ALKALOIDS ISOLATED FROM THE BARK OF *ALSEODAPHNE PEDUNCULARIS* (WALL. EX NEES) MEISN AND THE ROOTS OF *ALSEODAPHNE CORNERI* KOSTERM

SITI NOR FADZILAH BINTI MOHAMMAD

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

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ABSTRACT

The objectives of this study are to extract and isolate the alkaloids from two species of *Alseodaphne*; *Alseodaphne peduncularis* (Wall. ex Nees) Meisn from Kluang-Mersing, Johor and *Alseodaphne corneri* Kosterm from University Malaya, Kuala Lumpur. The extraction process of the plant material started by cold percolation process using hexane to remove non-polar organic compounds, waxes and fats. The plant material then re-extracted by dichloromethane using soxhlet extractor followed by acid-base extraction to get the alkaloid crude extract. The isolation and purification of alkaloids from the crude extract were done by using various chromatographic techniques including column chromatography and preparative thin layer chromatography. The elucidation of the isolated alkaloids were determine by using various spectroscopic methods such as 1D NMR (^{1}H, ^{13}C and DEPT) and 2D NMR (COSY, HMQC and HMBC), ultraviolet (UV), infrared (IR) and mass spectrometry (MS). The structures were further confirmed by comparison with other literature data. Isolation and purification of alkaloids from the bark of *Alseodaphne peduncularis* (KL 5165) yielded four aporphines; boldine **69**, norpredicentrine **90**, norlirioferine **91** and norboldine **78**. In addition, seven alkaloids were successfully isolated from the roots of *Alseodaphne corneri* (KL 4928). The isolated alkaloids include two aporphines; laetanine **30**, boldine **69** and five bisbenzylisoquinolines; gyrolidine **47**, stephasubine **92**, 2-norobaberine **93**, 3′,4′-dihydrostephasubine **94** and *O*-methyllimacusine **95**. The bioactivity study on the bark crude extract of *Alseodaphne peduncularis* and three isolated aporphines; boldine **69**, norlirioferine **91** and norboldine **78** showed good to moderate antiplasmodial activity against *Plasmodium falciparum* after compared to the standard (chloroquine; 0.087 μg/ml) with IC_{50} value of 2.135, 1.067, 2.786 and 2.228 μg/ml, respectively. It was found that boldine **69** showed the most potent activity with an IC_{50} value of 1.067 μg/ml and it showed potential for antiplasmodial drug. The extraction and isolation of alkaloids from this species will be continued to determine various type of alkaloids contents and new alkaloids findings. Moreover, the isolated alkaloids should be tested with other biactivity test such as cytotoxicity, antibacterial and antifungal activities for new drugs discovery.

Alkaloid dari Kulit Kayu Pokok *Alseodaphne peduncularis* (Wall. ex Nees) Meisn dan Akar Pokok *Alseodaphne corneri* Kosterm

ABSTRAK

Objektif kajian ini adalah mengestrak dan mengasingkan sebatian alkaloid daripada dua spesies *Alseodaphne*; *Alseodaphne peduncularis* (Wall. ex Nees) Meisn dari Kluang-Mersing, Johor dan *Alseodaphne corneri* Kosterm dari Universiti Malaya, Kuala Lumpur. Pengekstrakan sampel tumbuhan dimulakan dengan proses serapan sejuk menggunakan heksana untuk mengeluarkan sebatian organik tidak polar, lilin dan lemak. Sampel tumbuhan kemudiannya diekstrak semula oleh diklorometana menggunakan pemerah *soxhlet* diikuti oleh pengekstrakan asid-bes untuk mendapatkan ekstrak mentah alkaloid. Pengasingan dan penulenan alkaloid dari ekstrak mentah telah dilakukan dengan menggunakan pelbagai teknik kromatografi termasuk kromatografi turus dan kromatografi lapisan nipis. Struktur alkaloid dikenalpasti dengan kaedah kombinasi spektroskopi seperti resonan magnet nukleus satu dimensi; 1D NMR (^{1}H, ^{13}C dan DEPT), dua dimensi; 2D NMR (COSY, HMQC dan HMBC), ultralembayung (UV), inframerah (IR) dan spektrometri jisim (MS). Struktur-struktur alkaloid disahkan melalui perbandingan dengan data daripada kajian-kajian lepas. Pengasingan dan penulenan alkaloid daripada kulit kayu pokok *Alseodaphne peduncularis* (KL 5165) menghasilkan empat sebatian aporfina; boldina **69**, norpredicentrina **90**, norlirioferina **91** dan norboldina **78**. Tambahan pula, tujuh sebatian alkaloid berjaya diasingkan daripada akar pokok *Alseodaphne corneri* (KL 4928). Sebatian alkaloid tersebut termasuklah dua aporfina; laetanina **30**, boldina **69** dan lima bisbenzilisokuinolina; girolidina **47**, stephasubina **92**, 2-norobaberina **93**, 3´,4´-dihidrostephasubina **94** dan *O*-metillimacusina **95**. Kajian bioaktiviti ke atas ekstrak mentah kulit kayu daripada *Alseodaphne peduncularis* dan tiga sebatian aporfina; boldina **69**, norlirioferina **91** dan norboldina **78** menunjukkan aktiviti baik sehingga sederhana terhadap aktiviti antiplasmodial ke atas *Plasmodium falciparum* selepas dibandingkan dengan standard (chloroquine; 0.087 µg/ml) dengan nilai IC_{50} masing-masing iaitu 2.135, 1.067, 2.786 dan 2.228 µg/ml. Didapati boldina **69** menunjukkan aktiviti yang paling baik dengan nilai IC_{50} iaitu 1.067 µg/ml dan berpotensi digunakan sebagai ubat antiplasmodial. Proses pengekstrakan dan pengasingan alkaloid daripada spesies ini juga akan diteruskan untuk menentukan pelbagai jenis kandungan alkaloid di dalamnya termasuk penemuan sebatian alkaloid baru. Selain itu, alkaloid yang berjaya diasingkan juga perlu diuji untuk kajian bioaktiviti lain seperti kajian sitotoksik, antibakteria dan antikulat untuk digunakan dalam penemuan ubat-ubatan baru.

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ABBREVIATIONS

α	Alpha
β	Beta
λ	Maximum wavelength
δ	Chemical shift
g	Gram
kg	Kilogram
cm^{-1}	per centimeter
ml	Mililitre
nm	Nanometer
MHz	Mega Hertz
Hz	Hertz
UV	Ultraviolet
IR	Infrared
ppm	Part per million
MeOH	Methanol
CH_2Cl_2	Dichloromethane
$CHCl_3$	Chloroform
OCH_3	Methoxyl group
OH	Hydroxyl group
NH_3	Ammonia
HCl	Hydrochloric acid
Na_2SO_4	Sodium sulphate
$MgSO_4$	Magnesium sulphate
NaCl	Sodium chloride
KCl	Potassium chloride

$CDCl_3$	Deuterated chloroform
pH	Power of hydrogen
TLC	Thin layer chromatography
CC	Column chromatography
PTLC	Preparative thin layer chromatography
NMR	Nuclear magnetic resonance
J	Coupling constant
d	Doublet
dd	Doublet of doublet
s	Singlet
br s	Broad singlet
m	Multiplet
t	Triplet
1D NMR	One dimension nuclear magnetic resonance
2D NMR	Two dimension nuclear magnetic resonance
COSY	Correlation spectroscopy
HMQC	Heteonuclear multiple quantum coherence
HMBC	Heteronuclear multiple bond coherence
GCMS	Gas chromatography spectrometry
m/z	Mass to charge ratio

CHAPTER 1

INTRODUCTION

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1.1 General

In Southeast Asia, there are rich jungles in Cambodia and Malaysia region due to the hot climate and humid all year round and it supports some of the most complex and species-rich ecosystem on the globe (Croix, 2008). Malaysia known as a green country with 60% of the land surface covered by forest various type of flora and fauna. The forest of East Malaysia are estimated around 2,000 tree species and known as one of most biodiverse areas in the world with 240 difference species in every hectare (Wikipedia, 2014).

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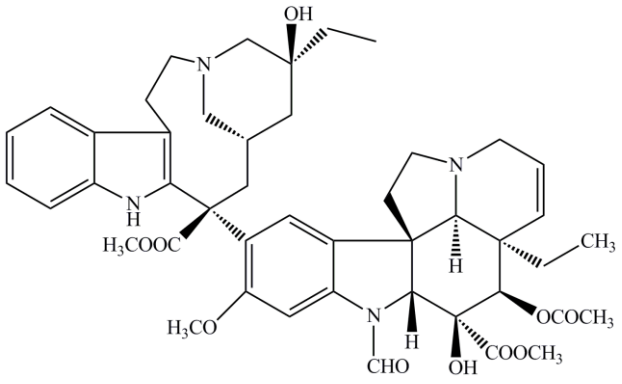
Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand for plant-based

medicines, health products, pharmaceuticals, food supplements and cosmetics. It was estimated that about 80% of all world's medicine are originally derived from plant sources (Cseke et al., 2006). Therefore, plants have contributed to the varieties of medicinal products since the past years.

Higher plants are important sources of natural products and are still used commercially to produce a wide range of chemicals as drugs, flavors, enzymes, perfumes, insecticides and emulsifying agents. Hence, many tropical plants from Malaysia has been extensively studied as well as their biological activity such as *Artocarpus* and *Actinodaphne* species (Hashim et al., 2012; Rachmatiah et al., 2009a).

Plants continue to be a major source of medicines. Among the most important are the physiologically active alkaloids including vincristine **1** and vinblastine **2** from *Catharanthus roseus*, codeine **3** and morphine **4** from *Papaver somniferum*, hyoscyamine **5** and scopolamine **6** from *Datura* species, quinine **7** and quinidine **8** from *Cinchona ledgeriana* and reserpine **9** from *Rauwolfia* species.



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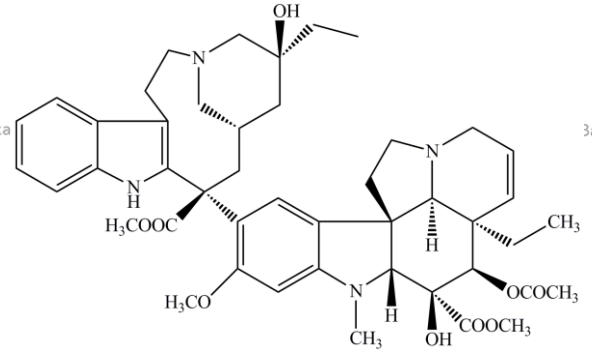

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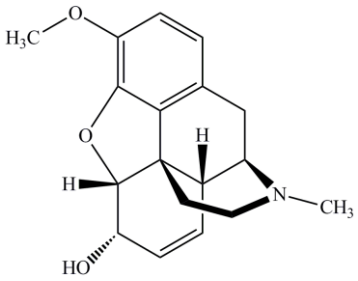

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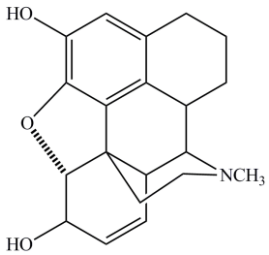

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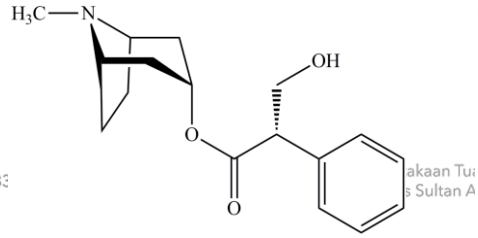

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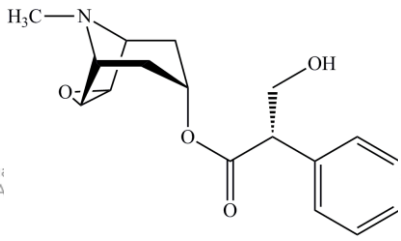

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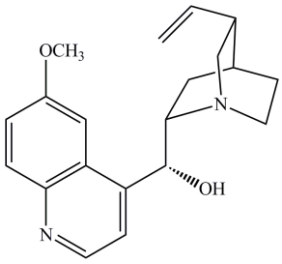

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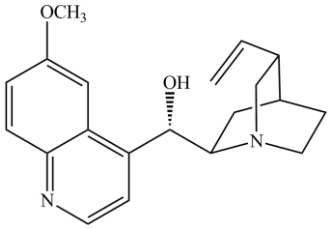

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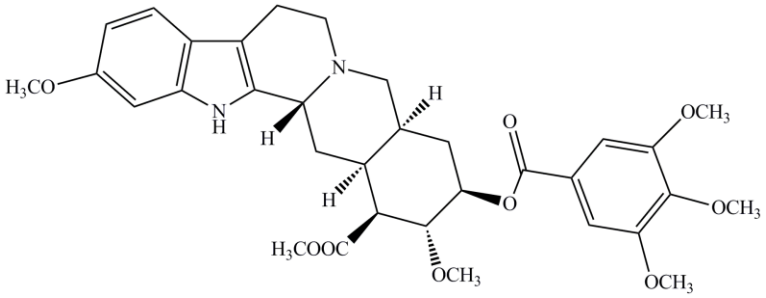

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1.2 Objectives of study

A research work on the species of *Alseodaphne*; *Alseodaphne peduncularis* and *Alseodaphne corneri* were carried out with the following objectives:

1) To extract and isolate the alkaloids.
2) To elucidate the structures of the isolated compounds using modern spectroscopic methods such as 1D-NMR, 2D-NMR, UV, IR and MS.
3) To test the antiplasmodial activity of crude extract and pure alkaloids.

1.3 Lauraceae

Lauraceae form a large family of woody plants with about 50 genera and 2,500 to 3,000 species distributed throughout tropical to subtropical latitudes (Chanderbali, Werff & Renner, 2001). There are 20 genera and more than 420 species of Lauraceae in China which are mainly distributed in areas south of the Qinling Mountain-Huaihe River (Kuo et al., 2012).

In Malaysia, Lauraceae also known as '*Medang*' or '*Tejur*'. About 16 genera and 213 species of Lauraceae family can be found in Malaysia (Omar et al., 2013). Lauraceae distributed in the lowland and becoming more abundant in the mountains between 1,200 and 1,600 m altitude. Major producing states in Peninsular Malaysia including Kelantan, Perak, Terengganu, Negeri Sembilan and Kedah (Gan & Lim, 2004).

The Lauraceae family is known to contain alkaloids which explain the positive alkaloid tests towards *Dehaasia caesia* (leaves 2+ and bark 3+) and *Litsea Elliptibacea* (leaves 2+ and bark 4+) (Ismail & Din, 1995a). Previous works showed that aporphine alkaloids have been isolated from 18 genera of these plants including *Actinodaphne*, *Alseodaphne*, *Beilschmiedi*, *Cassytha*, *Cinnamomum*, *Cryptocarya*, *Dehaasia*, *Laurus*, *Lindera*, *Litsea*, *Machilus*, *Mezilaurus*, *Nectandra*, *Neolitsea*, *Ocotea*, *Phoebe*, *Ravensara* and *Sassafras* (Kuo et al., 2012).

1.3.1 Anatomical features and wood characteristics of Lauraceae

Growth rings absent but the presence of terminal parenchyma in some species may stimulate growth rings. Vessels medium-sized, solitary, radial pairs and multiples of up to 4, tyloses usually present (Figure 1.1). Wood parenchyma mainly as incomplete border to the vessels with ill-developed aliform to confluent (Gan & Lim, 2004).

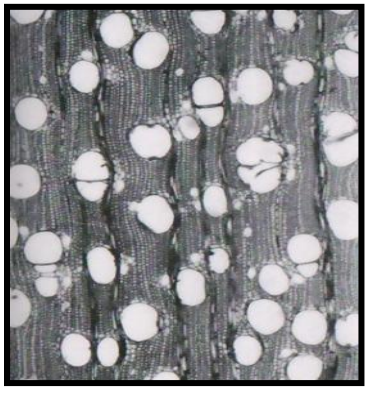

Figure 1.1. Anatomical features of Lauraceae. Adapted from "Common Commercial Timbers of Peninsular Malaysia," by K. S. Gan and S. C. Lim, 2004, *Research Pamphlet No. 125*, p. 38.

Some species with irregularly spaced bands. Rays fine or medium-sized and not distinct to naked eye. The characteristics of heartwood very variable, light-straw, red-brown to olive brown. Sapwood ill-defined and the surface is dull while the texture is moderately fine but even (Figure 1.2). Grain interlocked or wavy (Gan & Lim, 2004).

Figure 1.2. Wood characteristic of Lauraceae. Adapted from "Common Commercial Timbers of Peninsular Malaysia," by K. S. Gan and S. C. Lim, 2004, *Research Pamphlet No. 125*, p. 38.

1.3.2 Taxonomy of Lauraceae

The classification of Lauraceae illustrated as listed below:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Laurales

Family: Lauraceae

Genera:

Actinodaphne
Aiouea
Alseodaphne
Aniba
Apollonias
Aspidostemon
Beilschmiedia
Caryodaphnopsis
Cassytha
Chlorocardium
Cinnadenia
Cinnamomum
Cryptocarya
Dehaasia
Dicypellium
Dodecadenia
Endiandra
Endlicheria
Eusideroxylon
Gamanthera
Hexapora
Hufelandia
Hypodaphnis
Iteadaphne
Kubitzkia
Laurus
Licaria
Lindera
Litsea
Machilus
Malapoenna
Mezilaurus
Misanteca
Mocinnodaphne
Mutisiopersea
Nectandra
Neocinnamomum
Neolitsea
Notaphoebe
Nothaphoebe
Ocotea
Oreodaphne
Parasassafras
Parthenoxylon
Paraia
Persea
Phoebe
Phyllostemonodaphne
Pleurothyrium
Polyadenia
Potameia
Potoxylon
Povedadaphne
Ravensara
Rhodostemonodaphne
Sassafras
Schauera
Sextonia
Sinopora
Sinosassafras
Syndiclis
Tetranthera
Tylostemon
Umbellularia
Urbanodendron
Williamodendron

1.3.3 Uses of Lauraceae

In Sabah, a species namely, *Cinnamomum* from Lauraceae family had been used for traditional medicine. Dusun people of Sabahan called the *Cinnamomum* species as '*Lamau-Lamau*'. They boiled the roots and make as a tonic to heal the headache (Ismail & Din, 1995b).

One of the major product of Lauraceae is in timber industry (Gan & Lim, 2004). The timber classification of Lauraceae is light hardwood and it is a large family of medium-sized to large trees. Some species of Lauraceae family are commercial

importance in Malaysia. The important genera which produce the timber of '*Medang*' include *Actinodaphne, Alseodaphne, Beilschmiedia, Cinnamomum, Cryptocarya, Dehaasia, Litsea, Nothaphoebe* and *Phoebe.*

The wood density is within 400 to 800 kg/m^3 air dry. Its suitable for decorative work such as interior finishing, paneling, furniture and cabinet making. It also suitable for plywood manufacture and the heavier species are suitable for medium construction under cover (Gan & Lim, 2004).

Besides that, the family's great economic has another sources such as the high content of ethereal oils in the woods and leaves of many Lauraceae which are sources of perfumes, spices and flavourings such as camphor and cinnamon (Renner, 2011).

1.4 Genus *Alseodaphne*

From the *World Dictionary of Plant Names* state that *Alseodaphne Ness* (Lauraceae) origins name is from Greek. The *alsos* is "a grove" and *daphne* is "bay laurel" (Umberto, 2002). *Alseodaphne* is one of genus in Lauraceae family. It can be found in South East Asia countries such as China, Philippines, Borneo, Indonesia, Malaysia, New Guinea and Burma. *Alseodaphne* species also known as '*gemor*' in Indonesia and grows in Kalimantan Tengah and Kalimantan Selatan. Each year, it has become the major product of timber with about 250-300 kg/tree to 500-600 kg/tree (Budi & Andri, 2011).