

สารออกฤทธิ์ทางชีวภาพจากราเอ็นโดไฟต์จากใบเปล้าน้อย *Croton sublyratus*

นางสาว มณฑิกา ไพธัถาวร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาเทคโนโลยีชีวภาพ

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

Miss Monthika Pothavorn

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for the Degree Master of Science in Biotechnology

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งานวิจัยนี้ทำการแยกสารออกฤทธิ์ทางชีวภาพที่สร้างจากราเอนโดไฟต์ที่แยกได้จากใบเปล้าน้อย  
*Croton sublyratus* โดยนำส่วนของใบ จาก 4 แหล่งตัวอย่าง มาคัดแยกราโดยผ่านวิธีฆ่าเชื้อที่ผิวภายนอก และ  
 วางบน malt extract agar สามารถแยกราได้ทั้งหมด 75 ไอโซเลต จากการทดสอบเบื้องต้นในการสร้างสารออก  
 ฤทธิ์ทางชีวภาพของราเอนโดไฟต์ในการยับยั้งจุลินทรีย์ทดสอบโดยวิธี Dual culture agar diffusion technique  
 พบว่าราเอนโดไฟต์ไอโซเลต CsPr03 สามารถสร้างสารที่มีฤทธิ์ทางชีวภาพในการยับยั้งจุลินทรีย์ทดสอบได้ดีที่  
 สุด เมื่อทำการจัดจำแนกสายพันธุ์โดยศึกษาลักษณะทางสัณฐานวิทยาและวิเคราะห์ลำดับนิวคลีโอไทด์ใน  
 บริเวณ internal transcribed spacer ของ rDNA พบว่าราเอนโดไฟต์ไอโซเลต CsPr03 คือ *Bipolaris spicifera*  
 เมื่อทำการศึกษาเพื่อหาสารออกฤทธิ์ทางชีวภาพโดยเลี้ยงในอาหารเหลว malt extracts broth แยกสารบริสุทธิ์  
 จากน้ำหมักด้วยวิธีโครมาโตกราฟีและการตกผลึก และหาสูตรโครงสร้างของสารดังกล่าว โดยอาศัยสมบัติทาง  
 กายภาพและเทคนิคทางสเปกโตรสโกปี พบว่า ได้สารบริสุทธิ์ชนิดที่ 1 คือ Curvulin, สารบริสุทธิ์ชนิด ที่ 2 คือ  
 Emodin และ สารบริสุทธิ์ชนิดที่ 3 คือ สาร curvulin สังเคราะห์ นำสารบริสุทธิ์ที่แยกได้มาทดสอบฤทธิ์ทางชีว  
 ภาพในการยับยั้งจุลินทรีย์ทดสอบพบว่า สารบริสุทธิ์ชนิดที่ 1 คือ Curvulin มีฤทธิ์ยับยั้ง *Bacillus subtilis* ATCC  
 6633, *Saccharomyces cerevisiae* TISTR 5169 โดยมีค่า MIC เท่ากับ 15.62 และ 500 µg/ml ตามลำดับ  
 ส่วน สารบริสุทธิ์ชนิดที่ 2 คือ Emodin มีฤทธิ์ยับยั้ง *Candida albicans* ATCC 10231 โดยมีค่า MIC เท่ากับ  
 500 µg/ml. และสารชนิดที่ 3 มีฤทธิ์ในการยับยั้งเชื้อ *Staphylococcus aureus* ATCC 25923 ที่ความเข้มข้น  
 ต่ำสุด 125 µg/ml.

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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 SRIHANONTH, 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 µg/ml, respectively. Emodin showed against *Candida albicans* ATCC 10231 with MIC value 500 µg/ml and compound 3 showed against *Staphylococcus aureus* ATCC 25923 with MIC value 125 µg/ml.

Student's signature..... Monthika Pothavorn  
 Field of study.....Biotechnology.....Advisor's signature.....  
 Academic year.....2004..... Co-advisor's signature..... Prakit Sin Srihanonth

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## CONTENTS

	Page
Abstract in Thai.....	iv
Abstract in English.....	v
Acknowledgements.....	vi
Contents.....	vii
List of Tables.....	xi
List of Figures.....	xiii
List of Schems.....	xv
List of Abbreviations.....	xvi
Chapter 1 introduction.....	1
Chapter 2 Literature review.....	3
2.1 Endophytic fungi.....	3
2.2 Grass endophytes.....	5
2.3 Non-grass endophytes.....	6
2.4 Isolation technique of endophytic fungi.....	8
2.5 Botanical aspects of <i>Croton sublyratus</i> .....	9
2.5.1 The uses of <i>C.sublyratus</i> .....	9
2.5.2 Chemical constituents of <i>C.sublyratus</i> .....	10
2.6 Study of secondary metabolite from the endophytic fungi.....	12
Chapter 3 Materials and Methods.....	27
3.1 Sample collection.....	27
3.2 Fungal isolation and culture methods.....	27
3.3 Identification of the isolated endophytic fungi.....	28
3.3.1 Morphological identification.....	28
3.3.2 Molecular identification.....	28
3.4 Fungal cultivation.....	30
3.5 Evaluation of the antimicrobial activity.....	30

## CONTENTS (CONTINUED)

	Page
3.5.1 Test microorganisms for antimicrobial.....	30
3.5.2 Procedures for evaluation of the antimicrobial activity.....	30
3.6 Cultivation and metabolite extraction of endophytic fungus	
isolate CsPr03.....	31
3.6.1 Cultivation of endophytic fungus isolate CsPr03.....	31
3.6.2 Metabolite extraction of endophytic fungus	
isolate CsPr03.....	31
3.7 Application of culture broth extracted and mycelium extracted.....	32
3.8 Isolation of metabolites of culture broth from endophytic fungus	
isolate CsPr03.....	33
3.8.1 Isolation of crude culture broth from fraction	
code BE01.....	34
3.8.2 Isolation of crude culture broth from fraction	
code BE06.....	35
3.9 Isolation of metabolites of crude mycelium from	
endophytic fungus isolate CsPr03.....	36
3.9.1 Isolation of crude mycelium from fraction code ME04.....	37
3.10 Purification and properties of pure compounds from	
endophytic fungus isolate CsPr03.....	38
3.10.1 Purification and properties of pure compounds from	
fraction BE0121.....	38
3.10.2 Purification and properties of pure compounds from	
fraction ME0405.....	39
3.11 Biological activities test.....	40
3.11.1 Antimicrobial activity test	
3.11.1.1 Antimicrobial activity for the crude extracts	
and pool fractions.....	40

## CONTENTS (CONTINUED)

	Page
3.11.1.2 Antimicrobial activity of pure compounds.....	40
3.12 Cytotoxic test.....	41
3.13 Chromatographic techniques.....	42
3.14 Spectroscopy.....	43
3.14.1 Fourier Transform Infrared Spectrophotometer (FT-IR).....	43
3.14.2 Nuclear Magnetic Resonance Spectrometry (NMR).....	43
3.14.3 Optical rotation.....	43
3.14.4 Mass spectrometry (MS).....	43
3.14.5 Melting point.....	44
3.14.6 UV-VIS spectrometry.....	44
3.14.7 Solvent.....	44
Chapter 4 Results and discussion.....	45
4.1 Isolation of endophytic fungi.....	45
4.2 Identification of the isolated endophytic fungi .....	45
4.3 Enumeration of test microorganisms.....	50
4.4 Determination of antimicrobial activity.....	50
4.5 Identification of fungal endophyte CsPr03.....	57
4.5.1 Morphology identification.....	57
4.5.2 Taxonomy of bipolaris.....	58
4.5.3 Molecular identification.....	58
4.6 Cultivation and extraction.....	60
4.7 Isolation and purification of bioactive compounds in crude culture broth and crude mycelium.....	61
4.8 Structure elucidation of the pure compounds from endophytic fungus isolate CsPr03.....	61
4.8.1 Structure elucidation of compound 1.....	61
4.8.2 Structure elucidation of compound 2.....	69



## CONTENTS (CONTINUED)

	Page
4.9 Hydrolysis of compound 1.....	76
4.10 Biological activities.....	77
4.10.1 Antimicrobial activity of the culture broth of endophytic fungus isolate CsPr03.....	77
4.10.2 Antimicrobial activity of the crude extract from endophytic fungus isolate CsPr03.....	78
4.10.3 Antimicrobial activity of the fraction from crude extracts.....	79
4.10.4 Antimicrobial activity of pure compounds.....	81
4.10.5 Cytotoxic activity.....	83
Chapter 5 Conclusion.....	85
References.....	86
Appendices.....	94
Appendix A.....	95
Appendix B.....	97
Biography.....	116

## LIST OF TABLES

Table	Page
2.1 The chemical compounds, sources, biological activities of secondary metabolites of endophytic fungi.....	13
3.1 The results from separation of crude culture broth.....	33
3.2 The results of crude culture broth from fraction code BE01.....	34
3.3 The results of crude culture broth from fraction code BE06.....	36
3.4 The results from separation of crude mycelium.....	37
3.5 The results from the separation of the fraction code ME04.....	38
4.1 The number and isolates of endophytic fungi.....	45
4.2 Quantity of standardized inocula.....	50
4.3 Antimicrobial activities of endophytic fungi isolated from <i>C. sublyratus</i> leaves. ....	51
4.4 The number of endophytic fungi isolates in each inhibition groups.....	55
4.5 The IR absorption band assignment of compound 1.....	61
4.6 The HSQC spectral data of compound 1.....	63
4.7 The HSQC, HMBC and COSY spectral data of compound 1.....	63
4.8 The <sup>1</sup> H-NMR spectral data of compound 1 and curvulin.....	66
4.9 The <sup>13</sup> C-NMR spectral data of compound 1 and curvulin.....	67
4.10 The IR absorption band assignment of compound 2.....	69
4.11 The HSQC spectral data of compound 2.....	71
4.12 The HSQC and HMBC spectral data of compound 2.....	72
4.13 The <sup>1</sup> H-NMR spectral data of compound 2 and emodin.....	74
4.14 The <sup>13</sup> C-NMR spectral data of compound 2 and emodin.....	75
4.15 Antimicrobial activities of the crude extracts from endophytic fungus isolate CsPr03.....	78
4.16 Antimicrobial activities of each fractionated of culture broth extracts.....	79
4.17 Antimicrobial activities of each fractionated of mycelium extracts.....	80
4.18 Broth microdilution method for antimicrobial activities of pure compounds.....	81

## LIST OF FIGURES

Figure	Page
2.1 Vegetative growth in endophytic fungi of grasses.....	3
2.2 The five interaction components that can be involved in a symptomatic plant disease.....	4
2.3 Alternative asexual and sexual life cycle of <i>Epichloa featacae</i> in symbiosis with <i>Festuca</i> sp.....	5
2.4 <i>Croton sublyratus</i> .....	11
2.5 Structure of secondary metabolites of endophytic fungi.....	19
3.1 ITS regions of rDNA.....	30
4.1 Colonial morphology of endophytic fungus isolate, CsPr02.....	46
4.2 Colonial morphology of endophytic fungus isolate, CsPr03.....	46
4.3 Colonial morphology of endophytic fungus isolate, CsPr08.....	47
4.4 Colonial morphology of endophytic fungus isolate, CsPr24.....	47
4.5 Colonial morphology of endophytic fungus isolate, CsPr33.....	48
4.6 Colonial morphology of endophytic fungus isolate, CsPr36.....	48
4.7 Colonial morphology of endophytic fungus isolate, CsBkk05.....	49
4.8 Colonial morphology of endophytic fungus isolate, CsPt08.....	49
4.9 Dual culture agar diffusion technique for antimicrobial activity.....	54
4.10 Percentage of each groups inhibitor.....	55
4.11 A summary of the dual culture agar diffusion technique assay results for the antimicrobial activity of endophytic fungi.....	56
4.12 Endophytic fungal isolate CsPr03 (A) culture on MEA (B) Conidiophores.....	57
4.13 Nucleotide sequences of partial 18S region, complete ITS region of the isolate CsPr03.....	59
4.14 Alignment data of ITS region of isolate CsPr03 and 1 reference taxa.....	59
4.15 HMBC correlation of compound 1.....	64
4.16 COSY correlation of compound 1.....	64
4.17 NOESY correlation of compound 1.....	65
4.18 The structure of compound 1.....	68

## LIST OF FIGURES (CONTINUED)

Figure	Page
4.19 HMBC correlation of compound 2.....	73
4.20 The structure of compound 2.....	76
4.21 Antimicrobial activity of culture broth of endophytic fungi isolate CsPr03.....	77
4.22 Broth microdilution methods for antimicrobial activity.....	82

## LIST OF SCHEME

Scheme	Page
3.1 Extraction of culture broth and mycelium of endophytic fungus isolate CsPr03.....	32
4.1 Hydrolysis reaction of compound 1.....	76

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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
$\mu\text{l}$	= microliter
$\lambda_{\text{max}}$	= wavelength of maximum absorption

## LIST OF ABBREVIATIONS (CONTINUED)

$[M+H]^+$	= protonated molecular ion
m	= multiplet (for NMR spectral data)
MEA	= Malt extract agar
MHB	= Mueller- Hinton broth
MeOH	= methanol
MIC	= Minimum inhibitory concentration
mg	= milligram
$\mu\text{g}$	= microgram
MHz	= megahertz
ml	= millilitre
mm	= millimeter
$\nu_{\text{max}}$	= 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	= triplet (for NMR spectral data)
TLC	= thin layer chromatography
UV	= Ultraviolet