UNIVERSITI PUTRA MALAYSIA

BIOACTIVE COMPOUNDS FROM *PLOIARIUM ALTERNIFOLIUM* (THEACEAE) AND *CALOPHYLLUM MUCIGERUM* (GUTTIFERAE)

NG KIM NEE

FSAS 2001 15

BIOACTIVE COMPOUNDS FROM *PLOIARIUM ALTERNIFOLIUM* (THEACEAE) AND *CALOPHYLLUM MUCIGERUM* (GUTTIFERAE)

By

NG KIM NEE

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Science in the Faculty of Science Universiti Putra Malaysia

July 2001

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

BIOACTIVE COMPOUNDS FROM *PLOIARIUM ALTERNIFOLIUM* (THEACEAE) AND *CALOPHYLLUM MUCIGERUM* (GUTTIFERAE)

By

NG KIM NEE

July 2001

Chairman : Associate Professor Dr. Gwendoline Ee Cheng Lian

Faculty : Science and Environmental Studies

Chemical and cytotoxic studies were carried out on two plant species, *Calophyllum mucigerum* (Guttiferae) and *Ploiarium alternifolium* (Theaceae). The chemical investigations covered anthraquinones, triterpenes, xanthone and coumarins. These compounds were isolated using common chromatographic techniques and HPLC and identified using spectroscopic methods including 2-D NMR, GCMS, MS, IR and UV.

Ploiarium alternifolium provided emodin, ploiariquinone A, 1,8-dihydroxy-3-methyl-6-methoxy-anthraquinone, 3β-benzoyloxyolean-11-en-13β,28-olide and euxanmodin C. Emodin and 1,8-dihydroxy-3-methyl-6-methoxy-anthraquinone have not been reported from *Ploairium alternifolium*. *Calophyllum mucigerum* gave the common steroidal triterpenes friedelin and stigmasterol, a prenylated xanthone cudraxanthone C and two new coumarins mucigerin I and mucigerin II.

The crude n-hexane, ethyl acetate and ethanol stem bark extracts of both plants were screened for their larvicidal activity against the larvae of *Aedes aegypti*. The crude n-hexane, ethyl acetate and ethanol extracts for both of the plants were susceptible to the larvae of *Aedes aegypti* with LC_{50} values of 95.0 µg/ml, 129.4 µg/ml and 131.6 µg/ml, respectively for *Ploiarium alternifolium* whereas 87.9 µg/ml, 138.5 µg/ml and 147.4 µg/ml, respectively for *Calophyllum mucigerum*. Larvicidal activity on the pure compound, emodin gave an LC_{50} value of 2.79 µg/ml.

The cytotoxicity, antibacterial and antifungal activities test were also carried out on the three crude extracts of both plants and also on the pure compounds. Cytotoxicities were determined by performing the microtitration assay. All the crude extracts were weakly cytotoxic towards the CEM-SS cell line except hexane extracts from *C. mucigerum* and *P. alternifolium* which gave moderate activity with IC_{50} = 16.2 µg/ml and IC_{50} = 19.2 µg/ml, respectively. The pure compound euxanmodin C was the most sensitive against the cell line with IC_{50} = 5.9 µg/ml. The antimicrobial activity was tested using the modified disc diffusion method. The crude extracts from both plants also showed different antimicrobial activity against the growth of four bacteria; *Bacillus subtilis* mutant, *Bacillus subtilis* wild type, *Staphyloccus aures* and *Pseudomonas aeruginosa*. However, these crude extracts were weakly active against the bacteria with less than 10 mm diameter inhibition zone.

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

SEBATIAN BIOAKTIF DARI *PLOIARIUM ALTERNIFOLIUM* DAN *CALOPHYLLUM MUCIGERUM*

Oleh

NG KIM NEE

Julai 2001

Pengerusi : Professor Madya Dr. Gwendoline Ee Cheng Lian

Fakulti : Sains dan Pengajian Alam Sekitar

Kajian terhadap kulit batang pokok *Ploiarium alternifolium* and *Calophyllum mucigerum* dengan menggunakan pelbagai teknik kromatografi telah menghasilkan emodin, ploiariquinone A, 1,8-dihidroksi-3-metil-6-metoksi-antrakuinon, 3β-benzoiloksiolean-11-en-13β,28-olid dan euxanmodin C daripada pokok *P. alternifolium* dan stigmasterol, friedelin, mucigerin I, mucigerin II dan cudraxanthone C daripada pokok *C. mucigerum.* Penentuan struktur-struktur sebatian ini dilakukan dengan menggunakan 2-D RMN, skektrokopi jisim, spektroskopi inframerah dan ultralembayung.

Estrak menggunakan kulit pokok mentah heksana, etil asetat dan etanol dari *P. alternifolium* dan *C. mucigerum* telah dikaji secara teliti mengenai aktiviti pembunuh larva bagi larva *Aedes aegypti.* Ekstrak mentah heksana, etil asetat dan etanol dari kulit pokok menunjukkan aktiviti membunuh larva dengan nilai LC_{50}

95.0 μg/ml, 129.4 μg/ml dan 131.6 μg/ml masing-masing untuk pokok *P. alternifolium* dan nilai LC_{50} 87.9 μg/ml, 138.5 μg/ml and 147.4 μg/ml masing-masing untuk pokok *C. mucigerum.* Sebatian tulen, emodin memberi nilai LC_{50} 2.79 μg/ml.

Aktiviti sitotoksik, antibakteria dan antifungi telah dijalankan ke atas ekstrak mentah heksana, etil asetat dan etanol kulit batang pokok *P. alternifolium* dan *C. mucigerum*. Aktiviti-aktiviti ini juga dijalankan terhadap sebatian-sebatian tulen. Aktiviti sitotoksik ditentukan melalui pengasaian mikrotiter. Semua ekstrak mentah memberikan aktiviti lemah terhadap sel CEM-SS kecuali ekstrak mentah heksana daripada *C. mucigerum* dan *P. alternifolium* yang sederhana aktif dengan nilai IC_{50} masing-masing ialah16.2 μg/ml dan 19.2 μg/ml. Sebatian tulen, euxanmodin C adalah yang paling aktif terhadap sel dengan nilai IC_{50} = 5.9 μg/ml.

Aktiviti antimikrob dijalankan dengan menggunakan pengubahsuaian kaedah resapan disk. Ekstrak mentah daripada kedua-dua pokok menunjukkan aktiviti antimikrob terhadap pertumbuhan empat jenis bakteria; *Bacillus subtilis* mutan, *Bacillus subtilis* jenis liar, *Staphyloccus aures* dan *Pseudomonas aeruginosa.* Semua ekstrak mentah menunjukkan aktiviti lemah terhadap bakteria dengan garis pusat zon perencatan pertumbuhan yang kurang daripada 10 mm.

ACKNOWLEDGEMENTS

I would like to express my sincere and deepest appreciation to my supervisor, Dr. Gwendoline Ee Cheng Lian for her intellectual advice, suggestions and guidance throughout the course of this project and for reviewing this thesis with constructive criticisms. My sincere thanks and deepest gratitude is also extended to Prof. Dr. Mawardi Rahmani for permission to work in his lab and guidance during my thesis writing. I'm also indebted to Dr. Taufiq Yap, Assoc. Prof. Dr. Radzali Muse and Assoc. Prof. A. Manaf Ali for their invaluable guidance. Financial support from IRPA programmne is also greatly appreciated.

I also wish to thank my labmates Ratna, Tian Hai and Hassan for their help and advice throughout the course of my research. I am also grateful to En. Zainal Zahari Zakaria for assistance with NMR spectra, En. Zainuddin Samadi for helping me with HPLC work and En. Shamsuddin for recording mass spectra.

My special thanks also goes to Boon Keat and Maizura from Biotechnology Department for their help on bioassay work involving cytotoxicity and antimicrobial test.

Last but not least, I am also indebted to my parents, brother and friends whose encouragement and support have contributed towards the success of this project.

I certify that an Examination Committee met on 5th July 2001, to conduct the final examination of Ng Kim Nee on her Master of Science thesis entitled "Bioactive Compounds from *Ploiarium alternifolium* (Theaceae) and *Calophyllum mucigerum* (Guttiferae)" 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. Members of the examination Committee are as follows:

MOHD ASPOLLAH HJ. SUKARI, Ph.D.
Associate Professor
Department of Chemistry
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Chairman)

GWENDOLINE EE CHENG LIAN, Ph.D.
Associate Professor
Department of Chemistry
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

MAWARDI RAHMANI, Ph.D.
Professor
Department of Chemistry
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

TAUFIQ YAP YUN HIN, Ph.D.
Lecturer
Department of Chemistry
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

RADZALI MUSE, Ph.D.
Associate Professor
Department of Biochemistry and Microbiology
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

ABDUL MANAF ALI, Ph.D.
Associate Professor
Department of Biotechnology
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

MOHD GHAZALI MOHAYIDIN, Ph.D.
Professor
Deputy Dean of Graduate School
Universiti Putra Malaysia

Date: 06 AUG 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.

AINI IDERIS, Ph.D.
Professor,
Dean of Graduate School,
Universiti Putra Malaysia.

Date: 13 SEP 2001

DECLARATION

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

NG KIM NEE

Date: 31/7/01

TABLE OF CONTENTS

ABSTRACT ii
ABSTRAK iv
ACKNOWLEDGEMENTS vi
APPROVAL SHEETS vii
DECLARATION FORM ix
LIST OF TABLES xii
LIST OF FIGURES xiv
LIST OF ABBREVIATIONS xviii

CHAPTER **PAGE**

1 INTRODUCTION 1
1.1 The Genus of *Ploiarium* 1
1.1.1 Chemistry of *Ploiairum alternifolium* 4
1.1.2 Biological Activities 7
1.2 The Genus of *Calophyllum* 8
1.2.1 Chemistry of *Calophyllum* Species 9
1.2.2 Biological Activities 23
1.3 Objectives of Study 26

2 EXPERIMENTAL 27
2.1 Plant Material 27
2.2 Instruments 27
2.2.1 Infrared Spectroscopy (IR) 27
2.2.2 Mass Spectra (MS) 27
2.2.3 Melting Point 27
2.2.4 Ultra Violet (UV) 28
2.2.5 Nuclear Magnetic Resonance (NMR) 28
2.2.6 High Performance Liquid Chromatography (HPLC) 28
2.3 Chromatographic Methods 28
2.3.1 Thin Layer Chromatography (TLC) 28
2.3.2 Preparative Layer Chromatography (PLC) 29
2.3.3 Column-Chromatography 29
2.4 Extraction of *Ploiarium alternifolium* and *Calophyllum mucigerum* 30
2.4.1 *Ploiarium alternifolium* 31
2.4.2 *Calophyllum mucigerum* 37
2.5 Larvicidal Assay 42
2.6 Antimicrobial Activity 42
2.6.1 Preparation of Nutrient Broth Culture 43
2.6.2 Medium and Plates Preparations 43
2.7 Cytotoxicity assay 44

3 RESULTS AND DISCUSSION 45
3.1 Isolation of Bioactive Compounds from *Ploiarium alternifolium* 45

3.1.1 Characterisation of Ploiariquinone A (51)..... 47
3.1.2 Characterisation of 3β-benzoyloxyolean -11-en-13β,28-olide (52)..... 57
3.1.3 Characterisation of Emodin (53)..... 65
3.1.4 Characterisation of 1,8-dihydroxy-3-methoxy-6-methyl-anthraquinone (54)..... 73
3.1.5 Characterisation of Euxanmodin C (55)..... 81
3.2 Isolation of Bioactive Compounds from *Calophyllum mucigerum*..... 93
3.2.1 Characterisation of Friedelin (56)..... 95
3.2.2 Characterisation of Stigmasterol (57)..... 103
3.2.3 Characterisation of Cudraxanthone C (58)..... 109
3.2.4 Characterisation of Mucigerin I (59)..... 118
3.2.5 Characterisation of Mucigerin II (60)..... 137
3.3 Bioassay Results..... 151
3.3.1 Larvicidal Assay..... 151
3.3.2 Antimicrobial Assay..... 152
3.3.3 Cytotoxic Assay..... 153

4 CONCLUSIONS..... 155

BIBLIOGRAPHY..... 156

APPENDICES..... 161

Appendix 1..... 161

Appendix 2..... 163

Appendix 3..... 165

Appendix 4..... 167

Appendix 5..... 169

Appendix 6..... 171

Appendix 7..... 173

VITA..... 175

LIST OF TABLES

TABLE		PAGE
1.1	Results of the Preliminary Molluscicidal Screening of *Calophyllum* Extracts on Snails *Biomphalaria glabrata*	24
1.2	Molluscicidal Activity on Snails *Biomphalaria glabrata* of some Natural Neoflavonoids and Biogenetically Related Compounds.....	25
3.1	^{13}C NMR (100 MHz, $CDCl_3$) and ^{1}H NMR (400 MHz, $CDCl_3$) Assignments for Ploiariquinone A.....	49
3.2	^{1}H NMR (δ) Assignments and *J* (Hz) Values for 3β-benzoyloxyolean-11-en-13β,28-olide (400 MHz, $CDCl_3$).....	58
3.3	^{13}C NMR (δ) Assignments for 3β-benzoyloxyolean -11-en-13β,28-olide (100 MHz, $CDCl_3$).....	59
3.4	^{13}C NMR (100 MHz, DMSO) and ^{1}H NMR (400 MHz, DMSO) Assignments for Emodin.....	66
3.5	^{13}C NMR (100 MHz, $CDCl_3$) and ^{1}H NMR (400 MHz, $CDCl_3$) Assignments for 1,8-dihydroxy -3-methoxy-6-methyl-anthraquinone.....	74
3.6	^{13}C NMR (100 MHz, CD_3COCD_3) and ^{1}H NMR (400 MHz, CD_3COCD_3) Assignments for Euxanmodin C.....	83
3.7	^{13}C NMR (100 MHz, $CDCl_3$) and ^{1}H NMR (400 MHz, $CDCl_3$) Assignments for Friedelin.....	96
3.8	^{13}C NMR (100 MHz, $CDCl_3$) and ^{1}H NMR (400 MHz, $CDCl_3$) Assignments for Stigmasterol.....	104
3.9	^{13}C NMR (100 MHz, $CDCl_3$) and ^{1}H NMR (400 MHz, $CDCl_3$) Assignments for Cudraxanthone C.	110
3.10	^{1}H NMR (400 MHz, $CDCl_3$) Assignments for Mucigerin I.....	120

3.11	^{13}C NMR (100 MHz, $CDCl_3$) Assignments for Mucigerin I.....	121
3.12	Proton and Carbon Connectivities Obtained from ^{1}H-^{13}C HETCOR and HMBC Experiments for Mucigerin 1.....	122
3.13	^{13}C NMR (100 MHz, $CDCl_3$) and ^{1}H NMR (400 MHz, $CDCl_3$) Assignments for Mucigerin II.....	138
3.14	Proton and Carbon Connectivities Obtained from ^{1}H-^{13}C HETCOR and HMBC Experiments for Mucigerin 1I.....	139
3.15	Lethal Concentration 50% (LC_{50}) and Lethal Concentration 90% (LC_{90}) of Crude Extracts on *Aedes aegypti* Activity.....	151
3.16	Lethal Concentration 50% (LC_{50}) and Lethal Concentration 90% (LC_{90}) of Compounds on *Aedes aegypti* Activity.....	152
3.17	Diameter Inhibition Zone (mm) of the Crude Extracts and Pure Compound of *P. alternifolium* and *Calophyllum mucigerum*.....	152
3.18	Inhibition Concentration 50% (IC_{50}) Toward CEM-SS Cells of *P. alternifolium* and *C. mucigerum*.....	153
3.19	Inhibition Concentration 50% (IC_{50}) of Pure Compounds Toward CEM-SS Cells.....	154

LIST OF FIGURES

FIGURE		PAGE
1.1	Flowers of *Ploiarium alternifolium*.....	2
1.2	Young seeds of *Ploiarium alternifolium*.....	3
1.3	A cluster of young *Ploiarium alternifolium*.....	3
1.4	Close-up shot of leaves of *Calophyllum mucigerum*.....	9
1.5	Structures of xanthones and xanthones derivatives.....	14
1.6	Structures of coumarins and coumarin derivatives.....	16
1.7	Structures of neoflavonoid and biflavonoids.....	21
3.1	Compounds obtained from stem bark of *Ploiarium alternifolium*.....	46
3.2	Infrared spectrum of (51).....	50
3.3	^{1}H NMR spectrum of (51) (400 MHz, $CDCl_3$	51
3.4	^{1}H-^{1}H COSY spectrum of (51) (400 MHz, $CDCl_3$).....	52
3.5	^{13}C NMR spectrum of (51) (100 MHz, $CDCl_3$).....	53
3.6	DEPT spectra of (51).....	54
3.7	^{1}H-^{13}C HETCOR spectrum of (51) (expanded).....	55
3.8	^{1}H-^{13}C HETCOR spectrum of (51) (expanded).....	56
3.9	Infrared spectrum of (52).....	60
3.10	^{1}H NMR spectrum of (52) (400 MHz, $CDCl_3$).....	61
3.11	^{13}C NMR spectrum of (52) (100 MHz, $CDCl_3$).....	62
3.12	^{13}C NMR spectrum of (52) (100 MHz, $CDCl_3$) (expanded).....	63
3.13	EIMS spectrum of (52).....	64
3.14	Infrared spectrum of (53).....	67

3.15	^{1}H NMR spectrum of (53) (400 MHz, DMSO).....	68
3.16	^{1}H-^{1}H COSY spectrum of (53) (400 MHz, DMSO).....	69
3.17	^{13}C NMR spectrum of (53) (100 MHz, DMSO).....	70
3.18	^{1}H-^{13}C HETCOR spectrum of (53).....	71
3.19	EIMS spectrum of (53).....	72
3.20	Infrared spectrum of (54).....	75
3.21	^{1}H NMR spectrum of (54) (400 MHz, $CDCl_3$).....	76
3.22	^{13}C NMR spectrum of (54) (100 MHz, $CDCl_3$).....	77
3.23	DEPT spectra of (54).....	78
3.24	^{1}H-^{13}C HETCOR spectrum of (54).....	79
3.25	EIMS spectrum of (54).....	80
3.26	Infrared spectrum of (55).....	84
3.27	^{1}H NMR spectrum of (55) (400 MHz, CD_3COCD_3).....	85
3.28	^{1}H-^{1}H COSY spectrum of (55) (400 MHz, CD_3COCD_3) (expanded).....	86
3.29	^{1}H-^{1}H COSY spectrum of (55) (400 MHz, CD_3COCD_3) (expanded).....	87
3.30	^{13}C NMR spectrum of (55) (100 MHz, CD_3COCD_3).....	88
3.30	^{13}C NMR spectrum of (55) (100 MHz, CD_3COCD_3) (expanded).....	89
3.32	DEPT spectra of (55).....	90
3.33	^{1}H-^{13}C HETCOR spectrum of (55).....	91
3.34	CIMS spectrum of (55).....	92
3.35	Compounds obtained from stem bark of *Calophyllum mucigerum*.....	94
3.36	Infrared spectrum of (56).....	97
3.37	^{1}H NMR spectrum of (56) (400 MHz, $CDCl_3$).....	98

3.38	^{13}C NMR spectrum of (56) (100 MHz, $CDCl_3$).....	99
3.39	^{13}C NMR spectrum of (56) (100 MHz, $CDCl_3$) (expanded)	100
3.40	DEPT spectra of (56).....	101
3.41	EIMS spectrum of (56).....	102
3.42	^{1}H NMR spectrum of (57) (400 MHz, $CDCl_3$).....	105
3.43	^{13}C NMR spectrum of (57) (100 MHz, $CDCl_3$).....	106
3.44	DEPT spectra of (57).....	107
3.45	EIMS spectrum of (57).....	108
3.46	^{1}H NMR spectrum of (58) (400 MHz, $CDCl_3$).....	111
3.47	^{1}H-^{1}H COSY spectrum of (58) (400 MHz, $CDCl_3$).....	112
3.48	^{13}C NMR spectrum of (58) (100 MHz, $CDCl_3$).....	113
3.49	DEPT spectra of (58).....	114
3.50	^{1}H-^{13}C HETCOR spectrum of (58) (expanded).....	115
3.51	^{1}H-^{13}C HETCOR spectrum of (58) (expanded).....	116
3.52	EIMS spectrum of (58).....	117
3.53	Infrared spectrum of (59).....	123
3.54	^{1}H NMR spectrum of (59) (400 MHz, $CDCl_3$).....	124
3.55	^{1}H NMR spectrum of (59) (400 MHz, $CDCl_3$) (expanded).....	125
3.56	^{1}H NMR spectrum of (59) (400 MHz, $CDCl_3$) (expanded).....	126
3.57	^{1}H-^{1}H COSY spectrum of (59) (400 MHz, $CDCl_3$).....	127
3.58	^{13}C NMR spectrum of (59) (100 MHz, $CDCl_3$).....	128
3.59	DEPT spectra of (59).....	129
3.60	^{1}H-^{13}C HETCOR spectrum of (59).....	130
3.61	HMBC spectrum of (59) (100 MHz, $CDCl_3$) (expanded).....	131

3.62	HMBC spectrum of (59) (100 MHz, $CDCl_3$) (expanded).....	132
3.63	HMBC spectrum of (59) (100 MHz, $CDCl_3$) (expanded).....	133
3.64	EIMS spectrum of (59).....	134
3.65	CIMS spectrum of (59).....	135
3.66	Fragmentations Patterns of (59).....	136
3.67	Infrared spectrum of (60).....	140
3.68	^1H NMR spectrum of (60) (400 MHz, $CDCl_3$).....	141
3.69	^1H-^1H COSY spectrum of (60) (400 MHz, $CDCl_3$)...	142
3.70	^{13}C NMR spectrum of (60) (100 MHz, $CDCl_3$).....	143
3.71	DEPT spectra of (60).....	144
3.72	^1H-^{13}C HETCOR spectrum of (60).....	145
3.73	HMBC spectrum of (60) (100 MHz, $CDCl_3$).....	146
3.74	HMBC spectrum of (60) (100 MHz, $CDCl_3$) (expanded).....	147
3.75	HMBC spectrum of (60) (100 MHz, $CDCl_3$) (expanded).....	148
3.76	Fragmentation Patterns of (60).....	149
3.77	CIMS spectrum of (60).....	150

LIST OF ABBREVIATIONS

br	broad
β	beta
^{13}C	carbon-13
^{o}C	degree in Celsius
$CDCl_3$	deuterated chloroform
$CHCl_3$	chloroform
CIMS	Chemical ionization mass-spectroscopy
COSY	Correlated Spectroscopy
δ	chemical shift in ppm
d	doublet
dd	doublet of doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	dimethylsulphoxide
EIMS	Electron impact-mass spectroscopy
EtOAc	ethyl acetate
EtOH	ethanol
^{1}H	proton
HETCOR	Heteronuclear Chemical Shift-correlation
HMBC	Heteronuclear Multiple Bond Connectivity by 2D Multiple Quantum NMR
HPLC	High Performance Liquid Chromatography
Hz	Hertz
IC	Inhibition Concentration

IR	Infra Red
J	coupling constant in Hz
LC	Lethal Concentration
Lit	literature
m	multiplet
ml	mililitre
m.p	melting point
MeOH	methanol
MS	mass spectrum
nm	nanometer(s)
NMR	Nuclear Magnetic Resonance
PLC	Preparative Thin Layer Chromatography
s	singlet
TLC	thin layer chromatography
µg	microgram
UV	Ultra violet
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 The Genus of *Ploiarium*

The genus *Ploiarium* belongs to the Theaceae family. Two of the *Ploiarium* species are *Ploiarium alternifolium* and *Ploiarium pulcherrimum*. *Ploiarium* species are widely distributed at Southern Sarawak, Malaysia and they appear to be rather rare further north of Sarawak. However, they do not grow to timber size and are considered locally for domestic purposes such as pepper post and firewood. The wood is hard and heavy (Burkill, 1966).

Ploiarium alternifolium (Vahl.) Melanch is a cicada tree and it is locally known as "Jinggau" in Sarawak. "Jinggau" is one of the most common trees in secondary forests and on sandy and acid soils in Southern Sarawak, Malaysia. It is a small tree, attaining a maximum girth of two to three feet, with a narrow conical crown and the stem is without buttress. However on the swampy ground, the bark develops to be slender and almost perpendicular stilt roots. The young tree bark is rather smooth. The inner bark is yellow in colour and fibrous while the outer bark appears reddish brown. Normally the leaves are simple, stalkless, spirally arranged, smooth and rather fleshy with the length ranging from two to five inches long and the width ranging from half to almost two inches wide. The leaves are pale green or yellowish green at the base and tinged with pink colouration at the edge. Old leaves are either red or orange in colour. The flower's width is one inch wide. It is white in colour,

tipped with pink and with numerous fluffy stamens. The trees fruit a thin conical woody capsule, splitting from base to apex into five parts to expose a central column when they are ripe. The trees grow on swampy ground and they are very tolerant to poor soils although they seem to prefer dry and acid soils. The tree is evergreen and it flowers fairly frequently. Each year its girth increases about 0.5 inches. The leaves are eaten raw as salad and have a pleasant sharp taste. *P. alternifolium* is recognized as a hard heavy, red wood with indistinct soft tissue and rays. The wood is commonly used as fence and pepper posts. It is also popularly used as firewood (Burkill, 1966).

Figure 1.1: Flowers of *Ploiarium alternifolium*

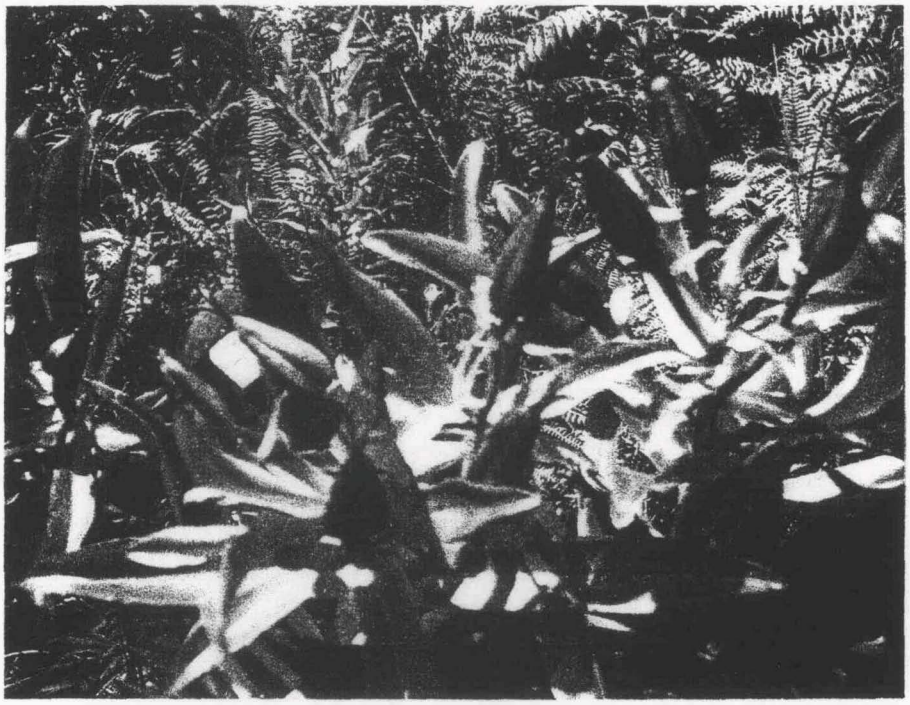


Figure 1.2: Young seeds of *Ploiarium alternifolium*

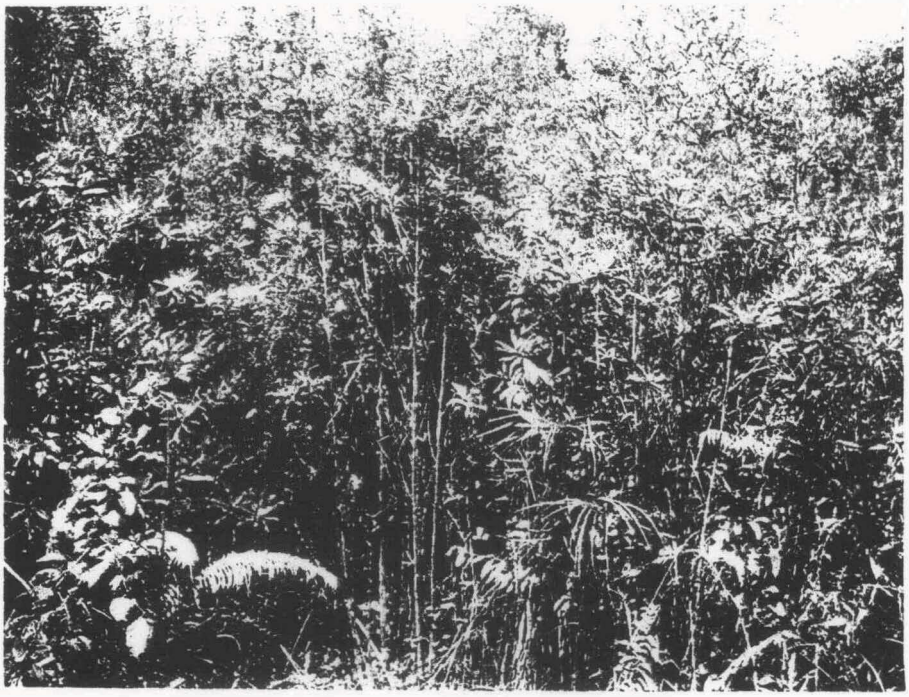


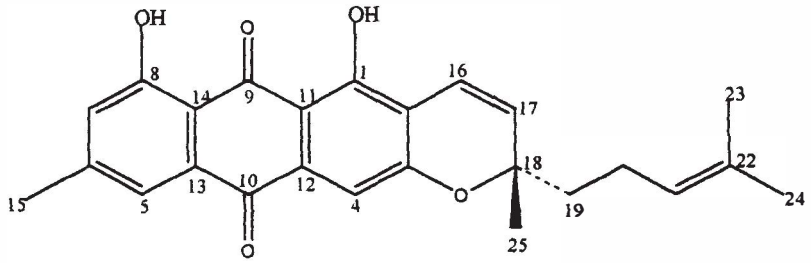
Figure 1.3: A cluster of young *Ploiarium alternifolium* tree

1.1.1 Chemistry of *Ploiarium alternifolium*

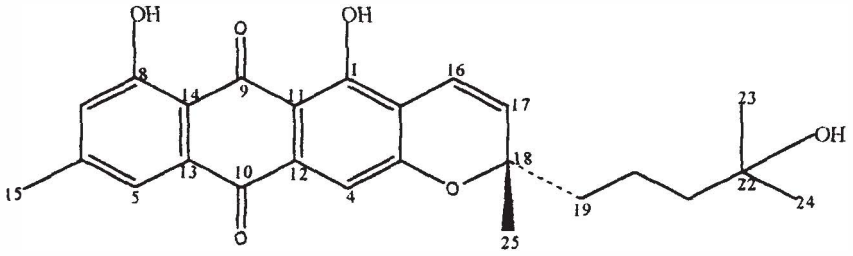
P. alternifolium has been found through phytochemical studies to contain secondary metabolites that can be grouped as geranyl anthraquinones, anthraquinonyl xanthones, triterpenoid benzoates and bixanthones. Anthraquinone is the main group in the quinones. It is widely found in liken, fungus and higher stage plants. Rubiaceae, Polygonacecae, Leguminosae and Liliaceae families are rich in anthraquinones. Besides the natural quinones, a dianthraquinone has also been isolated. This dianthraquinone was also synthesized from the oxidative coupling phenol reaction (Ahmad, 1993).

1.1.1.1 Geranyl Anthraquinones

The ethyl acetate soluble portion from the bark of *P. alternifolium* have provided two geranylated anthraquinones ploiariquinone A (1) and ploiariquinone B (2) (Graham *et al.*, 1991). Ploiariquinone A and B can be envisaged as arising by cyclization of 2-geranylemodin followed by oxidation of the resulted chroman.



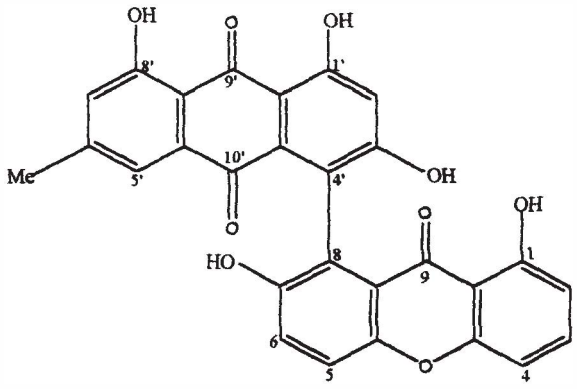
Ploiariquinone A (1)



Ploiariquinone B (2)

1.1.1.2 Antharaquinonyl Xanthones

Two anthraquinonyl xanthones from the shrub of *P. alternifolium* have been reported by Graham *et al.*, 1990. They were euxanmodin A (3) and euxanmodin B (4). This isolation was a great interest. *Ploiarium* is placed in the Bonnetiaceae or Theaceace family together with two South American genera, *Bonnetia* and *Archytaea*. The Bonnetiaceae has in the past been merged with either Theaceae or the Guttiferae. Isolation of xanthones supported a close link between the Bonnetiaceae and the Guttiferae. Euxanthone is known in the 16 genera of the Guttiferae and anthraquinones occur widely in one Guttiferae tribe, but neither have been isolated from the Theaceae.



Euxanmodin A (3)