CHAPTER 7

PURIFICATION OF SECONDARY METABOLITES FROM ENDOPHYTIC FUNGI

7.1 Introduction

Microorganisms have been shown to be the attractive sources of natural compounds for pharmaceuticals, industry and agriculture. In pharmaceutical applications, microbial secondary metabolites especially antimicrobial compounds are used for human and animal therapy (Bérdy, 2005). However, some antibiotic treatments causes increased antibiotics resistant pathogenic microorganisms and are toxic for human and animal, so the discovery for new antibiotics from new sources is necessary and important for clinical treatment. In agriculture, plant pathogenic fungi can cause plant diseases and much loss of crop yields (Bajwa *et al*., 2003). However, agrochemical treatment causes environmental pollution and destroyed diversity of non-target organisms. Microorganisms as biological control agents have high potential to control human pathogen, and plant pathogens and with out effect to the environment or other non-target organisms. There are several reports on the potential use of biocontrol agents as replacements for agrochemicals (Shimizu *et al.*, 2000).

Fungi are among the most important groups of eukaryotic organisms that are well known for producing many novel metabolites which are directly used as drugs or function as lead structures for synthetic modifications (Kock *et al*., 2001; Bode *et al*.,

2002; Donadio *et al*., 2002; Chin *et al*., 2006; Gunatilaka, 2006; Mitchell *et al*., 2008; Stadler and Keller, 2008). Endophytic fungi are capable of synthesizing bioactive compounds that can be used as potential sources of pharmaceutical leads. Endophytic fungi have been proven useful for novel drug discovery as suggested by the chemical diversity of their secondary metabolites. Many endophytic fungi have been reported to produce novel antibacterial, antifungal, antiviral, anti-inflammatory, antitumor, and other compounds belonging to the alkaloids, steroid, flavonoid and terpenoids derivatives and other structure types (Pongcharoen *et al*., 2008; Qin *et al*., 2009; Yu *et al*., 2010; Klaiklay *et al*., 2012; Zhang *et al*., 2013).

The study aimed to screening endophytic fungi as a potential antimicrobial agent against bacteria and fungal pathogen. In addition, the interesting chemical compounds extracted from both fungal endophytes, *Mycoleptodiscus terrestris* CMU-Cib179 and *Nodulisporium* sp. CMU-Cib1018 culture which isolated in Chapter 3 were purified, structure elucidated and investigated their antimicrobial activities.

7.2 Materials and methods

7.2.1 Test microorganisms

Bacterial strains

The bacteria *Bacillus subtilis* (LM-CMU03), *Escherichia coli* (ATCC35218), *Micrococcus luteus* (LM-CMU04), *Pseudomonas aeruginosa* (ATCC27853), *Staphylococcus aureus* (ATCC29213) and methicillin-resistant *S. aureus* (ATCC33591) obtained from Division of Microbiology, Department of Biology, Faculty of Science and

Faculty of Medicine, Chiang Mai University, Thailand. All bacterial strains were separately culture and maintained on nutrient agar (NA, Appendix A) at room temperature.

Fungal strains

The fungi, *Candida albicans* (ATCC90028), *Aspergillus flavus* (CMU-ASF037), *Colletotrichum gloeosporioides* (CMU-COLL029), *Penicillium digitatum* (CMU-PEN022) and *P. expansum* (CMU-PEN023) obtained from The Sustainable Development of Biological Resources Laboratory, Faculty of Science, Chiang Mai University, Thailand were culture and maintained at room temperature on PDA, except yeast was maintion on malt peptone agar (MPA).

Preparation of assay plate

Inoculum of all tested organism, were preparation for agar well or paper disk diffusion assay as follows. One loop of each bacterial and yeast isolate was scraped from the agar culture slants and inoculated into separate tube 18 × 180 mm containing 5 mL of nutrient broth and malt peptone broth, respectively. The tubes were then incubated for 12 h at room temperature on a reciprocal shaker at 125 rpm. For fungal tested, 0.1% Tween 80 was added to before inoculation with one loop of conidia. The mixture was then vortexed, and immediately used for assay plated. One minliter of inoculum of each test organism was added to 200 mL of appropriate medium at 50°C, poured into assay plated, and allowed to solidify.

7.2.2 Determination of fungal antimicrobial activity production

7.2.2.1 Screening of fungi for production of antimicrobial activity

Three different liquid fermentation media, F1 and F2 which modified from Cheeptham *et al*. (1999, Appendix A) and PDB were used in this experiment. Total 5,925 endophytic fungi isolated from *Cinnamomum bejolghota*, *C. zylanicum*, *Gnetum montanum* and *Lagerstroemia loudoni* (in Chapter 3) were grouped and 127 fungal strains were selected for screening of antimicrobial activities. Inocula of endophytic fungi were individually subculture onto PDA plates and incubated for 7 days. Mycelium plug (5 mm diameter) was cut from the growing edge of each colony and inoculated into test tubes 18 × 180 mm containing 5 mL fermentation media. The fermentation media were incubated at room temperature 27±2°C for 7 days on a reciprocal shaker at 125 rpm. The supernatant were filtered by passing through Miracloth (CALBIOCHE®, Germany), concentrated by lyophilization, resuspended with 1 mL dimethyl sulfoxide (DMSO) and used immediately to test for inhibitory activity.

7.2.2.2 Bioassay

Agar well diffusion assay

Forty microliters of resuspended fungal supernatant was separately loaded into 0.5 mm diameter wells cut into assay plates.

Paper disc diffusion assay

Concentrated fungal supernatant, extracted fungal supernatant, column chromatography eluted or pure compound were loaded into sterile paper discs (8 mm diam, ADVANTEC®, Japan) and allowed to air dry, before they were placed on assay plate. Paper disc were loaded with solvents and used as control. Moreover, minimum inhibitory concentration (MIC) for antimicrobial activity of pure compound was determined. The pure compounds were dissolved in methanol (MeOH) and test at 5, 10, 25 and 50 μg $disc^{-1}$.

All assay plates were then incubated at 37°C in the dark for one day for all bacterial tested strain, yeast incubated chamber at 30°C in the dark for one day, and fungal strain incubated at 27±2°C for 2–3 days. After incubation, the diameter of the bacterial and fungal growth inhibition zone was measured. For the antimicrobial production, the tests were conducted in triplicate and data generated from this experiment was analyzed by one-way analysis of variance (ANOVA) using SPSS version 16.0 for window. The antimicrobial activity (inhibition zone diameter) of 127 fungal endophytes strain against the test organisms was also qualitatively avaluated as: +++ = > 20 mm; ++ = 11–20 mm; + = 8–10 mm; ± = 7–5 mm; – = < 5 mm.

7.2.3 Fermentation of selected endophytic fungi for antimicrobial production

7.2.3.1 Fungal strain

Two selected fungal strains exhibiting the most inhibition agents tested organisms described in section 7.2.2 were used in this experiment. Identification of *Mycoleptodiscus*

terrestris CMU-Cib179 and *Nodulisporium* sp. CMU-Cib1018 was based on morphological characteristics. The morphological identification was used the traditional techniques (Chapter 3) and molecular identification was followed the method as described in Chapter 4.

7.2.3.2 Cultivation, extraction and purification

Mycoleptodiscus terrestris CMU-Cib179

The *M. terrestris* CMU-Cib179 was cultivated on PDA and incubated at room temperature for 7 days. Small pieces of the mycelium disc (5 mm diam) were then inoculated into 500 mL Erlenmeyer flask containing 200 mL of PDB media (125 flasks, 25L), followed by incubation at 27±2°C for 7 days on a reciprocal shaker at 125 rpm. After fermentation, the culture was harvested by filtration with Miracloth (CALBIOCHE®, Germany). The culture broths were extracted twice with equal volumes of ethyl acetate (EtOAc). The ethyl acetate layer was dried with Na_2SO_4 followed by evaporation under reduced pressure to obtain a crude ethyl acetate extract as green gum (1.55 g) used for separation (Figure 7.1).

Nodulisporium sp. strain CMU-Cib1018

The *Nodulisporium* sp. strain CMU-Cib1018 was cultivated on PDA and incubated at room temperature for 7 days. Small pieces of the mycelium disc (5 mm diameter) were then inoculated into 500 mL Erlenmeyer flask containing 200 mL of PDB media (100 flasks, 20L), followed by incubation at 27±2°C for 7 days on a reciprocal

shaker at 125 rpm. After fermentation, the culture was harvested by filtration with Miracloth (CALBIOCHE®, Germany). The culture broths were extracted twice with equal volumes of ethyl acetate (EtOAc). The ethyl acetate layer was dried with Na_2SO_4 followed by evaporation under reduced pressure to obtain a crude ethyl acetate extract as a brown gum (3.45 g) used for separation (Figure 7.2).

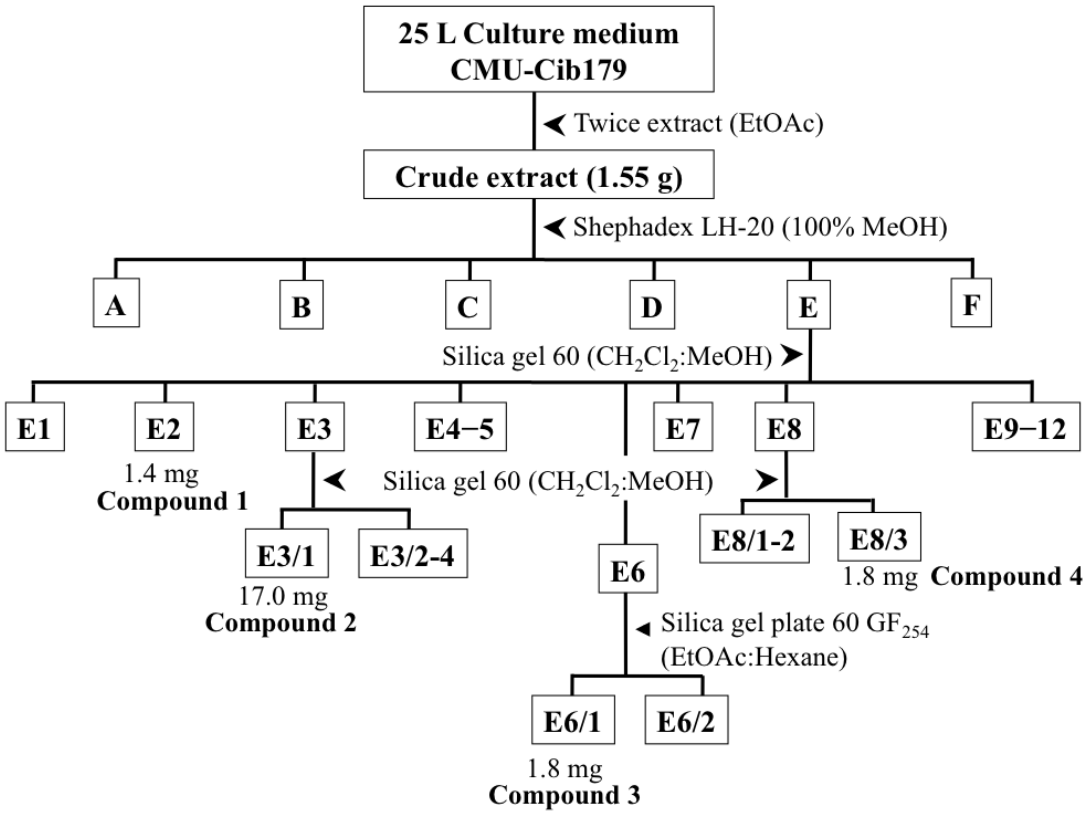


Figure 7.1 The diagram of the purification procedure of the crude extract from *Mycoleptodiscus terrestris* strain CMU-Cib179.

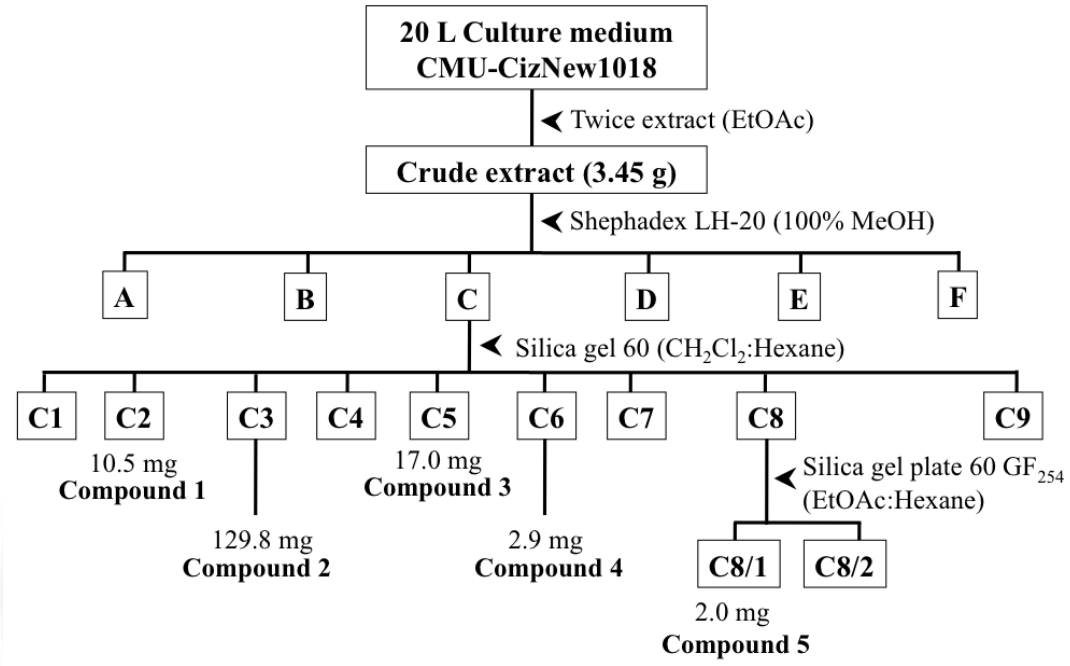


Figure 7.2 The diagram of the purification procedure of the crude extract from *Nodulisporium* sp. strain CMU-Cib1018.

7.2.4 General experimental procedures

Infrared spectra (IR) were recorded neat on a Perkin Elmer FTS FT-IR spectrophotometer. Ultraviolet spectra (UV) spectra were measured in MeOH with a Perkin Elmer UV-Vis spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz Bruker FTNMR Ultra Shield spectrometer in $CDCl_3$. Thin layer chromatography (TLC) and precoated TLC (PTLC) were performed on silica gel GF_{254} (Merck, Germany). Column chromatography (CC) was carried out on Sephadex LH-20 or silica gel (Merck, Germany) type 100 (70–230 Mesh ASTM).

7.3 Results

7.3.1 Screening for antimicrobial production

All fungal strain cultured in the different fermented media showed varied inhibition against tested organisms which depended on fungal strain and media. Potato dextrose broth showed the best media for promoting antimicrobial compounds production, followed by F1 and F2 media and yielded higher number of strain of endophytic fungi that strongly inhibited growth of the test microorganisms (Table 7.1 and Figure 7.3). The inhibition zone (≥8 mm diameter) against tested microorganism in different fermented media by each fungal endophyte strain was shown in Table 7.2−7.4. Four fungal endophyte strain *Chaetomium globosum* CMU-Cib222, *Fusarium solani* CMU-Cib216, *M. terrestris* CMU-Cib179 and *Nodulisporium* sp. CMU-Cib1018 in fermented PDB inhibited all tested microorganisms. The highest diameter of all tested microbial inhibition zone was found in fermented PDB by strain CMU-Cib179, followed by strain CMU-Cib1018 which both strains were selected for further experimentation.

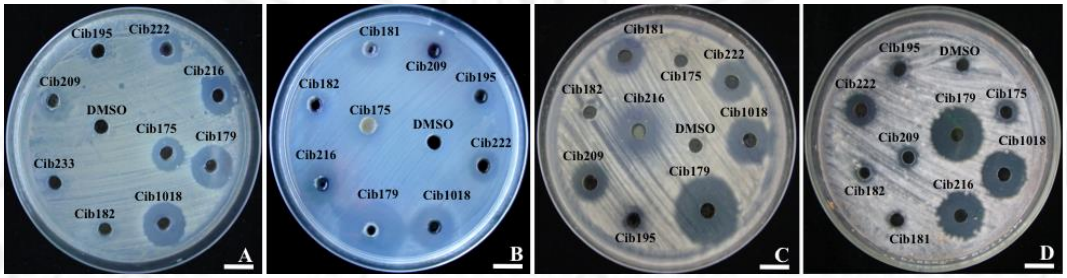


Figure 7.3 The inhibition zone of antimicrobial agent produce by some endophytic fungal strains in fermented potato dextrose broth by agar well diffusion assay. A. *Pseudomonas aeruginosa*, B. *Staphylococcus aureus*, C. *Aspergillus flavus* and D. *Colletotrichum gloeosporioides*. Bar = 10 mm.

Table 7.1 Qualitative antimicrobial activity of endophytic fungi culture in different fermented media.

Potential antimicrobial activity[a]	Number of isolates and percentage (%) of endophytic fungi inhibiting growth of test organisms										
	F1 medium										
	B. sub	***E. col***	***M. lut***	***Ps. aer***	***S. aur***	**MRSA**	***C. alb***	***A. fla***	***Co. glo***	***Pe. dig***	***Pe. exp***
+++	1 (0.79)	0 (0)	1 (0.79)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
++	3 (2.36)	1 (0.79)	2 (1.57)	2 (1.57)	4 (3.15)	2 (1.57)	1 (0.79)	2 (1.57)	3 (2.36)	1 (0.79)	1 (0.79)
+	7 (5.51)	3 (2.36)	11 (8.66)	2 (1.57)	9 (7.09)	13 (10.24)	3 (2.36)	0 (0)	4 (3.15)	7 (5.51)	5 (3.94)
±	21 (16.54)	15 (11.81)	37 (29.13)	3 (2.36)	17 (13.39)	13 (10.24)	0 (0)	12 (9.45)	9 (7.09)	4 (3.15)	3 (2.36)
-	95 (74.80)	108 (85.04)	76 (59.84)	120 (94.49)	97 (76.38)	99 (77.95)	123 (96.85)	113 (88.98)	111 (87.40)	115 (90.55)	118 (92.91)

Potential antimicrobial activity[a]	Number of isolates and percentage (%) of endophytic fungi inhibiting growth of test organism										
	F2 medium										
	B. sub	***E. col***	***M. lut***	***Ps. aer***	***S. aur***	**MRSA**	***C. alb***	***A. fla***	***Co. glo***	***Pe. dig***	***Pe. exp***
+++	1 (0.79)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
++	1 (0.79)	0 (0)	2 (1.57)	0 (0)	2 (1.57)	2 (1.75)	1 (0.79)	0 (0)	1 (0.79)	1 (0.79)	1 (0.79)
+	12 (9.45)	1 (0.79)	11 (8.66)	2 (1.57)	4 (3.15)	5 (3.94)	2 (1.57)	2 (1.57)	2 (1.57)	3 (2.36)	2 (1.57)
±	4 (3.15)	14 (11.02)	19 (14.96)	8 (6.30)	13 (10.24)	14 (11.02)	0 (0)	3 (2.36)	4 (3.15)	4 (3.15)	1 (0.79)
-	109 (85.83)	112 (88.19)	95 (74.80)	117 (92.13)	108 (85.04)	106 (83.46)	124 (97.64)	112 (96.06)	120 (94.49)	119 (39.70)	123 (96.85)

[a] +++ = > 20 mm; ++ = 11–20 mm; + = 8–10 mm; ± = 6–5 mm; – = < 5 mm.

B. sub = Bacillus subtilis, E. col = Escherichia coli, M. lut = Micrococcus luteus, Ps. aer = Pseudomonas aeruginosa, S. aur = Staphylococcus aureus, MRSA = methicillin-resistant *Staphylococcus aureus, Ca. alb = Candida albicans, A. fla = Aspergillus flavus, Co. glo = Colletotrichum gloeosporioides, Pe. dig = Penicillium digitatum* and *Pe. exp = Penicillium expansum.*

Table 7.1 (Continued).

Potential antimicrobial activity[a]	Number of isolates and percentage (%) of endophytic fungi inhibiting growth of test organisms										
	Potato dextrose broth										
	B. sub	***E. col***	***M. lut***	***Ps. aer***	***S. aur***	**MRSA**	***C. alb***	***A. fla***	***Co. glo***	***Pe. dig***	***Pe. exp***
+++	1 (0.79)	0 (0)	1 (0.79)	0 (0)	2 (1.57)	1 (0.79)	0 (0)	1 (0.79)	2 (1.57)	1 (0.79)	1 (0.79)
++	4 (3.15)	2 (1.57)	3 (2.36)	2 (1.57)	7 (5.51)	2 (1.57)	2 (1.57)	3 (2.36)	3 (2.36)	1 (0.79)	2 (1.57)
+	12 (9.45)	4 (3.14)	14 (11.02)	5 (3.93)	11 (8.66)	12 (9.45)	8 (6.30)	2 (1.57)	12 (9.45)	8 (6.30)	7 (5.51)
±	18 (14.17)	27 (21.26)	79 (76.37)	48 (37.79)	21 (16.54)	36 (28.35)	1 (0.79)	11 (8.66)	17 (13.39)	2 (6.30)	6 (4.72)
-	92 (72.44)	94 (74.01)	30 (23.62)	72 (56.69)	86 (67.72)	76 (59.84)	116 (91.34)	110 (86.61)	93 (73.23)	115 (90.55)	111 (87.40)

[a] +++ = > 20 mm; ++ = 11–20 mm; + = 8–10 mm; ± = 7–5 mm; – = < 5 mm.

B. sub = Bacillus subtilis, E. col = Escherichia coli, M. lut = Micrococcus luteus, Ps. aer = Pseudomonas aeruginosa, S. aur = Staphylococcus aureus, MRSA = methicillin-resistant *Staphylococcus aureus, Ca. alb = Candida albicans, A. fla = Aspergillus flavus, Co. glo = Colletotrichum gloeosporioides, Pe. dig = Penicillium digitatum* and *Pe. exp = Penicillium expansum.*

Table 7.2 The inhibition zone (≥8 mm diameter) against test organisms of endophytic fungi culture in F1 medium.

Taxa	Isolate number (CMU-)[a]	Inhibition zone (mm)[b]										
		Ba	***Es***	***Mi***	***Ps***	***St***	**MR**	***Ca***	***As***	***Co***	***Pe. d***	***Pe. e***
Chaetomium globosum	Cib222	16.7		13.5	10.7	14.5	10.0	9.5		13.0	10.6	9.6
Cladosporium cladosporioides	Ciz008			8.0								
Cladosporium cladosporioides	Ciz256			9.3								
Colletotrichum gloeosporioides	Cib146					8.0	9.5					
Colletotrichum gloeosporioides	Cib181					8.0	9.0					
Colletotrichum gloeosporioides	Gne443					9.5	9.0					
Eupenicillium javanicum	Ciz200	8.0								9.5		
Fusarium oxysporum	Ciz220	17.0		10.7	8.5	8.0	8.0			10.0	10.0	9.0
Fusarium sp. 1	Cib466	10.3		9.7							8.0	8.0
Guignardia mangiferae	Cib344						8.5					
Guignardia mangiferae	Cib422						10.0					
Humicola fuscoatra	Cib1657	10.5										
Mycoleptodiscus terrestris	Cib179	21.5	12.0	20.0	13.5	18.6	19.0	18.0	15.0	16.3	14.3	13.0
Nodulisporium sp.	Cib1018	19.0	10.7	16.7	11.0	17.0	15.5	10.5	11.3		9.0	10.3
Pestalotiopsis mangiferae	Cib044			9.3			10.5					
Pestalotiopsis microspora	Lag132	9.0		9.7								
Phomopsis sp.	Cib051			10.0		10.0	9.7					
Phomopsis sp.	Ciz630			9.0		9.5	9.5					
Talaromyces flavus	Cib275	9.5	9.3							10.0	10.3	
Trichoderma sp.	Cib223	9.7	9.7	10.5		9.5				13.0	8.3	10.0
Verticillium sp.	Gne985			10.0		13.0	9.5	8.0		10.0	8.0	
Xylaria sp.	Cib186	8.0		8.0		9.0	8.0					
Xylaria sp.	Cib1976					9.0	8.0					

[a] Cib = *Cinnamomum bejolghota*, Ciz = *C. zylanicum*, Gne = *Gnetum montanum* and Lag = *Lagerstroemia loudoni*.
[b] Means of inhibition zones surrounding each application point, three replicate.
Ba = Bacillus subtilis, *Es = Escherichia coli*, *Mi = Micrococcus luteus*, *Ps = Pseudomonas aeruginosa*, *St = Staphylococcus aureus*, MR = methicillin-resistant *Staphylococcus aureus*, *Ca = Candida albicans*, *As = Aspergillus flavus*, *Co = Colletotrichum gloeosporioides*, *Pe. d = Penicillium digitatum* and *Pe. e = Penicillium expansum.*

Table 7.3 The inhibition zone (≥8 mm diameter) against test organisms of endophytic fungi culture in F2 medium.

Taxa	Isolate number (CMU)[a]	Inhibition zone (mm)[b]										
		Ba	***Es***	***Mi***	***Ps***	***St***	**MR**	***Ca***	***As***	***Co***	***Pe. d***	***Pe. e***
Chaetomium globosum	Cib222	13.3		9.7	8.0	11.0	11.5	9.0	8.0	10.0		
Cladosporium cladosporioides	Ciz008						8.0					
Colletotrichum gloeosporioides	Cib181					9.0	8.7					
Colletotrichum gloeosporioides	Gen443					9.3	9.0					
Drechslera sp.	Ciz786				9.0			8.0				
Eupenicillium javanicum	Ciz200	9.7		9.0								
Fusarium oxysporum	Ciz220	8.0		10.0		8.5	8.0					
Fusarium oxysporum	Lag348	8.0		10.0		8.0	8.0					
Guignardia mangiferae	Cib344		8.7									8.5
Glomerella sp.	Cib002										8.3	
Glomerella sp.	Cib006										8.0	
Mycoleptodiscus terrestris	Cib179	20.3		13.5		11.7	11.5	12.0		12.5	12.0	12.0
Mycilia sterilia	Cib1388	8.7		8.0								
Mycilia sterilia	Cib2044	8.5		8.0								
Nigrospora oryzae	Cib080									10.3		
Phomopsis sp.	Cib051	10.3		9.7								
Phomopsis sp.	Ciz630	9.7		9.0								
Phomopsis sp.	Cib269	9.7		9.0								
Phomopsis sp.	Lag312	9.0		9.5								
Verticillium sp.	Gne985								9.0		8.0	
Xylaria sp.	Cib186	8.3		8.0								
Xylaria sp.	Cib1976	8.0		8.0								
Xylaria sp.	Gne553	8.0										

[a] Cib = *Cinnamomum bejolghota*, Ciz = *C. zylanicum*, Gne = *Gnetum montanum* and Lag = *Lagerstroemia loudoni*.
[b] Means of inhibition zones surrounding each application point, three replicate.
Ba = Bacillus subtilis, *Es = Escherichia coli*, *Mi = Micrococcus luteus*, *Ps = Pseudomonas aeruginosa*, *St = Staphylococcus aureus*, MR = methicillin-resistant *Staphylococcus aureus*, *Ca = Candida albicans*, *As = Aspergillus flavus*, *Co = Colletotrichum gloeosporioides*, *Pe. d = Penicillium digitatum* and *Pe. e = Penicillium expansum.*

Table 7.4 The inhibition zone (≥8 mm diameter) against test organisms of endophytic fungi culture in PDB medium.

Taxa	Isolate number (CMU)[a]	Inhibition zone (mm)[b]										
		Ba	***Es***	***Mi***	***Ps***	***St***	**MR**	***Ca***	***As***	***Co***	***Pe. d***	***Pe. e***
Alternaria alternata	Cib198			9.0						9.0		
Chaetomium globosum	Cib222	15.0	8.0	14.5	9.5	10.5	15.7	10.0	16.0	12.0	10.5	14.5
Cladosporium cladosporioides	Ciz008	10.3		8.3		9.0	10.5			8.0		
Cladosporium cladosporioides	Ciz256	9.3		9.0		9.0	10.0			8.3		
Colletotrichum gloeosporioides	Cib181	9.0		9.0		8.7			8.0			
Eupenicillium javanicum	Ciz200			8.0	9.0	10.5	10.5			8.0		
Eupenicillium shearii	Cib967		10.0	8.0	9.0	9.7	9.0			8.0		
Fusarium oxysporum	Ciz220	10.0				14.5	9.5					
Fusarium solani	Cib216	18.7	10.5	17.7	10.7	19.7	10.0	10.7	15.0	16.5	10.0	10.0
Glomerella sp.	Cib002					10.5			8.0			
Guignardia mangiferae	Cib344	8.3		9.0						8.3	9.5	9.0
Guignardia mangiferae	Cib422	8.3		9.0							9.0	9.0
Mycoleptodiscus terrestris	Cib179	27.5	17.5	24.0	15.5	27.0	29.5	19.0	23.0	21.5	20.5	21.0
Nodulisporium sp.	Cib1018	18.0	12.5	19.5	12.7	22.7	19.0	17.5	17.5	20.0	16.5	15.7
Nigrospora oryzae	Ciz861	10.5				14.0	10.0	10.5		9.0	9.5	8.0
Pestalotiopsis microspora	Lag132	8.0				9.5						
Pestalotiopsis theae	Lag376			10.0		9.0						
Phoma sp.	Cib159	9.5		9.0		10.5	9.5	9.0		9.5		
Phoma sp.	Cib166	9.5		9.3		10.0	9.5	9.0		9.5		
Phomopsis spp.	Cib269	9.0		10.5		10.0				10.7		
Sclerotium sp.	Cib175	13.0	10.5	10.5	10.3	15.0	10.5	10.7		14.7	10.7	9.0
Trichoderma sp.	Cib223	8.0		9.0		11.7	9.5	10.7		10.5	10.0	8.7
Verticillium sp.	Gne985					12.5	8.0				9.5	8.0
Xylaria sp.	Cib186							9.5		10.0		

[a] Cib = *Cinnamomum bejolghota*, Ciz = *C. zylanicum*, Gne = *Gnetum montanum* and Lag = *Lagerstroemia loudoni*.
[b] Means of inhibition zones surrounding each application point, three replicate.
Ba = Bacillus subtilis, *Es = Escherichia coli*, *Mi = Micrococcus luteus*, *Ps = Pseudomonas aeruginosa*, *St = Staphylococcus aureus*, MR = methicillin-resistant *Staphylococcus aureus*, *Ca = Candida albicans*, *As = Aspergillus flavus*, *Co = Colletotrichum gloeosporioides*, *Pe. d = Penicillium digitatum* and *Pe. e = Penicillium expansum.*

7.3.2 Taxonomy of selected fungal endophyte strains

7.3.2.1 *Mycoleptodiscus terrestris* CMU-Cib179

The identification of CMU-Cib179 was based on morphological characteristic and molecular data via partial sequencing of its ITS1, ITS2 5.8S rDNA. Fungal colonies were dark green to dark gray on PDA (Figure 7.4A) and white to cream on basal salts medium (Jackson *et al*., 2011) (Figure 7.4B). Sclerotia green to black spherical, subspherical, fusiform, irregular in shape, often elongated, or aggregated embedded, up to 4.5 mm (Figure 7.4C, 7.4B), tissues composed of hyphae with subspherical thick-walled cell 3.7–19.5 µm (Figure 7.4E). Spore and sporodochia were not observed.

Partial of ITS1, ITS2 5.8S rDNA sequence *Mycoleptodiscus terrestris* CMU-Cib179 was obtained and compared with GenBank database. The sequence is deposited in GenBank as KC911630 and analyzed phylogenetically with 17 sequences of allied genera and the out group (*Arthrobotrys conides* and *A. musiformis*) obtained from literature and the GenBank database. Parsimony analyses of the alignment yielded 57 most parsimonious trees with total length of 744 steps (CI=0.774, RI=0.849, RC=0.657 and HI=0.226), one of which is shown in Figure 7.5. Phylogenetic analysis indicated that *M. terrestris* CMU-Cib179 was related to *M. terrestris* EU364807 and JN711860 which from sister group with 100% bootstrap support.

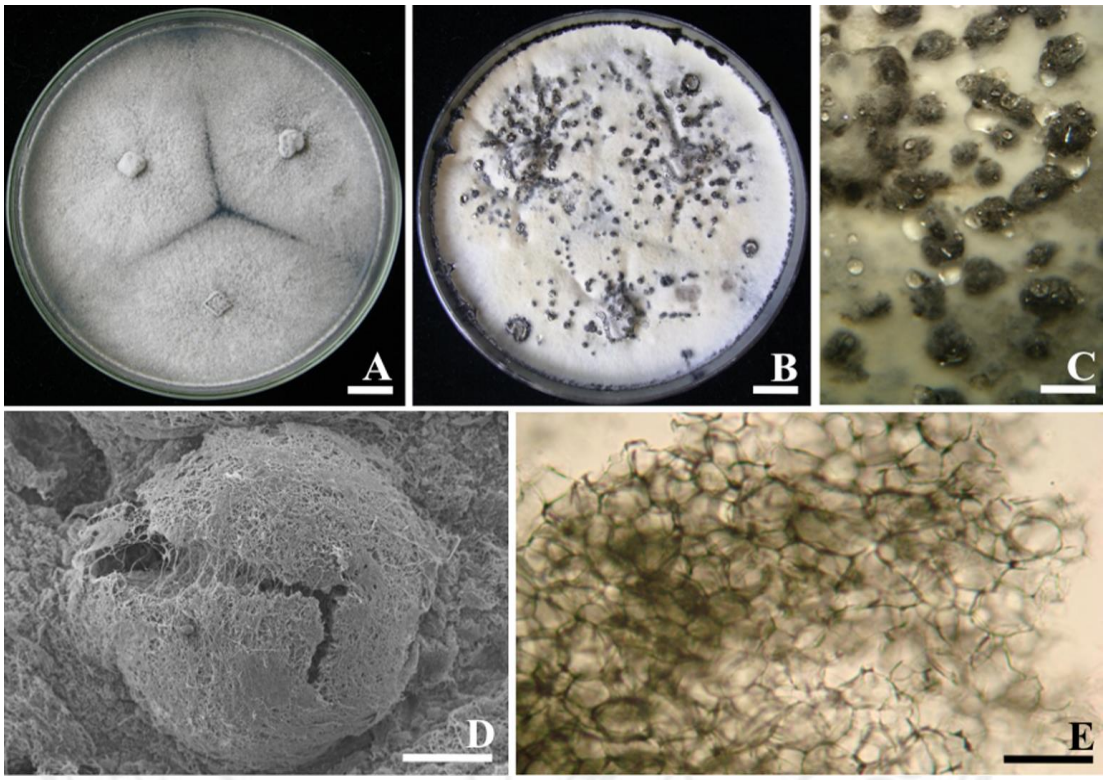


Figure 7.4 *Mycoleptodiscus terrestris* CMU-Cib179. A. Colonies grown 12 days at 25°C on PDA, B. Colonies grown 12 days at 25°C on basal salts medium, C. Sclerotia under stereo microscopy, D. Sclerotia under scanning electron microscopy, E. Spherical component cells of sclerotia. Bars: A, B = 10 mm, C = 2 mm, D = 0.5 mm, E = 5 μm.

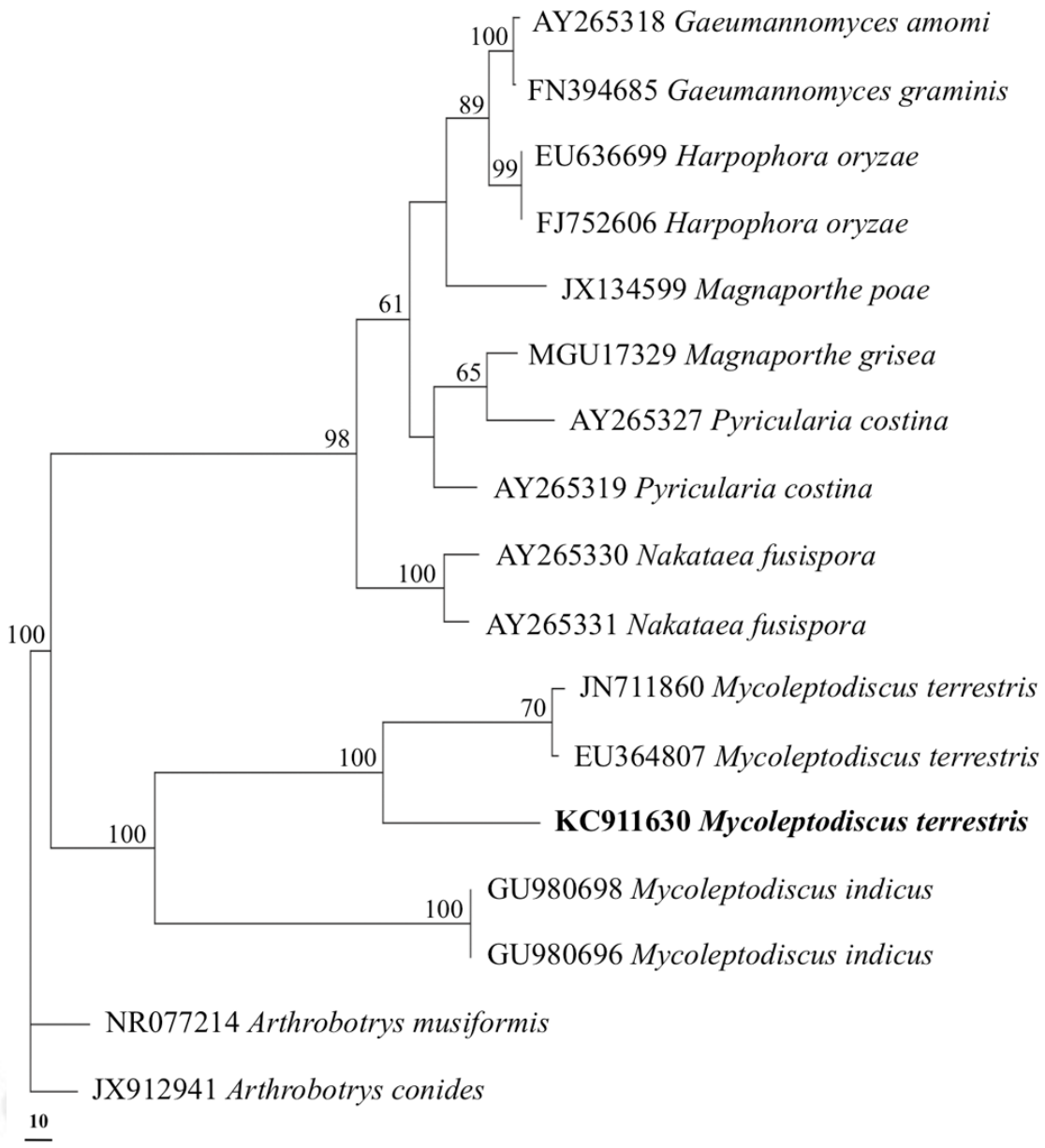


Figure 7.5 One of 57 most parsimonious trees inferred from a heuristic search of the ITS1, ITS2 5.8S rDNA sequence alignment of 17 sequences. *Arthrobotrys conides* and *A. musiformis* were used to root the tree. The size of the branches is indicated with a scale bar. Branches with bootstrap values ≥ 50% are shown at each branch.

7.3.2.2 *Nodulisporium* sp. CMU-Cib1018

The identification of CMU-Cib1018 was based on morphological characteristic and molecular data via partial sequencing of its ITS1, ITS2 5.8S rDNA. Fungal colonies were whiteon PDA (Figure 7.6A). Hyphae were 0.6–3.9 µm thick and branching. Conidiogenous cells were attached in irregular to verticillate patterns, arising singly or more often in groups, laterally or more frequently at each branch terminus (Figure 7.6B, 7.6C). Conidia were pale brown and smooth, 2.1–3.4 × 4.7–6.1 µm, single celled and ellipsoidal, with a flattened base indicating the former point of attachment to the conidiogenous cell (Figure 6.1D–6.1F). In morphological respects the fungus appeared to be a hyphomycetes, *Nodulisporium* sp.

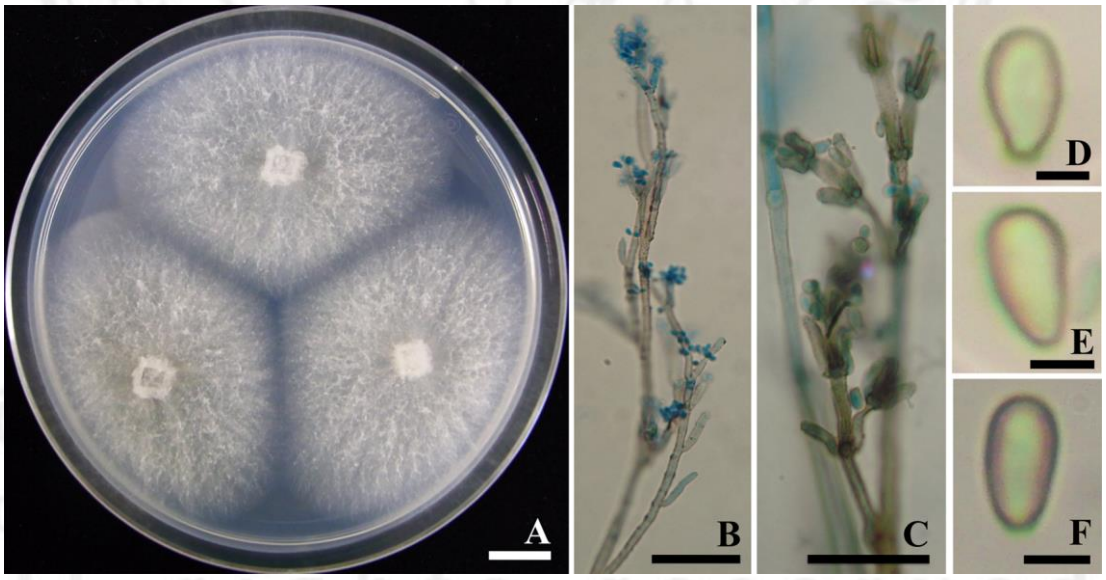


Figure 7.6 *Nodulisporium* sp. CMU-Cib1018. A. Colonies grown 7 days at 25°C on PDA, B and C. Conidiophores, D–F. Conidia. Bars: A = 10 mm, B = 15 µm, C–F = 5 µm.

Partial of ITS1, ITS2 5.8S rDNA sequence *Nodulisporium* sp. CMU-UPE34 was obtained and compared with GenBank database. The sequence is deposited in GenBank as JN558831 and analyzed phylogenetically with 24 sequences of allied genera and the out group (*Peziza badia* and *Taphrina sadebeckii*) obtained from literature and the GenBank database. Parsimony analyses of the alignment yielded 125 most parsimonious trees with total length of 700 steps (CI=0.630, RI=0.667, RC=0.420 and HI=0.369), one of which is shown in Figure 7.7 Phylogenetic analysis indicated that *Nodulisporium* sp. CMU-Cib1018 was closely related to *Nodulisporium* sp. JP807 and A4, which in turn are related to genus *Daldinia*.

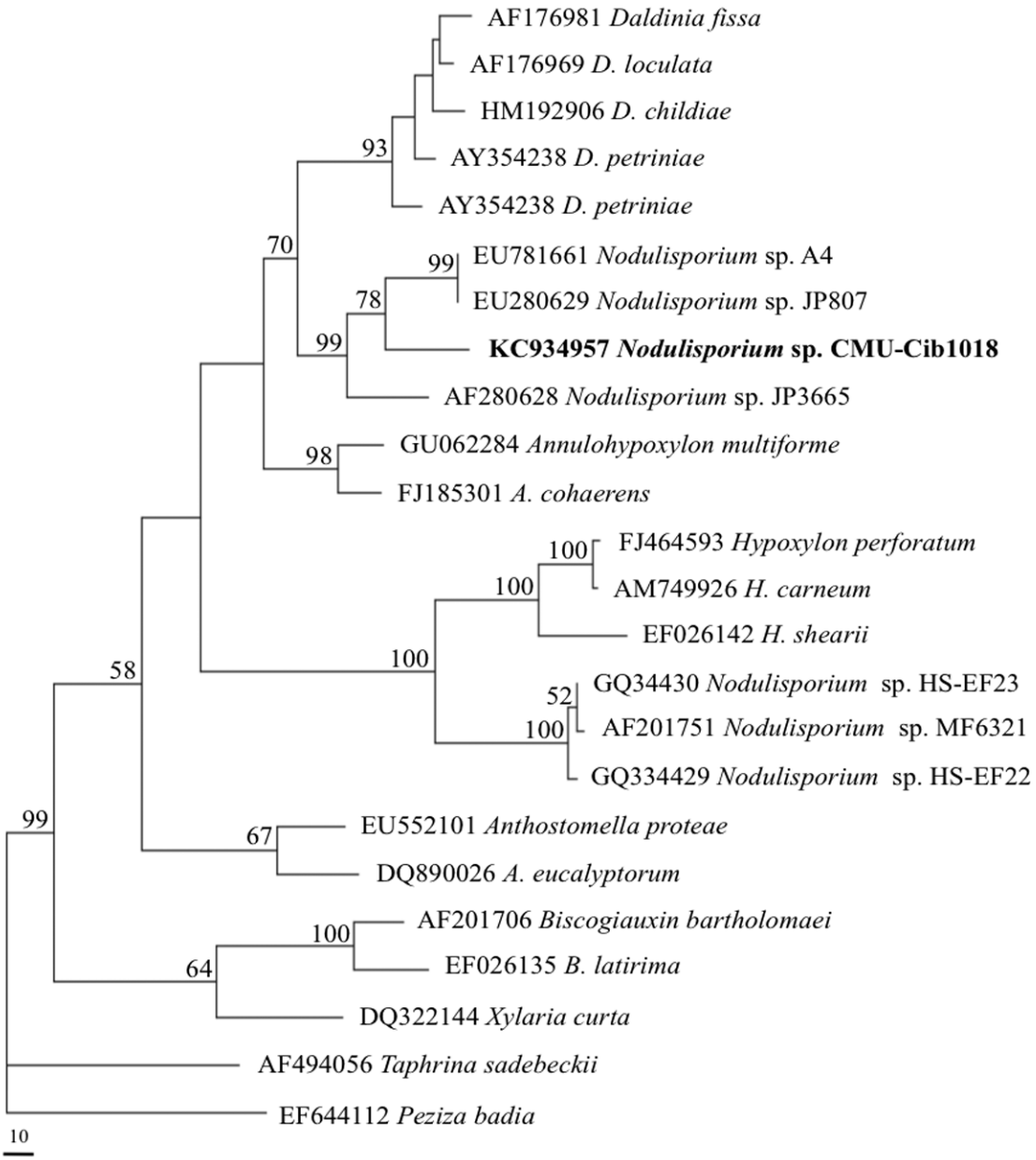


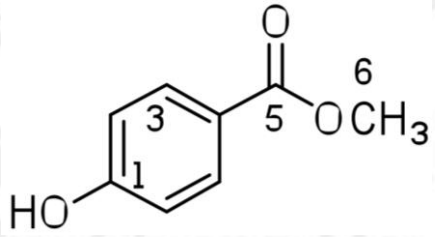
Figure 7.7 One of 125 most parsimonious trees inferred from a heuristic search of the ITS1, ITS2 5.8S rDNA sequence alignment of 24 sequences. *Peziza badia* and *Taphrina sadebeckii* were used to root the tree. The size of the branches is indicated with a scale bar. Branches with bootstrap values ≥ 50% are shown at each branch.

7.3.3 Structure elucidation of compounds

7.3.3.1 *Mycoleptodiscus terrestris* CMU-Cib179

The crude ethyl acetate extract was separated and purified using column chromatography over silica gel or Sephadex LH-20, leading to the isolation of compounds. Six fractions were evaporated to dynes, weighed and tested for antimicrobial activity. Fraction E (239.9 mg) was active against *S. aureus*, methicillin-resistant *S. aureus* (MRSA) and *A. flavus* in the initial antimicrobial assay. Four compounds, *p*-hydroxybenzoic acid methyl ester (**1**, 1.4 mg), puupehenol (**2**, 17.0 mg), 4-hydroxybenzoic acid (**3**, 1.8 mg) and 2,4-dihydroxyacetophenone (**4**, 1.8 mg) were obtained from *M. terrestris* CMU-Cib179. The structures of the pure compounds were determined from ^{1}H and ^{13}C NMR spectra and comparison of their spectroscopic data with those in the literatures.

***p*-Hydroxybenzoic acid methyl ester (1)**



1

Compound **1** was obtained as a brown gum. It shows a strong absorption under UV light at λ_{max} 254 nm. The ^{1}H NMR spectrum showed characteristic signals for four

aromatic protons of *p*-disubstituted benzene ring and methoxy protons (Figure 7.8 and Table 7.5).

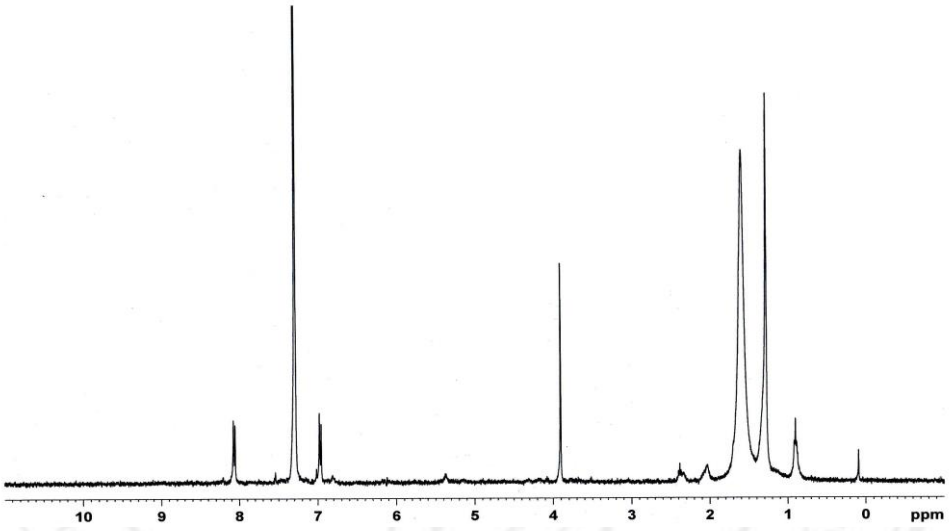
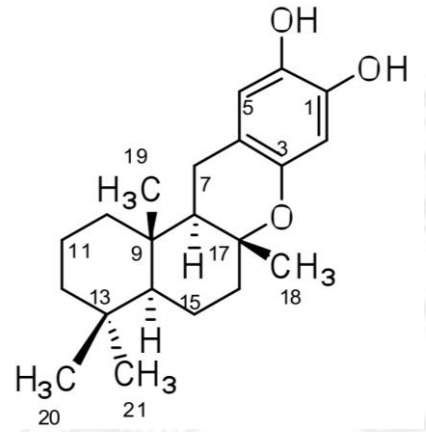


Figure 7.8 ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) of *p*-hydroxybenzoic acid methyl ester (**1**).

Table 7.5 ^{1}H NMR data of *p*-hydroxybenzoic acid methyl ester (**1**)

Position	**δ_H mult. (*J* in Hz)**
1	-
2	6.95 d (8.0)
3	7.95 d (8.0)
4	-
5	-
6	4.75 s

Puupehenol (2)



2

Compound **2** was obtained an orange-brown powder. It shows a strong absorption under UV light at λ_{max} 254 nm. The ^{1}H NMR and ^{13}C NMR spectra displayed two aromatic protons, six methylene groups, one methine proton and four singlet methyl units (Figure 7.9, 7.10 and Table 7.6). 2D NMR data (HMBC, HMQC, COSY) was indicated that compound **2** was a puupehenol.

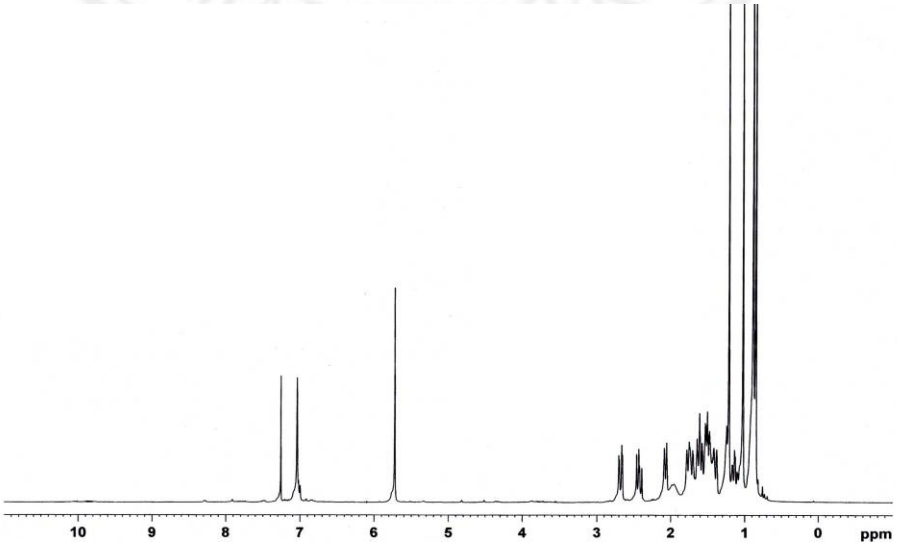


Figure 7.9 ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) of puupehenol (**2**).

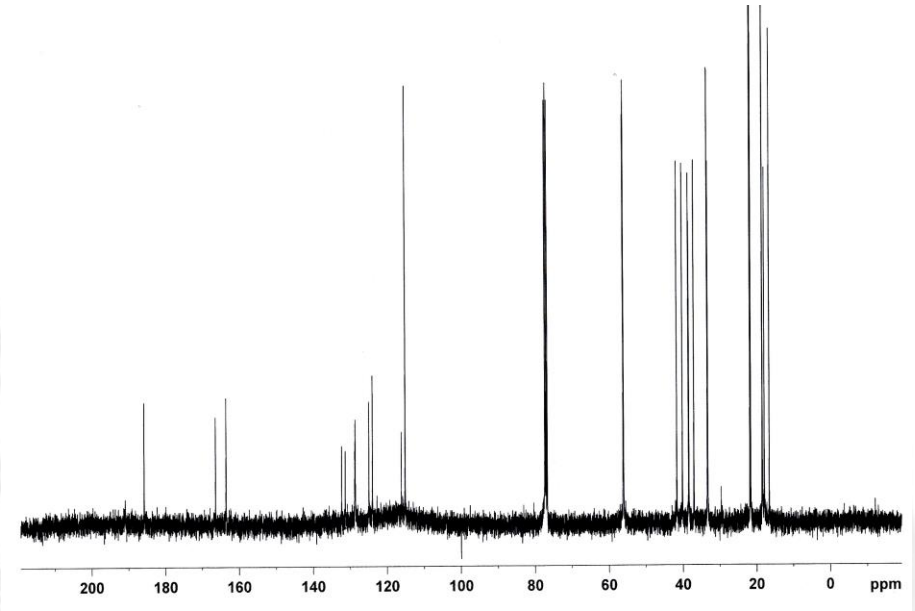


Figure 7.10 ^{13}C-NMR spectrum ($CDCl_3$, 100 MHz) of puupehenol (**2**).

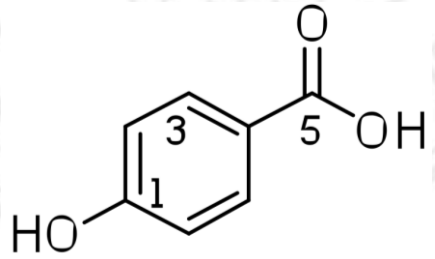
Table 7.6 ^{1}H and ^{13}C NMR data of puupehenol (**2**)

Position	**δ_H mult. (*J* in Hz)**	**δ_C mult.**
1	-	166.4 C
2	5.7 s	115.1 CH
3	-	163.5 C
4	-	124.0 C
5	7.04 s	131.30 CH
6	-	132.3 C
7	2.68 dd (16.4, 3.6)	16.6 CH_2
	2.44 dd (16.4, 12.0)	
8	0.82 m	55.98 CH
9	-	38.5 C
10	1.75 m	40.1 CH_2
11	1.75 m	18.5 CH_2
	1.70 m	
12	1.42 m	41.62 CH_2
	1.35 tm 14.2	

Table 7.6 (Continued).

Position	δ_H mult. (*J* in Hz)	δ_C mult.
13	-	33.2 C
14	1.50 m	40.11 CH
15	2.07 d (12.8)	37.0 CH_2
	1.61 tm (13.2)	
16	1.75 m	18.5 CH_2
	1.70 m	
17	-	79.0 C
18	1.24 s	21.8 CH_3
19	1.03 s	16.6 CH_3
20	0.85 s	21.6 CH_3
21	0.89 s	33.4 CH_3

4-Hydroxybenzoic acid (3)



3

Compound **3** was obtained as a cream powder. It shows a strong absorption under UV light at λ_{max} 254 nm. The ^{1}H NMR spectrum of compound **3** (Figure 7.11 and Table 7.7) was similar to that of compound **1** except for the absent of the methoxy pronton at δ 3.90. Thus, compound **3** was assigned as a 4-hydroxybenzoic acid.

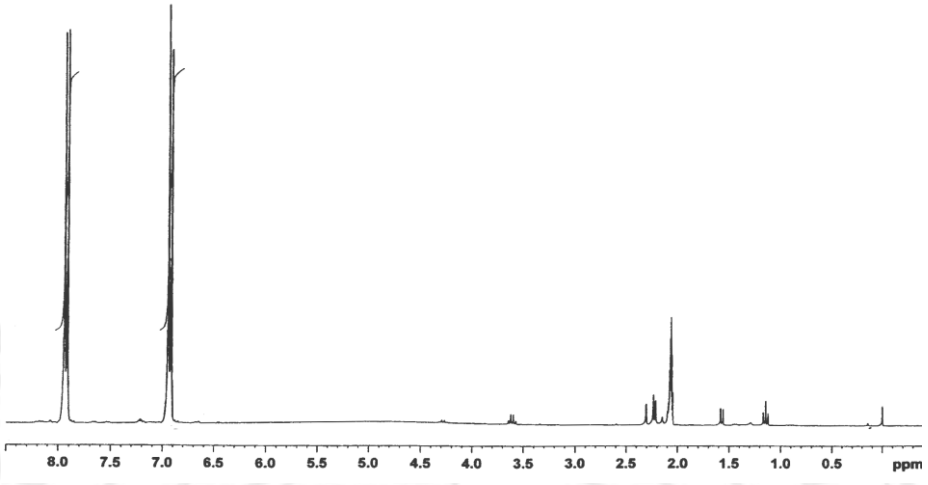
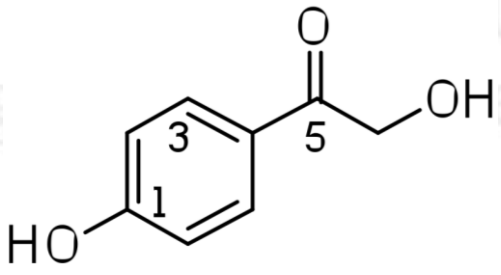


Figure 7.11 ^{1}H-NMR spectrum (Acetone-d_6, 400 MHz) of 4-Hydroxybenzoic acid (**3**).

Table 7.7 ^{1}H NMR data of 4-hydroxybenzoic acid (**3**)

Position	**δ_H mult. (*J* in Hz)**
1	-
2	6.85 d (8.4)
3	7.85 d (8.4)
4	-
5	-

2,4′-Dihydroxyacetophenone (4)



4

Compound **4** was obtained as a yellow powder. It shows a strong absorption under UV light at λ_{max} 254 nm. The ^{1}H NMR spectrum of compound **4** (Figure 7.12 and Table 7.8) was similar to that of compound **1** except for the replacement of the methoxy group in compound **1** with a signal of a hydroxymethyl unit (δ 4.75) in compound **4**. Consequently, compound **4** was identified as a 2,4′-dihydroxyacetophenone.

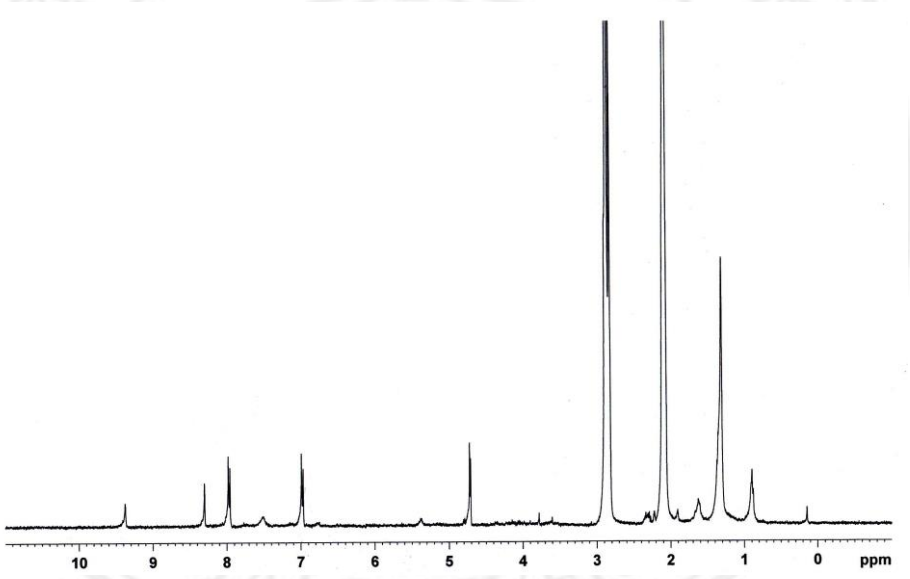


Figure 7.12 ^{1}H-NMR spectrum (Acetone-d_6, 400 MHz) of 2,4′-dihydroxyacetophenone (**4**).

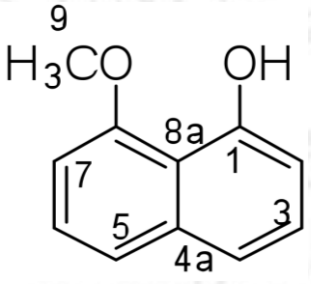
Table 7.8 ^{1}H NMR data of 2,4′-dihydroxyacetophenone (**4**)

Position	**δ_H mult. (*J* in Hz)**
1	-
2	6.95 d (8.0)
3	7.95 d (8.0)
4	-
5	-
6	4.75 s

7.3.3.2 *Nodulisporium* sp. CMU-Cib1018

The crude ethyl acetate extract was separated and purified using column chromatography over silica gel or Sephadex LH-20, leading to the isolation of five compounds. Six fractions were evaporated to dynes, weighed and tested for antimicrobial activity. Fraction C (610.0 mg) was active against *B. subtilis*, *E. coli*, *M. luteus*, *S. aureus*, methicillin-resistant *S. aureus* (MRSA), *A. flavus*, *C. gloeosporioides P. digitatum* and *P. expansum* in the initial antimicrobial assay. 8-methoxynaphthalen-1-ol (**5**, 10.5 mg), 5-hydroxy-2-methyl-4-chromanone (**6**, 129.8 mg), 5-hydroxy-2-methyl-4*H*-chromen-4-one (**7**, 17.0 mg), 1-(2,6-Dihydroxyphenyl) propan-1-one (**8**, 2.9 mg) and 1-(2,6-dihydroxyphenyl)ethanone (**9**, 2.0 mg) were obtained from *Nodulisporium* sp. CMU-Cib1018. The structures of the pure compounds were determined from ^{1}H and ^{13}C NMR spectra and comparison of their spectroscopic data with those in the literatures.

8-Methoxynaphthalen-1-ol (5)



5

Compound **5** was obtained as a yellow powder. It shows a strong absorption under UV light at λ_{max} 254 nm. The ^{1}H NMR and ^{13}C NMR spectra displayed six aromatic protons, one methoxyl group and a hydroxyl group. 2D NMR data (HMBC, HMQC,

COSY) of compound **5** was indicated that compound **5** was a 8-methoxynaphthalen-1-ol (Figure 7.13 and Table 7.9).

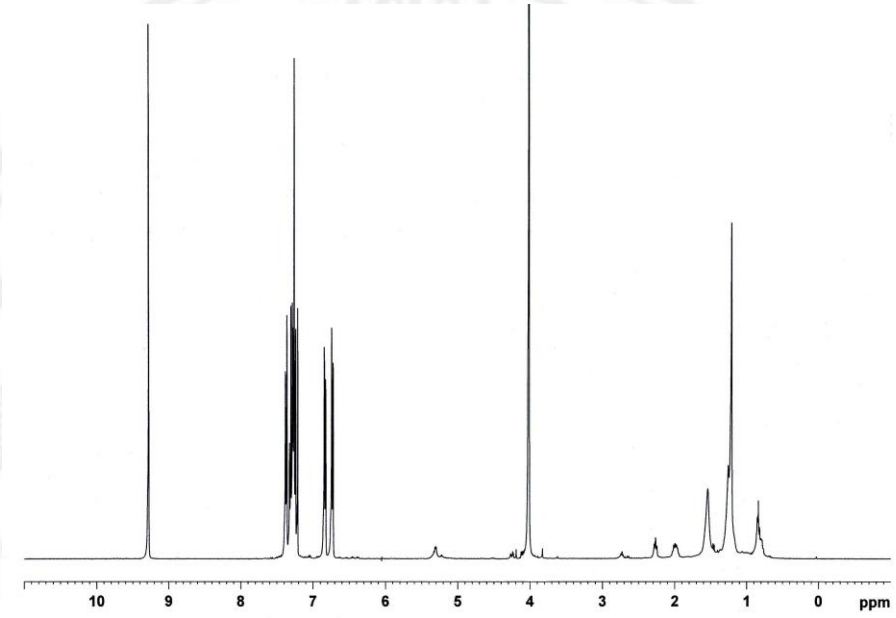
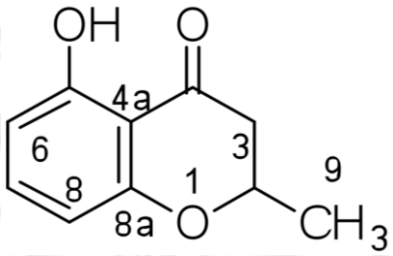


Figure 7.13 ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) of 8-methoxynaphthalen-1-ol (**5**).

Table 7.9 ^{1}H and ^{13}C NMR data of 8-methoxynaphthalen-1-ol (**5**)

Position	**δ_H mult. (*J* in Hz)**	**δ_C mult.**
1	-	154.50 C
1-OH	9.32 s	-
2	6.87 dd (8.1, 1.8)	110.42 CH
3	7.35 t (8.1)	127.72 CH
4	7.29 dd (8.1, 1.8)	118.87 CH
4a	-	136.76 C
5	7.41 d (7.8)	121.87 CH
6	7.30 t (7.8)	125.60 CH
7	6.76 d (7.8)	103.91 CH
8	-	156.17 C
8a	-	115.00 C
9	4.05 s	56.10 CH_3

5-Hydroxy-2-methyl-4-chromanone (6)



6

Compound **6** was obtained as a brown powder. It shows a strong absorption under UV light at λ_{max} 254 nm. The structure of compound **6** was analyzed using the ^{1}H and ^{13}C NMR spectra data in $CDCl_3$ (Figure 7.14 and Table 7.10). The proton signals were determined as 5-hydroxy-2-methyl-4-chromanone.

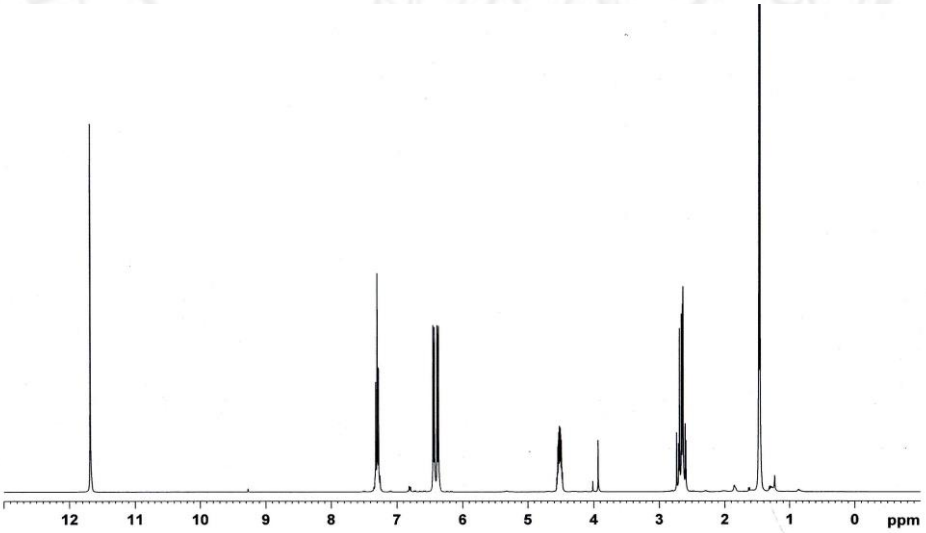
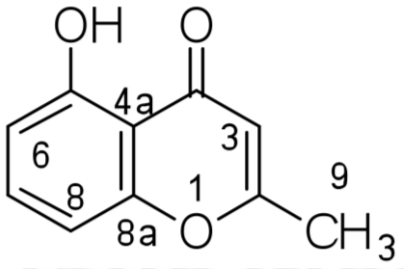


Figure 7.14 ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) of 5-hydroxy-2-methyl-4-chromanone (**6**).

Table 7.10 ^{1}H and ^{13}C NMR data of 5-hydroxy-2-methyl-4-chromanone (**6**)

Position	δ_H mult. (*J* in Hz)	δ_C mult.
1		
2	4.51 m	73.67 CH
3	2.65 dd (18.4, 10.0)	43.67 CH_2
4	-	198.38 C
4a	-	107.86 C
5-OH	11.68 s	161.93 C
6	6.44 d (8.4)	108.98 CH
7	7.30 t (8.4)	138.00 CH
8	6.38 d (8.4)	107.16 CH
8a	-	161.55 C
9	1.47 s	20.69 CH_3

5-Hydroxy-2-methyl-4*H*-chromen-4-one (7)



7

Compound **7** was obtained as a brown powder. It shows a strong absorption under UV light at λ_{max} 254 nm. The structure of compound **7** was analyzed using the ^{1}H and ^{13}C NMR spectra data in $CDCl_3$ (Figure 7.15 and Table 7.11). The ^{1}H NMR spectrum of compound **7** was similar to that of compound **6** except for the oxymethine and methylene

protons in compound **7** were replaced by a olefinic proton in compound **6.** Compound **7** was determined as a 5-hydroxy-2-methyl-4*H*-chromen-4-one.

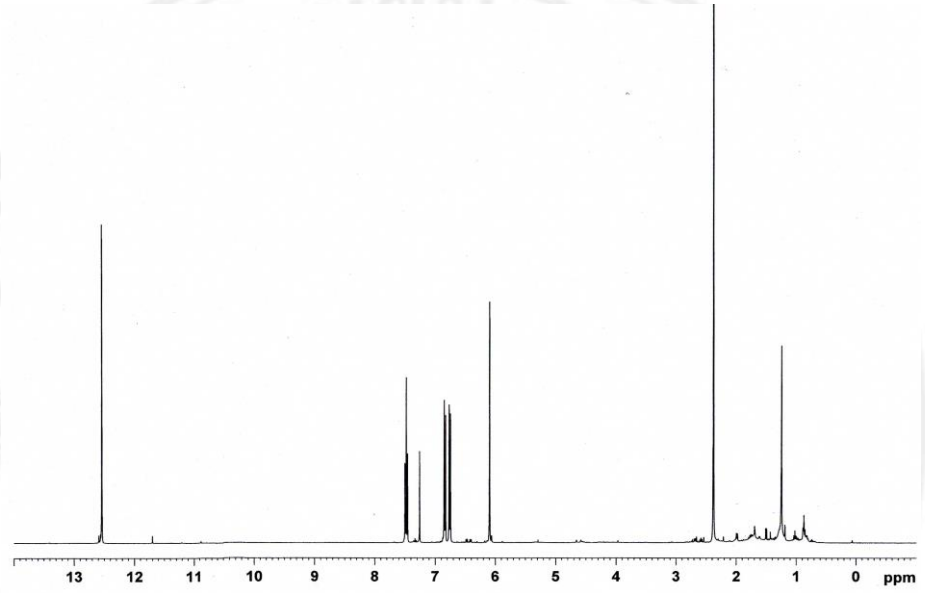
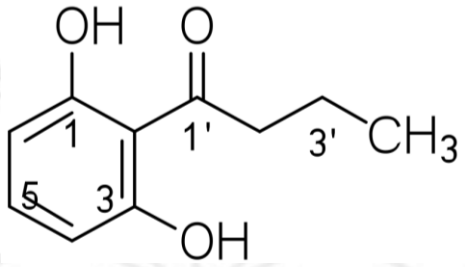


Figure 7.15 ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) of 5-hydroxy-2-methyl-4*H*-chromen-4-one (**7**).

Table 7.11 ^{1}H and ^{13}C NMR data of 5-hydroxy-2-methyl-4*H*-chromen-4-one (**7**)

Position	**δ_H mult. (*J* in Hz)**	**δ_C mult.**
1		
2	-	167.60 C
3	6.10 s	109.09 CH
4	-	183.48 C
4a	-	110.38 C
5-OH	12.54 s	160.74 CH
6	6.76 d (8.4)	111.17 CH
7	7.48 t (8.4)	135.06 CH
8	6.84 d (8.4)	106.79 CH
8a	-	156.73 C
9	2.38 s	20.61 CH_3

1-(2,6-Dihydroxyphenyl)propan-1-one (8)



8

Compound **8** was obtained as a yellow powder. It shows a strong absorption under UV light at λ_{max} 254 nm. The ^{1}H NMR spectrum displayed characteristic signals for three aromatic protons of a 1,2,3-trisubstituted benzene ring and 1-butanoyl fragment (Figure 7.16 and Table 7.12). From the 2D NMR data (HMQC, HMBC and COSY), compound **8** was assigned as a 1-(2,6-dihydroxyphenyl)propan-1-one.

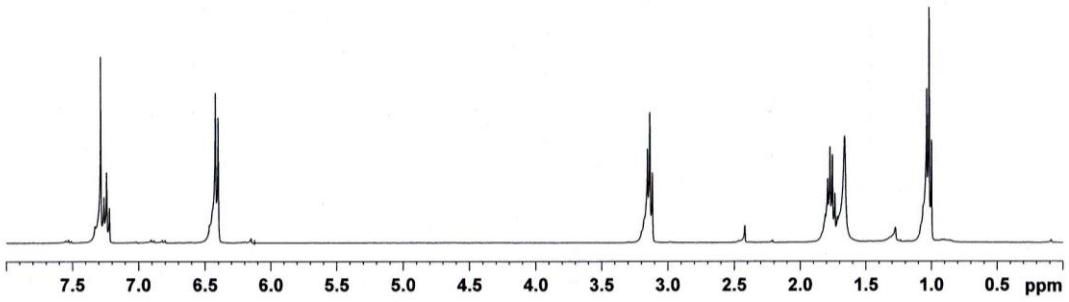
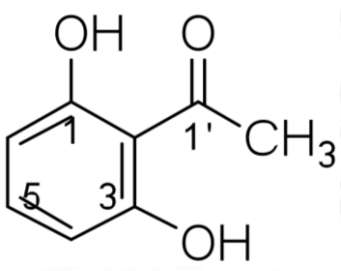


Figure 7.16 ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) of 1-(2,6-dihydroxyphenyl)propan-1-one (**8**).

Table 7.12 ^{1}H and ^{13}C NMR data of 1-(2,6-dihydroxyphenyl)propan-1-one (**8**)

Position	δ_H mult. (*J* in Hz)	δ_C mult.
1, 3	-	161.70 C
2	-	110.50 C
4, 6	6.48 d (8.1)	108.80 CH
5	7.26 t (8.1)	136.30 CH
1′	-	208.70 C
2′	3.17 t (7.3)	47.10 CH_2
3′	1.79 q (7.3)	18.30 CH_2
4′	1.04 t (7.3)	14.30 CH_2

1-(2,6-dihydroxyphenyl)ethanone (9)



9

Compound **9** was obtained as a brown powder. It shows a strong absorption under UV light at λ_{max} 254 nm. The ^{1}H NMR spectrum was similar to that of compound **8** except for the replacement of the 1-butanoyt unit in compound **8** with acetyl unit (Figure 7.17 and Table 7.13). Consequently, compound **9** was determined as a 1-(2,6-dihydroxyphenyl)ethanone.

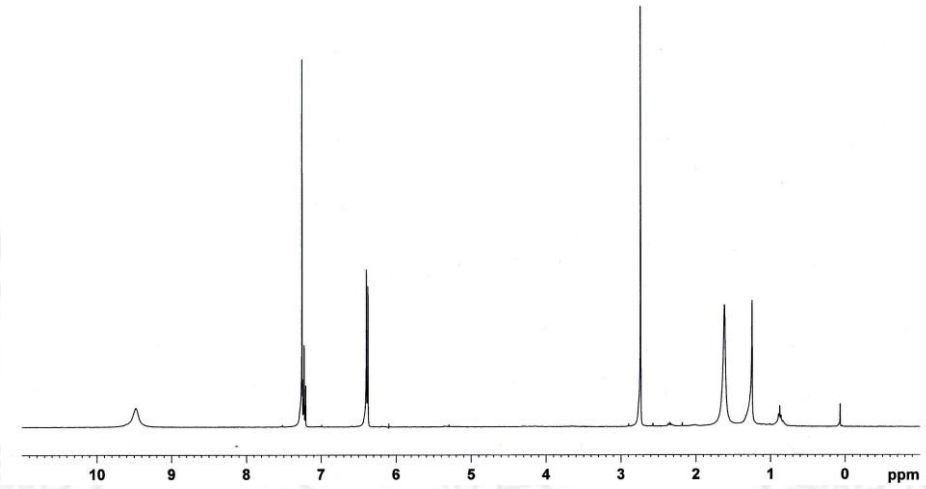


Figure 7.17 ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) of 1-(2,6-dihydroxyphenyl)ethanone (**9**).

Table 7.12 ^{1}H NMR data of 1-(2,6-dihydroxyphenyl)ethanone (**9**)

Position	**δ_H mult. (*J* in Hz)**
1, 3	-
2	-
4, 6	6.39 d (8.1)
5	7.25 t (8.1)
1′	-
2′	2.73 s

7.3.4 Bioassay

In this study, all pure compounds isolates from selected strain endophytic fungi, *M. terrestris* CMU-Cib179 and *Nodulisporium* sp. CMU-Cib1018 were tested against bacteria; *B. subtilis*, *E. coli*, *M. luteus*, *P. aeruginosa*, *S. aureus* and methicillin-resistant *S. aureus* (MRSA), and plant pathogenic fungi; *Ca. albicans*, *A. flavus*, *Co. gloeosporioides*, *P. digitatum* and *P. expansum*. The MIC value of each pure coumpound is shown in Table 7.14.

Table 7.14 Minimum inhibitory concentration for antimicrobial activity of pure compound **1**–**9** obtained from CMU-Cib179 and CMU-Cib1018

Test organism	MIC value of each compound (μg disc^{-1})[a]								
	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**
B. subtilis	50	>50	>50	>50	>50	>50	25	10	>50
E. coli	>50	>50	>50	>50	>50	>50	>50	25	>50
M. luteus	5	5	>50	>50	>50	>50	25	10	50
P. aeruginosa	>50	>50	>50	>50	>50	>50	>50	>50	>50
S. aureus	5	5	5	5	50	>50	25	5	50
MRSA	5	5	5	5	50	>50	50	10	50
C. albicans	>50	>50	>50	>50	>50	>50	>50	>50	>50
A. flavus	>50	>50	>50	>50	50	>50	>50	>50	10
C. gloeosporioides	>50	>50	>50	>50	>50	>50	>50	>50	>50
P. digitatum	>50	>50	>50	>50	>50	>50	>50	>50	25
P. expansum	>50	>50	>50	>50	>50	>50	>50	>50	25

[a]Means of MIC value of each pure compounds (three replicates for each treatment).

Compound **1** *p*-Hydroxybenzoic acid methyl ester and compound **2** puupehenol showed MIC value of 5 μg disc^{-1} against *M. luteus*, *S. aureus* and MRSA but it has no activity against Gram-negative bacteria and plant pathogenic fungi. Moreover, compound **2** showed MIC value 50 μg disc^{-1} against *B. subtilis*.

Compound **3** and **4** 4-hydroxybenzoic acid and 2,4′-dihydroxyacetophenone showed MIC value of 5 μg $disc^{-1}$ against *S. aureus* and MRSA but it has no activity against Gram-negative bacteria and plant pathogenic fungi.

Compound **5** 8-methoxynaphthalen-1-ol showed MIC value of 50 μg $disc^{-1}$ against *S. aureus* and MRSA but it has no activity against Gram- negative bacteria and 50 μg $disc^{-1}$ against only one fungal pathogen *A. flavus*.

Compound **6** 5-Hydroxy-2-methyl-4-chromanone not showed MIC value but it has no activity against bacteria and plant pathogenic fungi.

Compound **7** 5-hydroxy-2-methyl-*4H*-chromen-4-one showed MIC value of 25 μg $disc^{-1}$ against *M. luteus*, *S. aureus*, MRSA and *B. subtilis* but it has no activity against Gram-negative bacteria and plant pathogenic fungi.

Compound **8** 1-(2,6-Dihydroxy phenyl) propan-1-one showed MIC value of 10 μg $disc^{-1}$ against *B. subtilis*, *M. luteus*, *S. aureus* and MRSA, and 50 μg/disc against Gram-negative *E. coli*.

Compound **9** 1-(2,6-dihydroxyphenyl) ethanone showed MIC value of 50 μg $disc^{-1}$ against *B. subtilis*, *M. luteus*, *S. aureus* and MRSA, and 25 μg $disc^{-1}$ against fungal pathogen *A. flavus*, *P. digitatum* and *P. expansum* but it has no activity against Gram-negative bacteria.

7.4 Discussion

Fungi produce a diverse range of secondary metabolites which have no effect on the growth of the producer strain but can be strongly inhibitory against other microorganisms and many of these have important chemotherapeutic and other uses (Bhadury *et al*., 2006; Boustie and Grube, 2007). Endophytic fungi are widely recognized as prolific sources of bioactive secondary metabolites that might represent useful leads in the development of medicine, agriculture and industry (Yu *et al*., 2010; Guo *et al*., 2011; De Souza *et al*., 2011; Gutierrez *et al*., 2012). The present study has shown that all of fungal isolates tested produce antimicrobial agents and some endophytic fungi from selected medicinal plants have a promising prospect for production of useful bioactive compounds. The highest activity for producing antibacterial and antifungal substances was found on strain CMU-Cib179, followed by strain CMU-Cib1018 which isolated from *C. bejolghota*. The morphology and molecular characteristics identification indicated that strain CMU-Cib179 and strain CMU-Cib1018 were *Mycoleptodiscus terrestris* and *Nodulisporium* sp., respectively. The result was agreed with the previous studies that *M. terrestris* was an endophytic and pathogenic fungus of aquatic (e.g., *Ceratophyllum demersum, Myriophyllum spicatum, Hydrilla verticillata* and *Potamogeton cheesemanii*) and terrestrial plants (leguminous hosts and black pepper roots) (Watanabe *et al*., 1997; Hofstra *et al*., 2009; 2012). *Mycoleptodiscus terrestris* had been used as a bioherbicide for control aquatic plants (Shearer and Jackson, 2006; Linda *et al*., 2008). However, this the first reported of antimicrobial activity of this fungal culture. Recently, Rosa et al. (2012) reported that crude culture of *M. indicus* could inhibit the growth of *C.*

gloeosporioides. Furthermore, previously studied indicated that *Nodulisporium* spp. known to be endophytic fungi and produced secondary metabolites from a culture which showed antimicrobial activity (Dai *et al*., 2006; Nuangmek *et al*., 2008; Wu *et al*., 2010; Rehman *et al*., 2011).

Four compounds, *p*-hydroxybenzoic acid methyl ester (**1**), puupehenol (**2**), 4-hydroxybenzoic acid (**3**) and 2,4′-dihydroxyacetophenone (**4**) were obtained from *M. terrestris* culture. All four compounds showed antibacterial activity against *S. aureus* and MRSA. Moreover, compound **1** and **2** inhibited the growth of *M. luteus* and compound **1** has potent activity against *B. subtilis*. Previously studies reported that compound **1** that was antimicrobial agent isolated from *P. viridicatu*m and *A. sulphureus* (Tong and Draughon, 1985; Alverson and Cohen, 2002; Keena, 2005). Gordaliza (2010) and Alvarez-Manzaneda *et al*. (2005) reported that compound **2** isolated from marine sponges had an anti-tumor and anti-tuberculosis activities. In addition, previously studies had been reported the anti-oxidant and antimicrobial agent from compound **3** this compound isolated from *Triticun aestivum* (Jeong *et al*., 2001; Paliumbo *et al*., 2007; Jeong *et al*., 2010; Merkl *et al*., 2010). Recently, compound **4** was reported as a substrate for the antimicrobial and anti-oxidant compounds synthesis (Doan and Tran, 2011; Patil *et al*., 2012).

Five compounds, 8-methoxynaphthalen-1-ol (**5)**, 5-hydroxy-2-methyl-4-chromanone (**6)**, 5-hydroxy-2-methyl-4H-chromen-4-one (**7)**, 1-(2,6-dihydroxyphenyl) propan-1-one (**8**) and 1-(2,6-dihydroxyphenyl)ethanone (**9**) were isolated from *Nodulisporium* sp. CMU-Cib1018 culture. In this study, all five compounds have potent

activity against MRSA and *S. aureus*, except compound **6** had no antibacterial activity. Compound **7** and **8** have potent activity against *B. subtilis* and *M. luteus*. Only compound **8** has potent activity against *E. coli*. Compound **5** and **9** have potent activity against *A. flavus* and *C. gloeosporioides*. Moreover, compound **9** could inhibit the growth of *M. luteus, P. digitatum* and *P. expansum*. Rukachaisirikul *et al*. (2007) reported an antioxidant activity of compound **5** which this compound isolated from xylariaceous fungus PSU-A80. Nadeau and Sorensen (2011) isolated compound **5** and **7** from *Daldinia loculata* but not reported the biological activity. Dai *et al*. (2006) and Dai *et al*. (2009) reported that compound **5**, **6**, **7** and **8** were isolated from endophytic fungi, *Nodulisporium* sp. strain 7080 and strain 7093 which compound **5**, **6** and **7** showed antifungal and antialgae activities which have potent activity against *Microbotryum violaceum* and *Chlorella fusca*, as well as compound **7** could inhibit *Septoria trici* and compound **8** has potent antifungal activity against *M. violaceum* and *S. trici*. Compoud **9** could isolat from endophytic fungus, *Nodulisporium* sp. A4 culture (Wu *et al*., 2010) and Rezk *et al*. (2011) reported this compound was an anti-oxidant agent. In addition, some compound such as **5**, **6** and **7** were used for a chemotaxonomic identification of *Daldinia* and other *Xylariaceae* (Stadler *et al*., 2001; 2010a; 2010b; Bitzer *et al*., 2008).