

สารที่มีฤทธิ์เป็นพิษต่อเซลล์มะเร็งเม็ดเลือดขาวจากราเอนโดไฟต์ *Phomopsis* sp.  
ที่แยกได้จากซิงเฮา

นางสาวปุกัญญา งานกรณาธิการ



ห้องสมุดคณะเภสัชศาสตร์  
จุฬาลงกรณ์มหาวิทยาลัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต  
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คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย  
ปีการศึกษา 2556  
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ANTILEUKEMIC COMPOUNDS FROM AN ENDOPHYTIC FUNGUS *PHOMOPSIS* SP.  
ISOLATED FROM *ARTEMISIA ANNUA* L.

Miss Punyisa Ngankaranatikarn

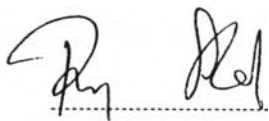


A Thesis Submitted in Partial Fulfillment of the Requirements  
for the Degree of Master of Science in Pharmacy Program in Pharmacognosy  
Department of Pharmacognosy and Pharmaceutical Botany  
Faculty of Pharmaceutical Sciences  
Chulalongkorn University  
Academic Year 2013  
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Thesis Title ANTILEUKEMIC COMPOUNDS FROM AN  
ENDOPHYTIC FUNGUS *PHOMOPSIS* SP. ISOLATED  
FROM *ARTEMISIA ANNUA* L.  
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Field of Study Pharmacognosy  
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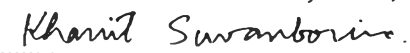
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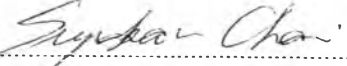
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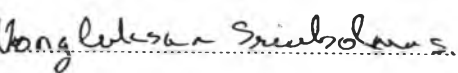
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ปญญา งานกรณาธิการ : สารที่มีฤทธิ์เป็นพิษต่อเซลล์มะเร็งเม็ดเลือดขาวจากราเอนโดไฟต์ *Phomopsis* sp. ที่แยกได้จากชิงเฮา. (ANTILEUKEMIC COMPOUNDS FROM AN ENDOPHYTIC FUNGUS *PHOMOPSIS* SP. ISOLATED FROM *ARTEMISIA ANNUA* L.) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ภก. ดร.คณิต สุวรรณบริรักษ์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ภญ. ดร.ทักษิณา ชวนอาษา, 72 หน้า.

ได้ทำการสกัดแยกสารเมแทบอไลต์ทุติยภูมิจากสารสกัดเอทิลอะซิเตทของน้ำหมักจากราเอนโดไฟต์ *Phomopsis* sp. AANN8 ที่แยกได้จากกิ่งของชิงเฮา (*Artemisia annua* L., family Asteraceae) โดยอาศัยฤทธิ์เป็นพิษต่อเซลล์มะเร็งเม็ดเลือดขาว ได้ทั้งหมด ๓ ชนิด ประกอบด้วยสารผสม ๒ diastereomers ของ *O*-1-(2-hydroperoxy-1,2-dimethylethyl)-*O*-1'-(mercaptoethyl-*S*-oxide)-peroxocarbonate ร่วมกับสารพวกฟีนอล ชื่อ tyrosol และสารที่พบในวัฏจักรเครบส์ ชื่อ succinic acid โครงสร้างทางเคมีของสารทั้งหมดที่แยกได้ถูกพิสูจน์โดยการวิเคราะห์ข้อมูลจาก อัลตราไวโอเลตสเปกโทรสโกปี อินฟราเรดสเปกโทรสโกปี แมสสเปกโทรเมทรี และนิวเคลียร์แมกเนติกเรโซแนนซ์สเปกโทรสโกปี ร่วมกับการเปรียบเทียบข้อมูลของสารที่เคยมีรายงานมาแล้ว นอกจากนี้สารที่แยกได้ยังถูกนำไปทดสอบฤทธิ์เป็นพิษต่อเซลล์มะเร็งเม็ดเลือดขาว THP-1 ในหลอดทดลองด้วยวิธีการทดสอบด้วยสาร sulforhodamine B โดยใช้สาร ellipticine เป็นสารควบคุมผลบวก มีค่า  $EC_{50}$  เท่ากับ ๗๙ ไมโครโมลาร์ ผลการทดสอบพบว่าสาร tyrosol และ succinic acid ไม่มีความเป็นพิษต่อเซลล์มะเร็งเม็ดเลือดขาว ในขณะที่สารผสม *O*-1-(2-hydroperoxy-1,2-dimethylethyl)-*O*-1'-(mercaptoethyl-*S*-oxide)-peroxocarbonate แสดงฤทธิ์เป็นพิษต่อเซลล์มะเร็งเม็ดเลือดขาวอย่างอ่อน มีค่า  $EC_{50}$  เท่ากับ ๑๓๑ ไมโครโมลาร์

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ภาควิชา เกษษเขตและเกษษพฤษศาสตร์  
สาขาวิชา เกษษเขต  
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# # 5476238433 : MAJOR PHARMACOGNOSY

KEYWORDS: PHOMOPSIS SP. AANN8 / ARTEMISIA ANNUA L. / ENDOPHYTIC FUNGUS / ANTILEUKEMIC ACTIVITY

PUNYISA NGANKARANATIKARN: ANTILEUKEMIC COMPOUNDS FROM AN ENDOPHYTIC FUNGUS *PHOMOPSIS* SP. ISOLATED FROM *ARTEMISIA ANNUA* L.. ADVISOR: KHANIT SUWANBORIRUX, Ph.D., CO-ADVISOR: ASST. PROF. TAKSINA CHUANASA, Ph.D., 72 pp.

Three secondary metabolites named a mixture of two diastereomers *O*-1-(2-hydroperoxy-1,2-dimethylethyl)-*O*-1'-(mercaptoethyl-*S*-oxide)-peroxocarbonate, together with a phenolic metabolite, tyrosol, and a Kreb's cycle metabolite, succinic acid, were isolated from the ethyl acetate extract of the fermentation broth of an endophytic fungus, *Phomopsis* sp. AANN8 by antileukemic-guided fractionation. The endophytic fungus was obtained from the twigs of a Thai medicinal plant, *Artemisia annua* L. (family Asteraceae). The identification and structure determination of the isolated compounds were elucidated by analyses of UV, IR, MS and NMR spectroscopic data along with comparison with previous publications. The isolated compounds were evaluated for *in vitro* antileukemic activity against THP-1 cell line by a sulforhodamine B colorimetric bioassay using ellipticine as a positive control with EC<sub>50</sub> 79 μM. The results showed that tyrosol and succinic acid had no antileukemic activity while the mixture of *O*-1-(2-hydroperoxy-1,2-dimethylethyl)-*O*-1'-(mercaptoethyl-*S*-oxide)-peroxocarbonate exhibited weak antileukemic activity with EC<sub>50</sub> 131 μM.



Department: Pharmacognosy and  
Pharmaceutical Botany

Field of Study: Pharmacognosy

Academic Year: 2013

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## ACKNOWLEDGEMENTS

I would like to express my deepest gratitude and sincere thanks to my thesis advisor, Dr.Khanit Suwanborirux and my thesis co-advisor, Assistant Professor Dr.Taksina Chuanasa of the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University. I greatly appreciate their valuable advice, useful guidance, kindness, constant encouragement, patience, and endless support throughout my research study.

I wish to express my thanks to the members of my thesis committee, including Professor Dr.Kittasak Likhitwitayawuid, Associate Professor Dr.Nongluksna Sriubolmas and Dr.Supakarn Chamni, for their critical review and constructive suggestions for this thesis.

I would also like to thank Assistant Professor Dr.Suthep Wiyakrutta and Mrs.Jitra Limthongkul of the Department of Microbiology, Faculty of Science, Mahidol University, and Associate Professor Dr.Nongluksna Sriubolmas of the School of Pharmacy, Eastern Asia University, for fungal material and antileukemic activity assay.

I would like to thank Professor Naoki Saito of the Meiji Pharmaceutical University, Japan, for mass measurement and the Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for supporting scientific equipment.

I am thankful to all staff members of the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for supplying chemicals and facilities.

I am grateful to all graduate students of the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for unforgettable friendship and kindness.

Finally, I would like to express my deepest appreciation to my family for their love, understanding and encouragement.



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## ABBREVIATIONS AND SYMBOLS

%	= Percentage
$\mu\text{g}$	= Microgram
$\mu\text{l}$	= Microliter
$\mu\text{M}$	= Micromolar
$^{13}\text{C-NMR}$	= Carbon-13 nuclear magnetic resonance
$^1\text{H-NMR}$	= Proton nuclear magnetic resonance
$^1\text{H-}^1\text{H COSY}$	= Homonuclear (Proton-Proton) correlation spectroscopy
A549	= Human lung adenocarcinoma epithelial cell line
Acetone- $d_6$	= Deuterated acetone
BC	= Human lymphoma cell line
BT220	= Human breast cancer cell line
br	= Broad (for NMR spectra)
Calc	= Calculated
CC	= Column chromatography
$\text{CD}_3\text{OD}$	= Deuterated methanol
$\text{CDCl}_3$	= Deuterated chloroform
$\text{CH}_2\text{Cl}_2$	= Dichloromethane
$\text{CHCl}_3$	= Chloroform
cm	= Centimeter



$\text{cm}^{-1}$	= Reciprocal centimeter (unit of wave number)
$\text{cm}^2$	= Square centimeter
$\text{CO}_2$	= Carbon dioxide
d	= Doublet (for NMR spectra)
DEPT	= Distortionless enhancement by polarization transfer
DMSO	= Dimethyl sulfoxide
dq	= Double quartets (for NMR spectra), doublet of quartets
$\epsilon$	= Molar absorptivity
$\text{EC}_{50}$	= Half maximal effective concentration
EIMS	= Electron impact mass spectrometry
ELISA	= Enzyme-linked immunosorbent assay
EtOAc	= Ethyl acetate
eV	= Electron volt
FABMS	= Fast atom bombardment mass spectrometry
g	= Gram
$\text{H}_2\text{O}$	= Water
HCT-116	= Human colorectal carcinoma cell line
HeLa	= Human cervical adenocarcinoma cell line
HepG2	= Human liver hepatocellular carcinoma cell line
HL251	= Human lung cancer cell line
HL-60	= Human promyelocytic leukemia cell line



HMBC	= $^1\text{H}$ -detected heteronuclear multiple bond correlation
HPLC	= High pressure liquid chromatography
HR	= High resolution
HSQC	= Heteronuclear single quantum coherence
HT-29	= Human colorectal adenocarcinoma cell line
Hz	= Hertz
IR	= Infrared
ITS	= Internal transcribed spacer
$J$	= Coupling constant
K562	= Human erythromyeloblastoid leukemia cell line
KB	= Human epidermoid carcinoma cell line
KBr	= Potassium bromide
l	= Liter
$m/z$	= Mass to charge ratio
MDA-MB-231	= Human breast adenocarcinoma cell line
MeOH	= Methanol
mg	= Milligram
MHz	= Mega Hertz
ml	= Milliliter
mm	= Millimeter
mM	= Millimolar



MS	= Mass spectrum
NCI-H187	= Human small-cell lung cancer cell line
nm	= Nanometer
NMR	= Nuclear magnetic resonance
NOESY	= Nuclear overhauser enhancement spectroscopy
nt	= Not tested
°C	= Degree Celsius
OD	= Optical density
P388	= Murine leukemia cell line
PC-3	= Human prostate adenocarcinoma cell line
PDA	= Potato dextrose agar
PDB	= Potato dextrose broth
ppm	= Part per million
psi	= Pound per square inch
s	= Singlet (for NMR spectra)
sp.	= Species
SRB	= Sulforhodamine B
t	= Triplet (for NMR spectra)
TCA	= Trichloroacetic acid
THP-1	= Human acute monocytic leukemia cell line
TLC	= Thin layer chromatography





UV	= Ultraviolet-visible
YSB	= Yeast extract sucrose broth
$\beta$	= Beta
$\delta$	= Chemical shift
$\lambda_{\max}$	= Wavelength at maximal absorption
$\nu_{\max}$	= Wave number at maximal absorption

