



UNIVERSITI PUTRA MALAYSIA

**CHEMICAL CONSTITUENT OF *MURRAYA PANICULATA*
(RUTACEAE) AND THEIR BIOLOGICAL ACTIVITIES**

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By

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LIST OF ABBREVIATIONS

br	broad
CC	column chromatography
CDCl ₃	deuterated chloroform
CHCl ₃	chloroform
d	doublet
DMSO	dimethylsulphoside
EtOAc	ethyl acetate
ml	millilitre
m.p	melting point
MeOH	methanol
MS	Mass Spectrum
m	multiplet
NMR	Nuclear Magnetic Resonance
PE	petroleum ether
PLC	Preparative Thin Layer Chromatography
q	quartet
IR	Infrared
s	singlet
TLC	Thin Layer Chromatography
t	triplet
UV	Ultraviolet
ppm	parts per million



Abstract of the thesis presented to the Senate of Universiti Putra Malaysia
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**CHEMICAL CONSTITUENTS OF *MURRAYA PANICULATA*
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Faculty : Science and Environmental Studies

An investigation on the plant of *Murraya paniculata*, employing extraction of the leaves and stem barks and various chromatographic isolation techniques have been used. The structure of the compounds were elucidated by using spectroscopic techniques such as IR, NMR, MS and also by comparison with the previous work. Extraction and isolation work of the leaves and stem barks of *Murraya paniculata* collected from Lenggong, Perak yielded gardenin E (28), 3',4',5,5',7-pentamethoxyflavone (29), 3',4',5,5',7,8-hexamethoxyflavone (30), gardenin A (31), sterol mixtures (32), 3',4',5,5',7-pentamethoxyflavanone (33) and gardenin C (34).



Further study on the leaves of the same plant collected from different location in Ipoh, Perak afforded four known coumarins ; auraptene (36), gleinadiene (37), 5,7-dimethoxy-8-(3-methyl-2-oxo-butyl)coumarin (38) and toddalenone (39).

The antibacterial and antifungi activities test were also carried out on crude PE, CHCl₃ and MeOH extracts of the leaves and stem barks and also towards pure compounds. This antibacterial activity was tested using the ‘ disk Diffusion’ method. One gram positive bacteria (*Bacillus cereus*) and four fungi (*Aspergillus ochraceous*, *Saccharomyces lipolytica*, *Saccharomyces cerevisiae* dan *Candida lipolytica*) were selected for this test. Chloroform extract of *Murraya paniculata* gave the highest average zone of inhibition, indicating the degree of its sensitivity. Meanwhile compound (37) showed moderate degree of toxicity towards the bacteria.



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**KANDUNGAN KIMIA DARI *MURRAYA PANICULATA* (RUTACEAE)
DAN AKTIVITI BIOLOGINYA**

Oleh

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Kajian terhadap pokok *Murraya paniculata* melibatkan pengekstrakan daun dan kulit batang dan pelbagai teknik kromatografi telah dijalankan. Struktur sebatian telah ditentukan dengan menggunakan teknik spektroskopi seperti infra merah, resonans magnet nukleus, spektroskopi jisim dan juga perbandingan dengan kajian lepas.

Kajian pengekstrakan dan pemencilan terhadap daun dan kulit batang pokok *Murraya paniculata* dari Lenggong, Perak menghasilkan gardenin E (28), 3',4',5,5',7-pentametoksiflavon (29), 3',4',5,5',7,8-heksametoksiflavon (30),



gardenin A (31), campuran sterol (32), 3',4',5,5',7-pentametoksiflavanon (33) dan gardenin C (34).

Kajian terhadap daun tumbuhan sama yang diambil dari Ipoh. Perak menghasilkan empat kumarin yang sudah dikenalpasti; aurapten (36), gleinadiena (37), 5,7-dimetoksi-8-(3-metil-2-okso-butil)kumarin (38) dan toddalenone (39).

Aktiviti antibakteria dan antifungi telah dijalankan ke atas ekstrak mentah petroleum eter, kloroform dan metanol (daun dan kulit batang) dan juga terhadap sebatian-sebatian tulin. Aktiviti ini dilakukan dengan menggunakan kaedah “Resapan Agar”. Satu bakteria gram positif (*Bacillus cereus*) dan empat fungi (*Aspergillus ochraceous*, *Saccharomyces lipolytica*, *Saccharomyces cerevisiae* dan *Candida lipolytica*) telah dipilih dalam ujian ini.

Ekstrak kloroform dari daun *Murraya paniculata* memberikan zon perencatan pertumbuhan yang tertinggi keamatannya. Manakala sebatian tulin (37) menunjukkan kesan ketoksikan yang sederhana terhadap bakteria yang diuji.

CHAPTER I

INTRODUCTION

Herbalism And Medicine

There are various plants that can cause problems to humans, such as skin rash and other discomforts. People usually view these as minor allergies that can be cured easily. Some of the skin rashes and discomforts however, have been known to cause grave illness and even death. Nevertheless, through the years with or without careful study and understanding, many of these types of plants have been brought into homes, gardens and public park. Logically, these moves can endanger the surrounding society but the number of these plants in our surrounding are increasing.

Some of the poisonous plants are however, very useful in making valuable medicines. Nearly all cultures from ancient times until the present day have been using plants as a source for medicine. A good example would be the common purple foxglove (*Digitalis purpurea*) grown in gardens : digitalis present in the leaves and seeds strengthens the heartbeat of an ill person (James, 1973). However, without a proper doctor's prescription on the proper dosage, the result of such treatment can be fatal. We can see now that like a common drug, the extracts from plants must be used in the proper way. We can also see that the plants can be dangerous in two ways:



1. giving illness, if you are in close vicinity or are in contact with it
2. fatal if the extracts from it are not used properly.

Nevertheless, these plants are valuable for their contents of powerful chemical ingredients that can be used to cure people. Therefore, we should understand this advantages and used it properly to help rather than create more problems and danger for us. Everyone can benefit from the use of herbal medicine, but unlike our grandmothers who have long years experience in understanding the correct mixtures of the herbs, which we do not. Therefore it would be greatly advisable to allow herbalist with their training and experience to do the mixings and prescribe the dosage.

Family Rutaceae

This is a large family of trees, shrubs and climbers recognized most easily from the resinous, aromatic or lime-like smell of the broken twigs or fruits or of the crushed leaves. The oil-glands in the leaves appear either as dark green spots or pimples on the under surface or as translucent spots when the leaf is held to the light. Many essential oils, such as citronella and bergamot, are obtain by distillation from plants of this family : and for this same oils, many species are used in native medicine.

The family is represented by about 161 genera and 1700 species, distributed throughtout the world and found mostly in warm countries (Mabberly,



1987). There are only 16 genera and 50 species occurred in Malaysia. mostly found in lowland areas. The lime-tree, pomelo (*Citrus*), kemuning and the curry bush (*Murraya*) provide the best introduction to the family. The Mediterranean Rue (*Ruta graveolens*) with ashen grey, pinnate leaves is occasionally grown as a potted.

Genus *Murraya*

Genus *Murraya* is from Angiosperma's division, Dicotilidon's Class, Sepindales's Order, Rutaceae's (Lemon) family and Aurantiodea's subfamili. *Murraya* is one the 161 genera from family Rutaceae in the world (Heywood, 1979). Genus *Murraya* was named after John Andrew Murray (1740-1791), a Sweedish botanist and a Professor of Medicine and Botany in the University of Gottingen. According to Huxley (1992), among the seven species from this genus, only *Murraya paniculata* has aromatic flowers. *M. paniculata* and *M. koenigii* are the only two species that have been detected to occur in Malaysia.

M.paniculata (Lynn) Jack is known as kemuning in Malaysia. It also given common names such as orange jasmine, orange jessamine, satinwood, cosmetic-bark tree and also known as Chinese box in America and Canada (Bailey, 1978). Other local names such as Cha Prik (Laos), Manchulati (Bombay), Machalla (Andamans), Kariveppilai (Tamil), Buis de Chine (French), Bisbar (Hindi), Shan Fan (Chinese) and others (Kirtikar and Basu, 1962).

M. paniculata is a shrub or small tree, (Plate 1) with smooth bark, yellowish white with slender branches. The leaves (Plate 2) are 3"-6" long, leaflets 2"-2.5" x 1"-1.5" rather leathery, dark shiny green, the terminal one is the largest and upstanding with upcurled sides.

The flowers (Plate 2) are white with five petals, very fragrant, campanulate, solitary or in terminal and axillary corymbs. The roots of the kemuning provides the pale yellow wood so much prized for making kris-handles and sheaths.

The wood of the trunk is hard and dense like box (Buxus). The flowers are sometimes found among those offered for decorating the hair in native shops. In appearance the kemuning looks like a Citrus. It is evergreen and apparently night flowering.



Plate 1 : *M. paniculata*



Plate 2: The leaves and flowers of *M. paniculata*

Some Uses of Genus *Murraya*

Among the Mundas, the ground bark of *M. paniculata* is used as a drink and as antidote in snake bites and rubbed on the bitten limb. The ground bark of the root is eaten and rubbed on body to cure bodyache. The powdered leaves is used as an application to fresh cuts, and decoction of the leaves is drunk in dropsy.

In Philipines, the leaves which are stimulant and astringent were used in the treatment of diarrhoea and dysentery. While the bark of both the stem and the root is also used as an antidiarrhoeal (Kirtikar and Basu, 1962).

M. koenigii or popular known as curry leaf (Rutaceae) is an important leafy vegetable and the leaves are widely used in Indian cooking for flavouring foodstuffs. Volatile oil or curry leaf oil, produced from the plants has been used widely in the soap industry (Joseph and Peter, 1985). Curry leaf is also used in many Indian ayurvedic and unani medical prescriptions. (Kirtikar et al, 1935).

Screening of Bioactive Compounds

One of the most successful strategies for investigation of medicinal agents from higher plants includes the pharmacological screening of plant extracts followed by a bioassay-guided fractionation of active extracts and leading to the isolation of the pure constituents (Vlietinck and Vanden, 1991).

Unfortunately, the goal of many today's phytochemists is simply to isolate, characterize and publish a plethora of natural substances without regard to bioactivities. To achieve applied meaning and significance, any work in natural product chemistry must incorporate bioassays (McLaughlin, 1990).

The tropical forest of Malaysia is blessed with more than 10,000 species of medicinal plants. (Burkill, 1996). Because plants contain of too many compounds, extract must be screen for biological activity. In 1992, Rahmani et al. have carried out screening procedure for the presence of bioactive compounds from plant sources and a number of plant species have been identified for further detail studies.

Three readily available techniques may be combined for the natural product chemists to follow to obtain bioactive compounds.

1. Separation techniques (chromatography)
2. Elucidation methods (spectroscopy)
3. Simple bioassays

Three simple effective bioassays may be useful to the researchers. They are brine shrimp lethality, crown gall tumor on potato disc and Lemna minor (duckweed).

Objectives

The objectives of this study are:-

- a. To isolate and purify the chemical constituents of the leaves and stem barks of *M. paniculata*.
- b. To elucidate the structure of the isolated compounds using spectroscopic methods.
- c. To determine the antimicrobial activity of plant *M. paniculata* .
- d. To identify the compounds which contribute to the antimicrobial activity.