



UNIVERSITI PUTRA MALAYSIA

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

MOHD ZULKHAIRI BIN AZID

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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

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in fulfillment of the requirement for the degree of Master of Science

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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 oligophylla*. 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 activity 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 IC₅₀ values of > 30 µg/ml except for chloroform extract, which displayed IC₅₀ value of 9.20 ± 0.02 µg/ml. Compounds (20) and (21) also exhibited moderate cytotoxic activity against CEM-SS cells with IC₅₀ 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 IC₅₀ 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 IC₅₀ 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 IC₅₀ value less than 15 µg/ml against HeLa cells whilst methanol extract showed IC₅₀

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 $15 \mu\text{g/ml}$.

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

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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-dimetoksifenil)-2-metoksinafto-1,4-kuinon (**20**), dan beta-sitosterol (**30**).

Aglaiia 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 *Aglaiia* 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 $IC_{50} > 30 \mu\text{g/ml}$ kecuali ekstrak kloroform yang menunjukkan nilai $IC_{50} 9.20 \pm 0.02 \mu\text{g/ml}$. Sebatian (**20**) dan (**21**) juga menunjukkan aktiviti sitotoksik yang sederhana terhadap sel CEM-SS dengan nilai $IC_{50} 25.96 \pm 0.94$ dan $28.34 \pm 0.39 \mu\text{g/ml}$. 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 $IC_{50} < 15 \mu\text{g/ml}$. Hampir kesemua ekstrak daripada kulit pokok *Aglaia oligophylla* tidak menunjukkan aktiviti sitotoksik terhadap sel kanser CEM-SS kecuali ekstrak metanol dengan nilai $IC_{50} 22.76 \pm 0.08 \mu\text{g/ml}$. 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 $\mu\text{g/ml}$ terhadap sel HeLa, manakala ekstrak metanol menunjukkan nilai IC_{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 $\mu\text{g/ml}$.

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*.

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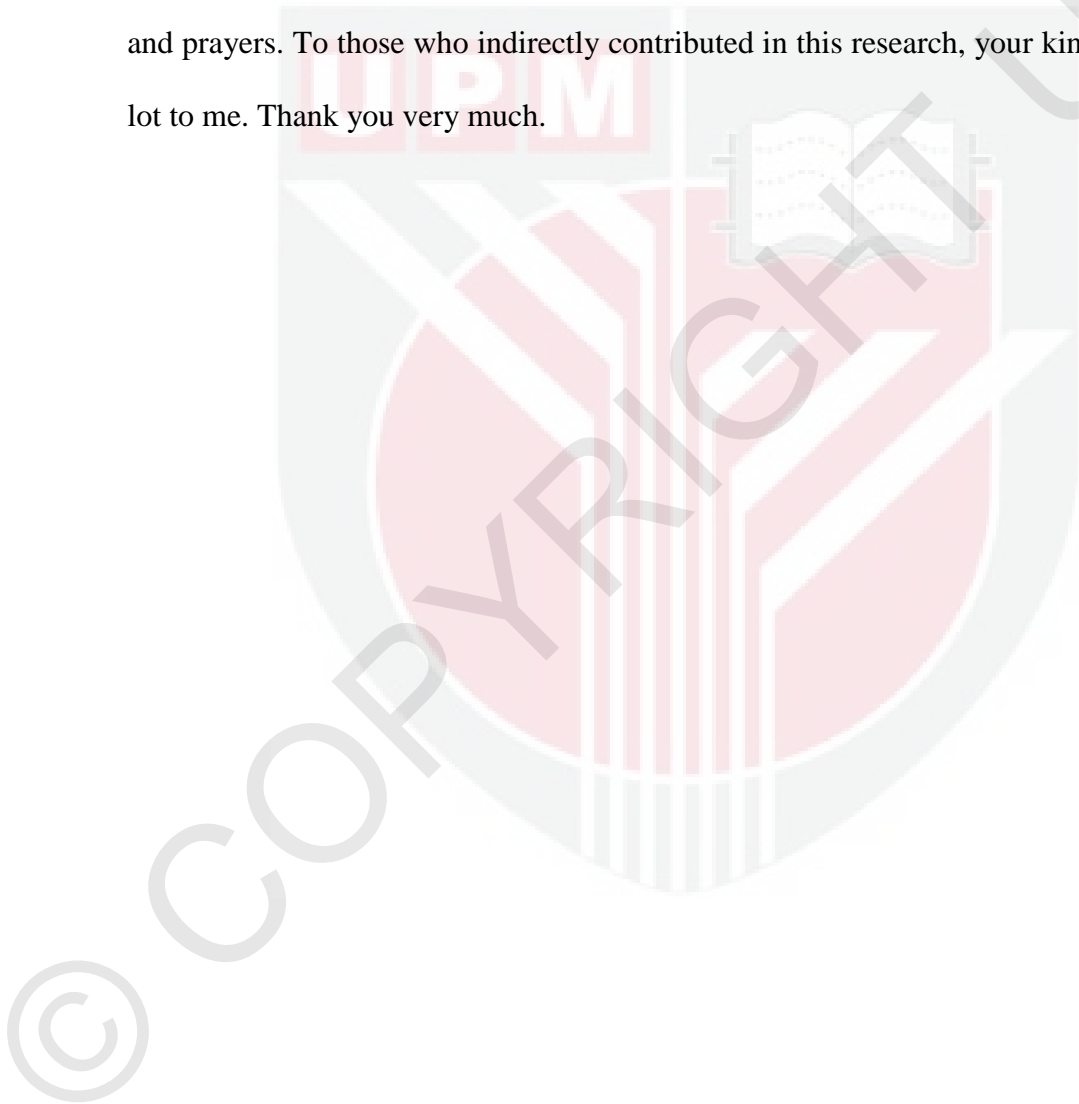
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APPROVAL

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).

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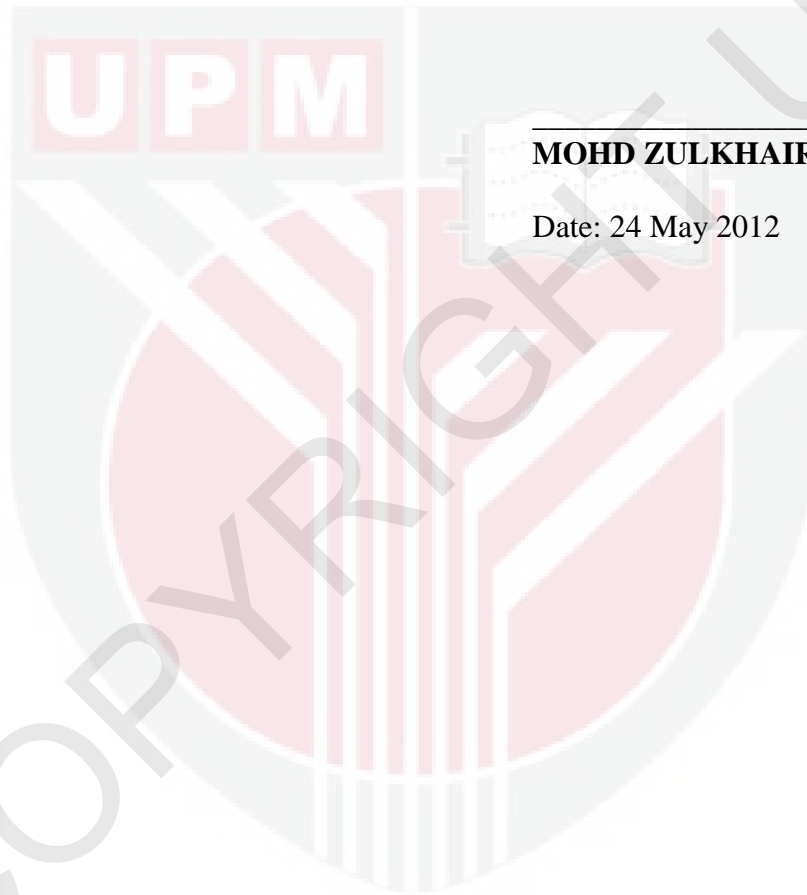
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DECLARATION

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



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LIST OF ABBREVIATIONS

| | |
|------------------------|---|
| α | Alpha |
| β | Beta |
| δ | Chemical shift in ppm |
| ^{13}C | Carbon-13 |
| CHCl_3 | Chloroform |
| $^\circ\text{C}$ | 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 |
| ^1H | 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 |
| <i>s</i> | Singlet |
| <i>t</i> | Triplet |
| TLC | Thin Layer chromatography |
| WHO | World Health Organization |



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