



UNIVERSITI PUTRA MALAYSIA

**PHYTOCHEMICALS FROM GARCINIA, MESUA AND JATROPHA
SPECIES AND THEIR BIOLOGICAL ACTIVITIES**

LIM CHAN KIANG.

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**PHYTOCHEMICALS FROM *GARCINIA*, *MESUA* AND
JATROPHA SPECIES AND THEIR BIOLOGICAL ACTIVITIES**

By

LIM CHAN KIANG

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
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Chairman : Associate Professor Gwendoline Ee Cheng Lian, PhD

Faculty : Science

Extensive studies on 5 plants, *Garcinia penangiana*, *Garcinia nitida*, *Mesua daphnifolia*, *Mesua beccariana* and *Jatropha podagrica* have resulted in the isolation of twenty one compounds. Out of these compounds two are new. All these compounds were isolated by means of chromatographic method and their structures derived on the basis of spectroscopic evidence, mainly 1D and 2D NMR spectroscopy.

Chemical investigations on the stem bark extracts of *Garcinia penangiana* yielded a flavonoid, catechin (86) and a steroidal triterpene, stigmasterol (85) whereas the stem bark extracts of *Garcinia nitida* yielded two triterpenoids, stigmasterol (85) and stigmasterol acetate (87) plus a total of five xanthenes, inophyllin B (88), osajaxanthone (89), 1,3,7-trihydroxy-2,4-bis(3-methylbut-2-enyl)xanthone (90), rubraxanthone (91) and 3-isomangostin (92). Meanwhile, studies on the stem bark extracts of *Mesua daphnifolia* yielded three triterpenoids, friedelin (93), friedelan-



1,3-dione (94) and lup-20(29)-en-3 β -ol (96), three known xanthenes, ananixanthone (95), cudraxanthone G (97) and euxanthone (52) and a new xanthone, daphnifolin (98). On the other hand, the stem bark extracts of *Mesua beccariana* gave two triterpenoids, stigmasterol (85) and friedelin (93) and a phenylcoumarin, isocalanone (99). *Jatropha podagrica* afforded two triterpenoids, β -sitosterol (61) and acetylaleuritic acid (77), a coumarin, fraxidin (101), a new ferulic acid ester, n-heptyl ferulate (100) and sucrose (102).

Acetylation on rubraxanthone (91) gave two new compounds which were never reported before. These are rubraxanthone monoacetate (103) and rubraxanthone diacetate (104). The known rubraxanthone triacetate (105) was also synthesized.

Cytotoxic assay was performed using CEM-SS (T-lymphoblastic leukemia) cell line. All the crude extracts of *Garcinia penangiana* and *Jatropha podagrica*, the crude hexane extract of *Mesua beccariana* and the crude hexane and chloroform extracts of *Garcinia nitida* were found to show significant growth inhibitory activities with IC₅₀ values of less than 30 μ g/ml.

Cytotoxic assays were also carried out on the pure compounds towards the CEM-SS (T-lymphoblastic leukemia), HeLa (cervical carcinoma), MDA-MB-231 (human estrogen receptor negative breast cancer) and CaOV3 (human ovarian cancer) cell lines. Cudraxanthone G (97) and friedelan-1,3-dione (94) were found to show strong inhibitory activities toward the HeLa cell line with IC₅₀ values of 4.0 and 4.6 μ g/ml respectively. Both cudraxanthone G (97) and rubraxanthone (91) gave moderate inhibitory activities with IC₅₀ values of 6.7 and 9.4 μ g/ml respectively towards the

CEM-SS cell line. The MDA-MB-231 cell line was found to be very susceptible towards most of the prenylated xanthenes tested: cudraxanthone G (97) ($IC_{50} = 1.3 \mu\text{g/ml}$), inophyllin B (88) ($IC_{50} = 1.4 \mu\text{g/ml}$), 1,3,7-trihydroxy-2,4-bis(3-methylbut-2-enyl)xanthone (90) ($IC_{50} = 2.2 \mu\text{g/ml}$) and ananixantone (95) ($IC_{50} = 4.6 \mu\text{g/ml}$). Meanwhile, euxanthone (52) gave a moderate activity ($IC_{50} = 9.0 \mu\text{g/ml}$) towards the CaOV3 cell line. Most of the compounds tested indicated selective activity towards the cancer cell lines except for cudraxanthone G (97) which was found to have a broad spectrum of activities.

Antimicrobial assays were carried out towards four pathogenic bacteria: Methicillin Resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Bacillus subtilis*. Most of the crude extracts tested against these microbes gave only moderate or weak activities except for the ethyl acetate extract of *Jatropha podagrica*, which was strongly active against the microbe *Pseudomonas aeruginosa* with an inhibition width of 15 mm which is close to that of the standard, streptomycin sulphate (16 mm).

Larvicidal tests were carried out against the larvae of *Aedes aegypti*. The larvae were strongly susceptible to the hexane and chloroform extracts of *Mesua daphnifolia* with LC_{50} values of as low as 9.7 and 6.0 ppm respectively while the other crude extracts gave LC_{50} values of more than 60 ppm. The pure compound, rubraxanthone (91) indicated a strong larvicidal activity with LC_{50} value of 15.5 ppm.

Antifungal assays were also carried out on the crude extracts of *Mesua daphnifolia* towards the microbes, *Candida albican*, *Aspergillus ochraceaus*, *Sacchoromyces cerevisiae* and *Candida lypolytica*. All the crude extracts of *Mesua daphnifolia* exhibited moderate activities towards the microbe *Candida lypolytica* but they were not active against the other targeted microbes.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**SEBATIAN-SEBATIAN FITOKIMIA DARIPADA SPESIS-SPESIS
GARCINIA, *MESUA* DAN *JATROPHA* DAN
AKTIVITI-AKTIVITI BIOLOGI MASING-MASING**

Oleh

LIM CHAN KIANG

October 2005

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Kajian terperinci ke atas pokok-pokok *Garcinia penangiana*, *Garcinia nitida*, *Mesua daphnifolia*, *Mesua daphnifolia* dan *Jatropha podagrica* telah menghasilkan dua puluh satu sebatian-sebatian semulajadi. Semua sebatian ini telah dipisahkan dengan menggunakan kaedah kromatografi dan struktur masing-masing telah diterbitkan berdasarkan bukti-bukti spektroskopi, terutamanya melalui penggunaan spektroskopi jenis 1D dan 2D RMN.

Kajian secara kimia ke atas ekstrak-ekstrak mentah bagi kulit pokok *Garcinia penangiana* telah menghasilkan sebatian-sebatian semulajadi iaitu catechin (86) dan stigmasterol (85) manakala ekstrak-ekstrak mentah bagi kulit pokok *Garcinia nitida* telah menghasilkan stigmasterol (85), stigmasterol asetat (87), inophyllin B (88), osajaxanthone (89), 1,3,7-trihydroxy-2,4-bis(3-methylbut-2-enyl)xanthone (90), rubraxanthone (91) dan 3-isomangostin (92). Selain daripada itu, kajian juga telah dijalankan ke atas kulit pokok *Mesua daphnifolia* dan berjaya menghasilkan friedelin

(93), friedelan-1,3-dione (94) and lup-20(29)-en-3 β -ol (96), ananixanthone (95), cudraxanthone G (97), euxanthone (52) dan satu sebatian baru iaitu daphnifolin (98). Manakala kulit pokok *Mesua beccariana* telah menghasilkan stigmasterol (85), friedelin (93) dan isocalanone (99). Bagi pokok *Jatropha podagrica*, ia menghasilkan β -sitosterol (61), asid asetilaleuritolik (77), fraxidin (101), satu sebatian baru iaitu n-heptil ferulat (100) dan sukrosa (102).

Tindakbalas pengasetilan ke atas rubraxanthone (91) telah menghasilkan dua sebatian baru yang tidak pernah dilaporkan iaitu rubraxanthone monoasetat (103) dan rubraxanthone diasetat (104). Rubraxanthone triasetat (105) turut dihasilkan dalam tindakbalas kimia tersebut.

Ujian sitotoksik telah dijalankan dengan menggunakan sel CEM-SS. Kesemua ekstrak yang diperolehi daripada pokok-pokok *Garcinia penangiana* dan *Jatropha podagrica*, ekstrak heksana daripada *Mesua beccariana* dan juga ekstrak-ekstrak heksana dan kloroform daripada *Garcinia nitida* telah menunjukkan aktiviti yang ketara iaitu dengan nilai IC₅₀ masing-masing yang kurang daripada 30 μ g/ml.

Ujian sitotoksik juga telah dijalankan ke atas sebatian-sebatian semulajadi dengan menggunakan sel-sel CEM-SS, HeLa, MDA-MB-231 dan CaOV3. Cudraxanthone G (97) dan friedelan-1,3-dione (94) menunjukkan aktiviti penghalangan yang kuat terhadap sel HeLa dengan nilai IC₅₀ masing-masing iaitu 4.0 dan 4.6 μ g/ml. Kedua-dua sebatian, cudraxanthone G (97) dan rubraxanthone (91) menunjukkan aktiviti penghalangan yang sederhana iaitu 6.7 dan 9.4 μ g/ml terhadap sel CEM-SS

manakala sebatian-sebatian seperti cudraxanthone G (97) ($IC_{50} = 1.3 \mu\text{g/ml}$), inophyllin B (88) ($IC_{50} = 1.4 \mu\text{g/ml}$), 1,3,7-trihydroxy-2,4-bis(3-methylbut-2-enyl)xanthone (90) ($IC_{50} = 2.2 \mu\text{g/ml}$) dan ananixantone (95) ($IC_{50} = 4.6 \mu\text{g/ml}$) didapati menunjukkan aktiviti penghalangan yang kuat terhadap sel MDA-MB-231. Sementara itu, euxanthone (52) menunjukkan aktiviti yang sederhana iaitu dengan nilai IC_{50} , $9.0 \mu\text{g/ml}$ terhadap sel CaOV3. Kebanyakan sebatian-sebatian yang diuji telah menunjukkan aktiviti penghalangan secara selektif terhadap sel-sel kanser kecuali sebatian cudraxanthone G (97) yang didapati mempunyai aktiviti penghalangan yang pelbagai.

Ujian antimikrob telah dijalankan dengan menggunakan bakteria-bakteria jenis Methicillin Resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* dan *Bacillus subtilis*. Kebanyakan ekstrak yang diuji menunjukkan keaktifan yang sederhana atau rendah terhadap bakteria-bakteria tersebut kecuali ekstrak etil asetat daripada *Jatropha podagrica* telah menunjukkan keaktifan yang tinggi terhadap mikrob *Pseudomonas aeruginosa* dengan diameter penghalangan iaitu 15 mm yang hampir sama dengan nilai piawai, streptomycin sulfat iaitu 16 mm.

Ujian larva telah dijalankan dengan menggunakan larva jenis *Aedes aegypti*. Dalam ujian ini, ekstrak heksana dan ekstrak kloroform daripada *Mesua daphnifolia* telah diuji dan menunjukkan aktiviti yang kuat iaitu dengan nilai LC_{50} serendah 9.7 dan 6.0 ppm manakala ekstrak-ekstrak yang lain menunjukkan nilai LC_{50} lebih daripada 60 ppm. Sebatian rubraxanthone (91) menunjukkan aktiviti yang kuat iaitu dengan nilai LC_{50} 15.5 ppm.

Ujian antifungi telah dijalankan ke atas ekstrak-ekstrak daripada *Mesua daphnifolia* dengan menggunakan mikrob-mikrob jenis *Candida albican*, *Aspergillus ochraceaus*, *Sacchoromyces cerevisiae* dan *Candida lypolytica*. Semua ekstrak daripada *Mesua daphnifolia* menunjukkan aktiviti yang sederhana terhadap mikrob *Candida lypolytica* tetapi tidak menunjukkan aktiviti terhadap mikrob-mikrob lain.

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I certify that an Examination Committee met on 27th October 2005 to conduct the final examination of Lim Chan Kiang on his Doctor of Philosophy thesis entitled "Phytochemicals from *Garcinia*, *Mesua* and *Jatropha* Species and Their Biological Activities" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

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



LIM CHAN KIANG

Date: 30/10/2005

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