



UNIVERSITI PUTRA MALAYSIA

**THE SYNTHESIS AND BIOACTIVITY STUDY OF
ANTHRAQUINONES, AND THE ISOLATION OF BIOACTIVE
COMPOUNDS FROM LEEA INDICA (BURM.F.) MERR.**

KOUSHIK SAHA.

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By

KOUSHIK SAHA

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

May 2005



***Dedicated to
My Dearest Parents;
Loving Wife, Jhumu and
Dearest Son, Ani.***



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

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KOUSHIK SAHA

May 2005

Chairman: Professor Nordin Hj. Lajis, PhD

Institute: Bioscience

Damnacanthal and nordamnacanthal were synthesized due to their interesting property as cytotoxic agents against several cancer cell-lines. Friedel-Craft condensation reaction has been used to prepare anthraquinone back-bone in view of its less stringent requirements with regards to reaction conditions (such as humidity), highly reactive reagents and lower cost. Various other synthetic methods were used to prepare their derivatives. A total 43 derivatives of anthraquinone have been successfully synthesized including damnacanthal and nordamnacanthal. A new rearrangement was observed when phthalic anhydride reacted with different halophenols leading to a few rearrangement products depending on the types of halophenol used. All the synthesized compounds were subjected to cytotoxicity assay against different cancer cell lines and antioxidant activities. A total 22 anthraquinone derivatives showed the cytotoxicity against the cell-lines used. Among the compounds, 2-bromomethyl-1,3-dimethoxyanthraquinone (A-34) was found as most cytotoxic against all the cell-lines. 3-Acetoxy-2-bromomethyl-1-methoxyanthraquinone (A-42) and damnacanthal (A-46) also showed strong cytotoxicity against different cancer cell-lines. On the other hand, only two



synthesized anthraquinones, damnacanthal (A-46) and nordamnacanthal (A-37) showed strong antioxidant activity comparable with vitamin E.

A preliminary antioxidant and nitric oxide inhibition activities screening of seven medicinal plants including *Lasianthus oblongus*, *Psychotria rostrata*, *Spermacoce exilis*, *Chasalia chartacea*, *Hedyotis verticillata*, *Spermacoce articularis* and *Leea indica* have been conducted properly. *Leea indica* (family: Leeaceae) has been selected for further investigation. Phytochemical study on this plant has resulted the isolation of a novel carotenoid, leeatene (L-7) and nine other known compounds including squalene (L-1), hexadecanoyl- β -amyirin (L-2), vitamin E (L-3), 1-tetratriacontanol (L-4), β -amyirin (L-5), 3-hydroxy-12-oleanen-28-oic acid (L-6), β -sitosteryl- β -D-glucopyranoside (L-8), 2 α ,3 α ,23-trihydroxy-12-oleanen-28-oic acid (L-9) and phloridzin (L-10). The chemical structure of the compounds was established based on spectral studies including ultraviolet-visible, infrared, one and two dimensional nuclear magnetic resonance and mass spectroscopies. All isolated compounds were tested for their antioxidant and cytotoxic activities. Three compounds including vitamin E (L-3), leeatene (L-7) and phloridzin (L-10) showed antioxidant activity whereas squalene (L-1), hexadecanoyl- β -amyirin (L-2), 1-tetratriacontanol (L-4), 3-hydroxy-12-oleanen-28-oic acid (L-6), leeatene (L-7), β -sitosteryl- β -D-glucopyranoside (L-8), 2 α ,3 α ,23-trihydroxy-12-oleanen-28-oic acid (L-9) were found cytotoxic against different cancer cell lines.



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

**SINTESIS DAN KEAKTIFAN BIOLOGI ANTRAKUINON, DAN
PEMENCILAN SEBATIAN AKTIF DARIPADA *LEEA INDICA* (BURM.F.)
MERR.**

Oleh

KOUSHIK SAHA

Mei 2005

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Damnakantal dan nordamnakantal telah disintesis kerana didapati mempunyai sifat keaktifan biologi yang menarik sebagai agen sitotoksik terhadap beberapa sel kanser. Kondensasi Fridel-Craft telah digunakan untuk menghasilkan gelang antrakuinon berdasarkan keperluan yang kurang ketat termasuk keadaan tindakbalas (seperti kelembapan) dan kereaktifan reagen yang tinggi. Sejumlah 43 terbitan antrakuinon telah berjaya disintesis termasuk damnakantal dan nordamnakantal. Satu kaedah baru penyusunan semula telah dijalankan apabila ftalik anhidrida ditindak balas dengan halofenol yang berbeza mengarah kepada beberapa hasil penyusunan semula bergantung kepada jenis halofenol yang digunakan. Kesemua sebatian yang telah disintesis diuji kesan sitotoksik terhadap beberapa talian sel kanser dan aktiviti antioksidan. Dua puluh dua terbitan antrakuinon didapati sitotoksik terhadap beberapa talian sel yang digunakan. Daripada semua sebatian, 2-bromometil-1,3-dimetoksiantrakuinon (A-34) didapati paling sitotoksik diikuti 3-asitoksi-2-bromometil-1-metoksiantrakuinon (A-42) dan damnakantal (A-46). Selain itu, hanya 2 antrakuinon yang telah disintesis iaitu damnakantal (A-46) dan

nordamnakantal (A-37) menunjukkan aktiviti antioksidan yang tinggi berbanding vitamin E.

Kajian awal aktiviti antioksidan dan perencatan nitrik oksida (NO) daripada 7 tumbuhan ubatan termasuk *Lasianthus oblongus*, *Psychotria rostrata*, *Spermacoce exilis*, *Chasalia chartacea*, *Hedyotis verticillata*, *Spermacoce articularis* and *Leea indica* telah dijalankan. *Leea indica* (keluarga: Leeaceae) telah dipilih untuk kajian lebih lanjut. Kajian fitokimia ke atas tumbuhan ini telah berakhir dengan pemencilan satu novel karotenoid, leeatene (L-7) serta sembilan sebatian yang telah diketahui termasuk skualena (L-1), heksadekanoil- β -amirin (L-2), vitamin E (L-3), 1-tetratriakontanol (L-4), β -amirin (L-5), asid 3-hidroksi-12-oleanen-28-oik (L-6), β -sitosteril- β -D-glukopiranosida (L-8), asid $2\alpha,3\alpha,23$ -trihidroksi-12-oleanen-28-oik (L-9) dan floridzin (L-10). Struktur kimia sebatian-sebatian tersebut telah dikenalpasti berdasarkan kajian spektroskopi termasuk spektroskopi ultra lembayung-nampak, spektroskopi inframerah, spektroskopi resonan magnetik nuclear satu dan dua dimensi dan spektroskopi jisim. Kesemua sebatian telah diuji aktiviti antioksidan dan sitotoksik menggunakan kaedah yang berbeza. Tiga sebatian termasuk vitamin E (L-3), leeatene (L-7) dan floridzin (L-10) telah menunjukkan aktiviti antioksidan manakala skualena (L-1), heksadekanoil- β -amirin (L-2), 1-tetratriakontanol (L-4), asid 3-hidroksi-12-oleanen-28-oik (L-6), leeatene (L-7), β -sitosteril- β -D-glukopiranosida (L-8), asid $2\alpha,3\alpha,23$ -trihidroksi-12-oleanen-28-oik (L-9) didapati sitotoksik terhadap beberapa talian sel kanser.

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I certify that an Examination Committee met on 5th May 2005 to conduct the final examination of Koushik Saha on his Doctor of Philosophy thesis entitled “The Synthesis and Bioactivity Study of Anthraquinones, and the Isolation of Bioactive Compounds from *Leea indica* (Burm.f.) Merr.” 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.

K. Saha.

KOUSHIK SAHA

Date: *18th May, 2005*

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