CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITY OF *CLAUSENA EXCAVATA* (RUTACEAE)

By

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Thesis Submitted to the School of Graduate Studies,
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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of requirement for the degree of Master of Science

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Chairman: Associate Professor Taufiq Yap Yun Hin, PhD

Faculty: Science

Ten chemical constituents were isolated from the investigations on the leaves of *Clausena excavata* (Rutaceae) collected from Jasin, Melaka. The structures of these chemical constituents were elucidated by using spectroscopic techniques, such as NMR, MS, GC, FTIR and UV, and also by comparison with the previous reports.

The fresh fruits and leaves were also hydrodistillated to obtain the essential oils. The chemical constituents and the composition of the essential oils were identified by GC-MS analysis. Compounds isolated from the leaves of *Clausena excavata* afforded one **new coumarin** from *Clausena excavata*, denoted as clauslactone-R (121), two known coumarins, clauslactone-B (96) and scopoletin (8), five known triterpenes, friedelin (115), stigmasterol (116), β-sitosterol (117), 5(6)-gluten-3-one (118) and glutinol (5), together with one carbazole alkaloid suggested as, 2, 6-dihydroxy-4-(1'hydroxy-1'-

methoxy-ethyl)-5-prenylcarbazole (120) and one triterpene proposed as, 23-ethyl-24-methyl-art-5,24-diene-3-one (119).

The crude extracts and essential oils obtained from these plant materials were evaluated for their larvicidal, antimicrobial, antifungal and cytotoxic activities. The main components found in essential oils obtained from the fruits were β-caryophyllene (22.89%) and germacrene-D (15.49%); whereas leaf's oil gave elemicin (65.02%) and methyl eugenol (12.95%) as its major components. Others minor components found in these essential oils were various terpenes.

The insecticidal test for the crude extracts and essential oils yielded was conducted on the mosquito larvae *Aedes aegypti* revealed that the leave's oil showed significant activity against the larvae, with LC_{50} value of 24.26μg/ml. The fruit's oil and crude chloroform extract were highly active, with LC_{50} values of 51.03 and 55.10μg/ml, respectively; followed by crude hexane extract and crude ethyl acetate extract, with LC_{50} values of 82.46 and 77.92 μg/ml, respectively, which categorized as active against the larvae of *Aedes aegypti*.

Both L-oil and F-oil were tested against four pathogenic bacteria, *Bacillus subtillis*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*; together with three fungi, *Candida albican*, *Aspergillus ochraceaus* and *Sacchoromyces serevisiae*. The antimicrobial and antifungal screening results showed that the fruit's oil was moderately active, while leave's oil was not active against all the microbes and fungi tested.

The cytotoxicity screening shown that glutinol (5), 5(6)-glutin-3-one (118), clauslactone-B (96), clauslactone-R (121) and 23-ethyl-24-methyl-art-5, 24-diena-3-on (119) isolated were found to be inactive against HL-60 cell-line. Both Leave's oil and Fruit's oil were bioassay against various cell-lines, *i.e.* T-cell Acute Lymphoblastic Leukaemia Cells (CEM-SS), Human Ovarian Cancer Cells ($CaOV_3$), Human Colon Adenocarcenoma Cells (HT 29), Hormone-Dependent Breast Cancer Cells (MCF-7) and Liver Cancer Cells (Hep-G2). However both essential oils were inactive against all these cell-lines used.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk ijazah Master Sains

KANDUNGAN KIMIA DAN AKTIVITI BIOLOGI DARIPADA *CLAUSENA EXCAVATA* (RUTACEAE)

Oleh

LIM GIN KEAT

Januari 2005

Pengerusi: Profesor Madya Taufiq Yap Yun Hin, PhD

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Sepuluh sebatian kimia telah terhasil atas kajian ke atas daun *Clausena excavata* (Rutaceae) dari Jasin, Melaka. Struktur sebatian-sebatian kimia ini telah dikenalpasti dengan menggunakan kaedah spektroskopi seperti NMR, MS, GC, FTIR dan UV, dan juga melalui perbandingan dengan laporan kajian yang terdahulu.

Penyulingan hidro juga telah dijalankan ke atas buah dan daun segar untuk mendapatkan minyak pati. Sebatian-sebatian kimia dan komposisi minyak pati tersebut telah dikenalpasti melalui analisis GC-MS. Pengekstrakan and pemencilan daripada ekstrak mentah daun *Clausena excavata* telah menghasilkan satu **koumarin baru** daripada *Clausena excavata*, ditandakan sebagai clauslactone-R (121), dua koumarin yang telah dikenali, clauslacton-B (96) dan scopoletin (8), lima triterpena yang telah dikenali, friedelin (115), stigmasterol (116), β-sitosterol (117), 5(6)-gluten-3-one (118), bersama-sama dengan satu sebatian karbazole alkaloids yang dicadangkan sebagai 2, 6-

dihydroxy-4-(1'hydroxy-1'-methoxy-ethyl)-5-prenylcarbazole (120) dan satu sebatian triterpena dicadangkan sebagai 23-ethyl-24-methyl-art-5,24-diena-3-on (119).

Ekstrak mentah dan minyak pati yang diperolehi telah dinilaikan aktiviti larvisidal, antimikrobial, antifungi dan sitotoksik. Komponen-komponen utama yang didapati dalam minyak pati yang diperolehi daripada buah ialah β-caryophyllene (22.89%) dan germacrene-D (15.49%); manakala minyak pati dari daun memberikan elemicin (65.02%) dan methyl eugenol (12.95%) sebagai kandungan utamanya. Komponen-komponen minor lain yang didapati dalam minyak pati tersebut adalah seperti linalool, safrol, terpinolena, α-humulena, α-terpinena dan β-elemena.

Pengaruh dan penentangan terhadap larva nyamuk *Aedes aegypti* untuk ujian insektisida ke atas ekstrak mentah dan minyal pati yang diperolehi menunjukkan bahawa minyak pati daripada daun menunjukkan aktiviti yang sangat aktif ke atas larva tersebut, dengan nilai LC_{50} 24.26 μg/ml. Manakala minyak pati daripada buah dan ekstrak mentah kloroform menunjukkan keaktifan yang tinggi, diikuti dengan ekstrak mentah heksana dan ekstrak mentah etil asitat, dengan nilai LC_{50} masing-masing 51.03, 55.10, 82.46 and 77.92 μg/ml.

Minyak pati daripada daun dan buah juga diujikan terhadap empat jenis bakteria patogenik, iaitu *Bacillus subtillis*, *Salmonella typhimurium*, *Staphylococcus aureus* dan *Pseudomonas aeruginosa*; bersama-sama dengan tiga jenis fungi, iaitu *Candida albican*, *Aspergillus ochraceaus* dan *Sacchoromyces serevisiae*. Aktiviti-aktiviti antimikrobial and

antifungal menunjukkan bahawa minyak pati buah adalah sederhana aktif, manakala minyak pati daun adalah tidak aktif terhadap kesemua mikrob dan fungi yang diuji.

Untuk ujian sitotosik, glutinol (5), 5(6)-glutin-3-one (118), clauslactone-B (96), clauslactone-R (121) dan 23-ethyl-24-methyl-art-5,24-diena-3-on (119) yang diperolehi daripada kajian ini didapati tidak aktif ke atas sel HL-60. Kedua-dua minyak pati daripada daun dan buah juga telah dilakukan dibio-cerakinan ke atas pelbagai sel, seperti Sel Leukimia Limfoblastik Akut (CEM-SS), Sel Kanser Ovari Manusia ($CaOV_3$), Sel Adenokarsinoma Kolon Manusia (HT 29), Sel Kanser Payu Dara Pergantungan Hormon (MCF-7) and Sel Kanser Hati (Hep-G2). Kedua-dua minyak pati didapati tidak aktif ke atas kesemua sel ini.

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I certify that an Examination Committee met on 19th January 2005 to conduct the final examination of Lim Gin Keat on his Master of Science thesis entitled "Chemical Constituents and Biological Activity of *Clausena excavata* (Rutaceae)" 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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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Master of Science. The members of the Supervisory 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 Universiti Putra Malaysia or other institutions.

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LIM GIN KEAT

Date:

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