



UNIVERSITI PUTRA MALAYSIA

***CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF
CALOPHYLLUM INOPHYLLUM L. AND CALOPHYLLUM SOULATTRI
BURM. EX F. MULL.***

MAH SIAU HUI

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**DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA**

2012

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CALOPHYLLUM INOPHYLLUM L. AND *CALOPHYLLUM SOULATTRI* BURM.
EX F. MULL.**

By

MAH SIAU HUI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

July 2012

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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July 2012

Chair : Professor Gwendoline Ee Cheng Lian, PhD

Faculty : Science

A chemical investigation of the stem bark of *Calophyllum inophyllum* and *Calophyllum soulattri* was carried out using various chromatographic and recrystallization techniques. All of the secondary metabolites successfully isolated were structurally characterized on the basis of spectroscopic evidence, such as 1D and 2D NMR, MS, IR and UV. Several biological assay screenings were also conducted on the crude extracts and pure metabolites.

Extensive chromatographic techniques applied to the dichloromethane extract of the stem bark of *Calophyllum inophyllum* resulted in two new xanthenes, namely inophinnin (**208**) and inophinone (**209**), along with three other xanthenes, pyranojacareubin (**132**), rheediaxanthone A (**213**), and macluraxanthone (**62**) and, a sterol, stigmasterol (**207**). The ethyl acetate extract of *C. inophyllum* gave a simple xanthone, which is 4-

hydroxyxanthone (**63**). In addition, two terpenoids, friedelin (**4**) and betulinic acid (**205**), as well as a sterol, lupeol (**206**), were also isolated from the non-polar *n*-hexane extract.

Meanwhile, the stem bark of *Calophyllum soulattri* afforded two new xanthenes, soulattrin (**210**) and phylattrin (**211**), and a new coumarin, soulamarin (**212**). Both new xanthenes were isolated from the dichloromethane extract, together with four other xanthenes, macluraxanthone (**62**), caloxanthone C (**52**), brasixanthone B (**123**) and trapezifolixanthone (**14**), a coumarin, calanolide E (**80**), and a sterol, stigmasterol (**207**). On the other hand, one new coumarin was obtained from the hexane extract, which also contains a sterol, β -sitosterol (**18**), and a terpenoid, friedelin (**4**).

Structural modifications were achieved using the acetylation process on two major constituents, namely, phylattrin (**211**) and macluraxanthone (**62**). The outcome was the successful conversion of the hydroxyl groups in the molecules into acetyl groups for both compounds. The acetylation reaction of phylattrin (**211**) and macluraxanthone (**62**) gave one and two acetate-substituted products, respectively.

Cytotoxicity screening (MTT Assay) was carried out on all of the crude extracts and pure compounds using nine human cancer cell lines, SNU-1 (stomach), HeLa (cervical), NCI-H23 (lung), Hep G2 (liver), K562 (leukemia), Raji (lymphoma), LS174T (colon), SK-MEL-28 (skin) and IMR-32 (neuroblastoma) cells. A new xanthone, soulattrin (**210**), exhibited strong anti-proliferative activity against all of the cell lines with IC₅₀ values less than 1.25 μ g/mL, except for the Hep-G2 cell line. Macluraxanthone (**62**) also showed significant cytotoxic effects against all of the cell lines with IC₅₀ values less than 2.74

$\mu\text{g/mL}$, except for the Hep-G2, LS174T and IMR-32 cell lines. Another two new xanthenes, phyllatrin (**211**) and inophinnin (**208**) are considered as strong cytotoxic agents of the HeLa, SNU-1 and NCI-H23 cell lines (IC_{50} values of 3.90, 4.15 and 4.43 $\mu\text{g/mL}$, respectively), and HeLa cell line (IC_{50} value of 3.90 $\mu\text{g/mL}$), respectively.

Caloxanthone C (**52**) possessed high inhibition rate against the Hep-G2 (IC_{50} value of 2.35 $\mu\text{g/mL}$) and HeLa (IC_{50} value of 2.60 $\mu\text{g/mL}$) cell lines. 4-Hydroxyxanthone (**63**), trapezifolixanthone (**14**), and calanolide E (**80**) were found to have strong cytotoxicity against the HeLa cell line with respective IC_{50} values of 2.54, 2.86 and 2.86 $\mu\text{g/mL}$. In addition, pyranojacareubin (**132**) and betulinic acid (**205**) possessed strong activity against the K562 and SK-MEL-28 cell lines, respectively. Stigmasterol (**207**) gave low IC_{50} values of 0.17 and 3.90 $\mu\text{g/mL}$ with respect to Raji and SK-MEL-28 cells indicating strong cytotoxic effects. Kaempferol and quercetin were used as standard drugs for comparison purposes of all these results.

Antioxidant properties of the crude extracts and pure compounds were tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method, and ascorbic acid was chosen as the standard agent. The results showed that the polar crude extracts exhibited higher activities with lower EC_{50} values. In other words, the methanol extracts for both plants showed the strongest activity, followed by the ethyl acetate and dichloromethane extracts. The non-polar *n*-hexane extracts were inactive. Among the pure compounds, soulattrin (**210**) and macluraxanthone (**62**) indicated strong activities with the same IC_{50} value of 11.72 $\mu\text{g/mL}$. Also, the total phenolic contents of all the crude extracts were measured using the Folin-Ciocalteu method and the methanol extracts of *Calophyllum*

soulattri and *Calophyllum inophyllum* possessed the highest values of 460.7 and 426.4 $\mu\text{g/mL}$ GAE, respectively, which contributed partly to the antioxidant activity.

Anti-inflammatory assay was carried out using the nitric oxide (NO) assay method and this revealed that both hexane extracts possessed the highest percentage of inhibition of NO. The new xanthone, inophinnin (**208**), together with macluraxanthone (**62**), showed strong activities in the assay with the IC_{50} values of 23.91 and 8.82 $\mu\text{g/mL}$.

Lastly, antibacterial tests were also carried out using four Gram positive and four Gram negative bacteria on the *Calophyllum inophyllum* extracts. The hexane and methanol extracts indicated some activities against the Gram positive bacteria, *Bacillus cereus*, *Micrococcus luteus*, Methicillin-sensitive *Staphylococcus aureus* (MSSA), and Methicillin-resistant *Staphylococcus aureus* (MRSA).

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

KANDUNGAN KIMIA DAN AKTIVITI BIOLOGI DARIPADA *CALOPHYLLUM INOPHYLLUM* L. DAN *CALOPHYLLUM SOULATTRI* BURM. EX F. MULL.

Oleh

MAH SIAU HUI

Julai 2012

Pengerusi : Profesor Gwendoline Ee Cheng Lian, PhD

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Kajian kimia telah dilakukan terhadap kulit batang *Calophyllum inophyllum* dan *Calophyllum soulattri* dengan menggunakan pelbagai jenis teknik kromatografi dan penghabluran semula. Semua struktur metabolit sekunder dikenalpasti dengan analisis spektroskopi seperti 1D dan 2D NMR, MS, IR dan UV. Pelbagai jenis kajian biologi turut dilakukan terhadap semua ekstrak mentah dan metabolit tulen.

Teknik kromatografi yang dilakukan terhadap ekstrak diklorometana daripada kulit batang *Calophyllum inophyllum* memberikan dua xanton yang baru, inophinnin (**208**) dan inophinone (**209**) bersama-sama dengan tiga xanton lain, pyranojacareubin (**132**), rheediaxanthone A (**213**) dan macluraxanthone (**62**) dan satu sterol, stigmasterol (**207**).

Ekstrak etil asetat dari *C. inophyllum* memberikan satu xanton mudah iaitu 4-hidroksixanthone (**63**). Walau bagaimanapun, dua terpenoid, friedelin (**4**) dan asid

betulinik (**205**) serta satu sterol, lupeol (**206**) juga dikenalpasti daripada ekstrak hesana yang tidak berkutub.

Sementara itu, kulit batang dari *Calophyllum soulattri* menghasilkan dua xanton yang baru, soulattrin (**210**) dan phylattrin (**211**) dan satu kumarin, soulamarin (**212**). Kedua-dua xanton baru diasingkan daripada ekstrak diklorometana bersama-sama dengan empat xanton lain, macluraxanthone (**62**), caloxanthone C (**52**), brasixanthone B (**123**) dan trapezifolixanthone (**14**), satu kumarin, calanolide E (**80**) dan satu sterol, stigmasterol (**207**). Sebaliknya, satu kumarin yang baru diperolehi daripada ekstrak heksana yang mengandungi minyak pati, satu sterol, β -sitosterol (**18**) dan satu terpenoid, friedelin (**4**).

Modifikasi struktur telah dicapai dengan menggunakan proses asetilasi terhadap beberapa komponen utama iaitu phylattrin (**211**) dan macluraxanthone (**62**). Hasilan ialah penukaran berjaya dari kumpulan hidroksi kepada kumpulan asetil untuk kedua-dua komponen. Proses asetilasi bagi phylattrin (**211**) dan macluraxanthone (**62**) masing-masing menghasilkan satu dan dua produk pengantian asetat.

Saringan sitotoksiti (kajian MTT) telah dilakukan terhadap semua ekstrak mentah dan komponen tulen dengan menggunakan sembilan sel kanser manusia, SNU-1 (perut), HeLa (serviks), NCI-H23 (paru-paru), Hep-G2 (hati), K562 (leukemia), Raji (limfoma), LS174T (kolon), SK-MEL-28 (kulit) dan IMR-32 (neuroblastoma) sel. Xanton baru, soulattrin (**210**) mempamerkan aktiviti anti-percambahan yang baik terhadap semua sel dengan nilai IC_{50} yang kurang daripada $1.25 \mu\text{g/mL}$ kecuali terhadap sel Hep-G2.

Macluraxanthone (**62**) juga menunjukkan kesan sitotoksik yang baik terhadap semua sel dengan nilai IC_{50} yang kurang daripada $2.74 \mu\text{g/mL}$, kecuali terhadap sel-sel Hep-G2, LS174T dan IMR-32. Dua lagi xanton baru, phylattrin (**211**) dan inophinnin (**208**) masing-masing dianggap sebagai agen sitotoksik yang kukuh bagi sel-sel HeLa, SNU-1 dan NCI-H23 (nilai IC_{50} masing-masing sebanyak 3.90, 4.15 dan $4.43 \mu\text{g/mL}$) dan HeLa sel (nilai IC_{50} sebanyak $3.90 \mu\text{g/mL}$).

Caloxanthone C (**52**) memiliki kadar perencatan yang tinggi terhadap sel-sel Hep-G2 (nilai IC_{50} sebanyak $2.35 \mu\text{g/mL}$) dan HeLa (nilai IC_{50} sebanyak $2.60 \mu\text{g/mL}$). 4-Hydroxyxanthone (**63**), trapezifolixanthone (**14**) dan calanolide E (**80**) telah didapati mempunyai sitotoksiti kukuh terhadap sel HeLa dengan nilai IC_{50} masing-masing sebanyak 2.54, 2.86 dan $2.86 \mu\text{g/mL}$. Di samping itu, pyranojacareubin (**132**) dan asid betulinic (**205**) masing-masing mempunyai aktiviti yang baik terhadap sel-sel K562 dan SK-MEL-28. Stigmasterol (**207**) memberi nilai IC_{50} yang rendah iaitu 0.17 dan $3.90 \mu\text{g/mL}$ terhadap sel-sel Raji dan SK-MEL-28 menunjukkan kesan sitotoksik yang kuat. Kaempferol dan quercetin telah digunakan sebagai dadah umum untuk tujuan perbandingan bagi semua keputusan.

Keupayaan anti-pengoksidaan untuk semua ekstrak mentah dan komponen tulen telah dikaji dengan menggunakan cara perencatan radikal DPPH (2,2-difenil-1-pikrihidrazil) dan asid askorbik telah dipilih sebagai agen umum. Keputusan menunjukkan ekstrak mentah yang berketub mempamerkan aktiviti yang lebih tinggi dengan nilai IC_{50} yang lebih rendah. Dengan erti kata lain, ekstrak metanol daripada kedua-dua tumbuhan

menunjukkan aktiviti yang paling baik dan diikuti oleh ekstrak etil asetat dan diklorometana. Ekstrak heksana yang tidak berkutub adalah tidak aktif. Di antara sebatian yang tulen, soulattrin (**210**) dan macluraxanthone (**62**) menunjukkan aktiviti yang memberansangkan dengan nilai IC_{50} yang sama iaitu $11.72 \mu\text{g/mL}$. Selain itu, jumlah kandungan fenolik untuk semua ekstrak mentah telah diukur dengan menggunakan cara Folin-Ciocalteu dan ekstrak metanol *Calophyllum soulattri* dan *Calophyllum inophyllum* masing-masing mempunyai nilai yang paling tinggi iaitu 460.7 dan $426.4 \mu\text{g/mL}$ GAE yang menyumbang sebahagiannya kepada aktiviti anti-pengoksidaan.

Kajian anti-inflamasi telah dilakukan dengan menggunakan kaedah kajian nitrik oksida (NO) dan ini mendedahkan bahawa kedua-dua ekstrak heksana memiliki peratusan yang paling tinggi terhadap perencatan NO. Xanton baru, inophinnin (**208**), bersama-sama dengan macluraxanthone (**62**), menunjukkan aktiviti yang kukuh dalam kajian tersebut dengan nilai IC_{50} sebanyak 23.91 dan $8.82 \mu\text{g/mL}$.

Akhir sekali, kajian antibakteria telah dilakukan dengan menggunakan empat Gram positif dan empat Gram negatif bakteria terhadap ekstrak *Calophyllum inophyllum*. Ekstrak heksana dan metanol menunjukkan aktiviti serdahana terhadap Gram positif bakteria iaitu *Bacillus cereus*, *Micrococcus luteus*, Methicillin-sensitive *Staphylococcus aureus* (MSSA) dan Methicillin-resistant *Staphylococcus aureus* (MRSA).

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I certify that a Thesis Examination Committee has met on 5th July to conduct the final examination of Mah Siau Hui on her thesis entitled “Chemical Constituents and Biological Activities of *Calophyllum inophyllum* Linn. and *Calophyllum soulattri* Burm. ex F. Mull.” in accordance with 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 Doctor of Philosophy.

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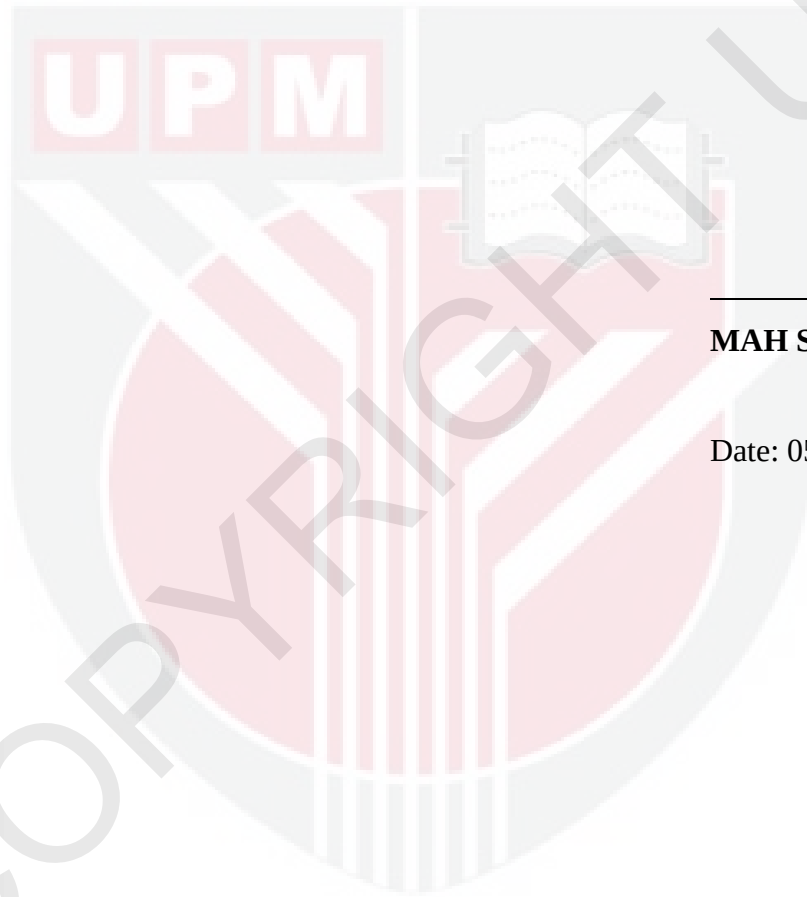
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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also 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.



MAH SIAU HUI

Date: 05 July 2012

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