PHYTOCHEMICALS AND BIOLOGICAL ACTIVITIES OF ELAEOCARPUS FLORIBUNDUS BLUME, BARRINGTONIA CONOIDEA GRIFF AND FIBRAUREA TINCTORIA LOUR

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By

RAHAYU UTAMI UMAR

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

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A present for my beloved Papa (Umar Adnan), Ibu (Mariana Murad), brothers (Kuswara Sumaamijaya, Rinaldi Afriyanto and Willian Sandra), sister-in law (Salina) and the one who own my heart, my nephew (Dzakwannafis Syafiq). Thank you for all the supports, prayers and patience.

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

PHYTOCHEMICALS AND BIOLOGICAL ACTIVITIES OF *ELAEOCARPUS FLORIBUNDUS* BLUME, *BARRINGTONIA CONOIDEA* GRIFF AND *FIBRAUREA TINCTORIA* LOUR

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September 2012

Chairman : Prof. Mohd. Aspollah Hj. Sukari, PhD

Faculty : Science

Phytochemicals and biological activity studies on three medicinal plants, *Elaeocarpus floribundus* Blume, *Barringtonia conoidea* Griff and *Fibraurea tinctoria* Lour were carried out. The chemical constituents of these plants were isolated using chromatographic methods, whilst the structure of the compounds were elucidated by using spectroscopic methods including infrared (IR), nuclear magnetic resonance (^{1}H, ^{13}C and 2D NMR), mass spectrometry (MS) and by comparison with previous data. Total phenolic content of methanolic extracts of plants was determined by colorimetric method using Folin-Ciocalteu reagent. Extracts and selected isolated compounds were subjected to biological activity studies of antimicrobial, antioxidant and cytotoxic activity.

The isolation work on the leaves of *Elaeocarpus floribundus* has yielded triterpenoids, friedelin **(38)** and epifriedelinol **(39)**. Mixture of α- **(41)** and β-amyrin

(42) were isolated from leaves and stem bark of *Barringtonia conoidea*. This is a first report on the phytochemical study of *Elaeocarpus floribundus* and *Barringtonia conoidea.* While furanolactone diterpene, clerodanine **(43)**, an ecdysteroid, 20-hydroxyecdysone **(37)** and an isoquinoline alkaloid, berberine **(30)** were obtained from the whole part of *Fibraurea tinctoria*. In addition, *β*-sitosterol **(40)** was isolated from these three plant species.

In this present study, all methanolic extracts of plants showed significant amount of phenolic content. The methanolic extract of leaves of *Elaeocarpus floribundus* exhibited the highest value of 503.08±16.71 mg GAE/g DW. In the screening of antimicrobial activities, extracts of leaves and stem bark of *Elaeocarpus floribundus* and *Barringtonia conoidea* were either possessed weak activity or inactive against gram positive and negative bacterial strains. Extracts of *Fibraurea tinctoria* were found to be inactive towards the bacterial strains. Similarly, none of extracts of the plants gave inhibition activity towards fungal strains *Candida albicans*, *Aspergillus ochraceaus* and *Saccharomyces cerevisiae.*

Antioxidant activity assay of extracts and isolated compounds have been conducted using DPPH free radical scavenging method. Only polar extracts of leaves and stem bark of *Elaeocarpus floribundus* as well as methanol extract of *Fibraurea tinctoria* afforded potential antioxidant activity. Clerodanine **(43)** was the only isolated compound that gave significant activity with IC_{50} value of 20.81±0.02 µg/ml. While, the others extracts as well as isolated compounds were found either to be weak or inactive.

As for the cytotoxic assay, extracts and selected isolated compounds were tested using microculture tetrazolium (MTT) assay against human T4-lymphoblastoid cell (CEM-SS) and human cervical cancer cell (HeLa). The results showed that chloroform extract of leaves of *Elaeocarpus floribundus* and methanol extract of *Fibraurea tinctoria* gave significant activity against CEM-SS cells with IC_{50} values of 25.6±0.06 and 16.13±0.04 µg/ml, respectively. Ethyl acetate extract of stem bark and chloroform extract of leaves of *Barringtonia conoidea* exhibited promising activity towards HeLa cells with IC_{50} values of 13.5±0.16 and 28.1±0.01 µg/ml, respectively.

Compounds **38**, **39**, **43** and mixture of **41** & **42** showed significant cytotoxic activity against the two cancer cells, with compound **38** gave the strongest activity with IC_{50} value of 3.54±0.30 µg/ml towards HeLa cells. Compound **43** was also found to be active against the two cancer cells with IC_{50} values of 12.49±0.10 µg/ml (CEM-SS) and 9.37±0.36 µg/ml (HeLa). This is the first study conducted on the cytotoxic activity of compound **43**.

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

KAJIAN FITOKIMIA DAN AKTIVITI BIOLOGI KE ATAS *ELAEOCARPUS FLORIBUNDUS* BLUME, *BARRINGTONIA CONOIDEA* GRIFF DAN *FIBRAUREA TINCTORIA* LOUR

Oleh

RAHAYU UTAMI UMAR

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Pengerusi : Prof. Mohd. Aspollah Hj. Sukari, PhD

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Kajian fitokimia dan aktiviti biologi ke atas tiga tumbuhan ubat-ubatan, *Elaeocarpus floribundus* Blume, *Barringtonia conoidea* Griff dan *Fibraurea tinctoria* Lour telah dijalankan. Juzuk kimia tumbuh-tumbuhan ini telah diasingkan dengan menggunakan kaedah kromatografi, manakala struktur sebatian telah dijelaskan dengan menggunakan kaedah spektroskopik termasuk inframerah (IR), resonans magnetik nuklear (^{1}H, ^{13}C dan 2D NMR), spektrometri jisim (MS) dan perbandingan dengan data sebelumnya. Kandungan jumlah fenol daripada ekstrak metanol pokok tumbuh-tumbuhan telah ditentukan oleh kaedah kolorimetrik menggunakan reagen Folin-Ciocalteu. Ekstrak dan sebatian terpencil yang terpilih tertakluk kepada kajian aktiviti biologi seperti antimikrob, antioksidan dan aktiviti sitotoksik.

Kerja pemencilan ke atas daun *Elaeocarpus floribundus* telah menghasilkan triterpenoids, friedelin **(38)** dan epifriedelinol **(39)**. Campuran α-**(41)** dan β-amyrin

(42) telah diasingkan daripada daun dan kulit kayu batang *Barringtonia conoidea*. Ini adalah laporan pertama kepada kajian fitokimia *Elaeocarpus floribundus* dan *Barringtonia conoidea*. Manakala diterpene furanolakton, clerodanine **(43)**, ecdysteroid, 20-hydroxyecdysone **(37)** dan yang isokuinolin alkaloid, berberine **(30)** telah diperolehi daripada kerja-kerja pengasingan pada bahagian seluruh *Fibraurea tinctoria*. Di samping itu, β-sitosterol **(40)** telah diasingkan daripada ketiga-tiga spesies tumbuhan.

Di dalam kajian ini, semua ekstrak metanolik daripada pokok tumbuhan menunjukkan jumlah kandungan fenolik yang ketara. Ekstrak metanolik daun *Elaeocarpus floribundus* mempamerkan nilai tertinggi 503.08±16.71 mg GAE/g DW. Dalam ujian aktiviti antimikrob, ekstrak daun dan kulit batang *Elaeocarpus floribundus* dan *Barringtonia conoidea* mempunyai aktiviti yang lemah atau tidak aktif terhadap strain bakteria gram positif dan negatif. Semua ekstrak daripada *Fibraurea tinctoria* telah didapati tidak aktif. Begitu juga, tiada ekstrak daripada tumbuh-tumbuhan ini memberikan aktiviti antifungal terhadap *Candida albicans*, *Aspergillus ochraceaus* dan *Saccharomyces cerevisiae*.

Ujian aktiviti antioksidan ekstrak dan sebatian terpencil telah dijalankan menggunakan kaedah DPPH free radical scavenging. Hanya ekstrak polar daun dan kulit batang *Elaeocarpus floribundus* serta ekstrak metanol *Fibraurea tinctoria* yang berpotensi memberikan aktiviti antioksidan. Clerodanine **(43)** adalah satu-satunya sebatian yang memberikan aktiviti yang signifikan dengan nilai IC_{50} 20.81±0.02 μg/ml. Ekstrak dan sebatian terpencil lainnya didapati memberikan aktiviti yang lemah atau tidak aktif.

Untuk ujian sitotoksik, ekstrak dan beberapa sebatian telah diuji menggunakan kaedah microculture tetrazolium (MTT) terhadap sel T4-lymphoblastoid manusia (CEM-SS) dan sel kanser serviks manusia (HeLa). Hasilnya menunjukkan bahawa ekstrak kloroform daun *Elaeocarpus floribundus* dan ekstrak metanol *Fibraurea tinctoria* memberi aktifiti yang signifikan terhadap sel CEM-SS dengan nilai IC_{50} 25.6±0.06 dan 16.13±0.04 µg/ml, masing-masing. Ekstrak etil asetat kulit batang dan ekstrak kloroform daun *Barringtonia conoidea* mempamerkan aktiviti sitotoksik yang menjanjikan ke atas sel HeLa dengan nilai IC_{50} 13.5±0.16 and 28.1±0.01 µg/ml, masing-masing.

Sebatian **38**, **39**, **43** dan campuran sebatian **41** & **42** memberikan aktiviti sitotoksik yang ketara terhadap kedua-dua sel-sel kanser, sebatian **38** memberikan aktiviti terkuat dengan IC_{50} nilai 3.54±0.30 µg/ml terhadap sel HeLa. Sebatian **43** juga didapati aktif terhadap kedua-dua sel-sel kanser dengan nilai IC_{50} 12.49±0.10 µg/ml (CEM-SS) dan 9.37±0.36 µg/ml (HeLa). Ini adalah kajian pertama dijalankan pada aktiviti sitotoksik sebatian **43**.

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I certify that a Thesis Examination Committee has met on **date on viva voce** to conduct the final examination of **Rahayu Utami** on her thesis entitled **"Phytochemicals and Biological Activities of *Elaeocarpus Floribundus* Blume, *Barringtonia Conoidea* Griff And *Fibraurea Tinctoria* Lour"** 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 (Name of relevant degree).

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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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RAHAYU UTAMI UMAR

Date: 20 September 2012

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LIST OF ABBREVIATIONS

α	Alpha
β	Beta
δ	Delta, chemical shift in ppm
µg	Microgram
µl	Microliter
Acetone-d_6	Deuterated Acetone
br	Broad
^{o}C	Degree in Celcius
^{13}C	Carbon-13
$CDCl_3$	Deuterated chloroform
CD_3OD	Deuterated methanol
COSY	Correlation Spectroscopy
cm	centimeter
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethylsulfoxide
DW	Dried Weight
d	Doublet
dd	Doublet of doublets
ddd	Doublet of doublets of doublets
EIMS	Electron Impact Mass Spectrum
eV	Electron volt
FTIR	Fourier Transform Infra-Red
GAE	Gallic Acid Equivalence
^{1}H	Proton
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple-Quantum Ceherence
Hz	Hertz
IC	Inhibition concentration
IR	Infrared
J	Coupling in Hz
Lit.	Literature
m	Multiplet
m/z	Mass per charge
MeOH	Methanol
MHz	MegaHertz
m.p.	Melting point
NCI	National Cancer Institute
NMR	Nuclear Magnetic Resonance
OCH_3	Methoxy
OH	Hydroxy
PBS	Phosphate Buffered Saline
ppm	part per million

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CHAPTER ONE

INTRODUCTION

1.1 Background of Research

Plant still remains as source of medicines and have been used traditionally for thousands of years. Phytochemical and bioassay studies on medicinal plants revealed that secondary plant metabolites responsible for that useful function. Phenolics, alkaloids, terpenoids, essential oils and others secondary metabolites produced by plants have acted as source for development of many modern medicines. Phenolic compounds were reported to have wide biological activities including anticarcinogenic, antioxidant, antimutagenic and ability modifiying the gene expression (Tapiero *et al.*, 2002). Some alkaloid compounds: vincristine, vinblastine, papaverine, codeine and morphine are well known as drug of choice for treating particular diseases such as leukemia, heart disease, analgesic and anasthetic (Vickery and Vickery, 1981). While, promising antiviral and antineoplastic activities that are represented by some terpenoid compounds especially lupane derivatives has become subjects of interest in recent years (Tolstikova *et al.*, 2006).

Elaeocarpus floribundus, *Barringtonia conoidea* and *Fibraurea tinctoria* are three species of plants that have been used as folk medicines in the treatment several diseases. *Elaeocarpus floribundus*, commonly known as medang teja, medang telur (Malay) is a tree that belongs to family Elaeocarpaceae (Burkill *et al.*, 1966). In Sumatera, infusion of its leaves and bark is used as a mouth-wash for inflamed gums

(Burkill *et al.*, 1966). Previous studies reported that alkaloids, flavonoids and terpenoids were successfully isolated from some species of genus *Elaeocarpus.* Some of these isolated compounds have also been found to possess antioxidant, cytotoxic and δ-opioid receptor binding affinity activities (Ray *et al.*, 1976; Cambie *et al.*, 1992; Ito *et al.*, 2002; Katavic *et al.*, 2007; Piao *et al.*, 2009). However, phytochemicals and biological activities of *E. floribundus* has been unexplored yet (Wiart, 2006). Similarly, *Barringtonia conoidea* or putat ayer is a mangrove plant with limited distribution, belonging to family Lecythidaceae. There was no previous study has been reported related to its chemical constituents as well as the bioactivities. Its fruits and young leaves are used to relieve the stomach ache and eaten as fresh vegetables. Research on chemical constituents of other *Barringtonia* species showed that terpenoids were successfully isolated (Subba Rao *et al.*, 1984; Hasan *et al.*, 2000; Gowri *et al.*, 2009).

On the other hand, *Fibraurea tinctoria* is another plant species with interesting medicinal purposes. This plant is belonging to family Menispermaceae, also known as one of four Khaminkhruea species besides *Arcangelisia flava* (L.) Merr., *Coscinium blumeanum* Miers and *C. fenestratum* (Gaertn.) Colebr. The stem and roots of *Fibraurea tinctoria* have been used as bitter tonic and treatment for dysentery, jaundice, diarrhea and skin abscess, for analgesic, antidote and diuretic effects. The chemical investigations have showed the presence of furanoterpenoids, steroids and alkaloids on this species (Ito and Furukawa, 1969; Itokawa *et al.*, 1986; Zakaria *et al.*, 1989). While, the biological studies demonstrated antioxidant, cytotoxic and anti malaria properties of crude extracts as well as anti-inflammatory and antiplasmodial activities of some isolated compounds of *Fibraurea tinctoria*

(Keawpradub *et al.*, 2005; Nguyen-Pouplin *et al.*, 2007; Su *et al.*, 2007; Su *et al.*, 2008).

1.2 Problem Statement

Elaeocarpus floribundus, *Barringtonia conoidea* and *Fibraurea tinctoria* are plant species that have been used as traditional medicines for treating several diseases. Despite all the applications in traditional medicinal practices, phytochemicals and biological activity study on *Elaeacarpus floribundus* and *Barringtonia conoidea* have not been investigated yet. While, medicinal purposes, interesting variety of chemical constituents and significant biological activities of *Fibraurea tinctoria* have prompted us to carry out details investigations on phytochemical and biological activity on this plant.

1.3 Objectives of Research

In view of the medicinal values and interesting chemical constituents of these species, the research was carried out with the following objectives:

1. To extract and isolate the chemical constituents, identify and elucidate the structures of the isolated compounds of *Elaeocarpus floribundus* Blume, *Barringtonia conoidea* Griff and *Fibraurea tinctoria* Lour using chromatographic methods and spectroscopic techniques.
2. To determine the total phenolic contents of the crude extracts
3. To examine the antimicrobial, antioxidant and cytotoxic activities of crude extracts and isolated compounds.

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