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PHYTOCHEMICAL STUDIES OF GARCINIA EUGENIFOLIA WALL., G. NITIDA PIERRE., G. MANGOSTANA L., AND MORINDA CITRIFOLIA L. AND THEIR BIOLOGICAL ACTIVITIES

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By

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PHYTOCHEMICAL STUDIES OF *GARCINIA EUGENIFOLIA* WALL., *G. NITIDA* PIERRE., *G. MANGOSTANA* L., AND *MORINDA CITRIFOLIA* L. AND THEIR BIOLOGICAL ACTIVITIES

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Chairman : Professor Gwendoline Ee Cheng Lian, PhD

Faculty : Science

Phytochemical studies were carried out on four plants, *Garcinia eugenifolia, Garcinia nitida, Garcinia mangostana* and *Morinda citrifolia*. The chemical investigation has resulted in the isolation of 22 compounds which covered xanthones, triterpenoids and quinones. These compounds were isolated using common chromatographic techniques and were identified using spectroscopic experiments such as NMR, MS, IR and UV.

The roots of *Garcinia eugenifolia* afforded six compounds comprising three triterpenoids, β-sitosterol **(145)**, magniferolic acid **(147)** and euphadienol **(148)**; two methanone, (3'-hydroxyphenyl)(2,4,6-trihydroxyphenyl)methanone **(149)** and (3,4-dihydroxyphenyl)(3-hydroxy-5-methoxyphenyl)methanone **(150);** and a xanthone, 1,6-dihydroxy-7-methoxy-6',6'-dimethyl-2H-pyrano[2',3':3,2]-xanthone **(146)**. (3,4-dihydroxyphenyl)(3-hydroxy-5-methoxyphenyl)methanone **(150)** was isolated as a new compound. Besides this, the major compound, magniferolic acid **(147)** was

isolated for the first time from *Garcinia eugenifolia*. Meanwhile, the roots of *Garcinia nitida* afforded a new compound 1,6-dihydroxy-5-methoxy-6,6-dimethyl-pyrano[2',3':2,3]-xanthone **(151)**, together with three known xanthones and two common triterpenoids, inophyllin B **(152)**, caloxanthone A **(154)**, rubraxanthone **(156)** stigmasterol **(153)** and friedelin **(155).**

Garcinia mangostana (roots) provided three pure compounds, α-mangostin **(157)**, β-mangostin **(158)** and cowagarcinone B **(160)**. β-mangostin was isolated as the major compound from the roots of *Garcinia mangostana*. Acetylation of β-mangostin **(158)** successfully yielded 1-hydroxy-3,7-dimethoxy-2,8-bis(3-methyl-2-butenyl)-9H-xanthen-9-one-6-acetate **(159)** as the product. Meanwhile, purification of the roots of *Morinda citrifolia* using chromatotron gave seven anthraquinones, damnacanthal **(161)**, nordamnacanthal **(162)**, sorandidiol **(163)**, rubiadin **(164)**, 1-hydroxy-2-methylanthraquinone **(165)**, 2-ethoxy-1-hydroxyanthraquinone **(166)** and rubiadin-1-methyl ether **(167)**.

Cytotoxic tests were carried out using HeLa, MCF-7 and HL-60 cell lines on the crude extracts of *Garcinia eugenifolia* and *Garcinia nitida*. The pure compounds from all the four plants were assayed against HT-29 (Human Colorectal Cancer) and A549 (Human Lung Cancer). *Garcinia eugenifolia* were found to show moderate cytotoxicity with IC_{50} values ranging from 11 to 77 µg/mL for HeLa and MCF-7 cell lines. Meanwhile, for the HL-60 cell line, the hexane and ethyl acetate extracts of *Garcinia eugenifolia* indicated strong cytotoxicities with IC_{50} values of 1.9 µg/mL and 2.5 µg/mL respectively. For *Garcinia nitida*, the leaf extracts showed strong cytotoxic activity with IC_{50} values of 4 and 7 µg/mL respectively towards the HeLa

and MCF-7 cell lines. However, the crude extract of the roots and twigs of *Garcinia nitida* was found to be inactive to MCF-7 activity test and weak cytotoxic activity against the HeLa cell line.

Twelve pure compounds were subjected to cytotoxic assays using HT-29 and A549 cell lines. Magniferolic acid **(147)** gave IC_{50} values of 4.8 and 4.7 µg/mL respectively towards HT-29 and A549 cell lines while euphadienol **(148)** gave an IC_{50} value of 2.7 µg/mL towards the A549 cell line but showed moderate inhibition to HT-29 cell line. Meanwhile, 2-ethoxy-1-hydroxyanthraquinone **(166)** gave strong cytotoxicities (IC_{50} = 4.5 µg/mL) towards HT-29 but moderate activities (11.2 µg/mL) against A549. Both damnacanthal **(161)** and nordamnacanthal **(162)** showed moderate inhibitory activities (IC_{50} <10 µg/mL) towards HT-29 cell line.

The antimicrobial activity test was also carried out using five pathogenic bacteria, namely, *Staphylococcus aures*, *Pseudomonas aeruginosa*, *Clostridium difficile*, *Streptococcus pyogenes* and *Escherichia coli*. It was observed that most of the crude extracts from both *Garcinia* species exhibited strong inhibitory activities against the microbes. The only weak activity observed was for the *Staphylococcus aures* microbe for both methanol and ethyl acetate extracts of *Garcinia nitida* with an inhibitory diameter ranging from 4 to 8 mm.

The DPPH antioxidant assay was carried out for both *Garcinia eugenifolia* and *Garcinia nitida*. Of all the tested extracts of *Garcinia eugenifolia* and *Garcinia nitida*, only the methanol extract from *Garcinia eugenifolia* stem bark and leaves exhibited moderate antioxidant activity (EC_{50} = 21.1 and 29.5 µg/L) when compared

to ascorbic acid with EC_{50} values of 15.32 µg/L. As for total phenolic content, the twigs of *Garcinia eugenifolia* had the highest total phenolic content of 0.2322 ± 0.059 µg GAE/mg extract, followed by the roots (0.2274 ± 0.067 µg GAE/mg extract) and stem (0.2138 ± 0.013 µg GAE/mg extract).

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

KAJIAN FITOKIMIA DAN AKTIVITI-AKTIVITI BIOLOGI DARIPADA *GARCINIA EUGENIFOLIA* WALL., *G. NITIDA* PIERRE., *G. MANGOSTANA* L., DAN *MORINDA CITRIFOLIA* L.

Oleh

VIVIEN JONG YI MIAN

Jun 2012

Pengerusi : Profesor Gwendoline Ee Cheng Lian, PhD

Fakulti : Sains

Kajian fitokimia telah dijalankan ke atas empat tumbuh-tumbuhan, *Garcinia eugenifolia*, *Garcinia nitida*, *Garcinia mangostana* dan *Morinda citrifolia*. Kajian kimia terperinci menghasilkan 22 sebatian semulajadi yang merangkumi xanthon, triterpenoid dan kuinon. Sebatian-sebatian ini telah diasingkan dengan menggunakan teknik-teknik kromatografi biasa dan telah dikenal pasti dengan menggunakan eksperimen spektroskopi seperti NMR, MS, IR dan UV.

Akar daripada *Garcinia eugenifolia* telah memberikan enam sebatian iaitu tiga triterpenoid, β-sitosterol **(145)**, asid magniferolik **(147)** dan euphadienol **(148)**; dua metanon, (3'-hidroksifenil)(2,4,6-trihidroksifenil)metanon **(149)** dan (3,4-di hidroksifenil)(3-hidroksi-5-metoksifenil)metanon **(150);** dan satu xanthon, 1,6-dihidroksi-7-metoksi-6',6'-dimetil-2H-pyrano[2',3':3,2]-xanthon **(146)**. (3,4-di hidroksifenil)(3-hidroksi-5-metoksifenil)metanon **(150)** telah diasingkan sebagai sebatian yang baru. Selain itu, sebatian utama, asid magniferolik **(147)** juga dihasilkan untuk kali yang pertama daripada *Garcinia eugenifolia*. Sementara itu,

akar *Garcinia nitida* memberikan satu sebatian baru iaitu 1,6-dihidroksi-7-metoksi-6',6'-dimetil-2H-pyrano[2',3':3,2]-xanthon **(151)**, bersama dengan tiga xanthon yang diketahui dan dua triterpenoid biasa, inophyllin B **(152)**, caloxanthone A **(154)**, rubraxanthone **(156)** stigmasterol **(153)** dan friedelin **(155)**.

Garcinia mangostana (akar) menghasilkan tiga sebatian tulen, α-mangostin **(157)**, β-mangostin **(158)** dan cowagarcinone B **(160)**. β-mangostin diasingkan sebagai sebatian utama daripada akar *Garcinia mangostana*. Pengasetilan β-mangostin **(158)** berjaya menghasilkan 1-hidroksi-3,7-dimetoksi-2,8-bis(3-metil-2-butenyl)-9H-xanthen-9-one-6-asetat **(159)** sebagai produk. Manakala penulenan akar *Morinda citrifolia* menggunakan chromatotron memberi tujuh antrakuinon, damnacanthal **(161)**, nordamnacanthal **(162)**, sorandidiol **(163)**, rubiadin **(164)**, 1-hidroksi-2-metilantrakuinon **(165)**, 2-etoksi-1-hidroksiantrakuinon **(166)** dan rubiadin-1-metil eter (**167**).

Ujian sitotoksik telah dijalankan menggunakan sel HeLa, MCF-7 dan HL-60 untuk ekstrak mentah *Garcinia eugenifolia* dan *Garcinia nitida*. Manakala sebatian tulen daripada semua empat tumbuhan dijalankan terhadap sel HT-29 (Kanser Kolorektal Manusia) dan A549 (Kanser paru-paru Manusia). *Garcinia eugenifolia* telah didapati menunjukkan sitotoksik yang sederhana dengan nilai IC_{50} antara 11 hingga 77 µg/mL untuk sel HeLa dan MCF-7. Sementara itu, bagi sel HL-60, ekstrak heksana dan etil asetat *Garcinia eugenifolia* menunjukkan aktiviti sitotoksik yang baik dengan nilai IC_{50} 1.9 µg/mL dan 2.5 µg/mL masing-masing. Bagi *Garcinia nitida*, ekstrak daun menunjukkan aktiviti sitotoksik yang baik dengan nilai IC_{50} 4 dan 7 µg/mL masing-masing terhadap sel HeLa dan MCF-7. Walau bagaimanapun, ekstrak

mentah akar dan ranting *Garcinia nitida* didapati tidak aktif untuk ujian aktiviti sel MCF-7 dan sitotoksik yang lemah terhadap sel HeLa.

Dua belas sebatian-sebatian tulen telah diuji sitotoksik menggunakan sel HT-29 dan A549. Asid magniferolik **(147)** memberi nilai IC_{50} sebanyak 4.8 dan 4.7 µg/mL masing-masing pada sel HT-29 dan A549 manakala euphadienol **(148)** memberi nilai IC_{50} sebanyak 2.7 µg/mL terhadap sel A549 tetapi menunjukkan perencatan yang sederhana pada sel HT-29. Sementara itu, 2-etoksi-1-hidroksiantrakuinon **(166)** memberi sitotoksik yang kuat (IC_{50} = 4.5 µg/mL) terhadap sel HT-29 tetapi aktiviti yang sederhana (11.2 µg/mL) terhadap sel A549. Kedua-dua damnacanthal **(161)** dan nordamnacanthal **(162)** menunjukkan aktiviti perencatan yang sederhana (IC_{50} <10 µg/mL) pada sel HT-29.

Ujian aktiviti antimikrob juga dijalankan dengan menggunakan lima bakteria patogenik, iaitu, *Staphylococcus aures*, *Pseudomonas aeruginosa*, *Clostridium difficile*, *Streptococcus pyogenes* dan *Escherichia coli*. Adalah diperhatikan bahawa sebahagian besar daripada ekstrak mentah dari kedua-dua spesies Garcinia mempamerkan aktiviti perencatan yang tinggi terhadap mikrob. Satu-satunya aktiviti lemah diperhatikan untuk mikrob *Staphylococcus aures* bagi kedua-dua ekstrak metanol dan etil asetat *Garcinia nitida* dengan diameter perencatan antara 4 hingga 8 mm.

Ujian antioksidan DPPH telah dijalankan untuk kedua-dua *Garcinia eugenifolia* dan *Garcinia nitida* untuk ujian DPPH. Daripada kesemua ekstrak yang diuji, hanya ekstrak metanol daripada batang kulit dan daun pokok *Garcinia eugenifolia*

menunjukkan aktiviti antioksida yang sederhana (EC_{50} = 21.1 dan 29.5 µg/L) berbanding dengan asid askorbik dengan nilai EC_{50} 15.32 µg/L. Bagi jumlah kandungan fenolik, ranting *Garcinia eugenifolia* mempunyai jumlah kandungan fenolik tertinggi sebanyak 0.2322 ± 0.059 µg GAE/mg ekstrak, diikuti oleh akar (0.2274 ± 0.067 µg GAE/mg ekstrak) dan batang (0.2138 ± 0.013 µg GAE/mg ekstrak).

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I certify that a Thesis Examination Committee has met on 6 June 2012 to conduct the final examination of Vivien Jong Yi Mian on her thesis entitled "**Phytochemical Studies of *Garcinia eugenifolia* Wall., *G. nitida* Pierre., *G. mangostana* L., and *Morinda citrifolia* L. 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 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 fulfillment 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 quotation and citations which have been duly acknowledged. I also declare that it has been previously and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

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VIVIEN JONG YI MIAN

Date: 6 June 2012

TABLE OF CONTENTS