UNIVERSITI PUTRA MALAYSIA

PHYTOCHEMICAL AND BIOACTIVITY STUDIES OF MANGIFERA PAJANG KOSTERM, AGLAIA ODORATISSIMA BLUME AND ACACIA ALBIDA DELILE

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PHYTOCHEMICAL AND BIOACTIVITY STUDIES OF *MANGIFERA PAJANG* KOSTERM, *AGLAIA ODORATISSIMA* BLUME AND *ACACIA ALBIDA* DELILE.

By

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PHYTOCHEMICAL AND BIOACTIVITY STUDIES OF *MANGIFERA PAJANG* KOSTERM, *AGLAIA ODORATISSIMA* BLUME AND *ACACIA ALBIDA* DELILE.

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

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Studies on the phytochemicals and bioactivities were carried out towards three plants species; *Mangifera pajang* Kosterm, *Aglaia odoratissima* Blume and *Acacia albida* Delile. Various classes of chemical constituents have been isolated and identified by spectroscopic method including infrared (IR), mass spectrometry, nuclear magnetic resonance (NMR) and by comparison with reported data. Some of the plant extracts and isolated constituents demonstrated potential activity on antibacterial, antioxidant and cytotoxic screening tests.

Chromatographic isolation of the kernel, stem bark and leaves extracts of *Mangifera pajang* Kosterm from Sabah has led to the isolation of an aromatic ester, methyl gallate **(28)**, mixture of benzaldehyde **(64)** and benzyl alcohol **(65)**, cycloartane triterpenes identified as mangiferonic acid **(10)**, 3*β*-hydroxy-cycloart-24-ene-26-oic acid **(66)** and 3*β*,23-dihydroxy-cycloart-24-ene-26-oic acid **(9)**, lupane triterpenes; lupeol **(59)** and lupenone **(58)**, steroids; *β*-sitosterol **(46)** and stigmasterol **(47)**, monoterpene; *trans*-sobrerol **(67)** and a flavonol glycoside identified as quercitrin **(68)**. However, isolation work towards extracts of *Aglaia odoratissima* also has led to the isolation of compounds **(46)**, **(47)** and **(59).** Chromatographic isolation towards extracts of stem bark of *Acacia albida* has afforded compounds **(47)** and **(59)** from its crude hexane and chloroform extracts. Meanwhile, sucrose **(69)** was obtained from methanol crude extract.

All of the crude extract from *Mangifera pajang* Kosterm, *Aglaia odoratissima* Blume and *Acacia albida* Delile were subjected to cytotoxic screening against various

cancer cell lines (MCF-7, HeLa and HT-29). Crude ethyl acetate and methanol extracts from kernel show strong cytotoxic activity towards MCF-7 and HeLa cells with IC_{50} less than 10 µg/mL while crude petroleum ether, chloroform and ethyl acetate extracts of the stem bark show strong to moderate activity against MCF-7, HeLa and HT-29 cancer cell lines with the IC_{50} values ranging from 5 to 30 µg/mL. Cytotoxic screening on the isolated compounds revealed high cytotoxic activity by compounds **(28)** and **(66)** for MCF-7 cell. Meanwhile, compounds **(10)**, **(59)**, **(66)** and **(68)** showed high cytotoxic activity towards HeLa cell, while compounds **(10)**, **(28)** and **(68)** were also active towards HT-29 cell.

However, only crude ethyl acetate and methanol extracts from kernel shows inhibition towards microbes in antimicrobial and antifungal assays. For DPPH assays, the kernel crude extracts show highest free radical scavenging activity with IC_{50} less than 10 µg/mL, while crude ethyl acetate and methanol extracts of leaves show weak activity with IC_{50} less than 150 µg/mL. Methyl gallate **(28)** was the only isolated compound which showed high antifungal activity towards MRSA and high radical scavenging activity with IC_{50} value of 6.24 ± 0.30 µg/mL.

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

KAJIAN FITOKIMIA DAN AKTIVITI BIOLOGI *MANGIFERA PAJANG* KOSTERM, *AGLAIA ODORATISSIMA* BLUME DAN *ACACIA ALBIDA* DELILE.

Oleh

SADIKAH BINTI AHMAD

Mei 2014

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

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Kajian fitokimia dan bio-aktiviti telah dijalankan terhadap tiga jenis spesis tumbuhan; *Mangifera pajang* Kosterm, *Aglaia odoratissima* Blume dan *Acacia albida* Delile. Penemuan dari kajian ini telah menunjukkan pelbagai jenis kelas juzuk kimia dipencilkan dan dikenalpasti dengan menggunakan kaedah spektoskopi termasuk inframerah (IR), spektrometri jisim, resonans magnetik nuklear (NMR) dan perbandingan dengan data yang sedia ada. Sesetengah daripada ekstrak tumbuhan dan sebatian pencilan menunjukkan aktiviti yang menarik dan berpotensi terhadap saringan ujian antimikrob, antioksidan dan sitotoksik.

Pemencilan melalui teknik kromatografi terhadap ekstrak biji, kulit batang dan daun daripada *Mangifera pajang* Kosterm dari Sabah berjaya memperolehi sebatian ester aromatik seperti metil gallat **(28)**, serta campuran benzaldehid **(64)** dan benzil alkohol **(65)**, kumpulan sikloartan triterpena yang dikenalpasti sebagai asid mangiferonik **(10)**, asid 3*β*-hidroksi-sikloartan-24-ene-26-oik **(66)**, dan asid 3*β*,23-dihidroksi-sikloartan-24-ene-26-oik **(9)**, triterpena lupane; lupeol **(59)** dan lupenone **(58)**, steroid; *β*-sitosterol **(46)** dan stigmasterol **(47)**,monoterpena; *trans*-sobrerol **(67)** dan satu glukosida flavonol yang dikenalpasti sebagai quercitrin **(68)**. Walaubagaimanapun, pemencilan terhadap ekstrak dari *Aglaia odoratissima* Blume berjaya memperolehi sebatian **(46)**, **(47)** dan **(59)**. Pemencilan kromatografi terhadap ekstrak kulit batang *Acacia albida* Delile pula turut memperolehi sebatian **(47)** dan **(59)** daripada ekstrak mentah heksana dan klorofom. Manakala, sukrosa **(69)** diperolehi dari ekstrak mentah metanol.

Kesemua ekstrak mentah daripada *Mangifera pajang* Kosterm, *Aglaia odoratissima* Blume dan *Acacia albida* Delile diuji terhadap aktiviti sitotoksik terhadap sel kanser MCF-7, HeLa dan HT-29. Ekstrak mentah daripada etil asetat dan metanol (biji) menunjukkan aktiviti sitotoksik yang kuat terhadap MCF-7 dan HeLa dengan nilai IC_{50} kurang daripada 10 µg/mL, manakala ekstrak mentah daripada petroleum eter, klorofom dan etil asetat daripada kulit batang menunjukkan aktiviti sitotoksik yang kuat kepada sederhana terhadap sel MCF-7, HeLa dan HT-29 dengan nilai IC_{50} 5 ke 30 µg/mL. Ujian sitotoksik terhadap sebatian yang telah dipencil menunjukkan aktiviti yang kuat oleh sebatian **(28)** dan **(66)** terhadap sel MCF-7. Manakala, sebatian **(10)**, **(59)**, **(66)** dan **(68)** menunjukkan aktiviti sitotoksik yang kuat terhadap sel HeLa. Sebatian **(10)**, **(28)** dan **(68)** pula aktif terhadap sel kanser HT-29.

Bagi ujian antibakteria dan antikulat, hanya ekstrak mentah daripada etil asetat dan metanol daripada biji *Mangifera pajang* Kosterm sahaja menunjukkan tindak balas terhadap ujian tersebut. Ujian terhadap aktiviti antioksidan juga menunjukkan hanya ekstrak mentah daripada etil asetat dan metanol daripada biji *Mangifera pajang* Kosterm sangat aktif dengan nilai IC_{50} kurang daripada 10 µg/mL. Manakala ekstrak mentah etil asetat dan metanol daripada daun *Mangifera pajang* Kosterm menunjukkan aktiviti antioksidan yang lemah dengan nilai IC_{50} kurang daripada 150 µg/mL. Sebatian **(28)** adalah satu-satunya sebatian yang aktif terhadap ujian antibakteria dan antikulat oleh MRSA mikrob dan menunjukkan nilai antioksidan yang tinggi dengan bacaan IC_{50} 6.24 ± 0.30 µg/mL.

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I certify that a Thesis Examination Committee has met on 16th of May 2014 to conduct the final examination of Sadikah Binti Ahmad on her thesis entitled "Phytochemicals and Bioactivity Studies of *Mangifera pajang* Kosterm , *Aglaia odoratissima* Blume and *Acacia albida* Delile" 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 Master of Science.

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This thesis was 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 were as follows:

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LISTS OF ABBREVIATIONS

α	Alpha
β	Beta
δ	Delta, chemical shift in ppm
Acetone-d_6	Deuterated acetone
br	Broad
°C	Degree in Celcius
^{13}C	Carbon-13
$CDCl_3$	Deuterated chloroform
CD_3OD	Deuterated methanol
COSY	Correlation spectroscopy
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethylsulfoxide
d	Doublet
dd	Doublet of doublets
ddd	Doublet of doublets of doublets
EI-MS	Electron Impact Mass Spectrum
eV	Electron volt
FTIR	Fourier Transform Infra-Red
^1H	Proton
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple-Quantum Correlation
Hz	Hertz
IC	Inhibition concentration
IR	Infrared
J	Coupling in Hz
m	Multiplet
m/z	Mass per charge
MeOH	Methanol
MHz	MegaHertz
M^+	Molecular ion
m.p.	Melting point
NMR	Nuclear Magnetic Resonance
OCH_3	Methoxy
OH	Hydroxy
s	Singlet
t	Triplet
TLC	Thin Layer Chromatography

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CHAPTER ONE

INTRODUCTION

1.1 Natural products

Natural products are organic compounds that are formed by living organisms including animals and plants. Natural products compounds can be classified into two major categories which are primary metabolites and secondary metabolites. Between these two categories, secondary metabolites compounds such as alkaloids, steroids, terpenoids, flavonoids, coumarins, phenolic compounds and others are the most interested compounds to natural products researchers because of their biological effect on other organisms (Hanson, 2003).

Natural products normally focussed on the study of medicinal or herbal plants that have been known and used traditionally over thousands years ago in treatment of diseases as they are known by the older folks in many medicinal values. For examples, studies have discovered that about 2000 plants utilized by Indian Ayurveda to cure many types of ailments. The Chinese system depends on 5757 plants listed in the "*Encyclopedia of Traditional Chinese Medicinal Substances"* while Japanese and Korean systems also depend on a large number of medicinal herbs. In overall, about 70,000 plants including the lower plants estimated have been used in medicine (Daniel, 2006).

Malaysia is a tropical country which are rich in their natural products sources and some of the large diversity of plant have never been exploited. Out of 12 000 species of vascular plants, 1200 species are reported to be consumed by three major races; Malays, Chinese and Indians as medicinal sources. Medicinal plants are the basis of health care for indigenous people and rural populations. Nowadays, herbal drug industry in Malaysia is a growing industry with large potential in the regional and global markets. Many of potential plants which traditionally used as health food and tonics are now been developed into large-scale production of functional foods to meet demand within and outside Malaysia (Shyun and Mat Ali, 2004).

The market for herbs and plant-based medicine in Malaysia worth about US$ 527 million in 2000 and expected to increase to more than US$ 2 billion in 2013. The government play important role to conserve and ensure large-scale planting of quality herbs for the local herbal manufacturers (Shyun and Mat Ali, 2004). Thus, research institutes like Agricultural Research and Development Institute (MARDI) and Forest Research Institute Malaysia (FRIM) would serve by supplying planting guidelines and research on quality planting materials. In collaboration with other government agencies such as Forestry Department, National University of Malaysia (UKM), University Malaya (UM), FRIM and NGOs such as the Malaysian Nature Society (MNS), yearly expeditions are held to collect medicinal plant materials. Apart from government researches institutes like MARDI and FRIM and other

government departments, Malaysian local universities such as University Science Malaysia (USM), National University of Malaysia (UKM), University Malaya (UM) and University Putra Malaysia (UPM) are actively conducting researches in natural products chemistry and drug discovery.

1.2 Natural products and drug discovery

Nature stands as a rich source of novel chemotypes and pharmacophores, and has been source of medicinal agents for thousands of years and through out researches, a large number of modern drugs found are derived from natural products source (Brahmachari, 2012). Natural products (plants, animals and minerals) play important roles in development of modern medicine nowadays. It start as the basis of treatment of human diseases which gradually developed over the years through researches. However, the basis of its development remains in the roots of traditional medicine and therapies. So many ancient wisdom and herbal uses in curing diseases remain as one important source of future medicine and therapeutics (Patwardhan, 2007). Thus, a lot of efforts have been done in discovering medicines from our plant diversity. For this research, *Mangifera pajang* Kosterm, *Aglaia odoratissima* Blume and *Acacia albida* Delile have been chosen for its chemical constituents and bioactivity studies.

1.3 Problem statements

In this research, three species were selected for phytochemicals and bioactivities investigation including *Mangifera pajang* Kosterm, *Aglaia odoratissima* Blume and *Acacia albida* Delile. To the best of our knowledge, there were no phytochemical studies reported from these plant species. Previous reports have confirmed the antioxidant, anti-malarial and anti-cancer activities of the plant isolates (Hassan *et al*., 2011; Tijani *et al*., 2010; Abu Bakar *et al*., 2010 and Abu Bakar *et al*., 2009).

1.4 Objectives

The objectives of this research are as follow:

1) To extract and isolate the chemical constituents of *Mangifera pajang* Kosterm, *Aglaia odoratissima* Blume and *Acacia albida* Delile.
2) To identify and elucidate the structure of isolated compounds using spectroscopic analysis (IR, MS and NMR).
3) To screen potential bioactivities (antioxidant, antimicrobial & antifungal and anti-cancer) of the crude extracts and isolated compounds.

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