



UNIVERSITI PUTRA MALAYSIA

**PHYTOCHEMISTRY AND BIOLOGICAL ACTIVITIES OF
ARTOCARPUS KEMANDO MIQ. (PUDAU) AND *ARTOCARPUS*
ODORATISSIMUS BLANCO (TERAP) (MORACEAE)**

**TEO SIOW HWA
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By

TEO SIOW HWA

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March 2010

Chairman : Gwendoline Ee Cheng Lian, PhD

Faculty : Science

Chemical investigation of *Artocarpus kemando* and *Artocarpus odoratissimus* have resulted in the isolation of ten compounds, three of which are new. All the pure metabolites were purified by means of chromatographic method and their structures were determined from modern spectroscopic identification such as one-dimensional nuclear magnetic resonance spectroscopy, two-dimensional nuclear magnetic resonance spectroscopy, infrared spectroscopy, ultraviolet spectroscopy and mass spectrometry. The two new compounds successfully isolated from the crude extracts of stem bark of the *Artocarpus kemando*, are artomandin (**161**) and kemandonin (**162**). Besides this, two triterpenoids were identified to be 24-methylenecycloartanyl acetate (**160**) and β -Sitosterol (**1**), while four flavonoids were authenticated as cycloartobiloxanthone (**8**), artoindonesianin C (**51**), artonol B (**73**) and artochamin A



(95). On the other hand, studies on the stem bark crude extracts of *Artocarpus odoratissimus* yielded a triterpenoid, traxateryl acetate (**163**), and a new flavonoid, artosimmin (**164**).

The biological studies on the crude extracts and pure compounds of *Artocarpus* species indicated both to have significant biological activity especially in the cytotoxic assay. Cytotoxic assay was performed using human promyelocytic leukemia, HL-60 and human breast adenocarcinoma, MCF-7 cell lines. All the stem bark extracts of *Artocarpus* species were found to show significant growth inhibition activities towards HL-60 cell line especially the chloroform, ethyl acetate and ethanol extracts of *Artocarpus odoratissimus*, which gave extremely low IC₅₀ values of 3.2, 3.7 and 2.9 µg/ ml, respectively. Moreover, the MCF-7 cell line was found to be sensitive against the chloroform, ethyl acetate and ethanol extracts of *Artocarpus odoratissimus* with IC₅₀ values of less than 30.0 µg/ ml. Artomandin (**161**) strongly inhibits the HL-60 and MCF-7 cell lines with IC₅₀ values of 2.4 and 3.1 µg/ ml, respectively. Both cell lines also showed similar degree of susceptibility toward artosimmin (**164**) with IC₅₀ values of 1.1 and 3.4 µg/ ml, respectively. However, the HL-60 cell line was found to be moderately susceptible toward cycloartobiloxanthone (**8**) and kemandonin (**162**) with IC₅₀ values of 5.7 and 6.9 µg/ ml, respectively. Meanwhile, the MCF-7 cell line was also observed to be far less sensitive to cycloartobiloxanthone (**8**) and kemandonin (**162**), which exhibited medium cytotoxicity with the IC₅₀ value of 13.4 and 13.1 µg/ ml, respectively.

Antioxidant assays were also carried out on the crude extracts and pure compounds of the stem bark of *Artocarpus kemando* and *Artocarpus odoratissimus* using free radical DPPH scavenging test. The crude chloroform, ethanol and methanol extracts of *Artocarpus kemando* gave the strongest antioxidant capacity in the free radical DPPH tests with extremely low IC₅₀ values of 27.1, 19.2 as well as 23.3 µg/ ml. artomandin (**161**) and artosimmin (**164**), showed the highest potential of scavenging effect towards the DPPH radical test with IC₅₀ values of 38.0 and 32.1 µg/ ml, respectively. Meanwhile, cycloartobiloxanthone (**8**) revealed a lower DPPH scavenging effect with an IC₅₀ value of 87.2 µg/ ml. Both artoindonesianin C (**51**) and artonol B (**73**) gave weak scavenging activity with IC₅₀ values of more than 90.0 µg/ ml.

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

**SEBATIAN-SEBATIAN KIMIA DAN AKTIVITI-AKTIVITI BIOLOGI
DARIPADA *ARTOCARPUS KEMANDO* MIQ. AND *ARTOCARPUS
ODORATISSIMUS* BLANCO**

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Kajian secara kimia ke atas pokok-pokok *Artocarpus kemando* dan *Artocarpus odoratissimus* telah menghasilkan sepuluh sebatian-sebatian semulajadi dengan tiga sebatian-sebatian semulajadi yang baru. Semua sebatian tulen ini telah dipisahkan dengan menggunakan kaedah kromatografi dan struktur masing-masing telah dikenalpasti berdasarkan bukti-bukti spektroskopi termasuk satu-dimensi resonans magnetic nuclear spektroskopi, dua-dimensi resonans magnetic nuclear spektroskopi, infra-merah spektroskopi, ultra-ungu spektroskopi dan jisim spektrosmetri. Dua sebatian semulajadi baru iaitu artomandin (**161**) dan kemandonin (**162**) telah berjaya ditemui daripada ekstrak-ekstrak mentah kulit pokok *Artocarpus kemando*. Sementara itu, dua sebatian triterpenoid telah masing-masing dikenalpasti sebagai 24-methylenecycloartanyl acetate (**160**) dan β -Sitosterol (**1**) manakala empat

flavonoid yang ditemui telah dikenali sebagai cycloartobiloxanthone (**8**), artoindonesianin C (**51**), artonol B (**73**) dan artochamin A (**95**) masing-masing. Selain daripada itu, kajian juga telah dijalankan ke atas ekstrak-ekstrak mentah kulit pokok *Artocarpus odoratissimus* dan ini menghasilkan satu triterpenoid, traxateryl acetate (**163**) dan satu sebatian flavonoid yang baru, artosimmin (**164**).

Kajian secara biologi ke atas ekstrak-ekstrak mentah dan sebatian-sebatian semulajadi daripada spesis *Artocarpus* menunjukkan kedua-duanya adalah aktif terutamanya dalam kajian sitotoksik. Kajian sitotoksik telah dijalankan dengan menggunakan sel-sel human promyelocytic leukemia, HL-60 dan human breast adenocarcinoma, MCF-7. Kesemuanya ekstrak mentah yang diperolehi daripada spesis *Artocarpus* telah menunjuk aktiviti yang ketara terutamanya ekstrak-ekstrak kloroform, etil asetat, dan etanol daripada pokok *Artocarpus odoratissimus*, dengan nilai IC_{50} yang terlampau rendah iaitu 3.2, 3.7 dan 2.9 $\mu\text{g}/\text{ml}$. Di samping itu, sel MCF-7 juga didapati sensitive kepada ekstrak-ekstrak kloroform, etil asetat, dan etanol daripada pokok *Artocarpus odoratissimus* iaitu dengan nilai IC_{50} kurang daripada 30.0 $\mu\text{g}/\text{ml}$ masing-masing. Sebatian artomandin (**161**) menunjukkan aktiviti penghalangan yang kuat terhadap sel-sel HL-60 and MCF-7 dengan nilai-nilai IC_{50} iaitu 2.4 dan 3.1 $\mu\text{g}/\text{ml}$. Kedua-duanya sel juga menunjukkan aktiviti penghalangan yang serupa dengan sebatian artosimmin (**164**) dengan nilai IC_{50} iaitu 1.1 dan 3.4 $\mu\text{g}/\text{ml}$. Manakala, sel HL-60 menunjukkan aktiviti penghalangan yang sederhana daripada sebatian-sebatian cycloartobiloxanthone (**8**) dan kemandonin

(162) dengan nilai IC_{50} 5.7 dan 6.9 $\mu\text{g}/\text{ml}$. Selain itu, sel MCF-7 juga didapati kurang sensitif kepada sebatian-sebatian cycloartobiloxanthone (8) dan kemandonin (162) iaitu dengan nilai-nilai IC_{50} , 13.4 and 13.1 $\mu\text{g}/\text{ml}$ masing-masing.

Ujian antioksidan telah dijalankan ke atas ekstrak-ekstrak mentah dan sebatian-sebatian semulajadi daripada *Artocarpus kemando* dan *Artocarpus odoratissimus* dengan menggunakan kaedah peresapan cakera bebas DPPH. Ekstrak-ekstrak kloroform, etanol dan metanol daripada pokok *Artocarpus kemando* telah menunjukkan kapasiti antioksidan yang paling tinggi dalam ujian cakera bebas DPPH dengan nilai IC_{50} yang terlampau rendah, iaitu 27.1, 19.2 dan 23.3 $\mu\text{g}/\text{ml}$. Sebatian-sebatian artomandin (161) and artosimmin (164) menunjukkan kesan potensi peresapan yang paling tinggi terhadap ujian cakera DPPH, iaitu dengan nilai IC_{50} , 38.0 and 32.1 $\mu\text{g}/\text{ml}$ masing-masing. Manakala, sebatian cycloartobiloxanthone (8) menunjukkan kesan peresapan cakera DPPH yang paling rendah dengan nilai IC_{50} iaitu 87.2 $\mu\text{g}/\text{ml}$. Kedua-duanya sebatian, artoindonesianin C (51) dan artonol B (73) menunjukkan aktiviti peresapan yang rendah dengan nilai IC_{50} lebih daripada 90.0 $\mu\text{g}/\text{ml}$.

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I certify that an Examination Committee met on 24th March 2010 to conduct the final examination of Teo Siow Hwa on his Master of Science thesis entitled “The Phytochemistry and Biological Activities of *Artocarpus kemando* Miq. (Pudau) and *Artocarpus odoratissimus* Blanco (Terap) (Moraceae)” in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Putra 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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DECLARATION

I 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 Universiti Putra Malaysia or at any other institution.

TEO SIOW HWA

Date:

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LIST OF ABBREVIATIONS

α	alpha
β	beta
γ	gamma
δ	chemical shift in ppm
ϵ	epsilon
λ_{\max}	wavelength maxima in nm
μ	micro
μg	microgram
μl	microliter
μM	micromolar
$\mu\text{g/ ml}$	microgram per milliliter
AlCl_3	Aluminium(III) Chloride
br	broad
$^{\circ}\text{C}$	degree celcius
1D	one-dimensional
2D	two-dimensional
3D	three-dimentional
^{13}C	carbon-13
CC	Column Chromatography
CDCl_3	deuterated chloroform



CD ₃ COCD ₃	deuterated acetone
CD ₃ OD	deuterated methanol
CHCl ₃	chloroform
CH ₃ CH ₂ OH	absolute ethanol/ ethanol
C ₂ H ₅ OC ₂ H ₅	diethyl ester
CH ₃ COCH ₃	acetone
CH ₃ OH	methanol
CI	Chemical Ionization
CO ₂	carbon dioxide
COSY	Correlated Spectroscopy
CPTLC	Centrifugal Preparative Thin Layer Chromatography
cm	centimeter
cm ⁻¹	per centimeter
d	doublet
dd	doublet of doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DMEM	Dulbecco's Modified Engle's Medium
DMAPP	dimethylallyl pyrophosphate
DPPH	1,1-diphenyl-2-picrylhydrazyl
DPPH-H	1,1-diphenyl-2-picrylhydrazine
EC	Effective Concentration
EI	Electron Ionization

