UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS OF VITEX NEGUNDO AND EVALUATION OF THEIR ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES

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By

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Chairman : Prof. Madya Dr. Khozirah Hj. Shaari , PhD

Institute : Bioscience

Leaves and stem of *Vitex negundo* were examined for phytochemicals using various techniques such as normal column chromatography, gel filtration on Sephadex LH-20 and radial chromatography. From the leaves, seven compounds were isolated and identified, by the use of various spectroscopic methods, to be mixture of the flavonoids luteolin, luteolin-3'-*O*-glucuronide, and isoorientin, the iridoid glycosides 2'-*p*-hydroxybenzoylmussaenosidic acid and agnuside, and *p*-hydroxyl benzoic acid as well as stigmasterol and *β*-sitosterol. Meanwhile, the stem yielded four lignans which were isolated for the first time from the plant, identified as 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde**,** vitedoin A**,** vitrofolal E and detetrahydroconidendrin**.**

Nitric oxide (NO) inhibitory assay using RAW 264.7 murine macrophage and soybean lipoxygenase inhibitory assay were carried out in the screening for anti-inflamatory properties of the crude methanolic extract, the hexane, dichloromethane and ethyl acetate soluble fractions of the plant. From the leaves, both the hexane and dichloromethane fractions were shown to strongly inhibit nitric oxide production with an IC_{50} of 14.00 μg/ml and 20.00 μg/ml respectively. Meanwhile, inhibition of soybean lipoxygenase activity was shown by the ethyl acetate fractions from both plant parts with IC_{50} of 56.38 μg/ml and 63.94 μg/ml respectively.

Further anti-inflammatory investigation on some of the isolated compounds showed that luteolin was significantly inhibited NO production with an IC_{50} of 41.50 μg/ml (145.10 μM), and inhibited formation of (9Z, 11E)-(13S)-13-hydroxyoctadeca-9,11-dienoate with an IC_{50} of 1.55 μg/ml (5.42 μM). Luteolin also exhibited high activity in PAF receptor binding assay with 70.20% inhibition at concentration of 18.2 μg/ml and xanthine oxidase assay with 98.20% inhibition at concentration of 100 μg/ml. The antioxidant evaluation using DPPH radical scavenging assay showed that luteolin and 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde at a concentration of 250 μg/ml exhibited significant inhibition at 96.2% and 94.7% respectively. The results indicated that luteolin may play a key factor in the plant's ability to reduce inflammation.

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

KOMPONEN KIMIA SERTA PENILAIAN AKTIVITI ANTI-INFLAMMASI DAN ANTIOKSIDAN DARIPADA *VITEX NEGUNDO*

Oleh

FADZUREENA JAMALUDIN

Disember 2008

Pengerusi : Prof. Madya Dr. Khozirah Hj. Shaari , PhD

Institut : Biosains

Kajian fitokimia telah dilakukan terhadap daun dan batang *Vitex negundo* melalui penggunaan berbagai teknik kromatografi seperti kromatografi turus biasa, filtrasi gel Sephadex LH-20 dan kromatografi radial. Tujuh sebatian telah berjaya dipencilkan dan dikenalpasti menggunakan pelbagai teknik spektroskopi daripada ekstrak daun iaitu daripada kumpulan flavonoid, luteolin, luteolin-3'-*O*-glukuronida dan isoorientin, daripada kumpulan iridoid glukosida, 2'-*p*-hidroksibenzoilmussaenosidik asid dan agnusida, juga sebatian yang dikenali sebagai *p*-hidroksibenzoik asid dan campuran stigmasterol and *β*-sitosterol. Daripada ekstrak batang, empat sebatian daripada kumpulan lignan berjaya dipencilkan pertama kali daripada tanaman ini yang dikenali sebagai 6-hidroksi-4-(4-hidroksi-3-metoksiphenil)3-hidroksimetil-7-metoksi-3,4-dihidro-2-naphthaldehid**,** vitedoin A**,** vitrofolal E and detetrahidroconidendrin**.**

Daripada penialaian sifat anti-nflammasi tanaman ini telah diuji dengan model inflamasi seluler dan asai kinetic enzimatik. Asai perencatan nitric oksida (NO) menggunakan makrofag murin monositik (RAW 264.7) dan asai lipoxigenase kacang soya telah digunakan sebagai penabiran awal bagi ekstrak kasar methanol, fraksi heksana, fraksi diklorometana dan fraksi etil asetat. Hasilnya fraksi heksana dan fraksi diklorometana daripada daun menunjukkan aktiviti yang kuat merencat penghasilan nitric oksida, dengan nilai 50% perencatan 14.00 μg/ml dan 20.00 μg/ml setiap satu. Manakala penekanan lipoksigenase kacang soya hanya diperlihatkan oleh fraksi etil asetat daripada kedua-dua bahagian daipada tanaman ini dengan nilai 50% perencatan 56.38 μg/ml dan 63.94 μg/ml setiap satu.

Penyelidikan anti-inflamasi keatas sebahagian daripada sebatian hasil penulinan menunjukkan luteolin secara signifikan menekan pembebasan nitric oksida dengan nilai 50% perencatan 41.50 μg/ml (145.10 μM), dan merencatan penghasilan (9Z, 11E)-(13S)-13-hydroksioktadeka-9,11-dienoat dengan nilai 50% perencatan 1.55 μg/ml (5.42 μM). Luteolin juga didapati memberi nilai perencatan yang tinggi daripada asai penggabungan reseptor PAF dengan peratus perencatan sebanyak 70.20% pada kepekatan 18.2 μg/ml dan pada asai xanthine oksida dengan peratus perencatan sebanyak 98.20% pada kepekatan 100 μg/ml.

Kajian anti-oksida yang telah dilakukan menggunakan asai pemusnah radikal DPPH mendapati dua sebatian iaitu luteolin and 6-hidroksi-4-(4-hidroksi-3-metoksiphenil)3-hidroksimetil-7-metoksi-3,4-dihidro-2-naphthaldehid pada

kepekatan 250 μg/ml secara signifikan memberi peratus perencatan sebanyak 96.22% dan 94.7% setiap satu. Keputusan yang dipeolehi menunjukkan sebatian luteolin yang berjaya dipencilkan memainkan peranan penting dalam tanaman ini bagi merencat kesan inflammasi.

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I certify that a Thesis Examination Committee has met on 10th December 2008 to conduct the final examination of Fadzureena binti Jamaludin on her thesis entitled "Chemical Constituents of *Vitex negundo* and the Evaluation of their Anti-inflammatory and Antioxidant Activities" in accordance with the Universities and University Colleges 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 Degree of Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were 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, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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FADZUREENA JAMALUDIN
Date :

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GLOSSARY OF ABBREVIATIONS

MRSA	Methicilin-resistant *Staphylococcus aureus*
MSSA	Methicilin-sensitive *Staphylococcus aureus*
PAMPs	Pathogen-associated molecular patterns
IL-1	Interleukin-1
TNF-α	Tumor necrosis factor-α
PG	Prostaglandin
LT	Leukotrien
PAF	Platelet-activating factor
LOX	Lipoxygenase
HLO	Human lipoxygenase
AA	Arachidonic acid
FLAP	5-lipoxygenase activating protein
5-HPETE	5S-hydroperoxy-6,8-trans-11,14-*cis*-eicosatetraenoic acid
LTA_4	Leukotriene A_4
LTB_4	Leukotriene B_4
LTC_4	Leukotriene C_4
LTD_4	Leukotriene D_4
LTE_4	Leukotriene E_4
NO	Nitric oxide
NOS	Nitric oxide synthase
XO	Xanthine Oxidase
ROS	Reactive oxygen species
UV	Ultraviolet
EIMS	Electron Spray Impact Mass Spectrometry
LCMS	Liquid Chromatography Mass Spectrometry
FTIR	Fourier Transform Infra Red
NMR	Nuclear Magnetic Resonance
COSY	Correlation Spectroscopy
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Spectroscopy

TLC	Thin Layer Chromatography
MTT	3-(4,5)-dimethyl-thiazol-2-yl)2,5-diphenyltetrazolium bromide
DPPH	1,2-diphenyl-2-picrylhydrazyl
AOP	Antioxidant Potential
NBT	Nitro blue tetrazolium
OD	Optical density