

สารออกฤทธิ์ทางชีวภาพจากลำต้นสักขี้ *Cudrania cochinchinensis*

นายสมพล ปรมายพันธ์



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชา เคมี ภาควิชาเคมี

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2543

ISBN 974-346-989-3

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

I 20508034

BIOACTIVE COMPOUNDS FROM THE STEMS OF
Cudrania cochinchinensis

Mr. Sompol Paramapojn

**A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Chemistry**

Department of Chemistry

Faculty of Science

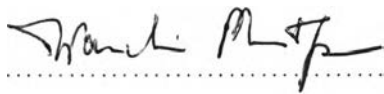
Chulalongkorn University

Academic Year 2000


ISBN 974-346-989-3

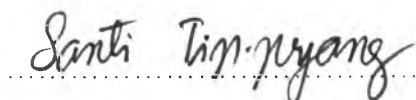
Thesis Title Bioactive Compounds from the Stems of *Cudrania cochinchinensis*
By Mr. Sompol Paramapojn
Field of Study Chemistry
Thesis Advisor Santi Tip-pyang, Ph.D.


Accepted by the Faculty of Science, Chulalongkorn University in Partial
Fulfillment of the Requirements of the Master's Degree

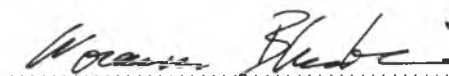
..... Dean of Faculty of Science
(Associate Professor Wanchai Phothiphichitr, Ph.D.)

Thesis committee:

..... Chairman
(Professor Udom Kokpol, Ph.D.)

..... Thesis Advisor
(Santi Tip-pyang, Ph.D.)

..... Member
(Associate Professor Siri Varothai, Ph.D.)

..... Member
(Assistant Professor Worawan Bhanthumnavin, Ph.D.)

สมพล ปรมาพจน์ : สารออกฤทธิ์ทางชีวภาพจากลำต้นสักชี (Bioactive compounds from the stems of *Cudrania cochinchinensis*). อ. ที่ปรึกษา: อ.ดร.สันติ ทิพยางค์; 117 หน้า
ISBN 974-346-989-3

การศึกษาสารออกฤทธิ์ทางชีวภาพจากลำต้นสักชี สามารถแยกสารทั้งหมดได้ 7 ชนิด ซึ่งเป็นสารใหม่ 1 ชนิด คือ 8,2',3'-trihydroxy-7,6'-dimethoxyisoflavan-4-ol (5) และสารที่เคยพบแล้วอีก 6 ชนิด ได้แก่ 5,7-dihydroxy-4'-methoxyisoflavone หรือ biochanin A (1), 7,3'-dihydroxy-2',4'-dimethoxy หรือ violanone (2), 5,7-dihydroxyflavanone หรือ pinnocembrin (3), 7-hydroxy-2',4'-dimethoxy isoflavanone หรือ sativanone (4), 7-hydroxy-4'-methoxyflavone (6), และ cycloartenone (7) โดยแยกได้จากสิ่งสกัดในชั้นเอทิลเอซ และไดคลอโรมีเทน สามารถพิสูจน์สูตรโครงสร้างของสารใหม่ด้วยวิธีทางสเปกโตรสโคปี

ผลการทดสอบฤทธิ์ทางชีวภาพของสารสกัดที่แยกได้พบว่า สาร 2 และสาร 5 มีฤทธิ์ต้านอนุมูลอิสระต่อ DPPH สูง (IC_{50} 138.8 และ 61.2 ไมโครโมลาร์) ในขณะที่สาร 1, 3 และ 4 แสดงฤทธิ์ความเป็นพิษต่อ KB cell lines นอกจากนี้สาร 5 ยังแสดงฤทธิ์การยับยั้ง NBT reduction (29.60 %) สูงสุดของสารที่แยกได้ทั้งหมดในพีชนี้ซึ่งใกล้เคียงกับ allopurinol (34.65%) ซึ่งใช้เป็นยารับประทานรักษาโรคเก๊าท์

ภาควิชา.....เคมี.....ลายมือชื่อนิสิต.....*สมพล ปรมาพจน์*.....

สาขาวิชา.....เคมี.....ลายมืออาจารย์ที่ปรึกษา.....*สันติ ทิพยางค์*.....

ปีการศึกษา.....2543.....

#4172471023 : MAJOR **CHEMISTRY**

KEY WORD *Cudrania cochinchinensis*, BIOACTIVE COMPOUND, ANTIOXIDANT /
SOMPOL PARAMAPOJN : BIOACTIVE COMPOUNDS FROM THE
STEMS OF *Cudrania cochinchinensis*. THESIS ADVISOR : SANTI TIP-
PYANG, Ph.D., 117 pp. ISBN 974-346-989-3

The investigation for bioactive compounds from the stems of *Cudrania cochinchinensis* Lour. led to the isolation of one new compound, 8,2',3'-trihydroxy-7,6'-dimethoxyisoflavan-4-ol (5) along with six known compounds, 5,7-dihydroxy-4'-methoxyisoflavone or biochanin A (1), 7,3'-dihydroxy-2',4'-dimethoxyisoflavanone or violanone (2), 5,7-dihydroxyflavanone or pinnocembrin (3), 7-hydroxy-2',4'-dimethoxy isoflavanone or sativanone (4), 7-hydroxy-4'-methoxyflavone (6), cycloartenone (7) from hexane and dichloromethane crude extracts. The structure of 8,2',3'-trihydroxy-7,6'-dimethoxyisoflavan-4-ol, new flavonoid, was elucidated by spectroscopic methods.

In addition, compound 2 and 5 showed significant free radicals scavenging activity on DPPH (IC_{50} 138.8 μ M and 61.2 μ M), while compound 1, 3 and 4 were found to be high in-vitro cytotoxicity against KB cell lines. Compound 5 also showed highest %NBT reduction inhibition (29.60) among the isolated compounds from this plant and its degree of inhibition activity was closed to allopurinol (34.65) which was used as an oral drug for treating gout.

Department... Chemistry..... Student's signature..... *Sompol Paramapojn*
Field of study..... Organic Chemistry..... Advisor's signature..... *Santi Tip-pyang*
Academic year..... 2000.....



ACKNOWLEDGEMENT

The author would like to express his appreciation to his advisor, Dr. Santi Tip-pyang for his assistance and encouragement in conducting this research. He wished to thank the members of his thesis committee, Professor Dr. Udom kokpol, Associate Professor Dr. Siri Varothai and Assistant Professor Dr. Worawan Bhanthumnawin for their valuable discussion and advice. He would like to acknowledge Miss. Pongpun Siripong (Natural Products Research Section, Research Division, National Cancer Institute, Thailand) for testing cytotoxic activity against KB cell lines, Mrs. Siriporn Stonsaovapak (Institute of Food Research and Product Development, Kasetsart University) for performing antibacterial bioassay, Miss Suratwadee Jiwajinda (Kampangsak, Kasetsart University) for providing xanthine oxidase bioassay method and Assistant Professor Dr. Rutt Sutthisri (Faculty of Pharmaceutical Science, Chulalongkorn University) for interpreting of compound 7.

Special appreciation is expressed to the staff of the Natural Products Research Unit, Department of Chemistry, Chulalongkorn University, for the support of chemicals and laboratory facilities throughout the course of study. Moreover, thanks are extended to the Faculty of science and Graduate School of Chulalongkorn University for financial support.

His deep appreciation is expressed to his parents and family members for their great support, love, understanding and encouragement throughout the entire course of his studies.

CONTENTS

	Pages
Abstract in Thai.....	iv
Abstract in English.....	v
Acknowledgement.....	vi
List of Figures.....	ix
List of Schemes.....	xii
List of Tables.....	xiii
List of Abbreviations.....	xiv
CHAPTER	
1. INTRODUCTION	1
1.1 Botanical Aspect and Distribution.....	10
1.2 Chemical constituents of <i>C.cochinchinensis</i>	12
1.3 Ethnomedical Informations and Biological Activities.....	14
2. EXPERIMENT	19
2.1 Plant material.....	19
2.2 Equipment.....	19
2.3 Dipping Reagent.....	20
2.4 Bioassay Procedures.....	20
2.4.1 Antibacterial Bioassay.....	20
2.4.2 The Inhibitory Effect for Tumor Cell Lines.....	20
2.4.3 Scavenging Effects on DPPH Radicals.....	20
2.4.4 $\cdot\text{O}_2^-$ Scavenging Activity.....	20
2.5 Extraction.....	21
2.6 Separation and Purification.....	21
3. RESULTS AND DISCUSSION	24
3.1 Primary Bioassay Screening Results of Crude Extracts.....	24
3.1.1 Antioxidant Test.....	24
3.2 Properties and Structural Elucidation of Isolated Compound.....	26
3.2.1 Compound 1.....	26
3.2.2 Compound 2.....	34
3.2.3 Compound 3.....	51
3.2.4 Compound 4.....	57

	Pages
3.2.5 Compound 5.....	75
3.2.6 Compound 6.....	91
3.2.7 Compound 7.....	98
3.3 Biological Activity of Isolated Compounds.....	107
4. CONCLUSION	112
Proposal for the Future Work.....	113
REFERENCES.....	114
VITA.....	117

List of Figures

Figures	Pages
1.1 Structure of DPPH and DPPHn.....	4
1.2 Xanthine oxidized to uric acid.....	4
1.3 Binding sites for trace metals.....	5
1.4 Scavenging of ROS (R [•]) by flavonoids.....	6
1.5 Flowers, leaf, and tree of <i>C. cochinchinensis</i>	11
1.6 Structures of flavonoids and xanthenes from <i>C. cochinchinensis</i>	16
3.1 Scavenging effect of crude extract.....	25
3.2 IR spectrum of compound 1.....	30
3.3 Mass spectrum of compound 1.....	30
3.4 ¹ H NMR spectrum of compound 1.....	31
3.5 ¹³ C NMR spectrum of compound 1.....	31
3.6 DEPT 90 and 135 spectrums of compound 1.....	32
3.7 NOE DIFF of compound 1.....	32
3.8 NOE DIFF of compound 1.....	33
3.9 IR spectrum of compound 2.....	39
3.10 Mass spectrum of compound 2.....	39
3.11 ¹ H NMR spectrum of compound 2.....	40
3.12 ¹³ C NMR spectrum of compound 2.....	41
3.13 DEPT 90 and 135 spectrums of compound 2.....	42
3.14 HMBC spectrum of compound 2.....	43
3.15 HMBC spectrum of compound 2.....	44
3.16 HMBC spectrum of compound 2.....	45
3.17 NOE DIFF of compound 2 (irradiated at 7.66 ppm).....	46
3.18 NOE DIFF of compound 2 (irradiated at 6.65 ppm).....	46
3.19 NOE DIFF of compound 2 (irradiated at 6.53 ppm).....	47
3.20 NOE DIFF of compound 2 (irradiated at 6.50 ppm).....	47
3.21 NOE DIFF of compound 2 (irradiated at 6.32 ppm).....	48
3.22 NOE DIFF of compound 2 (irradiated at 3.75 ppm).....	48
3.23 NOE DIFF of compound 2 (irradiated at 3.64 ppm).....	49

	Pages
3.24 NOESY of compound 2.....	49
3.25 NOESY of compound 2.....	50
3.26 IR spectrum of compound 3.....	54
3.27 Mass spectrum of compound 3.....	54
3.28 ¹ H NMR spectrum of compound 3.....	55
3.29 ¹³ C NMR spectrum of compound 3.....	56
3.30 DEPT 90 and 135 spectrums of compound 3.....	56
3.31 IR spectrum of compound 4.....	63
3.32 Mass spectrum of compound 4.....	63
3.33 ¹ H NMR spectrum of compound 4.....	64
3.34 ¹³ C NMR spectrum of compound 4.....	65
3.35 DEPT 90 and 135 spectrums of compound 4.....	65
3.36 DEPT 90 and 135 spectrums of compound 4.....	66
3.37 HMQC spectrum of compound 4.....	66
3.38 HMQC spectrum of compound 4.....	67
3.39 HMBC spectrum of compound 4.....	68
3.40 HMBC spectrum of compound 4.....	69
3.41 HMBC spectrum of compound 4.....	70
3.42 HMBC spectrum of compound 4.....	71
3.43 NOE DIFF of compound 4 (irradiated at 7.75 ppm).....	71
3.44 NOE DIFF of compound 4 (irradiated at 6.98 ppm).....	72
3.45 NOE DIFF of compound 4 (irradiated at 6.49 ppm).....	72
3.46 NOE DIFF of compound 4 (irradiated at 6.47 ppm).....	73
3.47 NOE DIFF of compound 4 (irradiated at 6.55 ppm).....	73
3.48 NOE DIFF of compound 4 (irradiated at 3.77 ppm).....	74
3.49 NOE DIFF of compound 4 (irradiated at 3.74 ppm).....	74
3.50 IR spectrum of compound 5.....	80
3.51 ¹³ C NMR spectrum of compound 5.....	80
3.52 Mass spectrum of compound 5.....	81
3.53 ¹ H NMR spectrum of compound 5.....	82
3.54 DEPT 90 and 135 spectrums of compound 5.....	83
3.55 HMBC spectrum of compound 5.....	83
3.56 HMBC spectrum of compound 5.....	84

	Pages
3.57 HMBC spectrum of compound 5.....	85
3.58 HMBC spectrum of compound 5.....	86
3.59 HMBC spectrum of compound 5.....	87
3.60 NOE DIFF of compound 5 (irradiated at 7.01 ppm).....	87
3.61 NOE DIFF of compound 5 (irradiated at 6.73 ppm).....	88
3.62 NOE DIFF of compound 5 (irradiated at 6.68 ppm).....	88
3.63 NOE DIFF of compound 5 (irradiated at 6.48 ppm).....	89
3.64 NOE DIFF of compound 5 (irradiated at 3.83 ppm).....	89
3.65 NOE DIFF of compound 5 (irradiated at 3.79 ppm).....	90
3.66 IR spectrum of compound 6.....	94
3.67 Mass spectrum of compound 6.....	94
3.68 ¹ H NMR spectrum of compound 6.....	95
3.69 ¹³ C NMR spectrum of compound 6.....	96
3.70 DEPT 90 and 135 spectrums of compound 6.....	96
3.71 NOE different of compound 6.....	97
3.72 IR spectrum of compound 7.....	102
3.73 Mass spectrum of compound 7.....	103
3.74 ¹ H NMR spectrum of compound 7.....	104
3.75 ¹³ C NMR spectrum of compound 7.....	105
3.76 DEPT 90 and 135 spectrums of compound 7.....	106
3.77 Scavenging effect of compound 2.....	109
3.78 Scavenging effect of compound 5.....	109
3.79 Scavenging effect of BHA.....	110

List of Schemes

Schemes	Pages
1.1 Illustrating the pathways to phenylalanine and acetyl CoA, and the following reaction steps leading to some flavonoid classes.....	7
2.1 The extraction procedure of the stems of <i>C. cochinchinensis</i>	22
2.2 Flow chart showed the separation of stems from <i>C. cochinchinensis</i>	23
3.1 Possible fragmentation patterns of compound 1.....	29
3.2 Possible fragmentation patterns of compound 2.....	38
3.3 Possible fragmentation patterns of compound 3.....	53
3.4 Possible fragmentation patterns of compound 4.....	62
3.5 Possible fragmentation patterns of compound 5.....	79
3.6 Possible fragmentation patterns of compound 6.....	93
3.7 Possible fragmentation patterns of compound 7.....	101

List of Tables

Tables	Pages
1.1 Chemical constituents of <i>C. cochinchinensis</i>	12
1.2 Ethnomedical Information.....	14
1.3 Biological Activities.....	15
3.1 Antioxidant Result from TLC autographic assay of various crude extracts.....	24
3.2 Antioxidant Result from UV absorbance at λ 516 nm of various crude extracts.....	24
3.3 The efficient concentration (IC_{50}) of crude extracts.....	25
3.4 ^{13}C NMR data of Compound 1 compared with Genistein.....	27
3.5 1H , ^{13}C and HMBC spectral data of Compound 2 in CD_3OD (500 MHz).....	36
3.6 ^{13}C NMR data of Compound 3 compared with Pinnocembrin.....	52
3.7 1H , ^{13}C and HMBC spectral data of Compound 4 in CD_3OD (500 MHz).....	60
3.8 ^{13}C NMR data of Compound 4 compared with Sativanone.....	61
3.9 1H , ^{13}C and HMBC spectral data of Compound 5 in CD_3OD (500 MHz).....	78
3.10 ^{13}C and 1H NMR data of compound 6.....	92
3.11 ^{13}C NMR of compound 7 compared with cycloart-24-ene-3 β ,28-diol (7a) and 21,24 (RS)-dihydroxycycloart-25-en-3-one (7b).....	99
3.12 The Biological Activity of Isolated Compounds from <i>C. cochinchinensis</i>	107
3.13 Cytotoxic Activity Against KB Cell Lines by MTT Assay.....	107
3.14 Free Radicals Scavenging Activity of Isolated Compounds on DPPH.....	108
3.15 Free Radicals Scavenging Activity of BHA on DPPH.....	110
3.16 IC_{50} on Free radical Scavenging Activity of Isolated Compounds on DPPH.....	110
3.17 Xanthine Oxidase Activity of Isolated Compounds on Xanthine-Xanthine Oxidase System.....	111

List of Abbreviations

CC, SiO ₂	= column chromatography using silica gel as absorbent
TLC	= thin layer chromatography
R _f	= retardation factor
m.p.	= melting point
°C	= degree celsius
w/w	= weight by weight
g	= gram
Kg	= kilogram
mg	= milligram
µg	= microgram
ml	= millilitre
nm	= nanometre
mM	= milimolar
UV	= ultra-violet
EIMS	= electron impact mass spectrometry
m/z	= mass per charge
M.W.	= molecular weight
IR	= infrared
ν _{max}	= wave number cause maximum absorption
FT	= fourier transform
NMR	= nuclear magnetic resonance
DMSO	= dimethylsulfoxide
CDCl ₃	= deuteratedchloroform
CD ₃ OD	= deuteratedmethanol
δ	= chemical shift
J	= coupling constant
Hz	= hertz
s	= singlet
d	= doublet
dd	= doublet of doublet
t	= triplet

q	= quartet
m	= multiplet
DEPT	= distortionless enhancement by polarization transfer
HMBC	= heteronuclear multiple bond connectivity by 2D multiple quantum NMR
HMQC	= ¹ H-detected heteronuclear multiple-quantum coherence <i>via</i> direct coupling
COSY	= two-dimension ¹ H correlation spectroscopy
NOE	= nuclear overhauser effect
NOE DIFF	= nuclear overhauser effect difference
NOESY	= nuclear overhauser enhancement spectroscopy
ppm	= part per million (or μg/g)
OMe	= methoxy
OH	= hydroxy
E ₇	= one-electron reduction potentials at pH 7
DPPH	= 2,2-diphenyl-1-picrylhydrazyl radical
BHA	= butylated hydroxyanisole