CHEMICAL CONSTITUENTS AND *ANTIPLASMODIAL* ACTIVITY OF INDOLE ALKALOIDS FROM *OCHROSIA OPPOSITIFOLIA*

MEHRAN FADAEI NASAB

FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2010

CHEMICAL CONSTITUENTS AND *ANTIPLASMODIAL* ACTIVITY OF INDOLE ALKALOIDS FROM *OCHROSIA OPPOSITIFOLIA*

MEHRAN FADAEI NASAB

DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF CHEMISTRY
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2010

ACKNOWLEDGEMENT

From my deepest heart and my greatest appreciation go to my supervisor Professor Dr Khalijah Awang for her kindly good supervision, advices, patience and guidance throughout the course in this study. I also would like to express my greatest thanks to Professor Datuk. Dr. A. Hamid A. Hadi and Professor Madya Dr. Mat Ropi Mukhtar for their help and encouragement in this research work.

I also wish to extend my thanks to Mr. Din, Mr Teo, Mr Hazri and Mr Rafli (staffs of the Herbarium), Ms Norzalida, Mr azizul and Mrs Fiona (for recording NMR data), Mr Siew (for recording MS data), my friends in the Phytochemistry Laboratory, especially: Dr Azlan, Dr Kartini, Azmi, Ibrahim, Omer Hmadi, Ahmad Kaleem, Yalda Kia, Chan ghomati, Chong Soon Lim, Dewi Rosmi, Hazrina, Vicky, Fadzly , Norsita, Aimi, Aza, and others for their help and friendship.

Finally, I would like express my special appreciation to my family (Mother and Father), brother and sisters.

CONTENTS

CHAPTER 6: EXPERIMENTAL

LIST OF SCHEMES

LIST OF FIGURES

LIST OF TABLES

ABBREVIATION

α	Alpha
β	Beta
λ	Maximum wave length
δ	Chemical shift
µM	Micromolar
µl	Microlitre
µg/ml	Microgram per mililitre
mM	Milimolar
mg/ml	Milligram per mililitre
g	Gram
kg	Kilogram
U/ml	Unit per mililitre
ml	Mililitre
m	Meter
MHz	Mega Hertz
Hz	Hertz
UV	Ultraviolet
IR	Infrared
mM	Milimolar
ppm	Part per million
eV	Electron Volt
MeOH	Methanol
$CHCl_3$	Chloroform
CH_2Cl_2	Dichloromethane

DMSO	Dimethylsulphoxide
OCH_2O	Methylenedioxy
CH_3	Methyl group
OCH_3	Methoxyl group
OH	Hydroxyl group
NH_3	Ammonia
pH	Power of Hydrogen
HCl	Hydrogen Chloride
TLC	Thin layer chromatography
PTLC	Preparative thin layer chromatography
CC	Column chromatography
NMR	Nuclear Magnetic Resonance
FT-NMR	Fourier Transform Nuclear Magnetic Resonance
cm^{-1}	Per centimeter
J	Coupling constant
d	Doublet
s	singlet
dd	Doublet of doublet
t	triplet
m	multiplet
BBIQ	Bisbenzylisoquinoline
1D-NMR	One Dimension Nuclear Magnetic Resonance
2D-NMR	Two Dimension Nuclear Magnetic Resonance
^{1}H	Proton NMR
^{13}C	13-Carbon NMR

COSY	^{1}H-^{1}H Correlation Spectroscopy
DEPT	Distortioness Enhancement by Polarization Transfer
HMQC	Heteronuclear Multiple Quantum Coherence
HMBC	Heteronuclear Multiple Bond Coherence
NOE	Nuclear Overhauser Enhancement
GC-MS	Gas Chromatography-Mass Spectroscopy
MS	Mass Spectroscopy
EIMS	Electron Impact Mass Spectroscopy
FAB	Fast Atomic Bombardment
ESI	Electrospray Ionization
m/z	Mass per charge
$CDCl_3$	Deuterated chloroform
MeOD	Deuterated methanol
OD	Optical density

ABSTRACT

The chemical constituents of *Ochrosia oppositifolia* have been studied. The compounds were extracted from the bark and leaves of this plant by using *n*-hexane, dichloromethane and methanol as solvents. The crudes were subjected to extensive chromatographic techniques like preparative thin layer chromatography, column chromatography and high performance liquid chromatography (HPLC). A total of six compounds were obtained. Structural elucidation was established through several spectroscopic methods, such as 1D-NMR (^{1}H, ^{13}C, DEPT, NOE), 2D-NMR (COSY, NOESY, HMQC, and HMBC), UV, IR, and MS (GCMS, LCMS and HRMS) and comparison with the published data.

Three compounds were isolated from the bark of *Ochrosia oppositifolia,* one indole alkaloid which is isoreserpiline **1** and two ferulic acid ester namely 2-propenoic acid, 3-(4-hydroxy-3,5-dimethoxyphenyl)-,methylester E **73** and 17-methoxy-carbonyl-14-heptadecaenyl- 4-hydroxy-3-methoxy cinnamate F **74.**

Isolation and purification of alkaloids from the leaves of *Ochrosia oppositifolia* afforded three indole alkaloids namely neisosposinine **2**, reserpinine **3** and alkaloid D **72**.

All crude extracts and pure compounds were tested for anti plasmodial activity, and the hexane crude of bark showed the highest activity with an IC_{50} of 0.0505 μg/mL. Among the alkaloids tested, alkaloid D **72** is the most potent compound with an IC_{50} of 0.0123 μmol L^{-1}

ABSTRAK

Kandungan kimia daripada *Ochrosia oppositifolia* telah dikaji. Sebatian kimia daripada batang dan daun daripada pokok ini telah diekstrak dengan menggunakan pelarut *n*- heksana, diklorometana dan methanol. Ekstrak- ekstrak tersebut telah disubjekkan kepada teknik- teknik kromatografi ekstensif seperti kromatografi lapisan nipis preparative, kromatografi turus dan kromatografi cecair prestasi tinggi (HPLC).

Sebanyak enam sebatian kimia telah diisolasikan daripada pokok ini; elusidasi struktur telah dilakukan melalui beberapa kaedah spektroskopi, seperti 1D NMR (^{1}H, ^{13}C, DEPT, NOE), 2D NMR (COSY, NOESY, HMBC and HSQC), UV, IR dan MS (GCMS, LCMS dan HRMS) serta perbandingan dengan data yang telah diterbitkan.

Tiga sebatian kimia telah diisolasikan daripada batang *Ochrosia oppositifolia*, satu alkaloid indola bernama isoreserpilina **1** dan dua asid ferulik ester bernama asid 2-propenoik, 3- (4-hidroksi- 3,5- dimetoksifenil)- metil ester E **73** dan 17- metoksi-karbonil- 14- heptadekaenil- 4- hidroksi- 3 metoksi sinnamat F **74**.

Isolasi dan penulenan alkaloid daripada daun *Ochrosia oppositifolia* berjaya mendapatkan tiga alkaloid indola, bernama neisosposinin **2**, reserpinin **3** dan alkaloid D **72.**

Kesemua ekstrak dan sebatian- sebatian tulen diuji bagi aktiviti antiplasmodial, dan ekstrak heksana daripada batang telah menunjukkan aktiviti yang paling tinggi, dengan IC_{50} 0.0505 μg/mL. Antara alkaloid- alkaloid yang telah diuji, alkaloid D **72** merupakan sebatian yang paling tinggi aktiviti dengan IC_{50} 0.0123 μmol L^{-1}.