UNIVERSITI PUTRA MALAYSIA

# CHEMICAL CONSTITUENTS AND BIOACTIVITIES OF *Clausena excavata*

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# CHEMICAL CONSTITUENTS AND BIOACTIVITIES OF *Clausena excavata*

**By**

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science**

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# CHEMICAL CONSTITUENTS AND BIOACTIVITIES OF *Clausena excavata.*

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**Mei 2008**

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*Clausena excavata* (Rutaceae) which is locally known as "Pokok Kemantu"(ghostly tree) or "Pokok Cemamar" (diarrhoea tree) is one of the Malaysian species of ulam with high anti-oxidant properties. This plant is used in folk medicine for treating ailments like colic, cough, wounds, rhinitis, and stomach trouble, fever and as a tonic. Detail investigations on the leaves, stem bark and roots of *Clausena excavata* (Rutaceae) resulted in the isolation of nine compounds. These compounds are from several groups of secondary metabolites such as limonoids, carbazole alkaloids, coumarins, and triterpenes. The structures of these compounds were elucidated using spectroscopic techniques such as NMR, GCMS, and IR and also by comparison with previously reported data.

The stem bark of *Clausena excavata* which was collected from Jabi and Pendang, Kedah in December 2006 , yielded a new limonoid, clausenolide 1-methylether **(133)**. This was obtained from the methanol extract together with a known limonoid, clausenarin **(8).** Besides that, two carbazole alkaloids, clausine–K **(43)** and 3-formyl-2,7-dimethoxycarbazole **(126)** were isolated from the crude chloroform extract and two triterpenes, stigmasterol **(131)** and β-sitosterol **(132)** were isolated from the hexane extract. Similar work on the roots led to the isolation of three known coumarins which were identified as xanthyletin **(64)** and dentatin **(130)**, together with one carbazole alkaloid, nordentatin **(54)**.

The crude extracts and pure compounds of *Clausena excavata* were subjected to cytotoxic activity test. The activities were expressed in terms of fifty percent inhibition concentration ($IC_{50}$) against HL60 cancer cell line (Human promyelocytic leukemia), MCF-7 cancer cell line (Human breast cancer), HT-29 cancer cell line (Human colon cancer), and HeLa cancer cell line. It was found that the crude hexane, chloroform, and acetone extracts exhibited very strong activities against HL-60, and MCF-7 cell lines with $IC_{50}$ values < 6μg/mL. However, the new limonoid, clausenolide 1-methylether **(133)** and clausenarin **(8)** which were isolated from the methanol extract of stem bark showed no significant cytotoxicity against all the cancer cell lines with $IC_{50}$ value >30μg/mL as compared to another 2 isolated compounds, xanthyletin **(64)** and dentatin **(130)** .

Most of the extracts and pure compounds were inactive towards all the fungi tested in the antifungal screening test. However, the chloroform and acetone extracts showed weak activity towards *S. Choleraesuis, B. Subtilis*, and *Pseudomonas aeruginosa*. Most of the pure compounds evaluated in the antimicrobial screening test were inactive towards all the bacterial strains tested except for nordentatin **(54)** and clausine-K **(43)** which exhibited very weak activity against *Staphylococcus aureus* (MRSA).

The phytochemical work carried out involve in chemical investigation on the roots of the *Clausena excavata*. Those, together with the cytotoxicity activity tests against various cancer cell lines were never been reported previously.

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

**KANDUNGAN KIMIA DAN AKTIVITI BIOLOGI DARIPADA *Clausena excavata***

Oleh

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*Clausena excavata* (Rutaceae) yang mana dikenali sebagai "Pokok Kemantu" (Pokok berhantu) atau "Pokok Cemamar" adalah salah satu jenis ulam-ulaman masyarakat Malaysia yang mempunyai kandungan antioksidan yang tinggi. Pokok ini juga digunakan sebagau sumber perubatan seperti bagi mengubati batuk, sakit perut, demam dan sebagai tonik. Penyelidikan lanjut yang dilakukan ke atas daun, kulit batang dan akar dari *Clausena excavata* (Rutaceae) menghasilkan sembilan jenis sebatian. Sebatian-sebatian ini terhasil daripada beberapa kumpulan yang terdiri dari metabolisme kelas kedua iaitu limonoid, karbazol alkaloid, coumarin dan triterpena. Struktur-struktur sebatian ini telah dapat dikenalpasti dengan menggunakan kaedah spektroskopi seperti NMR, GCMS, dan IR dan juga dari perbandingan dengan kajian laporan jurnal-jurnal yang lepas.

Kulit batang dari *Clausena excavata* yang mana dipungut dari Jabi dan Pendang, Kedah pada Disember 2006 menghasilkan satu limonoid baru, clausenolide 1-methyl ether **(133)** yang terhasil dari ekstrak methanol di samping satu limonoid yang telah dikenalpasti, clausenarin **(8).** Selain daripada itu, dua alkaloid karbazol, clausine-K **(43)** dan 3-formyl-2,7-dimethoxycarazole **(126)** telah dipencilkan dari ekstrak bahan mentah chloroform serta dua triterpina, stigmasterol **(131)** dan β-sitosterol **(132)** juga dipencilkan dari ekstrak hexane. Penyelidikan yang serupa dengan menggunakan akar menghasilkan tiga coumarin telah yang dikenalpasti sebagai xanthyletin **(64)**, dentatin **(130)** dan nordentatin **(54).**

Ekstrak bahan mentah dan sebatian tulen dari *Clausena excavata* disaring menggunakan aktiviti-aktiviti sitotoksik. Semua ekstrak bahan mentah dan sebatian tulen diuji ke atas potensi kepekatan lima puluh peratus $IC_{50}$ terhadap sel-sel kanser yang berbeza (HL60, MCF-7, HeLa dan HT-29). Ekstrak bahan mentah hexana, kloroform, aseton, dan metanol menunjukkan potensi yang sangat tinggi terhadap sel –sel kanser HL-60 dan MCF-7 dengan nilai $IC_{50}$ <6µg/mL. Walau bagaimanapun, sebatian baru dari ekstrak metanol yang dikenali sebagai clausenolide 1-methyether **(133)** dan clausenolide **(8)** tidak menunjukkan sebarang rangsangan sitotosik terhadap semua sel kanser dengan hanya memberikan nilai $IC_{50}$ >30µg/mL berbanding dua sebatian terpencil lain. Ini menunjukkan bahawa semua ekstrak bahan mentah memberikan kesan yang positif ke atas pelbagai aktiviti dari pokok *Clausena excavata* berbanding sebatian-sebatian tulen.

Semua ekstrak bahan mentah dan sebatian tulen tidak memberikan apa-apa kesan terhadap ujian penyaringan antimikrobial dan antifungi. Walau bagaimanapun, ekstrak kloroform dan aseton mempamerkan aktiviti yang lemah terhadap bakteria *S. Choleraesuis*, *B. Subtilis*, and *Pseudomonas aeruginosa*. Kebanyakan sebatian tulen yang diuji ke atas penyaringan antimicrobial adalah tidak aktif sama sekali terhadap semua bacteria kecuali Clausine-K **(43)** dan nordentatin **(54)** yang hanya menunjukkan potensi yang sangat lemah terhadap *Staphylococcus aureus* (MRSA).

Penyelidikan fitokimia ke atas pokok *Clausena excavata* melibatkan kajian kimia terhadap akar pokok *Clausena excavata*. Beserta dengan itu, kajian ini juga melibatkan kajian ujian sitotosik terhadap pelbagai sel-sel kanser yang tidak pernah dilaporkan lagi.

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## APPROVAL

This thesis was submitted to the Senate of University Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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## DECLARATION

I hereby 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 University Putra Malaysia or at any other institution.

_____

**NURUL WAZNAH BT. MUHAMAD SHARIF**

Date: 30 /7/ 09

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| α | Alpha |
| β | Beta |
| δ | Chemical shift in ppm |
| $^{13}C$ | Carbon-13 |
| $CHCL_3$ | Chloroform |
| $^{o}C$ | Degree in Celcius |
| $CDCl_3$ | Deutrated chloroform |
| COSY | Correlated spectroscopy |
| cm | Centimeter |
| *J* | Coupling constant in Hertz |
| *d* | Doublet |
| DEPT | Distortionless Enhancement by Polarisation Transfer |
| DMSO | Dimethylsulfoxide |
| EIMS | Electron Impact-MASS spectroscopy |
| EA | Ethyl Acetate |
| G | Gram |
| GC | Gas Chromatography |
| GC-MS | Gas Chromatography-Mass Spectroscopy |
| $^{1}H$ | Proton |
| HMBC | Heteronuclear Mutiple Bond Connectivity |
| HMQC | Heteronuclear Multiple Quantum Correlation |
| HPLC | High Performance Liquid Chromatogaphy |
| Hz | Hertz |
| OH | Hydroxy |
| IC | Inhibition Concentration |
| IR | Infrared |
| LC | Lethal concentration |
| *m/z* | Mass per charge |

| | |
|---|---|
| MS | Mass spectroscopy |
| MeOH | Methanol |
| OMe | Methoxy |
| Me | Methyl |
| m.p. | Melting point |
| mL | Milliliter |
| mm | Milimeter |
| µg | Microgram |
| µL | Microliter |
| MAE | Microwave-assisted extraction |
| mg | Milligram |
| $M^+$ | Molecular ion |
| *m* | Multiplet |
| nm | Nanometer |
| NMR | Nuclear Magnetic Resonance |
| ppm | Part per million |
| KBr | Potassium bromide |
| *s* | Singlet |
| *t* | Triplet |
| TLC | Thin layer chromatography |
| UV | Ultraviolet |
| WHO | World Health Organization |

# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction to Natural Product Chemistry

The term "natural products" refers to organic compounds found in various animals, plants, fungi, or micro-organisms. Some of these compounds are common to many different organisms, while others are found in only a few species. Among these compounds are terpenoids such as limonene, the active compound found in orange or lemon peel, steroids such as cholesterol, alkaloids such as caffeine, cocaine, or morphine, various phenolic compounds, plant pigments, etc. They perform various functions in nature, and many of them have interesting and useful biological activity (http:// www.ou.ac.lk.htm, 11-12-2007). Furthermore, the world market of herbal medicines which were developed by many countries for traditional or complementary based on traditional knowledge is estimated at US$ 60 thousand million. In fact, the use of medicinal plants contributes significantly to primary health care, especially in developing countries (WHO, 2003). The role of medicinal plants and traditional medicine for developing new drugs is incontestable (Rates, 2001).

Generally, the isolation of organic compounds from living organisms, most often plants are referred to the field of natural product chemistry. Organic substances are classified by their molecular structural arrangement and atoms present which are attached to the substances. It is also a branch of chemistry which deals with the isolation, identification, structure elucidation, and study of the chemical characteristics of chemical substances

produced by living organisms. The isolation processes are normally complex and a very tedious process because there are often many compounds in plants. (http://Cemistry Centre, Natural Products Chemistry.htm acc. on 21 July 2006).

Pharmaceutical chemistry is an area of chemistry focused on the development, production and delivery of drugs used to prevent, cure, and relieve symptoms of disease. Pharmaceutical chemists may synthesize new drugs, or modify older drugs so that they have improved therapeutic value which are less toxic, or have improved stability, includes tissue, cell and organ culture routes to compound production, understanding of nutraceuticals and compounds with psychoactive or immunoactive effect (http://www.usip.edu/chemistry/faculty/natprod.asp on 20 September 07).

Malaysia has about 12,000 species of flowering plants of which about 1,300 are claimed to have medicinal value, and only about a hundred have been investigated fully for their potential. The huge diversity of the Malaysian flora means that we can expect well diverse chemical structures from their secondary metabolises, and chemical diversity is one of the plus factors that make natural products excellent candidates for any screening programme (http:\\ www.arbec.com.my.html, on 11 December 2007).

### 1.1.1 Natural Product Research In Malaysia

Research on medicinal plant has started along time ago, but phytochemical studies on Malaysian plants were first reported by Douglas and Kiang in 1957. Since than, several other phytochemical studies including biological surveys were conducted. Rahmani *et al.*

(1985 & 1990) reported the phytochemical studies including biological survey of several plants families and evaluation of pesticide activities of some tropical plants were conducted by Sukari *et al.* (1992). While, Latiff *et al.* (1995) reported the phytochemical and toxicity screening of plants collected from Fraser Hill, while Jalil *et al.* (1995) have studied the phytochemical and bioactivity of aqueous extracts of medicinal plants.

The Malay community's comprehension of ailments such as cancer, hypertension and diabetes has contributed much information about plants which could be used for the treatment of these diseases. Plants reported to e beneficial in treating diabetes are *Andrographis paniculata* (Hempedu Bumi) and *Carchorus capsularis* (Kancing Baju) for treatment of cancer diseases several plants are used such as *Tabernaemontiona divaricata* (Susun Kelapa, Kembang Polong) and *Jasminium Sambae* (Bunga Manuru). Scientific studies have shown that several medicinal plants used in Malaysian traditional medicine contain compounds which have therapeutic effects or posses medicinal values. The study on isolation of anthraquinones from *Morinda citrifolia* (Mengkudu) was conducted by Ismail *et al.* (1992) and several biologically active compounds from Malaysian Rutaceae from Sirat *et al.* (1996). Currently, more results are due to be published in international scientific journals by Malaysian natural product chemists.

## 1.2 The Rutaceae Family

Rutaceae is a cosmopolitan family comprising approximately 160 genera and 1650 species (Jones, 1995), distributed largely in tropical and subtropical parts of the world with good representation in grid areas of warm temperature zone (Australia and South Africa). About 23 genera with 75 species of this family have so far been reported to occur in Sabah and Sarawak, of which 17 genera and 43 species are native trees and shrubs found in a wide range of natural habitats occurring in the lowlands, hills, mountains along coastal areas and on offshore islands.

The Rutaceae is known throughout the world for its several citrus fruit juices such as orange, lemon and grapes fruit (Sharma, 1993). The families also serve as a source of flavorings, essential oils, and traditional medicine (*Clausena, Glycosmis, Micromelum, Murraya*, etc.). The essential oils obtained from the leaves and fruits rind of various species of *Clausena, Citrus* and *Murraya* are popularly used in medicine and perfumery.

### 1.2.1 Genus *Clausena*

*Clausena* belongs to the Rutaceae family. It is a genus of about 14 species of evergreen trees, occurring mostly in India and Tropical Asia.