UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF SELECTED ARTOCARPUS SPP

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CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF SELECTED *ARTOCARPUS* SPP

By

NAJIHAH BINTI MOHD. HASHIM

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of requirements for the degree of Doctor of Philosophy

CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF SELECTED *ARTOCARPUS* SPP

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April 2011

Chairman: Professor Mawardi Rahmani, PhD

Faculty: Science

Thirty three extracts of stem barks of eleven *Artocarpus* species from Moraceae family were preliminary screened *in vitro* for their biological activities. Four potential plants, *Artocarpus kemando*, *A. melinoxylus*, *A. obtusus* and *A. rigidus* had been selected based on the preliminary bioassay screenings results for further phytochemical and biological studies. The species were subjected for detail isolation work which involves extraction and isolation of compounds by using several chromatographic techniques. The structural elucidations of the isolated compounds were carried out using spectroscopic methods such as UV, IR, NMR, MS and also by comparison with the literature data. These techniques have led to the isolation and identification of several compounds of different classes, the flavonoid derivatives, a coumarin, a dipeptide and a sterol. The crude extracts and some of the isolated compounds were screened for cytotoxic, antioxidant, antimicrobial and tyrosinase inhibitory activity using MTT (Microculture Tetrazolium

Salt), DPPH (1,1-diphenyl-2-picrylhydrazyl), disc diffusion and dopachrome methods, respectively. The cell lines used in the cytotoxic assay were human promyelocytic leukemia (HL60), human chronic myeloid leukemia (K562), human hepatocarcinoma (HepG2), human colon cancer (HT29), human cervical cancer (HeLa), human estrogen receptor (ER+) positive breast cancer (MCF7), human estrogen receptor (ER-) negative (MDA-MB 231), peripheral blood mononuclear cells (PBMC) and human non-tumorigenic breast cell line (MCF10A). The antimicrobial activity was tested against several selected pathogenic microbes, *Bacillus subtilis* (clinically isolated strain), *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, Methicillin resistant *Staphylococcus aureus* (MRSA) (ATCC 33591), *Micrococcus luteus* ATCC 10240, *Pseudomonas aeruginosa* (JCM 2412), *Salmonella typhimurium* S865B (IMR culture), *Staphylococcus aureus* ATCC 6538, *Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 1023 and *Saccharomyces cerevisiae* S617 (IMR culture).

Detail study on *A. obtusus* has led to the isolation of three new flavonoid derivatives, pyranocycloartobiloxanthone A **(112)**, dihydroartoindonesianin C **(113)** and pyranocycloartobiloxanthone B **(114),** and a sterol, β-sitosterol **(115).** Phytochemical work of *A. rigidus* yielded pyranocycloartobiloxanthone A **(112)**, artoindonesianin C **(34)** and β-sitosterol **(115).** However**,** the isolation work on *A. melinoxylus* has also afforded a similar compound as previously isolated from the first two plants, pyranocycloartobiloxanthone A **(112)** and a known xanthone, cycloartobiloxanthone **(24)**. Similar work on *A. kemando* has yielded four interesting compounds, cycloartobiloxanthone **(24),** dihydroartoindonesianin C **(113)**, 6, 7– dimethoxycoumarin

(116) and aurantiamide benzoate **(117)**. The coumarin and the dipeptide are new isolated compounds from the *Artocarpus* species.

Only three compounds, namely, pyranocycloartobiloxanthone A **(112)**, dihydroartoindonesianin C **(113)** and pyranocycloartobiloxanthone B **(114)** were subjected to bioassay screenings due to inadequate amount of isolated compounds. The compounds exhibited various interesting activities towards the assays. However, pyranocycloartobiloxanthone A **(112)** is the most potential bioactive compound towards the cytotoxic, DPPH free radical scavenging, tyrosinase inhibitory and a broad spectrum of antimicrobial activity compared to the other two compounds. The study on antiproliferative activity using ELISA BrdU assay against HL60 and MCF7 cell lines, showed that pyranocycloartobiloxanthone A **(112)** was able to impair the DNA synthesis and cell proliferation. The cytotoxic effect of pyranocycloartobiloxanthone A **(112)** on the HL60 and MCF7 cell lines was studied based on the morphological manner for over 72 hours. The microscopic observations, including inverted microscopy of live cultures and fluorescent microscopy of acridine orange-propidium iodide stained cultures, showed that both necrotic and apoptotic death occurred in pyranocycloartobiloxanthone A **(112)** treated cell populations at IC_{50} concentrations.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KANDUNGAN KIMIA DAN AKTIVITI BIOLOGI DARIPADA SPESIES *ARTOCARPUS* YANG TERPILIH

Oleh

NAJIHAH BINTI MOHD. HASHIM

April 2011

Pengerusi: **Profesor Mawardi Rahmani, PhD**

Fakulti: **Sains**

Tiga puluh tiga ekstrak kulit pokok daripada sebelas spesies *Artocarpus* dari famili Moraceae telah menjalani penyaringan awal secara *in vitro* untuk aktiviti-aktiviti biologi mereka. Empat pokok yang berpotensi iaitu *Artocarpus kemando, A. melinoxylus, A. obtusus* and *A. rigidus* telah dipilih berdasarkan keputusan penyaringan awal bioassay untuk kajian fitokimia dan biologi yang lebih lanjut. Spesies tersebut digunakan untuk kajian pemencilan yang lebih mendalam yang melibatkan pengekstrakan dan pemencilan sebatian dengan menggunakan beberapa teknik kromatografi. Pengenalpastian struktur sebatian-sebatian ini telah dijalankan dengan menggunakan kaedah-kaedah spektroskopi seperti UV, IR, NMR, MS dan juga perbandingan dengan data literature. Teknik-teknik ini telah membawa kepada pemencilan dan pengenalpastian beberapa sebatian daripada spesies-spesies tersebut seperti terbitan-terbitan flavanoid, kumarin, dipeptida dan sterol.

Ekstrak-ekstrak mentah dan sebahagian sebatian-sebatian yang telah dipencilkan telah diuji aktiviti sitotoksik, antioksidan, antimikrob dan aktiviti penghambatan tirosinase dengan menggunakan kaedah MTT (garam Mikrokultur Tetrazolium), DPPH (1,1-difenil-2-pikrilhidrazil), peresapan cakera dan kaedah dopakrom. Sel-sel yang digunakna untuk ujikaji sitotoksik adalah sel leukemia promeilositik manusia (HL60), leukemia meiloid manusia (K562), hepatokarsinoma manusia (HepG2), kanser usus manusia (HT29), kanser servik manusia (HeLa), kanser payudara reseptor positif estrogen (ER+) manusia (MCF7), kanser payudara reseptor negatif estrogen (ER-) manusia (MDA-MB 231), sel mononuclear darah periferi (PBMC) dan sel payudara bukan tumor manusia (MCF10A). Aktiviti antimikrob telah diuji ke atas beberapa mikrob patogenik yang telah dipilih seperti *Bacillus subtilis* (strain yang dipencilkan secara klinikal), *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* resistan Methicillin (MRSA) (ATCC 33591), *Micrococcus luteus* ATCC 10240, *Pseudomonas aeruginosa* (JCM 2412), *Salmonella typhimurium* S865B (IMR culture), *Staphylococcus aureus* ATCC 6538, *Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 1023 dan *Saccharomyces cerevisiae* S617 (kultur IMR).

Kajian terperinci ke atas *A. obtusus* telah membawa kepada pemencilan tiga terbitan baru flavonoid, pyranocycloartobiloxanthone A **(112)**, dihydroartoindonesianin C **(113)** dan pyranocycloartobiloxanthone B **(114),** serta sterol, β-sitosterol **(115)**. Kajian fitokimia ke atas *A. rigidus* telah menghasilkan tiga sebatian, pyranocycloartobiloxanthone A **(112)**, artoindonesianin C **(34)** and β-sitosterol **(115).** Namun begitu, pemencilan ke atas *A. melinoxylus* telah memberikan sebatian yang mirip

dengan sebatian yang telah dipencilkan terdahulu daripada dua pokok yang pertama, pyranocycloartobiloxanthone A **(112)** dan xanthone yang telah dikenali iaitu cycloartobiloxanthone **(24)**. Kajian yang serupa ke atas *A. kemando* telah menghasilkan empat sebatian yang menarik iaitu cycloartobiloxanthone **(24),** dihydroartoindonesianin C **(113),** 6, 7– dimethoxycoumarin **(116)** dan aurantiamide benzoate **(117)**. Koumarin dan dipeptida adalah merupakan sebatian yang baru dipencilkan dari spesies *Artocarpus*.

Hanya tiga sebatian iaitu pyranocycloartobiloxanthone A **(112)**, dihydroartoindonesianin C **(113)** telah menjalani penyaringan awal bioassay, ini disebabkan sebatian yang lain mempunyai amaun yang pemencilan yang sangat sedikit. Namun begitu, pyranocycloartobiloxanthone A **(112)** telah menunjukkan sebatian bioaktif yang sangat berpotensi terhadap ujian sitotoksik, penangkapan radikal bebas DPPH, penghambatan tirosinase dan aktiviti antimikrobial yang meluas berbanding dengan dua sebatian yang lain. Kajian ke atas aktiviti antiproliferatif dengan menggunakan ujian ELISA BrdU ke atas sel HL60 dan MCF7 menunjukkan pyranocycloartobiloxanthone A **(112)** telah mampu mengganggu sintesis DNA dan proliferasi sel. Kesan sitotoksik pyranocycloartobiloxanthone A **(112)** terhadap sel HL60 dan MCF7 telah dikaji berdasarkan morfologi untuk selama 72 jam**.** Pemerhatian secara mikroskopik termasuklah menggunakan mikroskop terbalikkan ke atas kultur hidup dan mikroskop floresen ke atas kultur yang diwarnakan dengan akridin oren-propidium iodide, menunjukkan kematian apoptosis dan nekrosis telah berlaku di dalam populasi yang telah dirawat dengan pyranocycloartobiloxanthone A **(112)** pada kepekatan IC_{50}.

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I certify that a Thesis Examination Committee has met on 4th April 2011 to conduct the final examination of Najihah binti Mohd. Hashim on her thesis entitled "Chemical Constituents and Biological Activities of Selected *Artocarpus* Spp" in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of **Doctor of Philosophy**. The members of the Supervisory Committee were 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 institutions.

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NAJIHAH BINTI MOHD.HASHIM

Date: 4th April 2011

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