PHYTOCHEMICAL STUDY ON *OCHROSIA OPPOSITIFOLIA* AND *KOPSIA SINGAPURENSIS* (APOCYNACEAE)

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UNIVERSITI PENDIDIKAN SULTAN IDRIS

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DECLARATION

I hereby declare that the work in this dissertation is own except for quotation and summaries which have duly acknowledged.

20 June 2012

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M20101000380

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ABSTRACT

The phytochemical study on two Malayan plants *viz*., *Ochrosia oppositifolia* and *Kopsia singapurensis* Ridl. (Apocynaceae) were investigated for their chemical constituents, which involves extraction and separation by using various chromatographic methods and structural elucidation by spectroscopic techniques such as ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR) including 1D-NMR (^{1}H, ^{13}C, and DEPT), 2D-NMR (^{1}H-^{1}H COSY, HSQC, and HMBC) and MS (GC-MS). The structures of the compounds were also elucidated by comparison with previous works. Isolation of chemical constituents on the leaves of *O. oppositifolia* yielded two new indole alkaloids with corynanthean (C-Type) skeleton namely *N*(4)-hydroxymethylisoreserpiline **189** and *N*(4)-acetylisoreserpiline **190**. Six known triterpenes were successfully isolated from the bark of *K. singapurensis*, which were identified as β-amyrin **191**, β-amyrin acetate **192**, β-amyrenone **193**, lupeol **194**, lupeol acetate **195** and stigmasterol **196**. These compounds have never been isolated and reported from this species.

ABSTRAK

Kajian fitokimia terhadap dua jenis tumbuhan Malaya, iaitu *Ochrosia oppositifolia* dan *Kopsia singapurensis* Ridl. (Apocynaceae) telah dikaji kandungan sebatian kimianya, melibatkan pengekstrakan dan pengasingan menggunakan pelbagai kaedah kromatografi dan pengenalpastian struktur dengan teknik-teknik spektroskopi seperti ultra-ungu (UV), infra-merah (IR), resonan magnet nukleus satu dimensi (NMR) termasuk 1D- NMR (^{1}H, ^{13}C, dan DEPT), 2D-NMR (^{1}H-^{1}H COSY, HSQC, dan HMBC) dan MS (GC-MS). Struktur sebatian yang telah dikenalpasti juga dibuat perbandingan dengan kajian-kajian lepas. Pengasingan sebatian ke atas daun pokok *O. oppositifolia* telah menghasilkan dua alkaloid indola baru dengan rangka jenis corinanthean (Jenis-C) dengan nama *N*(4)-hydroxymethylisoreserpilina **189** dan *N*(4)-acetilisoreserpilina **190**. Enam triterpena telah berjaya diasingkan dari batang pokok *K. singapurensis* yang dikenalpasti sebagai β-amirin **191**, β-amirin acetat **192**, β-amirenona **193**, lupeol **194**, lupeol acetat **195** dan stigmasterol **196**. Sebatian-sebatian ini tidak pernah diasingkan dan dilaporkan daripada spesis ini.

CONTENTS

Page Number

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

ABBREVIATIONS

α	Alpha
β	Beta
λ	Maximum Wave Length
δ	Chemical Shift
g	Gram
kg	Kilogram
M	Molar
mM	Milimolar
mL	Mililitre
μL	Microlitre
m	Meter
mm	Milimeter
μm	Mircometer
nm	Nanometer
MHz	Mega Hertz
Hz	Hertz
UV	Ultraviolet
IR	Infrared
ppm	Part per Million
eV	Electron Volt
MeOH	Methanol
$CHCl_3$	Chloroform
CH_2Cl_2	Dichloromethane

CH_3	Methyl group
OCH_3	Methoxyl group
C=O	Ketone group
OH	Hydroxyl group
NH_3	Ammonia
HCl	Hydrochloric Acid
$CDCl_3$	Deuterated Chloroform
CD_3OD	Deuterated Methanol
pH	Power of Hydrogen
TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography
CC	Column Chromatography
NMR	Nuclear Magnetic Resonance
cm^{-1}	Per Centimeter
$cm\ s^{-1}$	Centimeter per Second
min	Minute
°C	Degree Celsuis
$°C\ min^{-1}$	Degree Celsius per Minute
J	Coupling Constant
d	Doublet
dd	Doublet of doublet
t	Triplet
s	Singlet
m	Multiplet
dbh	Diameter at Breast Height

TIC	Total Ion Current
amu	Atomic Mass Unit
1D-NMR	One Dimension Nuclear Magnetic Resonance
2D-NMR	Two Dimension Nuclear Magnetic Resonance
^{1}H	Proton NMR
^{13}C	13-Carbon NMR
^{1}H-^{1}H COSY	Correlation Spectroscopy
DEPT	Distortioness Enhancement by Polarization Transfer
HSQC	Heteronuclear Single Quantum Correlation
HMBC	Heteronuclear Multiple Bond Correlation
GC-MS	Gas Chromatography-Mass Spectroscopy
MS	Mass Spectroscopy
m/z	Mass per Charge
ASTM	American Society for Testing and Materials

CHAPTER 1

INTRODUCTION

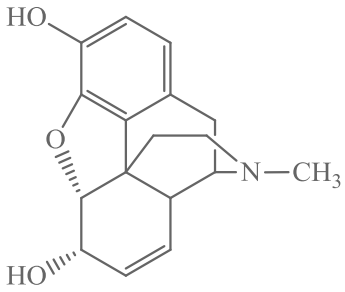
1.1 General Introduction

Natural resources such as plants are species that produce organic molecules naturally as their defense substance against herbivores, plant competitors and abiotic stress (Korkina, 2007). These chemical substances play an important role in the survival of these organisms in such a competitive and continuous changing environment. The secondary metabolites are the chemical substances produced by plants that exert pharmacological actions in human and animals.

The discovery of pharmacological properties in plants laid the foundation of medicinal plants used by humankind as a source of medicines since time immemorial. Using plants as drug in the treatment of various illness is recorded in the oldest medical text come from ancient Mesopotamia in 2600 B.C. (Ji, Li, & Zhang 2009;

Newman, Cragg, & Snader, 2000). Besides, the ancient civilizations of Chinese, Indians and North Africans provide written evidences for the use of natural resources for curing various diseases (Philipson, 2001). According to Musselman (1999), in the Bible and the Quran, about 125 and 20 plants are mentioned, respectively, as being used as medicinal agents to treat various ailments.

Modern Chemistry has provided us a new era for the study and use of natural products. In 1805, the analgesic morphine **1** was isolated from opium poppy (*Papaver omniferum*) by a German pharmacist Friedrich Wilhelm Sertüner (Croteau, Kutchen, & Lewis, 2000; Newman et al., 2000) and first commercially available in 1826 (Ji et al., 2009). Other effective drugs derived from natural products include quinine **2** (Philipson, 2001; Seeman, 2007), paclitaxel (Taxol®) **3** (Heinig & Jennewein, 2009), camptothecin **4** and topotecan **5**, a derivative of camptothecin (Wang et al., 2008).



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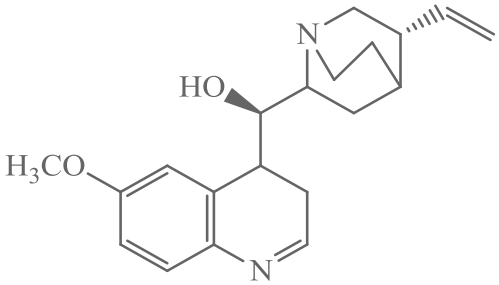

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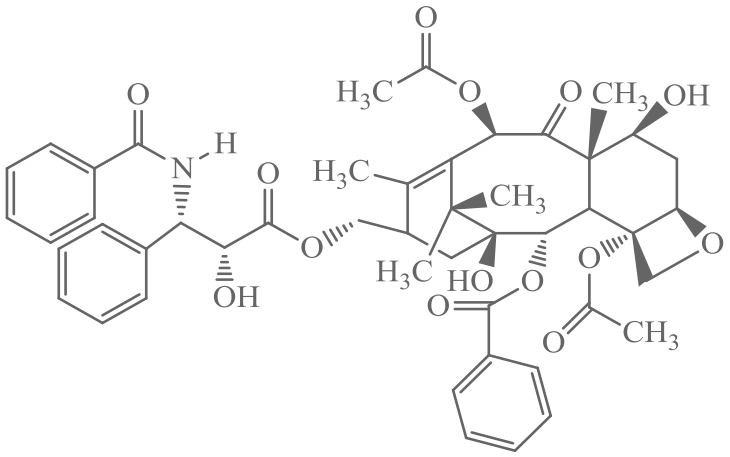

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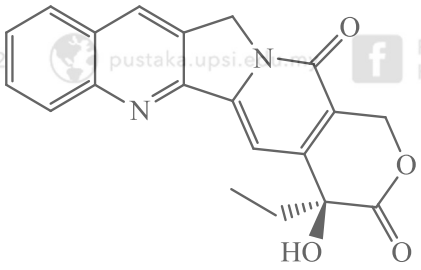

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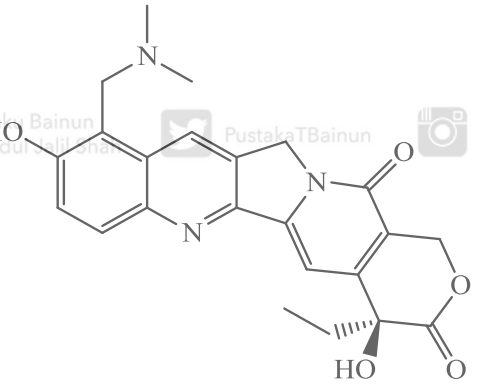

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According to the World Health Organization (WHO, 2008), up to 80 % of population in Asian and African countries relies on the traditional medicine for their primary health care. Therefore, the medicinal plants are particularly important to mankind. The awareness on the important of the natural resources as source of medicines is increasing. To date, about 15,000 species of the medicinal plants are globally threatened (Hamilton, 2008). The extinction of these plants results a serious mankind health problem. With every species lost, we may lose a remedy for global health problems.

Despite its rapid urban development, Malaysia is still remaining wealthy in her medicinal plants from its tropical rain forests. 2,000 species from 14,500 flowering plants have been reported to contain medicinal properties and many have been scientifically proven (Rizwana, Nazlina, Razehar, Noraziah, & Ling, 2010). Natural resources from tropical forests are a promising source of biologically active compounds and potential alkaloids, which posses interesting biological activities, are still waiting to be explored with the aim to discover new drugs.

1.2 Objectives of Study

For the above reasons, the author has started the study of the chemical constituents of *Ochrosia oppositifolia* and *Kopsia singapurensis* Ridl.. The objectives of this study are as described belows:

1. To extract, isolate and purify the chemical constituents from *Ochrosia oppositifolia* and *Kopsia singapurensis* Ridl.
2. To elucidate and identify the structure of the isolated compounds using modern spectroscopic methods such as NMR, UV, IR and MS.

1.3 Apocynaceae

1.3.1 General Appearance and Morphology

The Apocynaceae, also known as the dogbane family, is a family of flowering plants. Many of them are woody climbers, vines, perennial herbs, trees or shrubs. The leaves are simple and usually opposite. The plants usually have regular flowers that are conspicuous, slightly fragrant and pure white. The fruit is a characteristic pair of berries, drupes or follicles. The seed occasionally winged or tufted in splitting fruit (Wiart, 2002). Many of these plants have milky latex, sometimes clear (Wikipedia, 2010).

Plants of the Apocynaceae are often poisonous and rich in alkaloids or glycosides. Some species are valuable sources of medicine, insecticides, fibres and rubber (Tao, Leeuwenberg, & Middleton, 1995). For example, *Catharanthus roseus* and *Rauvolfia serpentine*.

1.3.2 Classification

The Apocynaceae family belongs to the order Gentianales and as currently recognized has 1,500 species classified into 424 genera distributed among five subfamilies: Rauvolfioideae (Plumerioideae), Apocynoideae, Periplocoideae, Secamonoideae and Asclepiadoideae (Endress, 2004; Middleton, 2009). About 80 species of plants classified within this family are medicinal and are often used to treat gastrointestinal

ailments, reduce fever and pains, and treat diabetes and infectious diseases (Wiart, 2006). The most remarkable characteristics and more specifically of the subfamily Rauvolfioideae further divided into four tribes: Plumerieae, Tabernaemontaneae, Rauvolfieae and Carissae. Each of the tribes consists of several genera. The genus *Ochrosia* and genus *Kopsia* are members of the tribe Rauvolfieae (Middleton, 2009).

1.3.3 Distribution and Habitat

The Apocynaceae distributed primarily in the tropics and subtropics, poorly represented in the temperate regions (Tao et al., 1995). However, in Malaysia, nearly 120 species of this family were found and classified into 32 genera are distributed over lowlands and mountains (Corner, 1952).

In the rainforests and swamps of Malaya small to very tall evergreen trees up to 80 m tall, often with buttress roots, such as *Alstonia costulata* and *Dyera laxiflora*. The family is also best known for its showy garden plants, many of which have been introduced and are widely cultivated such as the genus *Plumeria*.

1.4 The Genus *Ochrosia*

According to Middleton (2009), the genus *Ochrosia* is placed in the tribe Rauvolfieae of the subfamily Rauvolfioideae. Based on the most recent revision of the genus as cited in The Plant List (2010), a total of 43 species are recognized. In Malaysia, there is only one species in this genus namely *Ochrosia oppositifolia* (Whitmore, 1973).

1.4.1 *Ochrosia oppositifolia*

Ochrosia oppositifolia, also known as cork wood tree or lacally known as ‘Dhun' buri’ is an evergreen tree that grows in forests along the sandy shores of the tropical regions. It is mainly distributed in the tropical and even subtropical islands (Buot, Jr Okitsu, & Aguilar, 1997). It is a shrub or tree that can grow to a height of 6 m and 45 cm dbh (Figure 1.3.1.1) (Wiart, 2006). This species produces clusters of five-petal leathery white flowers with yellowish centre near the sepals (Figure 1.3.1.2). The fruits drupes, always in pairs, oblong in shape (about 7 cm in length and has a girth of 4 cm), turns yellow when it is ripe (Figure 1.3.1.3) (Whitmore, 1973; Wiart, 2006).

Figure 1.4.1.1. The Stem Bark of *O. oppositifolia*

Figure 1.4.1.2. The Flower of *O. oppositifolia*

Figure 1.4.1.3. The Fruit and Leaves of *O. oppositifolia*

1.5 The Genus *Kopsia*

The genus *Kopsia* is placed in the tribe Rauvolfieae of the subfamily Rauvolfioideae (Middleton, 2004). Twenty four species of the plants of this genus are recognized (The Plant List, 2010) and widely distributed over Southeast Asia, India and China. About 18 species are distributed in Malaysia (Awang et al., 2008; Kam, Yoganathan, & Chuah, 1997). It is most diverse in Peninsular Malaysia and Sarawak (Middleton, 2004). According to Whitmore (1973), this species mainly found at the lowland forests and a few species grow on limestone. All species of *Kopsia* are shrubs or small trees as part of the understory vegetation in forests, at forest edges, or in the open (Middleton, 2004).

1.5.1 *Kopsia singapurensis* Ridl.

Kopsia singapurensis Ridl., also known as white *kopsia* or locally known as ‘selada’, is a evergreen tree can grows up to 12 m tall and 24 cm dbh. The bark of this species is grayish buff to pale silvery brownish (Figure 1.4.1.1). It has shiny dark green leaves (Figure 1.4.1.2) with a red eye flower which grow in cluster on stalks (Figure 1.4.1.3). This species is endemic only to Peninsular Malaysia and Singapore. In Malaysia, it distributed commonly in lowland swampy forests (Middleton, 2004).

Figure 1.5.1.1. The Stem Bark of *K. singapurensis*

Figure 1.5.1.2. The Leaves of *K. singapurensis*

Figure 1.5.1.1. The Flower of *K. singapurensis*