EXTRACTION AND ISOLATION OF ALKALOIDS FROM THE LEAVES OF *Alseodaphne corneri* (LAURACEAE)

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ABSTRACT

This phytochemical study is aimed to extract, isolate and identify alkaloids from *Alseodaphne corneri* leaves. The crude alkaloid was extracted by cold percolation followed by acid-base extraction until Mayer test showed negative result. Then, it was separated by conventional chromatographic methods using column chromatography and preparative thin layer chromatography. The structural elucidation of isolated alkaloids was then carried out by spectroscopic techniques; UV, IR, NMR and HRESIMS. The structures of the compounds were also determined by comparison with previous study. Isolation of alkaloids yielded one new benzylisoquinoline; (6,7-dimethoxyisoquinolinyl)-(2',5'-dihydroxy-4'-methoxyphenyl)-methanone **68**, one bisbenylisoquinoline; obamegine **49,** one oxoaporphines; atheroline **71**, and five aporphine; isocorydine **48**, *N*-methyllaurotetanine **51**, laurotetanine **53**, lirioferine **69** and norlirioferine **70.** The benzylisoquinoline type alkaloid, lirioferine **69** and atheroline **71** were first reported in *Alseodaphne* genus while norlirioferine **70** was first isolated from *Alseodaphne corneri* species. These findings are very important as the elucidated alkaloids can be referred in future especially in identification of new alkaloid compound.

PENGEKSTRAKAN DAN PEMENCILAN ALKALOID DARIDAPA DAUN *Alseodaphne corneri* (LAURACEAE)

ABSTRAK

Kajian fitokimia ini bertujuan mengekstrak, memencil dan mengenal pasti alkaloid daripada daun *Alseodaphne corneri*. Alkaloid mentah diekstrak dengan menggunakan perkolasi sejuk diikuti pengekstrakan asid-bes sehingga ujian Mayer menunjukkan keputusan negatif. Seterusnya, alkaloid mentah dipencilkan melalui kaedah kromatografi konvensional dengan menggunakan kromatografi kolom dan kromatografi lapis tipis preparatif. Pengenalpastian struktur alkaloid yang dipencil dilakukan dengan teknik-teknik spektroskopi seperti UV, IR, NMR dan HRESIMS. Struktur sebatian yang dikenalpasti turut dibandingkan dengan kajian-kajian lepas. Pengasingan alkaloid telah menghasilkan satu benzilisokuinolina; (6,7-dimetoksiisokuinolinil)-(2',5'-dihidroksi-4'-metoksifenil)-metanon **68**, satu bisbenzilisokuinolina; obamegina **49**, satu oxoaporphina; aterolina **71,** serta lima aporphina; isocoridina **48,** *N*-metillaurotetanina **51,** laurotetanina **53,** lirioferina **69** dan norlirioferina **70.** Alkaloid jenis benzilisokuinolina, lirioferina **69** dan aterolina **71** adalah kali pertama dilaporkan dalam genus *Alseodaphne* manakala norlirioferina **70** adalah kali pertama diekstrak daripada spesis *Alseodaphne corneri*. Penemuan ini amat penting kerana data alkaloid yang dikenal pasti boleh dijadikan rujukan untuk masa depan terutamanya dalam pengenalpastian sebatian alkaloid 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
M	Molar
mM	Milimolar
ml	Mililitre
m	Meter
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
Me	Methyl group
OMe	Methoxyl group

OH	Hydroxyl group
NH_3	Ammonia
pH	Power of Hydrogen
HCl	Hydrogen chloride
TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography
CC	Column Chromatography
NMR	Nuclear Magnetic Resonance
cm^{-1}	Per centimeter
J	Coupling constant
d	Doublet
dd	Doublet of doublet
t	Triplet
dt	Doublet of triplet
s	Singlet
m	Multiplet
˚C	Degree Celsius
1D-NMR	One Dimension Nuclear Magnetic Resonance
2D-NMR	Two Dimension Nuclear Magnetic Resonance
^{1}H	Proton NMR
^{13}C	13-Carbon NMR
COSY	^{1}H-^{1}H Correlation Spectroscopy
DEPT	Distortioness Enhancement by Polarization Transfer
HMQC	Heteronuclear Multiple Quantum Correlation
HMBC	Heteronuclear Multiple Bond Correlation

NOESY	Nuclear Overhauser Effect Spectroscopy
LC-MS	Liquid Chromatography Mass Spectrometry
HRESIMS	High Resolution Electrospray Ionization Mass Spectrometry
m/z	Mass per charge
$CDCl_3$	Deuterated chloroform

CHAPTER 1

INTRODUCTION

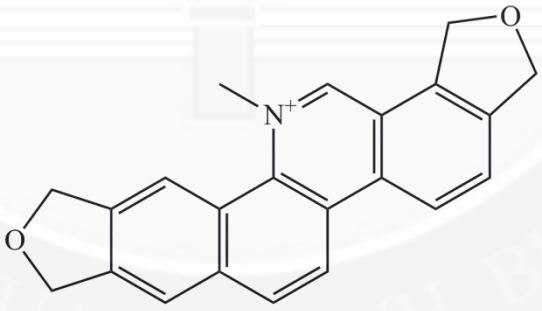
1.1 Introduction

Malaysia is located near the Equator confers on it a typically tropical climate which contributes to the tropical rainforests. This country is rich in flora and estimated to have 17,631 species of plants; including 61 gymnosperm, 337 of algea, 1,387 bryophyte, 1,600 species ferns and its families, 4,180 monocotyledons and 10,026 dicotyledons, thus, the 2001 Global Diversity Outlook recognized Malaysia as one of 12 mega diversity countries in the world (Department of Statistics, Malaysia, 2012).

Plants are usually used as traditional medicines or herbs for treatment, recovery and health maintenance of many diseases since ancient times. During Ming Dynasty, S.Z, Li had been compiled and written traditional Chinese medicine book titled 'Compendium of Materia Medica'. This compendium recorded the plants,

animals, minerals and other objects which believed to have medicinal properties ("Ben Cao Gang Mu (Compendium of Materia Medica)," 2011). In Malaysia, there are some common traditional medicinal plants used to cure many diseases such as diabetes treatment using *misai kucing* (*Orthosiphon stamineus*) (Ching, Zakaria, Paimin, & Jalalian, 2013). Besides traditional medicines, many plants have been proven the presence of biologically active compounds; for example, Sanguinarine **1** from Papaveracea family exhibit anticancer properties (Lu, Bao, Chen, Huang, & Wang, 2012) .

The natural resources (especially from plants) provide rich source of new pharmaceuticals, agrochemicals, industrial raw material and other economically important products. Recently, "Back to Nature" becomes a trend for the public. Therefore, natural products are one of the most promising sources for discovery of future drugs in various therapeutic areas.



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1.2 Problem Statement

The phytochemical studies on *Alseodaphne corneri* have been published since 2008. Twenty-two compounds, mainly isoquinoline type alkaloids were reported on this species and nine known compounds were isolated from the leaves of *A. corneri.* From the preliminary screening, the leaves of this species have high content of alkaloids. Therefore, it is a potential plant for extraction and isolation followed by structural elucidation of alkaloids especially the new compound as reference in future.

1.3 Objectives

The objectives of this study are as follows:

i. To extract alkaloid from the leaves of *Alseodaphne corneri*.

ii. To isolate alkaloids from the crude samples using chromatographic techniques.

iii. To carry out structural elucidation of isolated alkaloids.

1.4 Lauraceae

Lauraceae, named from the famous laural cultivated from centuries in Europe, usually appear as tropical evergreen shrubs or trees except in temperate zones which is deciduous. In past, its foliage was used by the ancient Greeks to crown victors in athlete events (Dharmananda, 2004). Later on, it was used in the conferring of academic honors. Nowadays, Lauraceae becomes important sources of traditional

medicines (e.g. *Lindera aggregata*), timber, spices (e.g. *Cinnamomum* trees), nutritious fruits (e.g. *Persea americana*) and perfumes (Xiwen et al., 2000).

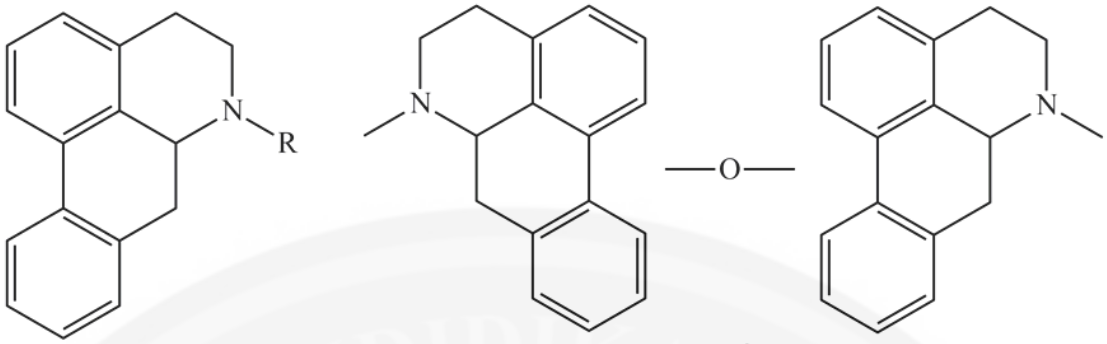
The laurel order of flowering plants contains 7 families, 91 genera and about 2900 species. They are found in tropical or warm temperate climates, abundant in regions with moist equable climates. The members of Laurales are trees, shrubs or woody vines and they are characterized by woodiness, aromatic parts and a single strand of conducting tissues continuing from the stem into the leaf. Among seven families, both Lauraceae and Monimiaceae constitute most of the genera in this order. Lauraceae consists of 67 genera distributed throughout tropical and subtropical regions, principally Southeast Asia and tropical America, particularly Brazil. In 1970, Keng (as cited in Perumal, Petol, Mat-Salleh, & Latiff, 1998) reported that Lauraceae belongs to ten most extensive genera in Malaysia.

Leaves of Lauraceae family usually alternate, occasionally opposite or subopposite or verticillate, simple, usually entire, rarely lobed. The flowers are perigynous and cup-like structure around the base of ovary or around ovary. In addition, they usually exist as axillary. Drupe or berry like fruit usually surrounded at base by enlarge and often persistant perianth tube seated on a large receptacle or pedicel (Xiwen et al., 2000).

1.5 The Genus: *Alseodaphne*

Alseodaphne is a member of family Lauraceae and distributed in tropical belt of India, Cambodia, China, Indonesia, Laos, Malaysia, Myanmar and Philipines. Most of these species are locally known as "Medang" or "Tejur" in Malaysia. There are 23 out of 56 species are found in Malaysia (Nafiah, 2009).

The plants are source of wood (contributes in house and boat building) and furniture such as plywood tea-chest. Many researchers have been explored this species. However, very less species have been reported their chemical composition. Recently, chemical constituents were reported from the isolation of some species such as *A. archboldina*, *A. andersonii*, *A. corneri*, *A. hainensis*, *A. pendulifolia*, *A. penduncularis*, *A. perenkensis* and *A. semicarpifolia*. This genus were reported rich to contain aporphines **2**, bisbenzylisoquinolines **3**, phenanthrenes **4**, lactones **5** and morphinandienones **6**; which could be of great biological importance. Although the fruits of *A. corneri* were known to be poisonous, there are still no report on biological study and medicinal value of this species (Thakur et al., 2012).



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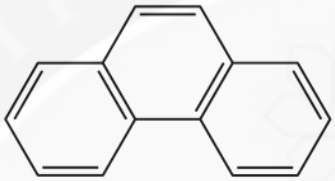

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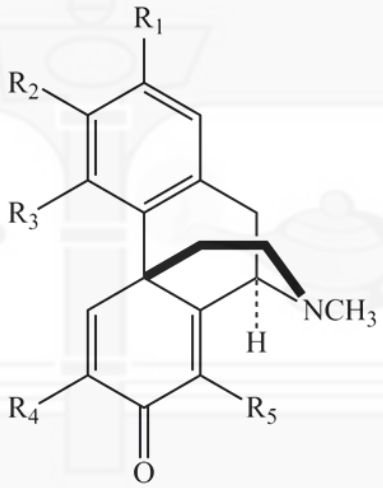

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Alseodaphne is small to medium sized tree of wet evergreen tropical forests which distributed from Yunnan to West Malaysia. It normally 5-35 meters height (Nafiah et al., 2011). The leaves are broad and alternate which mostly clustered near apex of branchlets. Terminal buds are scaly whereas inflorescences are axillary, paniculate or racemose. Flowers are bisexual, trimerous and short perianth tube with six lobes which are sub-equal and absent in fruit. There are nine fertile stamens in

three whorls with filaments of first and second whorls are glandless. Anthers are four celled. The ovary is partly immersed into shallow perianth tube. The style is as long as ovary. The stigma is small and inconspicuous. The fruits are green when unripe and purplish black when mature with ovoid, oblong or sub-globose in shape (Nafiah, 2009; Thakur et al., 2012; Zahari, 2010).

1.5.1 *Alseodaphne corneri*

Alseodaphne corneri (Figure 1.1) is a small tree up to 6 m tall which is found in Malaysia, Singapore and Indonesia. Terminal bud covered with many 1 cm long glabrous scales. The twigs are stout and grey colour with prominent leaf scars. The leaves (Figure 1.2) are closely, spirally arranged at end of twigs, stalk is about 3-4 cm long and blade thickly leathery with obovade to elliptic shape and about 28-52 x 12-16 cm. The apex is pointed but the base is tapered and glaucous below. The midrib rose on the upper surface consists of about 22 pairs of secondary nerves which rise on both surfaces. Tertiary nerves reticulate, visible on both surfaces (Thakur et al., 2012).

The flowers of this species are glaucous up to 14 mm long. Perianth lobes equal, oblong with 5 mm long. The fruits (Figure 1.2) of this species are ellipsoid which up to 3 x 2 cm, seated on very thick, rough, warted and pedicel with persistent perianth lobes. *Alseodaphne corneri* is very rare and the plant specimens are from lowland in East Malaysia especially in east Johor (Nafiah, 2009; Zahari, 2010).

Figure 1.1. *Alseodaphne corneri* Kosterm.

Figure 1.2. The leaves and fruit of *Alseodaphne corneri* Kosterm.

CHAPTER 2

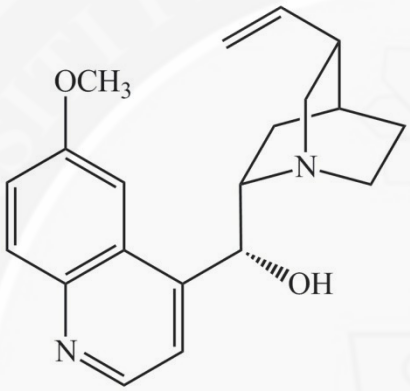
LITERATURE REVIEW

2.1 Introduction

Since ancient times, plants are always consumed for health purposes. After the plants have been extracted and studied, scientists found compounds and related them to the medicinal treatment. These compounds are clinically useful drug such as antimalarian agents (e.g. quinine **7**), agents promoting blood circulation in the brain (e.g. vincamine **8**), in Parkinson's disease treatment (e.g. apomorphine **9)** and anticancer agents (e.g. taxol **10**) (Aniszewski, 2007; Bently, 1998). The drug discovery from natural product experienced an evolution in the last decade and now it is widely accepted due to its potential in many therapeutic areas (Genilloud & Vicente, 2012).

In 1804, morphine **11** had been isolated from opium poppy. It is the first alkaloid that isolated and crystallized from plant. Then, the medicinal properties of

morphine were studied and researcher found that morphine **11** is a powerful narcotic used to relief pain. Later on, many alkaloids were reported and their medicinal properties were identified such as quinine **7** from genus *Cinchona* is used to treat arrhythmias or irregular rhythms of the heartbeat, ephedrine **12** from *Ephedra* species act as blood-vessel constrictors and so on ("Alkaloid," 2013; Nafiah, 2009).



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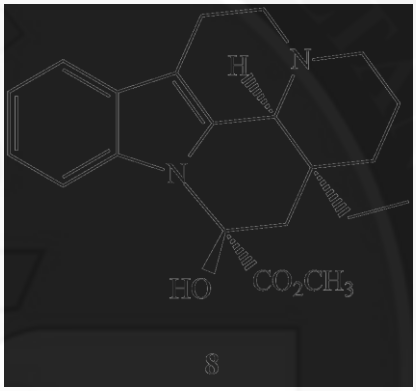

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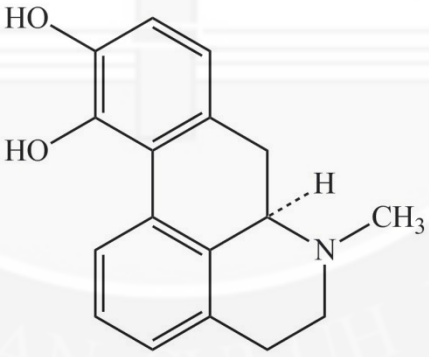

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