

**BIOACTIVITY-GUIDED ISOLATION OF CYTOTOXIC COMPOUNDS FROM
*GARCINIA UROPHYLLA***

By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
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Chairman : Professor Md. Nordin Hj Lajis, PhD

Institute : Bioscience

From a selective cytotoxic activity screening program on fifteen species of plants using MTT cytotoxic assay, *Garcinia urophylla* showed the most potent cytotoxic activity. The methanolic extract was tested on MCF-7 cells (hormone dependent breast cancer cell line), and showed the IC₅₀ of 2.7 µg/mL. *Garcinia urophylla* or locally known as “Kandis hutan”, is a small fruiting tree usually scattered through out the hills in Malaysia and Sumatra. Up to now, there have not been any studies conducted on *Garcinia urophylla*.

The methanolic extract of *Garcinia urophylla* was fractionated into hexane, dichloromethane (CH₂Cl₂), ethyl acetate (EtAOc) and butanol (BuOH) fractions. The fractions were tested for cytotoxic activity, and CH₂Cl₂ extract was found to be the most active, with IC₅₀ = 3 µg/mL. The active CH₂Cl₂ fraction gave 7 compounds including lupeol (**57**), gaudichaudione H (**58**), nor-deoxymorellin (**59**), 1,2,5-trihydroxy-3-methoxy-4-(3-methyl-2-butenyl)xanthone (**60**), 1,7-dihydroxy-3-methoxy-2-(3-methyl-2-butenyl)xanthone (**62**), 1,5-dihydroxy-3-methoxy-2-(3-methyl-2-butenyl)xanthone (**63**)

and 1,3,7-trihydroxy-2-(3-methyl-2-butenyl)xanthone (**64**). All compounds except for lupeol showed cytotoxic activity ($IC_{50} < 100\mu\text{g/ml}$) towards MCF-7. The IC_{50} values on MCF-7 for each compounds are; $> 100 \mu\text{g/ml}$ for **57**, $3.7 \pm 0.7 \mu\text{g/ml}$ for **58**, $25.0 \pm 4.9 \mu\text{g/ml}$ for **59**, $22.6 \pm 4.1 \mu\text{g/ml}$ for **60**, $21.6 \pm 3.4 \mu\text{g/ml}$ for **62**, $2.0 \pm 0.5 \mu\text{g/ml}$ for **63** and $5.5 \pm 2.4 \mu\text{g/ml}$ for **64**. The potent activity of compound **63** and **64** was probably due to the presence of a prenyl group at C-2 and has oxygen-linked substituents at C-1 and C-3. This structure activity relationship was modified from Ito *et al.* (2003), which stated that the essential feature for the activity of the xanthenes is the presence of two prenyl side chain at C-2 and C-8 positions in a xanthone skeleton that has oxygen-linked substituents at C-1, C-3, C-6 and C-7. Compound **59** was a new caged structure xanthonoid, due to a hydroxyl group at C-7. Compound **60** was a new tetraoxygenated xanthone due to occurrence of hydroxyl group at C-1, C-2 and C-5 and a methoxyl group at C-3.

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

**PENGEKSTRAKAN DAN PENGASINGAN SEBATIAN-SEBATIAN
SITOTOKSIK DARI *GARCINIA UROPHYLLA***

Oleh

ROZIDA MOHD KHALID

Januari 2006

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Ekstrak lima belas spesies pokok telah dijalankan ujian sitotoksik MTT, dan *Garcinia urophylla* telah menunjukkan aktiviti sitotoksik paling aktif. Ekstrak metanol pokok ini telah diujikan ke atas sel MCF-7 (sel kanser payu dara), dan telah menunjukkan aktiviti IC_{50} sebanyak 2.7 $\mu\text{g/ml}$. *Garcinia urophylla*, atau dikenali sebagai Kandis hutan, adalah pokok berbuah yang didapati di kawasan tinggi di Malaysia dan Sumatera. Sehingga kini, tiada kajian telah dilaporkan mengenai *Garcinia urophylla*.

Ekstrak metanol dari *Garcinia urophylla* telah difraksinasi menjadi fraksi heksana, dikloromethana (CH_2Cl_2), etil asetat (EtOAc) and butanol (BuOH). Kesemua fraksi di jalankan ujian sitotoksik MTT, dan CH_2Cl_2 telah menunjukkan aktiviti paling tinggi, iaitu $IC_{50} = 3 \mu\text{g/ml}$. Fraksi CH_2Cl_2 memberi 7 sebatian iaitu lupeol (**57**), gaudichaudione H (**58**), nor-dioksimorellin (**59**), 1,2,5-trihidroksi-3-metoksi-4-(3-metil-2-butenil)xanthon (**60**), 1,7-dihidroksi-3-metoksi-2-(3-metil-2-butenil)xanthon (**62**), 1,5-dihidroksi-3-metoksi-2-(3-metil-2-butenil)xanthon (**63**) and 1,3,7-trihidroksi-2-(3-metil-2-butenil)xanthon (**64**). Kesemua sebatian kecuali lupeol menunjukkan aktiviti sitotoksik,

dengan nilai $IC_{50} < 100 \mu\text{g/ml}$ ke atas MCF-7. Nilai IC_{50} bagi semua sebatian adalah seperti berikut; $> 100 \mu\text{g/ml}$ untuk **57**, $3.7 \pm 0.7 \mu\text{g/ml}$ untuk **58**, $25.0 \pm 4.9 \mu\text{g/ml}$ untuk **59**, $22.6 \pm 4.1 \mu\text{g/ml}$ untuk **60**, $21.6 \pm 3.4 \mu\text{g/ml}$ untuk **62**, $2.0 \pm 0.5 \mu\text{g/ml}$ untuk **63** and $5.5 \pm 2.4 \mu\text{g/ml}$ untuk **64**. Aktiviti sitotoksik yang tinggi pada **63** dan **64** adalah berkemungkinan kerana adanya kumpulan prenil pada C-2 dan mempunyai kumpulan oksigen pada C-1 dan C-3. Hubungan struktur dan aktiviti ini telah diambil dari Ito *et al.* (2003), yang menyatakan bahawa xanthone perlu mempunyai kumpulan prenil pada C-2 dan C-8 serta kumpulan oksigen pada C-1, C-3, C-6 dan C-7, untuk mempunyai aktiviti sitotoksik yang tinggi. Sebatian **59** adalah sebatian xanthonoid baru, kerana mempunyai kumpulan hidroksil pada C-7. Sebatian **60** juga adalah baru, kerana mempunyai kumpulan hidroksil pada C-1, C-2 dan C-5 dan kumpulan metoksil pada C-3.

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I certify that an Examination Committee met on 17th January 2006 to conduct the final examination of Rozida Mohd Khalid on her degree thesis entitled Bioactivity Guided Isolation of Cytotoxic Compounds from *Garcinia urophylla* in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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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 or concurrently submitted for any other degree at UPM or other institutions.

ROZIDA MOHD KHALID

Date: 24 April 2006

TABLE OF CONTENTS

| | Page |
|--|------|
| ABSTRACT | ii |
| ABSTRAK | iv |
| ACKNOWLEDGEMENTS | vi |
| APPROVAL | vii |
| DECLARATION | ix |
| LIST OF TABLES | x |
| LIST OF FIGURES | xiii |
| LIST OF ABBREVIATIONS | xvi |
| | |
| CHAPTER | |
| | |
| I. INTRODUCTION | 1 |
| | |
| II. LITERATURE REVIEW | |
| Medicinal Plants | 3 |
| Role of natural products in drug discovery | 3 |
| Anticancer agents from natural products | 5 |
| Guttiferae Family | 5 |
| The genus <i>Garcinia</i> (Guttiferae) | 6 |
| <i>Garcinia urophylla</i> | 7 |
| Phytochemical Studies of <i>Garcinia</i> species | 8 |
| Biological Activity Studies of <i>Garcinia</i> species | 10 |
| Tumour | 23 |
| Overview | 23 |
| Factors in cancer causation | 24 |
| Development of cancer | 25 |
| Drugs used for treatment of cancer | 27 |
| Conclusion | 29 |
| | |
| III. EXPERIMENTAL | |
| General Instrumentation | 30 |
| Chromatographic Methods | 31 |
| Chromatotron | 31 |
| Solvents | 31 |
| Isolation of Constituents from <i>Garcinia urophylla</i> | 32 |
| Plant material | 32 |
| Extraction and Isolation | 32 |
| Physical and Spectral Data of Compounds | 34 |
| Bioassay Procedures | 39 |
| Cell culture | 39 |
| Preparation of Medium | 39 |

| | | |
|------------|---|-----|
| | Cryopreservation of cells | 40 |
| | Maintenance of cell culture | 40 |
| | Cytotoxic Assay (MTT assay) | 40 |
| | Reviving of cell culture | 41 |
| IV. | RESULTS AND DISCUSSION | |
| | Structural Elucidation of Compounds | 43 |
| | Lupeol (57) | 43 |
| | Gaudichaudione H (58) | 51 |
| | Nor-deoxymorellin (59) | 62 |
| | 1,2,5-trihydroxy-3-methoxy-4-(3-methyl-2- butenyl)xanthone (60) | 74 |
| | 1,7-dihydroxy-3-methoxy-2- (3-methyl-2-butenyl)xanthone (62) | 85 |
| | 1,5-dihydroxy-3-methoxy- (3-methyl-2-butenyl)xanthone (63) | 94 |
| | 1,3,7-trihydroxy-2- (3-methyl-2-butenyl)xanthone (64) | 103 |
| | Cytotoxic Assay (MTT assay) | 112 |
| | Screening results for cytotoxic activity in various plants | 112 |
| | Results on bioassay guided fractionation | 114 |
| | Cytotoxic activity of the isolated compounds | 120 |
| | Structure activity relationship | 121 |
| V. | CONCLUSION | 122 |
| | REFERENCES | 124 |
| | APPENDICES | |
| | BIODATA OF THE AUTHOR | 128 |

