UNIVERSITI PUTRA MALAYSIA

ISOLATION AND BIOACTIVITY OF CHEMICAL CONSTITUENTS FROM ZINGIBER CASSUMUNAR ROXB. AND AGLAIA OLIGOPHYLLA MIQ.

MOHD ZULKHAIRI BIN AZID

FS 2012 52

ISOLATION AND BIOACTIVITY OF CHEMICAL CONSTITUENTS FROM *ZINGIBER CASSUMUNAR* ROXB. AND *AGLAIA OLIGOPHYLLA* MIQ.

MOHD ZULKHAIRI BIN AZID

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2012

ISOLATION AND BIOACTIVITY OF CHEMICAL CONSTITUENTS FROM *ZINGIBER CASSUMUNAR* ROXB. AND *AGLAIA OLIGOPHYLLA* MIQ.

By

MOHD ZULKHAIRI BIN AZID

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

May 2012

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

ISOLATION AND BIOACTIVITY OF CHEMICAL CONSTITUENTS FROM *ZINGIBER CASSUMUNAR* ROXB. AND *AGLAIA OLIGOPHYLLA MIQ.*

By

MOHD ZULKHAIRI BIN AZID

May 2012

Chairman : Professor Mohd Aspollah bin Sukari, PhD

Faculty : Science

Zingiber cassumunar Roxb. (Zingiberaceae) or locally known as "Bonglai" among Malay is traditionally used as medicines to treat, skin disease, inflammation and as one of the component in herbal spices. From the isolation worked on the rhizomes of *Zingiber cassumunar*, five compounds isolated were elucidated as *cis*-3-(3',4'-dimethoxyphenyl)-4-[(*E*)-3'",4'"-dimethoxystyryl]cyclo-hex-1-ene **(21)**, (*E*)-4-(3',4'-dimethoxyphenyl)but-3-en-1-ol **(12)**, 3,4-dimethoxybenzoic acid **(29)**, 8-(13,14-dimethoxyphenyl)-2-methoxynaphto-1,4-quinone **(20)**, and beta-sitosterol **(30)**.

Aglaia oligophylla Miq. belongs to the Meliaceae family which is a large tree up to 25 meter high and locally is known as "Bekak". *Aglaia* species have received a lot of attention lately due to its strong insecticidal and cytotoxic activity. Phytochemical studies on stem bark of *Aglaia oligophylla* has afforded dammarane acid type of compounds, namely 20*S*,24*R*-epoxy-25-hydroxy-2-methoxy-2,3-secodammarane-3-oic

acid **(77)** and 20*S*,24*S*-epoxy-25-hydroxy-2-methoxy-2,3-secodammarane-3-oic acid **(78)** while separation work from the trunk of *Aglaia oligophylla* successfully isolated one compound identified as silvaglin A **(47)** along with beta-sitosterol **(30)**. Compounds **(77)** and **(78)** have never been reported previously, while silvaglin A **(47)** was isolated for first time from *Aglaia oligophyalla*. Structure of the compounds were elucidated using various spectroscopic techniques such as GCMS, HRESIMS, FT-IR, 1D NMR and 2D NMR and comparison with the previous worked.

In vitro investigation on the cytotoxic activitiy of isolates of both plants have been carried out towards human T-lymphoblastic (CEM-SS) and human cervical (HeLa) cancer cells. All the extracts of rhizomes *Zingiber cassumunar* showed no activity towards (CEM-SS) with IC_{50} values of > 30 µg/ml except for chloroform extract, which displayed IC_{50} value of 9.20 ± 0.02 µg/ml. Compounds **(20)** and **(21)** also exhibited moderate cytotoxic activity against CEM-SS cells with IC_{50} values 25.96 ± 0.94 and 28.34 ± 0.39 µg/ml, respectively. Meanwhile, all crude extracts from *Zingiber cassumunar* displayed no cytotoxicity activity against HeLa cells. However, all compounds isolated showed significant cytotoxic activity against HeLa cell line with IC_{50} values < 15 µg/ml. Most of the crude extracts from stem bark of *Aglaia oligophylla* showed no cytotoxic activity towards CEM-SS cells except for methanol extract with IC_{50} value 22.76 ± 0.08 µg/ml. All extracts from trunk also did not give any activity towards CEM-SS cells. Meanwhile, petroleum ether, chloroform and ethyl acetate extracts from stem bark of *Aglaia oligophylla* showed moderate cytotoxic activity with IC_{50} value less than 15 µg/ml against HeLa cells whilst methanol extract showed IC_{50}

value of 22.93 ± 0.38. However, all extracts from trunk exhibit no cytotoxicity towards HeLa cells. Surprisingly, compounds (**47)**, **(77)** and **(78)** showed interesting cytotoxic activity towards HeLa cancer cell line with IC_{50} value less than 15 μg/ml.

Apart from the above activity, antimicrobial assay were also carried out on the isolates of the plants. Only certain extracts from *Zingiber cassumunar* and *Aglaia oligophylla* exhibit weak inhibition towards selected microbes and fungi while the rest were not active. Meanwhile, all extracts from both plants did not show any activity towards larvae of *Aedes aegypti*.

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

PENGASINGAN DAN AKTIVITI BIOLOGI KOMPONEN KIMIA DARIPADA *ZINGIBER CASSUMUNAR* ROXB. DAN *AGLAIA OLIGOPHYLLA* MIQ.

Oleh

MOHD ZULKHAIRI BIN AZID

Mei 2012

Pengerusi : Profesor Mohd Aspollah bin Sukari, PhD

Fakulti : Sains

Zingiber cassumunar (Zingiberaceae) Roxb. atau lebih dikenali sebagai "Bonglai" oleh masyarakat Melayu telah digunakan secara tradisional sebagai ubat untuk merawat penyakit kulit, keradangan dan salah satu komponen di dalam ramuan herba. Daripada kajian pengasingan ke atas rizom *Zingiber cassumunar*, 5 sebatian telah berjaya diasingkan yang dikenali sebagai *cis*-3-(3',4'-dimetoksifenil)-4-[(*E*)-3''',4'''-dimetoksistiril]siklo-hek-1-ena **(21)**, (*E*)-4-(3',4'-dimetoksifenil)but-3-en-1-ol **(12)**, asid 3,4-dimetoksibenzoik **(29)**, 8-(13,14-dimetoksifenill)-2-metoksinafto-1,4-kuinon **(20)**, dan beta-sitosterol **(30)**.

Aglaia oligophylla Miq. tergolong di dalam famili Meliaceae dan merupakan sebuah pokok besar yang boleh mencapai sehingga 25 meter tinggi dan dikenali sebagai "Bekak" dikalangan penduduk tempatan. Kebelakangan ini, spesies *Aglaia* telah mendapat banyak perhatian berikutan kekuatan aktiviti dari segi sifat sitotoksik dan

insektisidal spesies tersebut. Kajian fitokimia terhadap kulit pokok *Aglaia oligophylla* telah berjaya mengasingkan dua sebatian jenis asid damaran yang dinamakan sebagai asid 20*S*,24*R*-epoksi-25-hidroksi-2-metoksi-2,3-sekodammaran-3-oik **(77)** dan asid 20*S*,24*S*-epoksi-25-hidroksi-2-metoksi-2,3-sekodammaran-3-oik **(78)** dan manakala pengasingan daripada batang pokok pula telah berjaya memencilkan satu sebatian dinamakan sebagai silvaglin A **(47)** dan beta-sitosterol **(30)**. Sebatian **(77)** dan **(78)** belum pernah dilaporkan sebelum ini, manakala silvaglin A **(47)** pertama kali diasingkan daripada *Aglaia oligophylla*. Struktur sebatian telah dielusidasi menggunakan pelbagai teknik spektroskopi seperti GCMS, HRESIMS, FT-IR, 1D NMR dan 2D NMR serta perbandingan dengan kajian sebelum ini.

Kajian *in vitro* terhadap aktiviti sitotoksik daripada kedua-dua pokok tersebut telah dijalankan terhadap sel kanser manusia iaitu T-limfoblastik (CEM-SS) dan sel kanser serviks (HeLa). Kesemua ekstrak daripada rizom *Zingiber cassumunar* tidak menunjukkan aktiviti terhadap sel CEM-SS dengan nilai IC_{50} > 30 μg/ml kecuali ekstrak kloroform yang menunjukkan nilai IC_{50} 9.20 ± 0.02 μg/ml. Sebatian **(20)** dan **(21)** juga menunjukkan aktiviti sitotoksik yang sederhana terhadap sel CEM-SS dengan nilai IC_{50} 25.96 ± 0.94 dan 28.34 ± 0.39 μg/ml. Manakala, kesemua ekstrak mentah daripada rizom *Zingiber cassumunar* tidak menunjukkan aktiviti sitotoksik terhadap sel HeLa. Walaubagaimana pun, kesemua sebatian menunjukkan aktiviti sitotoksik yang signifikan terhadap sel HeLa dengan nilai IC_{50} < 15 μg/ml. Hampir kesemua ekstrak daripada kulit pokok *Aglaia oligophylla* tidak menunjukkan aktiviti sitotoksik terhadap sel kanser CEM-SS kecuali ekstrak metanol dengan nilai IC_{50} 22.76 ± 0.08 μg/ml. Kesemua

ekstrak daripada batang pokok juga tidak memberikan sebarang aktiviti terhadap sel CEM-SS. Manakala, ekstrak petroleum eter, kloroform dan etil asetat dari kulit pokok *Aglaia oligophylla* menunjukkan aktiviti sitotoksik yang sederhana dengan nilai IC_{50} kurang daripada 15 µg/ml terhadap sel Hela, manakala ekstrak metanol menunjukkan nilai $1C_{50}$ 22.93 ± 0.38. Manakala, kesemua ekstrak daripada batang pokok tidak menunjukkan aktiviti sitotoksik terhadap sel HeLa. Akan tetapi, sebatian **(47)**, **(77)** dan **(78)** menunjukkan aktiviti sitotoksik yang menarik terhadap sel kanser HeLa dengan nilai IC_{50} kurang daripada 15 µg/ml.

Selain daripada aktiviti di atas, kajian antimicrobial juga telah dijalankan terhadap isolat dari kedua-dua tumbuhan. Hanya sebahagian ekstrak daripada *Zingiber cassumunar* dan *Aglaia oligophylla* menunjukkan perencatan yang lemah terhadap mikrob dan fungi terpilih sedangkan yang lain adalah tidak aktif. Manakala, kesemua ekstrak dari kedua-dua pokok tidak menunjukkan sebarang aktiviti terhadap larva *Aedes aegypti*.

ACKNOWLEDGEMENT

In the name of Allah , the Most Gracious and Merciful

Alhamdulilah, all praises to Allah for the strengths and His blessing in completing this thesis. First and foremost, I offer my sincerest gratitude to my supervisor, Prof. Dr. Mohd Aspollah Sukari, who has supported me throughout my research with his patience, knowledge, financial assistant and also providing the opportunities to attend various seminars and conference throughout this research. Without him this thesis, would not have been completed or written. I would like to record my gratitude to my co-supervisors, Prof. Dr. Gwendoline Ee Cheng Lian and Dr. Ahmad Bustamam for their valuable advices during this research.

Many thanks to staff in UPM-MAKNA Cancer Research Laboratory for their assistance and providing the facilities in anticancer testing. Besides, assistance from Prof. Dr. Mohd Ambar Yarmo (UKM), Mr. Alefee (UKM) and Dr Nor Azah Mohamad Ali (FRIM) in obtaining experimental data is highly appreciated.

I would like to express my appreciation to Science officers and laboratory assistance Mr. Johadi Iskandar, Ms. Sharina, Mr. Fazley, Madam Rusnani Amirudin, Mr Zainal Kassim, Mr Ismail Yasin and Madam Rakina bt Manaf for their help and co-operation.

I would like to thank Ministry of Science, Technology and Innovation (MOSTI) for awarding me National Science Fellowship (NSF) during my study. I am grateful to be chosen as one of the recipient in the programme.

Sincere thanks to all my labmates, Dr Tang Sook Wah, Rahayu Utami Umar, Noorul Adawiyah Mustahil, Halimatul Saadiah Mohd Noor, Sadikah Ahmad and Nur Liyana Ithnin for their support and motivation. Thanks for the memories and the friendships.

Last but not least, I owe my deepest gratitude to my beloved parents, Mr Azid Kassim and Siti Zaleha Tugiman and also to my sister and brother for their endless love, support and prayers. To those who indirectly contributed in this research, your kindness means a lot to me. Thank you very much.

APPROVAL

I certify that an Examination Committee has met on **24th May 2012** to conduct the final examination of **Mohd Zulkhairi Bin Azid** on his **Master of Science** thesis entitled **Isolation and Bioactivity of Chemical Constituents from *Zingiber cassumunar* ROXB. and *Aglaia oligophylla* MIQ**. 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 (Name of relevant degree).

Members of the Examination Committee as were as follows:

Mohamad Zaki Ab Rahman, PhD
Professor
Centre of Foundation Studies for Agricultural Science
Universiti Putra Malaysia
(Chairman)

Mawardi Rahmani, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Siti Mariam Mohd Nor, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Nor Hadiani Ismail, PhD
Professor
School of Chemistry and Environmental Studies
Universiti Teknologi Mara, Puncak Alam, Shah Alam
Malaysia
(External Examiner)

_____
SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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

Mohd Aspollah Hj. Sukari, PhD
Professor
Faculty Of Science
Universiti Putra Malaysia
(Chairman)

Gwendoline Ee Cheng Lian, PhD
Professor
Faculty Of Science
Universiti Putra Malaysia
(Member)

Ahmad Bustamam Abdul, PhD
Associate Researcher
UPM-MAKNA Cancer Research Laboratory,
Institute of Bioscience
Universiti Putra Malaysia
(Member)

_____
BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

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

_____

MOHD ZULKHAIRI BIN AZID

Date: 24 May 2012

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

α	Alpha
β	Beta
δ	Chemical shift in ppm
^{13}C	Carbon-13
$CHCl_3$	Chloroform
^{o}C	Degree in Celcius
$CDCl_3$	Deuterated Chloroform
CD_3OD	Deuterated Methanol
COSY	Correlated spectroscopy
cm	Centimeter
J	Coupling constant in Hertz
d	Doublet
DEPT	Distortionless Enhancement by Polarisation Transfer
DMSO	Dimethylsulfoxide
EIMS	Electron Impact-Mass Spectroscopy
ESI-MS	Electrospray Ionization-Mass Spectroscopy
EtOAc	Ethyl acetate
G	Gram
GC	Gas-Chromatography
GC-MS	Gas-Chromatography-Mass-Spectroscopy
^{1}H	Proton
HMBC	Heteronuclear Multiple Bond Connectivity
HMQC	Heteronuclear Multiple Quantum Correlation
Hz	Hertz
OH	Hydroxy
IC	Inhibition Concentration
IR	Infrared
LC	Lethal Concentration
m/z	Mass per charge
MS	Mass Spectroscopy
MeOH	Methanol
OCH_3	Methoxy
m.p.	Melting point
ml	Mililiter
Mm	Milimeter
µg	Microgram
µM	Micromolar
mg	Microgram
M^+	Molecular ion
m	Multiplet
nm	Nanometer
NMR	Nuclear Magnetic Resonance

ppm	Part per million
KBr	Potassium Bromide
s	Singlet
t	Triplet
TLC	Thin Layer chromatography
WHO	World Health Organization

LIST OF APPENDICES

TABLE OF CONTENTS