

สารต้านจุลชีพของ *MICROMONOSPORA* SP. BTG10-2 จากดินป่าพรุนราธิวาส
และสารทุติยภูมิของราเอ็นโดไฟท์ *EXSEROHILUM ROSTRATUM* RNAS5

นางสาว นภวรรณ บุญถนอม

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชเวช ภาควิชาเภสัชเวช
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ANTIMICROBIAL METABOLITES OF *MICROMONOSPORA* SP. BTG10-2 FROM
NARATHIWAT PEAT SWAMP SOIL AND SECONDARY METABOLITES OF
THE ENDOPHYTIC FUNGUS *EXSEROHILUM ROSTRATUM* RNAS5

Miss Napannop Boonthanom

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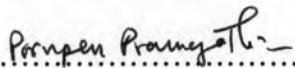
By Miss Napannop Boonthanom

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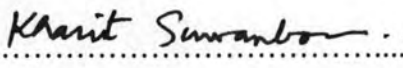
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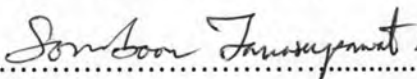
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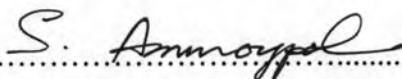
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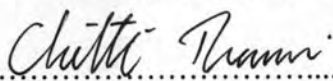
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 Thesis Advisor
(Khanit Suwanborirux, Ph.D.)

 Thesis Co-advisor
(Associate Professor Somboon Tanasupawat, Ph.D.)

 Member
(Associate Professor Surattana Amnuoypol, Ph.D.)

 Member
(Chitti Thawai, Ph.D.)

นภวรรณพ บุญถนอม: สารต้านจุลชีพของ *MICROMONOSPORA* SP.BTG10-2
 จากดินป่าพรุนราธิวาส และสารทุติยภูมิของราเอ็นโดไฟท์ *EXSEROHILUM*
ROSTRATUM RNAS5 (ANTIMICROBIAL METABOLITES OF
MICROMONOSPORA SP. BTG10-2 FROM NARATHIWAT PEAT
 SWAMP SOIL AND SECONDARY METABOLITES OF THE
 ENDOPHYTIC FUNGUS *EXSEROHILUM ROSTRATUM* RNAS5)
 อ.ที่ปรึกษา: อ. ดร. คณิต สุวรรณบริรักษ์ อ.ที่ปรึกษาร่วม: รศ.ดร. สมบูรณ์
 ธนาศุภวัฒน์; 126 หน้า

ในการศึกษาเพื่อหาสารออกฤทธิ์ทางชีวภาพจากแบคทีเรียที่แยกได้จากดินในสถานที่ต่างๆ
 ในประเทศไทย พบว่าสิ่งสกัดด้วยเอธิลอะซิเตทจากน้ำหมักของแบคทีเรีย *Micromonospora* sp.
 BTG10-2 ซึ่งแยกได้จากดินป่าพรุ จังหวัดนราธิวาส แสดงฤทธิ์ต้านแบคทีเรียแกรมบวก
Micrococcus luteus ATCC 9341 และ *Bacillus subtilis* ATCC 6633 รวมทั้งแบคทีเรียแกรม
 ลบ *Pseudomonas aeruginosa* ATCC 27853, *Eschericia coli* ATCC 25922 และ
Salmonella sp. เมื่อทำการแยกสารด้วยวิธีทางโครมาโตกราฟีโดยทำการทดสอบฤทธิ์ต้านจุลชีพ
 ควบคู่ไปด้วย และทำการวิเคราะห์โครงสร้างทางเคมีของ fraction ที่แยกได้โดยวิธีทาง NMR สเปค
 โตรสโคปี พบว่า fraction ที่มีฤทธิ์ต้านแบคทีเรีย ที่แยกได้เป็นสารในกลุ่มของ anthraquinones

นอกจากนี้ได้ศึกษาราเอ็นโดไฟท์ *Exserohilum rostratum* RNAS5 ที่แยกได้จากใบของ
 ต้นทองพันชั่ง (*Rhinacanthus nasutus*) พบว่าสิ่งสกัดด้วยเอธิลอะซิเตทจากน้ำหมักของราชนิดนี้
 มีคุณสมบัติในการเสริมฤทธิ์ยา ketoconazole ในการต้านรา *Candida albicans* ATCC 10231
 เมื่อทำการแยกสารด้วยวิธีทางโครมาโตกราฟี ทำให้สามารถแยกสารกลุ่ม diketopiperazines ที่
 สมมาตรได้ 2 ชนิด คือ rostratin A ซึ่งเป็นสารที่เคยมีรายงานมาแล้ว และอีก 1 ชนิด คือ
 exserohilin A ซึ่งเป็นสารชนิดใหม่ และยังแยกสารกลุ่ม diketopiperazine ชนิดใหม่ที่โครงสร้างไม่
 สมมาตรอีก 1 ชนิด คือ exserohilin B การพิสูจน์โครงสร้างของสารทำได้โดยการวิเคราะห์ข้อมูล
 สเปคโตรสโคปีของ ^1H , ^{13}C , 2D-NMR และ mass และข้อมูลทางผลึกของสาร รวมทั้งเปรียบเทียบ
 ข้อมูลในวารสารอ้างอิง

ภาควิชา ภาสัชเวช

สาขาวิชา ภาสัชเวช

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ลายมือชื่อนิสิต..... นภวรรณ บุญถนอม

ลายมือชื่ออาจารย์ที่ปรึกษา.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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KEY WORD : *MICROMONOSPORA SP.* / PEAT SWAMP SOIL / ANTIMICROBIAL ACTIVITY
/ *EXSEROHILUM ROSTRATUM* / ROSTRATIN

NAPANNOP BOONTHANOM: ANTIMICROBIAL METABOLITES OF
MICROMONOSPORA SP. BTG10-2 FROM NARATHIWAT PEAT SWAMP SOIL
AND SECONDARY METABOLITES OF THE ENDOPHYTIC FUNGUS
EXSEROHILUM ROSTRATUM RNAS5. THESIS ADVISOR: KHANIT
SUWANBORIRUX, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. SOMBOON
TANASUPAWAT, Ph.D. 126 pp.

In search for bioactive substances of bacteria from soil in Thailand, the ethyl acetate extract of *Micromonospora sp.* BTG 10-2 from Narathiwat peat swamp soil showed antimicrobial activities against not only the gram-positive bacteria, *Micrococcus luteus* ATCC 9341 and *Bacillus subtilis* ATCC 6633 but also the gram-negative bacteria, *Pseudomonas aeruginosa* ATCC 27853, *Eschericia coli* ATCC 25922, and *Salmonella sp.* Bioassay-guided fractionation of the extract resulted in the isolation of an active fraction. Analyses of proton and carbon NMR spectra of the fraction, demonstrated the characteristics of anthraquinones of the chemical composition.

The endophytic fungus *Exserohilum rostratum* RNAS5 was isolated from the leaf of the Thai medicinal plant, *Rhinacanthus nasutus*. The ethyl acetate extract of the fermentation broth possessed synergistic antifungal activity with ketoconazole against *Candida albicans* ATCC 10231. Using silica gel quick column and flash column chromatography, two highly symmetrical diketopiperazines, rostratin A and a new exserohilin A, along with a new asymmetrical diketopiperazine, exserohilin B, were isolated from the extract. The structures of those compounds were elucidated by analyses of ^1H , ^{13}C , 2D-NMR, mass spectra, and x-ray crystallography as well as comparison with the literature data.

Department Pharmacognosy
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Academic year 2006

Student's signature... *Napannop Boonthanom*
Advisor's signature... *Khanit Suwanborirux*
Co-advisor's signature... *Somboon Tanasupawat*

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ABBREVIATIONS

ATCC	=	American Type Culture Collection, Monassas, U.S.A.
br s	=	broad siglet
°C	=	degree Celsius
¹³ C-NMR	=	carbon-13 nuclear magnetic resonance
cm	=	centimeter
CFU	=	colony forming unit
COSY	=	correlation spectroscopy
δ	=	chemical shift
DEPT	=	distortionless enhancement by polarization transfer
d	=	doublet
g	=	gram
HMBC	=	¹ H-detected heteronuclear multiple bond correlation
HMQC	=	¹ H-detected heteronuclear multiple quantum coherence
¹ H-NMR	=	proton nuclear magnetic resonance
Hz	=	hertz
IR	=	infrared
<i>J</i>	=	coupling constant
L	=	liter
m	=	multiplet
MHz	=	megahertz
μg	=	microgram
mg	=	milligram
μl	=	microliter
ml	=	milliter
μm	=	micrometer
mm	=	millimeter
NCCLS	=	The National Commmittee for Clinical Laboratory Standards
NMR	=	nuclear magnetic resonance
NOSEY	=	nuclear overhouser effect correlation spectroscopy
OD	=	optical density

ppm	=	part per million
ppt	=	part per thousand
q	=	quartet
RPMI	=	Roswell Park Memorial Institute medium
s	=	singlet
SDA	=	Sabouraud dextrose agar
sp.	=	species
t	=	triplet
TLC	=	thin layer chromatography
UV	=	ultraviolet