

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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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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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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MOHD SYAFIK BIN MOHAMAD HAMDAN

Thesis submitted in fulfillment of the requirements
for the award of the degree of
Doctor of Philosophy

Faculty of Industrial Sciences and Technology
UNIVERSITI MALAYSIA PAHANG

FEBRUARY 2022

ACKNOWLEDGEMENTS

In the name of Allah S.W.T, the Most Gracious and the Most Merciful. Foremost, I would like to express my deep and sincere gratitude to my supervisor, Associate Professor Dr. Saiful Nizam Tajuddin for the continuous support of my PhD study and research. His wide knowledge, understanding, encouraging and personal guidance have provided a good basis for the present thesis and have been of great value for me.

I would like to thank Dr. Siti Hajar Noor Shaarani as my co-supervisor for her encouragement, insightful comments and suggestions. Special thanks to Mr Che Mohd Aizal for technical assistance with Gas-Chromatography additional analysis. I thank my fellow labmates in Bio Aromatic Research Centre (BIOAROMATIC) Laboratory for the stimulating discussions, and for all the fun we have had in the last few years. I am also grateful to the staff of Faculty of Industrial Sciences and BIOAROMATIC team for providing the facilities to carry out the study.

I convey my sincere gratitude towards the Ministry of Higher Education Malaysia for the financial support provided *via* MyBrain Scholarship and Research grant (FRGS) as well as Universiti Malaysia Pahang for the PGRS grant. I also would like to acknowledge the staff from the Institute of Postgraduate Studies, UMP for the cooperation given during the study period.

Last but not the least, I want to express my regards and blessings to my family and all of those who supported me in any respect during the completion of the journey. I hope the findings in this thesis will open more windows for the next generation and give significant impact to the nation.

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 pengenalanpastian 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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