UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITY OF FOUR MELICOPE SPECIES (RUTACEACE)

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By

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Chairman: **Professor Mawardi Rahmani, PhD**

Faculty: **Science**

Four *Melicope* species from Rutaceae family were phytochemically studied and screened for their biological activities. These species, *Melicope lunu-ankenda* (Gaertn.) T.G. Hartley, *Melicope latifolia* (D.C) T.G. Hartley, *Melicope clemensiae* (Merr.) Liu and *Melicope confusa* T.G. Hartley were collected from Sandakan, Sabah. The chemical studies on the leaves and stem bark of these species involves extraction using three organic solvents of different polarity and isolation of compounds by using several chromatographic techniques including vacuum liquid chromatography and gravity column chromatography. The structural elucidation of the isolated compounds was carried out using spectroscopic methods, UV, IR, NMR, MS and also by comparison with the literature data. These techniques have led to the isolation and identification of several compounds of different classes, furoquinoline alkaloids, flavanoid, lignan, cinnamic acid derivative and sterols. The crude extracts

and isolated compounds were screened for antimicrobial, antioxidant and cytotoxic activities using disc diffusion method, DPPH (1,1-Diphenyl-2-pircrylhydrazyl) and MTT (Microculture Tetrazolium Salt) assays, respectively. The microbes used for antimicrobial tests were methicillin resistant *S. aureus* (MRSA), *B. subtilis, P. aeruginosa, S. typhimurium, C. albicans, A. ochraceaus* and *S. cerevisiae*.

Detail study on the leaves of *Melicope lunu-ankenda* afforded 7-geranyloxycinnamic acid **(90)** and sesamin **(91)**. Sesamin **(91)**, a lignan was also found from the stem bark of the same plant together with pacyhpodol **(13)**, a flavanoid. Chemical investigations on the leaves of *Melicope latifolia* have resulted in the isolation of two alkaloids, dictamnine **(24)** and confusameline **(7)**. Isolation work on the leaves of *Melicope clemensiae* gave two compounds, sesamin **(91)** and β-sitosterol **(92)**. The latter compound, a sterol was also found to be the constituent of the stem bark of the species. Ecdysterone **(94)**, stigmasterol **(93)** and β-sitosterol **(92)** were isolated from the leaves of *Melicope confusa,* whereas from the stem bark, ecdysterone **(94)** and β-sitosterol **(92)** were isolated.

The leaf extracts of *Melicope clemensiae* possesses strong antimicrobial activity against MRSA (Methicillin resistant *Staphylococcus aureus*) whereas its stem bark extracts exhibited moderate activity against the same bacteria. Moderate activity was observed in both leaves and stem bark extracts of this species, towards targeted fungi. The methanol bark extract of *Melicope confusa* also showed moderate activity against *Pseudomonas aeruginosa* and *Candida albicans*. Other crude extracts and

isolated compounds, 7-geranyloxycinnamic acid **(90)** and sesamin **(91)** showed weak inhibition on the growth of the organisms.

Crude extracts of *Melicope lunu-ankenda, Melicope latifolia* and *Melicope clemensiae* exhibited free radical scavenging activity towards DPPH but no activity was observed in the crude extracts of *Melicope confusa*. As for the cytotoxic activity, all the crude extracts of *Melicope confusa and Melicope clemensiae* were not cytotoxic towards the HL 60 cells line. Meanwhile, strong cytotoxic activity was exhibited in chloroform and petroleum ether extracts of the leaves of *M. lunu-ankenda* and *M. clemensiae,* respectively.

Both isolated compounds, 7-geranyloxycinnamic acid **(90)** and sesamin **(91)** showed weak activity as antimicrobes and as free radical scavengers. Although dictamnine **(24)** shows a weak free radical scavenger, it possesses a moderate antimicrobial activity against some of the microbes. Confusameline **(7)** however, shows a moderate free radical scavenging activity. Cytotoxic activities were also tested on 7-geranyloxycinnamic acid **(90)**, sesamin **(91)** and dictamnine **(24)**. 7-Geranyloxycinnamic acid **(90)** exhibited a weak cytotoxic activity against HL 60 cells whereas sesamin **(91)** and dictamnine **(24)** show moderate activity towards the cells.

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

KANDUNGAN KIMIA DAN AKTIVITI BIOLOGI DARIPADA EMPAT SPESIES *MELICOPE* (RUTACEAE)

Oleh

NAJIHAH BT MOHD. HASHIM

April 2005

Pengerusi: **Profesor Mawardi Rahmani, PhD**

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Empat spesies *Melicope* daripada famili Rutaceae telah dikaji secara fitokimia dan diuji aktiviti biologi mereka. Spesies-spesies ini adalah *Melicope lunu-ankenda* (Gaertn.) T.G. Hartley , *Melicope latifolia* (D.C) T.G Hartley, *Melicope clemensiae* (Merr.) Liu dan *Melicope confusa* T.G. Hartley yang telah diperolehi daripada Sandakan, Sabah. Kajian kimia ke atas daun dan kulit batang spesies tersebut melibatkan pengekstrakan yang menggunakan tiga pelarut organik yang berbeza kekutubannya dan pemencilan sebatian dengan menggunakan beberapa teknik kromatografi termasuklah kromatografi vakum cecair dan kromatografi turus graviti. Pengenalpastian struktur sebatian ini telah dijalankan dengan menggunakan kaedah spektroskopi seperti UV, IR, NMR, MS dan juga perbandingan dengan data literature. Teknik-teknik ini telah membawa kepada pemencilan dan pengenalpastian

beberapa sebatian daripada spesies-spesies tersebut seperti alkaloid furokuinolin, flavanoid, lignan, terbitan asid sinamik dan sterol.

Ekstrak mentah dan sebatian yang telah dipencilkan telah diuji aktiviti antimikrob, antioksidan dan sitotoksik dengan menggunakan kaedah peresapan cakera, DPPH (1,1-Diphenyl-2-pircrylhydrazyl) and MTT (garam Mikrokultur Tetrazolium). Mikrob yang telah digunakan untuk ujian antimikrob ialah MRSA (*Staphylococcus aureus* yang resistan kepada methicillin), *B. subtilis, P. aeruginosa, S. typhimurium, C. albicans, A. ochraceaus* dan *S. cerevisiae*.

Kajian terperinci ke atas daun *M. lunu-ankenda* telah menghasilkan asid 7-geraniloksisinamik **(90)** dan sesamin **(91)**. Sesamin **(91)** adalah sebatian lignan yang juga telah ditemui daripada kulit batang spesies ini bersama-sama dengan pakipodol **(13)** iaitu sebatian flavanoid. Kajian kimia ke atas daun *M. latifolia* telah menghasilkan pemencilan dua alkaloid iaitu diktamnin **(24)** dan konfusamelin **(7)**. Kajian pemencilan ke atas daun *M. clemensiae* telah memberikan dua sebatian iaitu sesamin **(91)** dan β-sitosterol **(92)**. Sebatian β-sitosterol **(92)** adalah sejenis sterol yang juga telah ditemui daripada kulit batang spesies ini. Ekidisteron **(94)**, stigmasterol **(93)** dan β-sitosterol **(92)** telah dipencilkan daripada daun *M. confusa* dan β-sitosterol **(92)** telah dicirikan daripada kulit batangnya.

Ekstrak daun *M. clemensiae* memiliki aktiviti antimikrob yang kuat terhadap MRSA (*Staphylococcus aureus* yang resistan kepada methicillin) sementara kulit batangnya

telah mempamerkan aktiviti yang sederhana ke atas bakteria tersebut. Aktiviti sederhana telah diperhatikan di dalam ekstrak daun dan kulit batang spesies ini terhadap kulat *C. albicans, A. ochraceaus* dan *S. cerevisiae*. Ekstrak metanol kulit batang *M. confusa* juga menunjukkan aktiviti sederhana terhadap *Pseudomonas aeruginosa* dan *Candida albicans*. Ekstrak mentah yang lain dan beberapa sebatian yang dipencilkan seperti asid 7-geraniloksisinamik **(90)** dan sesamin **(91)** menunjukkan perencatan yang lemah ke atas pertumbuhan organisma-organisma tersebut.

Ekstrak mentah *M. lunu-ankenda*, *M. latifolia* dan *M. clemensiae* mempamerkan aktiviti antioksidan terhadap DPPH dan tiada aktiviti diperhatikan di dalam ekstrak mentah *M. confusa*. Untuk aktiviti sitotoksik pula, kesemua ekstrak mentah *M. confusa* dan *M. clemensiae* adalah tidak sitotoksik terhadap sel HL 60. Sebaliknya, ekstrak kloroform daripada daun *M. lunu-ankenda* dan ekstrak petroleum ether daripada daun *M. clemensiae* telah mempamerkan aktiviti sitotoksik yang kuat.

Kedua-dua sebatian iaitu sesamin **(91)** dan asid 7-geraniloksisinamik **(90)** telah menunjukkan aktiviti antimikrob dan antioksidan yang lemah. Walaupun diktamnin **(24)** menunjukkan aktiviti antioksidan yang lemah tetapi ia mempunyai aktiviti antimikrob dan sitotoksik yang sederhana ke atas beberapa mikrob dan sel HL 60, manakala konfusamelin **(7)** telah menunjukkan aktiviti antioksidan yang sederhana. Aktiviti sitotoksik juga telah dilakukan ke atas asid 7-geraniloksisinamik **(90)**, sesamin **(91)** dan diktamnin **(24)**. Asid 7-geraniloksisinamik **(90)** telah

mempamerkan aktiviti sitotoksik yang lemah terhadap sel HL 60 manakala sesamin **(91)** dan diktamnin **(24)** telah menunjukkan aktiviti yang sederhana terhadap sel tersebut.

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I certify that an Examination Committee met on 25th April 2005 to conduct the final examination of Najihah bt Mohd. Hashim on her Master of Science thesis entitled "Chemical Constituents and Biological Activities of Four *Melicope* Species (Rutaceae)" 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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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory 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 Universiti Putra Malaysia or other institutons.

NAJIHAH MOHD.HASHIM

Date: 17 JUNE 2005

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LIST OF ABBREVIATIONS

β	beta
δ	delta
δ	chemical shift in ppm
λ_{max}	maximum wavelength in mm
µg	microgram
µL	microliter
^{13}C	carbon -13
°C	degree celcius
$CDCl_3$	deuterated chloroform
CD_3OD	deuterated methanol
cm^{-1}	per centimeter
COSY	Correlated Spectroscopy
d	doublet
dd	doublet of doublet
ddd	doublet of doublet of doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	dimethyl sulphoxide
EtOAC	ethyl acetate
EIMS	Electron Impact Mass Spectrometry
g	gram
GC-MS	Gas Chromatography-mass spectroscopy

^1H	proton
HMBC	Heteronuclear Multiple Bond Connectivity by 2D Multiple Quantum
HSQC	Heteronuclear Single Quantum Coherence
IC_{50}	Inhibition Concentration at 50 percent
Id	Inhibition diameter
t	triplet
s	singlet
m	multiplet
Pet. ether	petroleum ether
MeOH	methanol
m.p	melting point
MS	Mass Spectrum
m/z	mass per charge
NMR	Nuclear Magnetic Resonance
OD	Optical density
TLC	Thin Layer Chromatography
IR	Infrared
UV	Ultraviolet
ε	molar absorptivity

CHAPTER I

INTRODUCTION

All cultures from ancient times to the present day have used natural products from plant extracts, animal products and mineral with therapeutic properties as a source of medicine. Today, according to the World Health Organization (WHO), as much as 80 % of the world's population depend on traditional medicine for their primary health care needs (Cordell, 1995). The major part of traditional therapy involves the use of plant extracts.

The importance of studies of natural products lies on the fact that it contributes directly to the well-being of human beings and the economy of the nation. Nature is an attractive source of new therapeutic compounds which is rich in chemical diversity and have been closely associated with human activity. The plant kingdom from which most of the natural products were derived, represents the major source of renewable resource to mankind. In addition, with the steady depletion of fossil fuel supplies and with the over-rising price of crude oil, the importance of plants as a new source of raw materials will definitely increase.

Antibiotics have saved countless lives but due to the indiscriminate use of antibiotics, such as regular treatment for long period of time has resulted in the emergence of a number of resistant bacteria. Bacterial strain such as *Staphylococcus aureus* is resistant to methicillin (MRSA) and the only established

treatment against MRSA is vancomycin. Therefore, to overcome the increasing resistance of nosocomial and community-acquired bacteria, more effective antimicrobial agents with novel modes of action must be developed.

Chemotherapy often causes unpleasant and life threatening side effects in most of cancer patients whereby the drugs are toxic to both tumour cells and proliferating normal cells especially the bone marrow (decrease production of blood), gastrointestinal (nausea and vomiting) and hair follicles (alopecia). Therefore new chemotherapeutic drugs need to be discovered from nature especially from plants in combating these complications.

With the arrival of modern chemistry and pharmacology during the nineteenth century, scientists were able to discover bioactive compounds from plants which have played an important role especially as a source of medicinal agent and in particular in the area of cancer and infectious diseases. Among the new approved drugs reported between 1983-1994, nearly 78 % of the antibacterial drugs and 61 % of the anticancer drugs are naturally derived from natural product parents (Cordell, 1995). Examples of important plant-derived drugs are digoxin from *Digitalis* spp., atropine from *Atropa beladona* and morphine from *Papaver somniferum*. Only then, we can make our heritage in traditional medicine or plant-derived compounds an asset for combating diseases and for improvement of health. There remains a great deal of work to be done to explore our abundant plant biodiversity systematically.

One of the important plant families intensively studied in UPM is the Rutaceae family. This family contains various compounds such as coumarin, alkaloids, flavanoids and sulphones. Some of these compounds have been proven biologically active. The genus *Melicope* species is one of the genus in the Rutaceae family (the genus was formally known as *Evodia* but had been categorized under *Melicope* recently). This genus is apparently rich in the above-mentioned compounds (Waterman and Grundon, 1983). It is characterized by its aromatic trees and opposite or whorled, trifoliate or unifoliate leaves. Several species of *Melicope* from various places have been phytochemically studied on certain parts of the plants and some bioactive secondary metabolites were discovered. For example the crude extracts of the stem barks and the essential oils of the *M. suberosa, M. latifolia and M. ridleyi* were found to possess antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus* (Mat Ali *et al.,* 1988). Whereas antifungal and antibacterial activity were reported from root-bark extracts and aerial parts of *M. lunu-ankenda* (McCormick *et al.,* 1996).

In this study, four *Melicope* species namely, *M. confusa* T.G. Hartley, *M. clemensiae* (Merr.) Liu, *M. lunu-ankenda* (Gaertn.) T.G. Hartley *and M. latifolia* (D.C) T.G. Hartley were investigated in detail. The purpose of this research are to extract and identify the chemical constituents *of* four *Melicope* species using chromatographic and modern spectroscopic methods. The crude extracts and isolated compound obtained were screened *in vitro* for antimicrobial and cytotoxicity to determine their biological activities. Some members of this genus have been investigated or studied in detail, however, many other *Melicope* species

especially from Hutan Sepilok, Sandakan have not been well studied. Thus, there is a need to identify bioactive compounds from this genus for effective drug development.

Objectives of Study

The objectives of this study are:

1. To extract and isolate compounds from the leaves and stem bark of *Melicope lunu-ankenda* (Gaertn.) T.G. Hartley, *M. clemensiae* (Merr.) Liu, *M. confusa* (T.G. Hartley), and leaves of *M. latifolia* (D.C) Hartley.
2. To elucidate the structures of the compounds using modern spectroscopic methods.
3. To screen the antimicrobial, cytotoxic and free radical scavenging activities of the crude extracts and isolated compounds.

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