vessels are often can be seen clearly and many in numbers. Beside that, multi solitary vessels are seen clearly and these multi solitary vessels are found in double, three or four vessels in a group in both species.

The measurement ranges of the vessels were from 2 – 6 μm in diameter. The small vessels are range from more than 3 μm and the large vessels are range from more than 4 μm. (See Plate 1 and Plate 2). Using One-way ANOVA analysis the diameter of the vessels is not significant. The diameter measurements for the two species were 2 – 6 μm.

4.2.2 Included phloem

In A. beccariana and A. microcarpa, the included phloem is common and can be seen very clearly. The measurement ranges from 3 – 60 μm in diameter. (See Plate 1 and 2). The length of the included phloem is significant between
two species and the analyzing is done by using One-way ANOVA analyses. The diameter measurement for two species were 3 – 60 μm. Results can be seen in Table 2.

Plate 1: Transverse section of the young twig of *A. beccariana* young stem showing A & B: Solitary vessel; C: Solitary vessel (small); D: Solitary vessel (multi solitary vessel); E: included phloem. Scale bar = 100μm.

Plate 2: Transverse section of the young twig of *A. microcarpa* young stem showing A: Solitary vessel; B: Solitary vessel (small); C: Solitary vessel (large); D: Multi solitary vessel; E: Included phloem. Scale bar = 100μm.
4.2.3 Vascular bundle arrangement

In *A. beccariana*, the vascular bundle of the petiole consists of one main bundle located in the central part surrounded by 6 – 7 sub vascular bundles. The types of vascular bundle are collateral. Collateral typed with xylem and phloem adjacent to each other. The shaped of the vascular bundle mostly look like ring – shaped. Crystals of rhomboid are commonly found in the xylem of the vascular bundle. (See Plate 3).

In *A. microcarpa*, the arrangement of the vascular bundle has a kidney - shaped with the end are connected together or consisted of a simple oval – shaped. The vascular bundle arrangement of *A. microcarpa* was different from *A. beccariana*. The type of the vascular bundle is amphivasal with xylem surrounding the phloem. (See Plate 4)

Plate 3: Vascular bundle of the of *A. beccariana* petiole. A: Cortex; B: Xylem; C: Phloem; D: Crystal inclusion; Scale bar = 100 μm