UNIVERSITI PUTRA MALAYSIA

ISOLATION, CHARACTERIZATION AND CYTOTOXICITY OF PHYTOCHEMICALS FROM SEKOBANG KECHIL (*ANAXAGOREA JAVANICA*)

SITI MARIAM BTE ZAKARIA

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ISOLATION, CHARACTERIZATION AND CYTOTOXICITY OF PHYTOCHEMICALS FROM SEKOBANG KECHIL (*ANAXAGOREA JAVANICA*)

By

SITI MARIAM BTE ZAKARIA

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

August 2009

DEDICATION

This thesis is dedicated to my beloved family

My father, Zakaria Bin Daud

My mother, Rose Bte Abdullah

My siblings
Mohd. Taufiq
Muhammad Hidhir
Ahmad Aqbar
Ahmad Yusran
Tsuraiya

Also to
Md Razak Bin Salleh
Asmah Bte Hassan

In loving memory of
My departed sister, Hidayah

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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Chairman : Professor Md Nordin Hj. Lajis, PhD

Institute : Bioscience

Thirty-one plant extracts were obtained from the extract bank of Natural Product Laboratory, Institute of Bioscience, Universiti Putra Malaysia. The whole plant extracts were tested for cytotoxic effect on human breast cancer (MCF-7), prostate cancer (DU-145) and lung cancer (H-460) cell lines using MTT assay. The results of the preliminary cytotoxicity tests showed that eight extracts exhibited very strong activity against one or more of the cell lines at 100 μg ml^{-1}, with cell viability of 10% or less. The methanolic extract of *Anaxagorea javanica* leaves exhibited the strongest activity against all three cell lines with cell viability of less than 2% and further dose-response tests against the MCF-7 cell line showed that it had an IC_{50} value of 2.4 μg ml^{-1}. This sample was thus selected and recollected in larger quantities for further phytochemical investigation.

The dichloromethane (DCM) fraction of the first collection was subjected to chromatographic purification from which a known flavonoid, 3',4',5-trihydroxy-3,7-dimethoxyflavone (**41**), was obtained. From the chromatographic separation of the DCM extract of the second collection batch, a mixture of long chain alkanes, predominated by nonacosane (**42**) and a mixture of stigmasterol (**43**) and β-sitosterol (**40**) were isolated, in addition to three pure phytochemicals, namely an aliphatic acid, hexadecanoic acid (**44**), and two alkaloids, 11-methoxyeupolauridine (**45**) and 4,11-dimethoxyeupolauridine (**46**). The latter two compounds were found to be new naphthyridine alkaloids with eupolauridine nuclei and reported for the first time for this species.

Compounds that had been obtained in sufficient quantities were tested for cytotoxic activity against the MCF-7 cell line. The samples assayed were 3',4',5-trihydroxy-3,7-dimethoxyflavone (**41**), nonacosane (**42**), stigmasterol (**43**) and β-sitosterol (**40**) mixture, and 11-methoxyeupolauridine (**45**). Only 3',4',5-trihydroxy-3,7-dimethoxyflavone (**41**) showed cytotoxic effect with an IC_{50} value of 3.4 μM, and this was its first report for this activity. Plausible biogenetic pathways of the new compounds were also discussed.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan Ijazah Sarjana Sains

PENGASINGAN, PENGENALPASTIAN DAN KESITOTOKSIKAN FITOKIMIA DARIPADA SEKOBANG KECHIL (*ANAXAGOREA JAVANICA*)

Oleh

SITI MARIAM BTE ZAKARIA

Ogos 2009

Pengerusi : Profesor Md Nordin Hj. Lajis, PhD

Institut : Biosains

Tiga puluh satu ekstrak pelbagai tumbuhan diperolehi daripada bank ekstrak di Laboratori Hasilan Semulajadi, Institut Biosains, Universiti Putra Malaysia. Keseluruhan ekstrak tumbuhan tersebut telah diuji aktiviti sitotosik menggunakan kaedah mikrotitratan (MTT) terhadap sel kanser payudara (MCF-7), kanser prostat (DU-145) dan kanser paru-paru (H-460). Kesimpulan daripada perbandingan hasil ujian saringan itu menunjukkan bahawa terdapat lapan ekstrak yang menunjukkan aktiviti yang tinggi terhadap satu atau lebih jenis titisan sel, dengan viabiliti sel sebanyak 10% atau kurang pada kepekatan 100 µg ml^{-1}. Ekstrak metanol daripada daun tumbuhan *Anaxagorea javanica* menunjukkan aktiviti yang sangat tinggi terhadap ketiga-tiga jenis sel dengan viabiliti sel kurang daripada 2% dalam ujian saringan. Seterusnya, ujian tindakbalas dos yang dijalankan terhadap sel MCF-7 menunjukkan aktiviti sitotoksik dengan IC_{50}

sebanyak 2.4 μg ml^{-1}. Sampel ini kemudian dipilih dan dikumpulkan dalam kuantiti yang lebih besar untuk penyelidikan fitokimia seterusnya.

Penulenan menggunakan kaedah kromatografi terhadap fraksi diklorometana daripada pengumpulan tumbuhan kali pertama telah menghasilkan sebatian flavonoid iaitu 3',4',5-trihidroksi-3,7-dimetoksiflavon (**41**). Pengasingan dan penulenan ekstrak diklorometana daripada pengumpulan tumbuhan kali kedua pula telah membawa kepada penemuan sebatian campuran alkana berantai panjang, didominasi oleh nonakosana (**42**), campuran stigmasterol (**43**) dan β-sitosterol (**40**), serta tiga fitokimia tulen, iaitu sebatian asid aliphatik, asid heksadekanoik (**44**), dan juga dua alkaloid, 11-metoksieupolauridina (**45**) dan 4,11-dimetoksieupolauridina (**46**) telah diperolehi. Kedua-dua sebatian alkaloid tersebut dikenalpasti buat kali pertama.

Sebatian yang telah diperolehi dalam kuantiti yang mencukupi telah diuji tahap aktiviti sitotoksikan terhadap sel MCF-7. Sampel yang diuji adalah 3',4',5-trihidroksi-3,7-dimetoksiflavon (**41**), nonakosana (**42**), campuran stigmasterol (**43**) dan β-sitosterol (**40**), dan 11-metoksieupolauridina (**45**). Hanya satu sebatian menunjukkan kesan sitotoksik iaitu 3',4',5-trihidroksi-3,7-dimetoksiflavon (**41**), dengan IC_{50} 3.4 μM. Ini adalah laporan pertama bagi kajian kesan sitotoksik (**41**) terhadap sel MCF-7. Cadangan tapak jalan biogenetik sebatian baru tersebut turut dibincangkan.

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have found in the Bioscience Postgraduate Club, thank you for the cooperation and motivation.

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To my parents, who have always supported me to pursue my interests and encouraged me through challenging moments, I owe a debt that I can never repay. I wish to thank my siblings for their continuous support and understanding, and my departed sister, who had always inspired me with her zest and ingenuity. The love that my family has provided transcends time and distance. To them, this thesis is dedicated.

I certify that a Thesis Examination Committee has met on 26 August 2009 to conduct the final examination of Siti Mariam Binte Zakaria on her thesis entitled "Isolation, Characterization and Cytotoxicity Evaluation of Phytochemicals from Sekobang Kechil (*Anaxagorea javanica*)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [(P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Master of Science.

Members of the Thesis Examination Committee were as follows:

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Date:

Saya mengesahkan bahawa satu Jawatankuasa Peperiksaan Tesis telah berjumpa pada 26 August 2009 untuk menjalankan peperiksaan akhir bagi Siti Mariam Binte Zakaria bagi menilai tesis beliau yang bertajuk "Pengasingan, Pengenalpastian Dan Kesitotoksikan Fitokimia Daripada Sekobang Kechil (*Anaxagorea javanica*)" mengikut Akta Universiti dan Kolej Universiti 1971 dan Perlembagaan Universiti Putra Malaysia [P.U.(A) 106] 15 Mac 1998. Jawatankuasa tersebut telah memperakukan bahawa calon ini layak dianugerahi Ijazah Sarjana Sains.

Ahli Jawatankuasa Peperiksaan Tesis adalah seperti berikut:

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Tarikh:

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 Supervisory Committee were as follow:

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Professor and Dean
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Date: 16 November 2009

DECLARATION

I hereby declare that the thesis is based on my original work except for quotation and citations which have been duly acknowledged. I also decree that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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SITI MARIAM BINTE ZAKARIA

Date: 18 January 2010

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LIST OF ABBREVIATIONS

δ	Chemical shift in ppm
°C	Degree in Celsius
^{13}C	Carbon-13
^{1}H	Proton
Acetone-d_6	Deuterated acetone
br	Broad
BuOH	Butanol
CC	Column chromatography
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
d	Doublet
dd	Doublet of doublets
ddd	Doublet of doublets of doublets
DCM	Dichloromethane
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethylsulfoxide
DMSO-d_6	Deuterated dimethylsulfoxide
DNP	Dictionary of Natural Products
DPPH	1,1-diphenyl-2-picrylhydrazyl
EIMS	Electron Impact Mass Spectrum
ESI	Electro-Spray Ionization
EtOAc	Ethyl acetate
eV	Electron volt
FTIR	Fourier Transform Infrared
GC-MS	Gas Chromatography-Mass Spectrometry
gHMBC	Gradient Heteronuclear Multiple Bond Correlation
gHSQC	Gradient Heteronuclear Single-Quantum Coherence
gCOSY	Gradient Correlation Spectroscopy
HPLC	High Performance Liquid Chromatography

Hz	Hertz
IC	Inhibition concentration
i.d.	Internal diameter of chromatographic column
IR	Infrared
J	Coupling in Hz
LHS	Laboratori Hasilan Semulajadi
Lit.	Literature
m	Multiplet
M	Molar
m/z	Mass per charge
MeOH	Methanol
MHz	MegaHertz
mp	Melting point
MS	Mass Spectrum/ Mass Spectrometry
nm	Nanometer
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Enhancement Spectroscopy
PTLC	Preparative Thin Layer Chromatography
s	Singlet
t	Triplet
THMF	3,5,7,4'-tetrahydroxy-2'-methoxyflavone
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
UV	Ultraviolet
UV-VIS	Ultraviolet-visible