Antibacterial and cytotoxic activities of endophytic fungus *Diatrypella* sp. MFLUCC 19-0492

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Abstract
Endophytic fungi are microorganisms living inside plant tissues without causing any disease symptoms in the plant. Various bioactive compounds have been produced and may be used as alternative drugs for controlling human pathogens. The aim of this work was to investigate antibacterial and cytotoxic activity potential of fungal crude extracts isolated from Barleria prionitis leaves. A total of 34 endophytic fungi were isolated. The crude ethyl acetate extracts of all fungi were preliminarily screened for their antibacterial activity. The acetate extract of isolate ANG4 showed significant antibacterial activity.

This isolate was further cultured to determine its minimum inhibition concentration (MIC). The crude ethyl acetate extracts of mycelia and culture broth from isolate ANG4 were active against all tested pathogens with low MIC values ranging from 0.39 mg/mL to 25.00 mg/mL when compared to the crude hexane extract of its mycelia. The cytotoxic effects of all crude extracts of isolate ANG4 were also determined using tetrazolium-based colorimetric (MTT and XTT) assays against T-lymphoblast, cholangiocarcinoma, liver hepatocellular and lung carcinoma cell lines. The crude ethyl acetate extract of mycelia showed the most significant activity inhibiting T-lymphoblast and lung carcinoma cell lines with 100 and 71% respectively whereas the crude hexane extract of mycelia showed good cytotoxic activity against the T-lymphoblast cell line with 74%. The fungus ANG4 was also identified by molecular and phylogenetic methods as Diatrypella sp. MFLUCC19-0492. Therefore, this study indicates that crude extracts of Diatrypella sp. MFLUCC19-0492, isolated from B. prionitis leaves, are potential sources of novel antibacterial compounds with good cytotoxic activity.

Keywords: Antibacterial activity, *Barleria prionitis*, cytotoxic activity, Diatrypella, endophytic fungi.

Introduction

Medicinal plants have played an important role as the principal natural product sources of bioactive compounds. They have been widely used in traditional treatments and studied extensively for their antimicrobial properties. Medicinal plants are also reported to host various bioactive compound-producing endophytic fungi[1,2]. Endophytic fungi are microorganisms that live inside host plants without causing any visible manifestation of diseases[3]. They reside in the host plant tissues by producing secondary metabolites to protect and resist external biotic and abiotic stress to the host plant by producing secondary metabolites with antagonistic activity[4].

New and novel bioactive compounds with various antimicrobial, anticancer, antimalarial and antioxidant activities, isolated from endophytic fungi of medicinal plants, have been reported such as alkaloids, terpenoids, steroids, quinones, flavonoids, phenols, phenolic acids and peptides[5,6]. Therefore, endophytic fungi are evaluated as alternative important sources of bioactive compounds that can be applied as new or novel drugs[7].

Antimicrobial resistance has been a major health problem and still poses a concern to health care systems globally. Many studies have shown that microbial pathogens have developed resistance to antibiotics via various molecular mechanisms[8]. Therefore, this global problem has led to the search for a new or novel drug from endophytic fungi, particularly those isolated from medicinal plants for their antimicrobial properties[9].

Barleria prionitis belongs to the Acanthaceae family and is a well-known medicinal plant and Ayurvedic herb. It is distributed in tropical Africa and Asia including India, Malaysia, the Philippines, Sri Lanka and Thailand[10]. This medicinal plant has been used for the treatment of fevers, toothaches, bronchial asthma, rheumatic affections, inflammation, glandular swelling, dysrhythmia and gastrointestinal disorders[11,12]. Several phytochemicals such as balarenone, pipataline, lupeol, prioniside A, prioniside B and prioniside C have been reported to possess the following biological activities: antibacterial, antiviral, antioxidant, antifertility, anthelmintic, cytoprotective, antidiabetic, anti-diarrheal, hepatoprotective and antifungal activity[13-15].

Due to the reported antimicrobial potential of various endophytic fungi, this research focused on the antibacterial and cytotoxic potential of crude extracts of endophytic fungi isolated from *B. prionitis* leaves where the most potent endophytic fungus could be used as an antibacterial agent against human pathogenic bacteria. These fungi, which are suggested to be associated with the production of compounds with medical value, could be used as alternative antibiotics.

Material and Methods

Plant material: Fresh plant materials of *B. prionitis* were collected from the flower market located in Chiang Rai Province, Thailand in October 2018. The plant material was brought to the laboratory in sterile plastic bags and processed within a few hours of sampling. Fresh, healthy leaves of *B. prionitis* were used for the isolation of endophytic fungi to reduce the chance of contamination.

Isolation of endophytic fungi: The healthy *B. prionitis* leaves were washed under running tap water several times to remove debris and soil. The leaf sterilization and the isolation of endophytic fungi utilized the methods described by Atiphasaworn et al.[16] The explant was immersed in 70% ethanol for 10 s followed by 1% sodium hypochlorite (NaOCl) for 30 s and washed with sterile water four times. The surface-sterilized leaves were then allowed to dry under aseptic conditions. The sterile blades were sectioned into 0.5 cm^2 pieces of leaf and placed on potato dextrose agar (PDA) medium plates containing chloramphenicol antibiotic (100 mg/mL) to inhibit bacterial growth.

The plates were sealed with parafilm and incubated at room temperature (25 ºC), protected from light, for five days until the growth of the hypha tip was visible. Only fungal hyphae emerging from the leaf pieces were transferred to PDA plates through hyphal tipping. A culture plug was further transferred to new PDA plates and cultured at room temperature (25 ºC) as soon as they outcrop from the medium, until pure colonies were formed**.**

Extraction of chemical compounds: All endophytic fungi were cultured in a liquid surface fermentation system followed by an ethyl acetate extraction using the experiment described previously[16,17]. Five pieces of mycelial agar plugs, 6 mm in diameter were inoculated into 150 mL potato dextrose broth (PDB). All fungi were individually incubated at room temperature (25 °C) for 28 days. After the incubation period, the broth culture was filtered with Whatmann filter paper no. 3 under vacuum using a Buchner funnel to separate the culture broth from the mycelia. The formed mycelial mat was ground using a mortar and pestle and macerated with 500 mL of ethyl acetate and further sonicated using ultrasound-assisted extraction (Crest/690DAE; GuangDong GT Ultrasonic, Guangdong, China) at room temperature (25 ºC) for 5 h.

In addition, 500 mL of ethyl acetate was added to the broth culture. After 24 h, the ethyl acetate extract was separated from the culture broth using a separatory funnel and Whatmann no. 1 filter paper. The extraction process was repeated in triplicate. All extracts were combined and concentrated by using a rotary evaporator (Buchi Rotavapor Model R114, BUCHI). The obtained extracts were subsequently stored at 4 °C until use.

Antibacterial activity screening: The ethyl acetate extracts were screened for their antibacterial activity against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, which were representatives of gram-positive and -negative bacteria respectively using the disk diffusion method described by Razmavar et al.[18] Both bacteria were obtained from the Department of Medical Science, Ministry of Health, Bangkok, Thailand. The bacterial suspensions were prepared from 24 h old culture of the test bacteria. They were standardized to contain approximately 1.5×10^8 CFU/mL based on the 0.5 McFarland Standard. All bacteria were inoculated on the dried surface of a Mueller-Hinton agar. The inoculum was incubated at 37 °C for 5 min to ensure the confluent lawn of bacterial growth before applying the test discs.

The dried crude extracts were dissolved in ethyl acetate to a concentration of 50 mg/mL. Each crude fungal extract (30 µL) was dropped on a sterilized 6 mm diameter paper disc (Whatmann Paper No. 1, USA) and allowed to dry prior to incubating at 37 °C for 24 h. The clear zone diameter of inhibition was determined. Paper disks eluted with 30 µL of ethyl acetate and chloramphenicol (5 mg/mL) were used as negative and positive controls respectively. Each experiment was carried out in triplicate.

Extraction of various crude extracts of fungus ANG4: Due to the highest antibacterial activity of endophytic fungus ANG4 against selected bacterial pathogens, a large-scale (20 L) fermentation of isolate ANG4 was performed using PDB culture medium at room temperature (25 °C) for 28 days. For the isolation of ANG4, the mycelia of the fungus were separated from the culture broth by filtration and macerated sequentially with methanol (400 mL) and dichloromethane (400 mL) for two days before concentrating and partitioning with hexane and ethyl acetate respectively.

Both hexane and ethyl acetate extracts were collected and dried over anhydrous sodium sulfate before solvent evaporation using a rotary evaporator to give crude extracts of 0.444 and 0.147 g from the hexane and ethyl acetate extracts respectively. The culture broth was partitioned with ethyl acetate three times (400 mL) and further concentrated using a rotary evaporator to obtain the crude ethyl acetate extract of 1.086 g.

Antibacterial activity assay of various crude extracts of ANG4: Eight human pathogenic bacteria were selected for this study. Four gram-positive bacteria, including *S. aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus pyogenes* ATCC 12384 and *Enterococcus faecium* ATCC 29212 and four Gram-negative bacteria including *E. coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* ATCC 13311 were used as test pathogens.

All strains were obtained from the Department of Medical Science, Ministry of Health, Bangkok, Thailand. The antibacterial activity assay was performed as described

above. The dried crude extracts were dissolved in hexane or ethyl acetate to concentrations of 50.00, 25.00, 12.50, 6.25, 3.12, 1.56, 0.78 and 0.39 mg/mL.

In addition, hexane and ethyl acetate were used as negative controls. Chloramphenicol was used as a positive control and prepared at concentrations of 500, 250, 125, 62.50, 31.25, 15.62, 07.81 and 3.91 µg/mL. The clear zone diameter of inhibition and minimum inhibition concentration (MIC) were measured and determined.

Cytotoxicity assay: Cytotoxic activity of all crude extracts of endophytic fungus ANG4 was investigated against non-adhesive T-lymphoblast (Molt-3) and adhesive cell lines including human cholangiocarcinoma (HuCCA-1), liver hepatocellular carcinoma (HepG2) and lung carcinoma (A549) based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 2,3-bis-2-methoxy -4-nitro-5-sulfonylphenyl]-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT) assays using a modified method by Thongnest et al.[19] Cells (5×10^3 cells/well) were seeded into 96-well plates in complete medium and incubated at 37 ºC. After 24 h of cell seeding, the cell lines were treated with crude extracts at a concentration of 30 µg/mL and incubated at 37 ºC for 48 h.

After the incubation period, 20 µL of a stock MTT solution were added to each well and incubated at 37 ºC under 5% CO_2 for 4 h before processing. The supernatant was removed and dimethyl sulfoxide was added to the cell pellet. The absorbance of solutions was measured at 540 nm using a microtiter plate reader (VersaMax tunable multi-well plate reader). Doxorubicin was used as the positive control for the HuCCA-1, A549, Molt-3 and HepG2 cell lines whereas etoposide was used as the positive control for the Molt-3 and HepG2 cell lines. All experiments were carried out three times. The percentages of cytotoxicity were calculated spectrophotometrically as:

$$\left[100 - \left(\frac{(A_{540}\text{ of treated cells}) - (A_{540}\text{of blank cells})}{(A_{540}\text{ of controlled cells }) - (A_{540}\text{of blank cells})} \text{x}100\right)\right]$$

Identification of endophytic fungus ANG4: A pure culture of isolate ANG4 was selected to confirm the fungal genus according to its antibacterial and cytotoxic activities compared to other fungal extracts. Pure fungus strain mycelium was scraped from the PDA surface and pulverized with a mortar and pestle to obtain a fine powder. Analysis of genomic DNA was conducted using the cetyltrimethyl ammonium bromide method[20]. The fungus was amplified with ITS barcoding using universal primers, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5-TCCTCCGCTTATTGATAT GC-3'). Polymerase chain reaction (PCR) was performed at 95 °C for 5 min followed by 40 cycles of 95 °C for 50 s, 52 °C for 50 s, 72 °C for 50 s and finally 72 °C for 10 min on a PeqSTAR 2x thermal cycler (Peqlab, Germany). The PCR-amplified products were analyzed by gel electrophoresis on 1% agarose gels stained with ethidium bromide under UV light and purified using NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel, Germany).

The DNA sequencing was performed on an automated sequencing system at 1st BASE Laboratories (Malaysia). The obtained sequences were used as a query to search for similar sequences in GenBank using the BLAST program (Basic Local Alignment Search Tool, https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm the classification of unidentified species. The phylogenetic analysis was performed using MEGA v.6.0. This isolate was stored at the Institute of Excellence in Fungal Research, Mae Fah Luang University as code MFLUCC19-0492 and further submitted to the NCBI Genbank (accession number MN715786**).**

Statistical analysis: Experiments were performed in triplicate. All data were statistically analyzed using an Analysis of Variance. Statistical significance was analyzed by Student's *t*-test and confidence limits were set to $P < 0.05$. Results were expressed as means with an error bar of standard deviations.

Results and Discussion

Isolation of endophytic fungi: Thirty-four endophytes were isolated from healthy leaves of *B. prionitis* collected from Chiang Rai Province, Thailand. Colony of isolated cultures is depicted in fig. 1. In recent years, various human pathogens have developed antibiotic resistance due to indiscriminate use of commercial antibiotics. Therefore, natural products that possess antimicrobial properties are needed as alternatives to antibiotic drugs. It has been discovered that endophytic fungi are an alternative source of bioactive compounds with biological activities[21,22]. Moreover, the populations of endophytic fungi may be dependent on host species and planting area.

Medicinal plants are considered an important source for bioactive compound-producing endophytes[23]. Medicinal plant *B. prionitis* is an Ayurvedic herb used in herbal medicines for the treatment of toothaches, catarrhal affections, whooping cough, inflammation, glandular swelling, urinary infections, jaundice, fever, gastrointestinal disorders and as a diuretic and tonic[24]. A wide range of bioactive compounds isolated from different parts of *B. prionitis* has been reported such as balarenone, pipataline prionisides, barlerinoside, verbascoside, acetylbarlerin, lupulinoside and scutellarein[25].

These isolated compounds have also been reported to possess various pharmacological properties including; antimicrobial, anthelmintic, antifertility, antioxidant, antidiabetic, anti-inflammatory and anti-arthritic activities without any toxic effects[26]. In addition, endophytes colonizing medicinal plants are reported to possess biological activities. Therefore, the medicinal properties of a

plant may be correlated to their endophytes. Although phytochemical and pharmacological screenings of endophytic fungi of medicinal plants have progressed, there is little information about the bioactivity of endophytic fungi associated with *B. prionitis*. All isolates were cultured for further antibacterial and cytotoxic investigation.

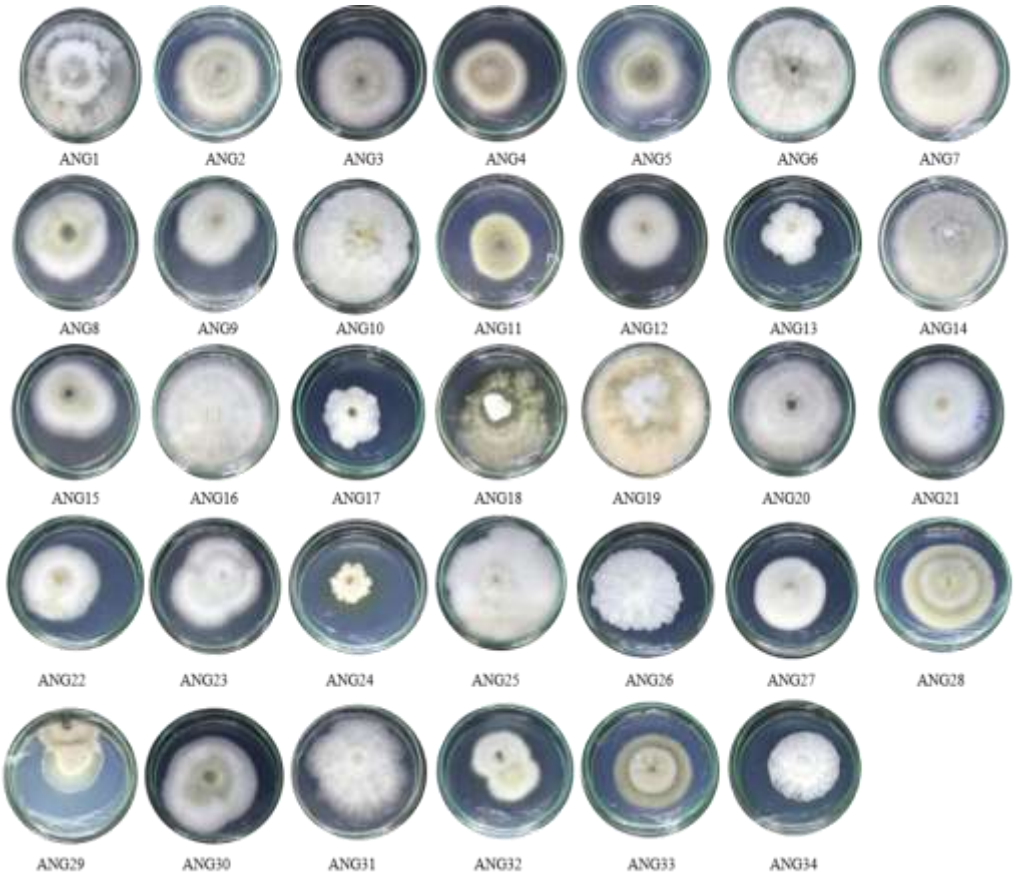

Fig. 1: Colony of endophytic fungi isolated from *B. prionitis* leaves

Table 1
Antibacterial activity of crude extracts of endophytic fungi isolated from *B. prionitis* leaves (50 mg/mL) and chloramphenicol (5 mg/mL) against *S. aureus* and *E. coli*

Isolate	*S. aureus*	*E. coli*	Isolate	*S. aureus*	*E. coli*
ANG1	++++	++	ANG19	+	-
ANG2	+++	+	ANG20	-	+
ANG3	++	-	ANG21	-	-
ANG4	+++++	+++++	ANG22	+	++
ANG5	++	++	ANG23	++	++
ANG6	++	++	ANG24	++	-
ANG7	+	+++	ANG25	++	+
ANG8	++	-	ANG26	++	++
ANG9	++	-	ANG27	++	+
ANG10	-	-	ANG28	+	+
ANG11	+++	+	ANG29	-	++
ANG12	-	++	ANG30	+	-
ANG13	-	+	ANG31	++	-
ANG14	-	-	ANG32	-	-
ANG15	-	+	ANG33	-	-
ANG16	++	+	ANG34	+	++
ANG17	+	-	Chloramphenicol	+++++	+++++
ANG18	+	++			

-not detected

Antibacterial activity assay: The crude extracts were obtained from extraction with ethyl acetate solvent due to several pieces of research reported that crude extract from ethyl acetate solvent showed a broad spectrum of antimicrobial activities[1,2,27]. The crude extracts were preliminarily screened for their antibacterial activities against the growth of *S. aureus* and *E. coli* which are representative of gram-negative and gram-positive bacterial pathogens respectively. The antibacterial activity of all crude extracts (50 mg/mL) and chloramphenicol (5 mg/mL) is demonstrated in table 1.

In the present study, 34 fungal isolates were isolated from *B. prionitis*, of which 94% (32 isolates) demonstrated antibacterial activity in preliminary screening against *S. aureus* and *E. coli*. The agar-based screening assay was used in this study because it is easy to identify active isolates for further studies and has been recommended as a rapid method for the determination of antibacterial activity in natural product research[28].

One of the isolates, ANG4, was the isolate with the highest antibacterial activity. It was selected for further investigation to determine its antibacterial and cytotoxic activities. The fungus ANG4 was further cultured and the bioactive compounds were extracted using different solvents including ethyl acetate and hexane. Ethyl acetate is the preferred solvent used to extract bioactive compounds from natural products because of its medium polarity.

However, hexane was also used to extract nonpolar bioactive compounds according to other studies that reported use of hexane to extract nonpolar bioactive compounds[29,30]. The antibacterial activity assay was performed using eight concentrations of crude ethyl acetate extract of culture broth, crude ethyl acetate extract of mycelia and crude hexane extract of mycelia. MIC values of all crude extracts against tested human pathogens are presented in table 2. The antibacterial activities were observed in various values with inhibition zone diameters ranging from 6.27±0.09 mm to 11.22±0.14 mm. The MIC values of all crude extracts of fungus ANG4 were investigated in the range of 0.39 mg/mL to 50.00 mg/mL. Among these concentrations, MIC value of 1.56 µg/mL was the most active against various tested pathogens. The crude ethyl acetate extracts of mycelia and culture broth of isolate ANG4 were active against all tested pathogens with low MIC values ranging from 0.39 mg/mL to 25.00 mg/mL when compared to the crude hexane extract of mycelia. However, the lowest MIC (0.39 mg/mL) was detected in the crude ethyl acetate extract of mycelia against *K. pneumoniae*.

Conversely, the crude hexane extract of mycelia showed weak antibacterial activity in the range of 12.50 mg/mL to 50.00 mg/mL. Among the tested pathogens, the crude hexane extract of mycelia showed no activity against *S. typhimurium* as shown in table 1. Chloramphenicol, which was used as a positive control, had MIC values ranging from 62.50 to 500.00 µg/mL. In this study, ethyl acetate crude extracts from mycelia and culture broth of fungus ANG4 showed potent antibacterial activity against all tested pathogens, while the hexane extract of mycelia of fungus ANG4 showed less inhibition against these same organisms.

Cytotoxic activity assay: The cytotoxic effects of crude ethyl acetate extract of culture broth, crude ethyl acetate extract of mycelia and crude hexane extract of mycelia were evaluated by MTT and XTT assays against four selected cell lines including Molt-3, HuCCA-1, A549 and HepG2 against doxorubicin and etoposide as reference drugs. The results of the cytotoxicity investigations are presented in table 3.

The cytotoxicity percentages of the extracts less than 50% were reported as no activity. Results showed that crude ethyl acetate extract of culture broth was the least active with no cytotoxic activity against all tested human cell lines.

The crude ethyl acetate extract of mycelia showed the most significant activity, inhibiting the Molt-3 and A549 cell lines with 100 and 71% respectively.

Table 2
Antibacterial activity of crude extracts of ANG4 and chloramphenicol against tested human pathogens

Extract/Drug	MIC (mg/mL)							
	Zone inhibition diameter [mm] (mean ±SD)							
	Gram-negative				Gram-positive			
	K. pneumoniae	***S. typhimurium***	***P. aeruginosa***	***E. coli***	***E. faecium***	***S. pyogenes***	***S. epidermidis***	***S. aureus***
Ethyl acetate-broth	3.12	12.50	6.25	12.50	1.56	25.00	12.50	3.12
	6.41±0.28	8.88±0.07	10.48±0.18	7.45±0.28	6.37±0.29	6.36±0.15	6.80±0.06	6.65±0.07
Ethyl acetate-mycelia	0.39	1.56	1.56	0.78	1.56	0.78	1.56	0.78
	7.95±1.25	10.49±0.30	6.57±0.26	8.55±0.31	6.37±0.29	7.48±0.39	6.62±0.37	6.77±0.47
Hexane-mycelia	25.00	-	25.00	50.00	50.00	50.00	25.00	12.50
	7.34±0.16		8.60±0.25	11.22±0.14	6.51±0.27	6.27±0.09	6.59±0.18	7.22±0.15
Chloramphenicol*	62.50	500.00	125.00	125.00	62.50	125.00	500.00	500.00
	9.42±0.27	12.47±0.37	10.54±0.35	10.5±0.35	9.44±0.27	10.57±0.35	9.52±0.19	9.52±0.19

*MIC unit is µg/mL
- not detected

In addition, the crude hexane extract of mycelia showed cytotoxic activity against the Molt-3 cell line with 74%. However, doxorubicin and etoposide exhibited IC_{50} values ranging from 0.013 to 0.5 µg/mL against all tested cell lines and 0.024 to 29.35 µg/mL against Molt-3 and HepG2 respectively.

In this study, the cytotoxicity of all crude extracts of ANG4 was investigated against four human cancers. The crude ethyl acetate extract of mycelia from ANG4 was found to be cytotoxic to cancer cell lines with stronger growth inhibition against Molt-3 compared to A549, HuCCA-1 and HepG2 cancer cell lines. High cytotoxic activity against tested cell lines was observed for ethyl acetate and hexane extracts of ANG4 mycelia especially against the Molt-3 cell line.

Thus, each extract contained different bioactive compounds with various potential activities that are specific to a particular cell line. Various crude extracts of endophytic fungi isolated from medicinal plants have been reported to possess cytotoxic activity against many human cancer cell lines. However, the crude extracts of endophytic fungi isolated from *B. prionitis* have not previously been reported and their bioactive compounds have also not been identified. This study describes the antibacterial and cytotoxic activities of the crude extracts of endophytic fungi isolated from *B. prionitis* against human pathogens and cancer cell lines.

Moreover, endophytic fungus ANG4 was determined to produce bioactive compounds having cytotoxic activities. Many studies reported the potential of antimicrobial compounds isolated from endophytic fungi to be toxic to human cancers[31].

Molecular identification: Results from molecular and phylogenetic analyses were used to characterize the endophytic fungus ANG4 taxonomically. Partial sequences obtained from ITS1-5.8S-ITS2 of the strain were compared to ITS sequences of organisms represented in the database Genbank. The highest score sequences were collected from the databases and aligned with the ITS sequences of this endophytic fungus. Based on morphological characteristics, fig. 2 shows the phylogenetic tree of the endophytic fungus ANG4 deposited at the Institute of Excellence in Fungal Research, Mae Fah Luang University as code MFLUCC19-0492.

Table 3
Percentage of cytotoxicity of crude extracts of ANG4 (30 µg/mL), doxorubicin and etoposide against four human cancer cell lines.

Extract/Drug	Molt-3	HuCCA-1	A549	HepG2
Ethyl acetate-broth	-	-	-	-
Ethyl acetate-mycelia	100	66	71	54.17
Hexane-mycelia	74	-	-	-
Doxorubicin (IC_{50}, µg/mL)	0.013±0.006	0.5±0.028	0.32±0.035	0.32±0.01
Etoposide (IC_{50}, µg/mL)	0.024±0.006	-	-	29.35±0.63

Molt-3 (acute lymphoblastic leukemia), HuCCA-1 (lung cholangiocarcinoma), A549 (lung carcinoma) and HepG2 (hepatocellular liver carcinoma), the percentage of cytotoxicity of crude extracts that less than 50% is reported as no activity (-).

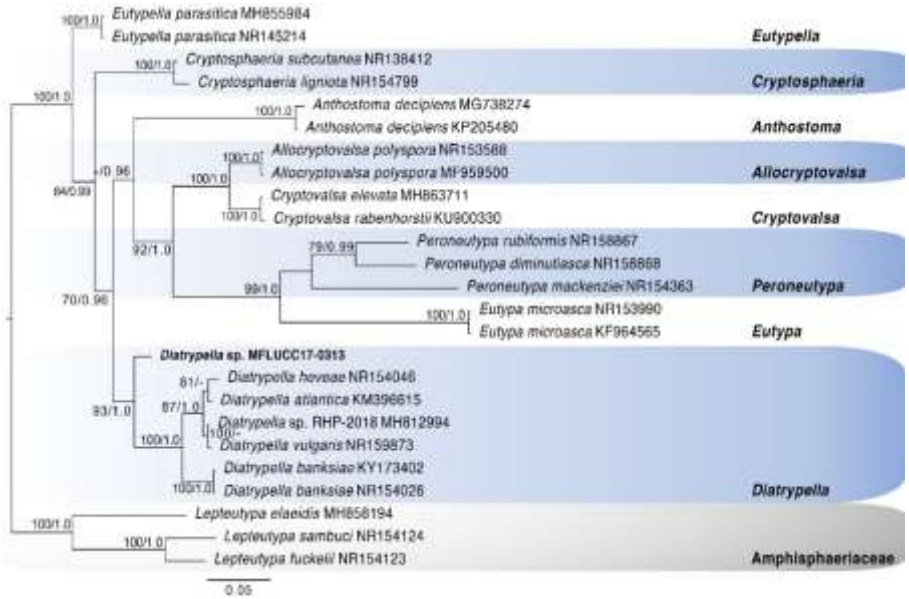

Fig. 2: Phylogenetic tree of endophytic fungus *Diatrypella* sp. MFLUCC19-0492. The values at each node represent the percentage of bootstrap values and the Bayesian posterior probability values per nucleotide

The bootstrap percentage of the fungus ANG4 into family classification was 93% Diatrypella. The abundance distribution of fungal species was evaluated to be highly tilted with various frequent and incidental species. Based on the data, this fungus was identified in the genera of *Diatrypella* as *Diatrypella* sp. MFLUCC19-0492.

Conclusion

This work demonstrates that *Diatrypella* sp. MFLUCC19-0492, isolated from *B. prionitis* leaves, has bioactive properties, highlighting its *pharmacological* potential as antibacterial and cytotoxic against human pathogens and cancer cell lines. The crude ethyl acetate extract of *Diatrypella* sp. MFLUCC19-0492 mycelia of this plant inhibits the growth of various human pathogenic bacteria and cancer cell lines on a highly active level.

Thus, there could be the possibility of new bioactive compounds in the crude extract which may provide new bioactive compounds to replace antibiotics from the *Diatrypella* genus. This study presents new antimicrobial fungal extracts that could be potential therapeutic candidates in the future. Further research needs to be conducted to identify the antibacterial and cytotoxic compounds produced by this fungus.

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