UNIVERSITI PUTRA MALAYSIA

ANTIMICROBIAL AND CYTOTOXIC COMPOUNDS OF *SCORODOCARPUS BORNEENSIS* (OLACACEAE) AND *GLYCOSMIS CALCICOLA* (RUTACEAE)

CHRISTOPHE WIART

FSAS 2001 28

ANTIMICROBIAL AND CYTOTOXIC COMPOUNDS OF *SCORODOCARPUS BORNEENSIS* (OLACACEAE) AND *GLYCOSMIS CALCICOLA* (RUTACEAE)

By

CHRISTOPHE WIART

Thesis Submitted in Fulfillment of the Requirement for the Degree of Doctor in Philosophy in the Faculty of Science and Environmental Studies Universiti Putra Malaysia

March 2001

To my beloved wife Mazdida, to my beloved mother Flora, my father Patrice, my sister Vanessa, my sons Adam and Pierre,
In memory of my beloved and greatly missed grand parents Renée and José Monllor...
In memory of my mentors Pr. Loic Girre, Dr. Lucille Allorge, Dr. Kochumen..

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor in Philosophy

ANTIMICROBIAL AND CYTOTOXIC COMPOUNDS OF *SCORODOCARPUS BORNEENSIS* (OLACACEAE) AND *GLYCOSMIS CALCICOLA* (RUTACEAE)

By

CHRISTOPHE WIART

March 2001

Chairman: **Professor Mawardi Rahmani, Ph.D**

Faculty: **Science and Environmental Studies**

The extracts of plants collected from the forest of Northern Malay Peninsula were tested against bacteria, fungi and CEM-SS leukemia cell line. Gram-positive *Bacillus cereus* and Gram-negative *Pseudomonas aeruginosa* which are commonly involved in skin infections, have been used for screening antibacterial activity whereas *Candida lipolytica*, *Saccharomyces cerevisae*, *Saccharomyces lipolytica*, and *Aspergillus ochraceous* have been used for screening antifungal activity.

The crude extract from the seeds of *Scorodocarpus borneensis* showed a strong cytotoxic activity against CEM-SS leukemia cell-line and antimicrobial activities. A bioassay-guided separation had been carried out to separate the active constituents of the seeds. This resulted in the isolation and identification of an aliphatic sulfur compound, *bis*-(methylthiomethyl)disulfide, and a new indole

alkaloid, 13-docosenoyl serotonine from the petroleum ether extract A new sesquiterpene, scopotin, has been isolated from the chloroform extract of the seeds Another sesquiterpene, cadalene-β-carboxylic acid, has been isolated from the bark of this species

bis-(Methylthiomethyl)-disulfide appeared as the most active compound and the major constituent of the seeds extract of *Scorodocarpus borneensis* It acted significantly on a methicillin resistant strain of *Staphylococcus aureus* and showed a strong cytotoxic activity against CEM-SS leukemia and KU812F chronic myelogenous leukemia cell lines 13-Docosenoyl serotonine showed a moderate cytotoxic activity against CEM-SS leukemia cell line Scopotin showed a moderate antimicrobial activity and a strong cytotoxic effect against CEM-SS leukemia cell line

The crude petroleum ether extract and *bis*-(methylthiomethyl)disulfide strongly inhibited the growth of pathogen fungi and was formulated in external preparation by using commercial paraffin as excipient This preparation exhibited drastic antifungal activities *in vitro* and *in vivo* The acute toxicity of the crude petroleum ether extract was found to be inferior to phenobarbital in mice with LD_{50} at 275 mg/kg The skin irritancy test was performed on rabbits and it showed that this preparation had an irritancy level acceptable by the FDA

Antimicrobial and cytotoxic activities were also performed on methylgerambullin and desmethoxyzanthophylline which were previously isolated from *Glycosmis calcicola* (Rutaceae). Methylgerambullin showed a strong cytotoxic activity against CEM-SS leukemia, melanoma HACC1, and colon cancer HT29 cell lines whereas desmethoxyzanthophylline has moderate activity against *Bacillus cereus*, *Pseudomonas aeruginosa* and CEM-SS leukemia cell line.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untunk ijazah Doktor Falsafah

ANTIMICROBIAL AND CYTOTOXIC COMPOUNDS OF *SCORODOCARPUS BORNEENSIS* (OLACACEAE) AND *GLYCOSMIS CALCICOLA* (RUTACEAE)

Oleh

CHRISTOPHE WIART

Mac 2001

Pengerusi: Profesor Mawardi Rahmani

Fakulti: Sains dan Pengajian Alam Sekitar

Ekstrak tumbuh - tumbuhan yang dikumpul dari hutan Utara Semenanjung Malaysia telah diuji keberkesanannya terhadap bakteria, fungi dan sel leukemia CEM-SS. Bakteria Gram-positif *Bacillus cereus* dan Gram-negatif *Pseudomonas aeruginosa* yang biasa terlibat di dalam jangkitan kulit telah digunakan untuk kajian aktiviti antibakteria. Manakala fungi *Candida lipolytica*, *Saccharomyces cerevisae*, *Saccharomyces lipolytica* dan *Aspergillus ochraceous* telah digunakan pula untuk aktiviti antifungi.

Didalam kajian ini, ekstrak daripada bahagian buah pokok *Scorodocarpus borneensis* telah mempamerkan aktiviti sitotoksik yang kuat terhadap sel leukemia CEM-SS dan juga terhadap aktiviti antimikrobial. Kerja pemisahan biocerabinaan-kawalan telah dijalankan terhadap ekstrak ini untuk mencari sebatian yang aktif. Satu sebatian sulfur alifatik, *bis*-(methylthiomethyl)disulfide

dan satu alkaloid indol yang baru, 13-docosenoyl serotonine, telah ditemui daripada ekstrak petroleum eter. Manakala satu sesquiterpene yang baru, scopotin, telah dipisahkan daripada ekstrak klorofom terhadap buah. Selain daripada itu, satu sesquiterpene asid kadalena-β-karboksilik telah ditemui dari bahagian kulit pokok ini.

Didapati, *bis*-(methylthiomethyl)disulfide muncul sebagai sebatian yang paling aktif dan merupakan sebatian major daripada ekstrak buah *Scorodocarpus borneensis* ini. Ia bertindak kuat terutamanya terhadap *Staphylococcous aureus* yang resisten kepada methicillin. Sebatian ini juga menunjukkan sitotoksik yang kuat terhadap sel leukemia CEM-SS dan KU812F. Manakala sebatian 13-docosenoyl serotonine pula menunjukkan sitotoksik terhadap sel leukemia CEM-SS. Begitu juga dengan sebatian scopotin yang menunjukkan aktiviti antimikrobial sederhana tetapi memberi kesan sitotoksik yang kuat terhadap sel leukemia CEM-SS.

Ekstrak mentah petroleum eter dan sebatian *bis*-(methylthiomethyl)disulfide pada khususnya menghalang kuat pertumbuhan fungi patogen dan telah diformulasikan sebagai kegunaan luar dengan menggunakan paraffin sebagai asasnya. Penyediaan ini telah mempamerkan aktiviti antifungal yang amat memberangsangkan dalam *in vitro* dan *in vivo*. Didapati ketoksikan ekstrak petroleum eter ini terhadap tikus adalah lemah berbanding dengan fenobarbital dengan LD_{50} sebagai 275 mg/kg. Ujian rangsangan kulit terhadap

arnab telah dijalankan dan didapati formulasi ini adalah pada kadar rangsangan yang diterima oleh FDA.

Ujian antimikrobial dan sitotoksik juga telah dijalankan ke atas sebatian methylgerambullin dan desmethoxyzanthophylline yang mana telah dipencilkan daripada pokok *Glycosmis calcicola* (Rutaceae). Sebatian methylgerambullin menunjukkan aktiviti sitotoksik yang kuat terhadap sel leukemia CEM-SS, melanoma HACC1 dan sel kolon kanser HT29. Manakala sebatian desmethoxyzanthophylline pula menunjukkan aktiviti yang sederhana terhadap bakteria *Bacillus cereus*, *Pseudomonas aeruginosa* dan sel leukemia CEM-SS.

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Professor Dr. Mawardi Rahmani, Chairman of my Supervisory committee, for his guidance and encouragement throughout the thesis research. Special thanks are also extended to the other members of the committee, Associate Professor Dr. Abdul Manaf Ali and Associate Professor Dr. Mohd. Aspollah Sukari, for their assistance.

Thanks are due to Ms Chew Yean Ling for her work on *Glycosmis calcicola.*

Thanks are also due to lab technicians and graduate students for their support.

Finally, my deepest thanks to my wife and my sons for the sacrifices, understanding and support they have given me throughout this work.

I certify that an Examination Committee met on 25th March 2001 to conduct the final examination of Christophe Wiart on his Doctor of Philosophy thesis entitled "Antimicrobial and Cytotoxic Compounds from *Scorodocarpus borneensis* (Olacaceae) and *Glycosmis calcicola* (Rutaceae)" 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:

Taufiq Yap Yun Hin, Ph.D.
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Chairman)

Mawardi Rahmani, Ph.D.
Associate Professor
Faculty of Science and Environmental Studies
(Member)

Abdul Manaf Ali, Ph.D.
Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

Aspolla Sukari, Ph.D.
Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

A. Hamid A. Hadi, Ph.D.
Professor
Deputy Dean
Faculty of Science
University Malaya
(Independent Examiner)

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

Date: 25 MAY 2001

This Thesis was submitted to the Senate of Universiti Putra Malaysia and was accepted as fulfilment of the requirements for the degree of Doctor of Philosophy.

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

Date: 14 JUN 2001

DECLARATION

I hereby declare that the thesis is based on my original work excepts 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 UPM or other institutions.

Candidate
Christophe Wiart

Date: 25th May 2001

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

$CHCl_3$	chloroform
$CDCl_3$	deuterated chloroform
cm	centimeter
COSY	Correlation Spectroscopy
d	doublet
dd	doublet of doublet
EtOH	ethanol
FDA	Food and Drug Administration
g	gram
GC	Gas Chromatography
kg	kilogram
HETCOR	Heteronuclear Correlation
IC_{50}	Inhibiting Concentration 50%
IR	Infra Red
LD_{50}	Lethal Dose 50%
l	liter
m	multiplet
Me	methyl
MeOH	methanol
mg	milligram
MIC	Minimum Inhibiting Concentration
ml	milliliter
mm	millimeter
m.p.	melting point
MS	Mass Spectrum
NCI	National Cancer Institute
NMR	Nuclear Magnetic Resonance
PE	Petroleum Ether
ppm	part per million
rpm	rotation per minute
s	singlet
TLC	Thin Layer Chromatography
UI	Unit International
µl	microliter
µg	microgram
µM	micromolar

CHAPTER I

INTRODUCTION

Widely recognized as being of serious and immediate concern is the crisis of new and re-emerging infectious diseases for which no effective therapies are available and the development of resistance of many pathogens, such as *Staphylococcus aureus* to methicillin Penicillins discovered in the forties as well as more recent antibiotics, are now totally ineffective in common bacterial infections

Another concern is the need to develop new cancer chemotherapeutic agents with activity against the disease-types still resistant to current therapies and to overcome the development of multidrug resistance, which is increasingly observed in the treatment of many tumors Death rates and side effect are still high and new chemotherapeutic drugs need to be discovered

Thus, there is an urgent need to identify novel antimicrobial and cytotoxic molecules as leads for effective drug development Such active molecules may be synthesized by organic chemists or extracted from bacteria, fungi, animals, or plants The plant kingdom has been described by Farnworth as a "virtually untapped reservoir of novel drugs awaiting imaginative and progressive organizations"(Cordell, 1995) The author also estimated that five to fifteen percent of the approximately two hundred and fifty thousand species of higher

plant have been systematically investigated for the presence of bioactive compounds (Cordell, 1995)

Bioactive compounds of plant origin have played, and continue to play an invaluable role in the drug discovery process related to all disease types and, in particular, in the area of cancer and infectious diseases For example, national prescription audit data for a period of fifteen years between 1959-1974 showed that over 25% of US Prescriptions dispensed in 1973 contained active ingredients derived from higher plants, while 13 3% and 2 7% were derived from microbial and animal sources, respectively (Cordell, 1995) Among the new approved drugs reported between 1983 and 1994, drugs of natural origin predominate in the area of antibacterial (78%), while 61% of the anticancer drugs are naturally derived or modeled on natural product parents Of the eighty seven approved anticancer drugs four are of plant origin Of the two hundred and ninety nine anticancer drug candidates nine are of plant higher origin (Cordell, 1995)

Higher plants which are able to produce new drugs are mainly found in primary and secondary tropical rain forests of Africa, Asia and especially South and Central America and Southeast Asia The biodiversity there has forced plants to protect themselves against microorganisms, animals or insects by producing a great variety of chemical weapons In Southeast Asia, our Malaysian forest is endowed with one of the oldest and richest flora in the world (Ridley, 1922) This