PHYTOCHEMISTRY AND BIOACTIVITY STUDIES OF SELECTED MALAYSIAN *CALOPHYLLUM* AND *GARCINIA* SPECIES

BY

SUHAIB IBRAHIM ALKHAMAISEH

A dissertation in fulfilment of the requirement for the degree of Doctor of philosophy in Pharmacy

Kulliyyah of Pharmacy

International Islamic University
Malaysia

APRIL 2013

ABSTRACT

Natural products from plant origin are well known to have important biological activities against many illnesses for several decads. *Guttiferae* is distributed in tropical area, with 27 genera and almost 1090 species. Many isolated compounds from *Guttiferae* showed significant biological properities. Therefore, this research aimed to investigate the presence of new potential anti-proliferative, anti-microbial and anti-oxidant compounds from selected species of Malaysian *Calophyllum* and *Garcinia* genera. In the phytochemistry study, *Calophyllum canum*, *C. rubiginosum, C. depressinervosum* and *Garcinia malaccensis* were extracted successively by using two methods, i.e., maceration and soxhlet extractor, using different polar solvents, followed by purification by using several chromatographic techniques. The purification yielded ten pure compounds from the selected species; and elucidated by using spectroscopic techniques, i.e., Nuclear Magnetic Resonance (NMR), Mass Spectrometry (MS), Infrared (IR) and Ultraviolet (UV). *C. canum* yielded five pure compounds, identified as trapezifolixanthone (**96**), taraxerone (**97**), taraxerol (**98**), 1,1'',3,7''-tetrahydroxy-7-methyl-8,6'',8''-tris(3-methyl-2-butenyl)-3'''',3''''-dimethyl-2H,6H-pyrano[3'',2'']bisxanthen-9,9''-dione (**99**) and 1,6-dihydroxy-3',3'-dimethyl-4-(3''-methyl-2''-butenyl)-2H,6H-pyrano[3,2]xanthen-9-one (**100**). *C. rubiginosum* and *C. depressinervosum* yielded same compound, identified as friedelin (**101**). *G. malaccensis* yielded four compounds, identified as β-mangostin (**102**), cycloart-24-en-3β-ol (**103**), α-mangostin (**104**) and 1,3,5-trihydroxy-7methoxy-2,8-bis(3-methyl-2-butenyl)xanthen-9-one (**105**). The biological activity studies were aimed to evaluate the activity of isolated compounds by using several assays. Cytotoxicity, anti-microbial and anti-oxidant tests were employed. The anti-proliferative activity of **96**, **98**, **100**, **102**, **104** and **105** against A549 cell line showed significant effect (IC_{50}: 119.3, 59.5, 49.9, 43.6, 22.6 and 20.7 μM) respectively. Compound **96**, **101**, **102**, **104** and **105** showed significant effect against MCF-7 cell line (IC_{50}: 88.4, 55.0, 14.0, 24.3 and 18.4 μM) respectively. In addition, **98**, **100** showed significant effect against C33A cell line (IC_{50}: 126.5 and 150.2 μM) respectively. Compound **102** showed significant effect against HSC-3 and K562 cell line (IC_{50}: 0.31 and 0.38 μM) respectively. While, compound **105** showed activity also against K562 cell line (IC_{50}: 0.64 μM). The morphology of treated cell lines indicated to observe an apoptosis and triggering necrosis cell death using scanning electron microscope. Compound **104** showed considerable activity against *P. aeruginosa* (MIC: 0.3 mM and MBC: 0.61 mM). The purified compounds were showed moderate antioxidant activity like quercetin. In conclusion, the present study has isolated two new compounds (**99** and **105**). As well as, the significant anti-proliferative potential and moderate anti-bacterial and anti-oxidant agents of Malaysian *Calophyllum* and *Garcinia* genera. The isolated compounds that showed new anti-proliferative activity need for further investigation that may lead to development of new anticancer agent.

خلاصة البحث

مما لا شك فيه ان النباتات ومنذ بداية الخلق كانت مصدرا للمركبات الطبيعية النافعة المعروفة بفاعليتها الحيويه ضد الميكروبات والعديد من مسببات الامراض. وفي بحثي هذا توخيت فيه تحقيق الاهداف التاليه: استخلاص وعزل المركبات الرئيسيه الفعاله. ايجاد التركيب الكيميائي وفحص فاعليته .وفي ذلك اخترت اصنافا من النباتات من فصيلة ال(*Calophyllum canum, Calophyllum rubiginosum and Calophyllum depressinervosum* و*Garcinia malaccensis*) التي استخلصتها بواسطة اداة الاستخلاص (soxhlet) بإستخدام عدة محاليل عضوية مختلفة القطبية. وقمت بتنقية الخامات المستخلصة بإستخدام تقنية (chromatography الكروماتوغرفي) وحللت المركبات النقية بإستخدام طريقة التحليل الطيفي حيث حصلت على عشر مركبات اثنان منها تعد جديده، وهي.- Trapezifolixanthone (96), Taraxerone (97), Taraxerol (98), 1,1'',3,7''-tetrahydroxy-7-methyl-8,6'',8''-tris(3-methyl-2-butenyl)-3'''',3''''-dimethyl-2H,6H-pyrano[3'',2'']bisxanthen-9,9''-dione (99) and 1,6-dihydroxy-3',3'-dimethyl-4-(3''-methyl-2''-butenyl)-2H,6H-pyrano[3,2]xanthen-9-one (100). *C. rubiginosum* and *C. depressinervosum* Friedelin (101). *G. malaccensis* β-mangostin (102), Cycloart-24-en-3β-ol (103), α-mangostin (104) and 1,3,5-trihydroxy-7methoxy-2,8-bis(3-methyl-2-butenyl)xanthen-9-one (105). وقمت بفحصها لإيجاد الفاعلية الحيويه لها ومن تلك الفحوص التي اجريتها فحص مضادات الاكسدة في ثلاث فحوص: DPPH Iron chelating Reducing Power ومضادات الميكروبات الذي تضمن فحص البكتيريا والفطريات وفحص السميه وذلك بإستخدام MTT. بالاضافة الى دراسة التغيرات السطحية للخلايا بإستخدام المنظار الماسح الالكتروني. وقد اظهرت نتائج بحثي فاعلية ايجابية عند استخدام المواد الخام، وفي المقابل كانت الفاعلية قد انخفضت الى النصف بالنسبة لمضادات الاكسدة ومضادات البكتيريا وذلك عند استخدام المركبات النقية، وكانت السميه عالية عند استخدام المركبات النقية على عدة خلايا سرطانية، مما يدل على فاعلية هذه المركبات، بالاضافة الى انه قد شوهد موت منظم للخلايا. وخلاصة هذا البحث تعتبر تقديم جديد بإعتبار ان النباتات المستخدمة فيه لم تدرس من قبل، والفاعليه كانت ايجابية خصوصا من ناحية السميه على الخلايا. الامر الذي يدعونا للتوصية بمتابعة الدراسة وتوسيعها على مختلف المستويات للوصول الى الالية المفسرة لفاعلية هذه المركبات.

ABSTRAK

Clusiaceae/Guttiferae ialah sejenis tumbuh-tumbuhan renek atau pepohon malar hijau dan boleh didapati secara meluas di kawasan tropika panas dan lembap di dunia. Ia dianggap sebagai salah satu spesies daripada famili tumbuh-tumbuhan tinggi yang terdiri daripada 27 genus dan hampir 1090 spesies. Famili ini dikenali sebagai sumber xanton terutamanya xanton diprenilasi. Lebih 175 xanton telah diasingkan daripada famili *Clusiaceae*/*Guttiferae*. Kompaun daripada famili ini mempunyai nilai perubatan yang tinggi, seperti aktiviti sitotoksik terhadap leukemia P338, kolon C6, dan titisan sel mamari CD. Kompaun tersebut juga menunjukkan aktiviti antiradang dan antimikrob. Kajian ini dijalankan untuk mencapai dua objektif utama, iaitu kajian aktiviti fitokimia dan biologi. Kajian fitokimia bertujuan untuk mengekstrak, mengasingkan, dan menstrukturkan elusidasi kompaun terasing. Tumbuh-tumbuhan yang dipilih daripada genus *Calophyllum canum*, *Calophyllum Rubiginosum*, dan *Calophyllum depressinervosum* dan *Garcinia malaccensis* telah diekstrak berturut-turut dengan menggunakan dua kaedah; pengekstrakan maserasi dan soxhlet dengan menggunakan pelarut organik yang berbeza dengan kepolaran bertingkat. Ekstrak mentah ditulenkan dengan menggunakan beberapa teknik kromatografi; dan sebelas kompaun tulen diasingkan daripada spesies terpilih. *Calophyllum canum* menghasilkan lima kompaun tulen iaitu satu bixanton, dua xanton, dan dua lanostan triterpenoid. Manakala *Calophyllum rubiginosum* dan *Calophyllum depressinervosum* menghasilkan satu kompaun iaitu triterpenoid. *Garcinia malaccensis* menghasilkan empat kompaun iaitu tiga xanton dan satu triterpenoid. Kompaun yang ditulenkan dikenal pasti dengan menggunakan teknik spektroskopi (UV, IR, EIMS, dan NMR). Kajian aktiviti biologi bertujuan untuk menilai aktiviti kompaun terasing dengan menggunakan beberapa cerakin. Kajian antitoksiti, antimikrob, dan antioksidan dijalankan. Hasilnya, kompaun **96**, **98**, **100**, **103**, **105,** dan **106** dalam kajian ini masing-masing telah menunjukkan kesan yang signifikan terhadap titisan sel A549 dengan nilai IC_{50} masing-masing 45.25, 27.73, 18.93, 18.55, 9.31, dan 8.48 μg/mL. Manakala kompaun **96**, **101**, **103**, **105**, dan **106** masing-masing menunjukkan aktiviti yang signifikan terhadap titisan sel MCF-7 dengan nilai IC_{50} 33.53, 23.5, 5.97, 9.98, dan 7.55 μg/mL. Kompaun **98** dan **100** masing-masing menunjukkan aktiviti yang signifikan terhadap titisan sel C33a dengan nilai IC_{50} masig-masing 59.03 dan 56.93 μg/mL. Kompaun **103** menunjukkan aktiviti yang ketara berbanding titisan sel HSC-3 dan K562 yang masing-masing menunjukkan nilai IC_{50} 0.13 dan 0.16 μg/mL. Kompaun **105** juga menunjukkan aktiviti terhadap titisan sel K562 dengan nilai IC_{50} 0.19 μg/mL. Morfologi titisan sel yang dirawat menunjukkan bahawa sel-sel menjalani apoptosis yang boleh dilihat dengan menggunakan mikroskop imbasan elektron.

APPROVAL PAGE

The thesis of Suhaib Ibrahim Muha'd Alkhamaiseh has been approved by the following:

_____
Muhammad Taher
Supervisor

_____
Farediah Ahmad
Co-Supervisor

_____
Qamar Uddin Ahmed
Internal Examiner

_____
Ibrahim Jantan
External Examiner

_____

Chairman

DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Suhaib Ibrahim Moha'd Alkhamaiseh

Signature …… Date ……..

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

DECLARATION OF COPYRIGHT AND AFFIRMATION OF FAIR USE OF UNPUBLISHED RESEARCH

PHYTOCHEMISTRY AND BIOACTIVITY STUDIES OF SELECTED MALAYSIAN *CALOPHYLLUM* AND *GARCINIA* SPECIES

Affirmed by Suhaib Ibrahim Moha'd Alkhamaiseh

…….. ……..

Signature Date

I dedicated this work especially to my father Mr. Ibrahim Alkhamaiseh, who is my ideal person in my life, my mother, brothers, sisters, son and my beloved wife

ACKNOWLEDGEMENTS

Praise be to ALLAH who bestowed on purposefulness and helped me to complete this research. Also, I cannot turn back without acknowledging those who helped me through this research.

I come and sincere thanks and gratitude to my supervisor Assoc. Prof. Dr. Muhammad Taher and co-supervisor Assoc. Prof. Dr. Farediah Ahmad for their help in completing this research and provide the opportunity to well understanding. Thank you for everything and may ALLAH (swt) reward and bless you.

Indeed, I do not forget great thanksgiving to my great parents and beloved brothers, who have given the intangible and tangible support.

I also would like to extend thanks to my lovely wife and dearly loved Ibrahim, who were stand by and supportive during the years of study.

Finally, thanks to my dearest brothers, Dr. Osama and Dr. Haitham as well as all my friends and everyone who contributed and participated in the completion of this research.

I cannot conclude this acknowledgment without expressing my appreciation to Research Mangment Center (IIUM) for the financial support and cannot forget the supporting came from Menistry of Higher Education Malaysia through the grant number (FRGS 0409-103).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

DCM	Dichloromethane
MeOH	Methanol
EtOH	Ethanol
EtOAc	Ethyl acetate
DEE	Diethyl ether
PE	Petroleum ether
CC	Column chromatography
PTLC	Preparative thin layer chromatography
NCI	American national cancer institute
IR	Infra red
UV/Vis	Ultra violet visible
EIMS	Electron impact Mass spectroscopy
NMR	Nuclear magnetic resonance
δ ^{1}H	Proton chemical shift
δ ^{13}C	Carbon chemical shift
HPLC	High performance liquid chromatography
TLC	Thin layer chromatography
VLC	Vacuum liquid chromatography
HMQC	Heteronuclear Multiple-Quantum Correlations
HMBC	Heteronuclear Multiple Bond Correlations
COSY	Correlation Spectroscopy
Rf	Retintion factor
MIC	Minimum inhibition concentration
MBC	Minimum bacterial concentration
ROS	Reactive oxigyen species
LDL	Low density lipoprotein
HDL	High density lipoprotein
RC_{50}	Minimum concentrations can scavenge 50 % of radical
DNA	Deoxyribonucleic acid
SEM	Scanning electron microscope
HMDS	Hexamethyldisiloxane
m.p	Melting point
BHT	Butylated hydroxytoluene
DPPH	Diphenylpicrylhydrazyl
MTT	Tetrazolium dye
DBE	Double bond equivalents

LIST OF UNITS

µg	Microgram
mg	Milligram
g	Gram
kg	Kilogram
µL	Micro liter
L	Liter
h	Hour
m/z	Mass over charge
ppm	Part per million
nm	Nanometer
Hz	Hehrtez
µM	Micro molar