

ฟลาโวนอยด์ที่มีฤทธิ์ทางชีวภาพจากเมล็ดมันแกวและเปลือกต้นชะเงาะ



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จุฬาลงกรณ์มหาวิทยาลัย

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BIOACTIVE FLAVONOIDS FROM  
*PACHYRRHIZUS EROSUS* SEEDS AND *MILLETTIA LEUCANTHA*  
VAR. *LEUCANTHA* STEM BARK

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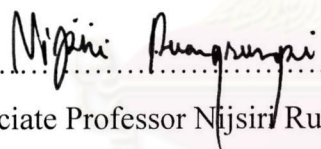
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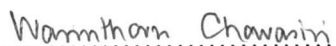
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จากการศึกษาทางพฤกษเคมีของเมล็ดมันแกว สามารถแยกสารที่เคยมีรายงานมาแล้วได้  
 ทั้งหมด 8 ชนิด คือ dehydroneoteneone, dolineone, (+)-12a-hydroxydolineone, (+)-12a-  
 hydroxypachyrrhizone, (-)-12a-hydroxyrotenone, neoteneone, pachyrrhizin และ (+)-pachyrrhizone  
 และได้ทำการพิสูจน์พร้อมทั้งรายงานตำแหน่งทาง NMR ของสารที่แยกได้อย่างสมบูรณ์ สำหรับการ  
 การศึกษาทางพฤกษเคมีของเปลือกต้นขะจาสามารถแยกสารได้ 11 ชนิดซึ่งเป็นสารใหม่ 2 ชนิด  
 คือ 2,4,6,β-tetramethoxy-3',4'-methylenedioxychalcone และ 2',4',6'-trimethoxy-3,4-methylene  
 dioxydihydrochalcone และสารที่พบครั้งแรกในธรรมชาติอีก 2 ชนิด คือ 2',4'-dimethoxy-3,4-  
 methylenedioxychalcone และ 2',4',6'-trimethoxy-3,4-methylenedioxychalcone นอกจากนี้ยังพบ  
 สารที่มีรายงานมาแล้วอีก 7 ชนิด ได้แก่ desmethoxykanugin, dihydromilletteneone methyl ether, 2'-  
 hydroxy-3,4,4',6'-tetramethoxychalcone, karanjin, lanceolatin B, 3',4'-methylenedioxy-5,7-  
 dimethoxyflavone และ 3',4'-methylenedioxy-7-methoxyflavone การพิสูจน์โครงสร้างทางเคมีของ  
 สารทั้งหมดที่แยกได้อาศัยการวิเคราะห์เชิงสเปกตรัมของ UV, IR, MS และ NMR ร่วมกับการ  
 เปรียบเทียบข้อมูลของสารที่ทราบโครงสร้างแล้ว นอกจากนี้ ยังได้นำสารที่แยกได้ไปทดสอบฤทธิ์  
 ทางชีวภาพ ได้แก่ ฤทธิ์ต้านจุลชีพ, ฤทธิ์ต้านไวรัสริบ, ฤทธิ์ยับยั้งเอนไซม์ cyclooxygenase-2 และ  
 ฤทธิ์ความเป็นพิษต่อเซลล์ พบว่า (+)-12a-hydroxydolineone และ (+)-12a-hydroxypachyrrhizone  
 จากเมล็ดมันแกว และ dihydromilletteneone methyl ether และ 2',4',6'-trimethoxy-3,4-methylene  
 dioxydihydrochalcone จากเปลือกต้นขะจาจะมีฤทธิ์ปานกลางในการยับยั้งเชื้อไวรัสริบ ในขณะที่  
 desmethoxykanugin มีฤทธิ์ปานกลางในการยับยั้งเอนไซม์ cyclooxygenase-2 และยังพบว่า 2',4'-  
 dimethoxy-3,4-methylenedioxychalcone และ 2',4',6'-trimethoxy-3,4-methylenedioxychalcone มี  
 ฤทธิ์เป็นพิษต่อเซลล์มะเร็ง NCI-H460 อย่างมีนัยสำคัญ ส่วนการตรวจสอบฤทธิ์ต้านจุลชีพนั้น  
 พบว่า ไม่มีสารใดแสดงความสามารถในการต้านการเจริญของจุลชีพทดสอบ

ภาควิชาเภสัชเวท

สาขาวิชาเภสัชเคมีและผลิตภัณฑ์ธรรมชาติ

ปีการศึกษา 2545

ลายมือชื่อนิสิต.....*อ.วิมลมาศ ลิปิพันธ์*.....

ลายมือชื่ออาจารย์ที่ปรึกษา.....*นิจศิริ เรืองรังษี*.....

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ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....*ดร. วิมลมาศ ลิปิพันธ์*.....

# # 4176961933 MAJOR: PHARMACEUTICAL CHEMISTRY AND NATURAL PRODUCTS

KEY WORD: *PACHYRRHIZUS EROSUS*/ *MILLETTIA LEUCANTHA*/ FLAVONOIDS/ ANTI-HERPES SIMPLEX VIRUS ACTIVITY/ CYCLOOXYGENASE-2 INHIBITORY ACTIVITY/ CYTOTOXIC ACTIVITY

AMPAI PHRUTIVORAPONGKUL: BIOACTIVE FLAVONOIDS FROM *PACHYRRHIZUS EROSUS* SEEDS AND *MILLETTIA LEUCANTHA* VAR. *LEUCANTHA* STEM BARK. THESIS ADVISOR: ASSOCIATE PROFESSOR NIJSIRI RUANGRUNGSI, Ph.D., THESIS CO-ADVISOR: ASSOCIATE PROFESSOR VIMOLMAS LIPIPUN, Ph.D., PROFESSOR TSUTOMU ISHIKAWA, Ph.D., 221 pp. ISBN 974-17-2001-7

Phytochemical study on the seeds of *Pachyrrhizus erosus* revealed the presence of eight known compounds including dehydroneotene, (+)-dolineone, (+)-12a-hydroxydolineone, (+)-12a-hydroxypachyrrhizone, (-)-12a-hydroxyrotenone, neotene, pachyrrhizin and (+)-pachyrrhizone. All isolates were identified and completed  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data. Additionally, eleven compounds were obtained from the phytochemical investigation of the stem bark of *Millettia leucantha* var. *leucantha*. These included two new compounds, 2,4,6, $\beta$ -tetramethoxy-3',4'-methylenedioxychalcone and 2',4',6'-trimethoxy-3,4-methylenedioxydihydrochalcone, two new natural products, 2',4'-dimethoxy-3,4-methylenedioxychalcone and 2',4',6'-trimethoxy-3,4-methylenedioxychalcone, and other seven known compounds, namely desmethoxykanugin, dihydromillettene methyl ether, 2'-hydroxy-3,4,4',6'-tetramethoxychalcone, karanjin, lanceolatin B, 3',4'-methylenedioxy-5,7-dimethoxyflavone and 3',4'-methylenedioxy-7-methoxyflavone. The structure determination of all isolates were accomplished by spectroscopic analyses (UV, IR, MS and NMR) and compared with the literary data of known compounds. The isolated compounds were also subjected for biological activities evaluation, involving antimicrobial, anti-Herpes Simplex Virus (HSV), cyclooxygenase-2 (COX-2) inhibitory and cytotoxic activities. (+)-12a-Hydroxydolineone and (+)-12a-hydroxypachyrrhizone from *P. erosus*, and dihydromillettene methyl ether and 2',4',6'-trimethoxy-3,4-methylenedioxydihydrochalcone from *M. leucantha* showed moderate anti-HSV activity, whereas desmethoxykanugin demonstrated moderate COX-2 inhibitory activity. Furthermore, 2',4'-dimethoxy-3,4-methylenedioxychalcone and 2',4',6'-trimethoxy-3,4-methylenedioxychalcone showed significant cytotoxic activity against NCI-H460 cell line. This work verified that no isolated compound showed antimicrobial activity.

Field of Study Pharmaceutical Chemistry and Natural Products

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## LIST OF ABBREVIATIONS AND SYMBOLS

$\alpha$	=	Alpha
$[\alpha]_D^{27}$	=	Specific rotation at 27° and sodium D line (589 nm)
$\beta$	=	Beta
BSA	=	Bovine serum albumin
°C	=	Degree Celsius
calcd	=	Calculated
CA	=	Chemical Abstract
CC <sub>50</sub>	=	50% Cytotoxic Concentration
CDCl <sub>3</sub>	=	Deuterated chloroform
C <sub>5</sub> D <sub>5</sub> N	=	Deuterated pyridine
CF <sub>3</sub> COOH	=	Trifluoroacetic acid
CFU	=	Colony forming unit
CHCl <sub>3</sub>	=	Chloroform
CH <sub>2</sub> Cl <sub>2</sub>	=	Dichloromethane
cm <sup>-1</sup>	=	Reciprocal centimeter (unit of wave number)
<sup>13</sup> C NMR	=	Carbon-13 Nuclear Magnetic Resonance
CO <sub>2</sub>	=	Carbon dioxide
COX	=	Cyclooxygenase
COX-1	=	Cyclooxygenase-1
COX-1 <sup>-/-</sup>	=	Cyclooxygenase-1 null cell
COX-2	=	Cyclooxygenase-2
COX-2 <sup>-/-</sup>	=	Cyclooxygenase-2 null cell
CPMA	=	Count per minute average
2-D NMR	=	Two Dimensional Nuclear Magnetic Resonance
<i>d</i>	=	Doublet (for NMR spectra)
<i>dd</i>	=	Doublet of doublets (for NMR spectra)
DEPT	=	Distortionless Enhancement by Polarization Transfer
DMEM	=	Dulbecco's modified medium
DMSO	=	Dimethyl sulfoxide
DMSO- <i>d</i> <sub>6</sub>	=	Deuterated dimethyl sulfoxide
$\delta$	=	Chemical shift

**LIST OF ABBREVIATIONS AND SYMBOLS (continued)**

EIMS	=	Electron Impact Mass Spectrometry
ESIMS	=	Electrospray Ionization Mass Spectrometry
EtOAc	=	Ethyl acetate
EtOH	=	Ethanol
Et <sub>3</sub> SiH	=	Triethylsilane
g	=	Gram
GC	=	Gas Chromatography
hr	=	Hour
<sup>1</sup> H NMR	=	Proton Nuclear Magnetic Resonance
HMBC	=	<sup>1</sup> H-detected Heteronuclear Multiple Bond Coherence
HMQC	=	<sup>1</sup> H-detected Heteronuclear Multiple Quantum Coherence
<sup>3</sup> H-PGE <sub>2</sub>	=	Tritium prostaglandin E <sub>2</sub>
H <sub>2</sub> O	=	Water
HRFABMS	=	High Resolution Fast Atom Bombardment Mass Spectrometry
HSV-1	=	Herpes Simplex Virus type 1
HSV-2	=	Herpes Simplex Virus type 2
Hz	=	Hertz
IC <sub>50</sub>	=	Median Inhibitory Concentration
IR	=	Infrared Spectrum
<i>J</i>	=	Coupling constant
Kg	=	Kilogram
KH <sub>2</sub> PO <sub>4</sub>	=	Potassium biphosphate
K <sub>2</sub> HPO <sub>4</sub>	=	Potassium phosphate
l	=	Liter
μg	=	Microgram
μl	=	Microliter
μM	=	micromolar
λ <sub>max</sub>	=	Wavelength at maximal absorption
ε	=	Molar absorptivity

## LIST OF ABBREVIATIONS AND SYMBOLS (continued)

$M^+$	=	Molecular ion
m	=	Multiplet (for NMR spectra)
MHA	=	Mueller Hinton agar
MeOH	=	Methanol
mg	=	Milligram
$[M+H]^+$	=	Protonated molecular ion
MHz	=	Megahertz
min	=	Minute
ml	=	Milliliter
MW	=	Molecular weight
$m/z$	=	Mass to charge ratio
MS	=	Mass Spectrometry
NaCl	=	Sodium chloride
NaHCO <sub>3</sub>	=	Sodium bicarbonate
nm	=	Nanometer
NMR	=	Nuclear Magnetic Resonance
NOE	=	Nuclear Overhauser Effect
NS-398	=	N-(2-[Cyclohexyloxy]-4-nitrophenyl)methanesulfonamide
NSS	=	Normal saline solution
PFU	=	Plaque forming unit
PGE <sub>2</sub>	=	Prostaglandin E <sub>2</sub>
ppm	=	Part per million
pyridine- <i>d</i> <sub>5</sub>	=	Deuterated pyridine
RIA	=	Radioimmunoassay
SDA	=	Sabouraud dextrose agar
spp.	=	Species
$\nu_{\max}$	=	Wave number at maximal absorption
s	=	Singlet (for NMR spectra)
TLC	=	Thin Layer Chromatography
UV-VIS	=	Ultraviolet and Visible Spectrophotometry