UNIVERSITI PUTRA MALAYSIA

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

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By

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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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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-\(\text{O}\)-\(\beta\)-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-\(\text{O}\)-\(\alpha\)-rhamnopyranosyl-7-\(\text{O}\)-\(\beta\)-glycopyranoside (47), kaempferol 3-\(\text{O}\)-\(\alpha\)-rhamnopyranoside (48), kaempferol 4'-\(\text{O}\)-\(\beta\)-glycopyranoside (49), quercetin 3-\(\text{O}\)-\(\alpha\)-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-dihydroxybenzoeic 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$_{50}$ 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$_{50}$ 72 and 60 µg/ml), whereas fractions from L. pumila and O. chinensis and hexane fraction of H. superba showed poor inhibition (IC$_{50}$ >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. & TL) WARDB) DAN PAKU LAYAR (ODONTOSORIA CHINENSIS) (L.) J. SM.)

Oleh

NABIL ALI NAJI AL-MEKHLAFI

August 2011

Pengerusi: Profesor Md. Nordin Hj. Lajis, PhD
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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, asidlemak, 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-\(\beta\)-glikopyranosida (40), (-)-loliolida (41), stigmasterol (42), 4-hidroksifeniletilamina (43), asid 3,4,5-trihidroksibenzoik (44), asid 3,4-dihidroksibenzoik (27), (+)-katekin (45), (-)-epikatekin (46), kaempferol 3-\(\alpha\)-ramnosil-7-\(\beta\)-glikopiranosa (47), kaempferol 3-\(\alpha\)-ramnopiranosa (48), kaempferol 4-\(\beta\)-glikopiranosa (49), kuersetin 3-\(\alpha\)-ramnopiranosa (50). Semua sebatian ini telah dipencilkan buat kali pertama dari *Labisia pumila*.

Kajian fitokimia terhadap ekstrak methanol 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'-metoksisiflavan (51), (-)-5,6-dihidroksi-6-undekil-2\(H\)-piran-2-one (52), (-)-5,6-dihidro-6-tridekil-2\(H\)-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-metoksi-siflavan (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-trihidroksiflavon (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-pentadekilbenzena (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 µM, 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 µg/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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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.

______________________________
NABIL ALI NAJI AL-MEKHLAFI

Date: 23 August 2011
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