

สารออกฤทธิ์ทางชีวภาพจากฝักพุดทุ่ง *Holarrhena curtisii* และผลแดงแพะ *Gymnema
griffithii*



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BIOACTIVE COMPOUNDS FROM *Holarrhena curtisii* PODS AND *Gymnema griffithii*
FRUITS

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A Dissertation Submitted in Partial Fulfillment of the Requirements
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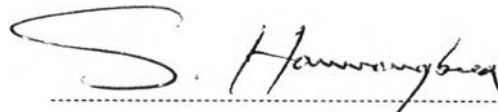
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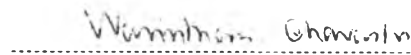
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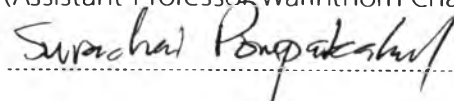
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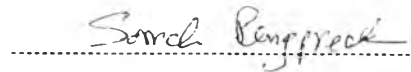
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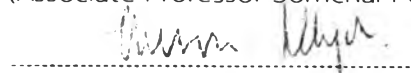
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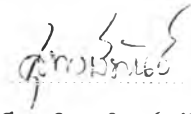
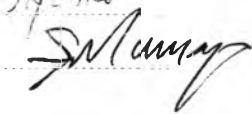


สุพงษ์พันธ์ ศรีสุริฉันทน์ : สารออกฤทธิ์ทางชีวภาพจากฝักพุดทุ่ง *Holarrhena curtisii* และผลแดงแพะ *Gymnema griffithii*. (BIOACTIVE COMPOUNDS FROM *Holarrhena curtisii* PODS AND *Gymnema griffithii* FRUITS) อ.ที่ปริกษาวิทยานินพนธ์หลัก: รศ. ดร.สุรัชย์ พรภคกุล , 252 หน้า.

งานวิจัยนี้ได้ศึกษาองค์ประกอบทางเคมีของแดงแพะ *G. griffithii* และพุดทุ่ง *H. curtisii* ซึ่งตั้งอยู่ในวงศ์ตีนเป็ด ในการแยกสารโดยเทคนิคทางโครมาโทกราฟีจากสารสกัดเมทานอลของผลแดงแพะ สามารถแยกสารใหม่ในกลุ่มของสเตอรอยด์ไกลโคไซด์ที่มีหมู่เออร์โธอะซิเตทบนโครงสร้างเพรกเนนสเตอรอยด์ได้ 8 สาร คือ gymnemogriffithoside A-H การพิสูจน์โครงสร้างทางเคมีของสารบริสุทธิ์ที่แยกได้โดยวิธีการทางสเปกโทรสโกปี (1D, 2D NMR, HR-ESIMS และ FTIR) พบว่าสารที่แยกได้นั้นมีโครงสร้างในส่วนสเตอรอยด์อยู่ 2 ลักษณะ สาร gymnemogriffithoside A-F มีโครงสร้างสเตอรอยด์แบบ dihydrosarcostin-7,14,18-orthoacetate และสาร gymnemogriffithoside G และ H มีโครงสร้างสเตอรอยด์แบบ dihydrosarcostin-14,17,18-orthoacetate การพิสูจน์โครงสร้างสัมบูรณ์ส่วนสเตอรอยด์ของสาร gymnemogriffithoside A โดยวิธีการทางสเปกโทรสโกปีร่วมกับวิธีการของ Mosher พบว่าสารมีลักษณะโครงสร้างสัมบูรณ์แบบ 3S*, 5S*, 8S*, 9R*, 10S*, 12R*, 13R*, 14R*, 17S*, 20S* สารสเตอรอยด์ไกลโคไซด์ทั้งหมดที่แยกได้จากผลแดงแพะ มีน้ำตาลไตรแซ็กคาไรด์ 2 ลักษณะ คือ $O\beta$ -D-thevetopyranosyl-(1 \rightarrow 4)- $O\beta$ -D-oleandropyranosyl-(1 \rightarrow 4)- $O\beta$ -D-digitoxopyransyl และ $O\beta$ -D-thevetopyranosyl-(1 \rightarrow 4)- $O\beta$ -D-canaropyranosyl-(1 \rightarrow 4)- $O\beta$ -D-digitoxopyranosyl ต่อเข้ากับคาร์บอนตำแหน่งที่ 3 ของสเตอรอยด์ จากการนำสารบริสุทธิ์ที่แยกได้ไปทำการทดสอบความเป็นพิษต่อเซลล์มะเร็ง 5 ชนิด (มะเร็งเต้านม BT 474, มะเร็งปอด Chago, มะเร็งตับ Hep-G2, มะเร็งกระเพาะอาหาร KATO-III และ มะเร็งลำไส้ SW620) พบว่า สาร gymnemogriffithoside C และ F ที่มีหมู่ ทิกโกอิล แทนที่อยู่บนคาร์บอนอะตอมตำแหน่งที่ 20 ของสเตอรอยด์ แสดงความเป็นพิษต่อเซลล์มะเร็งเล็กน้อยในช่วงความเข้มข้น 40-70 ไมโครโมลาร์ ในขณะที่สารตัวอื่นไม่แสดงความเป็นพิษต่อเซลล์มะเร็ง แสดงให้เห็นว่าหมู่ทิกโกอิลมีความสัมพันธ์ต่อความเป็นพิษต่อเซลล์มะเร็งของสารในกลุ่ม สเตอรอยด์ไกลโคไซด์ นอกจากนี้ยังได้นำสารบริสุทธิ์ที่แยกได้ไปทำการทดสอบฤทธิ์ในการยับยั้ง เอมไซม์แอลฟา กลูโคซิเดสโดยพบว่า สารที่แยกได้ไม่มีฤทธิ์ในการยับยั้งเอมไซม์แอลฟา กลูโคซิเดส ในขณะที่สารสเตอรอยด์ ที่ได้จากการตัดสายน้ำตาลออกจาก gymnemogriffithoside A และ G จะมีฤทธิ์ในระดับปานกลาง แสดงให้เห็นว่าโครงสร้างในส่วนของน้ำตาลจะส่งผลให้ฤทธิ์ในการยับยั้งเอมไซม์แอลฟา กลูโคซิเดสลดลง

ในการแยกสารจากฝักพุดทุ่ง สามารถแยกสารใหม่ในกลุ่มของไตรเทอร์ปีนอยด์ได้ 2 ชนิด คือ 3β -hydroxy-11 α -hydroperoxyursan-12-en-28-oic acid และ 3β -hydroxy-11 α -hydroperoxyolean-12-en-28-oic acid และสารที่มีการรายงานมาก่อน 12 ชนิดคือ squalene, α -amyrin acetate, β amyrin acetate, lupeol acetate, lupeol, cyclooleucalenol, 24-methylenepollinastanol, lanosta-7,24-dien- 3β ol, ursolic acid, oleanolic acid, (-)-catechin และ (-)-gallocatechin จากการนำสารที่แยกได้จากฝักพุดทุ่งไปทดสอบฤทธิ์การยับยั้ง เอมไซม์แอลฟา กลูโคซิเดสพบว่าสาร ursolic acid, oleanolic acid, 3β -hydroxy-11 α -hydroperoxyursan-12-en-28-oic acid และ 3β -hydroxy-11 α -hydroperoxyolean-12-en-28-oic acid มีฤทธิ์ในการยับยั้งเอมไซม์แอลฟา กลูโคซิเดสที่ดี ในช่วงความเข้มข้น 10-80 ไมโครโมลาร์ เมื่อเทียบกับสารมาตรฐานที่ใช้ในการทดสอบ acarbose (IC₅₀ = 884.6 ไมโครโมลาร์)

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SUPHONGPHAN SRISURICHAN: BIOACTIVE COMPOUNDS FROM *Holarrhena curtisii* PODS AND *Gymnema griffithii* FRUITS. ADVISOR: ASSOC. PROF. SURACHAI PORNPAAKAKUL, Ph.D., 252 PP.

In a phytochemical investigation of bioactive compounds from Apocynaceae family, *Gymnema griffithii* Craib. and *Holarrhena curtisii* King & Gamble. were selected to investigate their phytochemical components. The chromatographic separation of methanolic extract of *G. griffithii* fruits was performed and led to the isolation of 8 new pregnane-type steroidal glycosides substituted with orthoacetate groups, named gymnemogriffithoside A-H. Their structures were established by spectroscopic analysis (1D and 2D NMR, HR-ESIMS and FTIR). The steroidal skeleton of gymnemogriffithoside A-F was deduced to be a dihydrosarcostin-8,14,18-orthoacetate while the steroidal skeleton of gymnemogriffithoside G and H was deduced to be a dihydrosarcostin-14,17,18-orthoacetate. The absolute stereochemistry of the steroidal skeleton of gymnemogriffithoside A was established as 3S*, 5S*, 8S*, 9R*, 10S*, 12R*, 13R*, 14R*, 17S*, 20S* using both spectroscopic and Mosher's method. All eight steroidal glycosides isolated from *G. griffithii* fruits had two types of trisaccharide moieties, O-β-D-thevetopyranosyl-(1→4)-O-β-D-oleandropyranosyl-(1→4)-O-β-D-digitoxopyransyl and O-β-D-thevetopyranosyl (1→4)-O-β-D-canaropyranosyl-(1→4)-O-β-D-digitoxopyranosyl at the C-3 of their aglycones. All compounds were evaluated for their in vitro cytotoxic effects against five human tumor cell lines (BT 474, Chago, Hep-G2, KATO-III and SW620). Gymnemogriffithoside C and F, containing a tigloyl moiety at C-20, showed a slight cytotoxicity against all tested cell lines in 40-70 μM range while the others were inactive at 100 μM, suggesting that the presence of the tigloyl moiety influenced the cytotoxic activity of the compounds in this type. In addition, the α-glucosidase inhibitory activity of all compounds was also tested. However, only aglycone of gymnemogriffithoside A and G showed moderate α-glucosidase inhibitory activity.

The pods of *H. curtisii* provided 2 new triterpenoids identified as 3β-hydroxy-11α-hydroperoxyursan-12-en-28-oic acid and 3β-hydroxy-11α-hydroperoxyolean-12-en-28-oic acid, along with 12 know compounds, squalene, α-amyrin acetate, β-amyrin acetate, lupeol acetate, lupeol, cycloeucalenol, 24-methylenepollinastanol, lanosta-7,24 dien-3β-ol, ursolic acid, oleanolic acid, (-)-catechin and (-)-gallocatechin. All compounds, except squalene, were evaluated for their α-glucosidase inhibitory activity. Among of them, ursolic acid, oleanolic acid, 3β-hydroxy-11α-hydroperoxyursan-12-en-28-oic acid and 3β-hydroxy-11α-hydroperoxyolean-12-en-28-oic acid, processed with pentacyclic triterpenoid acid skeleton showed significant α-glucosidase inhibitory activity in the range of 10-80 μM comparable to standard control acarbose (IC₅₀ = 884.6 μM).

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LIST OF ABBREVIATIONS

$[\alpha]_D^{25}$	Specific rotation at 25 °C and using the wavelength of light at 589 nm (sodium D line) for the observation
δ	Chemical shift (NMR)
δ_C	Chemical shift of carbon (NMR)
δ_H	Chemical shift of proton (NMR)
λ_{\max}	Wavelength of maximum absorption (UV)
μL	Microliter (s)
μg	Microgram (s)
μM	Micromolar
μm	Micrometer (s)
ν_{\max}	Reciprocal wavelength at the highest signal in IR spectroscopy
°C	Degree Celsius
^1H NMR	Proton nuclear magnetic resonance spectroscopy
^{13}C NMR	Carbon-13 nuclear magnetic resonance spectroscopy
2D NMR	Two-dimensional nuclear magnetic resonance spectroscopy
Ac	Acetyl
amu	Atomic mass unit
A_0	Absorbance of the control
A_1	Absorbance of the test sample
ATR-FTIR	Attenuated total reflectance-Fourier transformed infrared
$\text{Ba}(\text{OH})_2$	Barium hydroxide
br	broad (NMR)
Bz	Benzyl
c	Concentration
calcd	Calculated
Can	Canarsoe

cat.	Catalyst
CC	Column chromatography
CDCl ₃	Deuterated chloroform
CD ₃ OD	Deuterated methanol
Ce(SO ₄) ₂	Cerium(IV) sulfate
CH ₂ Cl ₂	Dichloromethane
cm	Centimeter (s)
cm ⁻¹	Unit of reciprocal wavelength (or wavenumber) in IR
COSY	Correlation spectroscopy (NMR)
d	Doublet (NMR)
D	Dextrorotatory rotation (turned clockwise of the plane of polarization)
D ₂ O	Deuterium oxide
dd	Doublet of doublet (NMR)
ddd	Doublet of doublet of doublet (NMR)
Dig	Digitoxose
DMF	<i>N,N</i> -dimethylformamide
DMFCl	Chloride- <i>N,N</i> -dimethylformamide
DMSO	Dimethyl sulfoxide
dq	Doublet of quartet (NMR)
dt	Doublet of triplet (NMR)
EtOH	Ethanol
EtOAc	Ethyl acetate
v/v	Volume per volume
g	Gram (s)
h	Hour (s)
H ₂ SO ₄	Sulfuric acid
H ₂ O	Water
Hz	Hertz (s)

HMBC	Heteronuclear multiple-bond correlation spectroscopy
HRESIMS	High-resolution electrospray ionization mass spectrometry
HPLC	High-performance liquid chromatography
HSQC	Heteronuclear single quantum correlation spectroscopy
sp.	Species
sp ³	sp ³ hybridisation
MTPA	α -methoxy- α -trifluoromethylphenylacetic acid
MTPACL	α -methoxy- α -trifluoromethylphenylacetyl chloride
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
IC ₅₀	Half maximal inhibitory concentration
<i>J</i>	Coupling constant
L	Liter (s)
L	Levorotatory rotation
lit	Literature
m	Multiplet (NMR)
m	Meter (s)
M	Molar
MeOH	Methanol
mg	Milligram (s)
MHz	Megahertz (s)
mm	Millimeter (s)
mM	Millimolar (s)
mmol	Millimole (s)
mp.	Melting point
<i>m/z</i>	Mass per charge ratio
[M+H] ⁺	Protonated molecule ion
[M+Na] ⁺	Pseudo-molecular ion
nm	Nanometer (s)

$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$	Ammonium molybdate
NOESY	Nuclear Overhauser effect spectroscopy
Ole	Oleandrose
PNPG	<i>p</i> -nitrophenyl- α -D-glucopyranoside
ppm	Parts per million
q	Quartet (NMR)
qd	Quartet of doublet (NMR)
RP-18	Reverse phase C18 column
<i>R</i>	<i>Rectus</i> for right (configuration)
rt	Room temperature
s	Singlet (NMR)
<i>S</i>	<i>Sinister</i> for left (configuration)
t	Triplet (NMR)
td	Triplet of doublet (NMR)
Thv	Thevetose
Tig	Tigloyl
TLC	Thin layer chromatography
t_R	Retention time
U	Unit