STUDY ON ALKALOIDS FROM THE ROOTS OF *Murraya koenigii*

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CONTENTS

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ABSTRACT

The aims of this study were to determine the phytochemical constituents from the roots of *Murraya koenigii* and to test the antidiabetic activities. The isolation and purification of the chemical compounds involves extraction and separations by using various chromatographic methods and structural determination by specttroscopic techniques such as ultraviolet (UV), infrared (IR), mass spectroscopy (MS) and nuclear magnetic resonance (NMR) including 1D-NMR (^{1}H, ^{13}C and DEPT) and 2D-NMR (COSY, HMQC and HMBC). The structure of the isolated compounds were compared with previous work done by other researcher. Isolation studies on the roots of Malayan *M. koenigii* collected from Jerantut, Pahang involving hexane, dichloromethane and methanol extract yielded eight compounds. Hexane crude extracts gave two compounds; girinimbine and murrayafoline A, while five compounds were successfully isolated from the dichloromethane crude extracts known as mahanimbine, murrayanine, 3-formylcarbazole, *N*-methoxy-3-hydroxymethyl-9H-carbazole and koenoline. Futher investigation on the methanol crude extracts afforded one new indole alkaloid named 2-hydroxy-3,5-dimethoxyindole. In a parallel investigation, the antidiabetic activity were also tested on all crude extracts and isolated compounds. It showed negative results on antidiabetic activity test. The implication of the data obtained from this study can be use as a reference for other researchers to conduct studies on *M. koenigii*. In conclusion, the study successfully isolated and purified eight compounds from roots of *M. koenigii* and tested for their antidiabetic activity.

ABSTRAK

Kajian ini bertujuan menentukan kandungan fitokimia daripada akar *Murraya*

koenigii dan menguji aktiviti antidiabetik. Pemencilan dan penulenan sebatian kimia melibatkan pengekstrakan dan pengasingan menggunakan pelbagai kaedah kromatografi dan pengenalpastian struktur dengan teknik-teknik spektroskopi seperti ultraviolet (UV), inframerah (IR), resonan magnet nuklear (RMN) termasuk 1D-RMN (^{1}H, ^{13}C dan DEPT) dan 2D-RMN (COSY, HMQC dan HMBC). Struktur sebatian yang dipencilkan dibandingkan dengan kajian lepas yang dilakukan oleh penyelidik terdahulu. Kajian pengasingan ke atas akar pokok Malayan *Murraya koenigii* daripada Jerantut, Pahang melibatkan ekstrak mentah heksana, diklorometana dan metanol telah menghasilkan lapan sebatian. Ekstrak mentah heksana menghasilkan dua sebatian; girinimbina dan murrayafolina A, manakala lima sebatian telah berjaya diasingkan daripada ekstrak mentah diklorometana dikenali sebagai mahanimbina, murrayanina, 3-formilkarbazol, N-metoksi-3-hidroksimetil-9H-karbazol dan koenolina. Kajian seterusnya dilakukan ke atas ekstrak mentah metanol memberikan satu sebatian indola baru dinamakan 2-hidroksi-3,5-dimetoksiindol. Dalam kajian yang sama, aktiviti antidiabetik turut diuji pada semua ekstrak mentah dan sebatian terpencil. Ia menunjukkan hasil yang negatif ke atas ujian aktiviti antidiabetik. Implikasinya, data yang diperolehi daripada kajian ini dapat digunakan sebagai rujukan kepada penyelidik lain yang akan menjalankan kajian terhadap *M. koenigii*. Kesimpulannya, kajian ini berjaya mengasingkan dan menulenkan lapan sebatian dari akar *M. koenigii* dan diuji untuk aktiviti antidiabetik.

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LIST OF ABBREVIATIONS

cm	centimeter
CC	Column Chromatography
$CDCl_3$	deuterated chloroform
DCM	dichloromethane
g	gram
Hz	Hertz
IR	Infrared
J	coupling constant (Hz)
kg	kilogram
MS	Mass Spectrum
min	minutes
NMR	Nuclear Magnetic Resonance
nm	nanometres
PTLC	Preparative Thin Layer Chromatography
ppm	parts per million
TLC	Thin Layer Chromatography
UV	Ultraviolet
^{13}C	13-carbon NMR
^{1}H	proton NMR
δ	Chemical shifts
^{o}C	degree Celsius

CHAPTER 1

INTRODUCTION

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1.1 General Introduction

Nature has been a source of medicinal products for millennia, with many useful drugs developed from plant sources (Cragg & Newman, 2013). In nature, chemical compound or substances produced by a living organism called natural products. In other words, natural products include any substance produced by life. All animals and plants can produce primary metabolites but plants also can produce secondary metabolites. Primary metabolites is the process which are used for energy purpose whereas secondary metabolites serves other useful purpose for the plants (Drewes, 2012).

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Natural products have been the major sources of chemical diversity for starting materials while driving pharmaceutical discovery over the past century. Historically, pharmaceutical companies have utilized plant extracts to produce relatively therapeutic formulations but with the advancement of antibiotics in the mid-twentieth century, drug formulations of fairly purified compounds have become more typical (Mishra & Tiwari, 2011). Plants can be synthesized thousands of metabolites for their growth and development, reproduction, defense against attack by many different kinds of organisms and survival in often harsh and ever changing environments (Desai, Parikh & Parikh, 2010).

The alkaloids are one of the most diverse groups of secondary metabolites found in living organism and have an array of structure types, biosynthesis pathways and pharmacological activities. Alkaloids are any of a group of organic basic and containing nitrogen atoms and usually in a heterocyclic ring (Facchini, 2001). Carbazole alkaloid is one of the types of alkaloids. Several types of carbazole alkaloids are pyranocarbazole type, pyranocarbazole alkaloid of special type, *N*-substituted hexacyclic pyranocarbazole type, cyclic pyranocarbazole type, bicyclic pyranocarbazole type, carbazole type, quinone type, *N*-substituted carbazole alkaloid and coumarins (Nayak, 2010).

1.2 Background of Study

Curry leaf (*Murraya koenigii*) or curry patte (Hindi) are derived from an aromatic more or less deciduous shrub or small tree which is found almost throughout India and Andaman Islands up to an altitude of 1500 meters. *M. koenigii* belongs

to family Rutaceae, commonly called “curry leaf”. It is commonly found in forests, often as a gregarious growth along the foot of the Himalayas up to Sikkim and Assam. It is also found in West Bengal, Madhya Pradesh and in the South and South Western states, namely, Maharashtra, Tamil Nadu, Kerala and Andhra Pradesh. It is much cultivated for its aromatic leaves and for ornamental value throughout India. It is native to Ceylon but universally found in the dry regions. It is also found in cultivation in Sri Lanka, Burma, Indo-China, South China and Hawaii. It is widely used in Kerala and in South Indian states as an important ingredient in Indian cuisine (Chandy, 2007).

Various parts of *M. koenigii* have been used in traditional or folk medicine for the treatment of rheumatism, traumatic injury and snake bite and it has been reported to have antioxidant, anti-diabetic and anti-dysenteric activities (Dineshkumar et al., 2010). The leaves, barks and roots of the plant are used in indigenous medicine as tonic, stomachic, stimulant and carminative. An infusion of the roasted leaves is used to prevent vomiting. The green tender leaves are eaten raw for the cure of dysentery. The juice of the root is taken to relieve pain associated with kidney ailments (Nayak et al., 2010).

In 2011, Vats, Singh & Sardana reported the view of the presence of various phytochemical constituents and pharmacological properties of *M. koenigii* which is widely used in indigenous medicine. The coarse powder on extraction with petroleum ether, chloroform and acetone showed the presence of alkaloids, coumarins and investigated for antibacterial and antifungal activity. Medicinally,

these leaves found use in diarrhea, dysentery and to prevent vomiting (Vats, Singh & Sardana, 2011).

The source of essential oil which finds in leaves and fruits are use as a fixative for heavy type of soap perfume. Leaves, root and bark are tonic, stomachic and carminative. Fresh juice of the root is taken to relieve pain associated with kidney. The aqueous extracts of leaves when administered parenterally to female guinea pigs, not only raised the phagocytic index but also mobilized a greater number of leucocytes to take part in phagocytosis (Chandy, 2007).

Carbazole alkaloids represent a new and interesting variant in the number of existing indolent alkaloids which in the past have yielded several important drugs. A rich and rewarding source of carbazole alkaloids has been the Indian curry leaf plant *M. koenigii* (Rutaceae). All parts of this plant including root, stem, leaves and fruits yielded carbazole alkaloids. It has been reported that carbazole alkaloids possess various biological activities such as anti-tumor, anti-oxidative and anti-mutagenic activities (Itoigawa et al., 2006, Dineshkumar, Mitra & Mahadevappa, 2010).

1.3 Problem Statement of Study

Diabetis mellitus is one of the most common non-communicable diseases globally. It is the fourth leading causes of death in the most developed countries. This will create a serious threat within 21st century (Arumugam, Manjula & Paari,

2013). Carbazole alkaloids have been reported for their various pharmacological activities such as anti-tumor, anti-viral, anti-inflammatory, anti-convulsant, diuretic and anti-oxidant activities (Dineshkumar, Mitra & Mahadevappa, 2010, Knolker & Reddy, 2008). Therefore, the present studies was undertaken to identify more carbazole alkaloids and their antidiabetic activities in the roots of *M. koenigii* from different places. The roots of *M. koenigii* have been collected at Pahang which is a different location from the previous researcher which may resulted different carbazole alkaloid. Therefore, hopefully the carbazole alkaloid that found can be used in antidiabetic activities and also proves that different place will have different carbazole alkaloid.

1.4 Objectives of Study

The objectives of the study are:

1) to extract and isolate chemical compound from the roots of *Murraya koenigii*
2) to determine the structure of the isolated chemical compound from the roots of *Murraya koenigii*
3) to evaluate antidiabetic activity of crude extract and isolated compounds

1.5 Significance of Study

This study will provide a lot of benefits to others. As it is proven that the carbazole alkaloids possess various biological activities such as anti-tumor, anti-

oxidative and anti-mutagenic activities, so it can help many people to avoid or decrease the ability to get the harmful diseases.

M. koenigii was chosen since it is one of the most widely acclaimed remedies for the treatment of diabetes. *M. koenigii* are used as flavorings, condiment and folk medicine for the treatment of various metabolic and infectious diseases. The leaves, barks, roots and fruits are used intensively in indegenious system of medicine from ancient time, as a tonic for stomache and stimula. Phytochemical screening of *M. koenigii* leaves revealed the presence of some vitamins, carbazole alkaloid, terpenoids, phenolic compounds and mineral content such as calcium, iron, zinc and vanadium. In addition, carbazole alkaloid present in *M. koenigii* leaves were reported to have antioxidant and antidiabetic activities (Thembhurne & Sakarkar, 2009). There are several biological activities of *M. koenigii* leaves reported for its anti-hypercholesterolemic as well as its efficacy against colon carcinogensis, anti-microbial and antioxidant (Thembhurne & Sakarkar, 2009). Therefore, hopefully from this research, it can help many people to maintain their health from antidiabetic activities.

CHAPTER 2

LITERATURE REVIEW

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2.1 The Plant- *Murraya* koenigii (Linn.) Spreng

2.1.1 Taxonomy of Plant

Kingdom: Plantae

Sub-kingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliospida

Subclass: Rosidae

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Order: Sapindales

Family: Rutaceae

Genus: *Murraya* J.Koening ex L.

Species: *Murraya koenigii* L. Spreng

2.1.2 *Murraya koenigii*

Murraya koenigii, belongs to the family Rutaceae, commonly known as curry-leaf tree which represent more than 150 genera and 1600 species (Vandana, Munira & Kirti, 2012). It shares aromatic nature, deciduous shrub or tree up to 6 m in height and 15-40 cm in diameter with short trunk, thin smooth grey and dense shady crown. Most part of plant is covered with fine down and has strong peculiar smell.

Leaves are bipinnately compound, 15-30 cm long each bearing 11-25 leaflets alternate on rachis, 2.5-3.5 cm long ovate lanceolate with an oblique base. Flowers are bisexual, white, funnel shaped sweetly scented. Fruits are generally biseeded. Seeds generally occur in spinach green colour, 11 mm long, 8 mm in diameter (Shruthi, Pandith & Handral, 2012).

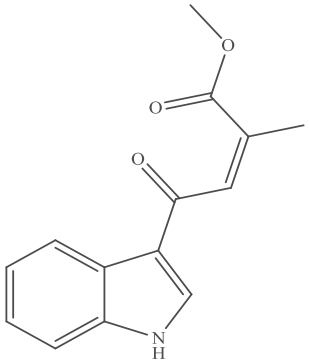
2.2 Chemical Aspects

2.2.1 Carbazole Alkaloid

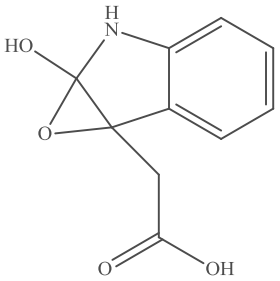
An intense search of literature has revealed that the stems, leaves, roots and seeds are potiential sources of carbazole alkaloids of common and lesser known skeleton (Nayak et al., 2010). There are ten types of carbazole alkaloids skeleton including pyranocarbazole type alkaloid, pyranocarbazole alkaloid type of special type, *N*-substituted haxacyclic pyranocarbazole type, cyclic pyranocarbazole type, bicyclic pyranocarbazole type, carbazole type, pentacyclic pyranocarbazole type, quinone type, *N*-substituted carbazole alkaloid and coumarins.

2.2.2 Previous Work on *Murraya* Species

Previous study shows many chemical constituents have been reported from the extraction and isolation of various parts of *Murraya* species by using different types of solvent such as n-hexane, chloroform, petroleum ether, dichloromethane, methanol and others. In 2012, Ng et al., reported two indole alkaloids, murrayacarine **(1)** and murrayaculatine **(2)** from roots, barks and fresh flowers of *Murraya paniculata*.

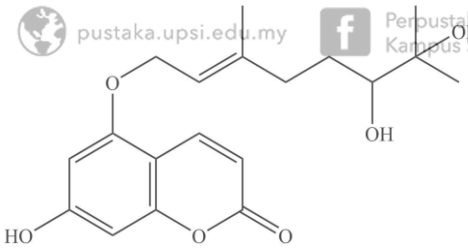


(1)

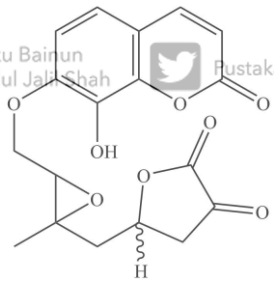


(2)

Three new coumarins, murrayacoumarins A **(3)**, murrayacoumarins B **(4)** and murrayacoumarins C **(5)** were isolated from the leaves of *Murraya siamensis* species of acetone crude extract (Ito et al., 2005).



(3)



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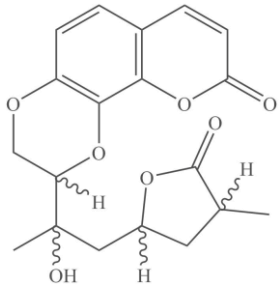
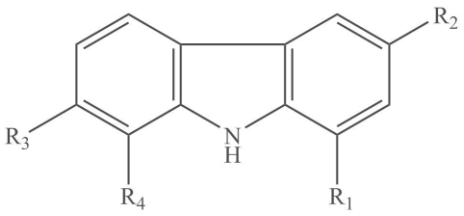
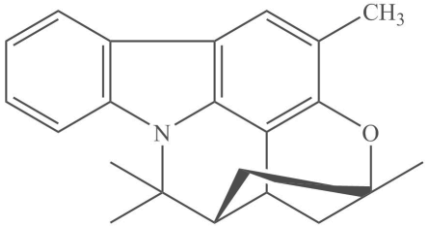


(5)

The dried root bark of *Murraya euchrestifolia* collected in Taiwan was extracted with ethanol under reflux yielded nine carbazole alkaloids; Murrayafoline-A **(6)**, Murrayafoline-B **(7)**, Murrayanine **(8)**, 3-methylcarbazole **(9)**, 1-hydroxy-3-methylcarbazole **(10),** (+)-murrayazoline **(11)**, Cyclomahanimbine **(12)**, Girinimbine **(13)** and Mahanimbine **(14)** and four novel carbazolequinone alkaloids; Murrayaquinone-A **(15)**, Murrayaquinone-B **(16)**, Murrayaquinone-C **(17)** and Murrayaquinone-D **(18)** (Furukawa et al., 1985).



	R_1	R_2	R_3	R_4
(6)	OCH_3	CH_3	H	H
(7)	OH	CH_3	OCH_3	prenyl
(8)	OCH_3	CHO	H	H
(9)	H	CH_3	H	H
(10)	OH	CH_3	H	H



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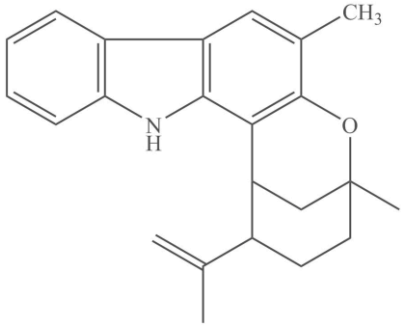

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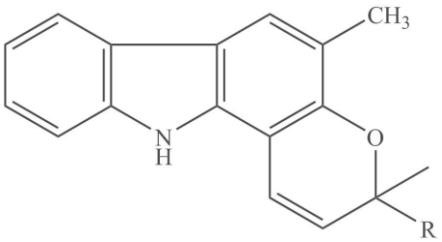

R

(13) CH_3

(14) $CH_2CH_2CH=C(CH_3)_2$