UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *CLAUSENA EXCAVATA* AND SOME CITRUS SPECIES (RUTACEAE)

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Chairman : Dr. Taufiq Yap Yun Hin

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Investigations on the leaves and stem bark of *Clausena excavata* (Rutaceae) have resulted in the isolation of twelve compounds. The structures of these compounds were elucidated by using spectroscopic techniques such as NMR, MS, IR and UV and also by comparison with previous reports.

The stem bark of *C. excavata* collected from Jabi, Kedah yielded two new carbazole alkaloids, 1,8-dihydroxy-3-formyl-4-prenylcarbazole and 3-carbomethoxy-2-hydroxy-7-methoxycarbazole, three known carbazole alkaloids, clausine-H, clausine-K and clausine-B together with one coumarin, scopoletin, two limonoids (clausenarin and CEA 10), and one triterpene, stigmasterol. From the leaves of *C. excavata*, a known carbazole alkaloid, 3-formyl-2,7-dimethoxycarbazole together with β-sitosterol and safrole were isolated.

The essential oils were obtained by hydrodistillation using fresh leaves and analysed using GC-MS spectrometry. The main essential oil components from *Clausena excavata* (Jabi A) were safrole and α-terpinolene, while Jabi B gave safrole and terpinolene as major constituents. The essential oils from several species of Citrus of Malaysia have also been investigated. Citronellal and citronellol were the major components of *C. hystrix*. The major constituents of *C. aurantifolia* were limonene, (z)-citral and (e)-citral. The major components of the oils of *C. sinensis* were found to be linalool and β-elemene. Germacrene-D, elemol and β-eudesmol were the major constituents of the oil of *C. microcarpa*. The oil of *C. maxima* (Kedah) contained significant amounts of sabinene, γ-terpinene and β-caryophyllene. However, β-phellandrene, *trans*-β-ocimene and β-caryophyllene are major constituents of *C. maxima* (Johor).

1,8-dihydroxy-3-formyl-4-prenylcarbazole, 3-carbomethoxy-2-hydroxy-7-methoxycarbazole and Clausine-K gave very strong activities against CEM-SS cells line with IC_{50} of 2.1, 8.2 and 5.1 μg/ml, respectively. However, crude hexane and EA extracts from the stem bark of *C. excavata* gave significant cytotoxic effect with IC_{50} of 3.27 and 2.47 μg/ml, respectively. However, All essential oils gave significant cyctoxic activity with IC_{50} value around 3 μg/ml.

The antimicrobial activity test against four types of bacteria: *Bacillus subtilis* mutant, *Bacillus subtilis* wild type, *Staphylococcus aureus* and *Pseudomonas aeruginosa* also carried out on crude extracts and essential oils. All the stem bark crude extracts of *C. excavata* and essential oil of *Citrus microcarpa* showed active against all the bacteria used.

The susceptibility or resistance of mosquito larvae of *Aedes aegypti* to insecticide test on crude extracts of *C. excavata* revealed that the hexane extracts of the stem bark, leaves and EA extract of leaves were strongly cytotoxic to the mosquito larvae. All the essential oils showed very strong cytotoxicity to the mosquito larvae with an extremely low LC_{50} value less than 50 μg/ml.

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

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

Oleh

PEH TIAN HAI

Januari 2001

Pengerusi : Dr. Taufiq Yap Yun Hin

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Kajian ke atas ekstrak mentah daun dan kulit batang *Clausena excavata* (Rutaceae) telah menghasilkan dua belas komponen. Struktur sebatian-sebatian ini telah dapat dikenalpasti dengan menggunakan kaedah spektroskopi seperti NMR, MS, IR dan UV dan juga perbandingan dengan kajian-kajian yang lepas.

Pengekstrakan dan pemencilan daripada kulit batang *C. excavata* telah menghasilkan dua alkaloid karbazole baru, 1,8-dihidroksi-3-formil-4-prenilkarbazole dan 3-karbometoksi-2-hidroksi-7-metoksikarbazole, dan tiga alkaloid karbazole yang telah dikenali, iaitu clausine-H, clausine-K dan clausine-B, satu koumarin, scopoletin, dua limonoid (clausenarin dan CEA 10), dan satu triterpene, stigmasterol. Daripada daun *C. excavata* pula, satu alkaloid karbazole yang telah dikenali, iaitu 3-formil-2,7-dimetoksikarbazole bersama dengan β-sitosterol dan safrole yang telah dipisahkan.

Minyak pati diperolehi dengan teknik penyulingan hidro yang menggunakan daun segar dan dianalisis dengan menggunakan kaedah spektrometri GC-MS. Komponen utama minyak pati daripada *Clausena excavata* (Jabi A) ialah safrole dan α-terpinolene. Manakala Jabi B pula mengandungi safrole dan terpinolene sebagai komponen utamanya. Kajian turut dilakukan ke atas minyak pati bagi beberapa spesies Citrus yang didapati di Malaysia. Citronellal and citronellol ialah komponen major bagi *C. hystrix*. Kandungan utama bagi *C. aurantifolia* adalah limonene, (z)-citral dan (e)-citral. Minyak pati daripada *C. sinensis* didapati major dalam linalool dan β-elemene. Germacrene-D, elemol dan β-eudesmol adalah komponen utama bagi minyak *C. microcarpa*. Minyak pati daripada *C. maxima* (Kedah) mengandungi amaun yang signifikan, iaitu sabinene, γ-terpinene dan β-caryophyllene. Manakala, β-phellandrene, *trans*-β-ocimene dan β-caryophyllene adalah komponen major bagi *C. maxima* (Johor).

1,8-dihidroksi-3-formil-4-prenilkarbazole, 3-karbometoksi-2-hidroksi-7-metokikarbazole dan clausine-K menunjukkan sangat aktif terhadap garisan sel CEM-SS dengan nilai IC_{50} = 2.1, 8.2 dan 5.1 μg/ml masing-masing. Manakala ekstrak mentah daripada kulit batang *C. excavata* memberi kesan sitotoksik yang signifikan terutamanya ekstrak mentah heksana dan EA (IC_{50} = 3.27 dan 2.47 μg/ml masing-masing). Manakala, Semua minyak pati memberi aktiviti sitotoksik yang signifikan di mana nilai IC_{50} di antara sekitar 3 μg/ml.

Aktiviti antimikrobial terhadap empat jenis bakteria: *Bacillus subtilis* mutan, *Bacillus subtilis* jenis liar, *Staphylococcus aureus* yang tahan metisillin dan *Pseudomonas aeruginosa* juga telah dijalankan ke atas ekstrak mentah dan minyak

pati. Semua ekstrak mentah daripada kulit batang *C. excavata* dan minyak pati daripada *Citrus microcarpa* memberi aktiviti terhadap semua bakteria yang digunakan.

Pengaruh atau penentangan larva nyamuk *Aedes aegypti* kepada ujian insektiside terhadap ekstrak heksana daripada kulit batang dan daun *C. excavata* serta ekstrak EA daripada daun menunjukkan sitotoksiksiti yang tinggi terhadap larva nyamuk. Semua minyak pati yang diuji telah menunjukkan sitotoksiksiti yang tinggi ke atas nyamuk *Aedes aegypti* dengan nilai LC_{50} yang tersangat rendah, iaitu kurang daripada 50 μg/ml.

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LIST OF ABBREVIATIONS

α	alpha
β	beta
δ	chemical shift in ppm
γ	gamma
µg	micro gram
brs	broad singlet
^{13}C	carbon-13
$CHCl_3$	chloroform
$CDCl_3$	deuterated chloroform
COSY-	Correlated Spectroscopy
d	doublet
dd	doublet of doublet
DEPT	Distortionless Enhancement by Polarisation Transfer
DMSO	dimethylsulfoxide
dt	doublet of triplet
EA	ethyl acetate
g	gram
GC	Gas Chromatography
GC-MS	Gas Chromatography-mass spectroscopy
^{1}H	proton
HETCOR	Heteronuclear Chemical Shift-correlation
HMBC	Heteronuclear Multiple Bond Connectivity by 2D Multiple Quantum
IC	Inhibition Concentration
IR	Infra Red
J	coupling constant in Hz
l	liter
LC	Lethal Concentration
LD	Lethal Dose
m	multiplet
ml	mili liter
mg	mili gram
Me_2CO	acetone
MeOH	methanol
m. p.	melting point
MS	Mass Spectrum/Spectra/Spectrometer/Spectroscopy
NMR	Nucleur Magnetic Resonance
pet. ether	petroleum ether
PLC	Preparative Thin Layer Chromatography
ppm	part per million
s	singlet
t	triplet
TLC	Thin Layer Chromatography
UV	Ultra Violet
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Extraction and Isolation of Chemical Constituents from *Clausena excavata*

1.1.1 *Clausena excavata* species

Clausena belongs to the Rutaceae family. It is a genus of about fourteen species of evergreen trees, occurring mostly in India and tropical Asia. *Clausena excavata* is a shrub with strong and rather objectionable smell, found from the Himalayas and China to and throughout Malaysia; particularly in the Peninsula. The local names of this species are "Cherek hitam", "Chemama" and "Kemantu hitam" (Burkill, 1966).

A slender tree to 10 m tall, its twigs are finely hairy. The leaves are pinnate, 60 cm long with 10-15 pairs of dark green narrowly oval oblique leaflets 3.5-7 cm long and with pointed tips. The leaflets have a characteristic curry-like smell when crushed. Small white flowers occur in terminal clusters, followed by translucent pink berries 7-10 mm across, each containing 1-2 seeds. (Swarbrick, 1997).

Clausena excavata is valued for its medicinal properties. It is also traditionally used in the treatment of snakebite, abdominal pain and as a detoxification agent. A decoction of the roots is drunk for bowel-complaints, chiefly colic. The pounded root is used as a poultice for sores including ulceration of the nose and the leaves are used also for poulticing. It is recorded that pounded they

may be applied to the head for headaches. The flowers and leaves may be boiled and the decoction taken for colic and a decoction of leaves is given after childbirth (Burkill, 1966).

The leaves of this plant are used as a traditional medicine to cure cold, abdominal pain, malaria and dysentery (Wu *et al.*, 1992). The dried and powdered rootstock can be used to treat decayed teeth, whereas its stem is given in colic with or without diarrhea (Kirtikar and Basu, 1933).

1.2 Essential Oils from the Leaves of *Clausena excavata* and Citrus Species

1.2.1 General

Essential oils are the odours of plants due to specific mixtures of volatile substances, which in general are liquid at ordinary temperatures. Essential oils are also called Ethereal or Volatile oils. They occur in small concentrations in special cells, glands or ducts, either in one particular organ of the plant or distributed over many parts e. g. leaves, barks, roots, flowers or fruits (Gupta, 1977).

Occasionally, they are present in combination with sugar as glycosides, *e.g.* amygdalin in better almonds and sinigrin in mustard seeds and are liberated when the glycosides are hydrolysed. Essential oils are insoluble in water but freely soluble in alcohol, ether, fatty oils and mineral oils. They are commonly liquid at ordinary temperatures and some of them deposit solid matters on standing. Most of the essential oils are optically active, are lighter than water and possess a high refractive

index. They are composed of a number of chemical compounds – hydrocarbons (terpenes, sesquiterpenes and diterpenes), alcohols, esters, aldehydes, ketons, oxides and lactones, and occasionally compounds of nitrogen and sulphur (Gupta, 1977).

The function of essential oils in the living plant tissue is not completely understood. Odours of flowers for instance may be directly associated with insect attraction or repulsion and it influences pollination and to some extent natural selection. Some are thought to act as a form of protection against parasites and other have such a repulsive odour as to give the plant protection from animal depredation (Lawrence, 1979).

Essential oils may be separated from plant materials by one or the other of following methods (Gupta, 1977):

i) Distillations (water, water and steam and live steam) are applicable to a wide range of materials.

ii) Extraction by volatile solvents, hot oils or fats (maceration) or cold neutral fats.

iii) Expression by hand or machinery, applicable especially to fruit rinds.

Citrus, Linn. A genus of fruit-trees of the family Rutaceae of Asiatic origin and cultivated throughout the warmer parts of the world. It may be stated at once that the aromatic substances in the rind develop best in dry subtropical climates, so that essential oil industries can scarcely be established competitively in the tropics. Different parts of the trees produce volatile oils, right from the seedling stage. These oils are not constant in composition but change in constitution after their first