

**PHYTOCHEMICAL STUDIES AND THE
BIOACTIVITIES OF THREE MELIACEAE
SPECIES**

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ABSTRACT

This thesis describes phytochemical studies on three species of Malaysian plants, *Chisocheton ceramicus*, *Chisocheton tomentosus*, and *Dysoxylum macrocarpum* from Meliaceae family, in which the latter two species have not been found in the literature.

The present work on these plants involves extraction, isolation and purification of compounds by using column chromatography followed by preparative TLC or RP-HPLC and structural elucidation, which has been done through several spectroscopic methods, notably UV, IR, MS (HRMS, GCMS, and LCMS), 1D, 2D-NMR (¹H NMR, ¹³CNMR, COSY, DEPT, HMQC, HMBC, NOESY, and single crystal X-ray diffraction analysis.

This work has resulted twenty new compounds including five limonoids while the rest were phenolic compounds, furthermore, three new compounds as crystals. In addition, twenty-six known compounds have been isolated and characterized for the first time from all of these plants.

The five new isolated compounds from the bark of *Chisocheton ceramicus* were chisomicine A **CC1**, chisomicine B **CC2**, chisomicine C **CC3**, chisomicine D **CC4**, and chisomicine E **CC5**, along with 14-deoxyxylocensin K **CC6**, which was new in its occurrence as natural product and as crystal. This compound has been synthesized from xylocensin K, and one known compound, proceranolide **CC7**.

Furthermore, fifteen new compounds, **CT5A**, **CT5C**, **CT5D**, **CT5K**, **CT5L**, **CT5M**, **CT5N**, **CT5O**, **CT5P**, **CT6B**, **CT6C**, **CT6L**, **CT6M**, **CT6N**, **CT6O** as well as one new as crystal, 7-hydroxy- β -sitostertol **CT1** were amongst the thirty eight compounds isolated from the bark of *Chisocheton tomentosus*, while the rest were known compounds; stigmasta-4,6-diene-3-one **CT2**, stigmasterol **CT3**, sitosterol **CT4**, hydroxy acid derivatives of ferulate **CT5B**, **CT5E**, **CT5F**, **CT5G**, **CT5H**, **CT5I**,

CT5G, *E*-alkyl-*p*-coumarate **CT6A**, **CT6D**, **CT6E**, **CT6F**, **CT6G**, **CT6H**, **CT6I**, **CT6G**, **CT6K**, ferulaldehyde **CT7**, and vanillin **CT8** and the last one was styryl lactone (goniothalamine) **CT9**.

While the bark and leaves of *Dysoxylum macrocarpum* yielded four compounds, two compounds from the leaves; 5-hydroxy-7-methoxy-2-methyl-4*H*-chromen-4-one (Eugenin) **DM1**, which was new as crystal and squalene **DM2**, while two more compounds were found from the bark; stigmasterol **CT3** and sitosterol **CT4**.

Five limonoids; chisomicine A **CC1**, chisomicine B **CC2** and C **CC3**, 14-deoxyxyloccensin K **CC6** and proceranolide **CC7**, were tested for their inhibitory activity against NO production. The results showed that only chisomicine A **CC1** inhibited NO production in J774.1 dose-dependently stimulated by LPS and also showed little effect on cell viability.

Three of above limonoids chisomicine A **CC1**, chisomicine B **CC2**, 14-deoxyxyloccensin K **CC6** have been tested for its cytotoxic effects against 8 types of cancer cell lines; only chisomicine A **CC1** showed positive results for colon (HT-29) cells.

7-Hydroxy- β -sitosterol **CT1**, hydroxy acid derivatives of ferulate **CT5A-P**, and ferulaldehyde **CT7** from *Chisocheton tomentosus* were tested for *in-vitro* cytotoxicity activity against MCF7 cells. The results showed 7-hydroxy- β -sitosterol **CT1** had the most potent cytotoxicity effect, the other compounds showed very weak or no cytotoxicity effect against MCF7 cells.

ABSTRAK

Tesis ini menerangkan kajian fitokimia ke atas tiga spesies pokok di Malaysia, *Chisocheton ceramicus*, *Chisocheton tomentosus* dan *Dysoxylum macrocarpum* daripada keluarga Meliaceae, di mana dua spesies yang terakhir tidak pernah dikaji sebelum ini.

Kajian ke atas pokok-pokok ini melibatkan proses pengekstrakan, pemisahan sebatian melalui kromatografi turus diiringi dengan preparatif TLC atau RP-HPLC dan ilusidasi struktur di mana beberapa kaedah spektroskopi telah digunakan, khususnya UV, IR, MS (HRMS, GCMS dan LCMS), 1D, 2D-NMR (¹H-NMR, ¹³C-NMR, COSY, DEPT, HMQC, HMBC, NOESY, dan analisis di fraksi X-ray hablur tunggal.

Kajian ini telah menghasilkan dua puluh sebatian baru; lima daripadanya adalah limonoid dan selebihnya adalah sebatian fenolik, di mana tiga daripadanya adalah dalam bentuk hablur. Tambahan pula, dua puluh enam sebatian diketahui juga telah dipisah dan dikenalpastikan buat kali pertama daripada pokok-pokok ini.

Lima sebatian baru telah dipisahkan daripada batang pokok *Chisocheton ceramicus*; chisomicine A **CC1**, chisomicine B **CC2**, chisomicine C **CC3**, chisomicine D **CC4** dan chisomicine E **CC5**, bersama-sama dengan 14-deoksixyloccensin K **CC6**, di mana ianya adalah sebatian semulajadi yang baru dan dipisahkan sebagai hablur, dan sebatian ini telah disintesiskan daripada xyloccensin K, serta satu sebatian diketahui, proceranolide **CC7**.

Seterusnya, lima belas sebatian baru, iaitu **CT5A**, **CT5C**, **CT5D**, **CT5K**, **CT5L**, **CT5M**, **CT5N**, **CT5O**, **CT5P**, **CT6B**, **CT6C**, **CT6L**, **CT6M**, **CT6N**, **CT6O** dan satu hablur baru; 7-hidroksi- β -sitostertol **CT1**, ialah antara tiga puluh lapan sebatian baru yang dipisahkan daripada batang pokok *Chisocheton tomentosus*, dan selebihnya adalah sebatian diketahui; stigmasta-4,6-diene-3-on **CT2**, sitosterol **CT4**, stigmasterol **CT3**,

terbitan asid hidroksi sebatian ferulat **CT5B, CT5E, CT5F, CT5G, CT5H, CT5I, CT5G**, *E*-alkil-*p*-coumarate **CT6A, CT6D, CT6E, CT6F, CT6G, CT6H, CT6I, CT6G, CT6K**, ferulaldehid **CT7**, vanillin **CT8** dan satu styrillakton; goniothalamine **CT9**.

Sementara itu, empat sebatian telah dipisahkan daripada daun dan batang pokok *Dysoxylum macrocarpum*, iaitu dua daripada bahagian daun; 5-hidroksi-7-metoksi-2-metil-4*H*-chromen-4-on (eugenin) **DM1**, di mana ia adalah hablur baru, dan skualene **DM2**, serta dua lagi sebatian ulangan daripada bahagian batang; stigmasterol **CT3** dan sitosterol **CT4**.

Lima limonoid, iaitu chisomicine A **CC1**, chisomicine B **CC2**, chisomicine C **CC3**, 14-deoksixyloccensin K **CC6** dan proceranolide **CC7** telah dikaji kesan penyekatannya ke atas produksi NO. Kajian mendapati hanya chisomicine A **CC1** memberi kesan penyekatan ke atas produksi NO dalam J774.1 yang bergantung kepada dos dan dirangsang oleh LPS serta memaparkan kesan yang sedikit terhadap kelangsungan hidup sel.

Tiga daripada limonoid di atas, iaitu chisomicine A **CC1**, chisomicine B **CC2** dan 14-deoksixyloccensin K **CC6** telah dipilih untuk menguji kesan sitotoksik terhadap lapan jenis sel kanser, dan hanya chisomicine A **CC1** memaparkan keputusan positif bagi sel kolon (HT-29).

Beberapa sebatian daripada *Chisocheton tomentosus*, iaitu 7-hidroksi- β -sitostertol **CT1**, terbitan asid hidroksi sebatian ferulat **CT5A-P**, dan ferulaldehid **CT7** telah dikaji kesan sitotoksik *in-vitro* terhadap sel MCF7. Keputusannya menunjukkan 7-hidroksi- β -sitostertol **CT1** adalah yang paling berpotensi dengan kesan sitotoksik, sebatian-sebatian lain menunjukkan kesan sitotoksik yang lemah atau tiada kesan langsung terhadap sel MCF7.

To

My Kurdish Nation and Kurdistan

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LIST OF ABBREVIATIONS

Symbols	terms
α	alpha
β	beta
λ_{\max}	maximum wave length
δ	chemical shift in ppm
μM	micromolar
μl	microlitre
$\mu\text{g/ml}$	microgram per mililitre
mM	milimolar
mg/ml	milligram per mililitre
g	gram
kg	kilogram
U/ml	unit per mililitre
ml	mililitre
m	meter
MHz	mega Hertz
Hz	Hertz
UV	ultraviolet
ϵ	UV extinction coefficient
IR	Infrared
mM	milimolar
ppm	part per million
eV	electron Volt
MeOH	methanol
CHCl_3	chloroform

CH ₂ Cl ₂	dichloromethane
CH ₃	methyl group
OCH ₃	methoxyl group
OH	hydroxyl group
KCl	potassium chloride
NaCl	sodium chloride
pH	power of hydrogen
HCl	hydrogen chloride
TLC	thin layer chromatography
PTLC	preparative thin layer chromatography
CC	column chromatography
NMR	nuclear magnetic resonance
FT-NMR	fourier transform nuclear magnetic resonance
cm ⁻¹	per centimeter
<i>J</i>	coupling constant
<i>d</i>	doublet
<i>s</i>	singlet
<i>dd</i>	doublet of doublets
<i>t</i>	triplet
<i>m</i>	multiplet
1D-NMR	one dimension nuclear magnetic resonance
2D-NMR	two dimension nuclear magnetic resonance
¹ H-NMR	proton nuclear magnetic resonance
¹³ C-NMR	carbon 13 nuclear magnetic resonance
COSY	2D homonuclear chemical shift correlation spectroscopy
DEPT	distortionless enhancement by polarization transfer

HMQC	heteronuclear multiple quantum coherence
HMBC	heteronuclear multiple bond coherence
NOE	nuclear overhauser enhancement
GC-MS	gas chromatography-mass spectroscopy
MS	mass spectroscopy
HRMS	high resolution mass spectroscopy
EIMS	electron impact mass spectroscopy
FAB	fast atomic bombardment
ESI	electrospray ionization
m/z	mass to charge ratio
CDCl ₃	deuterated chloroform
MeOD	deuterated methanol
[α] _D	optical rotation
HPLC	high performance liquid chromatography
RPHPLC	reverse phase high performance liquid chromatography
RPMI	Roswell Park Memorial Institute
CO ₂	carbon dioxide gas
PBS	phosphate buffer saline
EDTA	ethylenediaminetetraacetic acid
FBS	fetal bovine serum
rpm	rotate per minute
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DMSO	dimethyl sulfoxide
IC ₅₀	inhibition concentration for 50% killing
FITC	fluorescein isothiocyanate
PS	phosphatidyle serine

PI	propidium iodide
HMEC	human mammary epithelia cell
ATCC	American Type Culture Collection
DMEM	Dulbecco's modified Eagle's medium
XTT	2,3-bis(2-methoxyl-4-nitro-5-sulphonyl)-5- [(phenylamino)carboxyl]-2 <i>H</i> -tetrazolium hydroxide;
MTS	(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2- sulfonyl)-2 <i>H</i> -tetrazolium, inner salt
LPS	LipoPolySaccharide
V-FITC	Fluorecein isothiocynate
MCF7	Human Breast Adenocarcinoma