UNIVERSITI PUTRA MALAYSIA

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

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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

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

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

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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-3α, 25-diol (**35**), dymalol (**36**), 25-methoxycycloart-23-en-3β-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 µg/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

Fakulti : Fakulti Sains

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-3β-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 dinilaikan 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 biautografi-TLC kromatografi lapisan dengan garam Fast Blue B. Walau bagaimanapun, IC_{50} yang diperolehi dengan cara Ellman adalah tidak ketara (>200 µg/ml).

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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.

Members of the Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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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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ILYA IRYANI MAHMOD

Date: 27 September 2012

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