



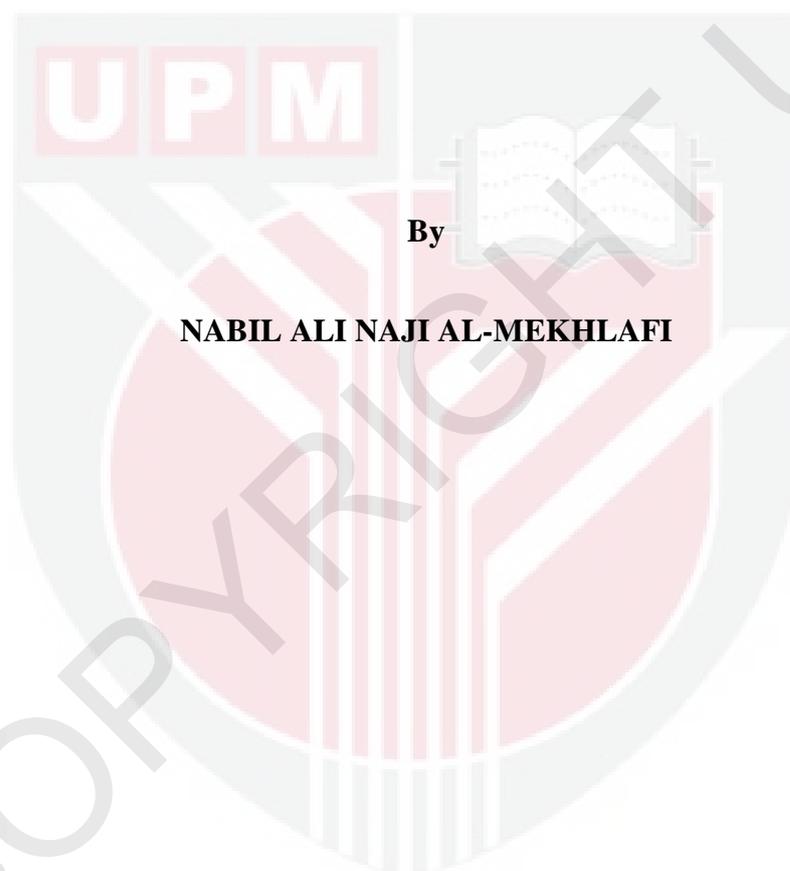
**UNIVERSITI PUTRA MALAYSIA**

**PHYTOCHEMICAL, CYTOTOXIC AND ACETYLCHOLINESTERASE  
INHIBITION IN KACIP FATIMAH (*LABISIA PUMILA* (BLUME) FERN.-  
VILL.), PENARAHAN (*HORSFIELDIA SUPERBA* (HK. F. & TH.) WARB)  
AND PAKU LAYAR (*ODONTOSORIA CHINENSIS* (L.) J. SM.) EXTRACTS**

**NABIL ALI NAJI AL-MEKHLAFI**

**IB 2011 26**

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By

**NABIL ALI NAJI AL-MEKHLAFI**

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

**August 2011**

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

**PHYTOCHEMICAL, CYTOTOXIC AND ACETYLCHOLINESTERASE INHIBITION IN KACIP FATIMAH (*LABISIA PUMILA* (BLUME) FERN.-VILL.), PENARAHAN (*HORSFIELDIA SUPERBA* (HK. F. & TH.) WARB) AND PAKU LAYAR (*ODONTOSORIA CHINENSIS* (L.) J. SM.) EXTRACTS**

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**August 2011**

**Chairman: Professor Md. Nordin Hj. Lajis, PhD**

**Institute: Bioscience**

In the present study, three Malaysian plants, specifically *Labisia pumila* (Myrsinaceae), *Horsfieldia superba* (Myristicaceae) and *Odontosoria chinensis* (Dennstaedtiaceae) were investigated for their chemical constituents. The plant materials were extracted and purified by chromatographic techniques. In total, forty two compounds were isolated, six of which are new compounds. These compounds can be classified into eight types based on their skeletons: alkyl phenols, dimeric 1,4-benzoquinone derivatives, terpenes, phenols derivatives, flavonoids, fatty acid, dihydrochalcone and  $\alpha,\beta$ -unsaturated lactones. The structures of these compounds were fully characterized by various physical (melting point, optical rotation) and spectral (UV, IR, ID and 2D NMR, EIMS, and ESIMS) techniques.

Phytochemical investigation on the leaves of *Labisia pumila* (Kacip Fatimah) (Myrsinaceae), an important medicinal herb in Malaysia, has led to the isolation of three new compounds, characterized as 1-acetoxy-4-hydroxy-2-methoxy-6-

(pentadec-10Z-enyl)benzene (**33**), labisiaquinone A (**34**) and labisiaquinone B (**35**). Along with these, sixteen known compounds including 1-acetoxy-4-hydroxy-2-methoxy-6-pentadecylbenzene (**36**), 1,3-dihydroxy-5-(pentadec-10Z-enyl)benzene (**37**), 1,3-dihydroxy-5-pentadecylbenzene (**38**), (9Z,12Z)-octadeca-9,12-dienoic acid (**39**), stigmasterol 3-*O*- $\beta$ -glycopyranoside (**40**), (-)-loliolide (**41**), stigmasterol (**42**), 4-hydroxyphenylethylamine (**43**), 3,4,5-trihydroxybenzoic acid (**44**), 3,4-dihydroxybenzoic acid (**27**), (+)-catechin (**45**), (-)-epicatechin (**46**), kaempferol 3-*O*- $\alpha$ -rhamnopyranosyl-7-*O*- $\beta$ -glycopyranoside (**47**), kaempferol 3-*O*- $\alpha$ -rhamnopyranoside (**48**), kaempferol 4'-*O*- $\beta$ -glycopyranoside (**49**), quercetin 3-*O*- $\alpha$ -rhamnopyranoside (**50**) were also isolated and identified. All the isolated compounds were reported for the first time from *Labisia pumila* species.

Phytochemical investigation of methanol extract of the bark of *Horsfieldia superba* led to the isolation of the nineteen compounds (**27**, **51-68**). Among these, three compounds (**51-53**) were found to be new, where compounds **52** and **53** were previously reported as a synthetic  $\alpha,\beta$ -lactone. These compounds were characterized as (-)-3,4',7-trihydroxy-3'-methoxyflavan (**51**), (-)-5,6-dihydro-6-undecyl-2*H*-pyran-2-one (**52**), (-)-5,6-dihydro-6-tridecyl-2*H*-pyran-2-one (**53**). Sixteen other known compounds were identified as (-)-viridiflorol (**54**), hexacosanoic acid (**55**),  $\beta$ -sitosterol (**56**), methyl 2,4-dihydroxy-6-methylbenzoate (methyl orsellinate) (**57**), methyl 2,4-dihydroxy-3,6-dimethylbenzoate (**58**), (-)-4'-hydroxy-7-methoxyflavan (**59**), (-)-4',7-dihydroxyflavan (**60**), (-)-4',7-dihydroxy-3'-methoxyflavan (**61**), (+)-3,4',7-trihydroxyflavan (**62**), (-)-catechin (**63**), (-)-epicatechin (**46**), (-)-7-hydroxy-3',4'-methylenedioxyflavan (**64**), 2',3,4-trihydroxy-4'-methoxydihydrochalcone (**65**),

3',4',7-trihydroxyflavone (**66**), (+)-4'-hydroxy-7-methoxyflavanone (liquiritigenin 7-methyl ether) (**67**), hexadecanoic acid (palmitic acid) (**68**).

Four pure aromatic compounds were isolated from the leaves of *Odontosoria chinensis* (Dennstaedtiaceae), which were characterized as 3,4-dihydroxybenzoic acid (**27**) 3,4-dihydroxybenzaldehyde (**28**), 4-hydroxy-3,5-dimethoxybenzoic acid (**70**) and 4-hydroxy-3-methoxybenzoic acid (**71**).

The cytotoxic and acetylcholinesterase (AChE) inhibitory activities of crude fractions and the isolated compounds from the *L. pumila*, *H. superba* and *O. chinensis* were studied. Compounds, including 1-acetoxy-4-hydroxy-2-methoxy-6-(pentadec-10Z-enyl)benzene (**33**) and 1-acetoxy-4-hydroxy-2-methoxy-6-pentadecylbenzene (**36**) showed strong activity against three cancer cell lines: MCF-7 (breast), HCT-116 (colon) and PC-3 (prostate) with IC<sub>50</sub> values 0.5-0.7 μM, whereas compounds (-)-5,6-dihydro-6-undecyl-2H-pyran-2-one (**52**) and (-)-5,6-dihydro-6-tridecyl-2H-pyran-2-one (**53**) showed moderate growth inhibition potency against the tested cell lines. The chloroform and ethyl acetate fractions of *H. superba* were found to exhibit moderate AChE inhibitory activity (IC<sub>50</sub> 72 and 60 μg/ml), whereas fractions from *L. pumila* and *O. chinensis* and hexane fraction of *H. superba* showed poor inhibition (IC<sub>50</sub> >100).

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

**FITOKIMIA, SITOTOKSIK DAN PERENCATAN  
ASETILKOLINESTERASE DALAM EKTRAK KACIP FATIMAH (*LABISIA  
PUMILA* (BLUME) FERN.-VILL.), PENARAHAN (*HORSFIELDIA  
SUPERBA*) (HK. F. & TH.) WARB) DAN PAKU LAYAR (*ODONTOSORIA  
CHINENSIS*) (L.) J. SM.)**

Oleh

**NABIL ALI NAJI AL-MEKHLAFI**

August 2011

**Pengerusi: Profesor Md. Nordin Hj. Lajis, PhD**

**Institut: Biosains**

Tiga tumbuhan Malaysia, iaitu *Labisia pumila* (Myrsinaceae), *Horsfieldia superba* (Myristicaceae) dan *Odontosoria chinensis* (Dennstaedtiaceae) telah dikaji kandungan fitokimianya. Ketiga-tiga tumbuhan itu telah diekstrak dan dipencilkan menggunakan teknik kromatografi. Secara keseluruhannya, empat puluh dua sebatian telah berjaya dipencilkan, termasuk enam sebatian baru. Sebatian ini dapat diklasifikasikan ke dalam lapan jenis berdasarkan struktur rangka iaitu alkilfenol, terbitandimer 1,4-benzokuinon, terpena, terbitanfenol, flavonoid, asid lemak, dihidrokalkon dan  $\alpha,\beta$ -lakton tak tepu. Struktur sebatian ini dikenalpasti berdasarkan sifat fizikal (takat lebur, putaran optik) dan menggunakan teknik spektroskopik (UV, IR, 1D dan 2D NMR, EIMS dan ESIMS).

Kajian fitokimia terhadap daun *Labisia pumila* (Kacip Fatimah) (Myrsinaceae), yang merupakan satu herba perubatan yang penting di Malaysia, telah berjaya memencilkan tiga sebatian baru, dikenalpasti sebagai 1-asitoksi-4-hidroksi-2-

metoksi-6-(pentadek-10Z-enil)benzena (**33**), labisiakuinon A (**34**) dan labisiakuinon B (**35**) dan enam belas sebatian yang telah diketahui termasuk 1-asitoksi-4-hidroksi-2-metoksi-6-pentadesilbenzena (**36**), 1,3-dihidroksi-5-(pentadek-10Z-enyl)benzena (**37**), 1,3-dihidroksi-5-pentadesilbenzena (**38**), asid (9Z,12Z)-octadeka-9,12-dienoik (**39**), stigmasterol 3-O- $\beta$ -glukopyranosida (**40**), (-)-loliolida (**41**), stigmasterol (**42**), 4-hidroksifeniletamina (**43**), asid 3,4,5-trihidroksibenzoik (**44**), asid 3,4-dihidroksibenzoik (**27**), (+)-katekin (**45**), (-)-epikatekin (**46**), kaempferol 3-O- $\alpha$ -ramnosil-7-O- $\beta$ -glukopiranosida (**47**), kaempferol 3-O- $\alpha$ -ramnopiranosida (**48**), kaempferol 4'-O- $\beta$ -glukopiranosida (**49**), kuersetin 3-O- $\alpha$ -ramnopiranosida (**50**). Semua sebatian ini telah dipencilkan buat kali pertama dari *Labisia pumila*.

Kajian fitokimia terhadap ekstrak metanol kulit *Horsfeldia superba* telah berjaya memencilkan sembilan belas sebatian (**27**, **51-68**). Daripada semua sebatian ini tiga sebatian adalah sebatian baru dan sebatian **52** dan **53** telah dilaporkan sebelum ini sebagai sebatian sintetik  $\alpha,\beta$ -lakton. Tiga Sebatian baru ini ialah (-)-3,4',7-trihidroksi-3'-metoksiflavan (**51**), (-)-5,6-dihidroksi-6-undekil-2H-piran-2-one (**52**), (-)-5,6-dihidro-6-tridekil-2H-piran-2-one (**53**). Enam belas sebatian lain yang telah diketahui iaitu (-)-viridiflorol (**54**), asid heksakosanoik (**55**),  $\beta$ -sitosterol (**56**), metil 2,4-dihidroksi-6-metilbenzoate (metal orsellinat) (**57**), metil 2,4-dihidroksi-3,6-dimetilbenzoat (**58**), (-)-4'-hidroksi-7-metoksiflavan (**59**), (-)-4',7-dihidroksiflavan (**60**), (-)-4',7-dihidroksi-3'-metoksi flavan (**61**), (+)-3,4',7-trihidroksiflavan (**62**), (-)-katekin (**63**), (-)-epikatekin (**46**), (-)-7-hidroksi-3',4'-metilenedioksiflavan (**64**), 2',3,4-trihidroksi-4'-metoksi dihidrocalkon (**65**), 3',4',7-trihidroksiflavan (**66**), (+)-4'-hidroksi-7-metoksi flavanon (liquiritigenin 7-metileter) (**67**) dan asid heksadekanoik (asid palmitik) (**68**).

Empat sebatian aromatik telah berjaya dipencilkan dari daun *Odontosoria chinensis* (Dennstaedtiaceae) dan dikenalapasti iaitu asid 3,4-dihidroksibenzoik (**27**) 3,4-dihidroksibenzaldehid (**28**), asid 4-hidroksi-3,5-dimetoksibenzoik (**70**) dan asid 4-hidroksi-3-metoksibenzoik (**71**).

Aktiviti sitotoksik dan perencatan enzim asetilkolinesterase (AChE) terhadap fraksi dan sebatian yang dipencilkan dari *L. pumila*, *H. superba* dan *O. chinensis* telah dikaji. Sebatian termasuk 1-asitoksi-4-hidroksi-2-metoksi-6-(pentadec-10Z-enil)benzena (**33**) dan 1-asitoksi-4-hidroksi-2-metoksi-6-pentadakilbenzena (**36**) menunjukkan aktiviti sitotoksik yang kuat terhadap tiga talian sel kanser: MCF-7 (payudara), HCT-116 (kolon) dan PC3 (prostat) dengan nilai  $IC_{50}$  0.5-0.7  $\mu$ M, manakala sebatian (-)-5,6-dihidroksi-6-undekil-2*H*-piran-2-on (**52**) dan (-)-5,6-dihidro-6-tridekil-2*H*-piran-2-on (**53**) menunjukkan aktiviti yang sederhana.

Fraksi kloroform dan etil asetat bagi *H. Superba* menunjukkan aktiviti perencatan AChE yang sederhana ( $IC_{50}$  72 dan 60  $\mu$ g/ml), manakala fraksi lain dari *L. pumila* dan *O. chinensis* dan fraksi heksana bagi *H. superba* menunjukkan aktiviti perencatan yang rendah ( $IC_{50} > 100$ ).

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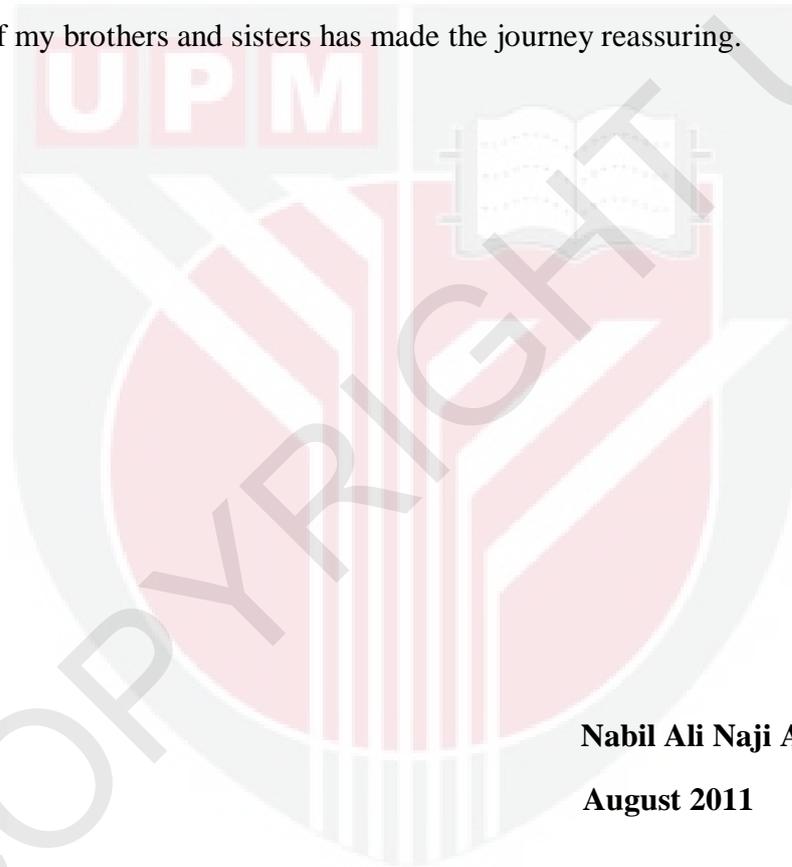
I am very grateful to Prof. Dr Khozirah Shaari, a member of my supervisory committee, for her kindness and helpful suggestions about structural elucidation. I gratefully acknowledge the useful ideas provided by Assoc. Prof. Dr. Faridah Abas, who is a member of my supervisory committee as well. I really can not thank them enough.

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**Nabil Ali Naji Al-Mekhlafi**

**August 2011**



I certify that an Examination Committee has met on 23 August 2011 to conduct the final examination of Nabil Ali Naji Al-Mekhlafi on his Doctor of Philosophy thesis entitled “Phytochemical and Bioactivity Studies On the Constituents Isolated From Kacip Fatimah (*Labisia pumila*), Penarahan (*Horsfieldia superba*) and Puku Layar (*Odontosoria chinensis*)” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the degree of Doctor of Philosophy.

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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 Doctor of Philosophy. The members of Supervisory Committee were as follows:

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## DECLARATION

I declare that the thesis is 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 other institutions.



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**NABIL ALI NAJI AL-MEKHLAFI**

Date: 23 August 2011

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