



**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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**Institute : Bioscience**

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<sub>50</sub> = 11 µg/mL). The extract also exhibited inhibition of NO production (IC<sub>50</sub>=

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 [2*E*,6*E*,10*E*]-(+)-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)-9*H*-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-enyl)benzoate (**89**), garcinisidone-A (**90**) and 3-(1-methoxycarbonyl-4,6-dihydroxyphenoxy)-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  $IC_{50}$  value of  $16.3 \mu\text{M} \pm 3.0$ . 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  $G_1$  phase. Furthermore, compound **85** was subjected to *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  $\alpha$ -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  $\alpha$ -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.



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

**PENGASINGAN, PEGENALPASTIAN DAN PENILAIAN PHARMACOLOGY  
AWAL SEBATIAN-SEBATIAN DARI *GARCINIA COWA* ROXB**

Oleh

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Kajian awal terhadap aktiviti sitotoksik dan perencatan penghasilan nitrik oksida (NO) telah dilakukan ke atas 14 ekstrak pelbagai bahagian dari lapan *Garcinia* spesies. Ekstrak 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 ( $IC_{50} = 11 \mu\text{g/mL}$ ). Ekstrak ini juga didapati menunjukkan aktiviti perencatan produksi NO ( $IC_{50} = 25 \mu\text{g/mL}$ ) 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 [2*E*,6*E*,10*E*]-(+)-4 $\beta$ -hidroksi-3-metil-5 $\beta$ -(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)-9*H*-xanten-9-on (**87**) and 1,3,6-trihidroksi-7-metoksi-4-(4-asetoksi-3-metil-2-butenil)-8-(3,7-dimetil-2,6-octadienil)xanton (**88**). Empat sebatian lainnya dicirikan sebagai rubraxanton (**5**), cowanin (**52**),  $\alpha$ -mangostin (**81**) and 6-hidroksikalabaxanton (**86**). Tiga sebatian telah diasingkan dari daun *G. cowa* dan dicirikan sebagai metil 2,4,6-trihidroksi-3-(3-metilbut-2-enil)benzoat (**89**), garsinisidon-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 diasingkan 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 dan 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 ( $IC_{50}=16.3 \mu M \pm 3.0$ ).

Analisis kitaran sel sebatian **85** terhadap sel NCI-H460 telah dilakukan dengan kaedah aliran sitrometri. Sebatian **85** didapati menghambat kitaran sel NCI-H460 pada fasa G<sub>1</sub>. Uji terhadap sebatian **85** dilanjutkan ke kajian *in vivo*. Menariknya, sebatian ini dapat melambatkan selama 5 hari pertumbuhan sel NCI-H460 yang ditanamkan ke tikus percubaan, berbanding kawalan.

Rubraxanton (**5**),  $\alpha$ -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.  $\alpha$ -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.

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**FATMA SRI WAHYUNI**

Date: November 2009



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## LIST OF ABBREVIATIONS

$\alpha$	Alpha
$\beta$	Beta
$\gamma$	Gamma
$\delta$	Delta, chemical shift in ppm
$\mu\text{g}$	Microgram
$\mu\text{l}$	Microliter
$\mu\text{M}$	Micromolar
br	Broad
$^{13}\text{C}$	Carbon-13
<i>d</i>	Doublet
<i>dd</i>	Doublet of doublets
<i>ddd</i>	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

