UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS OF *MURRAYA KOENIGII* (RUTACEAE) AND THEIR BIOLOGICAL ACTIVITIES

KARTINI BINTI AHMAD

FSAS 1999 25

CHEMICAL CONSTITUENTS OF *MURRAYA KOENIGII* (RUTACEAE) AND THEIR BIOLOGICAL ACTIVITIES

KARTINI BINTI AHMAD

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

1999

CHEMICAL CONSTITUENTS OF *MURRAYA KOENIGII* (RUTACEAE) AND THEIR BIOLOGICAL ACTIVITIES

By

KARTINI BINTI AHMAD

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Science in the Faculty of Science and Environmental Studies, Universiti Putra Malaysia

May 1999

ACKNOWLEDGEMENTS

In the name of Allah, most Gracious, most Merciful. I would like to convey my gratitude to my supervisors, Associate Professor Dr Mohd Aspollah Hj. Sukari, Associate Professor Dr Mawardi Rahmani and Associate Professor Dr Abdul Manaf Ali, for their guidance, concern, understanding and support throughout the development of this project.

I would like to thank all the staff of Chemistry Department for bearing with my demands and readily assisting me in moments of need.

My heartfelt thanks to all my friends, Kak Pah, Kak Ratna, Pak Sugeng, Gaber and officemates for the precious help, encouragement and confidence they gave me during the years of my studies.

Grateful appreaciation is also extended to my beloved husband (Mohd Azlan Nafiah), my parents, brothers and sisters who were responsible in completing my study in Universiti Putra Malaysia.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

br	broad
CC	column chromatography
$CDCl_3$	deuterated chloroform
$CHCl_3$	chloroform
d	doublet
dd	double doublet
ddd	doublet of doublet of doublet
t	triplet
s	singlet
m	multiplet
DMSO	dimethyl sulphoxide
Pet.ether	petroleum ether
MeOH	methanol
m.p	melting point
MS	Mass Spectrum
NMR	Nuclear Magnetic Resonance
TLC	Thin Layer Chromatography
IR	Infrared
UV	Ultraviolet

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia
in fulfilment of requirements for the degree of Master of Science

CHEMICAL CONSTITUENTS OF *MURRAYA KOENIGII* (RUTACEAE) AND THEIR BIOLOGICAL ACTIVITY

By

KARTINI BINTI AHMAD

May 1999

Chairman : Associate Professor Mohd. Aspollah Hj. Sukari, Ph.D.

Faculty : Science and Environmental Studies

The study on *Murraya koenigii* (curry leaf) involves extraction, separations by using various chromatographic methods and structural determination by spectroscopic techniques such as IR, NMR including 2D-NMR and MS. The structures of the compounds were also elucidated by comparison with the previous work. Isolation works on stem bark of the plant yielded mahanimbine (31), girinimbine (32), murrayanine (47), murrayazoline (48), murrayacine (49) and sucrose (50). From the leaves, five compounds were isolated and elucidated. Two of them were long chain compounds, 11-decyl-henecosane (51a) and methoxydotriacontane (51b), isolated as a mixture and the

other compounds were mahanimbine (31), ethyl octadecanoate (52) and mahanine (53).

Further study on the roots of the same plant afforded four compounds including girinimbine (32), murrayanine (47), together with 3-methylcarbazole (54) and murrayafoline A (55).

Crude extract and isolated compounds from various part of this plant were screened for antimicrobial activity using disc diffusion method, cytotoxic activity using microtitration method and antitumor promoting activity using Epstein Barr virus activation assay. The crude extracts of the roots and pure isolated compound including mahanimbine (31), girinimbine (32), mahanine (53) and murrayafoline A (55) exhibited significant cytotoxicity activity against CEM-SS cell line with IC_{50} 3 μg/ml. Girinimbine (32), although did not show any cytotoxic activity against CEM-SS cell line, the compound however inhibited EBV-activation (100%) in the antitumor promoting assay. The pet.ether, $CHCl_3$ and MeOH crude extracts of leaves, the methanol extract of stem bark, mahanimbine (31) and girinimbine (32) failed to show significant antimicrobial activity, while the pet.ether and the chloroform crude extracts of the stem bark exhibited weak antibacterial activity against *Bacillus cereus*.

Abstrak tesis ysng dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KANDUNGAN KIMIA DARI *MURRAYA KOENIGII* (RUTACEAE) DAN ACTIVITI BIOLOGINYA

Oleh

KARTINI BINTI AHMAD

Mei 1999

Pengerusi : Profesor Madya Mohd Aspollah Hj. Sukari, Ph.D.

Fakulti : Sains dan Pengajian Alam Sekitar

Kajian terhadap pokok *Murraya koenigii* (daun kari) melibatkan pengekstrakan dan pengasingan menggunakan pelbagai kaedah kromatografi dan pengenalpastian struktur dengan teknik-teknik spektroskopi seperti inframerah, resonans magnet nukleus termasuk resonans magnet nukleus dua dimensi dan spektroskopi jisim. Struktur sebatian yang dipencilkan juga telah dibuat perbandingan dengan kajian lepas. Pengasingan keatas kulit batang pokok ini telah menghasilkan mahanimbin (31), girinimbin (32), murayanin (47), murayazolin (48), murayasin (49) dan sukros (50). Lima sebatian telah dipencilkan dan diasingkan daripada daun pokok yang sama. Dua daripadanya ialah sebatian rantai panjang, 11-dekil-henekosana (51a) dan

metoksidotriakontana (51b), diasingkan sebagai campuran dan sebatian lain adalah mahanimbin (31). etil oktadekanoat (52) dan mahanin (53).

Kajian seterusnya dilakukan keatas akar pokok yang sama memberikan empat komponen termasuk girinimbin (32), murayanin (47), bersama dengan 3-metilkarbazol (54) dan murayafolin A (55).

Ekstrak mentah dan sebatian yang telah diasingkan dari pelbagai bahagian pokok tersebut juga telah diuji untuk aktiviti-aktiviti antimikrob menggunakan kaedah resapan cakera, sitotoksik dengan kaedah mikrotitratan dan anti promosi barah menggunakan kaedah cerakinan pengaktifan virus Epstein Barr. Ekstrak mentah dari akar dan sebatian yang telah dipencilkan termasuk mahanimbin (31), girinimbin (32), mahanin (53) dan murayafolin A (55) menunjukkan aktiviti- aktiviti sitotoksik yang kuat keatas sel CEM-SS dengan IC_{50} 3 μg/ml. Walaubagaimanapun, girinimbin (32) tidak menunjukkan sebarang aktiviti sitotoksik keatas sel CEM-SS, sebatian ini telah menunjukkan aktiviti anti promosi barah dengan 100% perencatan. Ekstrak mentah pet.eter, kloroform dan metanol dari daun, ekstrak metanol dari kulit batang, mahanimbin (31) dan girinimbin (32) gagal untuk menunjukkan sebarang aktiviti antimikrob, manakala ekstrak mentah pet.eter dan kloroform dari kulit batang menunjukkan aktiviti antimikrob yang lemah terhadap *Bacillus cereus*.

CHAPTER I

INTRODUCTION

Murraya koenigii and the Uses

Murraya koenigii is a member of the large Rutaceae family and represented by about 150 genera and 1600 species. However, in Malaysia, nearly 60 species of this family can be found including two species of *Murraya*; namely *Murraya koenigii* and *Murraya paniculata*. Other *Murraya* species are *M. alata*, *M. exotica*, *M. kwangsiensi* and *M. microphylla*, consisting mainly of trees or shrubs and a very small proportion of herbs all of which flourish in the tropics and subtropics.

M. koenigii (curry leaf) is one of the most widely used plants whose leaves are added to curries to improves flavour. It is usually cultivated for its aromatic smell which are used as natural flavourings in curries and souces as reported by Sastri in 1952 and Brandis in 1971. The intensive pungent, aromatic leaves are best when fresh, but adequately retain their potency for some time after picking.

This plant is originated from Tarai of Uttar Pradesh, India and distributed throughout India and Andaman Islands (Sastri, 1952). Curry leaf odorns every houseyard of South India and is usually propagated by seeds and roots suckers. However, attempts to propagate by air layering have not been successful (Phillip *et al.* 1981).

This plant is an unarmed, semideciduous, aromatic, shrub or small tree of about 3-5 meter high with strong woody stem. The branches are covered with dark grey bark. The woody stem has a closely crowded, shady crown. Leaves are 15-20 mm long, have slightly pungent, bitter and feebly acidulous taste. The flowers are small, 1 cm long and the small fruits occur in close clusters. It turned from green to red and ultimately black on ripening (Joseph and Peter, 1985).

Various parts of this plant have many medicinal uses and values as raw material for traditional medicine and popular in India (Kirthikar and Basu, 1935). It have been reported that the leaves and the roots can cure piles and heat removal of the body, imflamation and itching, while the powdered leaf is used to aid healing of fresh cuts.

Drury (1978) reported that the green leaves could be eaten in raw for the treatment of dysentery. As an external application, barks and roots are used to relieve skin eruptions and the bites by poisonous animals (Dasturs, 1970).

Generally, this plant has also been used in the industry, where fresh leaves are steam distilled under reduced pressure to yield volatile oils (curry leaf oil) which can be used as a fixative for a heavy type of soap perfume (Joseph *et al.* 1985).

Murraya paniculata or locally known as "kemuning" is a shrub or small tree, grow wild in many parts of Malaysia, found mainly in the drier parts of the North and the East coast, or on limestone hills and also often planted in gardens. In appearance the "kemuning" looks like a citrus on account of the dark green, upstanding leaflets. It is evergreen and apparently night-flowering (Corner, 1988).

Screening of Bioactive Compounds

In Malaysia the plant remain a very important source of therapeutic traditional medicine practices among the various ethnic groups. The tropical forest of Malaysia comprises of more than 15,000 plants species many of which have been claimed to possess medicinal properties (Ali *et al.* 1995).

Among the species commonly used in the preparation of traditional medicines are those belonging to the families of Annonaceae, Apocynaceae, Araceae and Rutaceae. Lately, a number of Rutaceous species have been reported to posses pharmacological activities, which then lead to the isolation and characterization of their active compounds (Ontengo *et al.* 1993). The study of biologically active compound from plants must be related to their medicinal values.

Biological activities of Malaysian plants were first reported by Nakashini *et al.* in 1965. More recently, a more systematic screening of medicinal plants for antimicrobial activity against bacteria and fungi was reported by Ali *et al.* (1995). Much effort in previous studies has been devoted in testing the bioactivities of phytochemicals in the search of new and useful compounds.

The most important factor in the search of new bioactive substances is the convenience and reliability of the bioassay system. Bioassay for screening purpose must be inexpensive and rapid, have broad applications to numerous target organisms, be reproducible and statistically valid. It also must require small amount of the test substance.

In order to achieve meaningful and significant results, future works in natural product chemistry must incorporate bioassays (Mc Laughlin, 1990). Rahmani *et al.* have carried out a screening procedure of tropical plants for the presence of bioactive compounds by using brine shrimp in 1992.

Objectives

The objectives of this research were;

1. To extract, isolate and purify chemical components from stem bark, leaves and roots of *Murraya koenigii*.
2. To identify and elucidate the structure of the isolated compounds using modern spectroscopic methods.
3. To determine the antimicrobial, cytotoxicity and antitumor promoting activities againts the extracts and the isolated compounds from *Murraya koenigii*.
4. To identify the compounds which contribute to the toxicity.

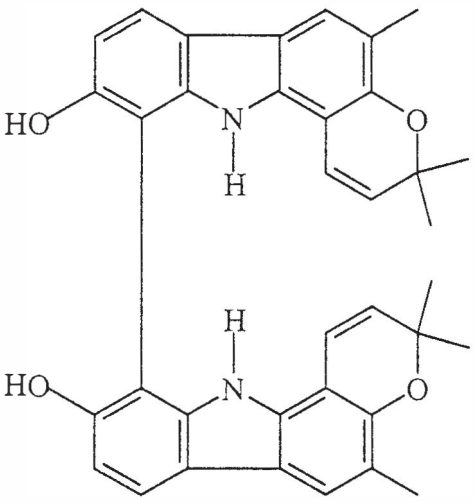
CHAPTER II

LITERATURE REVIEW

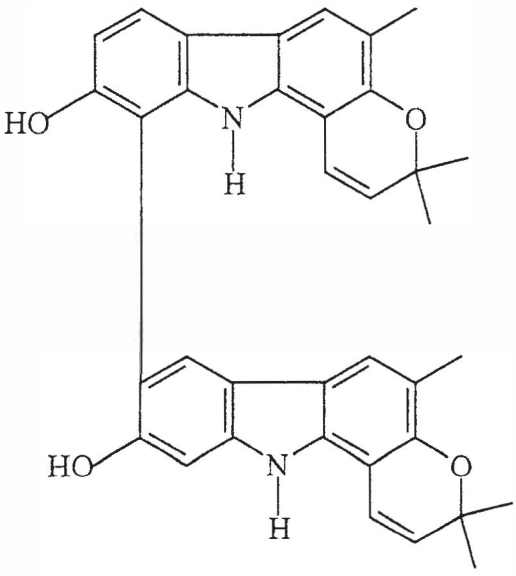
Previous Works on *Murraya* Species

Many constituents have been reported from the extraction of various part of *Murraya* species using different type of solvents such as petroleum ether, chloroform, methanol and others. Several coumarins (Gray, 1978; Wu *et al.* 1980; and Wu, 1981), carbazole alkaloids (Ganguly *et al.* 1978) and flavanoids (Dreyer, 1968) have been isolated. *Murraya koenigii* has also been proven to be a rich source of carbazole alkaloids.

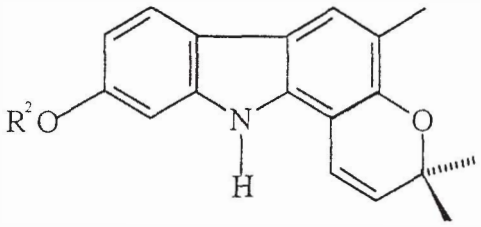
Previous work by Furukawa *et al.* (1985) reported the isolation of several novel alkaloids including simple carbazole alkaloids, dimeric carbazole alkaloids and carbazole quinone from the roots and stem bark of *Murraya euchrestifolia*. Futher study on the leaves constituents of the same plant by Wu *et al.* (1991) has resulted in the isolation of two carbazole alkaloids, bis-7-hydroxygirinimbine-A (1) and -B (2). In the same year Wu also isolated a few other carbazole alkaloids, including murrayamine-A (3), -B (4), and -C (5). Murrayamine-A showed significant cytotoxicity in the KB tissue culture assay at 3.0 $\mu g\ ml^{-1}$.



(1)



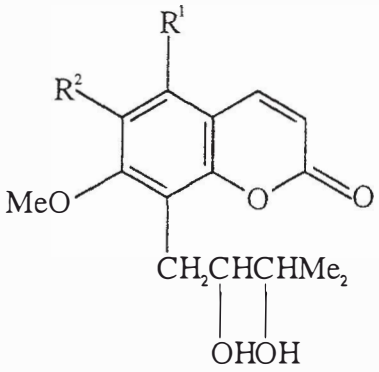
(2)



R^2

(3) : H
(4) : Me
(5) : OAc

In 1984, Mahinda *et al.* reported the isolation and identification of coumarins, murraglenin (6), mexoticine (7), sibrin (8) and pheabalosin (9) from the petrol extract of *Murraya glenei* leaves, together with known compound such as meranzine hydrate (10).



	R^1	R^2
(6)	OMe	OMe
(7)	OMe	H
(10)	H	H