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Biodiversity and antibacterial activity of endophytic fungi isolated from jambu bol (*Syzygium malaccense*)

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Abstract. Hapida Y, Elfita, Widjajanti H, Salni. 2021. Biodiversity and antibacterial activity of endophytic fungi isolated from jambu bol (*Syzygium malaccense*). *Biodiversitas* 22: 5668-5677. Infectious diseases caused by pathogenic bacteria are still high. The existence of bacterial resistance to antibiotics makes it necessary to search for new antibacterial substances from plants and microorganisms. *Syzygium malaccense* (L.) Merr and Perry (Myrtaceae), known as jambu bol, has been used as a traditional medicine in Indonesia and other countries. It is used to treat pathogenic bacterial infections. Therefore, this study aims to determine the diversity of endophytic fungi and their potential as antibacterial compound sources compared to their host plants. Specifically, screening for endophytic fungi that have antibacterial activities is intended. In this study, endophytic fungi were isolated from the leaves, stem bark, and root bark of the host plants. Each isolate was identified morphologically and cultivated in potato dextrose broth (PDB) medium for four weeks. After the incubation period, the liquid culture was extracted with ethyl acetate and evaporated to obtain a concentrated extract. The Kirby-Bauer method was used to evaluate the antibacterial activity of each endophytic fungal extract and the host plant parts extract. The concentration used was 400 µg/mL and the antibiotic was used as a positive control (Tetracycline) is 30 µg/mL. Seven endophytic fungi were isolated from the leaves (YD1, YD2, YD3, YD4, YD6, YD7, Y2D1), seven from the stem bark (YB1, YB2, YB3, YB4, YB5, Y2B5, and Y2B6), and six from the root bark (YA1, YA2, YA3, YA4, YA5, Y2A2). The endophytic fungi were morphologically identified, and the isolates were shown to be dominated by genera from the Ascomycota (e.g., *Poaceascaoma*, *Cladorrhinum*, *Penicillium*, *Madurella*, *Phytophthora*, *Phialemonium*, *Monascus*, *Trichoderma*, *Ramophialophora*, *Gliocladium*, *Wiesneriomyces*) and the Zygomycota (e.g., *Mucor*, *Mortierella*, and *Gongronella*) phyla. The fungi isolates Y2D1, YB1, and YA4 were shown to have the highest (strong) antibacterial activity equivalent to their hosts in the performed screening. Endophytic fungi have significant and continued antibacterial activities that have been identified molecularly.

Keywords: Antibacterial, endophytic fungi, *Syzygium malaccense*

INTRODUCTION

Plant specimens from the *Syzygium* genera, in which the jambu bol plants (*Syzygium malaccense* (L.) Merr and Perry (Myrtaceae) included, used by people in South Sumatra as a traditional medicine for various diseases, including pathogenic bacterial infections (Sonavane 2017; Fernandes and Rodrigues 2018). According to the results found in the literature, jambu bol plants have been used by people in other countries for treating various diseases in traditional medicine. In Brazil, jambu bol is used to treat diabetes (Freitas et al. 2015). In India, some parts of the plant such as fruit, seeds and bark are used in treating dysentery, diabetes, and gastric ulcers (Bairy et al. 2005). In Nigeria, these plants are used to treat diabetes, anti-inflammatory, and antimicrobial activities (Oyinlade 2014). Leaf extract was shown to be a growth inhibitor of *Staphylococcus aureus* and *Streptococcus pyogenes* at a 100 g/mL concentration (Locher et al. 1995). Some compounds from *S. malaccense* leaves and bark (e.g.,

5,7,3',4',5'-pentahydroxy flavonol compounds; 5,7,3',5'-tetrahydroxy-4'-methoxy flavonol; 5-trihydroxybenzoic acid; 3-acetyl-urs-12-en-28-oic acid; and 3β-O-acetyl-urs-12-en-28-oic acid) and the napin peptide from the seeds were shown to be new potential anti-*Staphylococcus* substances (da Silva Dantas et al. 2014).

Plants with ethnobotanical historical use in treating certain diseases are promising candidates for obtaining bioactive compounds to control high-activity endophytic fungi (Strobel and Daisy 2003; Abdalla and McGaw 2018). The current prospect of bioactive compounds and the high demand for medicinal ingredients have spurred the search for new bioactive metabolites inside medicinal plant endophytic fungi (Zhao et al. 2010). Endophytic fungi are promising and abundant sources of natural products with varied chemical structures, significant biodiversity, and many exciting bioactivities (Manganyi and Ateba 2020; El-Bondkly et al. 2021). Natural products derived from endophytic fungi are considered the most relevant sources for discovering new molecular drug (Singh et al. 2021). It

was indicated in several studies that most bioactive metabolites obtained in endophytic fungi carry unique chemical structures. These fungi can produce metabolites similar to those produced by their host plants. [56] 1, different new compounds have been discovered (Cruz et al. 2020; El-Hawary et al. 2020). The diversity of endophytic fungi obtained will be useful as an alternative to replace the supply shortage of plant resources, shorten the regeneration cycle, and use industrial fermentation to produce natural active compounds, such as antimicrobial and other bioactive compounds, for mass production at a low cost and without pollution through biotechnology, so its potential still needs to be explored (Du et al. 2020)

This scenario represents a significant biotechnological potential for the discovery of new drugs in microbial groups. Research regarding the antibacterial pharmacological effects of bioactive compounds obtained from the endophytic fungi hosted by *S. malaccense* is still in its initial stages. Nevertheless, by reviewing the documentation of endophytic fungal diversity, new insights into the possibilities for exploration and development may be provided. Therefore, the classification of pharmacological functions from extracts obtained from endophytic fungi hosted by *S. malaccense* specimens may be assisted by antibacterial pharmacological assays. With such assays, bioprospection information of endophytic fungi as biological entities may be provided, ensuring their continued use to improve human quality of life.

58 MATERIALS AND METHODS

Plant material

Samples from leaves, stem bark, and root bark of healthy *S. malaccense* plants were obtained in Palembang, South Sumatra, Indonesia in February 2021. This plant was identified by the Indonesian Institute of Sciences (LIPI) Plant Conservation Center, Botanical Gardens Purwodadi, East Java, Indonesia with no: B-302/III/KS. 01.03/1/2021.

80 Isolation of endophytic fungi

The surface of the plant samples (leaves, stem bark and root bark) was sterilized, later, they were cut longitudinally using an aseptic technique. Additionally, the samples were included in a petri dish containing PDA (Potato dextrose agar) medium. Incubation was conducted at 37°C for 3-10 days. Observations were made every day until the fungus was able to be seen. Fungal colonies growing on PDA (Potato Dextrosa Agar) medium showed different morphological that it was purified. Observations were made every day until it was possible to see the fungus. Fungal colonies growing on PDA medium had different morphological features, indicating that the samples were purified (Fadhillah et al. 2019).

Morphological identification of endophytic fungi

The identification of fungi was conducted based on three main characteristics: colonies, microscopic characteristics, and macroscopic characteristics. Macroscopic identification of endophytic fungi included observations on surface and reverse colonies, pattern,

texture, topography, concentric and radiated circle. Microscopic identification by observing hyphae, spore type, shape of spores and other specific characteristics by making culture slides first and the medium used is PDA (Pitt and Hocking 2009; Watanabe 2010; Walsh et al. 2018).

Cultivation and extraction

Each endophytic fungi was isolated during cultivation. Six pieces of PDA media (approximately 5 mm diameter) inoculated with pure fungal culture were placed into three erlenmeyer flasks containing 300 mL of potato dextrose broth (PDB) medium. Incubation was conducted at room temperature for approximately 33 weeks. The medium containing secondary metabolites was partitioned into ethyl acetate and evaporated to obtain a concentrated ethyl acetate extract (Habisukan et al. 2021).

Extraction of the host plant *Syzygium malaccense*

Approximately 100 g of fresh leaves, bark, and root bark of the plants were obtained. The samples were then dried at room temperature and macerated using methanol as a solvent for 24 hours with two repetitions to obtain the extracts. Later, the extract was filtered with filter paper and evaporated with a rotary evaporator (Metasari et al. 2020). Methanol used as a polar solvent more effective in extracting phytochemical compounds in natural materials such as plants to increase their biological activity (Nawaz et al. 2020).

55 Antibacterial activity test

An antibacterial activity test was performed using the disc diffusion method, also known as Kirby-Bauer, in a petri dish containing NA (Nutrien Agar) medium. The sample fungi extracts were put on standard disc paper as a test sample (400 µg/mL) with standard antibiotics (tetracycline 30 µg/mL). The test bacteria used were *Salmonella typhi* (ATCC1408), *Escherichia coli* (Ina CCB4), *Staphylococcus aureus* (Ina CCB5), and *Bacillus subtilis* (Ina CCB4). Observations and measurements of the inhibition zone were conducted for 24 h. The percentage was determined using the following formula (Ding et al. 2019).

$$\text{weak: } \frac{A}{B} \times 100\% < 50\%; \text{ moderat: } 50\% < \frac{A}{B} \times 100\% < 70\%; \text{ strong: } \frac{A}{B} \times 100\% > 70\%$$

Where: A is the zone of inhibition (mm) of the test samples and B is the zone of inhibition (mm) of the standard antibiotic samples.

74 RESULTS AND DISCUSSION

Isolation of endophytic fungi

In Figure 1, the isolated fungi have hyphae, which is indicative of endophytic fungi growth. Endophytic fungi were observed growing around the leaves, stem bark, and root bark. Surface sterilization of plants and antibiotics on PDA medium was conducted during the isolation of

endophytic fungi. Endophytic fungi were observed growing around the sliced leaf, stem bark, and root bark. In observations made three days after media inoculation, it was possible to detect colony differences caused by different fungi, and purification was conducted.

After purification and isolation of the endophytic fungi, 20 fungi isolates were obtained. There were seven isolates from the leaves, seven from the bark, and six from the root bark. In Figure 1, it can be seen that the fungal hyphae indicated the growth of endophytic fungi because they were found around the leaves, stem bark, and root bark after surface sterilization and put of antibiotics on PDA media during the isolation of endophytic fungi.

On the third day after inoculation, there were differences in the fungi colonies, so purification could be conducted. With the purification of the endophytic fungi, 20 isolates were obtained. There were seven isolates on the leaves, seven from the bark, and six from the root bark. Macroscopic and microscopic colonies of endophytic fungi on the leaves, stem bark and root bark of *S. malaccense* are shown in Figures 2. The characteristics of macroscopic and microscopic its endophytic fungi are shown in tables (1, 2, and 3).

84 Cultivation and extraction

With the cultivation of endophytic fungi from each part of *S. malaccense* in PDB medium, 30 bottles of 300 mL each were obtained. From these bottles 10 g of ethyl acetate extract was obtained from the leaves, stem bark, and root bark. On leaves: YD1, YD2, YD3, YD4, YD6, YD7 and Y2D1. On the stem bark: YB1, YB2, YB3, YB4, YB5, Y2B5 and Y2B6. On root bark: YA1, YA2, YA3, YA4, YA5, and Y2A2.

Extraction of the host plant *Syzygium malaccense*

The samples of fresh plant parts (100 g) were dried and macerated. Then, methanol was used as a solvent, and the solution was evaporated. With the methanol extract obtained from the leaves, stem bark, and root bark, 8.5 g, 7.4 g, and 6.9 g of extract were obtained, respectively.

Antibacterial activity test

Each endophytic fungi extract was evaluated for its antibacterial activity using the Kirby-Bauer method at a concentration of 400µg/disk. The extracts obtained from

the host plant parts were also assessed. Antibacterial activity with the different extracts was performed at the same concentration to compare the strength of their antibacterial activity. The standard antibiotic used was tetracycline (30 µg/disk) which had broad-spectrum activity. The measurement of the inhibition zone and its strength is shown in Tables 4, 5, and 6.

Regarding the antibacterial activity test results on four bacteria, the fungal extract Y2D1 and the methanol extract from the leaves of *S. malaccense* were potential inhibitors of bacterial growth, considering strong criteria. Other groups of endophytic fungi isolates, including YD2 and YD3, also inhibited bacterial growth with moderate criteria. Finally, endophytic fungi isolates YD1, YD4, and YD7 can inhibit bacteria with weak criteria. Antibacterial activity was determined by comparing it with tetracycline antibiotics. The isolate YB1 and YB3 endophytic fungal isolates from the stem bark of *S. malaccense* and methanol extract had antibacterial activities with stronger criteria than tetracycline antibiotics. Another group of endophytic fungal isolate extracts, including YB4, YB5, Y2B5, and Y2B6, had antibacterial activities with moderate criteria, while the YB2 isolate extract had weak criteria for antibacterial activity. The methanol extract and isolates of endophytic fungi obtained from the root bark of *S. malaccense*, including YA4 and YA5, could inhibit bacteria with strong criteria. The other isolate groups, such as YA1, YA3, and Y2A2, had antibacterial activities with moderate criteria. Isolate YA2 had antibacterial activities with weak criteria. The percentage values of each isolate are shown in Table 2 and Figure 3.

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The diversity values of endophytic fungi on the leaves, stem bark, and root bark of *S. malaccense* were determined using PAST 3, as shown in Figure 4 and Table 3. As the dominance value (D) of fungi in the leaves, stem bark, and root bark ranged from 0-0.5, regarding the Simpson index, it is an indication that there are no dominating genera in these plant parts. The Shannon diversity index is in the 1 > H'3 range, which is the moderate requirement, and the Simpson diversity index is in the 0.6-1 moderate diversity range (Arzamani et al. 2018).

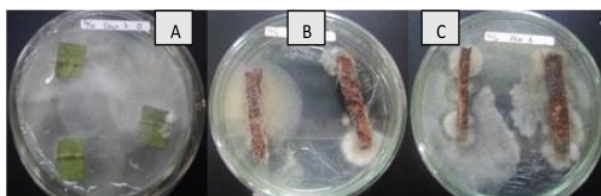


Figure 1. Hyphae from endophytic fungi that began to showed after day 3 around each organ. A) leaves, B) stem bark and C) root bark of jambu bol (*Syzygium malaccense*)

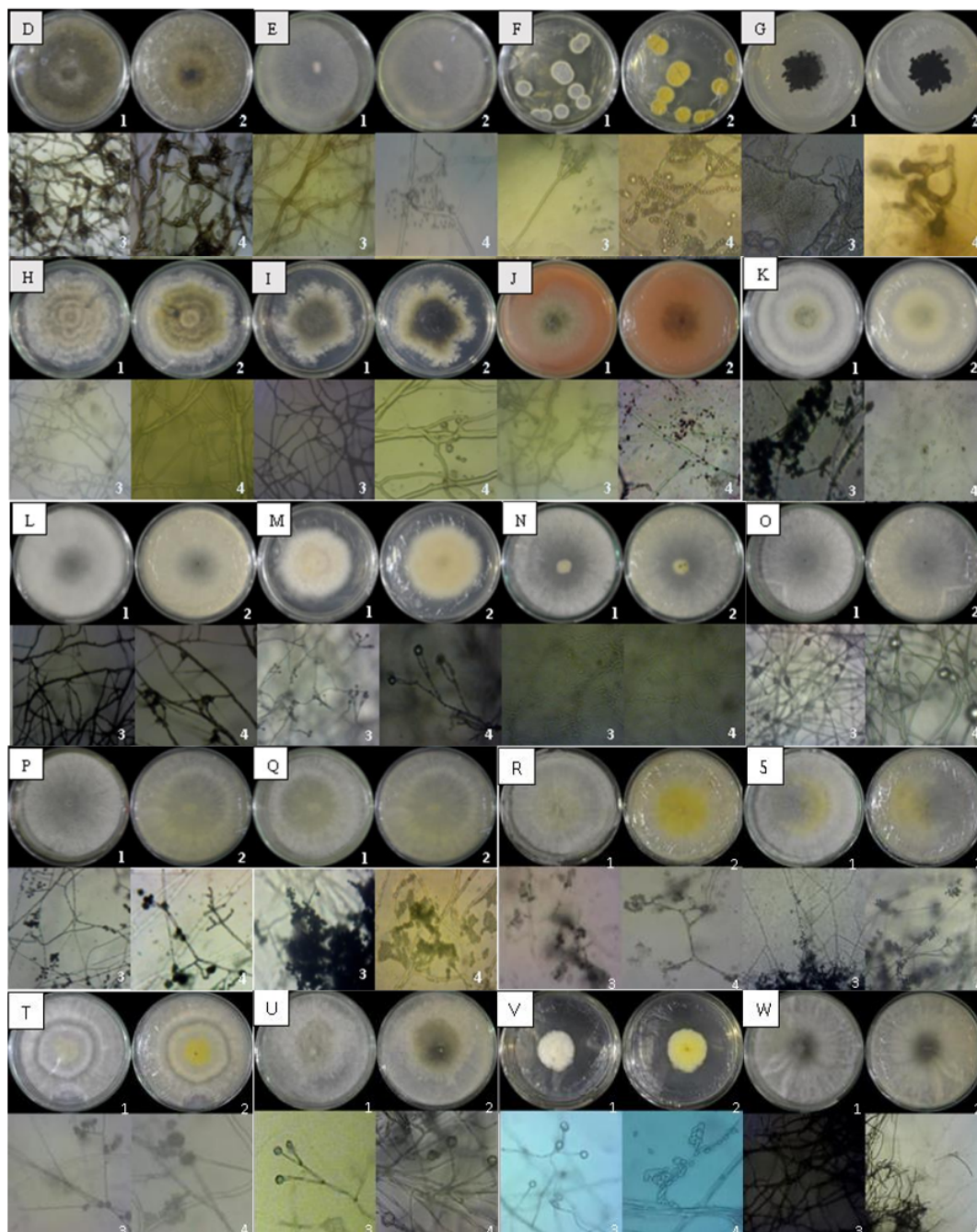


Figure 2. Endophytic fungi isolate on leaf organs, D. YD1, *Poaceascoma* sp., E. YD2, *Cladorrhinum* sp., F. YD3, *Penicillium* sp., G. YD4, *Madurella* sp., H. YD6, *Phytophthora* sp., I. YD7, *Phialemonium* sp., J. Y2D1, *Monascus* sp., On the stem bark. K. YB1, *Trichoderma* sp., L. YB2, *Ramophialophora* sp., M. YB3, *Gliocladium* sp., N. YB4, *Mucor* sp., O. YB5, *Gonatobotrys* sp., P. Y2B5, *Trichoderma* sp., Q. Y2B6, *Trichoderma*. On root bark, R. YA1, *Trichoderma* sp., S. YA2 *Trichoderma* ⁴⁹ *harzianum*. Q. YA3, *Trichoderma* sp., U. YA4, *Mortierella verticillata*. V. YA5, *Gonatobotrys* sp., W. Y2A2, *Wiesneriomyces* sp.

Table 1. Macroscopic and microscopic characteristics of the endophytic fungal isolate of the leaf (*), stem bark (**), and root bark (***) *Syzygium malaccense*

Isolate	Macroscopic characteristics					Microscopic characteristics			Genus / species	
	Colony color	Reverse colony color	Texture	Topography	Concentric circle	Type of spore	Shape of spore	Hyphae		Specific characteristic
YD1*	Yellowish white	Yellowish White	Cottony	Flat	✓	Klamidospora	Globose	Septate, non aerial	Hyaline to dark brown chlamydospores with a thicker and darkened wall, narrower around septa, and cylindrical to piriform shape.	<i>Poaceoscoma</i> sp.
YD2*	White	White, spreading	Cottony	Umbonate	✓	Conidia	Globose	Septate, non aerial	Phialides terminal dan lateral berbentuk labu. Konidia enteroblastik, hialin, berunding halus dan tipis, bulat.	<i>Cladorrhinium</i> sp.
YD3*	Dark green with marginal white	Light-yellow	Velvety	Rugose	-	Conidia	Globose	Septate, aerial	Conidiophores hyaline, erect, branching with 2-3 metula, each metula has a verticillate phialid, and each phialid dangles conidia.	<i>Penicillium</i> sp.
YD4*	Black	Black	Velvety	Rugose	-	Conidia	Globose	Septate, non aerial	The hyphae appear to be made up of chains of spherical cells, indicating that they are budding. There are also thinner, cylindrical, branching hyphae	<i>Madurella</i> sp.
YD6*	White-gray marginal yellow	Greenish-yellow	Cottony	Flowery	✓	Zoospora	Obovate, ellipsoide	Septate	Inside sporangia, zoospores form. Sexual organs do not develop. Globose hyphae	<i>Phytophthora</i> sp.
YD7*	Dark green marginal white	Dark green marginal light	Cottony	Verrucose	✓	Conidia	Verticillate	Septate, aerial	Without a septum at the base to demarcate the hyphae, phialides grow singly along them. Conidia are hyaline and single-celled.	<i>Phialemonium</i> sp.
YD2*	Greenish white	White greens and red pigment secretion	Cottony aerial	Verrucose	✓	Conidia	Globose	Septate	Dark brown hyphae, branching	<i>Monascus</i> sp.
YB1**	Yellowish white	White, spreading	Cottony	Flat	✓	Conidia	Sub-globose	Septate, aerial	Hyaline conidiophores, have an apical spore mass in phialides. Phylophorous conidia. Chlamydospores	<i>Trichoderma</i> sp.
YB2**	White Dark centred	White, spreading	Cottony	Flat	✓	Conidia	Globose	Septate, non aerial	Conidiophores are reduced to conidiogen cells. Lateral or terminal phialides of aerial mycelia.	<i>Ramaphialophora</i> sp.
YB3**	White	White, spreading	Velvety	Flat	✓	Conidia	Ellipsoid	Septate, aerial	Conidiophores hyalin, erect, bearing spore masses on phialides at the apex: phialides verticillate	<i>Gliocladium</i> sp.
YB4**	White	Yellowish white	Cottony	Umbonate	-	Sporangiospore hialin	Ellipsoid	Septate, aerial	Sporangiophores hyaline, erect, branched sympodial, sporangia terminal, rizblack, hitam, ovate	<i>Mucor</i> sp.
YB5**	White	White	Cottony	Umbonate	✓	Conidia	Globose	Septate, aerial	Conidiophores dark brown, erect, bearing spore masses in apical part nodes 4 positions. Botryblastosporous type	<i>Gonatobotrys</i> sp.

Y2B5 **	White	White	Cottony	Flat	✓	Conidia	Sub-globose	Septate, aerial	Hyalin conidiophore, phialides have apical spore masses. Fialosorus conidia, clamidospores light brown, subglobose.	<i>Trichoderma</i> sp.
Y2B6 **	Yellowish white	white	Cottony	Flat	✓	Conidia	Sub-Globose	Septate	Hyalin conidiophore, phialides have apical spore masses. Fialosorus conidia, clamidospores light brown, subglobose.	<i>Trichoderma</i> sp.
YA1 ***	Yellowish white	White, spreading	Cottony	Flat	✓	Conidia	Sub-globose	Septate, aerial	Hyalin conidiophore, phialides have apical spore masses. Fialosorus conidia, clamidospores light brown, subglobose.	<i>Trichoderma</i> sp.
YA2 ***	White	White, spreading	Cottony	Flat	✓	Conidia	Sub-globose	Septate, non aerial	Hyalin conidiophore, phialides have apical spore masses. Fialosorus conidia, clamidospores light brown, subglobose.	<i>Trichoderma</i> sp.
YA3 ***	White	White, spreading	Cottony	Flat	✓	Conidia	Sub-globose	Septate, aerial	Hyalin conidiophores erect, 1-3 branches, tapering gradually towards the apex. Sporangia non columella	<i>Mortierella</i> sp.
YA4 ***	White, dark centred	White, spreading	Cottony	Flat	-	Sporangiospore	Globose	Septate, aerial	76	
YA5 ***	White, dark centred	Light yellow	Glaborous	Rugose	✓	Clamydospore	Globose	Septate, aerial	Sporangiophores hyaline, erect, branched, septate near sporangia, rhizoidal basal. Sporangia globose, gray, columellate, and conspicuous apophysate.	<i>Gongronella</i> sp.
Y2A2 ***	White, dark centred	White, spreading	Cottony	Flat	✓	Conidia	Ovate, ellipsoid	Septate, aerial	Hyalin conidiophore, phialides have apical spore masses. Fialosorus conidia, clamidospores light brown, subglobose.	<i>Wiesneriomyces</i> sp.

Table 2. Percentage antibacterial activity of *Syzygium malaccense* leaf extract and its endophytic fungal extract (at a concentration of 400 µg/disk) compared standard antibiotic tetracycline (30 µg/disk)

Sampels	Extract	Antibacterial activity ^a			
		<i>Salmonella typhi</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Leaves <i>Syzygium malaccense</i> endophytic fungi	Methanol	18.3 ± 1.2	16.5 ± 1.7	17.4 ± 1.3	17.5 ± 2.3
	YD1	7.5 ± 1.2	7.6 ± 1.9	9.4 ± 0.8	7.3 ± 1.9
	YD2	12.7 ± 1.7	10.7 ± 1.4	12.9 ± 2.1	11.0 ± 2.2
	YD3	17.5 ± 2.5	17.5 ± 1.6	11.1 ± 1.9	16.8 ± 2.8
	YD4	9.9 ± 1.2	8.0 ± 1.7	8.4 ± 1.3	10.6 ± 1.4
	YD6	10.2 ± 1.4	10.8 ± 1.6	13.5 ± 1.8	12.5 ± 1.8
	YD7	7.9 ± 2.1	7.4 ± 1.8	8.5 ± 1.3	7.6 ± 2.2
	Y2D1	18.9 ± 2.3	16.7 ± 1.2	16.8 ± 1.5	17.2 ± 2.3
Stem bark <i>Syzygium malaccense</i> endophytic fungi	Methanol	17.9 ± 1.8	17.2 ± 1.2	17.4 ± 1.4	16.7 ± 1.7
	YB1	17.1 ± 1.5	15.8 ± 1.7	16.2 ± 1.8	17.3 ± 1.5
	YB2	8.3 ± 1.2	7.7 ± 2.2	7.2 ± 0.8	8.2 ± 2.2
	YB3	18.2 ± 1.3	16.2 ± 1.4	15.7 ± 1.2	15.7 ± 1.2
	YB4	13.6 ± 1.6	11.2 ± 1.8	10.7 ± 1.8	13.6 ± 0.9
	YB5	12.4 ± 2.2	11.7 ± 1.3	11.4 ± 2.5	6.6 ± 2.3
	Y2B5	9.1 ± 2.1	11.8 ± 0.83	9.3 ± 0.9	8.9 ± 2.9
	Y2B6	10.6 ± 2.17	11.7 ± 0.83	10.5 ± 1.1	9.7 ± 2.91
Root bark <i>Syzygium malaccense</i> endophytic fungi	Methanol	16.3 ± 1.1	17.2 ± 1.1	17.5 ± 1.6	16.7 ± 0.7
	YA1	13.6 ± 2.1	15.9 ± 2.6	12.8 ± 2.4	15.9 ± 3.1
	YA2	9.00 ± 1.6	9.7 ± 1.4	8.5 ± 1.3	8.3 ± 1.8
	YA3	14.6 ± 1.1	16.3 ± 1.6	15.9 ± 1.4	14.4 ± 2.1
	YA4	16.6 ± 1.3	15.2 ± 1.7	16.9 ± 1.9	15.6 ± 1.5
	YA5	17.2 ± 1.4	15.9 ± 2.5	15.9 ± 1.9	15.1 ± 1.8
	Y2A2	11.7 ± 1.6	15.7 ± 1.4	11.2 ± 2.4	12.6 ± 0.8
Antibiotic ^b	Tetracycline	22.2 ± 1.14	20.5 ± 1.32	27.3 ± 1.22	21.6 ± 1.25

Note: a. Percentage of antibacterial activity extract compared to standard antibiotics (%): zone of inhibition (mm) extract/ zone of inhibition (mm) tetracycline: *** strong (≥ 70%), **moderate (50-70%), and *weak (< 50%) against *Salmonella typhi*, *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*, b. positive control (30 g/disk), (-) no inhibition zone

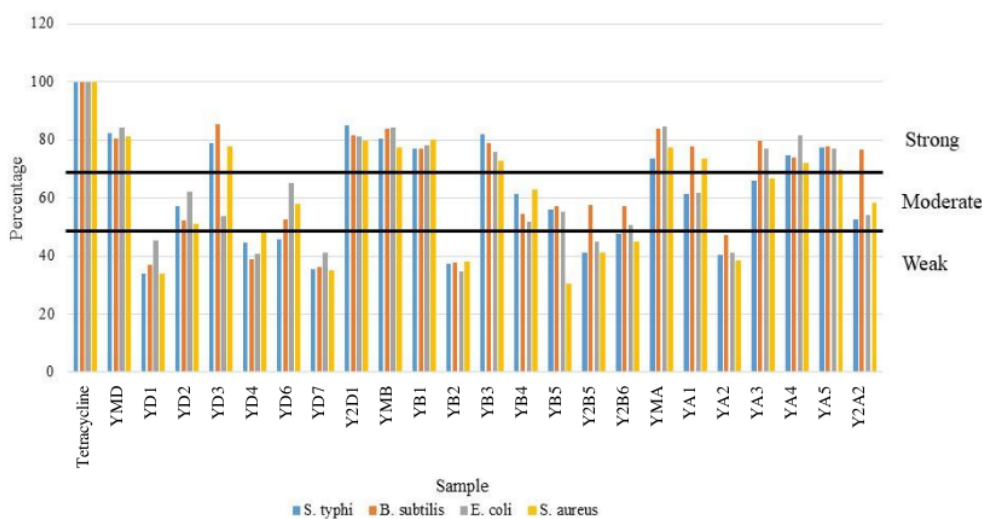


Figure 3. Antibacterial activity of *Syzygium malaccense* leaf methanol extract and its endophytic fungus extract (concentration 400 µg/mL) as a percentage of strength against *Salmonella typhi*, *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus* in comparison to tetracycline (30 µg/mL)

Table 3. Diversity of endophytic fungi isolated from *Syzygium malaccense*

Genera	Part of plat <i>Syzygium malaccense</i>			Total
	Leaf	Stem bark	Root bark	
<i>Poaceascoma</i>	1	0	0	1
<i>Cladorrhinum</i>	1	0	0	1
<i>Penicillium</i>	1	0	0	1
<i>Madurella</i>	1	0	0	1
<i>Phytophthora</i>	1	0	0	1
<i>Phialemonium</i>	1	0	0	1
<i>Monascus</i>	1	0	0	1
<i>Trichoderma</i>	0	3	3	6
<i>Ramophialophora</i>	0	1	0	1
<i>Gliocladium</i>	0	1	0	1
<i>Mucor</i>	0	1	0	1
<i>Mortierella</i>	0	0	1	1
<i>Gongronella</i>	0	0	1	1
<i>Gonatobotrys</i>	0	1	0	1
<i>Wiesneriomyces</i>	0	0	1	1
Jumlah is 48 fungi	7	7	6	20
Simpson index (D)	0.1429	0.2653	0.3333	0.125
Simpson index of diversity (1-D)	0.8571	0.7347	0.6667	0.875
Shannon Index of diversity (H)	1.946	1.475	1.242	2.458

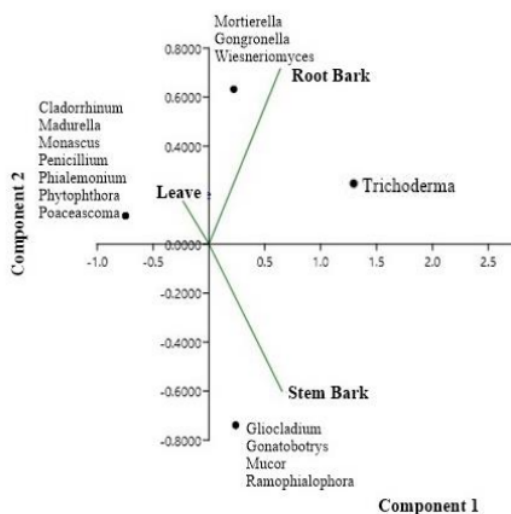


Figure 4. PCA (Principal Component Analysis) *Syzygium malaccense* endophytic fungi isolates in different organs. In components 1 and 2 have a variation of 60% diversity

In Figure 4 and Table 3, isolates of the same endophytic fungus, *Trichoderma* sp., apparently of the same species, were isolated from stem bark and root bark isolates of *S. malaccense*. *Trichoderma* can be found in a number of settings and seasons. This fungus genus contains a diverse range of species that have been isolated from soils, acidic

soils, bark, varied substrates, and tree bark. *Trichoderma* is distinguished by the shape of its conidia, which can range from globose to ellipsoidal, obovoidal, or short cylindrical with a truncate and tapering basal tip. Smooth to rough-walled, wing-like projections, obovoid, oval, or shortly cylindrical, sub-globose single-celled conidia, colorless, green, gray, or brownish; smooth to rough-walled, wing-like projections, may Intercalary or terminal chlamydo-spores, elliptical circular, greenish, yellowish, or colorless (Misra and Gupta 2009; Sun et al. 2012; Sharma et al. 2019).

Discussion

Endophytic fungus isolated from the leaves, stem bark, and root bark of *S. malaccense* plants from Palembang, South Sumatra, produced various antibacterial substances depending on their taxonomic groupings. These three plant parts could have varied functions in supporting host growth and nutritional needs in pathogen protection (Selim et al. 2012; Fitriarni and Kasiamdari 2018). Endophytic fungi create secondary compounds that protect host plants against pathogens, consequently, plant parts can produce a variety of endophytic fungal isolates (Fadiji and Babalola 2020). Endophytic fungi on leaves were mainly found in the lamina, and colonization increased during the rainy season (Chutulo and Chalannavar 2018). Several factors, including ecology, nutrition, host physiology, and fungal colonization sites, are thought to be correlated with the establishment and amount of secondary metabolites produced by endophytic fungi (Chauhan et al. 2019).

In the antibacterial activity test at a 400 µg/mL concentration, *S. typhi*, *B. subtilis*, *E. coli*, and *S. aureus* bacteria were inhibited by the ethyl acetate extract of endophytic fungi isolates and plant extract from *S. malaccense*. This result is presumably because endophytic fungi form the same secondary metabolites as their hosts (Zhao et al. 2010; Jia et al. 2016; Pelo and Mavumengwana 2020). Endophytic fungi are believed to imitate, duplicate, and modify the secondary metabolite compounds of their hosts, which is a complex process (Gouda et al. 2016). The group of fungi that had a substantial percentage of antibacterial activity as their host was found in the leaf methanol extract and fungi extract Y2D1. Still, antibacterial activities were observed in stem bark methanol extracts, such as YB1 and YB3, and fungi extracts from root bark methanol extract, such as fungi extract YA4 and YA5. Natural products derived from endophytic fungi are considered the most relevant discovery sources and molecular diversity for new drugs. It was shown in several studies that most bioactive metabolites obtained from endophytic fungi have unique chemical structures. These fungi are capable of producing metabolites similar to those produced by their hosts or different new compounds. This represents a substantial biotechnological potential of endophytic fungi for the discovery of new drugs (Cruz et al. 2020; El-Hawary et al. 2020). Endophytic fungi can duplicate and modify compounds produced by their host plants. Endophytic fungi can duplicate and change compounds by making more complex and increasing their function (Verma et al. 2009).

There are some secondary metabolites (e.g., phenolics, flavonoids, and carotenoids) in the different parts of *S. malaccense*, significantly in its highest leaves, which are helpful as nutrition and pharmacology substances (Batista et al. 2017). Secondary metabolites are hypothesized to work as antibacterial substances in host plants and endophytic fungi by preventing cell wall development, modifying membrane permeability, inhibiting nucleic acid production, and decreasing the formation of essential metabolites in bacterial cells (Chatterjee et al. 2019). With the results of macroscopic and microscopic identification in Figure 3 (YD3 and Y2D1), (YB1 and YB3), and (YA4 and YA5), it is suggested that endophytic fungal isolates are antibacterial agents from the Ascomycota and Zygomycota phyla. In the Zygomycota phylum, the obtained genera were *Mucor*, *Mortierella*, and *Gongronella*. Regarding the Ascomycota phylum, the obtained genera were *Monascus*, *Penicillium*, *Gliocladium*, and *Trichoderma*, including isolate Y2D1 from the genus *Monascus*, which was the best group of endophytic fungal isolates that could suppress the tested bacteria. It is thought that the species from the *Monascus* genus produce monascidin A, a substance with antibacterial activities that inhibit bacterial and fungal strains (Ferdeş et al. 2004). The species *Monascus purpureus* is a multicolored fungus with several biological properties, including antibacterial activities (Bi and Gajalakshmi 2018). *Penicillium* species produce more than 280 chemicals with antibacterial, anticancer, antiviral, antioxidant, anti-inflammatory, antiparasitic, immunosuppressive, antidiabetic, antiobesity, antifibrotic, neuroprotective, insecticidal, and biocontrol properties (Toghueo and Boyom 2020). Under microaerophilic circumstances, the species from the *Gliocladium* genus may break down plant cellulose, generating complex hydrocarbons (Ahamed and Ahring 2011). The fungi species from the genus *Trichoderma* live in the soil, potentially forming endophytic relationships with host plants that protect them from harmful fungi (Ruangwong et al. 2021). As an endophytic fungus, species from the *Mortierella* genus aid plants in absorbing P and Fe elements and protecting host plants from infections. (Ozimek and Hanaka 2021).

6 In methanol extracts of host plant tissues and endophytic fungi isolated from leaves, stem bark, and root bark, *S. malaccense* secondary metabolites had antibacterial activity. Furthermore, the species of endophytic fungi and their compounds from these potential secondary metabolites must be investigated.

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