UNIVERSITI PUTRA MALAYSIA

ISOLATION AND BIOLOGICAL ACTIVITY OF NATURALLY OCCURRING COMPOUNDS FROM MELICOPE LUNU ANKENDA (GAERTN) T-HARTLEY, MELICOPE BONWICKII(F-MUELL) T-HARYLEY AND TETRADIUM SAMBUCINUM (BL) HARTLEY

ISMIARNI KOMALA.

FS 2005 30
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By

ISMIARNI KOMALA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for Degree of Master of Science

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ISOLATION AND BIOLOGICAL ACTIVITY OF NATURALLY OCCURRING COMPOUNDS FROM MELICOPE LUNU ANKENDA (GAERTN) T-HARTLEY, MELICOPE BONWICKII (F-MUELL) T-HARTLEY AND TETRADZUM SAMBUCINUM (BL) HARTLEY

By

ISMIARNI KOMALA

June 2005

Chairman : Professor Mawardi Rahmani, PhD
Faculty : Science

Detailed investigation on three Rutaceous plants have resulted in the isolation and structural elucidation of a number of compounds. The structures of these compounds were elucidated by using spectroscopic methods such as UV (ultraviolet), IR (infrared), MS (mass spectra), NMR (Nuclear Magnetic Resonance) and also by comparison with previous reports. The crude extracts and isolated compounds were evaluated for their antioxidant, cytotoxicity and antimicrobial activities using DPPH (1,1-diphenil-2-picrylhydrazyl), MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and disc diffusion methods, respectively.

The phytochemical study on leaves of Melicope lunu-ankenda (Gaertn) T. Hartley afforded p-O-geranylcoumaric acid (153) sesamin (109), melisemine (154) and a new compound given tentative structure of 7,7”-digeranyloxy-2”,3”-epoxycinnamic anhydride (155). The melisemine (154) showed weak activity as a radical scavenger with an IC50 value of 75 µg/mL. Meanwhile the 7,7”-digeranyloxy-2”,3”-
epoxycinnamic anhydride (155) was found to be active against cervical cancer (Hela) cell line with an IC\(_{50}\) value of 34 \(\mu\)g/mL.

The investigations on leaves of *Melicope bonwickii* (F. Muell) T. Hartley have resulted in the isolation of three known furoquinoline alkaloid, 7-(2',3'-epoxypropoxy)-4-methoxyfuroquinoline (156), evellerine (157) and kokusaginine (11) together with a new 7-(2'-hydroxy-3'-chloropropoxy)-4-methoxyfuroquinoline (158) and a known amide compound aurantiamide acetate (159). Two compounds 7-(2',3'-epoxypropoxy)-4-methoxyfuroquinoline (156) and 7-(2'-hydroxy-3'-chloropropoxy)-4-methoxyfuroquinoline (158) were found to be toxic to cervical cancer (Hela) cell line with IC\(_{50}\) values of 6.0 and 11.4 \(\mu\)g/mL, respectively.

Detailed extraction and separation of bark and leaves of *Tetradium sambucinum* (Bl) Hartley have led to the isolation of decarine (160), rutaecarpine (122) 7-hydroxycoumarin (141) and aurantiamide acetate (159). Decarine (160) was active against the cervical cancer cell lines (Hela) with an IC\(_{50}\) value of 14.6 \(\mu\)g/mL, while rutaecarpine (122) showed weak a radical scavenger activity with an IC\(_{50}\) value of 75\(\mu\)g/mL.
Abstrak tesis yang dikemukakan kepada Senat Universti Putra Malaysia sebagai memenuhi keperluan ijazah Master Sains

PEMENCILAN DAN AKTIVITI BIOLOGI SEBATIAN SEMULAJADI DARIPADA MELICOPE LUNU ANKENDA (GAERTN) T-HARLEY, MELICOPE BONWICKII (F. MUELL) T-HARTLEY DAN TETRADIUM SAMBUCINUM (BL) HARTLEY

Oleh

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Jun 2005

Pengerusi : Professor Mawardi Rahmani, PhD
Fakulti : Sains

Kajian terhadap tiga tumbuhan dalam famili Rutaceae telah menghasilkan pemencilan beberapa sebatian. Struktur dari sebatian-sebatian ini dikenal pasti dengan menggunakan kaedah spektroskopi seperti IR, UV, NMR, MS dan juga perbandingan dengan kajian-kajian lepas. Ekstrak mentah dan sebatian-sebatian yang telah dipencilkan dari tumbuhan ini diuji untuk aktiviti antioksidan, sitotoksik dan antimikrob dengan menggunakan kaedah DPPH (1,1-diphenil-2-picrylhydrazyl), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dan peresapan cakera.

Kajian fitokimia ke atas daun Melicope lunu-ankenda (Gaertn) T. Hartley telah menghasilkan dua sebatian yang telah dikenali sebagai p-O-geranylcoumaric acid (153) sesamin (109) melisemine (154) dan struktur sementara daripada 7,7"- digeranyloxy-2","3"-epoxycinnamic anhydride (155). Melisemine (154) telah menunjukkan aktiviti yang lemah sebagai antioksidan dengan nilai IC50 75 μg/mL.
Manakala sebatian 7,7\(^{-}\)-digeranyloxy-2\(^{-}\),3\(^{-}\)-epoxycinnamic anhydride (155) telah menunjukkan kesan aktiviti terhadap kanser rahim (Hela) dengan nilai IC\(_{50}\) 34 \(\mu\)g/mL.

Pengkajian terhadap daun *Melicope bonwickii* (F. Muell) T. Hartley telah menghasilkan 3 alkaloid furoquinoline yang telah dikenali: 7-(2',3'-epoxyprenyloxy)-4-methoxyfuroquinoline (156) evellerine (157) dan kokusagine (11) bersama dengan sebatian baru 7-(2-hydroxy-3'-chloroprenyloxy)-4-methoxyfuroquinoline (160) dan sebatian amide yang telah dikenali, aurantiamide acetate (159). Dua sebatian yang telah dipencilkan daripada daun *Melicope bonwickii* (F. Muell) T. Hartley yaitu 7-(2',3'-epoxyprenyloxy)-4-methoxyfuroquinoline (158) dan 7-(2-hydroxy-3'-chloroprenyloxy)-4-methoxyfuroquinoline (160) telah diuji aktivitinya dengan sel kanser rahim (Hela), hasilnya menunjukkan kedua-dua sebatian ini mempunyai kesan sitotoksik yang masing-masing mempunyai nilai IC\(_{50}\) 6.0 dan 11.4 \(\mu\)g/mL.

Pengestrakan dan pemisahan daripada kulit batang dan daun *Tetradium sambucinum* (BI) Hartley telah membawa kepada pemencilan sebatian-sebatian decarine (160), rutaecarpine (122) 7-hydroxycoumarin (141) dan aurantiamide acetate (159). Decarine menunjukkan aktiviti yang sederhana terhadap sel kanser rahim (Hela) yang mempunyai nilai IC\(_{50}\) 34 \(\mu\)g/mL, manakala rutaecarpine (122) menunjukkan aktiviti yang lemah sebagai antioksidan dengan nilai IC\(_{50}\) 75\(\mu\)g/mL.
ACKNOWLEDGEMENTS

All praises do to Allah, Lord of the universe. Only by his grace and mercy this thesis can be completed.

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Finally, my deepest thanks to my parents, sister and brother for their prayers, continuous moral support and unending encouragement.
I certify that an Examination Committee met on 20th June 2005 to conduct the final examination of Ismiarni Komala on her Master of Science thesis entitled “Isolation and Biological Activity of Naturally Occuring Compounds from Melicope lunu ankenda (Gaertn) T-Hartley, Melicope bonwickii (F-Muell) T-Hartley and Tetradium sambucinum (BL) Hartley” 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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Date: 11 AUG 2005
DECLARATION

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

ISMIARNI KOMALA

Date 29/7/2005
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- Sesamin (109)
- Melisemin (154)
- 7,7″-Digeranyloxy-2″,3″-epoxycinnamic anhydride (155)

Characterization of Isolated Compounds from Leaves of *Melicope bonwickii* (F.Muell) T.Hartley

- 7-(2″,3″-Epoxyprenyloxy)-4-methoxyfuroquinoline (156)
- Evellerine (157)
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- Aurantiamide acetate (159)
- Kokusaginine (11)

Characterization of Isolated Compounds from Barks of *Tetradium sambucinum* (Bl) Hartley

- Decarine (160)

Characterization of Isolated Compounds from Leaves of *Tetradium sambucinum* (Bl) Hartley

- Rutaecarpine (122)
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<td>α</td>
<td>alpha</td>
</tr>
<tr>
<td>β</td>
<td>betha</td>
</tr>
<tr>
<td>δ</td>
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<tr>
<td>γ</td>
<td>gamma</td>
</tr>
<tr>
<td>λ&lt;sub&gt;max&lt;/sub&gt;</td>
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<td>Ar</td>
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<td>adenosine tri phosphate</td>
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<tr>
<td>br</td>
<td>broad</td>
</tr>
<tr>
<td>13C</td>
<td>carbon-13</td>
</tr>
<tr>
<td>°C</td>
<td>degree celcius</td>
</tr>
<tr>
<td>CFU</td>
<td>colony forming unit</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>chloroform</td>
</tr>
<tr>
<td>CDCl₃</td>
<td>deuterated chloroform</td>
</tr>
<tr>
<td>cm⁻¹</td>
<td>per centimeter</td>
</tr>
<tr>
<td>COSY</td>
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<td>d</td>
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<td>dd</td>
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<tr>
<td>DPPH</td>
<td>1,1-diphenil-2-picrylhydrazyl</td>
</tr>
<tr>
<td>DEPT</td>
<td>Distortionless Enhancement by Polarization Transfer</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxides</td>
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xx
EIMS  Electron Impact Mass Spectroscopy
ELISA  Enzym-Linked Immunosorbent Assay
FCS  Fetal Calf Serum
g  gram
GCMS  Gas Chromatography-Mass Spectroscopy
h  hour
'H  proton
HMBC  Heteronuclear Multiple Bond Connectivity by 2D Multiple Quantum
HSQC  Heteronuclear Single Quantum Coherence
Hz  hertz
IC₅₀  Inhibition Concentration at 50 percent
IR  Infra red
J  coupling constant value
KBr  Kalium bromida
Kg  kilogram
M⁺  molecular ion
m  multiplet
mg  milligram
mL  milliliter
mm  millimeter
MeOH  methanol
MHz  MegaHertz
m.p.  melting point
MTT  Microculture tetrazolium

3-[(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
m/z mass per charge
Na Natrium
NA Natrium agar
nm nanometer
NMR Nuclear Magnetic Resonance
o ortho
OD Optical density
p para
PBS Phosphate Buffered Saline
Pet.ether Petroleum ether
ppm part per million
RPMI Roswell Park Memorial Institute
s singlet
t triplet
TLC Thin Layer Chromatography
TMS Tetramethilsilane
UV ultra violet
WHO World Health Organization.
CHAPTER I

INTRODUCTION

The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main sources of drug. Even today, plants are the almost exclusive source of drug for the majority of the world’s populations.

About 25% of drugs prescribed worldwide come from plants and 121 such active compounds are currently being used. Of the 252 drugs considered as basic and essential by the World Health Organization (WHO), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors (Rates, 2001). This is particularly true as shown in cancer and infectious diseases, where over 60% and 75% of the drugs were known to be of natural origin, respectively (Newman et al., 2003).

After centuries of empirical use of herbal preparation, the isolation of active principles such as morphine, strychnine, quinine etc. in the early 19th century marked a new era in the use of medicinal plants, and the beginning of modern medicinal plant research. Plant metabolites were mainly investigated from a phytochemical and chemotaxonomic viewpoint during this period. Over the last decade, however, interest in drugs of plant origin has been growing steadily. Consumption of medicinal plants has almost doubled in Western Europe during that period (Hamburger and Hostettman, 1991).
It is currently estimated that there are at least 250,000-500,000 different plants species, up to 30 millions species of insects, 1.2 millions species of fungi and similar number of algae and prokaryotes in existence throughout the world (Pimm et al., 1995). All of the species coexist in ecosystem and interact with each other in several ways in which chemistry plays a major role, for example in defense, symbiosis and pollination. In basic term, these organisms all share a similar biochemical process necessary for living cell, but in addition to that they also produce a wide variety of the so called secondary metabolites that are involved in interactions between organisms. Considering the number of organisms, and the almost infinite number of interactions possible, it is not surprising that an enormously wide variety of secondary metabolites have evolved within organism (Vepoorde, 1998).

The potential of higher plants as sources for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plants species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller (Rates, 2001). Plants contain hundreds or thousands of metabolites. It is clear that plants provide an enormous potential for the discovery of new bioactive compounds.

*Melicope* species is one of the genus in Rutaceae family. *Melicope* have similar characteristic with *Tetradium* Lour and *Euodia* and recently Hartley reestablished the genus *Tetradium* Lour that was formerly placed under *Euodia* J.R. & G. Forst by Engler (Engler, 1931). Hartley further indicated that many other species formerly placed in *Euodia* were to be transferred to *Melicope* J.R & G. Forst. (Hartley, 1981)