UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS OF *MESUA FERREA* L. AND *PIPER NIGRUM* L. AND THEIR LARVICIDAL ACTIVITIES

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

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In the memory of Niko,

Thank you for believing in me and supporting me all the way. You are small, but have a heart of an angel. Missing and loving you forever……

17th April 2005 – 8th October 2006

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

CHEMICAL CONSTITUENTS OF *MESUA FERREA* L. AND *PIPER NIGRUM* L. AND THEIR LARVICIDAL ACTIVITIES

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May 2006

Chairman : Associate Professor Gwendoline Ee Cheng Lian, PhD

Faculty : Science

Chemical and biological studies were carried out on two plants, *Piper nigrum* L. (Piperaceae) and *Mesua ferrea* L. (Guttiferae). The chemical investigations covered alkaloids, triterpenoids, carboxylic acids and anthraquinones. These compounds were isolated using common chromatographic techniques and were identified by using spectroscopic experiments such as NMR, MS, IR and UV.

From the stem bark of *Mesua ferrea* L. two main components, betulinic acid and 1,8-dihydroxy-3-methoxy-6-methyl-anthraquinone were found in all crude extracts. In addition, stigmasterol and sitosterol were also isolated from the methanol extract. The fresh blossoms of *Mesua ferrea* L. gave Lup-20(29)-en-3β-ol, long chain hydrocarbons and carboxylic acids as major compounds. Meanwhile, studies on *Piper nigrum* L (roots) produced six alkaloids, piperlactam A and piperlactam D, one oxoarporphine (cepharadione A), piperine, sylvamide, 2,4 tetradecadienoic acid isobutyl amide, together with four carboxylic acids, tetracosanoic acid, p-hydrocinnamate ester, 2-

butenedioc acid, cinnamic acid, tetracosanoic acid and benzoic acid. Two triterpenoids, stigmasterol and sitosterol were also isolated from *Piper nigrum* L.

From the stem bark of *Mesua ferrea* L.*,* although 1,8-dihydroxy-3-methoxy-6-methyl-anthraquinone **(82)** (Kua *et al.,* 2006), betulinic acid **(83)** (Cao *et al.,* 1997) and lup-20(29)-en-3β-ol **(84)** (Cao *et al.,* 1997) were previously isolated from the family of Guttiferae, it is the first time that this class of compound has been isolated from this plant.

From the roots of *Piper nigrum* L., there are some isolated compounds that were new to the family and few of the which are new to the species. Sylvamide **(38)** (Banerji *et al.,* 1982), Cepharadione A **(41)** (Desai *et al.,* 1988), Piperolactam A **(46)** (Desai *et al.,* 1988) and Piperolactam D **(49)** (Olsen *et al,* 1993) has been not been isolated from *Piper nigrum,* but have been previously been discovered from some species of the genus *Piper.* This is the first time that 2,4 tetradecadienoic acid isobutyl amide **(89)**, tetracosanoic acid, p-hydroxycinnamate esters **(88)** and 2-butenedioic acid, mono(2-methylpropyl) ester **(87)** been successfully isolated from the family of Piperaceae. All this discovery might be chemotaxonomically significant to the family.

The larvicidal tests were performed against the larvae of *Aedes aegypti* following the WHO (1981) standard procedures with slight modifications. The crude hexane, chloroform, ethyl acetate, methanol extracts and pure piperine of *Piper nigrum* L showed very high toxicity activities towards the larvae by giving LC_{50} values of less than

2.5 µg/ml. However, the hexane, chloroform and methanol extracts of *Mesua ferrea* L. demonstrated lesser impact on the larvae with LC_{50} values higher than 150 µg/ml.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KANDUNGAN KIMIA DAN AKTIVITI LARVA DARI *MESUA FERREA* L. AND *PIPER NIGRUM* L.

LIM SOOI KIM

Mei 2006

Pengerusi : Profesor Madya Gwendoline Ee Cheng Lian, PhD.

Fakulti : Sains

Kajian kimia dan biologi telah dilakukan ke atas dua jenis tumbuhan iaitu *Piper nigrum* L. (Piperaceae) dan *Mesua ferrea* L. (Guttiferae). Kajian kimia merangkumi sebatian alkaloid, triterpenoid, asid karboksilik dan antrakuinon. Sebatian-sebatian ini diasingkan dengan menggunakan pelbagai teknik kromatografi dan dikenalpasti dengan menggunakan eksperimen spektroskopi seperti NMR, MS, IR dan UV.

Dari kulit batang *Mesua ferrea* L diperolehi dua komponen utama iaitu asid betulinik and 1,8-dihidro-3-metoksi-6-metil-antrakuinon. Sementara itu, kajian terhadap bunga *Mesua ferrea* L menghasilkan Lup-20(29)-en-3β-ol, hidrokarbon rantai panjang dan asid karboksilik sebagai komponent utama. Pada masa yang sama, kajian terhadap akar *Piper nigrum* L. menghasilkan enam alkaloid; piperlaktam A dan piperlaktam D, satu oksoaporphin (cepharadione A), piperin, silvamida, asid 2,4 tetradekadienoik isobutil amida (dari ekstrak etanol menggunakan kaedah asid/bes), bersama empat asid karbosilik iaitu asid tetrakosanoik, asid 2-butendiok, asid sinnamik, asid tetrakosanoik

dan asid benzoik. Dua triterpenoids, stigmasterol dan sitosterol juga dikenalpasti dari *Piper nigrum* L.

Dari kulit batang., didapati 1,8-dihidro-3-metoksi-6-metil-antrakuinon **(82)** (Kua *et al.,* 2006), asid betulinik **(83)** (Cao *et al.,* 1997) dan Lup-20(29)-en-3β-ol **(84)** (Cao *et al.,* 1997) pernah diekstrak dari keluarga Guttiferae. Walaubagaimanapun, ini merupakan kali pertama komponen ini berjaya diekstrak dari pokok *Mesua ferrea*.

Kajian terhadap akar *Piper nigrum* L. menghasilkan beberapa komponen yang baru kepada keluarga and ada komponen yang baru kepada spesis. Silvamida **(38)** (Banerji *et al.,* 1982), Cepharadione A **(41)** (Desai *et al.,* 1988), piperlaktam A **(46)** (Desai *et al.,* 1988) and piperlaktam D **(49)** (Olsen *et al,* 1993) tidak pernah diekstrak dari *Piper nigrum,* akan tetapi pernah diperolehi daripada genus *Piper* yang lain. Bagi 2,4 tetradekadienoik isobutil amida **(89)**, asid tetrakosanoik **(88)** and 2-butendiok **(87)**, ini merupakan kali pertama komponen ini diperoleh dari keluarga Piperaceae.

Ujian ke atas larva telah dijalankan dengan menggunakan larva jenis *Aedes aegypti* mengikut prosedur-prosedur piawai WHO (1981) dengan sedikit pengubasuaian. Ekstrak mentah dari heksana, klorofom, etil asitat, metanol dan piperin tulen dari *Piper nigrum* L. menunjukkan toksik yang sangat tinggi terhadap larva dengan nilai LC_{50} kurang dari 2.5 µg/ml. Manakala, ekstrak mentah klorofom dan metanol dari *Mesua ferrea* L. memberikan aktiviti yang lemah terhadap larva dengan tahab toksik LC_{50} lebih 150 µg/ml.

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I certify that an Examination Committee has met on 19 May 2006 to conduct the final examination of Lim Sooi Kim on her Master of Science thesis entitled "Chemical Constituents of *Mesua Ferrea* L. and *Piper nigrum* L and their Larvicidal Activities." in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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Date: 21 SEPT 2006

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

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Date:

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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LIM SOOI KIM

Date:

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LIST OF ABBREVIATIONS

α	alpha
β	beta
δ	chemical shift in ppm
γ	gamma
μg	micro gram
br s	broad singlet
br t	broad triplet
^{13}C	carbon-13
CC	column chromatography
$CHCl_3$	chloroform
$CDCl_3$	deuterated chloroform
COSY	Correlated Spectroscopy
d	doublet
dd	doublet of doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	dimethylsulfoxide
dt	doublet of triplet
EA	ethyl acetate
EIMS	Electron ionisation mass spectrometry
gm	gram
GC	Gas Chromatography