UNIVERSITI PUTRA MALAYSIA

DETECTION OF ANTIOXIDANT CONSTITUENTS FROM *Persicaria hydropiper* USING LC-UV-DAD-ESIMS/MS

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By

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

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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

DETECTION OF ANTIOXIDANT CONSTITUENTS FROM *Persicaria hydropiper* USING LC-UV-DAD-ESIMS/MS

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Chairman: Dr. Faridah Abas, PhD

Institute: Bioscience

The antioxidant activity of the crude methanolic extract and fractions of the aerial parts of *Persicaria hydropiper* or 'kesum' were investigated to evaluate their potential application as health supplement. Dried aerial part of the plant samples were extracted with methanol and the methanol crude extract was fractionated by liquid-liquid extraction into five different fractions. All fractions were analyzed for total phenolic content utilizing Folin Ciocalteau assay. The antioxidative potential of all fractions were also evaluated using 1,1-diphenyl-2-picryhydrazyl radical (DPPH), ferric thiocyanate (FTC) and xanthine oxidase inhibition (XO) methods. Furthermore, the metabolite profiling of the bioactive antioxidant fractions was accomplished using liquid chromatography coupled with mass spectrometry (LCMS). The use of LCMS techniques is an important approach for online identification of plant constituents.

The amount of the total phenolics, varied widely in the fractions and ranged from 0.00 to 224.4 mg GAE/100 g dry extract. The highest level was found in butanol fraction with 224.4 mg GAE/100 g dry extract, followed by ethyl acetate (68.9 mg GAE/100 g dry extract), dichloromethane (8.7 mg GAE/100 g dry extract), aqueous (6.4 mg GAE/100 g extract) and hexane (4.3 mg GAE/100 g dry extract) fractions. The DPPH assay revealed that the ethyl acetate and butanol fractions of *P. hydropiper* were the most active fractions with IC_{50} values of 25.5 and 28.6 µg/ml, respectively. Dichloromethane fraction showed moderate activity with IC_{50} value of 94.2 µg/ml. The lower radical scavenging activity was observed in aqueous and hexane fractions with IC_{50} values ranged from 110 to 380 µg/ml. In comparing the antioxidant activity using ferric thiocyanate assay, all fractions effectively inhibited higher lipid oxidation compared to α-tocopherol. Except for the hexane fraction, the inhibition of the other fractions were comparable to the known synthetic antioxidant, butylated hydroxytoluene (BHT). Of the fractions assayed for xanthine oxidase inhibitory activity, butanol fraction showed the most active with IC_{50} value of 32.3 µg/ml followed by ethyl acetate fraction with IC_{50} value of 166.9 µg/ml.

The ethyl acetate and butanol fractions exhibited good antioxidant activity in all the assay tested. The LC-DAD-ESIMS chemical profiling method was developed for the identification of phytochemical constituents in the antioxidative fractions of *P. hydropiper*. The phytochemical profile of ethyl acetate fraction has identified fourteen compounds together with two unidentified compounds. The compounds were identified as quercetin (**1**), quercetin-3-*O*-glucoside (**3**), galloyl quercetin-3-*O*-glucoside (**5**), galloyl quercetin-3-*O*-rhamnoside (**6**), quercetin-3-*O*-rhamnoside (**7**), kaempferol-3-*O*-glucoside (**13**), galloyl kaempferol-3-*O*-glucoside (**14**),

hydropiperoside (**31**), vanicoside A (**34**), vanicoside B (**35**), vanicoside D (**38**), rhamnetin (**87**), apigenin-3-*O*-glucoside (**89**) and kaempferol rutinoside (**90**). From the ethyl acetate fraction, six compounds; **1**, **3**, **5**, **7**, **87** and 3,5-dihydroxy-4-methoxybenzoic acid (**88**) have been isolated and identified using NMR spectroscopy.

Phytochemical investigation of the bioactive butanol fraction of *P. hydropiper* using LC-DAD-ESIMS/MS led to the identification of nine compounds including rhamnazin-3-sulphate (**91**), quercetin-3-*O*-glucuronide (**8**), luteolin-3-*O*-glucoside **(92),** kaempferol-3-*O*-sulphate (**93**), quercetin-3-*O*-rhamnoside **(7)**, apigenin-3-*O*-glucoside **(89)**, quercitrin 7-sulphate (**94**), luteolin-7-sulphate **(95)** and isorhamnetin-3-sulphate (persicarin) (**96**). All compounds were relatively identified based on the MS/MS and UV data in comparison with those of the standard compounds.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan Ijazah Master Sains

PENGESANAN SEBATIAN ANTIOKSIDAN DARI *Persicaria hydropiper* MENGGUNAKAN LC-UV-DAD-ESIMS/MS

Oleh

NOOR HASLINDA BINTI NOOR HASHIM

Jun 2011

Pengerusi: Dr. Faridah Abas, PhD

Institut: Biosains

Aktiviti antioksida bagi ekstrak mentah metanol dan fraksi daripada kesum atau *Persicaria hydropiper* diuji untuk mengenalpasti potensi aktiviti antioksidannya. Sampel kesum yang telah kering diekstrak dalam metanol dan ekstrak mentah metanol dilakukan fraksinasi menggunakan kaedah pengekstrakan cecair-cecair menghasilkan lima fraksi yang berbeza. Kesemua fraksi dianalisa untuk jumlah kandungan fenol menggunakan kaedah 'Folin Ciocalteau'. Potensi antioksidan bagi semua fraksi juga diuji menggunakan radikal bebas difenil-2-pikrilhidrazil (DPPH), ferrik tiosianat (FTC) dan kaedah perencatan zantina oksidase (XO). Tambahan lagi, profil metabolit bagi fraksi yang aktif antioksidan dicapai menggunakan kromatografi cecair gandingan spektroskopi jisim.

Amaun jumlah kandungan fenol berubah-ubah secara meluas dalam fraksi dan berkisar antara 0.00-224.4 mg GAE/100 g ekstrak kering. Tingkat tertinggi terdapat

pada fraksi butanol dengan 224.4 mg GAE/100 g ekstrak kering, diikuti oleh etil asetat (68.9 mg GAE/100 g ekstrak kering), diklorometana (8.7 mg GAE/100 g ekstrak kering), akues (6.4 mg GAE/100 g ekstrak kering) dan fraksi heksana (4.3 mg GAE/100 g ekstrak kering). Ujian DPPH menunjukkan bahawa fraksi etil asetat dan butanol bagi *P. hydropiper* adalah fraksi yang paling aktif dengan nilai IC_{50} adalah 25.5 dan 28.6 µg/ml. Fraksi diklorometana menunjukkan aktiviti sederhana dengan nilai IC_{50} adalah 94.2 µg/ml. Aktiviti radikal bebas yang rendah diamati dalam fraksi akues dan heksana dengan nilai IC_{50} berkisar di antara 110 hingga 380 µg/ml. Dalam membandingkan aktiviti antioksidan dengan menggunakan ujian ferrik tiosianat, semua fraksi menunjukkan aktiviti perencatan pengoksidaan lipid lebih tinggi berbanding dengan α-tokoferol, kecuali untuk fraksi heksana, perencatan pengoksidaan lipid daripada fraksi lain setanding dengan antioksidan sintetik, butil hidroksitoluena (BHT). Daripada fraksi ujian aktiviti perencatan zantina oksidase, fraksi butanol menunjukkan yang paling aktif dengan nilai IC_{50} adalah 32.3 µg/ml diikuti oleh fraksi etil asetat dengan nilai IC_{50} adalah166.9 µg/ml.

Fraksi etil asetat dan butanol menunjukkan aktiviti antioksidan yang baik bagi kesemua ujian. Kajian LC-DAD-ESIMS telah dibangunkan untuk mengenalpasti sebatian fitokimia dalam fraksi antioksidan dari *P. hydropiper*. Profil fitokimia fraksi etil asetat telah mengenalpasti empat belas sebatian bersama-sama dengan dua sebatian yang tidak dikenalpasti. Sebatian dikenalpasti sebagai kuersetin **(1)**, kuersetin-3-*O*-glukosida **(3)**, galloyl kuercetin-3-*O*-glukosida **(5)**, galloyl kuersetin-3-*O*-ramnosida **(6)**, kuersetin-3-*O*-ramnosida **(7)**, kaempferol-3-*O*-glukosida **(13)**, galloyl kaempferol-3-*O*-glukosida **(14)**, hidropiperosida **(31)**, vanikosida A **(34)**, vanikosida B **(35)**, vanikosida D **(38)**, ramnetin **(87)**, apigenin-3-*O*-glukosida **(89)**

dan kaempferol rutinosida **(90)**. Daripada fraksi etil asetat, enam sebatian; **1**, **3**, **5**, **7**, **87** dan asid 3,5-dihidroksi-4-metoksibenzoik **(88)** telah dipencilkan dan dikenalpasti dengan menggunakan spektroskopi NMR.

Kajian fitokimia menggunakan LC-DAD-ESIMS/MS ke atas fraksi bioaktif butanol daripada *P. hydropiper* membawa kepada pengenalpastian sembilan sebatian. Sebatian tersebut adalah ramnazin-3-sulfat **(91)**, kuersetin-3-*O*-glukuronat **(8)**, luteolin-3-*O*-glukosida **(92)**, kaempferol-3-*O*-sulfat **(93)**, kuersetin-3-*O*-ramnosida **(7)**, apigenin-3-*O*-glukosida **(89)**, kuersitrin-3'-sulfat **(94)**, luteolin-7-sulfat **(95)** dan isorhamnetin-3-sulfat (persicarin) **(96)**. Kesemua sebatian dikenalpasti secara tentatif berdasarkan data MS/MS dan UV melalui perbandingan dengan sebatian rujukan.

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I certify that a Thesis Examination Committee has met on 22 June 2011 to conduct the final examination of Noor Haslinda Binti Noor Hashim on her thesis entitled "Detection Of Antioxidant Constituents From *Persicaria hydropiper* Using LC-DAD-ESI-MS/MS" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

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The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for the quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

NOOR HASLINDA BT. NOOR HASHIM

Date: 19 September 2011

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