



UNIVERSITI PUTRA MALAYSIA

**CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF
SELECTED CINNAMOMUM SPECIES (LAURACEAE) AND
MELICOPE CF. HOOKERI T.G. HARTLEY (RUTACEAE)**

NOR AZAH BINTI MOHAMAD ALI.

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By

NOR AZAH MOHAMAD ALI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

June 2004



Dedicated to my parents, my husband, Mokhlis Maizan and my children



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: Professor Mawardi Rahmani, Ph.D.

Faculty: Science and Environmental Studies

The essential oils of nine *Cinnamomum* species (*C. sintoc*, *C. pubescens*, *C. impressiscostatum*, *C. subavenium*, *C. microphyllum*, *C. scortechinii*, *C. rhyncophyllum*, *C. aureofulvum* and *C. verum*) were investigated by means of gas chromatography (GC) and combination of gas chromatography/mass spectrometer (GC/MS). The chemical components of the essential oils (leaf, stem bark and twig oils) were identified by co-chromatography with authentic samples on three columns of different polarity, comparison with Kovats retention indices, capillary GC/MS and proton NMR on selected isolated components. The essential oils were made up of one, two or all three of the following class of compounds; monoterpenes and sesquiterpenoids, phenylpropanoids and benzyl esters. Some of the chemical components observed in the oils are (*E*)-methyl cinnamate, safrole, benzyl benzoate, linalool, terpinen-4-ol and camphor which are commercially important chemicals in the flavour and pharmaceutical industries. The distribution and occurrences of



specific compounds in different parts of the plants among the different species may be used as a chemotaxonomic marker for species identification.

Cinnamomum species (*C. impressicostatum*, *C. pubescens*, *C. microphyllum*) and *Melicope* cf. *hookeri* were selected for a study of their chemical constituents and biological properties. All of these species have not been reported previously on their chemical constituents. Phytochemical analysis of the bark and stem parts of *C. impressicostatum* yielded safrole (15), (*E*)-methyl cinnamate (17), (*E*)-piperonylprop-2-enal (116), cinnamic acid (117) and β -sitosterol (118). Similar analysis on the bark of *C. pubescens* also afforded safrole (15), (*E*)-methyl cinnamate (17), (*E*)-piperonylprop-2-enal (116) and β -sitosterol (118) as well as (*E*)-piperonylprop-2-enol (119). Antimicrobial test using the disc diffusion method showed that the chloroform and the hexane extract of the two species contained almost solely (*E*)-methyl cinnamate, which were active against fungus. The presence of (*E*)-piperonyl-2-enal (116) and (*E*)-piperonylprop-2-enol (119) were reported for the first time from the genus *Cinnamomum*.

Phytochemical investigation on the stem and bark of *Cinnamomum microphyllum* resulted in the isolation of a coumarin; scoparone (120), benzyl benzoate (4), β -sitosterol (118) and mixtures of pinoresinol-type lignans; pinoresinol (121), syringaresinol (37) and medioresinol (122). The lignan mixture was found to possess significant antioxidant activity against three antioxidant assays;



autooxidation of linoleic acid, xanthine/xanthine oxidase superoxide scavenging assay and DPPH radical scavenging activity.

Phytochemical investigation on the leaves and bark of *Melicope cf. hookeri* T.G. Hartley resulted in the isolation of three flavonoids, two coumarins and sterols. The three flavonoids; ayanin (**57**), ombuin (**123**) and kumatakenin (**124**) together with β -sitosterol (**118**) were isolated from the leaves. The bark extract afforded ayanin (**57**), β -sitosterol (**118**), umbelliferone (**102**) and scopoletin (**125**). Crude extracts of *M. cf. hookeri* were screened for antimicrobial activity, antioxidant activity and TPA-induced ear oedema assay. The extracts failed to show significant antimicrobial or antioxidant activity. However, the leaf ethyl acetate extract showed strong anti-inflammatory activity with the chloroform extract (95%) and methanol extract (92%) showing the highest inhibitions as compared to the petroleum ether extract (70%). The presence of flavonoid compounds in the species probably contribute to the anti-inflammatory activity of the plant extracts.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**KOMPONEN KIMIA DAN AKTIVITI BIOLOGI TERHADAP SPESIES
TERPILIH *CINNAMOMUM* (LAURACEAE) DAN *MELICOPE* CF.
HOOKERI T.G. HARTLEY (RUTACEAE)**

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Kandungan minyak pati sembilan spesies *Cinnamomum* (*C. sintoc*, *C. pubescens*, *C. impressiscostatum*, *C. subavenium*, *C. microphyllum*, *C. scortechinii*, *C. rhyncophyllum*, *C. aureofulvum* dan *C. verum*) telah dikaji dengan menggunakan kromatografi gas (GC) dan gabungan kromatografi gas/spectrometer jisim (GC/MS). Komponen-komponen bahagian minyak pati tersebut (daun, kulit kayu dan ranting) telah dicamkan dengan kaedah ko-kromatografi dengan sampel tulen menggunakan tiga turus kapilari yang mempunyai polariti yang berbeza, perbandingan dengan Indeks Penahanan Kovats, perbandingan spektrum jisim dengan spektrum jisim rujukan dan pengecaman proton NMR terhadap komponen yang berjaya dipisahkan. Kandungan minyak pati yang dikaji didapati mengandungi satu, dua atau ketiga-tiga sebatian kimia berikut; monoterpenoid dan seskuiterpenoid, fenilpropanoid dan benzilik ester. Antara komponen kimia yang sering dijumpai dalam minyak pati *Cinnamomum* ialah metil sinnamat, safrol, benzil benzoat, linalool, terpinen-4-ol dan kamfor iaitu bahan kimia penting yang digunakan secara komersil dalam industri



perisa dan farmaseutikal. Taburan dan kewujudan komponen kimia tertentu dalam bahagian pokok yang berbeza di kalangan spesies *Cinnamomum* yang berbeza boleh digunakan sebagai penunjuk kemotaksonomi dalam pengecaman spesies.

Spesies *Cinnamomum* (*C. impressicostatum*, *C. pubescens*, *C. microphyllum*) dan *Melicope* cf. *hookeri* telah dipilih untuk kajian kimia dan aktiviti biologi. Kandungan komponen kimia kesemua spesies ini belum pernah dilaporkan. Kajian fitokimia yang dijalankan terhadap kulit dan batang *Cinnamomum impressicostatum* menghasilkan safrol (15), (*E*)-metil sinamat (17), (*E*)-piperonilprop-2-enal (116), asid sinamik (117) dan β -sitosterol (118). Kajian pemencilan terhadap kulit *C. pubescens* pula telah menghasilkan sebatian kimia yang sama; safrol (15), (*E*)-metil sinamat (17), (*E*)-piperonilprop-2-enal (116), β -sitosterol (118) dan (*E*)-piperonilprop-2-enol (119). Kajian antimikrob menggunakan kaedah peresapan cakera terhadap kulat (*C. albicans*, *C. lipolytica*) menunjukkan bahawa ekstrak heksana dan kloroform yang mengandungi komponen (*E*)-metil sinamat adalah sangat aktif. Kehadiran (*E*)-piperonilprop-2-enal (116) dan (*E*)-piperonilprop-2-enol (119) pertama kali dilaporkan dari genus *Cinnamomum*.

Kajian terperinci terhadap kulit kayu *Cinnamomum microphyllum* menghasilkan pengecaman sebatian kumarin, skoparon (120), benzil benzoat (4), β -sitosterol (118) dan sebatian lignan iaitu pinoresinol (121), siringaresinol (37) dan medioresinol (122). Gabungan sebatian lignan ini telah didapati memberikan aktiviti antioksidan yang signifikan terhadap tiga esei antioksidan; autooksidasi asid

linoleik, ujian penyingkiran superoksida zantina/zantina oksida dan ujian penyingkiran radikal bebas DPPH.

Kajian fitokimia terhadap daun dan kulit *Melicope cf. hookeri* T.G. Hartley telah menghasilkan pemencilan tiga sebatian flavonoid, dua koumarin dan sterol. Tiga sebatian flavonoid; ayanin (57), ombuin (123) dan kumatakenin (124) bersama-sama β -sitosterol (118) telah dipencilkan daripada bahagian daun. Bahagian kulit batang pula telah menghasilkan sebatian ayanin (57), β -sitosterol (118) umbeliferon (102) dan skopoletin (125). Ekstrak mentah *M. cf. hookeri* telah diuji terhadap esei antimikrob, antioksidan dan anti radang. Kesemua ekstrak yang dikaji gagal menunjukkan keputusan aktiviti antimikrob dan antioksidan yang signifikan. Walau bagaimanapun, ekstrak kloroform daun *M. cf. hookeri* menunjukkan aktiviti anti radang yang tertinggi (95%) berbanding dengan ekstrak metanol (92%) dan petroleum eter (70%). Kehadiran sebatian flavonoid di dalam spesies ini berkemungkinan mempengaruhi aktiviti anti-radang ekstrak tumbuhan tersebut.

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LIST OF ABBREVIATIONS

α	Alpha
β	Beta
δ	Delta Chemical shift in ppm
γ	Gamma
λ_{\max}	Maximum wavelength in nm
μg	Microgram
μl	Microliter
$\mu\text{g}/\mu\text{l}$	Microgram/microliter
br	Broad
brs	Broad singlet
BHT	Butylated hydroxy toluene
^{13}C	Carbon-13
$^{\circ}\text{C}$	Degree celcius
cm^{-1}	Centimeter
CDCl_3	Deuterated chloroform
CHCl_3	Chloroform
COSY	Correlated spectroscopy
CD_3OD	Deutrated methanol
CD_3COCD_3	Deuterated acetone
DEPT	Distortionless enhancement by polarisation transfer
d	Doublet
dd	Doublet of doublet
ddd	Doublet of doublet of doublet
DMSO	dimethylsulfoxide
DPPH	1,2-Diphenyl-2-picrylhydrazyl
eV	Electron volt
EC	Effective concentration
ED	Effective dose
EDTA	Ethylenediamine tetracetic acid
EIMS	Electron impact mass spectrometry
ELISA	Enzyme –linked Imunosorbent assay
EtOH	Ethanol
FCS	Fetal calf serum
g	Gram
m	Multiplet
FCS	Fetal calf serum
GC	Gas chromatography
GC/MS	Gas chromatography/mass spectrometer
^1H	proton
Hex	Hexane
HMBC	Heteronuclear Multiple Bond Connectivity by 2D multiple quantum

