UNIVERSITI PUTRA MALAYSIA

SECONDARY METABOLITES FROM PEPPER (PIPER NIGRUM) AND TAHITIAN NONI (MORINDA CITRIFOLIA) AND THEIR BIOLOGICAL ACTIVITIES

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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Chairman : Associate Professor Gwendoline Ee Cheng Lian, PhD

Faculty : Science

Piper nigrum from the Piperaceae family and *Morinda citrifolia* from the Rubiaceae family were researched in this study. Detailed phytochemical investigations on the roots of these plants afforded ten pure compounds, which consists mainly of alkaloids and anthraquinones, and an acid. The structures of these compounds were elucidated based on ^{1}H NMR, ^{13}C NMR, COSY, DEPT, 2D NMR (HSQC and HMBC), mass spectrometry (MS), and Infrared spectroscopy (FTIR) analysis. Meanwhile, mixtures of compounds were identified through GCMS (gas chromatography-mass spectrometry).

From the roots of *Piper nigrum*, the alkaloids obtained are piperine, pellitorine and aristolactam AII. Besides that, the acid, 3,4-methylenedioxy benzoic acid was also present in the roots of this plant. Meanwhile, anthraquinones and their derivatives such as 1-hydroxy-2-methylanthraquinone, damnacanthal, nordamnacanthal, 2-formyl-1-hydroxyanthraquinone, 2-ethoxy-1-hydroxyanthraquinone and morindone-6-methylether were obtained from the roots of *Morinda citrifolia.* Among these

anthraquinones, 2-ethoxy-1-hydroxyanthraquinone is a new anthraquinone, while morindone-6-methylether is reported for the first time from the plant.

In the larvicidal test against the larvae of *Aedes aegypti*, the ethyl acetate extract of the roots of *Piper nigrum* showed good activity against the larvae. Pellitorine, on the other hand, gave a significant activity followed by piperine which only gave a moderate activity. The acid and aristolactam AII gave negative results which indicated that they are not biologically active towards the larvae of *Aedes aegypti*. For *Morinda citrifolia*, only the chloroform extract and the two anthraquinones, 1-hydroxy-2-methylanthraquinone and damnacanthal are strongly active.

From the cytotoxic activity, where tests were conducted using the HL-60 (Acute Promyelocytic Leukaemia) and MCF7 cell lines (Human Breast Adenocarcinoma), the ethyl acetate extract of *Piper nigrum* gave weak activity with an IC_{50} value of more than 30 µg/ml against the HL-60 cell line. However, in the tests against the same cell line using piperine and pellitorine, IC_{50} value of 7.5 µg/ml and 1.5 µg/ml, respectively, were obtained indicating the individual compounds to be strongly cytotoxic. Aristolactam AII gave weak activity with an IC_{50} value of more than 30 µg/ml when tested on the MCF-7.

Compared to the extract and alkaloids from *Piper nigrum*, the extracts and anthraquinones from *Morinda citrifolia* gave better results against both HL-60 and MCF7 cell lines. Three extracts amongst the five tested showed significant bioactivity against the HL-60 cell. These are the hexane extract, chloroform extract and petroleum ether extract, with IC_{50} values of 1.70 µg/ml, 9.3 µg/ml and 11.0

μg/ml, respectively. The methanol and acetone extracts showed moderate activities against the same cell line (HL-60). Damnacanthal, nordamnacanthal and 1-hydroxy-2-methylanthraquinone which were from the hexane and chloroform extracts gave IC_{50} values of 1.6 μg/mL, 4.4 μg/mL and 15.0 μg/mL, respectively, against the same cell line (HL-60). A comparison of the IC_{50} values of different extracts against the two cell lines indicated that these extracts did not give satisfying results against the MCF7 cell lines with only the chloroform and petroleum ether extracts giving a moderate activity.

The antifungal and antibacterial activities of the extracts and some compounds from the two plants were also evaluated. Fungi such as *Aspergilius ochraceaus* and *Sacchoromyces cerevisiae* were used in the antifungal screening. Meanwhile, MRSA, *Pseudomonas aeruginosa*, *Salmonella choleraesuis* and *Bacillus subtilis* were the microbes used in the antibacterial screenings. None of the extracts or alkaloids from *Piper nigrum* showed any inhibition in both screening.

As for *Morinda citrifolia*, only the chloroform and methanol extracts, and damnacanthal exhibited medium inhibition for *Bacillus subtilis* in the antibacterial screening. However, in the screening against *Salmonella choleraesuis*, only the chloroform extract and damnacanthal showed weak inhibition. Meanwhile, nordamnacanthal is the only sample tested that showed a very weak inhibition. In the antifungal screening, the two microbes were shown to be lightly susceptible to the chloroform extract and damnacanthal.

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

METABOLIT SEKUNDER DARIPADA *PIPER NIGRUM* DAN *MORINDA CITRIFOLIA* SERTA AKTIVITI BIOLOGINYA

Oleh

WEN YIN PING

Mac 2009

Pengerusi : **Associate Professor Gwendoline Ee Cheng Lian, PhD**

Fakulti **: Sains**

Piper nigrum dari famili Piperaceae dan *Morinda citrifolia* yang dari famili Rubiaceae telah diselidiki dalam kajian ini. Kajian fitokimia mendalam terhadap akar kedua-dua jenis tumbuhan ini telah menghasilkan sebanyak 10 sebatian tulen kebanyakannya adalah terdiri daripada alkaloid dan anthrakuinon, dan satu asid organik. Sebatian-sebatian ini telah dikenalpastikan berdasarkan analisis ^{1}H NMR, ^{13}C NMR, COSY, DEPT, 2D NMR (HSQC and HMBC), spektrometri jisim (MS), dan spektroskopi inframerah (IR). Manakala untuk sebatian-sebatian dalam campuran telah dikenalpasti melalui kaedah GCMS (kromatografi gas-spektrometri jisim).

Dari akar *Piper nigrum*, alkaloid yang diperolehi ialah piperin, pellitorin dan aristolaktam AII. Di samping itu, asid 3,4-metilenedioksi benzoik juga terkandung dalam akar tumbuhan ini. Manakala anthrakuinon dan juga sebatian-sebatian terbitannya seperti 1-hidroksi-2-metilanthrakuinon, damnacanthal, nordamnacanthal, 2-formil-1-hidroksianthrakuinon, 2-etoksi-1-hidroksianthrakuinon dan 6-metileter-morindon telah diperoleh daripada *Morinda citrifolia.* Antara anthrakuinon-

anthrakuinon ini, 2-etoksi-1-hidroksiantrakuinon merupakan satu anthrakuinon yang baru sedangkan 6-metileter-morindon dilaporkan buat pertama kalinya daripada *Morinda citrifolia*.

Dalam ujian larvisidal terhadap nyamuk *Aedes aegypti*, ekstrak mentah etil asetat daripada akar *Piper nigrum* telah menunjukkan aktiviti yang baik. Manakala pellitorin mempamerkan keputusan yang nyata baik, diikuti oleh piperin yang hanya memberi keputusan yang sederhana. Asid dan aristolaktam AII pula memberikan keputusan negatif dalam ujian ini yang membawa makna bahawa mereka tidak aktif terhadap larva *Aedes aegypti*. Untuk *Morinda citrifolia*, hanya ekstrak mentah klorofom serta dua anthrakuinon, 1-hidroksi-2-metilanthrakuinon dan damnacanthal sahaja yang menunjukkan kebioaktifan yang kuat.

Daripada aktiviti sitotoksik yang dijalankan menggunakan sel HL-60 (Acute Promyelocytic Leukaemia) dan sel MCF7 (Human Breast Adenocarcinoma), ekstrak etil asetat *Piper nigrum* menunjukkan aktiviti yang lemah terhadap sel HL-60 dengan nilai IC_{50} yang melebihi 30 µg/mL. Akan tetapi dalam ujian terhadap sel yang sama, dua sebatian yang diekstrak daripada ekstrak ini, piperin dan pellitorin, menunjukkan keputusan yang memuaskan dengan nilai IC_{50} masing-masing 7.5 µg/mL dan 1.5 µg/mL. Aristolaktam AII pula menunjukkan keputusan negatif terhadap sel MCF-7 dengan nilai IC_{50} yang melebihi 30 µg/mL.

Jika dibandingkan dengan ekstrak dan alkaloid daripada *Piper nigrum*, ekstrak dan anthrakuinon daripada *Morinda citrifolia* telah memberikan keputusan yang lebih positif terhadap kedua-dua sel tersebut. Daripada lima ekstrak yang dikaji, tiga

daripadanya menunjukkan bioaktiviti yang jelas terhadap sel HL-60. Tiga ekstrak ini ialah ekstrak heksana, ekstrak klorofom dan ekstrak petroleum-eter dengan nilai IC_{50} masing-masing, 1.70 µg/mL, 9.3 µg/mL dan 11.0 µg/mL. Manakala, ekstrak metanol dan aseton menunjukkan keaktifan yang sederhana terhadap sel yang sama (HL-60). Damnacanthal, nordamnacanthal dan 1-hydroksi-2-metilanthrakuinon yang diasingkan daripada ekstrak heksana dan klorofom memberi nilai IC_{50} masing-masing, 1.6 µg/mL, 4.4 µg/mL dan 15.0 µg/mL terhadap jenis sel-sel yang sama. Perbandingan antara kesan sitotoksik ekstrak-ekstrak terhadap kedua-dua jenis sel menunjukkan bahawa ekstrak-ekstrak ini tidak memberikan keputusan yang bagus terhadap sel MCF-7. Hanya ekstrak klorofom dan petroleum-eter yang memberikan aktiviti sederhana terhadap sel ini.

Aktiviti antifungal dan antibakteria untuk ekstrak dan sebatian-sebatian juga telah diuji. Fungi seperti *Aspergilius ochraceaus* dan *Sacchoromyces cerevisiae* telah digunakan dalam penyaringan antifungal. Manakala, MRSA, *Pseudomonas aeruginosa*, *Salmonella choleraesuis* dan *Bacillus subtilis* adalah mikrob yang digunakan dalam penyaringan antibakteria. Tiada sebarang tanda rencatan yang ditunjukkan oleh ekstrak mahupun alkaloid daripada *Piper nigrum* dalam kedua-dua penyaringan ini.

Manakala untuk *Morinda citrifolia*, hanya ekstrak klorofom dan metanol dan damnacanthal menunjukkan rencatan yang sederhana terhadap *Bacillus subtilis* dalam penyaringan antibakteria. Akan tetapi, dalam penyaringan terhadap *Salmonella choleraesuis*, hanya ekstrak klorofom dan damnacanthal menunjukkan aktiviti rencatan yang sederhana. Manakala nordamnacanthal merupakan satu-satu

sampel yang menunjukkan rencatan yang lemah. Dalam penyaringan antifungal, kedua-dua jenis mikrob telah menunjukkan sensitiviti yang lemah terhadap ekstrak klorofom dan damnacanthal.

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I certify that a Thesis Examination Committee has met on 6th March 2009 to conduct the final examination of Wen Yin Ping on her thesis entitled "Secondary Metabolites from Pepper (*Piper nigrum*) and Tahitiam Noni (*Morinda citrifolia*) and Their Biological Activities" in accordance with the 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 degree of Master of Science.

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I certify that a Thesis Examination Committee has met on 6th March 2009 to conduct the final examination of **Wen Yin Ping** on her thesis entitled "**Secondary Metabolites from Piper nigrum and Morinda citrifolia and Their Biological Activities**" in accordance with the 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 degree of Master of Science.

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α	alpha
β	beta
δ	chemical shift in ppm
λ_{max}	wavelength maxima in nm
ν_{max}	wavenumber maxima in cm^{-1}
µg	microgram
br	broad
^{13}C	carbon-13
$CDCl_3$	deuterated chloroform
$CHCl_3$	chloroform
COSY	Correlated Spectroscopy
d	doublet
dd	doublet of doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	deuterated dimethylsulfoxide
EIMS	Electron ionization mass-spectroscopy
EtOAc	ethyl acetate
$FeCl_3$	Ferric chloride
FTIR	Fourier Transform Infra Red
^{1}H	proton
HMBC	Heteronuclear Multiple Bond Connectivity by 2D Multiple Quantum
HMQC	Heteronuclear Multiple Quantum Coherence
Hz	hertz
IC	Inhibition Concentration