



UNIVERSITI PUTRA MALAYSIA

**BIOASSAY-GUIDED IDENTIFICATION OF ANTI-CHOLINESTERASE AND
ICHTHYOTOXIC COMPOUNDS FROM *WALSURA CHRYSOGYNE* (MIQ.)
BAKH.F.APUD VAN STEENIS**

ILYA IRYANI MAHMOD

FS 2012 9

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**MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA**

2012

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AND ICHTHYOTOXIC COMPOUNDS FROM *WALSURA*
CHRYSOGYNE (MIQ.) BAKH.F.APUD VAN STEENIS**

By

ILYA IRYANI MAHMOD

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirements for the Degree of Master of Science**

September 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment
of the requirement for the degree of Master of Science

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ILYA IRYANI MAHMUD

September 2012

Chairman : Intan Safinar Ismail, PhD

Faculty : Faculty of Science

Phytochemical studies on *Walsura chrysogyne* leaves and barks have resulted in the isolation of ten compounds including two new compounds which namely chrysura (**39**) and 20*R*,24*S*-epoxy-25-hydroxydammaran-3-one (**43**). The other eight compounds are cycloart-23-ene-3 α , 25-diol (**35**), dymalol (**36**), 25-methoxycycloart-23-en-3 β -ol (**37**), eichlerianic acid (**38**), foveolin A (**40**), viridiflorol (**41**), cabraleadiol (**42**) and methyl eichlerianate (**44**). The structures of the isolated compounds were elucidated using spectroscopic techniques including Nuclear Magnetic Resonance (NMR), Mass Spectrometry (MS), Fourier Transform Infrared (FT-IR), *X-ray* crystallography and by comparison with the reported data from literatures.

Based on a bioautographic Thin Layer Chromatography (TLC) profile of anti-acetylcholinesterase activity and ichthyotoxic assay, ethyl acetate fraction from leaves and hexane fraction from bark were further fractionated by several chromatographic techniques. The EtOAc-soluble part from leaves, which gave the strongest activity in ichthyotoxicity and TLC bioautography of acetylcholinesterase enzyme assay, was further fractionated and resulted in purification of six triterpenes (**35**, **36**, **37**, **38**, **39** and **40**). Repeated chromatography of the hexane bark fraction on normal phase silica gel led to the purification of a sesquiterpene (**41**) and three triterpenes (**42**, **43**, and **44**).

All of the isolated compounds from leaves and barks, except for **35** and **37** which amounts are too small, were then subjected to ichthyotoxic assay at a concentration of 40 ppm each. Only eichlerianic acid (**38**) and viridiflorol (**41**) exhibited strong toxicity against zebrafish (*Danio rerio*). The median tolerance limit ($TL_M = IC_{50}$) for **39** and **41** was evaluated by straight line graphical interpolation and resulted in the values of 6.7 and 15 ppm respectively. Compound **36**, **38**, **39** and **41** showed positive result on TLC-bioautography with fast blue B salt. However, the obtained IC_{50} for them by using Ellman's method was not significant ($>200 \mu\text{g/ml}$).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai
memenuhi keperluan untuk ijazah Master Sains

**PENGENALPASTIAN SEBATIAN BERPANDUKAN BIOCERAKINAN
BERDASARKAN ANTI-CHOLINESTERASE DAN ICHTHYOTOXIC
DARIPADA *WALSURA CHRYSOGYNE* (MIQ.) BAKH.F.APUD VAN
STEENIS**

Oleh

ILYA IRYANI MAHMOD

September 2012

Pengerusi : Intan Safinar Ismail, PhD

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Kajian kimia terhadap daun dan kulit batang *Walsura chrysogyne* telah menghasilkan sepuluh sebatian termasuk dua sebatian baru yang dinamakan chrysura (**39**) 20*R*,24*S*-epoxy-25-hydroxydammaran-3-one (**43**). Lapan sebatian lain ialah cycloart-23-ene-3 β , 25-diol (**35**), dymalol (**36**), 25-methoxycycloart-23-en-3 β -ol (**37**), eichlerianic acid (**38**), foveolin A (**40**), viridiflorol (**41**), cabraleadiol (**42**) dan methyl eichlerianate (**44**). Struktur sebatian yang diasingkan telah dikenalpasti dengan menggunakan teknik spektroskopik termasuk, spektroskopi resonans magnet nukleus (NMR), spektrometri jisim (MS), spektroskopi inframerah (FT-IR), *X-ray* kristalografi dan berdasarkan kepada perbandingan dengan penemuan data yang terdahulu.

Berdasarkan profil bioautografik kromatografi lapisan nipis (TLC) bagi aktiviti perencatan acetylcholinesterase dan ichthyotoxic, pecahan etil asetat daripada daun dan pecahan heksana daripada kulit batang dipisahkan seterusnya oleh beberapa teknik kromatografi. Bahagian etil asetat daripada daun yang memberikan aktiviti tertinggi dalam ichthyotoxic dan bioautografi TLC, enzim acetylcholinesterase telah dipisahkan dan telah menghasilkan enam triterpena (**39**, **40**, **41**, **42**, **43** and **44**). Kromatografi diulangi dengan menggunakan fasa gel silica normal pada pecahan heksana kulit batang, membawa kepada penulenan sesquiterpena (**41**) dan tiga triterpena (**42**, **43**, and **44**).

Semua sebatian yang diasingkan daripada daun dan kulit batang, kecuali sebatian **35** dan **37** yang jumlahnya terlalu sedikit, telah diuji dengan ichthyotoxic pada kepekatan 40 ppm setiap satu. Hanya eichlerianic acid (**38**) dan viridiflorol (**41**) menunjukkan ketoksikan yang tinggi terhadap ikan zebra. Had toleransi median ($TL_M = IC_{50}$) untuk **38** dan **41** telah dinilai dengan *Danio rerio* interpolasi garis lurus grafik dan masing-masing memberikan nilai TL_M 6.7 dan 15 ppm. Sebatian **36**, **38**, **39** dan **41** menunjukkan keputusan positif pada bioautografi-TLC kromatografi lapisan dengan garam Fast Blue B. Walau bagaimanapun, IC_{50} yang diperolehi dengan cara Ellman adalah tidak ketara ($>200 \mu\text{g/ml}$).

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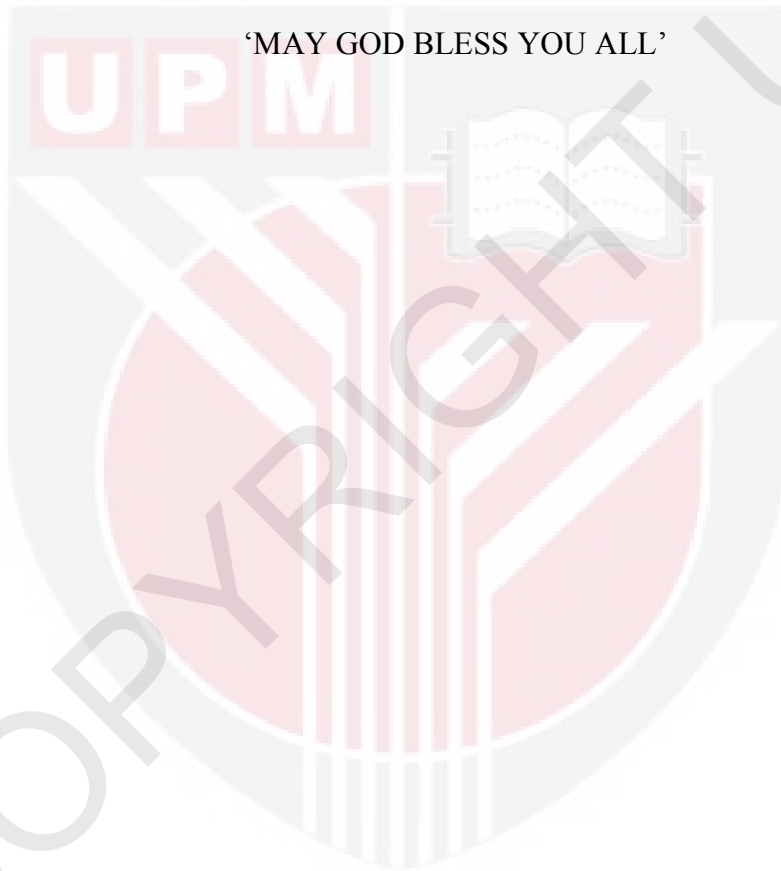
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I certify that an Examination Committee has meet on 27th September 2012 to conduct the final examination of Ilya Iryani Mahmod on her Master of Science thesis entitled 'Bioassay-Guided Identification of Anti-Cholinesterase and Ichthyotoxicity of *Walsura chrysogyne*' in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Master of Science.

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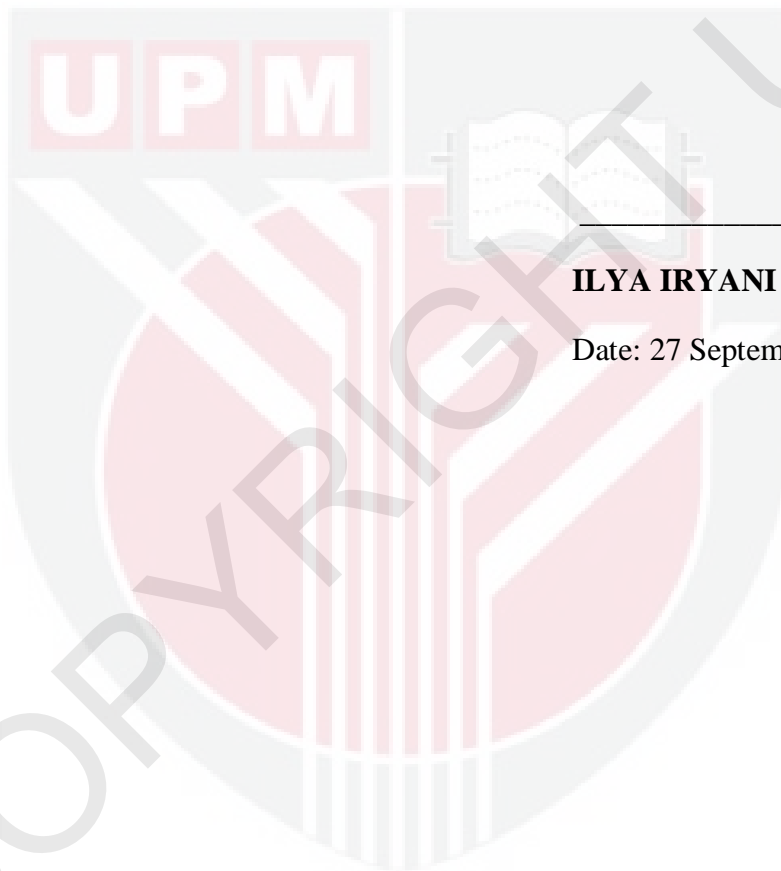
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DECLARATION

I declare that the thesis is on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



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Date: 27 September 2012

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