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TOPOISOMERASE I INHIBITORY ACTIVITY FROM THAI MEDICINAL PLANTS IN
YEAST CELL-BASED ASSAY

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Pharmacognosy

Department of Pharmacognosy and Pharmaceutical Botany

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ปริณดา อุปธารปริชา: การทดสอบฤทธิ์ยับยั้งโทโปไอโซเมอเรส I จากพืชสมุนไพรไทยโดยใช้เซลล์ยีสต์. (TOPOISOMERASE I INHIBITORY ACTIVITY FROM THAI MEDICINAL PLANTS IN YEAST CELL-BASED ASSAY) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ร.ต.อ.หญิง ดร.สุชาดา สุขหรั่ง, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ.อารีรัตน์ ลออปักษา, 103 หน้า.

เอนไซม์โทโปไอโซเมอเรส I เป็นเป้าหมายหนึ่งของการรักษาโรคมะเร็งด้วยวิธีเคมีบำบัดในปัจจุบัน งานวิจัยนี้จึงมุ่งหมายที่จะค้นหาสารออกฤทธิ์จากธรรมชาติที่มีฤทธิ์ยับยั้งโทโปไอโซเมอเรส I จากสมุนไพรไทยที่มีการใช้สืบทอดกันมาโดยภูมิปัญญาชาวบ้านมากกว่าก่อนว่ามีฤทธิ์เป็นพิษต่อเซลล์ โดยใช้เซลล์ยีสต์ ยีสต์ที่ใช้ในการคัดกรองเป็นยีสต์ที่ได้รับการถ่ายโอนยีนโทโปไอโซเมอเรส I จากพืช *Arabidopsis thaliana* ซึ่งสามารถควบคุมการแสดงออกของยีนโทโปไอโซเมอเรส I ได้ จากการคัดกรองฤทธิ์ยับยั้งโทโปไอโซเมอเรส I ของสมุนไพรไทยจำนวน 27 ชนิด พบว่าสารสกัดเอทานอลจากส่วนของสมุนไพรไทยจำนวน 6 ชนิด ได้แก่ ใบและรากของทองพันชั่ง ต้นพญามุตติ หัวบอระเพ็ด พุงช้าง เหง้าขมิ้นชัน และเหง้าขมิ้นอ้อย มีฤทธิ์ยับยั้งโทโปไอโซเมอเรส I สารสกัดจากต้นพญามุตติ ถูกเลือกเพื่อทำการศึกษาต่อให้ทราบถึงสารสำคัญที่มีฤทธิ์ดังกล่าวเนื่องจากยังไม่เคยมีรายงานถึงฤทธิ์การยับยั้งโทโปไอโซเมอเรส I ของต้นนี้มาก่อน

จากการศึกษาองค์ประกอบทางเคมีของต้นพญามุตติ โดยวิธีคัดเลือกจากสิ่งสกัดที่มีฤทธิ์ยับยั้งโทโปไอโซเมอเรส I โดยใช้เซลล์ยีสต์ สามารถแยกสารกลุ่ม sesquiterpene lactone ได้ 2 ชนิด คือ (-)-frullanolide และ (-)-7 α -hydroxyfrullanolide การพิสูจน์โครงสร้างทางเคมีของสารทั้งสองอาศัยเทคนิคทางสเปกโตรสโกปีร่วมกับการเปรียบเทียบข้อมูลจากงานวิจัยที่มีรายงานมาก่อน สารทั้งสองชนิดมีฤทธิ์ต้านเซลล์มะเร็งในช่องปากชนิด KB เซลล์มะเร็งเต้านมชนิด MCF-7 และเซลล์มะเร็งปอดชนิด NCI-H187 ในหลอดทดลอง ข้อมูลที่ได้เป็นการรายงานครั้งแรกถึงกลไกการออกฤทธิ์ในการเป็นพิษต่อเซลล์ของสารสำคัญสองชนิดจากต้นพญามุตติ

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PARINDA UPPATANPREECHA: TOPOISOMERASE I INHIBITORY ACTIVITY FROM THAI MEDICINAL PLANTS IN YEAST CELL-BASED ASSAY. THESIS ADVISOR: ASST. PROF. SUCHADA SUKRONG, Ph.D., THESIS COADVISOR: ASSOC. PROF. AREERAT LAORPAKSA, 103 pp.

Topoisomerase I is an important therapeutic target in cancer chemotherapy. We are interested in the continuing discovery of new topoisomerase I-targeted agents from Thai medicinal plants with previously reported as cytotoxicity by using Yeast cell-based assay. The transformant yeast containing *Arabidopsis thaliana* topoisomerase I gene was developed to achieve controllable expression of the topoisomerase I enzyme. From the screening of 27 Thai medicinal plants, 6 ethanolic plant-part extracts; root and leaf of *Rhinacanthus nasutus*, whole plant of *Grangea maderaspatana*, caudex of *Stephania suberosa*, rhizome of *Curcuma longa* and rhizome of *Curcuma zedoaria*, showed inhibitory activities of enzyme topoisomerase I. The extract of *G. maderaspatana* was selected for further study to determine the bioactive compounds since it has never been reported for its topoisomerase I inhibitory activity before.

Bioassay-guided fractionation from whole plant of *G. maderaspatana* by yeast cell-based assay led to the identification of two bioactive compounds. They are sesquiterpene lactone namely, (-)-frullanolide and (-)-7 α -hydroxyfrullanolide. Their structures were determined by spectroscopic methods and compared with previous reports. These compounds exhibited topoisomerase I inhibitory activities by yeast cell-based assay. They also processed *in vitro* anti-cancer activities against human cell lines, KB oral cavity cancer, MCF7 breast cancer, and NC-H187 small lung cancer. This is the first report of cytotoxicity due to topoisomerase I inhibitory mechanism of two compounds from *G. maderaspatana*.

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LIST OF ABBREVIATIONS

%	= percent (part per 100); percentage
μg	= microgram(s)
μl	= microliter(s)
μM	= micromolar
/	= per
λ_{max}	= wavelength at maxima absorption
ν_{max}	= wave number at maximum absorption
α	= alpha
β	= beta
ϵ	= molar absorptivity
$[\alpha]^{20}_{\text{D}}$	= specific rotation at 20°C and sodium D line (589 nm)
ATCC	= American Type Culture Collection
br	= broad (for NMR spectra)
C	= concentration
°C	= degree Celsius
CDCl_3	= deuterated chloroform
CFU	= colony forming units
CH_2Cl_2	= dichloromethane
cm	= centimeter(s)
$^{13}\text{C-NMR}$	= carbon-13 nuclear magnetic resonance
CPT	= camptothecin
d	= doublet (for NMR spectra)
dd	= doublet of doublet (for NMR spectra)
ddd	= doublet of doublet of doublet (for NMR spectra)
DEPT	= distortionless enhancement by polarization transfer
δ	= chemical shift

DMSO	= dimethylsulfoxide
DNA	= deoxyribonucleic acid
EIMS	= electron impact mass spectrometry
<i>et. al</i>	= et alii
EtOAc	= ethyl acetate
g	= gram(s)
¹ H-NMR	= proton nuclear magnetic resonance
hr	= hour(s)
Hz	= hertz
IC ₅₀	= inhibitory concentration 50%
IR	= infrared
<i>J</i>	= coupling constant
kg	= kilogram(s)
L	= liter(s)
m	= meter(s)
M ⁺	= molecular ion
mg	= milligram
MHz	= mega hertz
MeOH	= methanol
min	= minute(s)
ml	= milliliter
mm	= millimeter
mM	= millimolar
MS	= mass spectrum
MW	= molecular weight
m/z	= mass to charge ratio
O.D.	= optical density
pH	= the negative logarithm of the concentration of hydrogen ions
ppm	= part per million

q	= quartet (for NMR spectra)
R _f	= retention factor
s	= singlet (for NMR spectra)
S.C. ura ⁻ media	= synthetic complete media lacking uracil
t	= triplet (for NMR spectra)
TLC	= thin layer chromatography
top1	= topoisomerase I
UV	= ultraviolet light
v/v	= volume/volume (concentration)
w/v	= weight/volume (concentration)
w/w	= weight/weight (concentration)
YPD media	= Yeast Peptone Dextrose media