# Antioxidant and Antibacterial Activities of Endophytic Fungi Extracts of Syzygium zeylanicum

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Research Paper

# Antioxidant and Antibacterial Activities of Endophytic Fungi Extracts of *Syzygium zeylanicum*

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**Abstract**

*Syzygium zeylanicum*, one of the other therapeutic plants found in Indonesia, is used to treat various ailments, such as antimicrobial, anti-inflammatory, antioxidant, arthritis, antidiabetic, mosquitocidal, antitumor, and anti-rheumatic agents. The massive use of plant extracts has caused the development of isolation of bioactive compounds thru their endophytic. The present research aimed to obtain endophytic fungal isolates from the stem bark and leaves of Jambu nasi-nasi (*S. zeylanicum*) and analyze endophytic fungal extracts' antioxidant and antibacterial activity. Endophyte identification was performed morphologically, and isolates with high biological activity were molecularly characterized. The antibacterial activity was evaluated by the disc diffusion method, and the antioxidant activity was evaluated by the DPPH method. In total, 10 endophytic fungi were isolated and identified as *Phialemonium* sp. (Code SZT3), *Acremonium* sp. (Code SZT4), *Trichoderma aureoviridae* (Code SZT5), *Trichoderma koningi* (Code SZT7), *Phytium torulosum* (Code SZL1), *Phytium zingiberum* (Code SZL2), *Septonema* sp. (Code SZL3), *Lasiodiplodia pseudotheobromae* (Code SZL4), *Volutella cilliata* (SZL5), and *Trichophyton mentagrophytes* (Code SZL7). Isolate SZL4 gave activity the highest antioxidant (IC50 = 3.30 $\mu$g/mL) and strong antibacterial activity against four pathogens bacterial (*S. thypi, B. subtilis, S. aureus, and E. coli*). The potential endophytic fungi based on molecular analysis was *Lasiodiplodia pseudotheobromae* with accession number OK668257. These endophytic fungi can be developed into new sources of antibacterial and antioxidant bioactive compounds through further studies.

**Keywords**
Antibacterial Activity, Antioxidant Activity, Endophytic Fungi, *Syzygium zeylanicum*

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## 1. INTRODUCTION

The search for sources of biologically active compounds is constantly being carried out by many emerging diseases, including infectious diseases, cancer, and other dangerous diseases (Segaran and Sathiavelu, 2019; Teodoro, 2019; Zhao et al., 2010). Endophytic fungi have great potential in searching for new drug sources as they can produce bioactive compounds that can be processed into drugs (Bhardwaj and Agrawal, 2014; Lu et al., 2012; Omoarelojie and Van Staden, 2020; Uzma et al., 2019). Beneficial ondophytic fungi are easy to grow, have a short life cycle, and can produce large amounts of bioactive compounds by cultivation methods (Kumar et al., 2019).

Improving resistance through pathogenic microorganisms to business pills is a applicable trouble confronted by healthcare offerings and has become an extreme problem worldwide (Mane et al., 2018). Factors contributing to this resistance include the widespread and often inappropriate use of antibiotics, poor sanitation, the constant movement of tourists, and the growing number of immunocompromised patients and late diagnosis of infections. Fungi are easy to grow and have a short lifespan, and cultivation methods can produce many biologically active compounds (Manyi-Loh et al., 2018).

Accordingly, there is a need for an intensive search for novel antibacterial agents from various natural sources, including endogenous fungi (Kaul et al., 2012). In a recent study, antibacterial activity was measured against *Salmonella thypi* and *Eschericia coli* (gram-negative pathogenic bacteria); *Bacillus subtilis* and *Staphylococcus aureus* (gram-positive pathogenic bacteria).

Bacterial resistance can be severe when the cell body in damaged condition. The integrity of the cell can destroy by radicals in the body, and it can be solved using antioxidant agents (Pandey et al., 2014). Degenerative diseases such as cancer, atherosclerosis, diabetes, rheumatoid arthritis, and decreased immune response are caused by free radicals (Phaniendra et al., 2015; Sharma et al., 2018). Correlation damaged cell, free radical and bacterial resistance was the reason to be needed to find a new source with antibacterial and antioxidant potential.

Leaves of *S. zeylanicum* plant have been used by people in Indonesia as a medicinal plant related to pathogenic bacterial infections and the effects of free radicals in the body (Anoop and Bindu, 2014; Anoop and Bindu, 2015; Deepika et al., 2014; Vinodkumar, 2015). Endophytes, especially those that inhabit medicinal plant tissues, are often used as sources of bioactive compounds. Some plants can degrade the bioactive compounds they contain to endophytic microbes that grow in their tissues so that these endophytic microbes can produce the same compounds as their hosts. Our previous research Syarifah et al. (2021) found the antibacterial compound p-hydroxybenzaldehyde in endophytic fungi *Penicillium brefeldianum* (isolated from *S. zeylanicum* root bark). This p-hydroxybenzaldehyde compound is also produced by its host. To continue our research series, in this article, *S. zeylanicum* reports the potential bioactivity of endophytes from other parts of the plant, the bark of leaves and stems. Stem bark and leaves have higher presence and yield than flowers and seed (Figueiredo et al., 2008). Leaves and stems are the main organs responsible for accumulating active components with important medicinal value. Leaves are places for photosynthesis and play an important role in the plant's life. The leaves and stem can also be used as a synthetic and storage organs for secondary metabolites (Li et al., 2020). To the best of our knowledge, this paper is the first report to expose the potential of the endophytic fungal extract from stem bark and leaves of *S. zeylanicum* as antibacterial and antioxidant.

## 2. EXPERIMENTAL SECTION

### 2.1 Plant Materials

The stem bark and leaves of *S. zeylanicum* (L.) were collected from the PALI regency (Penukal Abab Lematang Ilir), South Sumatra. The plant has been registered in the botanical field of the BRIN (Badan Riset dan Inovasi Nasional) Cibinong Research Center for Biology with the number B-417/V/DI.05.07/10/2021. Sampling was carried out in a new state in July 2020. Sampling was carried out in a new state in July 2020. Isolation of endophytic fungi using young leaves and stem bark tissue. The leaves used are leaves in the third leaves position from the tip of the branch. The stem bark used is the bark from the central unit.

### 2.2 Isolation of Endophytic Fungi

Isolation of endophytic fungi begins by disinfecting the surface of leaves and stem bark of *S. zeylanicum*. Fresh plant offal was washed under running water until clean for ±5 min. Then soak in 70% alcohol for ±3 min. Then rinse with sterile distilled water for ±1 min, and then soak in 3% (w/v) sodium hypochlorite (NaOCl) for 1 min (Habisukan et al., 2021; Hanin and Fitriasari, 2019). Surface sterilized leaves were cut aseptically by ±2 cm, while the stem bark was cut ±3×0.5 cm. The samples were inoculated in PDA (Potato Dextrose Agar) plate, and incubated for 3-14 days at room temperature. Inspections were made every day until the fungi were visible. Fungal colonies grown on PDA plates with different morphological characteristics (color, size, and texture) were further purified. Purification was performed by transferring colonies to fresh PDA plate with single-spore isolation and then incubating at room temperature for 2×24 h. Purified fungal colonies are then transferred to the culture medium (in petri dishes) and stock cultures (in vitro) by culturing them on a PDA medium (Herlinda et al., 2021).

### 2.3 Endophytic Fungi Identification

Phenotypic characters both macroscopically and microscopically were used for endophytic fungi identification. Observation of colony characteristics included: colony color surface and reverse side; colony texture (cottony, granular, powdery, slimy); presence of exudate drops; presence of radial lines; presence of concentric circles. Microscopic characterization analysis using slide culture methods and observation of the hyphae, spores, color, and other specific characteristics under microscope until 500 x magnification. Both macroscopic and microscopic characterization then compared with fungi identification literature such as identification keys Pictorial Atlas of Soil and Seed Fungi (Watanabe, 2002), Fungi and Food Spoilage (Pitt and Hocking, 2009), Larone's medically necessary fungi (Walsh et al., 2018) and other fungi identification journal.

### 2.4 Endophytic Fungi Diversity Analysis

Diversity analysis of endophytic fungi from the leaves and stem bark of *S. zeylanicum* was estimated using the Simpson diversity index (Simpson, 1949) and Shannon–Weiner diversity index (Shannon and Weiner, 1949). The ecological interrelationships between the endophytic fungi species and the different organ were interpreted using Paleontological Statistics (PAST) Software to construct the Principal Component Analysis (PCA) (Hammer et al., 2001).

### 2.5 Molecular Identification

Potential endophytic fungi isolate continues with molecular identification based on the Internal Transcribed Spacer (ITS) area of DNA (rDNA). Primer ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') was used for the amplification process. Assembly of DNA sequences forward and reverse primers were compiled using the Bioedit program to cut the unnecessary sequences. The sequence assembly was then entered into the Basic Local Alignment Search Tool (BLAST) at the website address http://blast.ncbi.nlm.nih.gov/Blast.cgi. Next, the sequences from sample and database were aligned with the CLUSTAL W method using the MEGA11 program, and a phylogenetic tree was constructed using the Neighbor-joining tree method with a bootstrap value of 1000 (Tamura et al., 2021).

### 2.6 Extraction and Cultivation

All endophytic fungi isolates were cultured using 6 blocks of pure cultured agar (±6 mm in diameter) in 300 mL potato dextrose broth (PDB) medium. Each isolate was refined in an erlenmeyer flask up to 300 mL×5. The cultures were then incubated for 4 weeks at room temperature under static conditions. The medium and biomass were separated using filter paper. Then ethyl acetate solvent was added to the culture medium (1:1) and extracted by partition (repeated three times). The ethyl acetate extract was separated using a rotary evaporator to obtain the extract (Budiono et al., 2019; Habisukan et al., 2021). The extract was concentrated using an oven at 40°C. The concentrated extract was weighed on an analytical balance.

### 2.7 Antibacterial Activity

Antibacterial activity is analyses using Kirby-Bauer method with NA (Natrium Agar) medium. Bacterial tests used 2 gram-negative bacteria (*Escherichia coli* InaCCB5 and *Salmonella thypi* ATCC1048) and 2-gram-positive bacteria (*Staphylococcus aureus* InaCCB4 and *Bacillus subtilis* InaCCB1204). The blank disc paper was dripped to endophytic fungal extract with a concentration of 400 $\mu$g/disc. Dilution of fungi extracts using Dimethylsulfoxide (DMSO). A positive control using Tetracycline with a concentration 30 $\mu$g/disk. Disc paper test was placed on the MHA (Muller Hinton Agar) plate that had been inoculated with the bacteria. Plate then incubated for 1×24 hours at incubator with setting 37°C, then the inhibition zone was observed. The diameter of the inhibition zone formed was measured with a caliper. The criteria for determining the antibacterial activity of the test sample and the diameter of the inhibition zone were determined by the following formula (Elfita et al., 2019; Syarifah et al., 2021):

$$\text{Strong} : \frac{A}{B} \times 100\% > 70\%;$$

$$\text{Moderate} : 50\% < \frac{A}{B} \times 100\%; < 70\%;$$

$$\text{Weak} : \frac{A}{B} \times 100\% < 50\%$$

A: Inhibition zone (mm) of samples
B: Inhibition zone (mm) of antibiotic standard

### 2.8 Antioxidant Activity Analysis

Antioxidant activity was analyzed using the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method. Each endophytic fungi ethyl acetate extract was dissolved in methanol to concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.625 g/mL (three replications). At 0.2 mL of each concentration 3.8 mL of 0.5 mM DPPH solution was added. The mixture became homogenized and left in a darkish tube for 30 minutes. Absorption was measured the use of a UV-Vis spectrophotometer at $\lambda$max 517 nm (Fadhillah et al., 2019). In this study, ascorbic acid was used as the standard antioxidant. Antioxidant activity was calculated from the inhibition rate of DPPH absorption and the $IC_{50}$ value.

$$\%\text{Inhibition} = \frac{A_k - A_s}{A_s}$$

$A_k$ = Absorbance of control
$A_s$ = Absorbance of samples

## 3. RESULTS AND DISCUSSION

### 3.1 Endophytic Fungi of *Syzygium zeylanicum*

Isolation of endophytic fungi was characterized by the presence of fungal mycelium around the surface sterilized plant organs. The leaves and stem bark of *S. zeylanicum* dominated by white fungal colony (Figure 1). The frequency of endophyte establishment by whether young, mature or old segments (Dissanayake et al., 2015). Young leaves have lower concentrations of antifungal and anti-herbivorous substances compared to mature stems (stem bark), which may be the reason for the lower leaf colonization rate than stem bark. A total of 16 endophytic fungi were isolated from stem bark (4 isolates) and leaves (6 isolates) of *S. zeylanicum*.

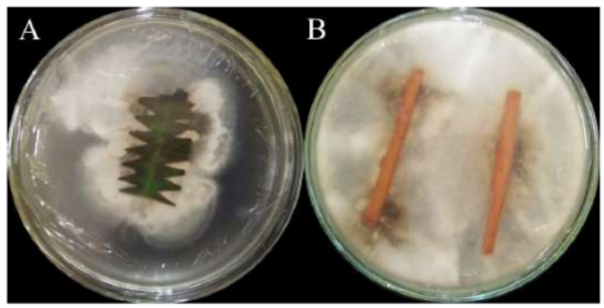


**Figure 1.** Endophytic Fungi from *Syzygium zeylanicum*. (A) Leaves (B) Stem Bark. Isolation Using PDA Medium after 7 Days Incubation in Room Temperature

Colony morphology of the 10 endophytic fungi isolates varied in shape and color (Figure 2) along with the shape of the hyphae and conidia which showed their characteristics (Figure 3). Colonies of endophytic fungus isolates showed various physical appearances. Large-scale hyphal growth occurred in bark and leaf samples, with predominant green, white, and black colonies. The results of the isolation of endophytic fungi from the bark obtained five isolates with codes SZT3 to SZT5 and SZT7 (Figure 2: A-D), and from leaf organs, seven isolates were obtained with codes SZL1 to SZL7 (Figure 2: E-J). The population structure of endophytes can be significantly affected by factors such as the host's genetic culture, age, and environmental conditions (Jia et al., 2016).

Endophytic fungi are facultative biotrophs, so they can complete their life cycle outside their host. The role of endophytic fungi can support the accumulation of bioactive compounds by helping to increase the production of secondary metabolites in the host plants (Khare et al., 2018; Sharma et al., 2018; Yuan et al., 2019). Endophytic fungi can produce bioactive compounds exclusively for their host plants, compounds can be the same or different from their hosts, this is very important to improve the adaptability of endophytic fungi and their host

**Table 1.** Colony Characteristics of Endophytic Fungi of Jambu Nasi-Nasi

| Code | Surface Color | Reverse Color | Texture | Elevation | Pattern | Exudate Drops | Radial Line | Concentric Circle |
|---|---|---|---|---|---|---|---|---|
| SZT3 | Yellowish white | Clear yellow | Cottony | Flat | Spread | – | – | √ |
| SZT4 | Grayish white | Unpigmented | Cottony | Flat | Spread | – | – | √ |
| SZT5 | Yellowish green | Clear yellow | Powdery | Flat | Spread | – | √ | √ |
| SZT7 | Yellowish white | Clear yellow | Cottony | Flat | Spread | – | – | √ |
| SZL1 | Milky white | White | Cottony | Flat | Zonate | – | – | – |
| SZL2 | Dusty white | Unpigmented | Cottony | Umbonate | Spread | – | – | √ |
| SZL3 | Cream and white | Cream | Velvety | Umbonate | Flowery | – | – | √ |
| SZL4 | Gray | Gray | Cottony | Raised | Spread | – | | – |
| SZL5 | Greenish gray | Dark green | Cottony | Flate | Zonate | – | – | √ |
| SZL7 | Milky white | White | Velvety | Flat | Zonate | – | – | √ |

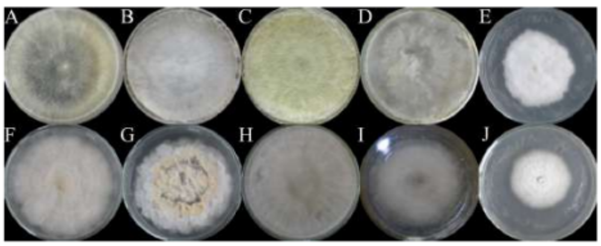

**Figure 2.** Colony Morphology of Endophytic Species; from Stem Bark (SZT3 (A); SZT4 (B); SZT5 (C); SZT7 (D)); and from Leaves (SZL1 (E); SZL2 (F); SZL3(G); SZL4 (H); SZL5 (I); SZL7 (J)). Colony Growth on Potato Dextrose Agar Medium after 5-7 Days

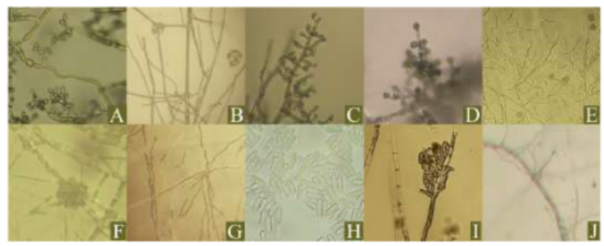

**Figure 3.** Microscopic Charcterization of Endophytic Fungal Isolates; from Stem Bark (SZT3 (A); SZT4 (B); SZT5 (C); SZT7 (D)); and from Leaves (SZL1 (E); SZL2 (F); SZL3 (G); SZL4 (H); SZL5 (I); SZL7 (J)). Observations Were Made on after 5-7 Days on PDA Medium Under Digital Microscope Magnification 400×

plants, such as tolerance to biotic and abiotic stresses (Jia et al., 2016).

### 3.2 Morphological Identification of Endophytic Fungi

Fungal identification begins by comparing morphologically known species. Observations can be made with the naked eye (macroscopic) or with a compound microscope (microscopic). Table 1 shows the characteristics of colonies from color to growth type. On the other hand, Table 2 shows the microscopic features from the shape of the spores to the specific features.

SZL4 isolate had colonies that proliferated throughout the disc with a cottony texture and grayish-white (Figure 2L; Table 1). The microscopic data supported identification where the isolates had sub-ovoid to ellipsoid conidia with wide rounded apex, 1 insulated and thick-walled (Figure 3L; Table 2). Based on these characteristics, SZL4 is close to the genus Lasidoplodia, which is by what was reported by Kapoor and Saxena (2014).

Endophytic fungi isolate SZT5 and SZT7 had the same colony shape with the yellowish-green colony and when compared with microscopic morphology (Figures 3C & 3D), both had setae like hyphae with globose-shaped conidia (Table 2). Phialid in SZT2 & SZT7 has a verticillate shape so it is close to the morphology of *Trichoderma koningi*. Meanwhile, the isolate SZT5 had verticillate, short, and thin phialid (Table 2). And the conidia from this isolate were in the form of hyaline and ovate phialosphorus, so they were identified as *Trichoderma aureoviridae*.

Isolates SZL1, SZL2, and SZL3 had yellowish-white colonies with a flowery pattern (Figure 2E-G). The isolate SZL2 (Figure 3F) showed coenocytic hyphae with lobate sporangiospores and coiling around the oogoniumphores (Table 2) so that SZL2 was identified as *Phytium zingiberum*. While SZL1 has a sporangia lobate form that forms vesicle formations so that this fungus is close to *Phytium torulosum*. And in SZL3 isolate the conidia were not completely differentiated, where the conidia grew directly on the hyphae and were blastosporous so that the SZL3 isolate was closer to *Septonema* sp.

SZT3 isolate had yellowish-white colonies that spread throughout the disc with a cotton-like texture (Figure 2A). SZT3 isolate was identified as *Phialemonium* sp. because microscopically (Figure 3A) it has septate hyphae and there are single short conidia along the hyphae. SZT4 colonies (Figure 2B) grew all over the disc, grayish-white in color with a cottony

**Table 2.** Microscopic Characteristics of Jambu Nasi-Nasi Endophytic Fungi

| Isolate Code | Type of Spore | Shape of Spore | Hyphae | Specific Characteristic | Species of Identification |
|---|---|---|---|---|---|
| SZT3 | Conidia | Peglike | Septate | Single-celled, shirt and peglike | *Phialemonium* sp. |
| SZT4 | Conidia | Oblong | Septate | Conidia are oblong, easily disrupted clusters | *Acremonium* sp. |
| SZT5 | Conidia | Globose | Septate | Conidiosphores branches, conidia phialosporous | *Trichoderma aureoviridae* |
| SZT7 | Conidia | Globose | Septate | Conidiosphores branches, conidia phialosporous | *Trichoderma koningi* |
| SZL1 | Oospore | Lobate | Coenocytic | Vesicle formation from lobate sporangia | *Phytium torulosum* |
| SZL2 | Antheredium spore | Lobate | Coenocytic | Coiling around oogoniumphores | *Phytium zingiberum* |
| SZL3 | Conidia | Blastosporous | Septate | Conidia is not well differentiated, truncate both ends | *Septonema* sp. |
| SZL4 | Conidia | Cylindrical | Septate | Bearing monoverticillate penicilla | *Lasiodiplodia pseudotheobromae* |
| SZL5 | Conidia | Cylindrical | Septate | Sporodochia subglobose, conidiophores phialosporous | *Volutella cilliata* |
| SZL7 | Conidia | Subglobose | Septate | Ovate conidia, short phialides, densely arranged | *Trichophyton mentagrophytes* |

texture. The microscopic characteristics of SZT4 (Figure 3B; Table 2) have a single erect and hyaline phialid, and oblong conidia and conidia form easily disturbed clusters at the ends of the phialides. The isolate SZT4 was identified as *Acremonium* sp. based on its morphological characteristics.

**Table 3.** Endophytic Fungi Diversity from *S. zeylanicum*

| Taxon (Genera) | Tissues of *Syzygium zeylanicum* | | |
|---|---|---|---|
| | Leaves | Stem Bark | Total |
| Phytium | 2 | 0 | 0 |
| Septonema | 1 | 0 | 1 |
| Lasiodiplodia | 1 | 0 | 1 |
| Volutella | 1 | 0 | 1 |
| Trichophyton | 1 | 0 | 1 |
| Trichoderma | 0 | 2 | 2 |
| Phialemonium | 0 | 1 | 1 |
| Acremonium | 0 | 1 | 1 |
| No. of total fungal isolates | 6 | 4 | 10 |
| Simpson's index (D) | 0.1837 | 0.4400 | 0.1389 |
| Simpson's index of diversity (1-D) | 0.8163 | 0.5600 | 0.8611 |
| Shannon index of diversity (H') | 1.7480 | 0.9503 | 2.0950 |

SZL5 isolates had greenish-gray colonies and dark reverse color (Figure 2I) supported microscopically (isolate 3I; Table 2) showing subglobose sporodochia and phialosphorous and cylindrical conidiophores. Based on the macroscopic and microscopic morphological characteristics, it was close to the isolate *Volutella ciliate*. SZL7 colonies were milky white with a velvety texture (Figure 2J) with microscopic morphology having septate hyphae and branching conidiophores and macroconidia attached to the hyphae (Figure 3J) so that SZL7 isolate was identified as *Trichophyton mentagrophytes*.

### 3.3 Endophytic Fungi Diversity Analysis

The stem bark of *S. zeylanicum* obtained four isolates and the leaves obtained six isolates. The diversity index values (Shannon and Weiner, 1949; Simpson, 1949) for these *S. zeylanicum* tissues are listed in Table 3. The $\alpha$-diversity values were acquired thru the PAST software (Sadeghi et al., 2019). The Shannon–Weiner and Simpson variety index values had been maximum in leaves (H'=1.748 and 1-D=0.8163, respectively) and lowest in root bark (H'=0.9503 and 1-D=0.56, respectively). In addition, the general variety values of the endophytic fungal populations had been H'=2.095 and 1-D=0.8611 (Table 3).

The two principal components or axes (1-2) obtained by PCA accounted for 78% of the total variability of the fungus (Figure 4). Analysis showed that some isolates showed affinity for specific tissues. There was no common genus of endophytes from the bark of leaves and stems, but the genus Trichoderma was found in the bark of the roots of *S zeylanicum* (Syarifah et al., 2021). Some isolated endophytic taxa prefer a particular tissue type. Some endophytic can invade the tree host through the root system and move from the soil to new niches within the plant (Gazis and Chaverri, 2010).

**Table 4.** Antibacterial Activity Percentage of Endophytic Fungi Extracts of *S. zeylanicum* (at a Concentration of 400 $\mu$g/disc) Compared to Tetracycline 30 $\mu$g/disc and Antioxidant Activity with Ascorbic Acid Antioxidant Standard and Weight of The Extract from Endophytic Fungi Cultivation in PDB Medium (5×300 mL)

| Isolate Code | Genus/Species of Identification | Fungal Extract Weight (Gram) | % Antibacterial ActivityAntioxidant Activity IC50 ($\mu$g/mL) | | | | Antioxidant Activity IC50 ($\mu$g/mL) |
|---|---|---|---|---|---|---|---|
| | | | *E. coli* | *B. subtilis* | *S. thypi* | *S. aureus* | |
| SZT3 | *Phialemonium* sp. | 5.2 | 81.0±3.19 *** | 72.8±5.40 *** | 80.8±2.56 *** | 77.7±0.23 *** | 28.5 *** |
| SZT4 | *Acremonium* sp. | 5.5 | 68.5±1.82 ** | 62.4±3.20 ** | 69.5±1.27 ** | 71.6±0.95 *** | 3.56 **** |
| SZT5 | *Trichoderma* sp. | 5.3 | 63.8±3.45 ** | 57.4±4.20 ** | 74.4±1.63 *** | 70.6±1.71 *** | 10.25 *** |
| SZT7 | *Trichoderma koningi* | 4.3 | 75.9±2.16 *** | 81.0±3.43 *** | 78.7±2.29 *** | 84.0±1.61 *** | 98.41 *** |
| SZL1 | *Phytium* sp. | 4.7 | 63.1±0.78 ** | 57.6±5.27 ** | 74.9±2.09 *** | 78.8±1.07 *** | 9.15 **** |
| SZL2 | *Phytium zingiberum* | 5.6 | 34.7±0.82 * | 40.6±1.97 * | 58.5±0.68 ** | 65.4±4.20 ** | 4.31 **** |
| SZL3 | *Septonema* sp. | 5.1 | 74.6±2.12 *** | 64.7±4.93 ** | 73.4±0.71 *** | 82.0±0.96 *** | 6.79 **** |
| SZL4 | *Lasiodiplodia pseudotheobromae* | 8.8 | 76.7±1.90 *** | 78.3±1.85 *** | 76.3±1.43 *** | 76.0±1.71 *** | 3.30 **** |
| SZL5 | *Volutella cilliata* | 4.6 | 59.1±1.86 *** | 50.6±0.66 *** | 57.2±0.35 *** | 62.5±0.85 *** | 6.18 **** |
| SZL7 | *Trichophyton mentagrophytes* | 4.7 | 46.6±1.48 * | 42.1±6.05 * | 75.3±1.72 *** | 69.1±5.58 ** | 27.69 *** |
| | Positive control | | Tetracycline 100±1.40 *** | Tetracycline 100±1.28 *** | Tetracycline 100±1.12 *** | Tetracycline 100±1.70 *** | Ascorbic Acid 2.73 **** |

Note: Antibacterial activity percentage: *** strong (≥70%), **moderate (50-70%), and *weak (<50%) [15,42]. Antioxidant activity IC50 ($\mu$g/mL): ****very strong <10 $\mu$g/mL ***strong <100 $\mu$g/mL; **moderat 100-500 $\mu$g/mL; *weak >500 $\mu$g/mL (Mbekou et al., 2021; Metasari et al., 2020).

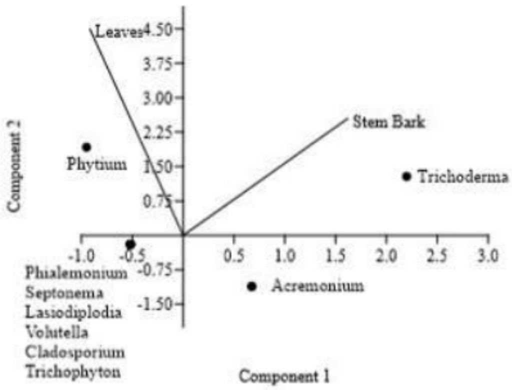


**Figure 4.** PCA of Endophytic Fungi Isolated from Leaves and Stem Bark of *S. zeylanicum*. Components 1 and 2 Described 78% of The Total Variation of The Fungi. The Percentages Shown Are Percentages of Variation Explained by The Components. The Data was Processed Using The PAST Software

### 3.4 Antibacterial and Antioxidant Activity of Endophytic Fungi Extract

Biological activity screening for potential endophytic fungus *S. zeylanicum* was carried out by analyzing its antibacterial and antioxidant activity. Antioxidant activity was carried out by DPPH activity to remove free radicals. Upon receiving an electron from an antioxidant compound, the DPPH radical is reduced to DPPH (Budiono et al., 2019; Praptiwi et al., 2018). The purple color of the DPPH radical changes to yellow (Pavithra and Vadivukkarasi, 2015). Overall endophytic fungi from leaves and bark showed vital to extreme antioxidant activity.

Endophytic fungi ethyl acetate extract from *S. zeylanicum* has potential as an antioxidant and antibacterial as shown in Table 4. There are 7 out of 10 extracts of endophytic fungi showing strong antibacterial activity (>70% inhibition) against *S. typhi* and *S. aureus* bacteria. While the antibacterial activity against *E. coli* showed as many as 5 fungal extracts that had strong activity and against *B. subtilis* bacteria as many as 4 isolates which had strong activity. All endophytic fungi extracts showed very strong antioxidant values (IC50 <10 $\mu$g/mL).

The data in Table 4 shows that all endophytic fungal extracts could inhibit the growth of the bacteria which was indicated by the formation of a clear zone. The majority of extracts gave moderate to strong antibacterial activity against the four tested bacteria. SZL1, SZL2 and SZL7 extract provided weak antibacterial activity. The same thing can be seen from the ability of endophytic fungi extracts to reduce DPPH free radicals. All extracts provide very strong antioxidant activity. Isolate SZL4 and SZL5 endophytic fungi from the leaves and stem bark of *S. zeylanicum* had very strong antioxidant and strong antibacterial against four pathogens bacterial. In this study, only one isolate was selected to be identified to the molecular stage with potential antioxidant and antimicrobial activity with a high yield of extract.

A total of 7 out of 10 extracts of the endophytic fungus *S. zeylanicum* showed a positive correlation with having strong antioxidant and antibacterial activity. The development of compounds that have dual activity as antioxidant and antibacterial is beneficial in the treatment of chronic infections such as Alzheimer's- infection is commonly associated with Gram-negative anaerobic bacteria (Dioguardi et al., 2020), Cystic Fibrosis, and other complex multigenic diseases (Martelli and Giacomini, 2018; Oset-Gasque and Marco-Contelles, 2018). We suggest that bioactive compounds with antioxidant and antibacterial activity include beta-lactam-based compounds, Dihydroquinolines, Quinolines, Piperidone-hydrazides, Isatin-thiosemicarbazones, Barbiturates, Indolophanes, Triazoles and substituted 3-indoles Martelli and Giacomini, 2018.

Antibacterials and antioxidant can be positively or negatively correlated. 70% *Syzygium zeylanicum* endophytic fungi extract showed a positive correlation where the antioxidant and antibacterial activity values were equally strong. In the study of Li et al. (2020) stated that natural flavonoids (i.e. quercetin, baicalin and rutin) have high antibacterial and antioxidant activity.

Not all endophytic fungi extract of *S. zeylanicum* showed a positive correlation, where 3 fungal extracts showed strong antioxidant activity but weak to moderate antibacterial activity. The negative correlation between antioxidant and antibacterial was also mentioned in another study where several tetradecane compounds, -palmitolactone and ethyl hydrocinnamate contributed to this correlation (Bittencourt et al., 2015). With the antibacterial and antioxidant screening of *S. zeylanicum* fungal extract, it can be used as a reference in obtaining alternatives to produce potential active compounds.

The low yield of bioactive substances is an obstacle in the development of these bioactive substances as candidates for medicinal ingredients because to go through a long research stage, many substances are needed. In this study, SZL4 isolates of endophytic fungi were found that produce twice the yield of other endophytic fungi, and have strong antibacterial and antioxidant activities. This discovery is very valuable so further research needs to be designed to make endophytic fungi as source of future medicinal raw materials.

### 3.5 Molecular Identification

The endophytic fungi isolates selected for the molecular identification were SZL4 fungi isolated from leaves. Extracts SZL4 fungi produce more yield than other extracts Fungi. The potential for endophytic fungi to be developed as a new source of medicinal raw materials, apart from the ability to biological activity, can also be viewed from the yield of the resulting extract. Molecular test results are presented in the form of a phylogenetic tree (Figure 5) to assist in the identification process.

Isolate SZL4 has a similarity of 100% and is in the same clade with *Lasiodiplodia pseudotheobromae* KP872340, isolate SZL4 has been registered in the NCBI Genbank with accession number OK668257.

The phylogenetic tree construction of the endophytic fungi isolates SZL4 in Figure 5 uses the Neighbor-Joining method. Endophytic isolates of *S. zeylanicum* indicated by an asterisk [*] were subjected to phylogenetic analysis with related species using neighboring phylogenetic trees (bootstrap value= 1000). Sequences were obtained from BLAST results. The value on the branch shows the bootstrap value (percentage of 1000× replication).

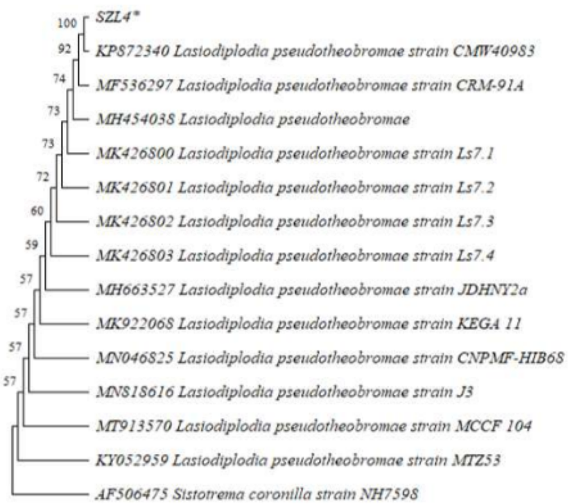


**Figure 5.** Phylogenetic Analysis of Endophytic Fungi Isolates SZL4 (*as The Subject of Phylogenetic Analysis with Related Species)

The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000) and are in the units of the number of base differences per site. This analysis involved 15 nucleotide sequences. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).

The fungus *Lasiodiplodia pseudotheobromae* is not only found in the genus Syzygium but this endophytic fungus is also found in the family Cucurbitaceae., Solanaceae, Leguminosae, Poaceae dan Valerianaceae (Dissanayake et al., 2015; Kapoor and Saxena, 2014; Adetunji et al., 2018). *Lasiodiplodia pseudotheobromae* has antibacterial activity against *Streptococcus* sp., *Bacteroides vulgates*, *Peptostreptococcus* sp., and *Veillonella parvula* Wei et al., 2014. This isolate also has activity potential in xanthine oxidase inhibition (Kapoor and Saxena, 2014) and as a herbicide with the discovery of the compound (R)-8-hydroxy-3-methylisochroman-1-one (known as Mellein) (Adetunji et al., 2018).

## 4. CONCLUSIONS

Ten endophytic fungi isolate were found from leaves and stem bark of *S. zeylanicum* and seven fungal extract showed strong antibacterial and antioxidant activity. Thus isolate can be used as potential candidates for producing antibacterial and antioxidant compounds in the pharmaceutical in the future. The endophyte *Lasiodiplodia pseudotheobromae* (SZL4) produces more bioactive substances and is more likely to be developed as a source of natural pharmaceutical ingredients. This discovery needs to be followed up through a series of follow-up studies to help supply medicinal ingredients.

## 5. ACKNOWLEDGMENT

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