

<b>Thesis Title</b>	Bioactive Compounds of Endophytic Fungi Isolated from <i>Cinnamomum loureiroi</i> , <i>Polyscias fruticosa</i> , <i>Barleria prionitis</i> , and Essential Oil from <i>Cuminum cyminum</i>
<b>Author</b>	Chutima Tanapichatsakul
<b>Degree</b>	Doctoral of Philosophy (Applied Chemistry)
<b>Advisor</b>	Assoc. Prof. Patcharee Pripdeevech, Ph. D.
<b>Co-Advisor</b>	Assoc. Prof. Prasat Kittakoop, Ph. D. Asst. Prof. Acharavadee Bunkoom, Ph. D.

## ABSTRACT

This research aimed to investigate the secondary metabolites and biological activities of endophytic fungi and the selected plant. The endophytic fungi were isolated from selected plants: *Cinnamomum loureiroi*, *Polyscias fruticosa*, *Barleria prionitis*, while the selected plant is *Cuminum cyminum*. The obtained results are divided into four parts according to their different activities.

Firstly, 11 endophytic fungi were isolated from healthy *C. loureiroi* leaves. All endophytic fungi of *C. loureiroi* were cultured for one month prior to analysis of their antioxidant and antibacterial activity against *Bacillus cereus* and *Staphylococcus epidermis*, and the chemical composition. Crude extracts of MFLUCC15-1130 and MFLUCC15-1131 showed the lowest minimum inhibitory concentration at 3.91 µg/mL. Significant antioxidant activity was detected in the crude extract of fungus MFLUCC15-1130 and MFLUCC15-1131 with half-maximal inhibitory concentration (IC<sub>50</sub>) of 22.92 ± 0.67 and 37.61 ± 0.49 µg/mL, respectively. The phylogenetic study

suggested MFLUCC15-1130 and MFLUCC15-1131 endophytes is identified as were identified as *Neopestalotiopsis* sp. and *Diaporthe* sp., respectively. The chemical composition of both crude extracts analyzed by gas chromatography-mass spectrometry (GC-MS) revealed eugenol, myristaldehyde, lauric acid, and caprylic acid as major components.

Secondly, 34 endophytic fungi isolates were isolated from the healthy leaves of *P. fruticosa*. *In vitro* antagonism of endophytic fungi isolates against the pathogenic fungus, *Athelia rolfsii* on tomatoes plant using a dual culture assay. The efficiency of different solvents for the extraction was determined using ethyl acetate, methanol, and hexane. Each crude extract was tested for antifungal activity. Especially, the hexane crude extract was showed the highest inhibition percentage. The phylogenetic study suggested MFLUCC17-0313 endophytes is identified as *Diatrype palmicol*. The bioactive compound was further purified using bioassay guidance. The bioactive compound was identified as 8-methoxynaphthalen-1-ol. The minimum inhibition concentration of 8-methoxynaphthalen-1-ol was 250 µg/mL against *A. rolfsii* and represented no phytotoxic effect on tomato leaves.

Thirdly, 34 endophytes were isolated from healthy leaves of *B. prionitis*. Antibacterial properties of all crude extracts were evaluated using disk-diffusion assay against human pathogenic Gram-positive and Gram-negative bacterial (*Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pyogenes*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*). The ethyl acetate crude extract of mycelia of isolate MFLUCC19-0492 was shown a significant inhibited growth all of bacteria with the lowest minimum inhibitory concentrations (MIC) values (0.39 mg/mL). The phylogenetic study suggested MFLUCC19-0492 endophytes is identified as *Diatrypella* sp. The cytotoxic activity was investigated by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) and 2,3-bis (2-methoxy-4-nitro-5-

sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide (XTT) assays against Molt-3, HuCCA-1, A549 and HepG2 compared to doxorubicin and etoposide, reference drugs. The ethyl acetate crude extract of mycelia showed significantly inhibited the Molt-3, HuCCA-1, A549, and HepG2 with 100, 66, 71, and 54.17%, respectively. On the other hand, the ethyl acetate crude extract of mycelia of isolate MFLUCC19-0492 displayed cytotoxic activity.

Lastly, the essential oil derived from *C. cyminum* was tested their antifungal activity against *Aspergillus aculeatus*. The chemical composition of *C. cyminum* essential oil were also identified by GC-MS. The main components were cuminaldehyde (33.94%) and  $\alpha$ -terpinen-7-al (32.20%). *C. cyminum* oil exhibited significant inhibition percentage of 95.08% against *A. aculeatus*. The effects of the reduction on conidia germination was detected in *C. cyminum* oil and cuminaldehyde treatment at a concentration of 1,000 and 100  $\mu\text{g/mL}$ , respectively. *In vivo* assay, the results showed the decrease of the severity (0.42%) and incidence (1.67%) probability of *A. aculeatus* on grape tested at 1,000  $\mu\text{g/mL}$  of *C. cyminum* oils than other treatments. In addition, grape treated with *C. cyminum* oil had slightly decreased weight loss and retained fruit firmness. The quality of grape fruit treated with *C. cyminum* oil was delayed including total soluble solids, total phenolic content, and antioxidant activity.

**Keywords:** Antibacterial, Antifungal, Endophytic fungi, Essential oil, *Cinnamomum loureiroi*, *Polyscias fruticosa*, *Barleria prionitis*, *Cuminum cyminum*