

สารฟลาวนอยด์จากต้นชำหุดและต้นช้างน้ำว

นางสาว รุ่งฤดี รุ่งเสรีชัย



สถาบันวิทยบริการ

จุฬาลงกรณ์มหาวิทยาลัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเกษตรศาสตรมหาบัณฑิต

สาขาวิชาเกษตรเขต ภาควิชาเกษตรเขต

คณะเกษตรศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2542

ISBN 974-334-507-8

ลิขสิทธิ์ของ 'จุฬาลงกรณ์มหาวิทยาลัย'

I 19230641

**FLAVONOIDS FROM *FISSISTIGMA POLYANTHOIDES*
AND *OCHNA INTEGERRIMA***



Miss Rungruedee Rungserichai

A Thesis Submitted in Partial Fulfilment of the Requirements

For the Degree of Master of Science in Pharmacy

Department of Pharmacognosy

Faculty of Pharmaceutical Sciences


Chulalongkorn University

Academic Year 1999

ISBN 974-334-507-8

Thesis Title Flavonoids from *Fissistigma polyanthoides* and *Ochna integerrima*
By Miss Rungruedee Rungserichai
Départment Pharmacognosy
Thesis Advisor Associate Professor Kittisak Likhitwitayawuid, Ph. D.
Thesis Co-Advisor Associate Professor Thatree Phadungcharoen, M. Sc. in Pharm.


Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in
Partial Fulfilment of the Requirements for the Master's Degree.


.....Dean of the Faculty of
Pharmaceutical Sciences
(Associate Professor Sunibhond Pummangura, Ph. D.)


Thesis committee


.....Chairman
(Associate Professor Rapepol Bavovada, Ph. D.)


.....Thesis Advisor
(Associate Professor Kittisak Likhitwitayawuid, Ph. D.)


.....Thesis Co-Advisor
(Associate Professor Thatree Phadungcharoen, M. Sc. in Pharm.)


.....Member
(Associate Professor Nijsri Ruangrunsi, Ph. D.)


.....Member
(Assistant Professor Vichien Jongbunprasert, M. Sc. in Pharm.)

รุ่งฤดี รุ่งเสรีชัย : สารฟลาโวนอยด์จากต้นข่าหุดและต้นข่าน้ำ (FLAVONOIDS FROM
FISSISTIGMA POLYANTHOIDES AND *OCHNA INTEGERRIMA*) อาจารย์ที่
ปรึกษา : รศ. ดร. กิตติศักดิ์ ลิขิตวิทยาวุฒิ, อาจารย์ที่ปรึกษาร่วม : รศ. ชาติรี ผดุงเจริญ,
140 pp. ISBN 974-334-507-8

จากการศึกษาสารฟลาโวนอยด์จากต้นข่าหุดและต้นข่าน้ำ สามารถแยกสารฟลาโวนอยด์ได้ 4
ชนิด สารแรกได้จากเปลือกต้นข่าหุด ได้แก่ 5,8-dihydroxy-6,7-dimethoxyflavone สารอีก 3 ชนิดเป็น
ฟลาโวนอยด์ใหม่ได้จากใบข่าน้ำ ได้แก่ 6- γ,γ -dimethylallyl taxifolin 7-O- β -D-glucoside, 2'',3''-
dihydroochnaflavone และ 2'',3''-dihydroochnaflavone 7''-O-methyl ether นอกจากนี้ได้ทำการศึกษา
แก้ไขโครงสร้างของสารอัลคาลอยด์จากเปลือกต้นข่าหุด (ALK1) ที่เคยมีการศึกษาไว้แล้ว การศึกษาโครงสร้าง
ทางเคมีของสารเหล่านี้ทำโดยการวิเคราะห์ข้อมูลทางสเปกโตรสโคปีชนิดต่างๆ ร่วมกับการเปรียบเทียบข้อมูลกับ
สารอื่นที่มีสูตรโครงสร้างทางเคมีสัมพันธ์กัน ได้ทำการทดลองฤทธิ์ในการยับยั้งเอ็นไซม์ไทโรซิเนสของสารฟลา
โวนอยด์ทั้ง 4 ชนิด พบว่าสารทั้งหมดไม่มีฤทธิ์

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

ภาควิชาเภสัชเวท
สาขาเภสัชเวท
ปีการศึกษา 2542

ลายมือชื่อนิติ..... รุ่งฤดี รุ่งเสรีชัย
ลายมือชื่ออาจารย์ที่ปรึกษา..... ชาติรี ผดุงเจริญ
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม..... ชาติรี ผดุงเจริญ

##4176579933

: MAJOR PHARMACOGNOSY

KEY WORD

: *FISSISTIGMA POLYANTHOIDES/OCHNA INTEGERRIMA*/
FLAVONOIDS

RUNGRUEDEE RUNGSEKICHAI:FLAVONOIDS FROM *FISSISTIGMA POLYANTHOIDES* AND *OCHNA INTEGERRIMA*. THESIS ADVISOR: ASSOCIATE PROFESSOR KITTISAK LIKHIWITAYAWUID. Ph.D., THESIS CO-ADVISOR:ASSOCIATE PROFESSOR THATREE PHADUNGCHAROEN, M. Sc. in Pharm. 140 pp. ISBN 974-334-507-8

Phytochemical studies of *Fissistigma polyanthoides* (DC.) Merr. and *Ochna integerrima* (Lour.) Merr. have resulted in the isolation of four flavonoids. A known flavonoid named 5,8-dihydroxy-6,7-dimethoxyflavone was isolated from the stem bark of *F. polyanthoides*, while three new flavonoids, namely 6- γ,γ -dimethylallyl taxifolin 7-*O*- β -D-glucoside, 2'',3''-dihydroochnaflavone and 2'',3''-dihydroochnaflavone 7''-*O*-methyl ether were isolated from the leaves of *O. integerrima*. In addition, the structure of a previously alkaloid isolated from *F. polyanthoides* (ALK1) was revised. The structures of these compounds were determined by analysis of their spectroscopic data and chemical evidence, as well as comparison with the data of other related compounds. All of the isolated flavonoids were tested for their tyrosinase inhibitory activity, but none of them showed activity.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

ภาควิชาเภสัชเวช
สาขาเภสัชเวช
ปีการศึกษา 2542

ลายมือชื่อนิติ.....รองศาสตราจารย์
ลายมือชื่ออาจารย์ที่ปรึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....



ACKNOWLEDGEMENTS

The author wishes to express her deepest gratitude to her thesis advisor, Associate Professor Dr. Kittisak Likhitwitayawuid of the Department Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for his advice, guidance and encouragement throughout her study.

The author would like to express her grateful thanks to her thesis co-advisor, Associate Professor Thatree Phadungcharoen, Department Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for her kindness and advice.

The author is grateful to Associate Professor Dr. Nijsiri Ruangrungsi, Department Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for his suggestion and help.

The author would like to thank Dr. Thawatchai Santisuk of the Royal Forest Department, Ministry of Agriculture and Co-operatives, Bangkok, for the identification of *Ochna integerrima* (Lour.) Merr..

The author would like to thank the Graduate School of Chulalongkorn University for granting partial financial support to conduct this investigation.

The author would like to thank to her teachers and her friends for their kindness and help.

Finally, the author wishes to express her infinite gratitude to her family for their love, understanding and encouragement.

CONTENTS

	Page
ABSTRACT (Thai).....	iv
ABSTRACT (English).....	v
ACKNOWLEDGEMENTS.....	vi
CONTENTS.....	vii
LIST OF TABLES.....	x
LIST OF FIGURES.....	xii
LIST OF SCHEME.....	xvi
LIST OF ABBREVIATIONS.....	xvii
CHAPTER	
I Introduction.....	1
II Historical	
1. Chemical Constituents of the Genus <i>Fissistigma</i>	5
2. Chemical Constituents of the Genus <i>Ochna</i>	11
III Experimental	
1. Sources of Plant Materials.....	30
2. General Techniques	
2.1 Analytical Thin-Layer Chromatography (TLC).....	30
2.2 Column Chromatography	
2.2.1 Vacuum Liquid Column Chromatography.....	31
2.2.2 Flash Column Chromatography.....	31
2.2.3 Gel Filtration Chromatography.....	31
2.3 Spectroscopy	
2.3.1 Ultraviolet (UV) Absorption Spectra.....	32

2.3.2 Infrared (IR) Absorption Spectra.....	32
2.3.3 Mass Spectra.....	32
2.3.4 Proton and Carbon-13 Nuclear Magnetic Resonance (¹ H and ¹³ C-NMR) Spectra.....	33
2.4 Physical Properties	
2.4.1 Melting Points.....	33
2.4.2 Optical Rotation.....	34
2.5 Solvents.....	34
3. Extraction and Isolation	
3.1 <i>Fissistigma polyanthoides</i>	
3.1.1 Extraction.....	34
3.1.2 Isolation	
3.1.2.1 Isolation of Compound FP-1.....	35
3.2 <i>Ochna integerrima</i>	
3.2.1 Extraction.....	36
3.2.2 Isolation	
3.2.2.1 Initial Separation.....	37
3.2.2.2 Isolation of Compound OC-1.....	43
3.2.2.3 Isolation of Compound OC-2.....	43
3.2.2.4 Isolation of Compound OC-3.....	43
4. Methylation of Pure Compounds	
4.1 Methylation of OC-2.....	44
4.2 Methylation of OC-3.....	44
5. Physical and Spectral data of Isolated Compounds and Methylation Products	
5.1 Compound FP-1.....	45
5.2 Compound OC-1.....	45

5.3 Compound OC-2.....	46
5.4 Compound OC-2-Me.....	46
5.5 Compound OC-3.....	47
5.6 Compound OC-3-Me.....	48
IV Results and Discussion	
1. Structure Determination	
1.1 <i>Fissistigma polyanthoides</i>	
1.1.1 Structure Determination of Compound FP-1.....	49
1.1.2 Rivision of Structure of Compound ALK1.....	52
1.2 <i>Ochna integerrima</i>	
1.2.1 Structure Determination of Compound OC-1.....	56
1.2.2 Structure Determination of Compound OC-2.....	60
1.2.3 Structure Determination of Compound OC-3.....	65
2. Tyrosinase Inhibitory Activity of Pure Compounds.....	69
V Conclusion.....	70
REFERENCES.....	71
APPENDIX.....	75
VITA.....	140

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF TABLES

Table	Page
1 Distribution of chemical constituents in the genus <i>Fissistigma</i>	5
2 Distribution of chemical constituents in the genus <i>Ochna</i>	11
3 Solvents in vacuum liquid column chromatography of hexane extract of <i>Fissistigma polyanthoides</i>	35
4 Combination of fractions from vacuum liquid column chromatography of hexane extract from <i>Fissistigma polyanthoides</i>	36
5 The percentage and volumes of solvents for vacuum liquid column chromatography of ethyl acetate extract of <i>Ochna integerrima</i>	37
6 Combination of fractions from vacuum liquid column chromatography of ethyl acetate extract of <i>Ochna integerrima</i>	39
7 The percentage and volumes of solvents for column chromatography of P-I, P-II and P-VI.....	40
8 Combination of fractions from column chromatography of P-I, P-II and P-VI.....	42
9 ¹ H and ¹³ C NMR spectral data of compound FP-1 (in CDCl ₃) and 5,8-dihydroxy-6,7-dimethoxyflavone (in CDCl ₃).....	51
10 ¹ H and ¹³ C NMR spectral data of compound ALK1 (in CDCl ₃) with long-range correlations observed in HMBC spectrum and ¹ H NMR data of (-)-thaipetaline (in CDCl ₃).....	54
11 ¹ H and ¹³ C NMR spectral data of compound OC-1 (in DMSO- <i>d</i> ₆) with long-range correlations observed in HMBC spectrum.....	58
12 ¹ H and ¹³ C NMR spectral data of compound OC-2 (in DMSO- <i>d</i> ₆) with long-range correlations observed in HMBC spectrum.....	63

- 13 ^1H and ^{13}C NMR spectral data of compound OC-3 (in $\text{DMSO-}d_6$)
with long-range correlations observed in COLOC spectrum..... 67



สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF FIGURES

Figure	Page
1 Structures of chemical constituents of <i>Fissistigma</i>	18
2 Structures of chemical constituents of <i>Ochna</i>	24
3 <i>Fissistigma polyanthoides</i> (DC.) Merr.	76
4 <i>Ochna integerrima</i> (Lour.) Merr.	77
5 EI mass spectrum of compound FP-1.....	78
6 300 MHz ^1H NMR spectrum of compound FP-1 (in CDCl_3).....	79
7 75 MHz ^{13}C NMR spectrum of compound FP-1 (in CDCl_3).....	80
8 75 MHz ^{13}C NMR, DEPT-90 and DEPT-135 spectra of compound FP-1 (in CDCl_3).....	81
9 EI mass spectrum of compound ALK1.....	82
10 500 MHz ^1H NMR spectrum of compound ALK1 (in CDCl_3).....	83
11 500 MHz ^1H NMR spectrum (partially expanded: δ_{H} 2.5-3.6 ppm) of compound ALK1 (in CDCl_3).....	84
12 125 MHz ^{13}C NMR spectrum of compound ALK1 (in CDCl_3).....	85
13 HMBC spectrum of compound ALK1 (in CDCl_3).....	86
14 HMBC spectrum of compound ALK1 (in CDCl_3) [δ_{H} 2.5-4.4 ppm, δ_{C} 22-64 ppm].....	87
15 HMBC spectrum of compound ALK1 (in CDCl_3) [δ_{H} 2.5-4.4 ppm, δ_{C} 102-152 ppm].....	88
16 HMBC spectrum of compound ALK1 (in CDCl_3) [δ_{H} 6.1-7.0 ppm, δ_{C} 22-62 ppm].....	89
17 HMBC spectrum of compound ALK1 (in CDCl_3) [δ_{H} 6.2-7.1 ppm, δ_{C} 100-152 ppm].....	90

18	UV spectrum of compound OC-1 (in MeOH).....	91
19	IR spectrum of compound OC-1 (KBr disc).....	92
20	FAB mass spectrum of compound OC-1.....	93
21	300 MHz ^1H NMR spectrum of compound OC-1 (in DMSO- d_6).....	94
22	75 MHz ^{13}C NMR spectrum of compound OC-1 (in DMSO- d_6).....	95
23	75 MHz ^{13}C NMR, DEPT-90 and DEPT-135 spectra of compound OC-1 (in DMSO- d_6).....	96
24	^1H - ^1H COSY spectrum of compound OC-1 (in DMSO- d_6).....	97
25	^1H - ^1H COSY spectrum (partially expanded: δ_{H} 1.0-8.0 ppm, δ_{H} 1.0-8.0 ppm) of compound OC-1 (in DMSO- d_6).....	98
26	HETCOR spectrum of compound OC-1 (in DMSO- d_6).....	99
27	HETCOR spectrum (partially expanded: δ_{H} 0-8.0 ppm, δ_{C} 10-140 ppm) of compound OC-1 (in DMSO- d_6).....	100
28	NOESY spectrum of compound OC-1 (in DMSO- d_6).....	101
29	NOESY spectrum (partially expanded: δ_{H} 1.0-7.5 ppm, δ_{H} 1.0-7.5 ppm) of compound OC-1 (in DMSO- d_6).....	102
30	HMBC spectrum of compound OC-1 (in DMSO- d_6).....	103
31	HMBC spectrum of compound OC-1 (in DMSO- d_6) [δ_{H} 1.5-7.0 ppm, δ_{C} 14-62 ppm].....	104
32	HMBC spectrum of compound OC-1 (in DMSO- d_6) [δ_{H} 1.5-7.0 ppm, δ_{C} 68-104 ppm].....	105
33	HMBC spectrum of compound OC-1 (in DMSO- d_6) [δ_{H} 1.5-7.0 ppm, δ_{C} 105-165 ppm].....	106
34	UV spectrum of compound OC-2 (in MeOH).....	107
35	IR spectrum of compound OC-2 (KBr disc).....	108
36	FAB mass spectrum of compound OC-2.....	109
37	300 MHz ^1H NMR spectrum of compound OC-2 (in DMSO- d_6).....	110

38	75 MHz ^{13}C NMR spectrum of compound OC-2 (in DMSO- d_6).....	111
39	75 MHz ^{13}C NMR, DEPT-90 and DEPT-135 spectra of compound OC-2 (in DMSO- d_6).....	112
40	^1H - ^1H COSY spectrum of compound OC-2 (in DMSO- d_6).....	113
41	^1H - ^1H COSY spectrum (partially expanded: δ_{H} 1.0-8.5 ppm, δ_{H} 1.0-8.5 ppm) of compound OC-2 (in DMSO- d_6).....	114
42	HETCOR spectrum of compound OC-2 (in DMSO- d_6).....	115
43	HETCOR spectrum (partially expanded: δ_{H} 5.0-9.0 ppm, δ_{C} 76-140 ppm) of compound OC-2 (in DMSO- d_6).....	116
44	HMBC spectrum of compound OC-2 (in DMSO- d_6).....	117
45	HMBC spectrum of compound OC-2 (in DMSO- d_6) [δ_{H} 5.4-7.9 ppm, δ_{C} 38-44 ppm].....	118
46	HMBC spectrum of compound OC-2 (in DMSO- d_6) [δ_{H} 5.4-7.9 ppm, δ_{C} 77-106 ppm].....	119
47	HMBC spectrum of compound OC-2 (in DMSO- d_6) [δ_{H} 5.4-7.9 ppm, δ_{C} 112-144 ppm].....	120
48	HMBC spectrum of compound OC-2 (in DMSO- d_6) [δ_{H} 5.4-7.9 ppm, δ_{C} 150-200 ppm].....	121
49	300 MHz ^1H NMR spectrum of OC-2-Me (in CDCl_3).....	122
50	El mass spectrum of OC-2-Me (in CHCl_3).....	123
51	NOESY spectrum of OC-2-Me (in CDCl_3).....	124
52	UV spectrum of compound OC-3 (in MeOH).....	125
53	IR spectrum of compound OC-3 (KBr disc).....	126
54	FAB mass spectrum of compound OC-3.....	127
55	300 MHz ^1H NMR spectrum of compound OC-3 (in DMSO- d_6).....	128
56	75 MHz ^{13}C NMR spectrum of compound OC-3 (in DMSO- d_6).....	129

57	75 MHz ^{13}C NMR, DEPT-90 and DEPT-135 spectra of compound OC-3 (in DMSO- d_6).....	130
58	^1H - ^1H COSY spectrum of compound OC-3 (in DMSO- d_6).....	131
59	^1H - ^1H COSY spectrum (partially expanded: δ_{H} 1.0-8.3 ppm, δ_{H} 1.0-8.3 ppm) of compound OC-3 (in DMSO- d_6).....	132
60	HETCOR spectrum of compound OC-3 (in DMSO- d_6).....	133
61	HETCOR spectrum (partially expanded: δ_{H} 2.0-8.9 ppm, δ_{C} 35-140 ppm) of compound OC-3 (in DMSO- d_6).....	134
62	NOESY spectrum of compound OC-3 (in DMSO- d_6).....	135
63	NOESY spectrum (partially expanded: δ_{H} 2.1-8.3 ppm, δ_{H} 2.1-8.3 ppm) of compound OC-3 (in DMSO- d_6).....	136
64	COLOC spectrum of compound OC-3 (in DMSO- d_6).....	137
65	COLOC spectrum (partially expanded: δ_{H} 5.0-8.3 ppm, δ_{C} 91-170 ppm) of compound OC-3 (in DMSO- d_6).....	138
66	300 MHz ^1H NMR spectrum of OC-3-Me (in CDCl_3).....	139

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF SCHEME

Scheme	Page
1 Mass fragmentation pattern of ALK1.....	55



สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF ABBREVIATIONS

Ag ₂ O	=	Silver oxide
approx.	=	Approximately
br	=	Broad (for NMR spectra)
<i>c</i>	=	Concentration
°C	=	Degree Celsius
CA	=	Chemical Abstract
CDCl ₃	=	Deuterated chloroform
CHCl ₃	=	Chloroform
CH ₃ I	=	Methyl iodide
cm	=	Centimeter
COLOC	=	Correlation spectroscopy via Long-range Coupling
¹³ C NMR	=	Carbon-13 nuclear magnetic resonance
COSY	=	Correlation spectroscopy
d	=	doublet (for NMR spectra)
dd	=	doublet of doublets (for NMR spectra)
DEPT	=	Distortionless Enhancement by Polarization Transfer
diam.	=	Diameter
DMSO- <i>d</i> ₆	=	Deuterated dimethylsulfoxide
δ	=	Chemical shift
EIMS	=	Electron Impact Mass Spectrum
FAB-MS	=	Fast Atom Bombardment Mass Spectrum
g	=	Gram

μg	=	Microgram
HETCOR	=	Heteronuclear Chemical Shift Correlation
$^1\text{H NMR}$	=	Proton nuclear magnetic resonance
HMBC	=	^1H -detected Heteronuclear Multiple Bond Correlation
Hz	=	Hertz
IR	=	Infrared spectrum
J	=	Coupling constant
Kg	=	Kilogram
L	=	Liter
L-DOPA	=	L-3,4-dihydroxyphenyl alanine
λ_{max}	=	Wavelength at maximal absorption
ϵ	=	Molar absorptivity
M^+	=	Molecular ion
m	=	Multiplet (for NMR spectra)
m	=	Meter
MeOH	=	Methanol
mg	=	Milligram
MHz	=	MegaHertz
min	=	Minute
ml	=	Milliliter
mm	=	Millimeter
m/z	=	Mass to charge ratio
nm	=	Nanometer
NMR	=	Nuclear magnetic resonance
NOESY	=	Nuclear Overhauser Effect Correlation Spectroscopy

ppm	=	part per million
ν_{\max}	=	Wave number at maximal absorption
s	=	Singlet (for NMR spectra)
TLC	=	Thin Layer Chromatography
UV	=	Ultraviolet



สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย