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

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By

ISMIARNI KOMALA

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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 (infra red), 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 IC_{50} 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 μg/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'-epoxyprenyloxy)-4-methoxyfuroquinoline **(156),** evellerine **(157)** and kokusaginine **(11)** together with a new 7-(2'-hydroxy-3'-chloroprenyloxy)-4-methoxyfuroquinoline **(158)** and a known amide compound aurantiamide acetate **(159).** Two compounds 7-(2',3'-epoxyprenyloxy)-4-methoxyfuroquinoline **(156)** and 7-(2'-hydroxy-3'-chloroprenyloxy)-4-methoxyfuroquinoline **(158)** were found to be toxic to cervical cancer (Hela) cell line with IC_{50} values of 6.0 and 11.4 μg/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 μg/mL, while rutaecarpine **(122)** showed weak a radical scavenger activity with an IC_{50} value of 75μg/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

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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 IC_{50} 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 μg/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 kokusaginine **(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 diujikan 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 μg/mL

Pengestrakan dan pemisahan daripada kulit batang dan daun *Tetradium sambucinum* (Bl) 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 μg/mL, manakala rutaecarpine **(122)** menunjukkan aktiviti yang lemah sebagai antioksidan dengan nilai IC_{50} 75μg/mL.

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α	alpha
β	betha
δ	delta (chemical shift in ppm)
γ	gamma
λ_{max}	maximum wavelength in nm
μg	microgram
μl	microliter
Ar	aromatic
ATP	adenosine tri phosphate
br	broad
^{13}C	carbon-13
^{o}C	degree celcius
CFU	colony forming unit
$CHCl_3$	chloroform
$CDCl_3$	deuterated choroform
cm^{-1}	per centimeter
COSY	Correlated Spectroscopy
d	doublet
dd	doublet of doublets
ddd	doublet of doublets of doublets
DPPH	1,1-diphenil-2-picrylhydrazyl
DEPT	Distortionless Enhacement by Polarization Transfer
DMSO	Dimethylsulfoxides

EIMS	Electron Impact Mass Spectroscopy
ELISA	Enzym-Linked Imunosorbent Assay
FCS	Fetal Calf Serum
g	gram
GCMS	Gas Chromatography-Mass Spectroscopy
h	hour
^1H	proton
HMBC	Heteronuclear Multiple Bond Connectivity by 2D Multiple Quantum
HSQC	Heteronuclear Single Quantum Coherence
Hz	hertz
IC_{50}	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-2yl)-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 19^{th} 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 (Vepoorte, 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)