สารออกฤทธิ์ทางชีวภาพจากราเอนโดไฟต์จากกระเบาใหญ่ *Hydnocarpus anthelminthicus* Pierre ex.

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BIOACTIVE COMPOUNDS FROM ENDOPHYTIC FUNGI FROM *Hydnocarpus anthelminthicus* Pierre ex.

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ราเอนโดไฟต์จำนวน 68 ไอโซเลต แยกได้จากกิ่งและใบของกระเบาใหญ่จากสวนพฤกษศาสตร์พุแค จังหวัดสระบุรี จากการศึกษาลักษณะทางสัณฐานวิทยาและการวิเคราะห์ลำดับนิวคลีโอไทด์ในบริเวณ ITS ของ rDNA พบว่าราเอ็นโดไฟต์ไอโซเลต HANT 7 และ HANT 25 มีความใกล้เคียงทางวิวัฒนาการกับสายพันธุ์ *Phomopsis* sp. เมื่อทำการแยกสารออกฤทธิ์ทางชีวภาพโดยเลี้ยงในอาหารเหลว Malt Czapek Broth แยกส่วนสกัดเอธิลแอซิเตตจากน้ำหมักของราเอนโดไฟต์ไอโซเลต HANT 25 ได้สารประกอบ 3 ชนิด คือ mycoepoxydiene (สารประกอบ **1**), สารผลิตภัณฑ์ธรรมชาติชนิดใหม่หนึ่งชนิด คือ 5-hydroxy-6-(8-methyl-9-oxa-bicyclo[4.2.1]nona-2,4-dien-7-yl)-5,6-dihydropyran-2-one (สารประกอบ **2**) และ สารอนุพันธุ์ใหม่หนึ่งชนิด คือ 2-(8-methyl-9-oxa-bicyclo[4.2.1]nona-2,4-dien-7-yl)-6-oxo-tetrahydro-2*H*-pyran-3-yl-acetate (สารประกอบ **3**) ส่วนราเอนโดไฟต์ไอโซเลต HANT 7 สามารถแยกสารได้หนึ่งชนิด คือ 2,5-furandimethanol (สารประกอบ **4**) เมื่อนำสารที่แยกได้มาทดสอบฤทธิ์ทางชีวภาพพบว่าสารประกอบ **1** มีฤทธิ์ยับยั้งเซลล์มะเร็ง HuCCA-1, KB, HeLA, MDA-MB231, T47D, H69AR, HepG2, A549, S102, HL-60, และ P388 โดยมีค่า IC_{50} เท่ากับ 0.30, 2.40, 2.10, 1.30, 2.00, 1.60, 2.25, 1.95, 2.80, 0.85, และ 0.80 μg/mL ตามลำดับ และยังมีฤทธิ์ยับยั้งเชื้อ *Mycobacterium tuberculosis* โดยมีค่า MIC เท่ากับ 50 μg/mL สารประกอบ **2** มีฤทธิ์ยับยั้งเซลล์มะเร็ง HepG2, A549, และ S102 โดยมีค่า IC_{50} เท่ากับ 1.45, 1.70, และ 0.87 μg/mL ตามลำดับ สารประกอบ **3** ไม่มีฤทธิ์ยับยั้งเซลล์มะเร็ง HepG2, A549, และ S102 ส่วนสารประกอบ **4** มีฤทธิ์ยับยั้งเซลล์มะเร็ง HuCCA-1, MDA-MB231, T47D, HL-60 และ P388 ในระดับปานกลาง

สาขาวิชา เทคโนโลยีชีวภาพ
ปีการศึกษา 2549

ลายมือชื่อนิสิต.....
ลายมือชื่ออาจารย์ที่ปรึกษา.....
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SURASAK PRACHYA: BIOACTIVE COMPOUNDS FROM ENDOPHYTIC FUNGI FROM *Hydnocarpus anthelminthicus* Pierre ex.: THESIS ADVISOR: ASSOC. PROF. Dr. NATTAYA NGAMROJANAVANICH, Ph.D, THESIS CO-ADVISOR: ASSIST. PROF. Dr. SUTHEP WIYAKRUTTA, Ph.D., 124 pp.

Endophytic fungi (68 isolates) were isolated from the twigs and leaves of the plant *Hydnocarpus anthelminthicus* Pierre ex. collected from the Central Botanical Garden (Pukae), Saraburi Province, Thailand. Based on the microscopic morphology and nucleotide sequencing of ITS regions of rDNA, endophytic fungal isolates HANT 7 and HANT 25 were identified as *Phomopsis* sp. In the present investigation, three compounds were isolated from MCz culture of the endophytic fungal isolate HANT 25 and one compound was isolated from the strain HANT 7. Fungal metabolites isolated were a new compound, 2-(8-methyl-9-oxa-bicyclo[4.2.1]nona-2,4-dien-7-yl)-6-oxo-tetrahydro-2*H*-pyran-3-yl-acetate, a new natural product, 5-hydroxy-6-(8-methyl-9-oxa-bicyclo[4.2.1]nona-2,4-dien-7-yl)-5,6-dihydropyran-2-one, and two known compounds, mycoepoxydiene and 2,5-furandimethanol. The isolated compounds **1**, **2**, **3**, and **4** were evaluated for their biological activities. Compound **1** was found to exhibit strong anticancer activity against HuCCA-1, KB, HeLA, MDA-MB231, T47D, H69AR, HepG2, A549, S102, HL-60, and P388 cancer cell lines with IC_{50} values of 0.30, 2.40, 2.10, 1.30, 2.00, 1.60, 2.25, 1.95, 2.80, 0.85, and 0.80 μg/mL, respectively, and also exhibited activity against *Mycobacterium tuberculosis* with MIC value of 50 μg/mL. Compound **2** was found to exhibit strong anticancer activity against HepG2, A549, and S102 cancer cell lines with IC_{50} values of 1.45, 1.70, and 0.87 μg/mL, respectively. Compound **3** was found inactive against HepG2, A549, and S102 cell lines. Compound **4** showed weak anticancer activity against HuCCA-1, MDA-MB231, T47D, HL-60, and P388 cancer cell lines.

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List of Abbreviations

s	singlet
d	doublet
t	triplet
q	quartet
brs	broad singlet
brd	broad doublet
m	multiplet
dd	doublet of doublet
δ	chemical shift
J	coupling constant
IR	infarred spectrum
m/z	a value of mass divided by charge
IC_{50}	50% inhibitory concentration
EC_{50}	50% effective concentration
°C	degree celcius
μg	microgram
mg	milligram
g	gram
mL	milliliter
L	liter
%	percent
cm^{-1}	reciprocal centimeter(wave number)
UV	ultraviolet
TMS	tetramethylsilane
Hz	hertz
MHz	megahertz
MIC	minimum inhibitory concentration
ν_{max}	wave number at maximum absorption
^{1}H-NMR	Proton Nuclear Magnetic Resonance
^{13}H-NMR	Carbon-13 Nuclear Magnetic Resonance
2D-NMR	two dimensional Nuclear Magnetic Resonance

COSY	homonuclear correlation spectroscopy
DEPT	distortionless enhancement by polarization transfer
NOESY	Nuclear Overhauser Enhancement Spectroscopy
HMQC	heteronuclear multiple quantum coherence
HMBC	heteronuclear multiple bond correlation
$CDCl_3$	deuterochloroform
CD_3COCD_3	deuteroacetone
DMSO	dimethylsulphoxide
MeOH	methanol
EtOAc	ethyl acetate
CH_2Cl_2	methylene chloride
PDA	Potato Dextrose Agar
SDA	Sabouraud's Dextrose Agar
MCz	Malt Czapek Agar
MEA	Malt Extract Agar
YCz	Yeast Czapek Agar
YES	Yeast Extract Sucrose
ppm	part per million
TLC	thin layer chromatography
λ_{max}	wavelength of maximum absorption
EIMS	Electron Impact Mass Spectrometry