

สารออกฤทธิ์ทางชีวภาพจากราเอนไดไฟต์จากใบเปล้าน้อย *Croton sublyratus*

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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BIOLOGICALLY ACTIVE COMPOUNDS FROM ENDOPHYTIC FUNGI  
IN *Croton sublyratus* LEAVES

Miss Monthika Pothavorn

A Thesis submitted in Partial Fulfillment of the Requirements  
for the Degree Master of Science in Biotechnology

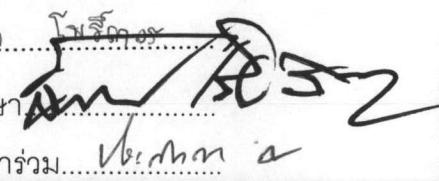
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งานวิจัยนี้ทำการแยกสารออกฤทธิ์ทางชีวภาพที่สร้างจากรากเอนโดยไฟต์ได้จากใบเปลือก Croton sublyratus โดยนำส่วนของใบ จาก 4 แหล่งตัวอย่าง มาคัดแยกโดยผ่านวิธีฆ่าเชื้อที่ผิวนอก และวางบน malt extract agar สามารถแยกได้ทั้งหมด 75 โถโดย เดลต์ การทดสอบเบื้องต้นในการสร้างสารออกฤทธิ์ทางชีวภาพของราเอนโดยไฟต์ใน การยับยั้งจุลทรรศ์ทดสอบโดยวิธี Dual culture agar diffusion technique พบว่าราเอนโดยไฟต์โดย CsPr03 สามารถสร้างสารที่มีฤทธิ์ทางชีวภาพในการยับยั้งจุลทรรศ์ทดสอบได้ที่สุด เมื่อทำการจัดจำแนกสายพันธุ์โดยศึกษาลักษณะทางสัณฐานวิทยาและวิเคราะห์ลำดับนิวคลีโอไทด์ในบริเวณ internal transcribed spacer ของ rDNA พบว่าราเอนโดยไฟต์โดย CsPr03 คือ *Bipolaris spicifera* เมื่อทำการศึกษาเพื่อหาสารออกฤทธิ์ทางชีวภาพโดยเลี้ยงในอาหารเหลว malt extracts broth และสารบิสูที จำนวนหนึ่งมักด้วยวิธีโครงสร้างและการตัดสินใจ พบว่า ได้สารบิสูทีชนิดที่ 1 คือ Curvulin, สารบิสูทีชนิดที่ 2 คือ Emodin และ สารบิสูทีชนิดที่ 3 คือ สาร curvulin สังเคราะห์ นำสารบิสูทีที่แยกได้มาทดสอบฤทธิ์ทางชีวภาพในการยับยั้งจุลทรรศ์ทดสอบพบว่า สารบิสูทีชนิดที่ 1 คือ Curvulin มีฤทธิ์ยับยั้ง *Bacillus subtilis* ATCC 6633, *Saccharomyces cerevisiae* TISTR 5169 โดยมีค่า MIC เท่ากับ 15.62 และ 500  $\mu\text{g}/\text{ml}$  ตามลำดับ ส่วน สารบิสูทีชนิดที่ 2 คือ Emodin มีฤทธิ์ยับยั้ง *Candida albicans* ATCC 10231 โดยมีค่า MIC เท่ากับ 500  $\mu\text{g}/\text{ml}$ . และสารชนิดที่ 3 มีฤทธิ์ในการยับยั้งเชื้อ *Staphylococcus aureus* ATCC 25923 ที่ความเข้มข้น ต่ำสุด 125  $\mu\text{g}/\text{ml}$ .

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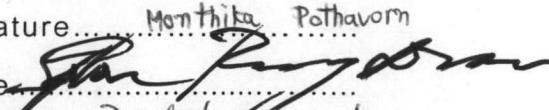
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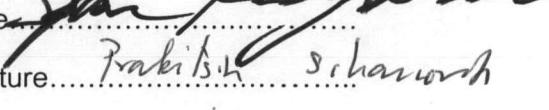
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MONTHIKA POTHAVORN : BIOLOGICALLY ACTIVE COMPOUNDS FROM ENDOPHYTIC FUNGI IN *Croton sublyratus* LEAVES. THESIS ADVISOR: PROF. SOPHON ROENGSUMRAN, Ph.D., THESIS COADVISOR: ASSOC. PROF. PRAKITSIN SRIHANÖNTH, Ph.D. 116 pp. ISBN974-17-6101-5.

The purpose of this research was to isolate bioactive compounds from endophytic fungi isolated from *Croton sublyratus* leaves. Plant samples were collected from 4 sources. Fungal endophyte were isolated from leaves by surface sterilization method and placed on malt extract agar. Seventy five fungal isolates were obtained and tested for the production of antimicrobial compounds by dual culture agar diffusion technique. Fungal isolate CsPr03 was chosen for the study of bioactive compounds because this isolate produced the compounds that were against a large number of test microorganisms. Based on morphology and nucleotide sequencing of ITS regions of rDNA, isolate CsPr03 was identified as *Bipolaris spicifera*. Chromatographic techniques and crystallization were used to isolate bioactive compounds from malt extract broth. Structure elucidation of the pure compounds were investigated using physical properties and spectroscopic techniques. Two compounds were isolated, compound 1 was curvulin, compound 2 was emodin and compound 3 was hydrolyzed curvulin. Antimicrobial activities of pure compounds were tested. It was found that a curvulin exhibited against *Bacillus subtilis* ATCC 6633 and *Saccharomyces cerevisiae* TISTR 5169 with MIC value of 15.62 and 500  $\mu\text{g}/\text{ml}$ , respectively. Emodin showed against *Candida albicans* ATCC 10231 with MIC value 500  $\mu\text{g}/\text{ml}$  and compound 3 showed against *Staphylococcus aureus* ATCC 25923 with MIC value 125  $\mu\text{g}/\text{ml}$ .

Student's signature..... Monthika Pothavorn

Field of study..... Biotechnology..... Advisor's signature..... 

Academic year..... 2004..... Co-advisor's signature..... 

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Finally, I would like to thank my parent, and my family for encouragement and moral support.

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## LIST OF ABBREVIATIONS

$[\alpha]_D^{20}$	= Specific rotation at 20° and Sodium D line (589 nm)
ATCC	= American Type Culture Collection, Maryland, U.S.A
br s	= broad singlet (for NMR spectral data)
°C	= degree Celsius
$^{13}\text{C-NMR}$	= carbon-13 nuclear magnetic resonance
$\text{CDCl}_3$	= deuterated chloroform
$\text{CHCl}_3$	= chloroform
$\text{CD}_3\text{OD}$	= deuterated methanol
cm	= centimeter
COSY	= $^1\text{H}$ - $^1\text{H}$ correlation spectroscopy
CFU	= Colony forming unit
$\delta$	= chemical shift
d	= doublet (for NMR spectral data)
dd	= doublet of doublet (for NMR spectral data)
dt	= doublet of triplets (for NMR spectral data)
$\epsilon$	= molar absorptivity
EIMS	= electron impact mass spectroscopy
Eq	= equatorial
EtOAc	= ethylacetate
g	= gram
HMBC	= $^1\text{H}$ - $^{13}\text{C}$ heteronuclear correlation
HMQC	= $^1\text{H}$ -detected heteronuclear multiple quantum coherence
$^1\text{H-NMR}$	= proton nuclear magnetic resonance
Hz	= hertz
IR	= infrared spectroscopy
l	= liter
$\mu$	= microliter
$\lambda_{\max}$	= wavelength of maximum absorption

## LIST OF ABBREVIATIONS (CONTINUED)

[M+H] <sup>+</sup>	= protonated molecular ion
m	= multiplet (for NMR spectral data)
MEA	= Malt extract agar
MHB	= Mueller- Hinton broth
MeOH	= methanol
MIC	= Minimum inhibitory concentration
mg	= miligram
μg	= microgram
MHz	= megahertz
ml	= millilitre
mm	= millimeter
V <sub>max</sub>	= wave number at maximum absorption
NMR	= nuclear magnetic resonance
No.	= Number
ppm	= part per million
s	= singlet (for NMR spectral data)
SEM	= scanning electron microscope
t	= tripel (for NMR spectral data)
TLC	= thin layer chromatography
UV	= Ultraviolet