UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS OF *MICROMELUM MINUTUM* AND *ACRONYCHIA LAURIFOLIA* (RUTACEAE)

YAP YUN HIN @ MD. TAUFIQ

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CHEMICAL CONSTITUENTS OF *MICROMELUM MINUTUM* AND *ACRONYCHIA LAURIFOLIA* (RUTACEAE)

By

YAP YUN HIN @ MD. TAUFIQ

Thesis Presented in Fulfilment of the
Requirements for the Degree of Master of Science
in the Faculty of Science and Enviromental Studies,
Universiti Pertanian Malaysia

1994

DEDICATIONS

To my beloved father and mother,

THE LATE YAP YOON SANG
ARWAH ABDULLAH LIM HENG FUI
SITI AISHAH CHIN SU KIAW

To my brothers and sister,

LIM YIT HIANG
NORAINI LIM FUNG KUN
ABDUL HALIM LIM YIT VUI

To my dearest friends

SAIDAH BTE MAMAT
NORIZAN BIN REMBUN

and the members of Nahwan Nur

WITHOUT WHOSE LOVE AND
CONTINUED SUPPORT THIS THESIS
WOULD NOT HAVE BEEN POSSIBLE.

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br.	broad
$CHCl_3$	chloroform
$CDCl_3$	deuterated chloroform
d	doublet
dt	doublet of triplet
dq	double of quartet
g	gram
IR	Infra Red
l	litre
LC	Lethal Concentration
m	medium
ml	mili litre
mg	mili gram
Me	methyl
MeOH	methanol
m.p.	melting point
MS	Mass Spectrum
NMR	Nucleur Magnetic Resonance
PE	petroleum ether
PLC	Preparative Thin Layer Chromatography
ppm	part per million
q	quartet
s	strong
TLC	Thin Layer Chromatography
UV	Ultra Violet
w	weak

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

CHEMICAL CONSTITUENTS OF *MICROMELUM MINUTUM* AND *ACRONYCHIA LAURIFOLIA* (RUTACEAE)

By

YAP YUN HIN @ MD. TAUFIQ

April 1994

Chairman : Associate Professor Dr. Mawardi Rahmani
Faculty : Science and Enviromental Studies

A chemotaxonomic investigation on the leaves and stems of *Micromelum minutum* (Rutaceae), has resulted in the isolation of six components. The structure of these compounds was elucidated by means of spectroscopic methods including by the extensive used of various NMR techniques and also comparison with previous studies. The use of High Field NMR is essential in structural determination of these complex molecules. With the aids of various NMR experimental techniques and other spectroscopic methods such as MS, UV and IR, the correct structures of the pure isolated compounds were established.

M. minutum collected from Kelantan yielded two novel coumarins, microminutinin and 6-methoxymicrominutinin, and a known coumarin,

scopoletin. From another separate collection of the plant from Pahang, a novel compound, MM5, squalene, and two known coumarin, micromelin and scopoletin have also been isolated. A similar study on the leaves and stems of *Acronychia laurifolia* collected from Pulau Singa Besar, Langkawi Island afforded three compounds, sitosterol, seselin and an unidentified compound.

The presence of bioactive compounds in these plants was detected by the use of brine shrimp (*Artemia salina*). With the aid of this bioassay system the activity of either the crude plant extracts or pure isolated compounds could be determined. The LC_{50} value of $CHCl_3$ and MeOH extracts of *M. minutum* (Pahang) gave no significant activity while $CHCl_3$ extracts of leaves and stems of *A. laurifolia* had LC_{50} of 2.4 and 22.6 ppm respectively. Microminutinin gave strong test result with LC_{50} of 34.7 ppm and MM5 with LC_{50} of 165.5 ppm.

A more specific bioassay was also carried out on the crude and pure compounds for development inhibition activity by using fruit fly, *Drosophila melanogaster* larvae. The MeOH extract of *M. minutum* (Kelantan), at concentration of 200 mg/2 g diet, completely retarded the larval growth. For the pure compounds, microminutinin has been shown to have a strong development inhibition activity. At only 4 mg/2 g diet, it caused 100% mortality against first/second instar larvae of the insect, whereas no activities was observed from MM5.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia bagi memenuhi keperluan Ijazah Master Sains.

KANDUNGAN KIMIA DARI *MICROMELUM MINUTUM* DAN *ACRONYCHIA LAURIFOLIA* (RUTACEAE)

Oleh

YAP YUN HIN @ MD. TAUFIQ

April 1994

Pengerusi : Professor Madya Dr. Mawardi Rahmani
Fakulti : Sains dan Pengajian Alam Sekitar

Kajian kimotaksonomi ke atas ekstrak mentah daun dan ranting *Micromelum minutum* (Rutaceae) telah memberikan enam komponen. Struktur sebatian-sebatian ini telah dapat dikenal pastikan dengan menggunakan kaedah spektroskopi termasuk penggunaan yang meluas pelbagai teknik NMR dan juga perbandingannya dengan kajian-kajian yang lepas. Penggunaan NMR berkuasa tinggi adalah amat diperlukan untuk menentukan struktur yang rumit. Dengan bantuan berbagai teknik ini dan juga kaedah spektroskopi yang lain seperti MS, UV dan IR, struktur yang betul bagi sebatian tulen yang dipencilkan dapat ditentukan.

M. minutum yang dikumpulkan dari Kelantan telah menghasilkan dua koumarin baru, mikrominutinin dan 6-metoksimikrominutinin dan satu

koumarin yang telah dikenali, skopoletin. Dari pengumpulan berasingan (Pahang) pokok yang sama telah memberikan satu sebatian baru, MM5, skualena dan dua koumarin yang telah dikenali, mikromelin dan skopoletin. Kajian yang serupa ke atas daun dan ranting *Acronychia laurifolia* yang dikumpulkan dari Pulau Singa Besar, Pulau Langkawi telah menghasilkan tiga sebatian, sitosterol, seselin dan satu sebatian yang belum dikenalpasti.

Kehadiran sebatian bioaktif dalam tumbuhan-tumbuhan ini dapat dikesan dengan menggunakan udang pepai (*Artemia salina*). Dengan bantuan sistem biocerakinan ini, aktiviti sama ada dari ekstrak mentah tumbuhan atau sebatian tulen dapat ditentukan. Nilai LC_{50} dari ekstrak $CHCl_3$ dan MeOH dari *M. minutum* (Pahang) tidak memberikan keputusan yang bermakna sementara ekstrak $CHCl_3$ bagi daun dan ranting *A. laurifolia* masing-masing memberikan LC_{50} 2.4 dan 22.6 ppm. Mikrominutinin memberikan keputusan yang kuat dengan LC_{50} 34.7 ppm dan MM5 dengan LC_{50} 165.5 ppm.

Kaedah biocerakinan yang lebih spesifik juga telah dijalankan ke atas ekstrak mentah dan sebatian tulen bagi aktiviti penghalang perkembangan dengan menggunakan larva *Drosophilamelanogaster*. Ekstrak MeOH bagi *M. minutum* (Kelantan) pada kepekatan 200 mg/2 g diet telah sepenuhnya menghalang perkembangan larva ini. Bagi sebatian tulen pula, mikrominutinin telah menunjukkan aktiviti penghalang perkembangan. Pada kepekatan hanya 4 mg/2 g diet, mikrominutinin telah menyebabkan 100% kematian terhadap larva

peringkat pertama dan kedua bagi serangga ini, sementara itu tiada aktiviti yang dikesan bagi MM5.

PART 1

EXTRACTION AND ISOLATION OF CHEMICAL COMPONENTS FROM *MICROMELUM MINUTUM* AND *ACRONYCHIA LAURIFOLIA* (RUTACEAE)

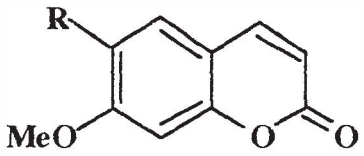
INTRODUCTION AND LITERATURE REVIEW OF *MICROMELUM MINUTUM* (FROST. f.) SEEM AND *ACRONYCHIA LAURIFOLIA* BLUME

Ten species of *Micromelum* have been found throughout South-Eastern Asia and the Pacific of which only two species occur in Peninsular Malaysia, *M. minutum* and *M. hirsutum*. *M. minutum* (synonymous with *M. pubescens* Blume) is a shrub or small tree of the family Rutaceae, which can grow up to 25 feet high. In Peninsular Malaysia, *M. minutum* which is locally known as "Chemama", "Cherek-cherek" or "Secherek" normally occurs in the hilly parts of the northern half and in the north-west area.

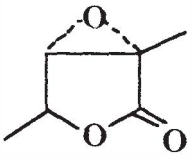
The plants are used medicinally. The leaves are normally pounded with tamarinds and salt and applied to the skin to relieve pain. In Peninsular Malaysia, it is traditionally used in the treatment of fever and giddiness and a poultice of the boiled roots is applied to treat ague (Burkill, 1966). The plant is also found widely throughout the Fiji Islands, where it is termed "Qiqila" and is also used by the Fijians in traditional folk medicine (Craft and Toia, 1989).

Previous phytochemical studies of *Micromelum* species revealed a number of prenylated coumarins, a pyranoquinoline alkaloid and polyoxygenated flavanoids. In 1966, Lamberton *et al.* isolated a neutral constituent, micromelin (**1**), $C_{15}H_{12}O_6$, m.p. 218-219.5°C from the MeOH extract of *M. minutum*. Another coumarin, osthol (**2**) , m.p. 82-83.5°C was

also found in the methylene chloride extract. Later Chatterjee *et al.* (1967) reported the isolation of coumarins, micropubescin, $C_{15}H_{14}O_4$ (**3**), m.p. 130-131°C and (**1**) from the stem bark of *M. pubescens*. In 1974, Joshi *et al.* isolated a novel coumarin, 6-(2,3-dihydroxy-3-methylbutyl)-7-methoxycoumarin or ulopterol (**4**), $C_{15}H_{18}O_5$, m.p. 139-140°C from *M. pubescens* Blume.



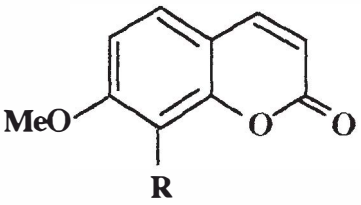
(**1**) R =



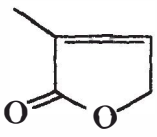
(**4**) R = $-CH_2CH(OH)C(OH)(CH_3)_2$

(**2**) R = $-CH_2CH{=}C(CH_3)_2$

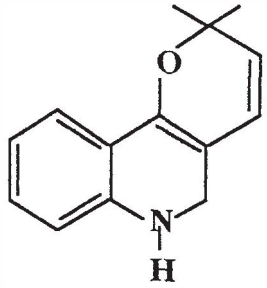
(**3**) R = $-\overset{\overset{O}{\|}}{C}-CH_2C(CH_3){=}CH_2$



(**5**) R =

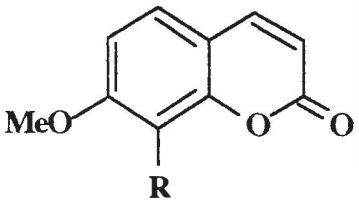


An investigation of *M. minutum* by Tantivatana *et al.* (1982) yielded a cytotoxic compound microminutin (**5**), and a pyranoquinoline alkaloid, flindersine (**6**). Microminutin, m.p. 154-155°C was found to be inactive in KB cytotoxicity test but was reported to show weak activity (ED_{50} 3.7 μg/mL) against the P-388 lymphocytic leukemia test system *in vitro*.



(**6**)

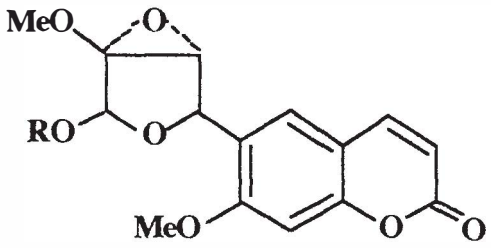
Further study on *M. minutum* collected in Diphu area of the Karbi Anglong District, Assam, India by Das *et al.* (1984) afforded four known coumarins, (**1**), (**2**), murralongin (**7**) and murrangatin (**8**); and four new coumarins, dihydromicromelin A and B, (**9a** and **9b**), acetyl dihydromicromelin A (**9c**), minumicrolin/mupanidin (**10**) and a new flavone, 7,12-ether of 5,7-dihydroxy-3,6,8,4'-tetramethoxyflavone (**11**).



(**7**) R = -C(CHO)=C(CH$_3$)$_2$

(**8**) R = -C(H)(OH)-C(H)(OH)-C(CH$_3$)=CH$_2$

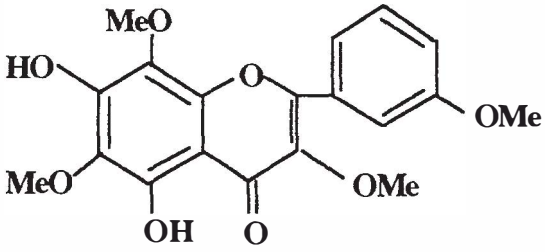
(**10**) R = -C(H)(OH)-C(OH)(H)-C(CH$_3$)=CH$_2$



(**9a**) R = α-OH

(**9b**) R = β-OH

(**9c**) R = α-OAc

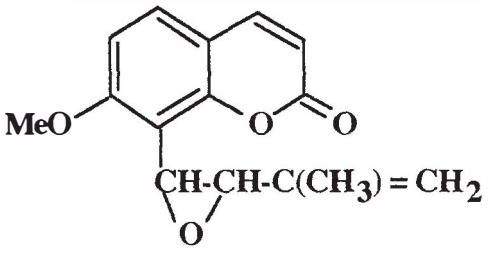


(**11**)

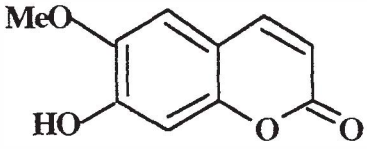
Tantishaiyakul *etal.* (1986) undertook the examination of the stem bark extract of *M. minutum* from which they reported the isolation of phebalosin (**12**), m.p. 120.5-121.5ºC. Phebalosin (**14**) was reported to be significantly toxic to brine shrimp (LC_{50} 47 ppm,) and to significantly inhibit the development of crown gall tumors on potato disc but had insignificant cytotoxic activity.

From the stem-leaf of *M. integerrimum*, Cassady *et al.* (1979) discovered the occurrence of two known coumarins, (**1**) and scopoletin (**13**), and both were demonstrated to have antitumor activities. 7-[4'-(4"-methyl-5"-

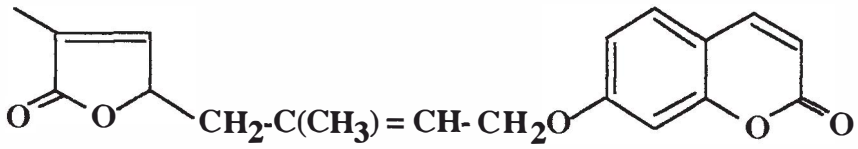
oxo-2",5"-dihydro-2"-furyl)-3'-methyl-2'-butenyloxy]coumarin (**14**), has been isolated by De Silva *et al.* (1980) from the PE extract of the leaves of *M. zeylanicum* Swingle.



(**12**)

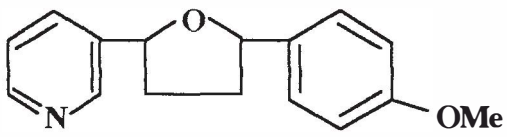


(**13**)

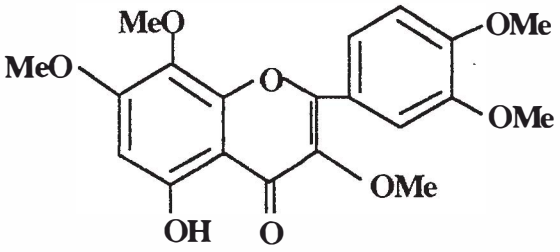


(**14**)

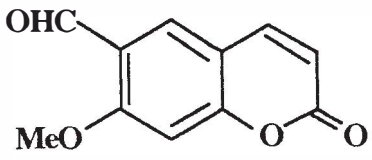
In another investigation by Bowen and Perera (1981), the leaves and stems of *M. zeylanicum* have yielded a new oxazole alkaloid, *o*-methylhalfordinol (**15**), coumarin (**1**) and 5-hydroxy-3,3',4',7,8-pentamethoxyflavone (**16**). In addition, the stems also contained 6-formyl-7-methoxycoumarin, or crenulatin (**17**) and the leaves contained the carbazole alkaloid koenigine (**18**) and β-sitosterol.



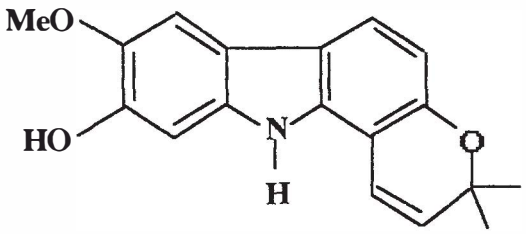
(**15**)



(16)



(17)



(18)

Another investigation by Kong *et al.* (1988) on the roots of *M. falcatum* has revealed the occurrence of the indole alkaloid, yuehchukene (**19**), the novel carbazole alkaloid, 5,6-pyranoglycozoline (**20**), as well as (**1**) and (**10**). The various chemical components present in *Micromelum sp.* are listed in Table 1.