CHARACTERISATION OF MALAYSIAN AGARWOOD SPECIES *(AQUILARIA MALACCENSIS*, *AQUILARIA HIRTA*, AND *AQUILARIA BECCARIANA)* BASED ON VOLATILE CHEMICAL PROFILE AND SOMATIC EMBRYOGENESIS DEVELOPMENT

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SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Doctor of Philosophy.

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ABSTRAK

Aquilaria malaccensis, Aquilaria hirta, dan *Aquilaria beccariana* merupakan spesies tumbuhan yang menghasilkan gaharu di Malaysia. Ia menghasilkan kayu resin dan minyak pati yang digunakan dalam industri wangian, perubatan, kosmetik dan upacara keagamaan. Spesies ini sukar untuk dikenal pasti dengan tepat kerana tiada piawaian sebatian kimia gaharu dan minyak pati bagi tumbuhan tersebut. Kajian ini bertujuan untuk mencirikan morfologi *A. malaccensis, A. hirta dan A. beccariana* melalui analisis mikroskopik imbasan elektron, dan untuk mengenal pasti sebatian kimia yang meruap dari kayu dan minyak pati menggunakan analisis kromatografi gas-pengesan pengionan nyala (GC-FID) dan Kromatografi Gas ¬Spektroskopi Jisim (GC-MS). Kemudian, kajian embriogenesis somatik dijalankan melalui teknik induksi kalus menggunakan analisis Mikroskopi Elektron Pengimbasan (SEM) dan Tindak Balas Berantai Polimerase (PCR). *A. hirta* dikenalpasti melalui struktur bulu pada bahagian atas daun dan tetulang daun. Analisis Pancaran Medan Mikroskopi Elektron Pengimbasan (FESEM) menunjukkan struktur lubang kapilari tanaman tidak dapat dilihat pada imej kayu resin kerana dilitupi resin dan mikroorganisma. *A. malaccensis* dan *A. beccariana* mempunyai struktur lubang vesel ringkas manakala *A. hirta* mempunyai vesel tidak berlubang dengan struktur apertur luaran. Sebanyak 19 sebatian kimia dikenal pasti dalam kayu *A. hirta* yang terdiri daripada 5.97 % seskuiterpena dan 20.32 % seskuiterpenoid. Terdapat 35 sebatian yang terdapat dalam minyak *A. hirta* yang mengandungi 0.45 % monoterpena, 23.51 % seskuiterpena dan 19.53 % seskuiterpenoid. Sebanyak 35 sebatian dikenal pasti dalam resin kayu *A. beccariana* yang terdiri daripada monoterpena 1.5 %, seskuiterpena 15.27 % dan 50.69 % seskuiterpenoid. Terdapat 32 sebatian yang terdapat dalam minyak pati *A. beccariana* yang mengandungi 36.69 % seskuiterpena dan 49.58 % seskuiterpenoid. Frekuensi pertumbuhan kalus tertinggi diperhatikan pada medium MS yang dilengkapi dengan BAP dan NAA bersama dengan parameter yang dioptimumkan. Analisis SEM menunjukkan ciri-ciri embrio pada sel-sel kalus yang kecil dan isodiametri. Hanya gen SERK berjaya digandakan untuk analisis bioinformatik. Kajian ini memberikan rujukan untuk pengenalpastian spesies gaharu di Malaysia khususnya *A. malaccensis, A. hirta* dan *A. beccariana* berdasarkan penilaian morfologi dan profil sebatian kimia dari minyak pati dan kayu gaharu. Selain itu, kajian embriogenesis somatik memberikan pengetahuan asas bagi usaha pemuliharaan genetik *Aquilaria sp*. ke arah penanaman gaharu yang lebih sistematik dan produktif di Malaysia.

ABSTRACT

Aquilaria malaccensis, Aquilaria hirta, and *Aquilaria beccariana* are known as Malaysian agarwood species. They produce unique resinous wood and essential oil used in perfumery, medicinal, cosmetic and religious ceremony. It is difficult to identify the species accurately since there is no standard to represent the chemical compounds of agarwood and essential oil. This study aims to characterise the morphology of *A. malaccensis*, *A. hirta* and *A. beccariana* via scanning electron microscopic analysis, and to identify the volatile chemical compounds of the wood and the essential oil using Gas Chromatography-Flame Ionisation Detector and Gas Chromatography-Mass Spectrometry analyses. Then, somatic embryogenesis study was developed by optimising callus induction techniques using Scanning Electron Microscope (SEM) and Polymerase Chain Reaction (PCR) analysis. *A. hirta* was identified by the presence of hirsute on the abaxial side of leaves and midrib. The FESEM analysis shows plant vessel pits cannot be seen in the resinous wood images due to resin and microorganisms coverage. Interestingly, *A. beccariana* has simple vessel pits structure while *A. hirta* has nonvestured vessel pits with slit-like outer pit apertures. A total of 19 compounds were identified in resinous *A. hirta* wood consists of 5.97 % sesquiterpenes and 20.32 % sesquiterpenoid. There were 35 compounds found in *A. hirta* oil contained 0.45 % monoterpenes, 23.51 % sesquiterpenes and 19.53 % sesquiterpenoid. A total of 35 compounds were identified in resinous *A. beccariana* wood consists of 1.5 % monoterpenes, 15.27 % sesquiterpenes and 50.69 % sesquiterpenoid. There were 32 compounds found in *A. beccariana* oil contained 36.69 % sesquiterpenes and 49.58 % sesquiterpenoid. The highest frequencies of callus induction were observed on MS medium supplemented with BAP and NAA together with optimized growth parameters. SEM analysis showed embryogenic characteristics in cells of the yellow friable calli evidenced by the presence of small and isodiametric cells. Only SERK gene was successfully amplified and enable to proceed to *in silico* analysis. This study provides a reference for Malaysia agarwood species identification specifically for *A. malaccensis*, *A. hirta* and *A. beccariana* based on morphology evaluation and volatile chemical compounds profile of the essential oil and chip wood towards standardizing the quality. In addition, the somatic embryogenesis study provides fundamental results for genetic conservation effort of *Aquilaria sp.* towards more systematic and productive agarwood plantation in Malaysia.

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