ISOLATION OF TERPENOID FROM ALSTONIA SPATHULATA AND KOPSIA SINGAPURENSIS (APOCYNACEAE)

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ABSTRACT

The aim of this study was to extract and identify the chemical compounds isolated from Alstonia spathulata and Kopsia singapurensis. The isolated compounds were tested for their biological activities include cytotoxicity (MCF-7 cell line), antibacterial (Bacillus cereus) and antioxidant (DPPH). The separation of the chemical components from both species was carried out using different chromatographic techniques (column chromatography and thin laver chromatography). The structures of isolated compounds have been elucidated through spectral analysis including 1D-NMR (¹H, ¹³C and DEPT), 2D-NMR (¹H-¹H COSY, HSQC/HMQC and HMBC), IR, UV, MS (GCMS) and also by comparison with previous literature data. Studies on the chemical constituents of the bark of Alstonia spathulata has led to the isolation of five triterpenes; β -amyrin 113, β -amyrin acetate 114, β -sitosterol 115, lupeol acetate 116, stigmasterol 117 and a mixture of three triterpenes (lupeol 118, α -amyrin 119 and β -amyrin 113). Four known tritepenes were also successfully isolated from the root of Kopsia singapurensis; β-amyrin 113, βamyrin acetate 114, lupeol acetate 116 and stigmasterol 117. These compounds were isolated for the first time from both species. Compound 113, 114, 116 and 117 exhibited cytotoxic effects against MCF-7 cell line with IC₅₀ values of 15.5, 22.5, 26.0 and 14.5 µg/mL, respectively. All triterpenes showed weak antioxidant activity with $IC_{50} > 500 \ \mu g/mL$ and were not active against the gram positive bacteria, *Bacillus* cereus. Both species have been traditionally used to cure diseases however there are not many publications on triterpenes extracted from this plants species. Isolation of chemical constituents from these two species has potential for producing new drugs.

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PEMENCILAN TERPENOID DARIPADA POKOK ALSTONIA SPATHULATA DAN KOPSIA SINGAPURENSIS (APOCYNACEAE)

ABSTRAK

Kajian ini bertujuan mengekstrak dan mengenalpasti sebatian kimia yang dipencil dari pokok Alstonia spathulata dan Kopsia singapurensis. Sebatian kimia yang dipencil diuji aktiviti biologi termasuk sitotoksisiti (MCF-7 cell line), antibakteria (Bacillus cereus) dan antioksidan (DPPH). Pemisahan komponen kimia dari kedua-dua spesis ini telah dijalankan menggunakan teknik kromatografi yang berbeza (kromatografi turus dan kromatografi lapisan nipis). Struktur sebatian kimia dikenalpasti melalui analisis spektrum termasuk 1D-NMR (¹H, ¹³C dan DEPT), 2D-NMR (¹H-¹H COSY, HSQC / HMQC dan HMBC), IR, UV, MS (GCMS) dan juga dibandingkan dengan data daripada kajian lepas. Kajian ke atas kandungan sebatian kimia kulit batang Alstonia spathulata telah membawa kepada pengasingan lima triterpena; β-amirin 113, β-amirin asetat 114, β-sitosterol 115, lupeol asetat 116, stigmasterol 117 dan campuran tiga triterpena (lupeol 118, α -amirin-119 dan β -amirin 113). Empat tritepena juga berjaya dipencil dari akar Kopsia singapurensis; β-amirin 113, βamyrin asetat 114, lupeol asetat 116 dan stigmasterol 117. Semua sebatian kimia ini merupakan pertama kali dipencil dari kedua-dua spesis ini. Sebatian 113, 114, 116 dan 117 masing-masing menunjukkan kesan sitotoksik terhadap MCF-7 dengan nilai IC₅₀; 15.5, 22.5, 26.0 dan 14.5 µg/mL. Semua triterpena menunjukkan aktiviti antioksidan yang lemah dengan IC_{50} > 500 µg/mL dan tidak aktif terhadap bakteria gram positif, Bacillus cereus. Kedua-dua spesis telah digunakan secara tradisional untuk menyembuhkan pelbagai penyakit tetapi tidak banyak penerbitan mengenai triterpena yang diekstrak daripada kedua-dua spesis tumbuhan ini. Pengasingan sebatian kimia daripada kedua-dua spesis ini berpotensi bagi penghasilan ubat-ubatan baru.

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LIST OF SYMBOLS AND ABBREVIATIONS

	α	Alpha
	β	Beta
	λ	Maximum wave length
	δ	Chemical shift
	g	Gram
	kg	Kilogram
	М	Molar
	mM	Milimolar
	ml	Mililitre
	m	Meter
	MHz	Mega Hertz
	Hz	Hertz
	UV	Ultraviolet
	IR	Infrared
	ppm	Part per million
	eV	Electron Volt
	МеОН	Methanol
	CHCl ₃	Chloroform
	CH ₂ Cl ₂	Dichloromethane
	DMSO	Dimethylsulphoxide
	EA	Ethyl Acetate
	OCH ₂ O	Methylenedioxy
DI	CH3 DIKAN SULTAN IDRIS	Methyl group UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKA

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OCH ₃	Methoxyl group
ОН	Hydroxyl group
NH ₃	Ammonia
рН	Power of Hydrogen
HCl	Hydrogen chloride
TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography
СС	Column Chromatography
NMR	Nuclear Magnetic Resonance
FT-NMR	Fourier Transform Nuclear Magnetic Resonance
cm ⁻¹	Per centimeter
J	Coupling constant
d	Doublet
dd	Doublet of doublet
t	Triplet
dt	Doublet of triplet
\$	Singlet
m	Multiplet
<i>q</i>	Quartet
dbh	Diameter at Breast Height
°C	Degree Celsius
1D-NMR	One Dimension Nuclear Magnetic Resonance
2D-NMR	Two Dimension Nuclear Magnetic Resonance
$^{1}\mathrm{H}$	Proton NMR
¹³ C	13-Carbon NMR

COSY	1H-1H Correlation Spectroscopy
DEPT	Distortioness Enhancement by Polarization Transfer
HMQC	Heteronuclear Multiple Quantum Correlation
HSQC	Heteronuclear Single Quantum Correlation
HMBC	Heteronuclear Multiple Bond Correlation
NOESY	Nuclear Overhauser Effect Spectroscopy
GC-MS	Gas Chromatography Mass Spectrometry
LC-MS	Liquid Chromatography-Mass Spectrometry
MS	Mass Spectrometry
HRESIMS	High Resolution Electrospray Ionization Mass Spectrometry
m/z.	Mass per charge
CDCl ₃	Deuterated chloroform
CD ₃ OD	Deuterated methanol
FeCl ₃	Ferric chloride
DMF	Dimethylformamide

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Nature especially flora furnishes interesting and diverse types of molecular structures some of which provide the chemist and pharmacologist with model compounds to forms the design or prototype for synthetic or semi synthetic medicines, pesticides and other valuable compounds. Mention may be made of previous discoveries of important drugs from plant such as ephedrine 1, quinine 2, reserpine 3 and vincristine 4 which are used directly or have been modeled for modern pharmaceuticals. Furthermore, the most common insecticide currently used in households depends on the discovery of pyrethrins 5 of Pyrethrum flowers which kill a variety of insects but remain relatively harmless to mammalian life (U.S Department of health and human services, 2003). In recent times, viral diseases including AIDs and many resistant

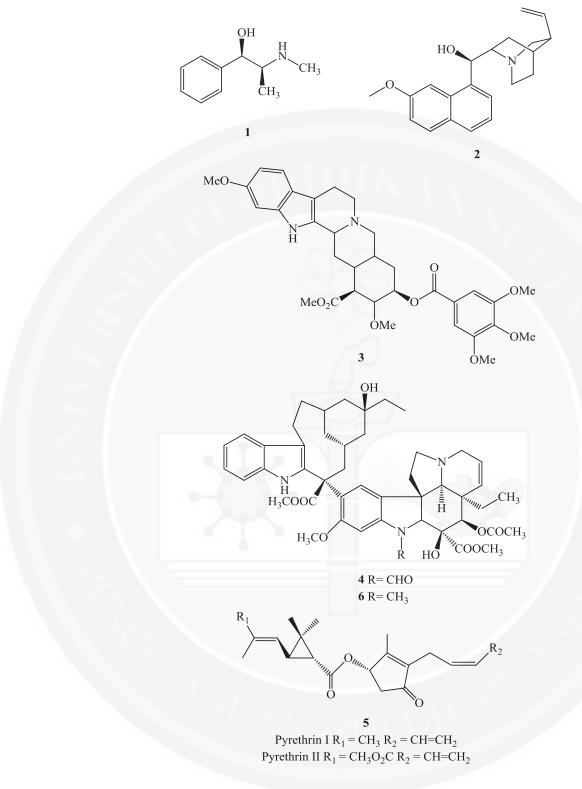
bacterial strains have appeared and the search for more natural products to be used asUNIVERSITI PENDIDIKAN SULTAN IDRISUNIVERSITI PENDIDIKAN SULTAN IDRISUNIVERSITI PENDIDIKAN SULTAN IDRISORISUNIVERSITI PENDIDIKAN SULTAN IDRISUNIVERSITI PENDIDIKAN SULTAN IDRISUNIVERSITI PENDIDIKAN SULTAN IDRIS

> new drug models has become more urgent. The number and variety of tropical plants provide immense opportunities for the discovery of new chemically and pharmacologically active principles. However, about 15000 species of the medicinal plants are globally threatened (Hamilton, 2008). Less than 1% of all tropical plants had been screened for possible pharmaceutical use and that habitats are being destroyed faster than scientists can research the plants. At current extinction rates, experts estimate that the Earth is losing at least one potential major drug every two years (Groombridge & Jenkins, 2002).

> The Malaysian flora is among the world's richest containing some of the primitive species originating from the earliest forms of angiosperms and gymnosperms of some 200 million years ago. The lack of major geologic upheavals in this region has allowed many of the floras maintain some of their primitive characteristic. It is therefore possible to find plants of great botanical interest apart from their potential as producers of useful natural products.

Malaysia has about 14500 species of flowering plants of which about 2000 have been reported to contain medicinal properties and many have been scientifically proven (Jaganath & Ng, 2000). The huge diversity of the Malaysian flora means that we can expect well diverse chemical structures from their secondary metabolites, and chemical diversity is one of the plus factors that make natural product excellent candidates for any screening program.





1.2 Objectives

This study aimed on investigating the chemical constituents of *Alstonia spathulata* Bl. and *Kopsia singapurensis* Ridl. The objectives guiding this inquiry were to:

- 1. Extract, isolate and purify the chemical constituents of the *Alstonia spathulata and Kopsia singapurensis*.
- 2. Elucidate and identify the structure of the isolated compounds using modern spectroscopic methods such as NMR, UV, IR and MS.
- 3. Determine the bioactivities of the compounds.

1.3 Significant of research

In this research study, isolate and purify the chemical constituents of the *Alstonia spathulata* and *Kopsia singapurensis* are determined. This is because plant natural products have long been and will continue to be important sources and models of spices, flavors and fragrances, vegetable oils, soaps, natural rubber, gums, resins, drugs, insecticides, and other industrial, medicinal, and agricultural raw materials. For example, many *Alstonia* species are commercial timbers. Trees from the section *Alstonia* produce light timber. However, since most plant species have never been described much less surveyed for chemical or biologically active constituents, it is reasonable to expect that new sources of valuable materials remain to be discovered. Furthermore, if the current trend of destruction of tropical forests continues at its

present rate. Phytochemists and other plant scientists may have only a few decades UNIVERSITI PENDIDIKAN SULTAN IDRIS VI PENDIDIKAN SULTAN IDRIS VI PENDIDIK

remaining in which to investigate much of the plant kingdom for useful chemicals (Balandrin, Klocke, Wurtele, & Bollinger, 1985). According to IUCN Red List of Threatened Species (Chua, 1998), *Kopsia singapurensis* was one of the threatened species and traditional used to poultice ulcerated nose in tertiary syphilis (Perry & Metzger, 1980). Thus, it is important to isolate and purify the chemical constituents of the plant species as well as from *Alstonia spathulata* and *Kopsia singapurensis*.

1.4 Apocynaceae

1.4.1 Botanical aspect of Apocynaceae

The Apocynaceae were first described by Jussieu (1789) as "Apocinae". It is a large family which currently recognized has 424 genera and 1500 species (Endress & Bruyns, 2000); cosmopolitan in distribution, but more common in the tropics (Bhattacharyya & Johri, 1998). In Malaysia, only 10 genera and 38 species are trees and shrubs; the remaining are climbers or scrambling shrubs except *Vallariopsis lancifolia* which is an epiphytic shrub (Whitmore, 1973). Plants of Apocynaceae are adapted to various habitats, from sea level to mountain tops, mainly on dry soils, but also on rocks or in flood areas, and sometimes river margins (Smith et al., 2004). Members of Apocynaceae are usually herbs, shrubs, vines or trees. Some of the members are large, stout and woody climbers (Bhattacharyya & Johri, 1998). The cardinal botanical features of Apocynaceae are an exudation of abundant milky latex (Wiart, 2006).

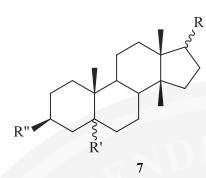
> Plants in the Apocynaceae usually have simple, opposite or whorled leaves. However, some genera of Apocynaceae are stem succulents with vestigial or no leaves (Hodgkiss, 2011). Flowers of Apocynaceae are bisexual and in clusters. They are usually showy, often pure white, salver-shaped and slightly fragrant flowers with five contorted lobes (Wiart, 2006). Fruits and seed pods are usually produced in pairs of follicle that split open at maturity. Seeds are flat and winged or have a tuft of hairs at one end.

> Economically, the family has limited importance. Some species are valuable sources of medicine, insecticides and timber. Plants of the Apocynaceae are often poisonous and are rich in indole alkaloids or glycosides, especially in the seeds and latex (Tao, Leeuwenberg & Middleton, 1995). Because of the profusion of secondary metabolites, the family is the important source of bioactive compounds. This family is known for plants that have a very high biological activity and medical properties like treatments of disorders of skin, liver diseases, leprosy, dysentery, ulcers, ear aches, tumors, malaria, fevers and hypertension (Perry & Metzger, 1980; Holdsworth, 1986; Ambasta, Ramchandran, Kashyapa & Chand, 1992). It has medical importance owing to the presence of these bioactive compounds such as vinblastine 6, vincristine 4 and cardiac glycosides 7 (Smith et al., 2004). Vincristine 4 found in Catharanthus roseus is the source of anticancer drugs. Rauwolfia serpentine roots are used medicinally as a cure for epilepsy, high blood pressure, insanity and cardiac diseases. Besides, some of the members of Apocynaceae also grown as ornamentals such as Nerium odorum and Thevetia peruviana. The wood of Wrightia tinctoria and Alstonia scholaris which is soft are used for wood carving. The members of Apocynaceae also contribute to

rubber and fibre yielding (Bhattacharyya & Johri, 1998).

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R = unsaturated lactone or pyrone ring system R' = H or OH R" = sugar moiety

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1.4.2 Classification of Apocynaceae

The Apocynaceae belong to the order Gentianales and have traditionally been divided into two subfamilies, the Rauvolfioideae (Plumerioideae) and the Apocynoideae (Smith, Mori, Henderson, Stevenson, & Heald, 2004). However, this family recently are divided into 5 subfamilies which are Rauvolfioideae (Plumerioideae), Apocynoideae, Periplocoideae, Secamonoideae and Asclepiadoideae (Sennblad & Bremer, 2002; Endress, 2004; Middleton, 2009). There are 17 newly recircumscribed tribes were also recognized in the family of Apocynaceae. Rauvolfioideae is further divided into nine tribes which are Alstonieae, Vinceae (Rauvolfieae), Willughbeieae, Tabernaemontaneae, Melodineae, Hunterieae, Plumerieae, Alyxieae and Carisseae. Wrightieae, Malouetieae, Apocyneae, Mesechiteaeand Echiteae are five tribes that classified in the Apocynoideae. While subfamilies of Periplocoideae and Secamonoideae do not consists of tribe (Sennblad & Bremer, 2002). Each tribe comprises of several genera. Figure 1.1 shows the subfamilies, tribes and examples of genus of the Apocynaceae.

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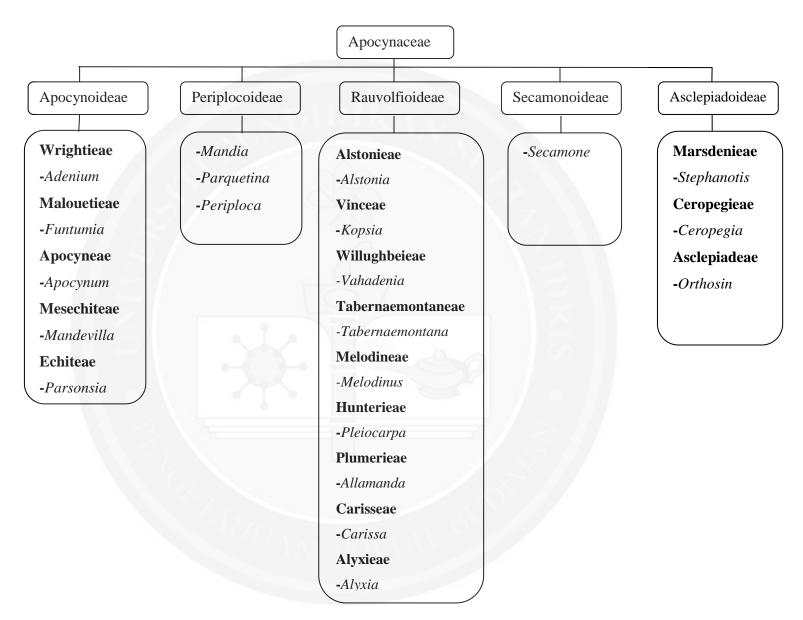


Figure 1.1. Classification of the Apocynaceae and sampling of taxa. Classifications are according to Endress and Bruyns (2000)