PHYTOCHEMICAL AND BIOLOGICAL ACTIVITY STUDIES OF *COSMOS CAUDATUS* AND *CURCUMA MANGGA* AND THE ONLINE CHARACTERIZATION OF BIOACTIVE FRACTIONS FROM *MELICOPE PTELEFOLIA*

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By

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PHYTOCHEMICAL AND BIOLOGICAL ACTIVITY STUDIES OF *COSMOS CAUDATUS* AND *CURCUMA MANGGA* AND THE ONLINE CHARACTERIZATION OF BIOACTIVE FRACTIONS FROM *MELICOPE PTELEFOLIA*

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Twelve species of Malay traditional vegetables were screened for antioxidant and nitric oxide (NO) inhibitory activities. Ferric thiocyanate (FTC), thiobarbituric acid (TBA) and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) methods were used for the antioxidant activity measurements, and the Griess assay on elicited murine peritoneal macrophages was used to assess NO inhibitory activity of the extracts. *Melicope ptelefolia*, *Cosmos caudatus,* and *Curcuma mangga* were selected for further study, based on the results of their biological activity evaluations.

Characterization of the NO inhibitory fractions of the *Melicope ptelefolia* using on-line high performance liquid chromatography (HPLC)-diode array detector (DAD)-mass spectrometry (MS), identified seven main constituents. The compounds were identified as kokusaginine (**1**), either kokusagine (**2a**) or 5-methoxymaculine (**2b**), 3-prenyl-2,4,6-trihydroxyacetophenone (**3**), 3-geranyl-2,4,6-trihydroxyacetophenone (**4**), 3-geranylgeranyl-2,4,6-trihydroxyaceto-phenone (**5**), 3-[4-*O*-(3,7-dimethyl-2,6-octadienyl)phenyl]-2-propenoic acid (**6**), and 3-farnesylgeranyl-2,4,6-trihydroxyacetophenone (**7**).

Phytochemical investigation of the methanolic extract of *Cosmos caudatus* led to the isolation of four known compounds namely, quercetin 3-*O*-*β*-arabinofuranoside (**17**), quercetin 3-*O*-α-rhamnoside (**18**), quercetin 3-*O*-*β*-glucoside (**19**), and quercetin (**20**). All four compounds isolated from *C. caudatus* showed strong antioxidant activity. The activity was in the order of **20 > 17 > 18 > 19 >** α-tocopherol (standard).

From the rhizomes of *C. mangga*, eleven compounds were isolated, namely a mixture of stigmasterol and *β*-sitosterol (**141**), demethoxycurcumin (**101**), bisdemethoxycurcumin (**102**), 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (**113**), 7-hydroxy-6-methoxycoumarin (**142**), curcumin (**100**), zerumin B (**143**), curcumanggoside (**144**), 4-hydroxycinnamic acid (**145**), labda-8(17),12-diene-

15,16-dial (**128**), and calcaratarin A (**146**). Curcumanggoside was identified as a new compound, while zerumin B and calcaratarin A were isolated for the first time from the genus *Curcuma*. The structures of these compounds were established based on spectral data and comparison with the literature. Four diarylheptanoids, demethoxycurcumin, bisdemethoxycurcumin, curcumin, and 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, showed strong antioxidant activity. Zerumin B showed strong and selective cytotoxic activity to four cell lines, including HL-60, HepG2, MCF-7 and DU-145 with IC_{50} values of 7.21, 25.33, 0.59 and 11.21 μM, respectively.

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

KAJIAN FITOKIMIA DAN AKTIVITI BIOLOGI DARIPADA *COSMOS CAUDATUS* DAN *CURCUMA MANGGA* DAN PENCIRIAN SECARA TERUS FRAKSI-FRAKSI BIOAKTIF DARIPADA *MELICOPE PTELEFOLIA*

Oleh

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Dua belas jenis ulam telah dikaji untuk menentukan aktiviti antioksidaan dan perencatan nitrik oksida (NO). Kaedah ferrik tiosianat (FTC), asid tiobarbiturik dan radikal bebas 1,1-difenil-2-pikrilhidrazil telah digunakan untuk menentukan aktiviti antioksidaan dan kaedah Griess digunakan untuk menentukan

perencatan NO. *Melicope ptelefolia*, *Cosmos caudatus* dan *Curcuma mangga* telah dipilih untuk kajian lebih lanjut berdasarkan keputusan penilaian aktiviti-aktiviti biologi.

Pencirian komponen fraksi-fraksi *M. ptelefolia* yang menunjukkan aktiviti perencatan NO menggunakan HPLC-DAD-MS/MS telah mengenalpasti tujuh sebatian kimia. Sebatian-sebatian itu dikenalpasti sebagai kokusaginina (**1**), samada kokusagina (**2a**) atau 5-metoksimakulina (**2b**), 3-prenil-2,4,6-trihidroksiasetofenon (**3**), 3-geranil-2,4,6-trihidroksiasetofenon (**4**), 3-geranilgeranil-2,4,6-trihidroksiasetofenon (**5**), asid 3-[4-*O*-(3,7-dimetil-2,6-oktadienil)fenil]-2-propenoik (**6**), and 3-farnesilgeranil-2,4,6-trihidroksiasetofenon (**7**).

Kajian fitokimia terhadap ekstrak metanol *C. caudatus* telah berjaya memencilkan empat sebatian yang diketahui, iaitu kuersetin 3-*O*-*β*-arabinofuranosida (**17**), kuersetin 3-*O*-α-ramnosida (**18**), kuersetin 3-*O*-*β*-glukosida (**19**), dan kuersetin (**20**). Semua sebatian yang telah dipencilkan dari *C. caudatus* menunjukkan aktiviti antioksidaan yang tinggi. Aktiviti mengikut turutan adalah **20 > 17 > 18 > 19 >** α-tokoferol (piawai).

Sebelas sebatian berjaya dipencilkan daripada rizom *Curcuma mangga*, iaitu campuran stigmasterol dan *β*-sitosterol (**141**), demetoksikurkumin (**101**),

bisdemetoksikurkumin (**102**), 1,7-bis(4-hidroksifenil)-1,4,6-heptatrien-3-on (**113**), 7-hidroksi-6-metoksikaumarin (**142**), kurkumin (**100**), zerumin B (**143**), curcumanggosida (**144**), asid 4-hidroksisinamik (**145**), labda-8(17),12-diene-15,16-dial (**128**), dan calcaratarin A (**146**). Curcumanggosida merupakan sebatian baru. Ini merupakan laporan yang pertama mengenai pemencilan zerumin B dan calcaratarin A daripada genus *Curcuma*. Struktur sebatian-sebatian ini dikenalpasti berdasarkan data spektroskopi dan perbandingan dengan literatur. Keempat-empat diarilheptanoid, iaitu demetoksikurkumin, bisdemetoksikurkumin, kurkumin dan 1,7-bis(4-hidroksifenil)-1,4,6-heptatrien-3-on telah menunjukkan aktiviti antioksidaan yang tinggi. Zerumin B didapati sitotoksik terhadap empat talian sel termasuk HL-60, HepG2, MCF-7 dan DU-145 dengan nilai 50% perencatan 7.21, 25.33, 0.59 dan 11.21 μM setiap satu.

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I certify that an Examination Committee met on 8th February 2005 to conduct the final examination of Faridah binti Abas on her Doctoral of Philosophy thesis entitled "Phytochemical and Biological Activity Studies of *Cosmos caudatus* and *Curcuma mangga* and the Online Characterization of Bioactive Fractions from *Melicope ptelefolia*" 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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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 UPM or other institutions.

_____

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