UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS OF *ANAXAGOREA JAVANICA* AND THEIR ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES

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By

KHAIRANA BINTI HUSAIN

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

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Chairman : Professor Nordin H. Lajis, PhD

Institute : Bioscience

Anaxagorea javanica Blume (synonym *A. scortechinii* King) or locally known as 'bunga pompun', 'kekapur', 'larak lecek', 'akar angin', 'kinchong' or 'atis' is an endermic plant in Malaysia. The plant belongs to Annonaceae family and is traditionally used for the treatment after childbirth.

Phytochemical investigation on the bark and root of this plant yielded seventeen pure compounds, comprising steroids, fatty acids, simple phenolics, alkaloids, flavonoids and sesquiterpenes. In total, seven alkaloids were isolated, out of which two new derivatives of copyrine alkaloid were 4,11-dimethoxyeupolauridine (**87**) and 2-methoxy-3-hydroxyeupolauridine (**88**) in addition to four known compounds, eupolauridine (**22**), 11-

methoxyeupolauridine (**86**), sampangine (**95**) and 3-methoxysampangine (**96**). Another known alkaloid came from oxoaporphine was lysicamine (**91**). The isolation of copyrine and oxoaporphine alkaloids was a first time from the genus. Other known compounds isolated, included a mixture of β-sitosterol (**49**) and stigmasterol (**94**), quercetin (**46**), 7α-hydroxystigmasterol (**92**), 7-oxostigmasterol (**93**), 4-hydroxybutanamide (**95**), tetradecanoic acid (**96**), syringic aldehyde (**97**) and 2,3-dihydroxy-1-(4'-hydroxy-3',5'-dimethoxyphenyl)-1-propanone (**98**). A novel sesquiterpene identified as 2,2,9-trimethyl-5-methylene-12-oxa-bicyclo[6.3.1]dodecane-4,9-diol (**85**) (nordine) was also isolated. All structures were elucidated by spectroscopic techniques and by comparison with data available from literature.

Studies on the biological activities related to inflammation were conducted using nitric oxide (NO) and 5-lipoxygenase (5-LOX) inhibitory assay as a priminary screening as well as antioxidant assay. The results showed that both hexane and dichloromethane extracts from the barks possessed weak inhibition on NO production at the highest concentration of 200 µg/ml without cytotoxic effect. Meanwhile, inhibition of lipoxygenase activity was shown only by methanolic and acetone extracts from roots at 100 µg/ml with an IC_{50} of 48.21 and 63.87 µg/ml, respectively. However, all the crude extracts from barks and roots are poor radical scavenger inhibitors. Further *in vitro* investigation on the selected pure compounds showed that only sampangine (**89**) and 3-

methoxysampangine (**90**) significantly inhibited NO production with IC_{50} values of 18.17 and 32.25 µg/ml, respectively, at the highest concentration of 25 µg/ml tested.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia

sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

SEBATIAN KIMIA KE ATAS *ANAXAGOREA JAVANICA* SERTA AKTIVITI ANTIOKSIDA DAN ANTI-INFLAMASI

Oleh

KHAIRANA BINTI HUSAIN

Disember 2008

Pengerusi : Professor Nordin H. Lajis, PhD

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Anaxagorea javanica Blume (sinonim *A. scortechinii* King) atau dikenali sebagai 'bunga pompun', 'kekapur', 'larak lecek', 'akar angin', 'kinchong' atau 'atis' merupakan tumbuhan endemik di Malaysia. Tumbuhan ini tergolong di dalam famili Annonaceae dan kegunaan tradisionalnya untuk merawat perempuan selepas bersalin.

Penyelidikan fitokimia ke atas bahagian kulit batang dan akar tumbuhan ini menghasilkan tujuhbelas sebatian tulen, yang mengandungi steroid, asid lemak, fenolik ringkas, alkaloid, flavonoid dan seskuiterpena. Sejumlah tujuh alkaloid yang telah dipencilkan, dua daripadanya merupakan terbitan baru daripada alkaloid kopirina yang dinamakan sebagai 4,11-dimetoksieupolauridina (**87**)

dan 2-metoksi-3-hidroksieupolauridina (**88)** bersama-sama dengan tiga sebatian yang diketahui, eupolauridina (**22**), 11-metoksieupolauridina (**86**), sampangina (**95**) dan 3-metoksisampangina (**96**). Sebatian alkaloid lain yang diketahui daripada alkaloid oxoaporfina dinamakan sebagai lisikamina (**91**). Sebatian lain yang diketahui yang telah ditulenkan, termasuklah campuran daripada β-sitosterol (**49**) and stigmasterol (**94**), kuersetin (**46**), 7α-hidroksistigmasterol (**92**), 7-oxostigmasterol (**93**), 4-hidroksibutanamida (**95**), asid tetradekanoik (**96**), aldehid siringik (**97**) dan 2,3-dihidroksi-1-(4'-hidroksi-3',5'-dimetoksifenil)-1-propanon (**98**). Seskuiterpena novel dikenalpasti sebagai 2,2,9-trimetil-5-metilena-12-oxa-bisiklo[6.3.1]dodekana-4-9-diol atau dinamakan sebagai nordina (**85**) turut juga dipencilkan. Struktur kesemua sebatian dielusidasi berdasarkan kaedah spektroskopi dan perbandingan dengan data spectrum daripada literatur.

Kajian penyaringan awal ke atas aktiviti biologi yang berkaitan inflamasi dilakukan dengan menggunakan asai perencatan nitrik oksida (NO), 5-lipoksigenase (5-LOX) dan antioksida. Keputusan ujian menunjukkan kedua-dua ekstrak heksana dan diklorometana daripada kulit batang menunjukkan aktiviti yang lemah ke atas pengeluaran nitrik oksida pada kepekatan tertinggi 200 μg/ml tanpa kesan ketoksikan. Manakala, aktiviti perencatan lipoksigenase pada 100 μg/ml menunjukkan hanya ekstrak metanol dan aseton daripada akar dengan nilai IC_{50} 48.21 dan 63.87 μg/ml, masing-masing. Walau bagaimanapun,

semua ekstrak mentah daripada bahagian kulit batang dan akar adalah lemah terhadap perencatan skavenger radikal. Kajian selanjutnya ke atas anti-inflamasi ke atas sebatian-sebatian tulen terpilih menunjukkan hanya sampangina (**89**) and 3-metoksisampangina (**90**) secara signifikan merencat pengeluaran NO pada kepekatan tertinggi 25 μg/ml dengan nilai IC_{50} 18.17 dan 32.25 μg/ml, masing-masing.

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I certify that a Thesis Examination Committee has met on 24th December 2008 to conduct the final examination of Khairana binti Husain on her thesis entitled "Chemical Constituents of *Anaxagorea javanica* and Their Antioxidant and Anti-inflammatory 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 Doctor of Philosophy.

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follow:

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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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KHAIRANA BINTI HUSAIN

Date:

TABLES OF CONTENTS

LIST OF TABLES

LIST OF FIGURES