



UNIVERSITI PUTRA MALAYSIA

***CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES
OF TWO RUTACEOUS PLANTS:
CLAUSENA EXCAVATA BURM. AND EVODIA MALAYANA RIDL.***

WINDA OKTIMA

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By

WINDA OKTIMA

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
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Chairman : Profesor Mawardi Rahmani, PhD

Faculty : Science

Phytochemical and biological activity studies on two Sumatran *Rutaceae* plants were carried out. Both *Clausena excavata* Burm. and *Evodia malayana* Ridl., were subjected to detail isolation work. The plants were extracted by using three organic solvents of different polarity, followed by isolation of the compounds by using several common chromatography techniques, such as gravity column chromatography, vacuum flash chromatography, radial chromatography and preparative thin layer chromatography. The structural elucidations of isolated compound were carried out by using various spectroscopic techniques including infrared (IR), mass spectroscopy (MS), nuclear magnetic resonance (NMR) and also by comparison with literature data. The phytochemical investigations have led to the isolation of several compounds of different

classes, including coumarins, alkaloid, flavonoid, lignan, acridin derivative and phytosterols. The crude extracts and some of the isolated compounds were screened for antimicrobial, antioxidant and cytotoxic activities, using disc diffusion method, DPPH (1,1-diphenyl-2-picrylhydrazyl) and MTT (Microculture Tetrazolium Salt) assay, respectively. The antimicrobial activity was tested against nine selected bacteria named *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*, respectively. The cell line used in cytotoxic assay was MCF-7, the human adenocarcinoma breast cancer cell line.

The phytochemical study on *Clausena excavata* Burm. has led to the isolation of four coumarins, seselin (**141**), imperatorin (**65**), xanthyletin (**37**), xanthotoxol (**142**), an alkaloid, murrayanin (**143**) and β -sitosterol (**140**). From the isolation work on *Evodia malayana* Burm. has yielded a lignan, sesamin (**145**), a flavonoid, pachypodol (**146**), an acridin derivative, 1,3-dimethoxy-10H-acridin-9-one (**147**) together with two phytosterols β -sitosterol (**140**) and stigmasterol (**144**). This acridin derivative was firstly reported to be isolated from natural origin and also new constituent to *E. malayana* Ridl.

Among the isolated compounds, imperatorin (**65**) and xanthotoxol (**37**) exhibited the moderate activity toward *Escherichia coli*, *Enterobacter aerogenes* and *Staphylococcus aureus*. In cytotoxic study, seselin (**141**), xanthotoxol (**142**) and sesamin (**145**) displayed remarkable potential activity against MCF-7 cell line, while potential radical scavenging activity was shown by xanthotoxol (**142**). Xanthotoxol (**142**) showed the excellent activities in all assays which have been conducted in this study, and becomes a strong reason for further drug discovery study.

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

**KANDUNGAN KIMIA DAN AKTIVITI BIOLOGI
DARIPADA DUA TUMBUHAN RUTACEOUS:
CLAUSENA EXCAVATA BURM. DAN *EVODIA MALAYANA* RIDL.**

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Kajian fitokimia dan aktiviti biologi ke atas dua tumbuhan *Rutaceae* dari Sumatra telah dijalankan. Kedua-dua tumbuhan, *Clausena excavata* Burm. dan *Evodia malayana* Ridl, digunakan untuk kajian pemencilan yang lebih mendalam. Tumbuhan-tumbuhan tersebut diekstrak dengan menggunakan tiga pelarut organik yang berbeza kekutubannya diikuti dengan pemencilan sebatian-sebatian dengan menggunakan beberapa teknik kromatografi biasa seperti kromatografi turus graviti, kromatografi turus vakum, kromatografi radial dan kromatografi lapisan nipis penyediaan. Pengenalpastian struktur sebatian yang telah dipencilkan ini telah dijalankan dengan menggunakan kaedah-kaedah spektroskopi seperti IR, MS, NMR dan juga perbandingan dengan data literatur. Kajian fitokimia telah membawa kepada pemencilan beberapa sebatian dalam kelas

yang berlainan, termasuk coumarin, alkaloid, flavonoid, lignan, terbitan acridin dan fitosterol. Ekstrak-ekstrak mentah dan sebahagian sebatian yang telah dipencilkan telah disaring untuk aktiviti antimikrob, antioksidan dan sitotoksik, menggunakan kaedah peresapan cakera, DPPH (1,1-difenil-2-pikrilhidrazil) dan MTT (garam Mikrokultur Tetrazolium). Aktiviti antimikrob telah diuji ke atas sembilan bakteria terpilih seperti *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Aspergillus niger* dan *Candida albicans*. Sel yang digunakan dalam ujikaji sitotoksik adalah MCF-7 iaitu sel kanser adenokarsinoma payudara manusia.

Kajian fitokimia ke atas *Clausena excavata* Burm. telah membawa kepada pemencilan empat coumarin; seselin (**141**), imperatorin (**65**), xantiletin (**37**), xantotoksol (**142**), alkaloid, murrayanin (**143**) dan β -sitosterol (**140**). Daripada kerja pemencilan ke atas *Evodia malayana* Ridl. telah menghasilkan lignin; sesamin (**145**), flavonoid; pakipodol (**146**), terbitan acridin; 1,3-dimetoksi-10H-akridin-9-on (**147**) bersama-sama dengan dua fitosterol; β -sitosterol (**140**) dan stigmasterol (**144**). Terbitan acridin ini dilaporkan sebagai yang pertama berhasil diasingkan dari sumber semula jadi dan juga merupakan konstituen baru untuk *E. malayana* Ridl.

Di antara sebatian-sebatian yang telah dipencilkan, imperatorin (**65**) dan xantotoksol (**142**) memiliki aktiviti sederhana terhadap *Escherichia coli*, *Enterobacter aerogenes* dan *Staphylococcus aureus*. Dalam kajian sitotoksik, seselin (**141**), xantotoksol (**142**) dan sesamin (**145**) mempamerkan potensi aktiviti yang menarik terhadap sel MCF-7, manakala potensi antioksidan ditunjukkan oleh xantotoksol (**142**). Xantotoksol (**142**) mempamerkan aktiviti-aktiviti yang sangat baik dalam semua ujian yang telah dijalankan dalam kajian ini, dan menjadi satu sebab kukuh untuk memperincinya dalam kajian penemuan obat selanjutnya.

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I certify that a Thesis Examination Committee has met on 8 of April 2013 to conduct the final examination of Winda Oktima on her thesis entitled “Chemical Constituents and Biological Activities of Two Rutaceous Plants: *Clausena excavata* Burm. and *Evodia malayana* Ridl.” in accordance with Universities and University Colleges Act 1971 and the Constitution of the university Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

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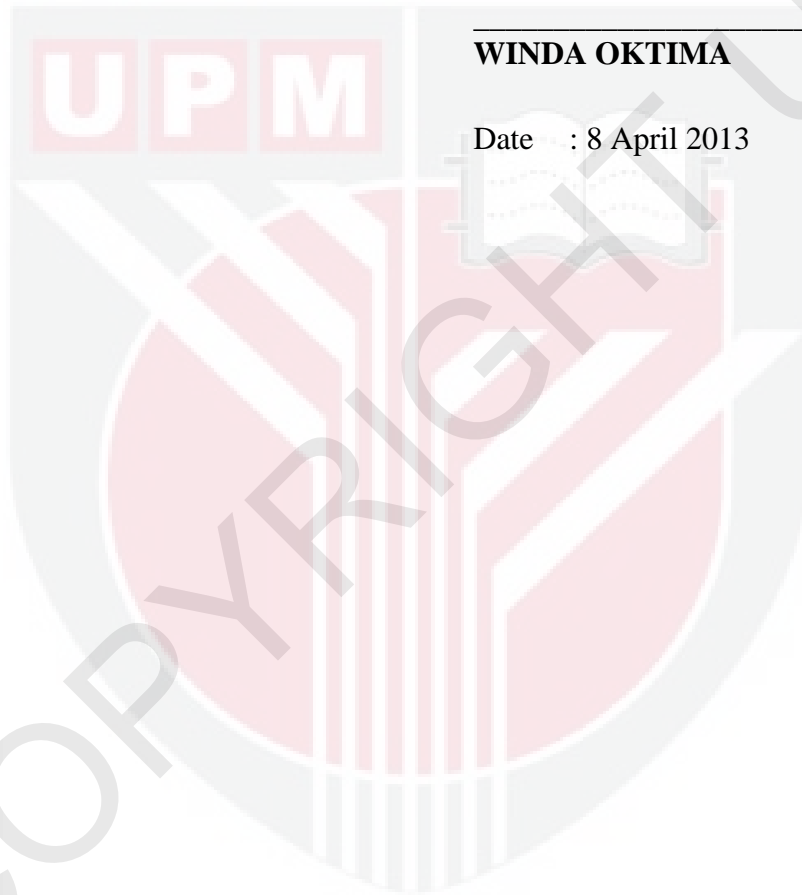
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DECLARATION

I 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, and is not concurrently, submitted for any other degree at University Putra Malaysia or at any other institutions.



WINDA OKTIMA

Date : 8 April 2013

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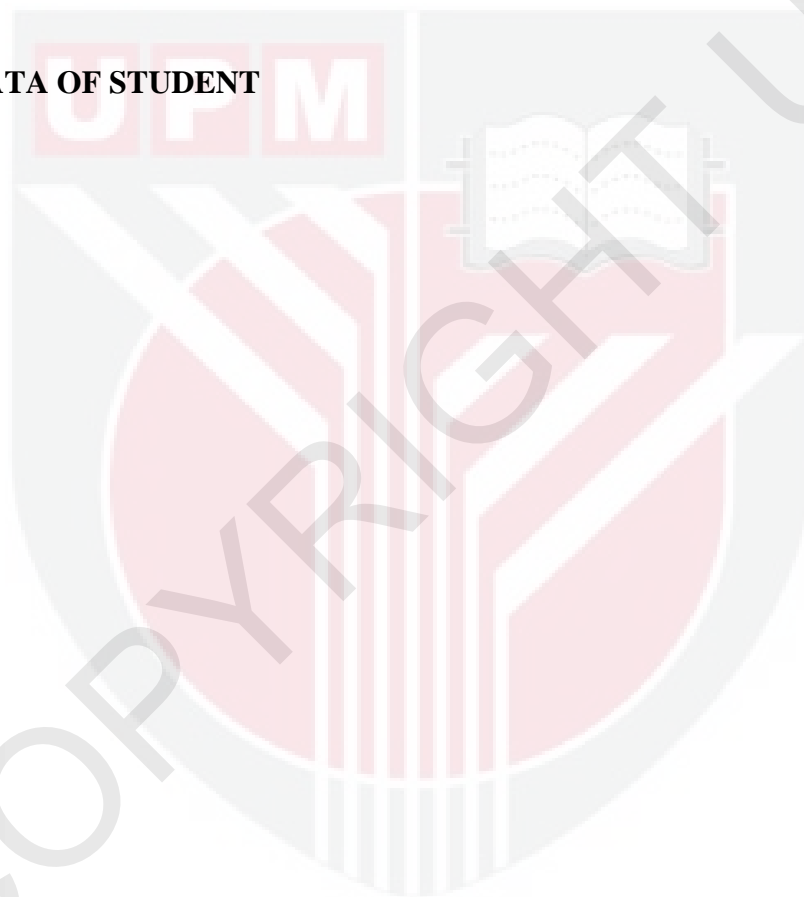
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LIST OF ABBREVIATION

α	alpha
β	beta
δ	delta (chemical shift in ppm)
γ	gamma
λ_{\max}	maximum wavelength in nm
μg	microgram
μl	microliter
br	broad
^{13}C	carbon-13
$^{\circ}\text{C}$	degree Celsius
CHCl_3	chloroform
CDCl_3	deuterated chloroform
cm^{-1}	per centimetre
COSY	Correlated Spectroscopy
<i>d</i>	doublet
<i>dd</i>	doublet of doublet
DPPH	1,1-diphenil-2-picrylhydrazyl
DEPT	Distortion less Enhancement by Polarization Transfer
DMSO	Dimethylsulfoxides

EtOAc	ethyl acetate
EIMS	Electron Impact Mass Spectrometry
g	gram
GC-MS	Gas Chromatography – Mass Spectrometry
^1H	proton
HMBC	Heteronuclear Multiple Bond Connectivity by 2D Multiple
HSQC	Heteronuclear Single Quantum Coherence
IC ₅₀	Inhibition Concentration at 50 percent
<i>t</i>	triplet
<i>s</i>	singlet
<i>m</i>	multiplet
MeOH	methanol
m.p	melting point
MS	Mass Spectrum
m/z	mass per charge
NMR	Nuclear Magnetic Resonance
OD	Optical Density
TLC	Thin Layer Chromatography
IR	Infra Red
UV	Ultraviolet
ϵ	molar absorptivity

CHAPTER I

INTRODUCTION

Traditional Medicinal Plants

Recently, there has been a steady increase in the market of herbal medicines due to the popularity of various herbs in the hope of promoting health and treating various diseases. The herbal medicine often been claimed to be nontoxic or generally regarded as safe, because of their natural origin and traditionally used in folk medicines. Nowadays, approximately 60% of world's populations still rely on plant-based drug for their primary healthcare. Thus, during the last ten years a significant shift has occurred in the strategic of approaching the traditional medicine and renewing the interest in phytomedicine. As the plants species still provide many biologically active constituents, the research on tropical plants has a special importance for the therapeutic interest. Currently many medicinal plant species are being screened for their pharmacological activities, to prove scientifically the traditional medicine systems which have been passed from generation to generation. (Cordell, 2011; Gautam *et al.*, 2007; Wu *et al.*, 2011).

Southeast Asia, the home of flora of the tropical rain forest is generally considered to be one of the richest medicinal sources in the world. Almost half of the world's flowering plant species can be found in the tropical forests, and continue to support a vast reservoir of potential drug agents. They continue to provide natural product chemists with invaluable compounds of starting points for the designing, developing and synthesizing of new drugs. This will lead to the understanding of structure-

activity relationship and obtaining the better knowledge about the drug's receptor (Fakim, 2006; Keng, 1978; Teo *et al.*, 1990).

Clausena excavata Burm. and *Evodia malayana* Ridl. are two important genus in the huge family of Rutaceae. In this study, both plants were collected from the Sumatran rain forest. Both plants are widely used as folk medicines for the treatment of various ailments ranging from cough and colds to parasitic infections and inflammations. The study was conducted to determine the bioactive constituents in both plants and evaluate their potentials as antimicrobes, antiproliferation and antioxidant agents.

Problem Statement

Clausena excavata Burm. and *Evodia malayana* Ridl. are two Rutaceous species which widely used as traditional medicines in the treatment of many ailments. As an effort to rationalize the traditional use of these plants, many phytochemical studies have been conducted on both *C. excavata* Burm. and *E. malayana* Ridl. which collected from various sources. It leads to the finding of abundance of secondary metabolites, especially coumarins, alkaloids, flavonoids and lignans and more. However, there has been no report on the phytochemical works conducted on Sumatran *C. excavata* Burm. and *E. malayana* Ridl., hence the current investigation is appropriate and further contribute to the knowledge, as well as to provide the better understanding of these plants.

Objectives of Study

The aims of this study are:

1. To extract and isolate the chemical constituents from the young trees (leaves and twigs) of *Clausena excavata* Burm. and the stem bark of *Evodia malayana* Ridl.
2. To identify and elucidate the structure of the isolated compounds using various spectroscopic techniques.
3. To determine the antimicrobial, cytotoxic and antioxidant activities of the crude extracts and isolated compounds.

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