UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS OF *GLYCOSMIS CRASSIFOLIA* AND *G. CHLOROSPERMA* (RUTACEAE) AND THEIR BIOLOGICAL ACTIVITIES

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By

MOHD AZLAN BIN NAFIAH

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Science in the Faculty and Environmental Studies
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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia
In fulfilment of requirement for the degree of Master of Science

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Chairman : Professor Dr. Mawardi bin Rahmani, Ph.D.

Faculty : Science and Environmental Studies

The phytochemical study on *Glycosmis crassifolia* (limau hutan) and *G. chlorosperma* (Rutaceae) involves extraction and separation by using various chromatographic methods, structural determination by spectroscopic techniques such as IR, NMR including 2D-NMR and MS. The structure of the compounds were elucidated by comparison with the previous works. Isolation work on roots of *G. crassifolia* yielded maculocidine (44). From the leaves, two more compounds were isolated and the structure elucidated. One of them was identified as a new alkaloid, glycofuranoxine (45) and the other one was stigmata-3,5,22-

trien-3-ol (46). Further study on the twigs of the same plant afforded two known compounds including ergosta-4,6-dien-3-ol (47) and stigmata-5-en-3-ol (48).

Detail study on the leaves extract of *Glycosmis chlorosperma* yielded two compounds. One of them was identified as a new compound, 5-hydroxy-7,4'-dimethoxy-8-(-3"-methylbut-2"-enyl)flavanone (49) and the other one was known as dambullin (50).

Crude extract from various plant were screened for antifungal activity using poison food method against *Sclerotium rolfsii*. However, the petroleum-ether, chloroform and methanol extract of the roots, leaves and twigs of the plant failed to show any activity in inhibition of mycelial growth and sclerotial germination.

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

KANDUNGAN KIMIA DARI *GLYCOSMIS CRASSIFOLIA* DAN *G. CHLOROSPERMA* (RUTACEAE) DAN AKTIVITI BIOLOGINYA

Oleh

MOHD AZLAN BIN NAFIAH

September 2001

Pengerusi : Profesor Dr. Mawardi bin Rahmani, Ph.D.

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Kajian terhadap pokok *Glycosmis crassifolia* (limau hutan) dan *G. chlorosperma* (Rutaceae) melibatkan pengekstrakan dan pengasingan menggunakaan pelbagai kaedah kromatografi dan pengenalpastian struktur menggunakan 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 ke atas akar pokok *Glycosmis crassifolia* ini telah menghasilkan makulocidin (44). Dari daun, dua sebatian telah dipencilkan dan diasingkan. Satu daripadanya ialah alkaloid, glikofuranoxin (45), satu sebatian

baru manakala yang satu lagi ialah stigmata-3,5,22-trien-3-ol (46). Kajian seterusnya dilakukan ke atas ranting pokok yang sama memberikan dua sebatian ergosta-4,6-dien-3-ol (47) dan stigmata-5-en-3-ol (48).

Kajian ke atas daun *G. chlorosperma* telah memberikan dua sebatian. Salah satu darinya adalah sebatian baru, 5-hidroksi-7,4'-dimetoksi-8-(-3"-metilbut-2"-enyl)flavanon (49) dan yang satu lagi adalah dambullin (50).

Ekstrak mentah yang telah diperolehi dari pelbagai bahagian pokok tersebut telah diuji untuk aktiviti antikulat menggunakan kaedah keracunan makanan ke atas kulat *Sclerotium rolfsii.* Walau bagaimana pun, ekstrak petroleum-eter, klorofom dan metanol gagal menunjukkan sebarang aktiviti terhadap kulat tersebut dalam perkembangan miselium dan pertumbuhan sclerotia.

ACKNOWLEDGEMENTS

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Grateful appreciation is also extended to my beloved wife (Kartini bt. Ahmad) my parents, brothers and sisters who were responsible in completing my study in Universiti Putra Malaysia.

I certify that an Examination Committee met on 29th October 2001 to conduct the final examination of Mohd Azlan bin Nafiah on his Master Science thesis entitled "Chemical Constituents of *Glycosmis crassifolia* and *G. chlorosperma* (Rutaceae) and Their Biological Activities" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Member of the Examination Committee are as follows:

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Date: 14 DEC 2001

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master Science.

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly, acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

MOHD AZLAN BIN NAFIAH

Date: 12/12/2001

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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

CHAPTER I

INTRODUCTION

Family Rutaceae

Rutaceae is a large family of woody shrubs or (small) trees. Member of the family are aromatic with opposite leaves containing oil glands. Surface features include stellate hairs and peltate scales. The leaves vary from simple to compound. Each flower has five sepals and petals, ten to twenty stamens, and a single pistil with a superior and lobed ovary. Member of this family is commonly from in tropical areas with species occurring in South Africa, Australia and South-East Asia. The family is represented by about 161 genera and 1700 species, distributed throughout the world and found mostly in warm country.

Glycosmis crassifolia and *G. chlorosperma* and the Uses

Glycosmis is a genus of forty species of small trees and shrubs from the family Rutaceae, found mostly in South-East Asia and South of China. Fourteen species are reported to occur in Peninsular Malaysia (Whitmore, 1983).

Other glycosmis species are *G. elmeri* Merr., *G. gracilis* Tanaka, *G. greenei* Elmer, *G. macrantha* Merr., *G. pentaphylla* (Retz.) and *G. triphylla* Wight. In Malaysia, *Glycosmis* species can be found in Langkawi, Perak, Selangor and Kelantan, which normally grow on limestone rocks and limestone hills. Members of the genus are aromatic and used medicinally, chiefly the roots (Burkill, 1966).

Glycosmis pentaphylla is used for the treatment of fever in India, while *Glycosmis cochinchinensis* found in the Dutch Indies is used to treat swollen spleen. A decoction of the leaves and roots of the latter, is also used as a stimulant for digestion. Previous works on the genus revealed the occurrence of a variety of compounds such alkaloids, coumarins, flavonoids, sulphones, terpenes etc. The present work deals with the isolation and characterization of the constituents from the petroleum ether and methanol extracts of the leaves and roots of *G. crassifolia* and *G. chlorosperma*.

Screening of Bioactive Compounds

The roots of Rutaceae in the subfamily Aurantioidea contain several classes of natural products and exhibit insecticidal or other biological activity. Among them are the coumarins, alkaloids, amides, flavonoids, limonoids, and terpenoids (Shapiro, 1991).

Species in the genus *Glycosmis* (Stone, 1985) contain a wide variety of compounds with potential biological activity. These include terpenoids (Chakravarty *et al.*, 1996), amides (Greger *et al.*, 1994, 1996; Hofer *et al.*, 1995, 1998), alkaloids (Wu *et al.*, 1983; Wurz *et al.*, 1993; Ono *et al.*, 1995), coumarins (Rahmani *et al.*, 1998), and flavonoids (Tian-Shung *et al.*, 1995). Compounds exhibiting antifungal and insecticidal activities (Greger *et al.*, 1996) have already been isolated from several *Glycosmis* species. Recently, a screening of foliage against the citrus leafminer, *Phyllocnistis citrell*a, demonstrated activity in both *Murraya koenigii* and *Glycosmis pentaphylla* (Jacas *et al.*, 1997). *Glycosmis pentaphylla* also exhibited antijuvenile hormone activity against the field cricket *Gryllus bimaculatus*, and activity was traced to the quinazolone alkaloid arborine (Muthukrishnan *et al.*, 1999).

OBJECTIVES OF STUDY

The objectives of this study are:

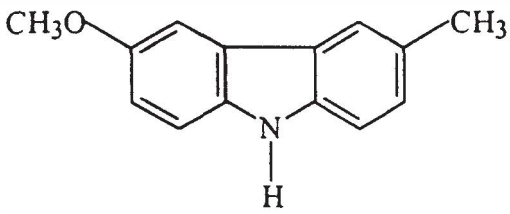
1. To extract and isolate compounds from *Glycosmis crassifolia* and *Glycosmis chlorosperma*.
2. To elucidate the structure of the compounds using modern spectroscopic methods.
3. To conduct bioassays on the crude extracts using poison food method.

CHAPTER II

LITERATURE REVIEW

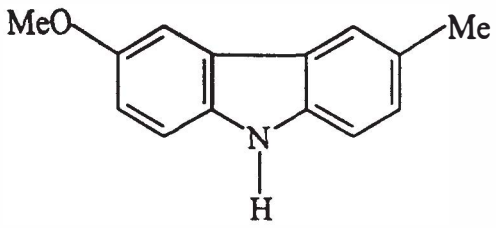
Previous Works on *Glycosmis* Species

One of the plants from the genus that had been exhaustively investigated was *G. pentaphylla* and it furnishes a number of acridone, carbazole and quinolone bases. In 1969, Chakraborty *et. al* isolated a known compound, glycozoline (1), $C_{14}H_{13}NO$, m.p. 181-182 °C.



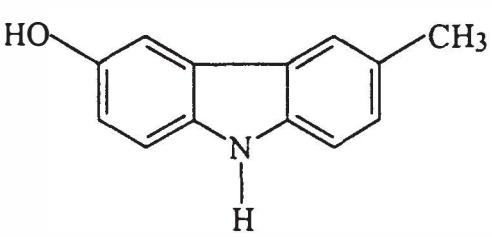
(1)

Mukherjee *et. al* (1983) undertook the examination of the seeds of the plant in which they reported the isolation and elucidation structure of glycozolinine (2) $C_{13}H_{11}NO$, m.p. 231 - 232 °C. From physical and chemical evidence, its structure was identified as 6-methoxy-3-methylcarbazole.



(2)

From the leaves of the same species, Bhattacharyya *et al.* (1984) discovered glycozolinol (3), $C_{13}H_{11}NO$, m.p. 230 °C. Another investigation by Bhattacharyya *et al.* (1985) on the roots of *G. pentaphylla*, a reputed Indian medicinal plant has revealed the presence of carbazole alkaloid, glycozolidal (4), $C_{15}H_{13}N_3$, m.p. 185 °C.



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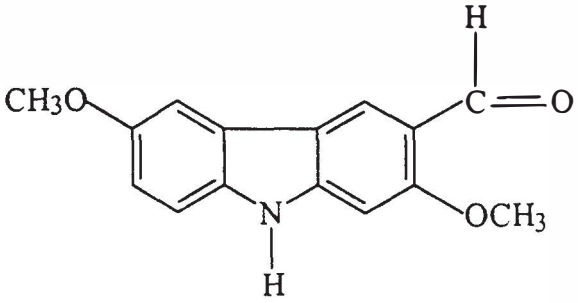
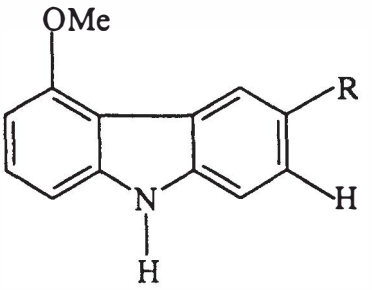


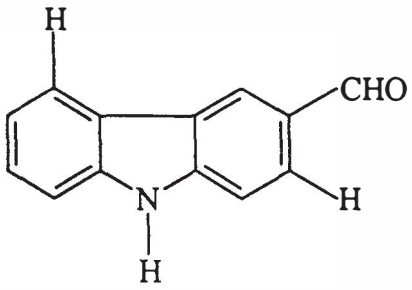
(4)

In an investigation of root bark of *G. pentaphylla*, Jash *et al.* (1992) isolated three carbazole alkaloids, glycozolicine (5), $C_{14}H_{13}NO$, m.p. 135 °C, glycosinine (6), $C_{14}H_{11}NO_2$, m.p. 185 °C and 3-formyl carbazole (7), $C_{13}H_9NO$, m.p. 158 °C.



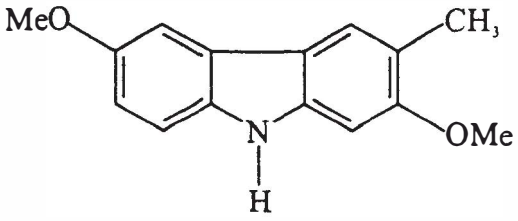
(5) R = Me

(6) R = CHO



(7)

In 1974, Chakraborty *et al.* reported the structural studies of carbazole alkaloids also from the root bark of *G. pentaphylla*, glycozolidine (8), m.p. 160 - 162 °C.



(8)