

UNIVERSITI TEKNOLOGI MARA

**PHYTOCHEMICAL STUDY OF
DRYOBALANOPS FROM
MALAYSIAN DIPTEROCARPACEAE
AND STRUCTURE-ACTIVITY
RELATIONSHIP STUDIES**

AGUSTONO WIBOWO

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

Faculty of Applied Sciences

July 2014

CONFIRMATION BY PANEL OF EXAMINERS

I certify that a Panel of Examiners has met on 14th May 2014 to conduct the final examination of Agustono Wibowo on his Doctor of Philosophy thesis entitled “Phytochemical study of *Dryobalanops* from Malaysian Dipterocarpaceae and structure-activity relationship studies” in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiners recommends that the student be awarded the relevant degree. The panel of Examiners was as follows:

Norashikin Saim, PhD
Professor
Fakulti Sains Gunaan
Universiti Teknologi MARA
(Chairman)

Datin Rohaya Ahmad, PhD
Professor
Ketua Pusat Pengajian Sains Kimia dan Persekitaran
Fakulti Sains Gunaan
Universiti Teknologi MARA
(Internal Examiner)

Khozirah Shaari, PhD
Professor
Laboratorium Hasil Semulajadi
Institut Biosains
Universiti Putra Malaysia
(External Examiner)

Geoffrey A. Cordell, PhD
Professor Emeritus
Department of Medicinal Chemistry and Pharmacognosy
College of Pharmacy
University of Illinois Chicago
(External Examiner)

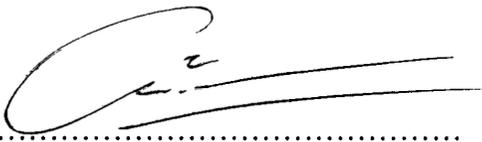
SITI HALIJAH SHARIFF, PhD
Associate Professor
Dean
Institute of Graduates Studies
Universiti Teknologi MARA
Date: 23th July, 2014

AUTHOR'S DECLARATION

I declare the work in this thesis/dissertation was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, Regulating the conduct of my study and research.

Named of student : Agustono Wibowo
Student I.D. No. : 2009390061
Programme : Philosophy Doctor in Science (AS990)
Faculty : Applied Sciences
Thesis/Dissertation Title : Phytochemical study of *Dryobalanops* from Malaysian Dipterocarpaceae and structure-activity relationship studies

Signature of Student : 
Date : July 2014

ABSTRACT

Dryobalanops is one of the genera in the Dipterocarpaceae family, which is distributed as major species in emergent canopy of Lambir Forest and Sarawak lowland dipterocarps forest. The genus is very unique, as there are only seven species available in the whole world, which confined to the tropical forests of West Malesia. The chemical constituents of Dipterocarpaceae are reported to possess various biological activities such as cytotoxicity, antiviral, antibacterial and anti-inflammatory activities. The aims of this study are to isolate secondary metabolites, to determine their antibacterial, DPPH scavenging and cytotoxic activities, to study structure-activity relationship, and to propose biogenesis pathway and chemotaxonomic significance in *Dryobalanops*. The dried powder of the stem bark of *D. aromatica*, *D. lanceolata*, *D. rappa* and *D. becarii* were macerated with acetone and evaporated under reduced pressure. The crude acetone extract was subjected to vacuum liquid chromatography to give several fractions. Purification of fraction with combination of several chromatography techniques gave four new oligostilbenoid derivatives; malaysianol A (1), B (2), C (3) and D (4), and a new galloylglucoside derivative; malaysin A (5), together with 15 known oligostilbenoid (6-20) and six known non-oligomeric compounds (21-26). The chemical structures of isolated compounds were elucidated based on the spectroscopic data evidences and comparison with reported authentic data. Biogenetically, the biosynthesis routes of non-oligomeric compounds were formed from the shikimate pathway, while oligomeric compounds were from the combination of shikimate and acetate malonate pathways. Based on the radical species and their condensation types, 19 oligostilbenoids isolated from this study were formed from the oxidative coupling reaction of two radicals with active site at carbons C-8 and C-14 (C8-C14 type), carbons C-8 and C-8 (C8-C8 type), carbons C-3 and C-8 (C3-C8 type), and oxygen O-13 and carbon C-8 (C7-C14 type). The finding of oligostilbenoids with the condensation types C3-C8 and C7-C14 are not commonly found in Dipterocarpaceae family. Based on the chemotaxonomic study, the presence of several compounds that were only found in the tribe Dipterocarpeae and never reported in the tribe Shoreae supported the previous studies on the morphological character that suggested the placement of *Dryobalanops* under the tribe Dipterocarpeae. In the antibacterial assay, flexuosol A (16) and upunaphenol D (18) showed moderately antibacterial activity against *S. epidermidis*, *S. aureus*, *S. xylosus* with MIC value of 50.0/16.7, 66.7/33.3 and 50.0/16.7 μM , respectively. In the cytotoxic assay, vaticanol C (20) were found to be moderately active against A549 cell line (IC_{50} 11.8 μM), as well as α -viniferin (11) and ampelopsin E (12) against MCF-7 cell line (IC_{50} 23.1 and 21.0 μM , respectively), while other compounds were either weak or not active. In the DPPH assay, malaysianol A (1), flexuosol A (16) and vaticanol B (19) displayed great scavenging activity with IC_{50} values 15.7, 15.0 and 11.8 μM , respectively. In the structure-activity relationship study, the scavenging activity of oligostilbenoid depend on the number of hydroxyl group and their stereochemistry, otherwise no definitive correlation between unit structures of oligostilbenoid and cytotoxicity was observed, but its conformation seem to be responsible for the cytotoxic properties.

ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious and the Most Merciful

Alhamdulillah, all praises to Allah for the strengths and His blessing in completing this thesis. I wish to express my sincere appreciation to those who have contributed to this thesis and supported me in one way or the other during this amazing journey.

First and foremost I want to thank my advisor Assoc. Prof. Dr. Norizan Ahmat. It has been an honor to be her first Ph.D. student. She has taught me, both consciously and un-consciously, how good experimental chemist is done. I appreciate her time, ideas, and funding to make my Ph.D. experience productive and stimulating. The joy and enthusiasm she has for her research was contagious and motivational for me, even during tough times in the Ph.D. pursuit. Not forgotten, my appreciation to my co-supervisor, Prof. Dr. Ahmad Sazali Hamzah for his support and knowledge.

I would like to thank the rest of my thesis committee: Prof. Dr Norashikin Saim (chairman), Prof. Datin Dr Rohaya Ahmat, Prof. Dr Khozirah Shaari, and Prof. Emeritus Dr Geoffrey A. Cordell, for their encouragement and insightful comments to improve of this thesis.

A very special thanks goes out to Dr. Haryoto and Dr. Muhtadi, without whose motivation and encouragement I would not have considered Natural Product Chemistry research as my graduate career.

I would like to thank administrative and technical staff members of the Faculty Applied Sciences who have been kind enough to advise and help in their respective roles. I thank Mr Ahmat Khambali, staff at Postgraduate Laboratory A409 for making life fun while working. Assoc. Prof. Dr. Mohd Rozi Ahmad who helped me in the first admission at Universiti Teknologi MARA. Encik Hisyam Abdul Hamid and Puan Noreen Hussein (Faculty Pharmacy, UiTM, 2013) and Assoc. Prof. Dr Latifah Saiful Yazan (Faculty Medic, UPM, 2014) who recruited me as a research assistant in their projects.

I would also like to thank my group member, Jamilul Nahry who helped me a lot in the submission of my thesis, Adila Sahida Sufian for the cytotoxicity assay, Anis Low Mohd Low for the antibacterial assay, and all my friends who have been supportive, and encouraging during the last four years and were always there to listen – there are too many to list but I hope you know who you are.

Lastly, I would like to thank my family and my host sister Noor Wini Mazlan for all their love and encouragement. For my parents, who raised me with a love of science and supported me in all my pursuits. Most of all, for my loving, supportive, encouraging and patient wife, Anita Parewang, whose faithful support during the final stages of this work are very much appreciated. Thank you.