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Histological observation of Gelam (*Melaleuca cajuputi* Powell) in different ecosystems of Terengganu

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Abstract
A histological and anatomical comparative analysis of Gelam, *Melaleuca cajuputi* Powell was conducted to determine the adaptability Gelam (*Melaleuca cajuputi* Powell, Family = Myrtaceae) in two different micro-ecosystems of Terengganu. The sites sampling chosen were Jambu Bongkok Amenity Forest (Formerly known as Rantau Abang Amenity Forest), a large swamp forests and prominent flooding site in the District of Marang, Terengganu and Taman Penyelidikan Alam, Bukit Kor - a hard top soil area of Marang, Terengganu. The objective of this study is to compare the anatomy and histology features of gelam in these two areas. Results show that gelam from both ecosystems exhibits morphological differences and variations in some tissues (in leaf, petiole, bark, and root anatomy such as in type of epicuticular waxes, bark anatomy, leaf anatomy, vascular bundle, bark fibre pits, vessel, phloem, xylem, cortex, epidermal cells and mesophyll cells). This initial observation may suggest that gelam can adapt well in different ecosystems of Terengganu. Its ability to response well toward Terengganu's different coastal environmental changes could make gelam a viable tree for conservation and landscape program.
Keywords: *Melaleuca cajuputi* Powell, Gelam; anatomy; histology; microscopy; Terengganu

1. Introduction

Melaleuca forests mostly occur in coastal regions, wetland, peatlands and lowlands. There are seven species that occur naturally in this world, namely *Melaleuca cajuputi*, *Melaleuca dealbata*, *Melaleuca leucadendra*, *Melaleuca nervosa*, *Melaleuca quinquenervia*, *Melaleuca stenostachya*, and *Melaleuca viridiflora* (Craven 1999).

In Terengganu, mangroves and Gelam (*M. cajuputi* Powell) forest are two main interconnected wetlands. Wetlands are important ecosystem supporting tremendous biodiversity for socio-economic activities, as well as upholding vital ecological functions. Extensive research and documentation of mangrove ecological functions are well covered (Erwin 2009, Robinson 2007, Kamaruzzaman, 2008). However less attention is given to Melaleuca forest as compare to other heath forest such as the mangrove.

M. cajuputi Powell is native to Peninsular Malaysia. This plant is belongs to Melaleuca species from Myrtacea family which is a large genus with more than 250 species have been described (Craven 1999, Brophy and Doran 1996). The connectivity of both mangroves and Gelam is very distinct where the health of one will eventually affect the other, both economical and ecological functions. Threat to these ecosystems mainly from fragmentation and land use changes are inevitable and are significantly reduced their coverage and ecological roles. Anthropogenic activities to the forests (e.g. land use change, logging and burning), particularly in Indonesia (Anderson and Bowen 2000), and Malaysia (Wetlands International – Malaysia 2010), are also another contributing factors to the changes of the wetlands. Land clearing and human habitation put significant pressure on *M. cajuputi* Powell.

Melaleuca forest can be found in abundance in Terengganu and part of the east coast of Peninsular Malaysia. The economic importance of Melaleuca forests have been well covered such as can provide timber for building and furniture and other traditional uses, such as for fuel wood, charcoal, tea-tree oil, and honey Gelam are still employed today [e.g. in Indonesia, Malaysia (Saberioon 2009), Thailand (Nuyim 1998), Cambodia (Hiramatsu *et al.*, 2007), and Vietnam (Duong *et al.*, 2005)]. Melaleuca forests can also store a large volume of soil organic carbon, especially in the peatlands (e.g. in Indonesia, Malaysia, Thailand, and Vietnam).

M. cajuputi, has the widest distribution and they dominated the current Melaleuca populations around the globe (Blake 1968). Most of the Melaleuca genus occurs in wetland and coastal regions, which are some of the most vulnerable locations in the world to future climate change. Furthermore, coastal and lowland regions will be the

areas most affected by global climate (Nicholls *et al*., 2007).
Although this plant grows across a wide range of sites characterized by soil moisture regime, such as semi-arid and swamp, the current distribution indicates that they have the ability to adapt to a wide range of climates. (Watt *et al.,* 2009, Blake 1968, Doran and Turnbull 1997). However less attention is being rendered to their anatomical and morphological characteristic in relation to their adaptation to the changing environment. Therefore, the examination of the plant part such as leaf, petiole, bark and root of *M. cajuputi* Powell species should be conducted using microscopy observation. The objectives of this study were to identify and describe some anatomical feature of Gelam and in adaptation to different ecosystem.

2. Materials and methods

Gelam Sampling Area

The plant samples were collected from the prominent sites as flooding site that is at Jambu Bongkok Amenity Forest formerly known as natural forest habitat in the District of Marang, Terengganu and at a rocky hill of Taman Penyelidikan Alam, Bukit Kor - a hard top soil area of Marang, Terengganu, Malaysia. Samples were collected randomly in each site for this study. Plant parts including leaf, petiole, bark and root that were used in this study and evaluated for anatomy, histology, thin or thick bark, vascular bundle, fiber pits bark, vessel, phloem, xylem, cortex, epicuticular waxes, leaf anatomy, epidermal cells and mesophyll cells.

Plant materials

M. cajuputi samples were collected from two different geographical sites. The plant root sample especially at Jambu Bongkok were collected in floodplains; the flooding water in the peat swamp was dark brown in color, and the root sample at Bukit Kor were collected in rough sand or rock hill of Bukit Kor. Ten sample was collected randomly in each site for this study. The entire sample were collected and placed in a controlled-environment room at 25–30 °C. The rest of the plant parts (leaf, bark, petiole and root) are directly cut using a sharp razor and fixed in Formalin-Acetic Acid-alcohol (FAA) immediately in order to avoid any traumatic change and artifact contamination as much as possible for future optical view. All samples were transported to and maintained in the Crop Science Laboratories, School of Food Science and Technology, Universiti Malaysia Terengganu.

Samples preparation

The samples were fixed in FAA for two days to penetrate the cells and their contents as nearly the living condition as possible or to retain their natural condition. Dehydration was carried out by treating specimens sequentially with 15%, 25%, 50%, 75%, 100% and dry ethanol, for 15 minute at each concentration. The specimens were trimmed into 4 to 5 mm or less in height, width and depth for infiltration in histoclear and wax. The leaf, petiole, and root samples were embedded in wax and the sample were sectioned at 8 µm thick with a rotary microtome (RM2235, Leica, Wetzlar, Germany).
Bark was process using maceration fluid. Maceration fluid is used to separate the xylem tissue so it will be easily broken up for vessel elements viewing. Before place the tissue slivers in vials with macerating fluid, the plant bark was cut into a small slivers and boiled it in water to aspirate it. Maceration fluid was prepared by adding 10% nitric acid with 10% chromic acid using Jeffrey's Method. The tissue was treated for one to two days in electric oven at 30˚C to 40˚C. The vials are cooled at room temperature and the tissues are crushed by shaking with glass beads. The macerated material was washed with water and the material was centrifuge between washed to speed the process. The tissue was stained with 1 % aqueous saffranin.
After that the semi thin sections were laid on slide and leave on hotplate to dry completely for overnight. The sections were stained with safranin O and fast green. After staining the section was mounted using DPX and the slide were leave on hotplate 60°C for overnight then examined at 4x, 10x, and 40x magnification under a microscope equipped with a digital camera Leica ICC50 HD (Leica DM750,Wetzlar, Germany).
Anatomy and histology such as the area occupied by intercellular spaces, anatomy, histology thin or thick bark, large or small leaf area, vascular bundle, fiber pits bark, vessel, phloem, xylem, cortex, epidermal cells and mesophyll cells are observe using Leica DM750. The images obtain using a digital camera Leica ICC50 HD are analyze and calculated the cross-sectional porosity by dividing the area of intercellular space by the total cross-sectional area.

3. Result and Discussion

Leaf anatomy

The leaf of Gelam covered with cuticle wax, palisade cell, sponge cell, and bundle sheet on a transverse section of gelam leaf view (Figure 1). Lamina area contains secretory oil cavities.

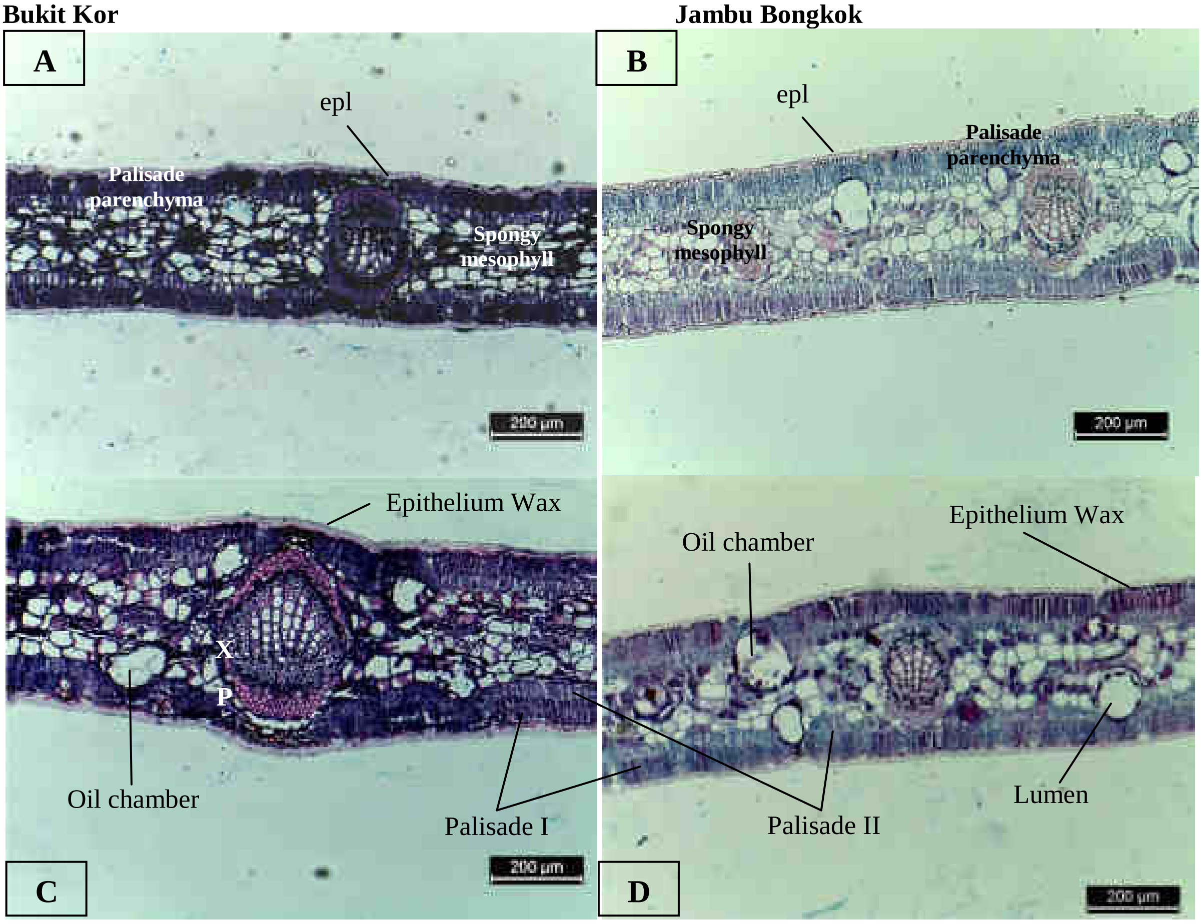


Figure 1. Comparative leaf anatomy in blade cross-sections of Gelam (*M. cajuputi* Powell) leaves grown under a hard top soil area (A, C) and a large swamp forests, prominent flooding site (B, D) conditions. Epithelium wax in Bukit Kor is ticker compare to Jambu Bongkok. Vascular bundle are bigger in size in A & C. (Magnification 10X) (epl, epidermis layer; x, xylem; p, phloem; cw, cuticle wax; ml, middle lamella; oil, oils; epi, epithelium).

This leaf cross section in figure 1 shows a large oil chamber. The outer area is made up of thin, flat epithelial cells; the inner wall of each is extremely thin. All the oil has drained out during specimen preparation, so a face view of the inside is empty. Brophy and Doran, 2004 state that the genus Melaleuca also contains hundreds of individual species with a myriad of oil constituents present in the leaf. Cross sections of Gelam leaves showed that in both site the palisade parenchyma of the mesophyll was composed of two parts, one being in contact with the upper epidermis and the other with the lower one. Palisade I consisted of one layers of relatively elongated cells, whereas palisade II consisted of one layer of short cells. The spongy parenchyma (between the two palisade parenchymas) contained large intercellular spaces, variously sized vascular bundles and individual or grouped long sclereid.

There were significant different in leaf anatomical characteristics between this two site. Leaves from Bukit Kor resulted in a significant decrease of the thickness of almost all histological components of the mesophyll, as well as of the entire lamina thickness. In stressed plants leaves grown under a hard top soil area (Bukit Kor) the chlorenchyma cells are denser than those in a large swamp forests and prominent flooding site of Jambu Bongkok (Fig. 1). As a consequence, the amount of intercellular spaces of water stressed leaves in Bukit Kor was lower than in control leaves of Jambu Bongkok. The lower amount of intercellular spaces in Bukit Kor leaf cells may result in the diffusion conductance in water stressed leaves in Bukit Kor.

Figure 1 (A and C) shows a spongy mesophyll that is quite compact compare to B, D. The feature different are usually good indicators of xerophytes. There is much less surface area in this A, C leaf compare to B, D leaf due to a dense arrangement of spongy mesophyll. So water will be loss from cells much more slowly. These results support the idea that direct relationship between leaf porosity and mesophyll conductance exists (Loreto et al., 1992). However, Syvertsen et al. (1995) showed that the mesophyll density is inversely correlated with the conductance through the liquid phase only. Quantitative analyses of anatomical parameters are reported in Table 1. Bar in µm.

Table 1.
Anatomical different in cross-section of fully expanded leaves/Gelam leaf (*Melaleuca cajuputi* Powell) of two different location.

Leaf histological component	**Jambu Bongkok**	**Bukit Kor**
Thickness (µm)		
Upper epidermis	4.652±1.692	3.526±1.244
Palisade I	50.703±12.457	44.854±10.895
Palisade II	36.307±4.254	34.995±5.762
Spongy	195.761±78.634	169.615±32.023
Lower epidermis	4.657±1.232	3.331±1.001
Total thickness	355.573±28.612	361.518±33.328

Petiole
The petiole had bundles of vascular bundle that is located in every center, jointed basal pulvinus for each end and thin undulating cuticle, vascular bundles orientated in a central of the marginal. Each vascular bundle consisted of phloem, xylem and sclerenchyma (Figure 2).

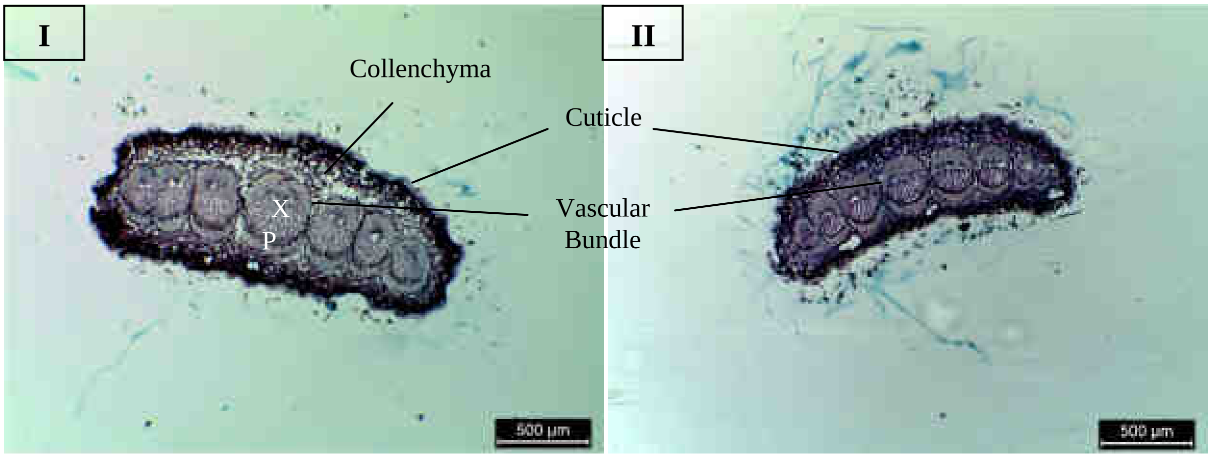


Figure 2. Petiole anatomy of Gelam (*Melaleuca cajuputi* Powell). General view of petiole at site I (Bukit Kor) and site II (Jambu Bongkok) with a thick undulating cuticle, vascular bundles orientated in the central. Each vascular bundle consists of phloem, xylem and sclerenchyma. (ep, epidermis; vb, vascular bundle; scl, sclerenchyma.; x, xylem; p, phloem).

The collenchyma cells of Gelam from Bukit Kor petiole are more fully develop than the Jambu Bongkok. The walls of collenchyma cell are much thicker, and the cell lumen appears round. The structural anatomy of phloem tissue in the vascular bundle determines the extant and speed of solute transport. The pores of sieve tubes in the phloem are small in size and less in number, it definitely transport less food material (sugar and ions) than the phloem tissue having more pores of larger size.

Similarly, the structure anatomy of xylem vessels determines the rates of water and nutrient uptake. The number and distribution of treachery elements are major determinants of water movement through xylem. Quantitative analyses of anatomical parameters are reported in Table 2. Bar in µm.

Table 2.
Anatomical different in cross-section view of petiole (*Melaleuca cajuputi* Powell) of two different places of Gelam

Petiole histological component	**Jambu Bongkok**	**Bukit Kor**
Thickness (µm)		
Vascular bundle	513.422±12.453	304.004±8.842
Phloem	375.098±11.658	202.841±9.989
Total thickness	787.610±12.436	509.042±10.345

Bark

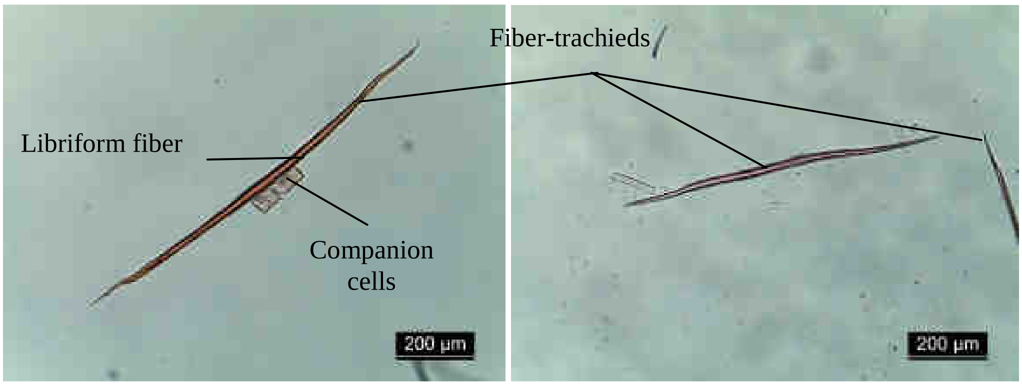


Figure 3. Maceration of gelam bark. The long, narrow cells are extraxylery fiber

Bark or the wood rings are porous to diffuse porous. The vessels are typically small and the vessel end-walls are simple. The vessels are with vestured pits; with spiral thickening, or without spiral thickening. The axial xylem with tracheids is commonly with vasicentric tracheids. Figure 3 shows a sclerenchyma fiber-trachieds of *M. cajuputi.* They are rather short for fibers, but they have pits with elongate inner apertures. The centers of several pits are bright and rather circular, indicating the there are small chamber, making this fibers-trachieds.

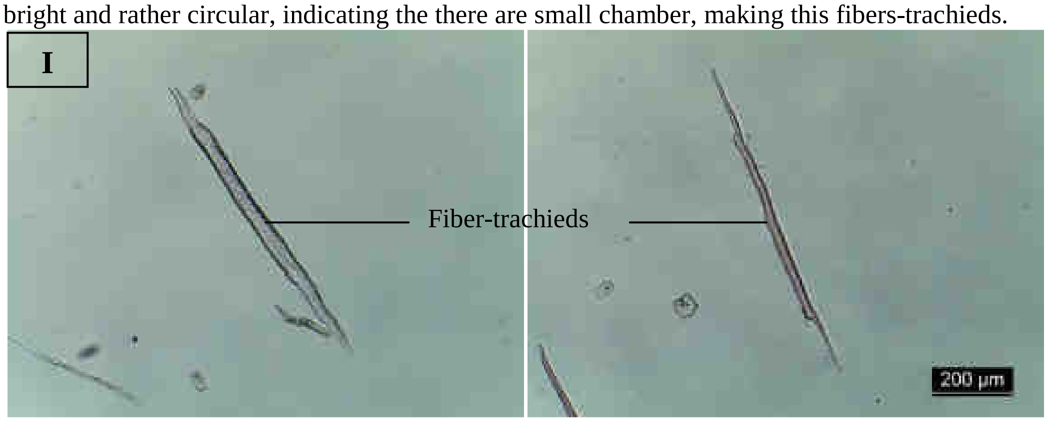


Figure 3.1 Sclerenchyma fiber-trachieds of *M. cajuputi.*

Note that in each cell the inner apertures of some pits on figure 3.1 are elongate in one direction and that the apertures of others ape perpendicular. This is because both the front and back wall was visible, and the two sets of pits appear superimposed. These cells were prepared by maceration: they were treated with acid until the middle lamella dissolved. Bark serves a function in nutrient storage.

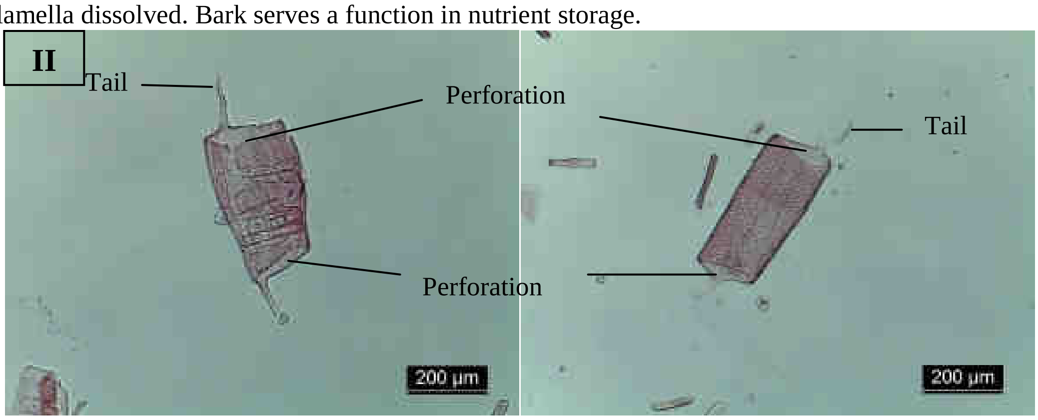


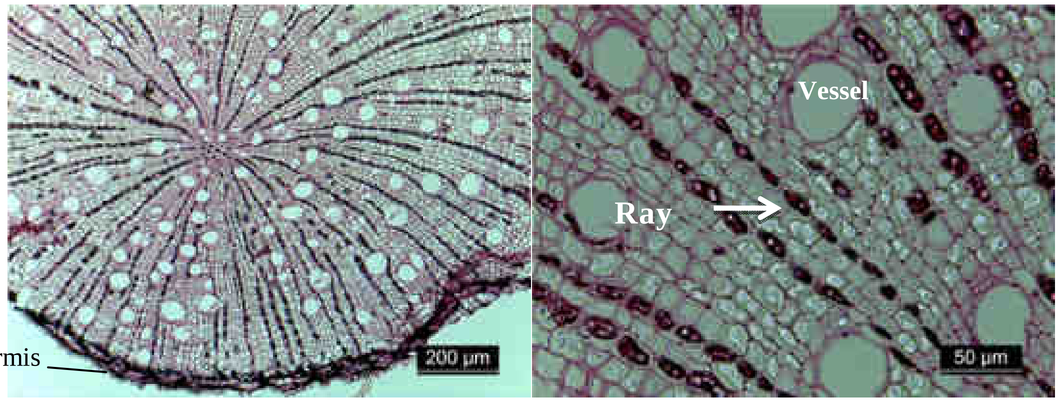
Figure 3.2 Vessel elements of gelam (*M. cajuputi* Powell). Vessel elements are slender, elongated and have foraminate perforation plates. The important features are they both have perforations. Obviously, the wider vessel element can conduct more water with less friction than can the narrower vessel elements.

Bark thickness, mean performance and range in variability for bark anatomical characters of the Gelam plant evaluated at the time of opening is given in table 3. The number of vessel rows range from 789.974 µm to 1563.897 µm with a mean of 1026.648 µm in Bukit Kor compare to 798.309 µm to 1155.470 µm with a mean of 937.060 µm in Jambu Bongkok. Between the two places, gelam at Bukit Kor exhibited significant superior number of vessel rows compare to gelam at Jambu Bongkok.

Table 3.
Different anatomical parameter of bark (*M. cajuputi* Powell) at two different places of Gelam

Bark anatomical parameters	**Bukit Kor**	**Jambu Bongkok**
Thickness (µm)		
Bark range	789. 974 to 1563.897	798.309 to 1155.470
Bark thickness	34.926	28.764
Mean	1026.648	937.060

Root
The root develops from the tuber and also from the stem base. From our observation, this root was fully lignified. A thick layer of epidermis developed as a protective layer.
Bukit Kor



Jambu Bongkok

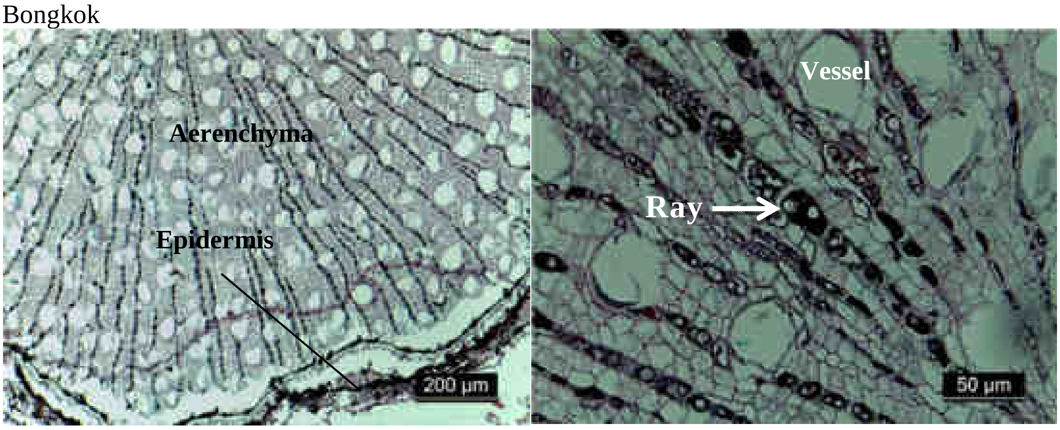


Figure 4. Transverse sections of *M. cajuputi* Powell root. Root cross section through light microscope showed a secretory duct in the root of gelam. The surrounding cells are others parenchyma cells of the cortex. Notice that root at site 1; Jambu Bongkok contained a high number of vessel compare to site 2; Bukit Kor. (epl, epidermis layer; x, xylem; p, phloem; r, ray; sd, secretory duct).
M. cajuputi is a long-lived, moderately fast-growing tropical tree adapted to both waterlogged and well drained soils. On soils subject to prolonged water logging (site 1; Jambu Bongkok) it root have more porous than root at Bukit Kor. The root systems are well-adapted to fluctuating water tables. The dense surficial roots are complemented by abundant vertical sinker roots that extend at least to the water table's deepest annual level (Geary and Woodall 1990).

4. Conclusion
The research showed that the anatomy of *M. cajuputi* Powell varied in different sites of coastal part of Terengganu due to natural regeneration ability in different sites in responses to different ecosystems of Terengganu especially their morphological and anatomical changes. Histology result of *M. cajuputi* Powell also shows that anatomies of gelam are variable between those two sites. Thin or thick bark, large or small leaf area and absent of special root. Results showed that gelam from both ecosystems exhibits morphological differences and variations in some tissues (in leaf, petiole, bark, and root anatomy such as in type of epicuticular waxes, bark anatomy, leaf anatomy, vascular bundle, bark fibre pits, vessel, phloem, xylem, epidermal cells and mesophyll cells.

In conclusion, Histology of gelam from two different sites is varied. This initial observation may suggest that gelam can adapt well in different ecosystems of Terengganu. The tree has the ability to response well toward Terengganu's different micro-climates and coastal environmental changes and the finding is essential to increase baseline knowledge of flora presence at the study area.

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