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ANTI-HEPATOTOXIC SUBSTANCES FROM *THUNBERGIA LAURIFOLIA* AND
PHYLLANTHUS AMARUS

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A Dissertation Submitted in Partial Fulfillment of the Requirements
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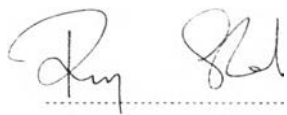
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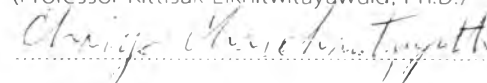
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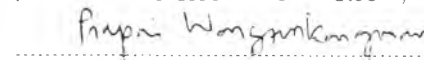
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ผกาภรณ์ เกตุมงคลสิทธิ์ : สารที่มีฤทธิ์ต้านความเป็นพิษต่อตับจากรางจืดและลูกใต้ใบ.
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การศึกษาของค้ประกอบทางเคมีจากใบรางจืดสามารถแยกสารที่เคยมีรายงานแล้วใน
กลุ่มฟีนอลิก ได้ 2 ชนิด คือ กรดคาเฟอิก และกรดโรสมารินิก โดยทำการพิสูจน์โครงสร้างทางเคมี
ด้วยการวิเคราะห์เชิงสเปกตรัมจาก MS และ NMR ร่วมกับการเปรียบเทียบจากข้อมูลที่เคยมี
รายงานมาก่อน จากนั้นได้ทำการศึกษาฤทธิ์ต้านความเป็นพิษต่อเซลล์มะเร็งระดับชนิด HepG2 ของ
กรดโรสมารินิกที่ถูกเหนี่ยวนำด้วยเอทานอล พบว่าการให้กรดโรสมารินิกก่อนเหนี่ยวนำด้วยเอทา
นอลเป็นเวลา 24 ชม.นั้น กรดโรสมารินิกแสดงฤทธิ์ปกป้องเซลล์ที่ถูกเหนี่ยวนำด้วยเอทานอลเป็น
เวลา 6 และ 12 ชม. นอกจากนี้ยังทำการศึกษาเซลล์มะเร็งระดับที่ถูกเหนี่ยวนำด้วยเอทานอล
ก่อนหลังจากนั้นจึงได้รับกรดโรสมารินิก พบว่าเมื่อเซลล์ถูกเหนี่ยวนำด้วยเอทานอลก่อนเป็นเวลา
12 ชม. กรดโรสมารินิกแสดงการรอดของเซลล์เพิ่มขึ้นใกล้เคียงกับกลุ่มควบคุมที่ไม่ได้ถูกเหนี่ยวนำ
ด้วยเอทานอล แสดงให้เห็นว่ากรดโรสมารินิกจากใบรางจืดเป็นสารที่มีฤทธิ์ต้านความเป็นพิษต่อ
ตับ

ทำการศึกษาของค้ประกอบทางเคมีของต้นลูกใต้ใบ สามารถแยกสารที่เคยมีรายงานแล้ว
ในกลุ่มลิแกน คือ ฟิลแลนทิน และเมื่อนำไปศึกษาฤทธิ์ต้านความเป็นพิษต่อเซลล์มะเร็งระดับชนิด
HepG2 ของสารฟิลแลนทินที่ถูกเหนี่ยวนำด้วยเอทานอล พบว่าการให้ฟิลแลนทินก่อนเป็นเวลา
24 ชม.นั้น ฟิลแลนทินแสดงฤทธิ์ปกป้องเซลล์ที่ถูกเหนี่ยวนำด้วยเอทานอลเป็นเวลา 12 ชม. อีก
ทั้งยังพบว่าเมื่อได้รับเอทานอลก่อนเป็นเวลา 12 ชม. ฟิลแลนทินแสดงการรอดของเซลล์เพิ่มขึ้น
ใกล้เคียงกับกลุ่มควบคุมที่ไม่ได้รับเอทานอล แสดงให้เห็นว่า ฟิลแลนทินจากต้นลูกใต้ใบเป็นสารที่
มีฤทธิ์ต้านความเป็นพิษของเอทานอลต่อตับ นอกจากนี้ยังสามารถนำฟิลแลนทินมาใช้เป็นสาร
มาตรฐานในการตรวจสอบสมุนไพรลูกใต้ใบและใช้ตรวจวิเคราะห์คุณภาพของผลิตภัณฑ์ทาง
การค้าจากสมุนไพรลูกใต้ใบ โดยใช้วิธี Thin Layer Chromatography Image Analysis เพื่อ
ความถูกต้องและประสิทธิภาพของสมุนไพร

ภาควิชา เภสัชเวทและเภสัชพฤกษศาสตร์

สาขาวิชา เภสัชเวท

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ลายมือชื่อนิสิต ผกาภรณ์ เกตุมงคลสิทธิ์

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PAKABHORN KETMONGKHONSIT: ANTI-HEPATOTOXIC SUBSTANCES FROM
THUNBERGIA LAURIFOLIA AND *PHYLLANTHUS AMARUS*. ADVISOR: ASSOC. PROF.
CHAIYO CHAICHANTIPYUTH, Ph.D., CO-ADVISOR: ASSOC. PROF. POL. CAPT.. SUCHADA
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Chemical investigation of *Thunbergia laurifolia* Linn. leaves (Family Acanthaceae) led to the isolation of two known phenolic compounds including, caffeic acid and rosmarinic acid. The chemical structures were determined by MS and NMR spectroscopic analyses and were compared with previously report data. In this study, anti-hepatotoxic activity of rosmarinic acid was investigated against ethanol-induced hepatotoxicity in HepG2 cell line. For the pretreatment study, rosmarinic acid exhibited protective effect on HepG2 cells induced by ethanol for 6 and 12 h. For the post-treatment study, HepG2 cells were treated with ethanol, and then with rosmarinic acid (1, 5, 10 μ M). Rosmarinic acid showed significant increased cell viability of HepG2 cells treated with ethanol for 12 h. These results suggested the anti-hepatotoxic activity of rosmarinic acid against ethanol-induced HepG2 cells damage.

From the whole plant of *Phyllanthus amarus* Schum.&Thonn. (Euphorbiaceae) one known lignan compound, phyllanthin, was isolated. In the present study, anti-hepatotoxic activity of phyllanthin was investigated against ethanol-induced hepatotoxicity in HepG2 cell line. For the pretreatment study, the protective effect of phyllanthin was observed on HepG2 cells induced by ethanol for 12 h. For the post-treatment study phyllanthin showed significant increased cell viability of HepG2 cells treated with ethanol for 12 h. These results suggested the potential anti-hepatotoxic activity of phyllanthin against ethanol-induced toxicity causing HepG2 cells damage. Additionally, phyllanthin, was found to be a suitable biochemical marker for the assessment of *P. amarus* plant materials and commercial herbal drugs quality. A TLC-image analysis method using a computer software technology was developed and validated for the quantitation of phyllanthin in *P. amarus* plant materials and its commercial herbal drugs.

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LIST OF ABBREVIATIONS

ADH	=	Alcohol dehydrogenase
ALD	=	Alcoholic Liver Disease
ALDH	=	Aldehyde dehydrogenase
α	=	Alpha
β	=	Beta
$^{\circ}\text{C}$	=	Degree Celsius
Calc	=	Calculate
CC	=	Column chromatography
CCl_4	=	Carbon tetrachloride
CDCl_3	=	Deuterated chloroform
CD_3OD	=	Deuterated methanol
CHCl_3	=	Chloroform
CH_2Cl_2	=	Dichloromethane
cm	=	Centimeter
cm^{-1}	=	Reciprocal centimeter (unit of wave number)
^{13}C NMR	=	Carbon-13 Nuclear Magnetic Resonance
CO_2	=	Carbondioxide
CYP2E1	=	Cytochrome P450 2E1
<i>d</i>	=	Doublet (for NMR spectra)
<i>dd</i>	=	Doublet of doublets (for NMR spectra)
DMSO	=	Dimethyl sulfoxide
δ	=	Chemical shift
ϵ	=	Molar absorptivity
ESI-MS	=	Electrospray Ionization Mass Spectrometry
EtOH	=	Ethanol
EtOAc	=	Ethyl acetate



g	=	Gram
h	=	Hour
HepG2	=	Human liver carcinoma cell line
^1H NMR	=	Proton Nuclear Magnetic Resonance
HPLC	=	High Performance Liquid Chromatography
HPTLC	=	High Performance Thin Layer Chromatography
HR	=	High Resolution
Hz	=	Hertz
J	=	Coupling constant
Kg	=	Kilogram
L	=	Liter
λ_{max}	=	Wavelength at maximal absorption
μg	=	Microgram
$\mu\text{g/ml}$	=	Microgram per milliliter
μl	=	Microliter
$[\text{M}]^+$	=	Molecular ion
$[\text{M}+\text{H}]^+$	=	Pseudomolecular ion
$[\text{M}+\text{Na}]^+$	=	Pseudomolecular ion
m	=	Multiplet (for NMR spectra)
MeOH	=	Methanol
mg	=	Milligram
MHz	=	Megahertz
min	=	Minute
ml	=	Milliliter
mm	=	Millimeter
MS	=	Mass Spectrometry
MW	=	Molecular weight
m/z	=	Mass to charge ratio



Na	=	Sodium
NADH	=	Nicotinamide adenine dinucleotide
nm	=	Nanometer
NMR	=	Nuclear Magnetic Resonance
PBS	=	Phosphate buffered saline
ppm	=	Part-per-million
ROS	=	Reactive Oxygen Species
s	=	Singlet (for NMR spectra)
TLC	=	Thin Layer Chromatography
TOF	=	Time of flight
UV	=	Ultraviolet

