UNIVERSITI PUTRA MALAYSIA

ISOLATION, CHARACTERIZATION AND PRELIMINARY PHARMACOLOGICAL EVALUATION OF CONSTITUENTS OF GARCINIA COWA ROXB

FATMA SRI WAHYUNI
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Kesempurnaan seseorang manusia itu dapat dilihat dari jauh dan dekatnya kepada Allah SWT. Selama ilmunya banyak dan sempurna, maka ia semakin dekat kepada Allah SWT, dan semakin menyerupai malaikat (Imam al-Ghazali)

To my daughter and son:
Meliannisa’ Afader and Muhammad Rizki Afader
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

ISOLATION, CHARACTERIZATION AND PRELIMINARY PHARMACOLOGICAL EVALUATION OF CONSTITUENTS OF GARCINIA COWA ROXB

By

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Preliminary screening for in vitro cytotoxic and inhibition nitric oxide (NO) production activities were carried out on fourteen extracts of different parts of eight Garcinia species. These extracts were evaluated for cytotoxic activity using microculture tetrazolium, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay by measuring the reduction of viability of cell cultures in the presence and absence of the extracts. Four type of cancer cell lines, HL-60 (leukemia), MCF-7 (breast), DU-145 (prostate) and NCI-H460 (non-small cell lung) were used for a preliminary picture of extracts selectivity profile. NO inhibition (in lipopolysaccharide (LPS)-activated macrophages) activity was evaluated using Griess assay.

Extract of the stem bark from G. cowa Roxb showed selective cytotoxicity towards NCI-H460 (IC\textsubscript{50} = 11 \mu g/mL). The extract also exhibited inhibition of NO production (IC\textsubscript{50} =
25 μg/mL) without being cytotoxic to RAW 264.7 cells. Based on these promising selective cytotoxicity towards NCI-H460 and NO inhibitory activities, *G. cowa* was selected for further studies on the isolation and identification its active components.

Bioassay-guided isolation of the stem extracts, yielded seven cytotoxic compounds. Utilising various spectroscopic (EI-MS, UV, IR, NMR and HRMS) analyses, three of them were identified as new compounds and characterized as [2E,6E,10E]-(+)-4β-hydroxy-3-methyl-5β-(3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraenyl-2-cyclohexen-1-one (85), 2-(3-methyl-2-butenyl)-1,5,6-trihydroxy-3-methoxy-4-(1,1-dimethyl-2-propenyl)-9H-xanthen-9-one (87) and 1,3,6-trihydroxy-7-methoxy-4-(4-acetoxy-3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl)xanthone (88). Four other known compounds were elucidated on the basis of their spectroscopy data and comparison with the literature. They were identified as rubraxanthone (5), cowanin (52), α-mangostin (81) and hydroxycalabaxanthone (86). Three compounds isolated from leaves of *G. cowa* including methyl 2,4,6-trihydroxy-3-(3-methylbut-2-etyl)benzoate (89), garcinisidone-A (90) and 3-(1-methoxycarbonyl-4,6-dihydroxyphenox)-6-methoxy-5,5-dimethyl-2-butenyl)-1,4-benzoquinone (91). Compounds 89 and 91 were identified as new compounds. In addition, rubraxanthone (5) and cowanin (52) were reisolated from roots of this plant together with 1,5-dihydroxyxanthone (92).

Compounds 81, 86, 87, 88, 89, 90 and 91 exhibited moderate to strong cytotoxic activity on tested cells. However, only compound 85 showed selectivity towards NCI-H460 cell
line with IC\textsubscript{50} value of 16.3 µM ± 3.0. The effect of compound 85 on the cell cycle progression of NCI-H460 cells was evaluated by using a flow cytometer. Compound 85 was found to significantly arrest cells growing, predominantly in the G\textsubscript{1} phase. Furthermore, compound 85 was subjected to \textit{in vivo} antitumor assay on NCI-H460 xenografted nude mice. Interestingly, compound 85 slowed the tumor growth by 5 days.

Compound 85 was subjected to Griess assay along with rubraxanthone (5) and α-mangostin (81). It was realised that the inhibition of NO production by compound 85 was most likely due to their cytotoxicity, with % inhibition of NO production value of 81% and 40% of cell viability. However, inhibition of NO production by α-mangostin 81 is high with 83% inhibition and 82% cell viability. Rubraxanthone 5 showed weak inhibition of NO production with 24% inhibition value and 77% of cell viability.
Kajian awal terhadap aktiviti sitotoksik dan perencatan penghasilan nitrik oksida (NO) telah dilakukan ke atas 14 ekstrak pelbagai bahagian dari lapan *Garcinia* spesies. Eksrak ini diuji aktiviti sitotoksiknya dengan kaedah 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Barisan sel kanser, HL-60 (leukemia), MCF-7 (kanser payudara), DU-145 (prostat) dan NCI-H460 (paru-paru) dipakai untuk kajian sitotoksik ekstrak. Kaedah Griess digunakan untuk menentukan perencatan pendhasilan NO.

Ekstrak kulit batang *G. cowa* Roxb didapati mempunyai aktiviti kesitotoksikan yang selektif terhadap NCI-H460 (IC\textsubscript{50} = 11 µg/mL). Ekstrak ini juga didapati menunjukkan aktiviti perencatan produksi NO (IC\textsubscript{50} = 25 µg/mL) tanpa menunjukkan kesitotoksikan.
terhadap sel RAW 264.7. *Garcinia cowa* Roxb telah dipilih untuk kajian lebih lanjut berdasarkan keputusan penilaian aktiviti-aktiviti biologi tersebut.

Pengasingan dan penulenan ekstrak kulit batang *G. cowa* berpandukan biocerakin telah membawa kepada tujuh penemuan sebatian. Daripada tujuh sebatian tersebut, tiga merupakan sebatian baru dan dicirikan sebagai \([2E,6E,10E]-(+)-4\beta\)-hidroksi-3-metil-5β-(3,7,11,15-tetrametil-2,6,10,14-heksadecatetraenil-2-sikloheksen-1-on (85), 2-(3-metil-2-butenil)-1,5,6-trihidroksi-3-metoksi-4-(1,1-dimetil-2-propenil)-9H-xanten-9-on (87) and 1,3,6-trihidroksi-7-metoksi-4-(4-asetoksi-3-metil-2-butenil)-8-(3,7-dimetil-2,6-octa\-dienil)xanton (88). Empat sebatian lainnya dicirikan sebagai rubraxanton (5), cowanin (52), α-mangostin (81) and 6-hidroksikalabaxanton (86). Tiga sebatian telah diaisingkan dari daun *G. cowa* dan dicirikan sebagai metil 2,4,6-trihidroksi-3-(3-metilbut-2-enil)benzoat (89), garsinisol-A (90) dan 3-(1-metoksikarbonil-4,6-dihidroksipenoksi)-6-metoksi-5,5-dimetil-2-butenil)-1,4-benzokuinon (91). Dua dari- padanya iaitu sebatian 89 dan 91 merupakan sebatian baru. Rubraxanton (5) and cowanin (52) telah diaisingkan kembali bersamaan dengan 1,5-dihidroksixanton (92) dari akar *G. cowa*.

Sebatian–sebatian tersebut diuji aktiviti sitotoksik menggunakan kaedah mikrotitratan (MTT) terhadap sel HL-60, MCF-7, DU-145 adan NCI-H460. Didapati Sebatian 81, 86, 87, 88, 89, 90 and 91 menunjukkan aktiviti baik hingga tinggi ke atas sel uji. Walaupun begitu, sebatian 85 memberikan aktiviti yang selektif kepada NCI-H460 (IC₅₀=16.3 µM ± 3.0).

Rubraxanton (5), α-mangostin (81) dan sebatian 85 dilakukan uji terhadap perencatan NO. Rubraxanton (5) menunjukkan perencatan NO dengan nilai peratus 24% dan 77% nilai kehidupan sel RAW 264.7. α-Mangostin (81) memberikan nilai peratus perencatan NO 83%, dan nilai kehidupan sel RAW masing-masingnya 82%. Perencatan NO oleh sebatian 85 memberikan nilai peratus 81% dengan 40% nilai kehidupan sel RAW 264.7.
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I certify that a Thesis Examination Committee has met on 11 Augustus 2009 to conduct the final examination of Fatma Sri Wahyuni on her thesis entitled “Isolation, characterization and preliminary pharmacological evaluation of constituents of *Garcinia cowa* Roxb” 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 Doctor of Philosophy.

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Date: 14 January 2010
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree at UPM or other institutions.

FATMA SRI WAHYUNI

Date: November 2009
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LIST OF ABBREVIATIONS

α  Alpha
β  Beta
γ  Gamma
δ  Delta, chemical shift in ppm
µg  Microgram
µl  Microliter
µM  Micromolar
br  Broad
$^{13}$C  Carbon-13
d  Doublet
dd  Doublet of doublets
ddd  Doublet of doublets of doublets
CDK  cyclin dependent kinase
COSY  Correlation Spectroscopy
DNA  deoxyribonucleic acid
DEPT  Distortionless Enhancement by Polarization Transfer
DMSO  Dimethylsulfoxide
EIMS  Electron Impact Mass Spectrum
EtOAc  Ethyl acetate
eV  Electron volt
FTIR  Fourier Transform Infra-Red