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Antibacterial activity of endophytic fungi isolated from the stem bark of jambu mawar (*Syzygium jambos*)

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Abstract. *'Aini K, Elfita, Widjajanti H, Setiawan A, Kurniawati AR. 2021. Antibacterial activity of endophytic fungi isolated from the stem bark of jambu mawar (*Syzygium jambos*). Biodiversitas 23: 521-532. Syzygium jambos* (jambu mawar) is widely used in South Sumatra as a traditional medicine to treat various diseases, including pathogenic bacterial infections. Literature studies report that parts of *S. jambos* have been used worldwide for treating diarrhea accompanied by fever, dysentery, sore throat, diabetes, and other infectious diseases. Endophytic fungi isolated from medicinal plants have high diversity, and the biological activity of their secondary metabolites is associated with their host. Therefore, this study aimed to determine the diversity of endophytic fungi from the stem bark of *S. jambos* and the antibacterial activity of secondary metabolites of endophytic fungi. Fungal isolates with high antibacterial activity were identified to obtain fungal species, and the structure of their compounds was determined. The disc diffusion method carried the antibacterial activity test against Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Escherichia coli* and *Salmonella typhi*) bacteria. Characteristics of endophytic fungi were identified microscopically and macroscopically. Active endophytic fungi were identified by molecular analysis of the internal transcribed region (ITS) of the ribosomal DNA. Isolation of antibacterial compounds using chromatographic techniques and determination of chemical structures using spectroscopy was performed. Eight endophytic fungi were obtained from the stem bark of *S. jambos*, namely SJ1–SJ8. The endophytic fungus SJ6 showed the highest activity and base on phylogenetic analysis, was identified as *Fusarium verticillioides*. Pure compounds isolated as yellowish-white solids showed good antibacterial activity against *S. aureus* and *S. typhi* with minimum inhibitory concentration values of 64 g/mL each. Based on 1D and 2D nuclear magnetic resonance spectroscopic analysis, compound 1 was identified as 3-hydroxy-4-(hydroxy(4-hydroxyphenyl)methyl)dihydrofuran-2-on.

Keywords: Antibacterial compound, endophytic fungi, *Fusarium verticillioides*, *Syzygium jambos*

INTRODUCTION

Most of the diseases that arise today are caused by microbial infections. Microbes that cause infection have become increasingly resistant to drugs over time, posing a major challenge to human health. New bioactive compounds from plants and microorganisms can provide the best alternative as a potential drug source to combat these infectious diseases (Sudha et al. 2016). Plants are the main source of bioactive compounds as antibacterial agents such as alkaloids, phenolics, polyphenols, flavonoids, tannins, terpenes (Katz and Baltz 2016; Anand et al. 2019). Endophytic fungi play an important role in discovering these new bioactive compounds. They live in healthy plant tissues without disease symptoms, protecting plants from herbivores and pathogens by producing bioactive secondary metabolites in host tissues (Rai et al. 2012, Martinez-Klimova et al. 2017; Abdel-Azeem et al. 2019).

In India, the fruit and leaves of *Syzygium jambos* are used as a traditional treatment for diuretics, toothaches, eye pain, rheumatism and as an expectorant. In Indo-China, its flowers are used to reduce fever, and its seeds are used to treat diabetes, diarrhea, and dysentery (Bonfanti et al. 2013, 2014; Sobeh et al. 2018; Rajkumari et al. 2018). Also, its leaf extract shows anti-herpes simplex virus activity and has antibacterial, antitoxic, antioxidant, and hepatoprotective properties (Athikomkulchai et al. 2008; Mohanty and Cock 2010; Ghareeb et al. 2016; Devakumar and Sudha 2017). A decoction of rose apple leaves and bark is used to treat diarrhea, rheumatism, asthma, bronchitis, and hoarseness and as an expectorant. Its leaf juice is used as a febrifuge. In some countries, such as Suriname, China, India, and Cambodia, its leaves, seeds, and fruit are used to remedy diarrhea, diarrhea with fever, dysentery, sore throat, diabetes, and other infectious diseases (Morton 1987; Lim 2012).

Endophytic fungi are widespread in many plant species. Many strains of endophytic fungi can produce various natural products that are active as antimicrobials. Endophytic fungi are a source of novel bioactive compounds with diverse biological activities. The bioactive compounds of endophytic fungi isolated from medicinal plants are known as volatile derivatives, such as esters, ethers, alkaloids, and phenolic compounds (Nisa et al.

2020). In the literature, several studies have been made on the endophytic fungi of the genus *Syzygium*, including *S. jambos*. *Chrysoporthe deuterocubines*, *Microthia havanensis*, *Celoporthe hauliensis* sp. nov., *C. hawaiiensis* sp. nov., *C. paradisiaca* sp. nov. found in the bark of *S. jambos* (Roux et al. 2020). However, there are no reports of its antibacterial activity and its secondary metabolites. Therefore, this study aimed to reveal the diversity of endophytic fungi from *S. jambos* from South Sumatra, Indonesia, their antibacterial activity, and their bioactive compounds.

MATERIALS AND METHODS

Plant material

The stem bark of *S. jambos* (L.) Alston. was collected at the Multipurpose Field, Jalan Sakura 3 Kompleks Kencana Damai, Kelurahan Sukamaju, Kecamatan Sako, and Kota Palembang. The samples were morphologically identified at the Biosystematics Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Indonesia. ID number: 233/UN9.1.7/4/EP/2021.

Isolation of endophytic fungi

The stem bark of *S. jambos* was washed thoroughly under running tap water. The surface of the sample was sterilized by dipping it into 70% ethanol for 2 min and then soaking it in sodium hypochlorite (NaOCl) for 1 min. Then, the sample was rinsed with sterile distilled water three times (2 min each time) and dried using sterile filter paper. Each sample explant (without epidermis) was aseptically cut to a size of 2 × 1 cm^2 and placed on potato dextrose agar (PDA) media (200 g/L potatoes, 20 g/L dextrose, and 20 g/L agar) to which 200 g of chloramphenicol was added to prevent bacterial growth. Samples were incubated in the dark at ±30°C for 1–2 weeks. The hyphal tips of several different colonies emerging from the segments were isolated and subcultured on new PDA media to obtain pure colonies (Elfita et al. 2019; Khalil et al. 2021).

Identification of endophytic fungi

Characteristics of endophytic fungal isolates were identified macroscopically and microscopically. Macroscopic characterization depends on the colony growth pattern, texture, color margin, and other features. Microscopic characterization was performed using the culture slide method in which one drop of lactophenol blue reagent was added. The slides were examined using a light microscope (Hirox MXB-2500REZ). The characteristic data are then compared with identification books for fungi (Habisukan et al. 2021), such as Larone's Medically Importan Fungi (Walsh et al. 2018), Pictorial Atlas of Soil and Seed Fungi (Watanabe 2010) and other journals of fungal identification.

Cultivation and extraction

Endophytic fungi culture (5-7 days) on PDA medium was cut into small pieces (±5mm in diameter), and six pieces were transferred into each 1-L culture bottle containing 300 mL of potato dextrose broth medium (ingredients: 200 g potato, 20 g dextrose in 1 L of water). The number of culture bottles for each endophytic fungal isolate is five. After incubation at room temperature for 4 weeks, fungal liquid culture was vacuum filtered and partitioned in a separating funnel with ethyl acetate (1:1) solvent three times. The ethyl acetate extract was evaporated to obtain a concentrated extract (Habisukan et al. 2021). The chemical procedure for obtaining organic constituents from plant tissue uses solvent ethyl acetate for more polar compounds (Wan 2017).

Antibacterial activity test

The antibacterial test was carried out using the disc diffusion method. Each sample of the fungal extract was dissolved in dimethyl sulfoxide (DMSO, Merck, Germany). Antibacterial activity was tested on Gram-positive bacteria (*Staphylococcus aureus* InaCCB4 and *Bacillus subtilis* InaCCB4) and Gram-negative bacteria (*Escherichia coli* InaCCB5 and *Salmonella typhi* ATCC 1408) on nutrient agar media. The extract concentration tested was 400 μg/disc, using tetracycline antibiotics with a concentration of 30 μg/disc as a positive control. The activity of the bacterial inhibition zone was measured after incubation at 37°C for 24 hours by measuring the clear zone formed around the paper disc. The clear zone formed indicates the bacteria's sensitivity to the test material's antibacterial material, which is expressed by the width of the diameter of the inhibition zone. The following equation determines the antibacterial activity of the test sample and the criteria for the inhibition zone diameter (Elfita et al. 2019).

The minimum inhibitory concentration (MIC) of pure compounds is determined by preparing a concentration series of 256 mg/mL to 1 g/mL. If the diameter of the inhibition zone is > 9 mm at a certain concentration series, the antibacterial activity at that concentration is positive. The lowest concentration of the concentration series positive for antibacterial activity is defined as the MIC value (Ding et al. 2019).

$$Weak: \frac{A}{B}x100\% < 50\%; Moderate: 50\% < \frac{A}{B}x100\% < 70\%; Strong: \frac{A}{B}x100\% > 70\%$$

Where:
A = zone of inhibition (mm) test sample
B = zone of inhibition (mm) standard antibiotic

Molecular analysis of active endophytic fungi

Molecular analysis were identified at Genetika Lab, PT Genetika Science Indonesia. Genomic DNA extraction method with Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005). PCR amplification with (2x) MyTaq HS Red Mis (Bioline, BIO-25048) using universal primers ITS1 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 5'-TCCTCCGCTTATTGATATGC-3' (White et al.

1990). The sequence results were identified of species using the Basic Local Alignment Search Tool (BLAST) at the website address http://blast.ncbi.nlm.nih.gov/Blast.cgi. Furthermore, multiple alignments were performed using the Mega 11 program (Tamura et al. 2021) use the CLUSTAL W method and building phylogenetic tree using the Neighbor-joining tree method with a bootstrap value of 1000 (Katoch and Pull 2017; Kuswytasari et al. 2019; Saitou and Nei 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the number of differences method (Nei and Kumar 2000) and are in the units of the number of base differences per sequence.

Isolation and identification of bioactive compound

The ethyl acetate extract, which showed strong antibacterial activity, was analyzed by thin-layer chromatography (TLC) of silica gel G-60 F 254, using solvents with various eluents to observe the staining pattern. The extract was subjected to column chromatography using a stationary phase of silica gel (70-230 mesh). The samples prepared by pre-absorption were eventually put into the chromatographic column and eluted using a gradient eluent. The eluate was collected in vials every 10 ml, and thin-layer chromatography was performed to group into column fractions based on the staining pattern. The fraction containing potential secondary metabolites was further chromatographed by column and rinsed with a suitable solvent to obtain a pure compound, namely compound 1. The structure of the pure compound (compound 1) was identified by spectroscopic methods, including ^{1}H-NMR, ^{13}C-NMR, HMQC, HMBC, and COSY (Fadhillah et al. 2019).

RESULTS AND DISCUSSION

Isolation of endophytic fungi

Endophytic fungi from the bark of *S. jambos* were obtained in eight isolates with codes SJ1 to SJ8 (Figure 1). Macroscopic characteristics were identified by the following criteria: color, texture, topography, pattern, exudate drops, radial line, and concentric circle. Microscopic characteristics were identified by the following criteria: spore type, spore shape, hyphae, and other specific characteristics. The results of this identification were compared with the literature and the key to determining fungi (Watanabe 2010).

Endophytic fungi SJ1: brownish-white and dark brown, cottony, raised, zonate, erect conidiophores of variable height, apical conidia, transverse and longitudinal septa, narrowed setae near the septa, identified as *Spegazzinia tessarthra* (Cole 1974; Watanabe 2010; Mena-Portales et al. 2017). Endophytic fungi SJ2: white and white-middle yellow, cottony, raised, patter arrowed, aerial mycelia abundant, hyphae hyaline, septate, branched, terminal or lateral arising hyaline conidiogenous cells, asci globose, identified as *Auxarthronopsis* sp. (Sharma et al. 2013a; Zhang et al. 2017).

Endophytic fungi SJ3: gray-green and yellowish-green, velvety, raised and flowered, ascospore, globose, septate, hyphae hyaline to brown, scattered, according to the characteristics of *Pectinotrichum* sp. (Varsavsky and Orr 1971; Zhang et al. 2017). Endophytic fungi SJ4: light brown, velvety, umbonate, radiated, concentric circle, ascospore, subglobose, septate, paraphyses hyaline, cylindrical, curved, thread-like, according to the characteristics *Phaeotrichosphaeria* (Sivanesan 1983; Watanabe 2010).

Endophytic fungi SJ5: white to gray with yellow edges and white and yellow around, cottony, raised, radiated, concentric circle, conidia, globose, septate, frequently intercalary in hyphae sole, identified as *Poaceascoma* (Phookamsak et al. 2015; Luo et al. 2016; Fors et al. 2020). Endophytic fungus SJ6: pink with a lighter periphery, cottony, zonate, conidiophores hyaline, erect, long, branched verticillately, rarely simple, conidia lunate shaped, long elliptical, 4-5 celled identified as *Fusarium verticillioides* (Walsh et al. 2018; Watanabe 2010).

Endophytic fungi SJ7: green-white around and dark green to gray, cottony, raised, spreading, conidia and globose spores, coenocytic hyphae, catenulate bearing conidia and chlamydospores apically, identified as *Thielaviopsis* sp. (Watanabe 2010; Álvarez et al. 2012; Majumdar and Mandal 2018). Endophytic fungi SJ8: black and white, cottony, umbonate, zonate, concentric circle, conidia and globose spores, conidiogenous cells giving rise to conidia, septate hyphae, these endophytes identified as *Nigrospora bambusae* (Wang et al. 2017). Macroscopic and microscopic identification of endophytic fungi isolated from the bark of *S. jambos* is shown in Tables 1 and 2, respectively.

Table 1. Macroscopic characteristics of endophytic fungi isolate SJ1 – SJ8

Isolate	Colony color	Reverse colony color	Texture	Topography	Pattern	Concentric circle
SJ1	Brownish white	Dark brown	Cottony	Raised	Zonate	-
SJ2	White	White-middle yellow yellow	Cottony	Raised	Arrowed	
SJ3	Grey-green	Yellowish green	Velvety	Raised	Flowery	-
SJ4	Light brown	Light brown	Velvety	Umbonate	Radiated	√
SJ5	white to gray with yellow edges	White and yellow around	Cottony	Raised	Radiated	√
SJ6	Pink and white around	Pink and white around	Cottony	Raised	Zonate	-
SJ7	Green-white around	Dark green to gray	Cottony	Raised	Radiated	
SJ8	Black and White around	Black and White around	Cottony	Umbonate	Zonate	√

Note: (-) = characteristic doesn't appear; (√) = characteristic appear

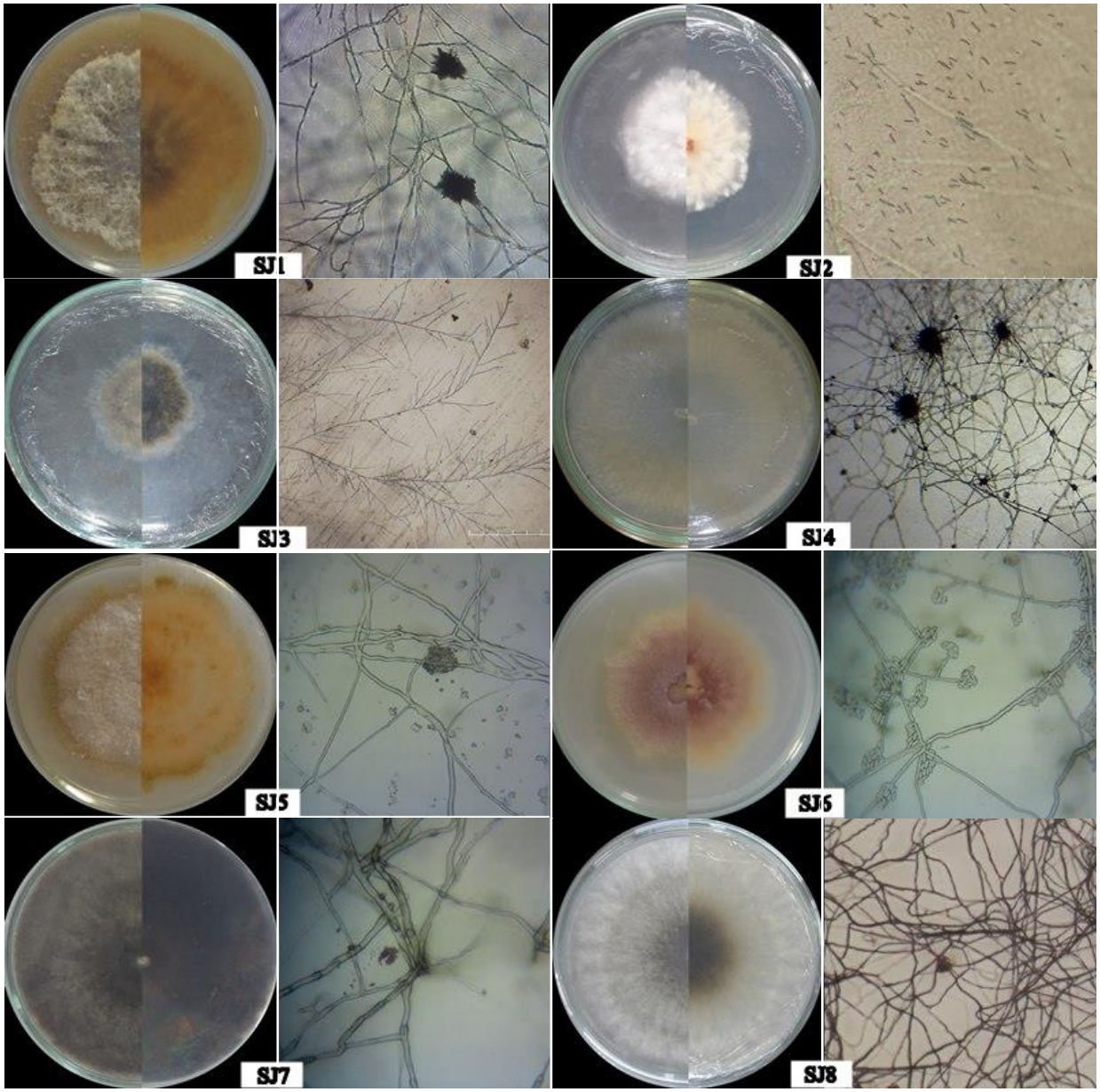

Figure 1. Morphological identification of endophytic fungi from the stem bark of *S. jambos* macroscopically and microscopically

Table 2. Microscopic characteristics of endophytic fungi isolate SJ1 – SJ8

Isolate	Type of spore	Shape of spore	Hyphae	Specific characteristic	Genus / species
SJ1	Conidia	Globose	Septate	Hyaline conidiophores, simple, cylindrical, apically narrowed.	*Spegazzinia tessarthra*
SJ2	Conidia	Globose	Septate	Hyphae hyaline to brown, branched, thin-walled aerial mycelia abundant.	*Auxarthronopsis* sp.
SJ3	Ascospore	Globose	Septate	Hyphae hyaline to brown, scattered	*Pectinotrichum* sp.
SJ4	Ascospore	Subglobose	Septate	Paraphyses hyaline, cylindrical, curved, thread-like	*Phaeotrichosphaeria* sp.
SJ5	Conidia	globose	Septate	frequently intercalary in hyphae sole	*Poaceascoma* sp.
SJ6	Conidia	Verticillate	Septate	Conidia lunate with a foot cell, long elliptical, 4-5 celled	*Fusarium verticillioides*
SJ7	Conidia	globose	Coenocytic	Bearing catenulate conidia and chlamydospores apically	*Thielaviopsis* sp.
SJ8	Conidia	Globose	Septate	conidiogenous cells giving rise to conidia Scale bars: d–g = 10 µm.	*Nigrospora bambusae*

Table 3. Antibacterial activity of ethyl acetate extract of the endophytic fungus *Staphylococcus jambos* (SJ1-SJ8) compared to standard antibiotics (tetracyclines)

Sample (400 µg/disc)	The comparison of the inhibition zone (mm) of the sample to the inhibition zone of tetracycline antibiotics (mm) expressed in % antibacterial activity [a]			
	Staphylococcus aureus	***Salmonella typhi***	***Escherichia coli***	***Bacillus subtilis***
SJ1	13.3±1.5	8.6±1.2	9.5±0.8	10.7±2.1
	56,9**	38.5*	49.0*	50.0**
SJ2	15.3±2.9	13.5±2.5	6.8±1.2	10.1±1.8
	65.4**	60.3**	35.2*	47.2*
SJ3	14.1±1.4	9.1±2.3	6.7±1.4	7.2±1.5
	60.3**	40.7*	34.8*	33.6*
SJ4	13.7±2.0	7.0±1.0	6.9±0.6	8.3±1.5
	58.6**	31.1*	35.7*	38.9*
SJ5	13.3±3.0	10.8±0.4	7.4±1.3	8.2±0.3
	56.8**	48.0*	38.1*	38.4*
SJ6	20.2±3.3	18.0±2.2	11.7±1.2	10.9±1.4
	86.2***	80.4***	60.7**	51.3**
SJ7	15.3±1.7	8.7±1.2	11.5±2.0	7.8±1.6
	65.3**	38,6*	59.7**	36.7*
SJ8	15.3±1.5	15.7±1.6	11.7±1.3	11.5±1.2
	65.2**	70.1***	60.5**	53.9**
Tetracycline	23,4±1.4	22.4±1.5	19.3±1.3	21.3±2.1
	100***	100***	100***	100***

Note: [a]: *** strong: inhibition ≥ 70%, ** moderate: inhibition 50-70%, * weak: inhibition < 50%, TM: no inhibition zone

Cultivation and extraction

Cultivation was carried out to increase endophytic fungi culture. A total of five bottles of endophytic fungi culture containing 300 mL, after an incubation period of 4 weeks, were extracted by partition in ethyl acetate solvent. The result of evaporation produces a concentrated extract of ethyl acetate, which weighs SJ1 6.5 g, SJ2 5.6 g, SJ3 5.8 g, SJ4 6.2 g, SJ5 5.9 g, SJ6 5.5 g, SJ7 5.3 g, and SJ8 5.0 g.

Antibacterial activity test

The activity of ethyl acetate extract of endophytic fungi from the bark of *S. jambos* was tested using the disc diffusion method by measuring the diameter of the growth inhibition zones at a concentration of 400 µg/disc. Endophytic fungi extract was tested on *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Salmonella typhi* (Table 3). The extract of endophytic fungi with strong antibacterial activity was subjected to continuous isolation to obtain the active compound. Endophytic fungi SJ6 had strong activity against *S. aureus* and *S. typhi* bacteria, moderate activity against *E. coli* and *B. subtilis* bacteria. Endophytic fungi SJ8 had strong activity against *S. typhi* bacteria and moderate activity against three other bacteria. For endophytic fungi SJ1-SJ5 and SJ7, it has moderate and weak activity against all four bacteria. Endophytic fungi SJ6 has the strongest antibacterial activity, then proceed with molecular analysis and isolation of secondary metabolite.

Molecular analysis

Molecular characterization combined with morphological identification can help mold taxonomy in differentiation to the species level (Poursafar et al. 2018). The results of PCR products using a pair of universal primers of the ITS rDNA area varied by±600bp, as shown in figure 2. Result of PCR amplification sequence assembly showed 543bp with CTTCCGTAGGTGAACCTGCGGAGGGATCATTACCG AGTTTACAACTCCCAAACCCCTGTGAACATACCAA TTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAA ACGGGACGGCCCGCCAGAGGACCCCTAAACTCTG TTTCTATATGTAACTTCTGAGTAAAACCATAAATA AATCAAAACTTTCAACAACGGATCTCTTGGTTCTG GCATCGATGAAGAACGCAGCAAAATGCGATAAGT AATGTGAATTGCAGAATTCAGTGAATCATCGAATC TTTGAACGCACATTGCGCCCGCCAGTATTCTGGCG GGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAG CCCAGCTTGGTGTTGGGACTCGCGAGTCAAATCGC GTTCCCCAAATTGATTGGCGGTCACGTCGAGCTTC CATAGCGTAGTAGTAAAACCCTCGTTACTGGTAAT CGTCGCGGCCACGCCGTTAAACCCCAACTTCTGAA TGTTGACCTCGGATCAGGTAGGAATACCCGCTGAA CTTAAGCATATCAATAAGCC. GenBank accession numbers is OK426382.

The ITS sequence obtain from isolated will be compared with the sequence available in the GenBank database to analyze the phylogenetic affiliation. For samples of microorganisms, when using 16S rRNA markers, it is said to be identical (similar) at the species level if the "percentage identity" value is above 97.5% (Stackebrandt and Goebel 1994). The neighbor-Joining method is used to unrooted trees is focused only on relationships among taxa rather than evolutionary change. Outgroup rooted method assumes that are divergent for one or more of taxa, branch linking of ingroup and outgroup becomes the starting point to evolutionary of subsequent (Kinene et al. 2016). Sequence reading results from each of the forward and reverse primers of fungi endophyte SJ6 identified as *F. verticillioides*, shown in phylogenetic tree (Figure 3). The outgroup rooted in this phylogenetic tree is *Colletotrichum acutatum.* ITS sequence of endophytic fungal SJ6 isolated from *S. jambos* showed the highest level of similarity with percentage identity 99% is closest to *F. verticillioides*. Bootstrasp value in next brach shown >70, it can be concluded that the endophytic fungus SJ6 was identified as *F. verticillioides.*

Isolation of bioactive compound

The concentrated extract of ethyl acetate (1.0 g) was separated using gravity column chromatography with silica gel as a stationary phase and a mixture of *n*-hexane and ethyl acetate with increasing polarity (10:0–0:10) and a mixture of ethyl acetate with methane (9:1). The results of the separation were accommodated using vials every 10 mL, and as many as 55 vials were obtained. The eluate was then analyzed using thin-layer chromatography (TLC) with a mixed eluent of *n*-hexane and ethyl acetate (5:5). TLC with similar chromatogram patterns was combined into one fraction. Based on the results of the chromatogram pattern,

three fractions, namely F1–F3, were obtained. In the F2 fraction, a yellowish-white solid was formed, and after being purified with *n*-hexane, a pure compound (compound 1) with a weight of 18 mg was obtained. The antibacterial activity test of compound 1 showed good antibacterial activity against *S. aureus* and *S. typhi*, with an MIC value of 64 g/mL for both (Table 4).

Determination of chemical structure

The chemical structure of compound 1 was determined based on 1D and 2D NMR analysis. The ^{1}H-NMR spectrum of compound 1 (Figure 4) shows the presence of six proton signals, including two doublet signals, on the aromatic chemical shift, namely δ_H 7.57 (1H, d, J = 8.5 Hz) and 8.21 ppm (1H, d, J = 8.5 Hz). In the spectrum, each of these signals has an integration of two protons with the ortho-lot constant (J=8.5 Hz). This indicates that compound 1 is a para-substituted aromatic compound, so it has two pairs of equivalent protons. Also, there are four signals in the chemical shift of δ_H 4.00–6.00 ppm, indicating the presence of four groups of sp^3 protons on oxygenated carbon and protons of methine. The four signals appear at δ_H 5.78 (1H, s), 5.33 (1H, d, J = 2.0 Hz), 4.16 (1H, m), and 4.01 ppm (2H, m). Based on the analysis of the ^{1}H-NMR spectrum, compound 1 is a para-substituted aromatic compound with nine protons bonded to a carbon atom. The solvent used in this measurement is $CDCl_3$ so that protons bound to heteroatoms do not appear in the spectrum.

The ^{13}C-NMR spectrum of compound 1 showed the presence of 11 signals. Four carbon signals appear in the aromatic region, namely at δ_C 164.4, 147.6, 126.8, and 123.8 ppm. A characteristic feature is the presence of two pairs of equivalent aromatic protons in the presence of two high-intensity signals at δ_C 126.8 and 123.8 ppm. It was also seen that the presence of aromatic oxyaryl carbon in the low field was C 164.4 ppm. Carbon in the lowest field appears at δ_C 175.6 ppm, which indicates that compound 1 has an ester group. Also, three oxygenated carbon signals appear in the δ_C 60.0-75.0 ppm area and one tertiary carbon signal at δ_C 55.6 ppm. The proton and carbon NMR spectra were confirmed by the data on the HMQC spectrum shown in Figure 5 and Table 5, namely the ^{1}H-^{13}C correlation through one bond. The HMQC spectrum showed six correlations consisting of two correlations on the aromatic ring, three correlations on oxygenated ^{1}H-^{13}C, and one correlation with proton methine.

The HMBC spectrum (Figure 6A) showed a ^{1}H-^{13}C correlation through two or three bonds. The aromatic proton signal at δ_H 8.21 ppm is correlated through three bonds with its equivalent aromatic carbon (δ_C 123.8 ppm) and quaternary aromatic carbon (δ_C 147.6 ppm). The aromatic proton at δ_H 7.57 ppm is correlated through three bonds with its equivalent aromatic carbon (δ_C 126.6 ppm) and oxygenated carbon (73.5 ppm) and correlated through two bonds with quaternary aromatic carbon (δ_C 147.6 ppm). Furthermore, oxygenated methine protons at δ_H 5.33 ppm were correlated through three bonds with equivalent aromatic carbon (δ_C 126.6 ppm) and two bonds correlated with quaternary aromatic carbon (δ_C 147.6 ppm). The correlation indicates that the oxygenated methine group is directly attached to the aromatic ring and is para-substituted with a hydroxyl group. In the spectrum, there is no correlation between the three protons (δ_H 4.01, 4.16, and 5.78 ppm). This may be due to the insufficient number of samples when measuring the spectrum. The proton hydroxyl signal does not appear on the spectrum because the pure compound is measured with the solvent $CDCl_3$. The 1D and 2D NMR spectral data for compound 1 are shown in Table 5.

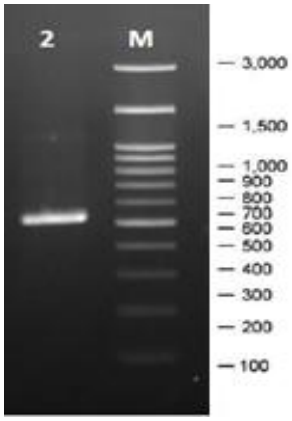

Figure 2. Electrophoresis of isolate SJ6. M (Marker 100 bp DNA ladder; 2 (sample isolate code SJ6 in ±600 bp)

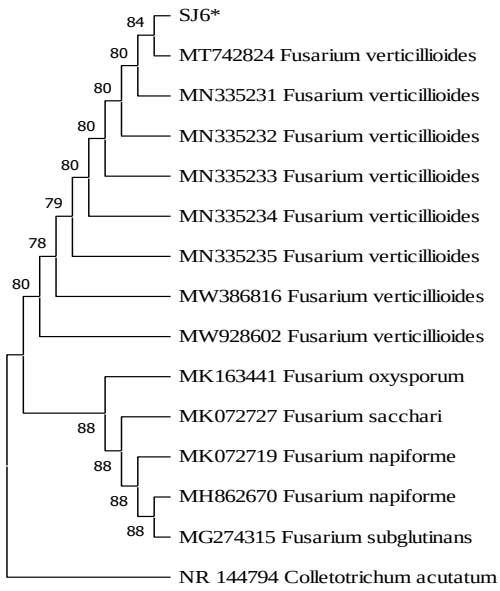

Figure 3. Phylogenetic position of endophytic fungal SJ6. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 3.00000000 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The analysis involved 20 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 148 positions in the final dataset

Table 4. MIC value of pure compound and tetracycline against four test bacteria

Sample	MIC Value (µg/mL)			
	Staphylococcus aureus	***Salmonella typhi***	***Escherichia coli***	***Bacillus subtilis***
Compound 1	64	64	> 256	> 256
Tetracycline	1	1	1	1

Note: Inhibition zone >9mm indicates that the compound has antibacterial activity at the appropriate concentration.

Table 5. The NMR data of Compound 1

No. C	δ_C ppm	δ_H ppm (ΣH. multiplicity. *J* (Hz))	HMBC	COSY
2	175.6			
3	66.2	5.78 (1H, s)		
4	55.6	4.16 (1H, m)		5.33
5	64.0	4.01 (2H, m)		
6	73.5	5.33 (1H, d, J=2.0 Hz)	126.8; 147.6	4.16
1'	147.6			
2'	126.8	7.57 (1H, d, J=8.5 Hz)	147.6; 126.8; 73.5	8.21
3'	123.8	8.21 (1H, d, J=8.5 Hz)	147.6; 123.8	7.57
4'	164.4			
5'	123.8	8.21 (1H, d, J=8.5 Hz)	147.6; 123.8	7.57
6'	126.8	7.57 (1H, d, J=8.5 Hz)	147.6; 126.8; 73.5	8.21

Table 6. Diversity of endophytic fungi from the *Syzygium* genera

Syzygium jambos	*Syzygium jambos* (Roux et al. 2020)	*Syzygium aqueum* (Habisukan et al. 2021)	*Syzygium cordatum* (Marsberg et al. 2014)	*Syzygium cumini* (Hanin and Fitriasari 2019; Nurhaida et al. 2019)	*Syzygium samarangense* (Budiono et al. 2019)
Spegazzinia tessarthra	*Chrysoporthe deuterocubensis*	*Chaetomium*	*Mycosphaerella markii*	*Collectotrichum*	*Lasiodiplodia venezuelensis*
Auxarthronopsis sp.	*Microthia havanensis*	*Cochliobolus*	*M. vietnamensis*	*Phomopsis* sp.	
Pectinotrichum sp.	*Celoporthe hauoliensis*	*Penicillium*		*Penicillium*	
Phaeotrichosphaeria	*Cel. hawaiiensis*	*Cylindrocladium*		*Pestalotiopsis*	
Poaceascoma	*Cel. paradisiaca*			*Fusarium*	
Fusarium verticillioides				*Aspergillus*	
Thielaviopsis sp.				*Myrothecium*	
Nigrospora bambusae				*Neofusicoccum parvum*	

The COSY spectrum in Figure 6B shows that the aromatic proton at δ_H 7.57 ppm correlates with the $^1H–^1H$ triple bonds with the aromatic proton at δ_H 8.21 ppm. Furthermore, there is a $^1H–^1H$ correlation of three hydrogenated protons of methine at δ_H 5.33 ppm with protons of methine at 4.16 ppm. This indicates that the two aromatic equivalent protons are in the ortho position. This reinforces the structural suggestion that compound 1 is a benzene ring directly bonded to the oxygenated methine carbon at the para position with a hydroxyl group.

Based on the spectral analysis of ^{1}H-NMR, ^{13}C-NMR, HMQC, HMBC, and COSY, it can be explained that compound 1 has a para-substituted benzene ring between the hydroxyl group and the oxygenated methine group. This oxygenated methine group binds to the 3-hydroxydihydrofuran-2-one ring. The proton at δ_H 5.78 ppm appears singlet, probably due to the transposition with the proton at δ_H 4.16 ppm (Figure 7), is in the farthest geometric position so that cleavage does not occur. Thus, it is proposed that the chemical structure of compound 1 is 3-hydroxy-4-(hydroxy(4-hydroxyphenyl)methyl)dihydrofuran-2-on, as shown in Figure 7.

Discussion

Eight endophytic fungi isolated from the stem bark of *S. jambos* have been found, namely *Spegazzinia*, *Auxarthronopsis*, *Pectinotrichum*, *Phaeotrichosphaeria*, *Poaceascoma*, *Fusarium*, *Thielaviopsis*, and *Nigrospora*. Seven endophytic fungi have not been found in other *Syzygium* genera, except *Fusarium* which is also found in *S. cumini* (Hanin and Fitriasari 2019). Based on the findings of endophytic fungi in this research and literature study from plants of the same genus, there are similarities in the diversity of endophytic fungi. The diversity of endophytic fungi from *S. jambos* and other species of the genus *Syzygium* is shown in Table 6.

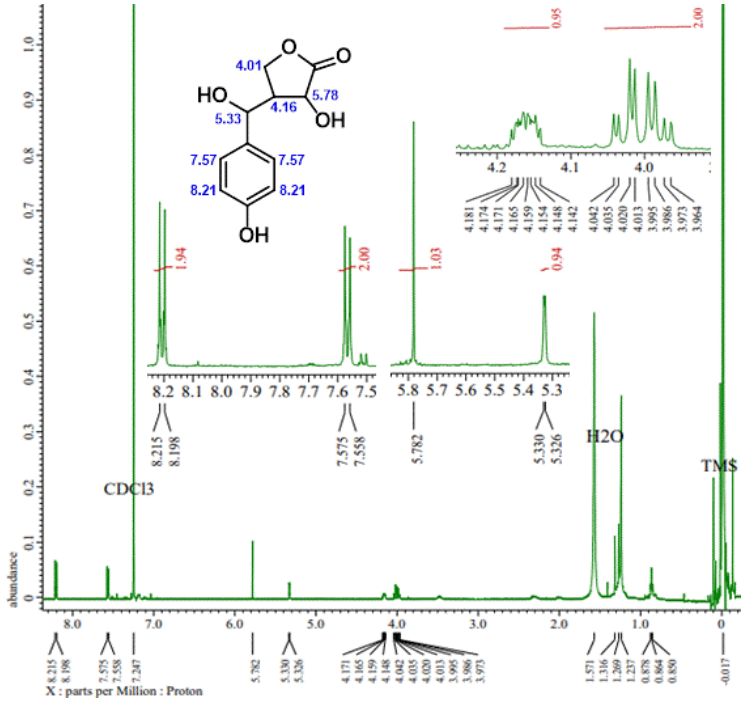

Figure 4. The [1]H-NMR spectrum of compound 1

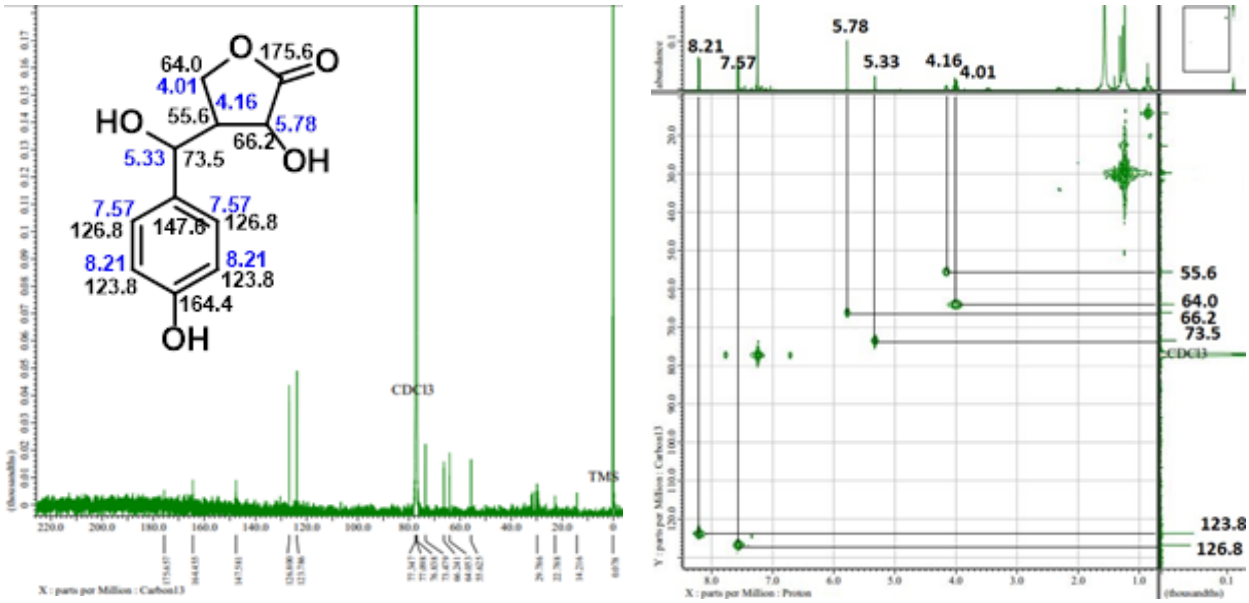

Figure 5. A Spectrum of [13]C-NMR (A) dan HMQC (B) of compound 1

The potential of *S. jambos* bark extract has been known to have antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis* (Murugan et al. 2011; Begum et al. 2015; Wamba et al. 2018), and *Propionibacterium acnes* (Sharma et al. 2013b). It has been reported that chemical compounds from the bark extract of *S. jambos* contain polyphenols, anthraquinones, tannins, triterpenes, steroids, saponins (Wamba et al. 2018), pentacyclic triterpene, sitosterol, triterpene derivative 3-nor-2,3-fridelan (Haque et al. 2015), jambosine alkaloids, pedunculagin ellagitannins, casuarinin, tellimagrandin I, strictinin, casuarictin, 2,3-HHDP glucose, and tellimagrandin II (Baliga et al. 2017). Some of these compounds are known to be antibacterial (Murugan et al. 2011). These antibacterial compounds are also produced by endophytic fungi, such as alkaloids, peptides, steroids, terpenoids, flavonoids, and quinines which can inhibit Gram-positive and Gram-negative bacteria (Manganyi and Ateba 2020).

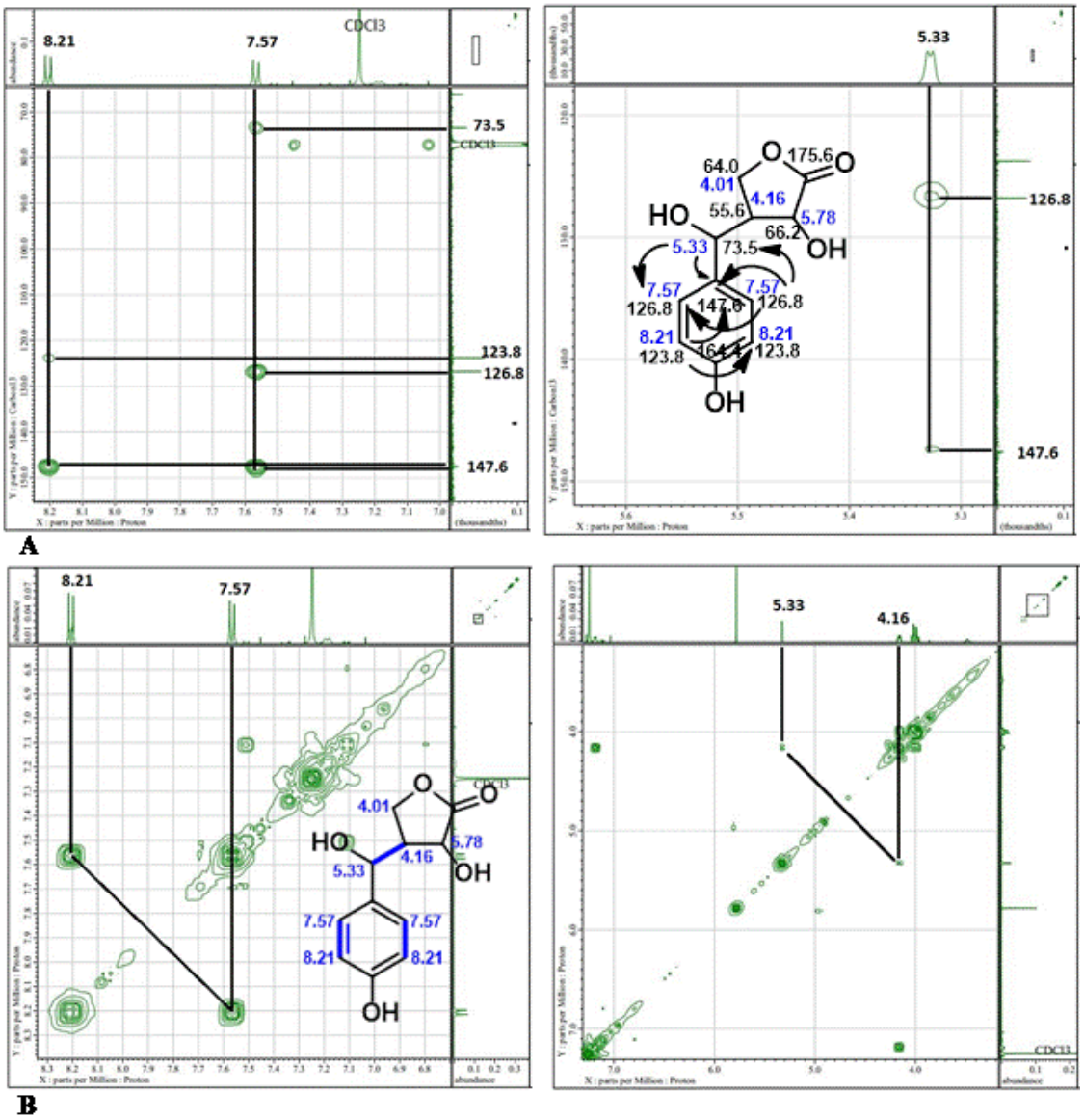

Figure 6. Spectrum of Compound 1 (A. Analysis HMBC, B. Analysis COSY)

The results of the antibacterial test showed that the ethyl acetate fungal extract had antibacterial activity against the four sample bacteria. Endophytic fungi SJ6 (*Fusarium* sp.) had the strongest activity against *S. aureus* and *S. typhi*, while against *E. coli* and *B. subtilis* had moderate activity. It is known that *Fusarium* sp has the strongest antibacterial activity. Previous studies have reported that a secondary metabolite of *Fusarium* sp species, namely fusariumin D, has the potent activity against *S. aureus* (Wei and Wu 2020). The chemical compound of pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) showed significant antibacterial activity against *B. subtilis*, *S. aureus*, *E. coli* (Putra and Karim 2020). Aliphatic carbonyl substituted compounds has antibacterial activity against *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. aureus*, *S. mutans*, and *S. typhi* (Yuniati and Rollando 2018). Antibiotic Y was isolated from *F. avenaceum* show very strong antibacterial activity against *B. subtilis* (Stępień et al. 2020).

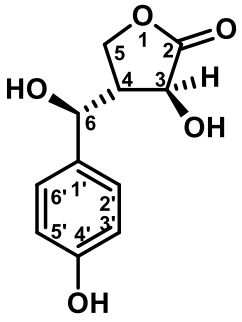

Figure 7. The chemical structure of compound 1 is 3-hydroxy-4-(hydroxy(4-hydroxyphenyl)methyl) dihydrofuran-2-on

The biochemical diversity of secondary metabolites produced by *Fusarium* species, namely trichothecenes, fumonisins, zearalenone, enniatins and beauvericins, fusaric acid, fusarins, moniliformin (Stępień et al. 2020). The chemical compounds from the endophytic fungus *Fusarium* sp. have also been found whose structure was identified as fusaric acid (5-butylpicolinic acid, FA), alkaloid fungerin contains an imidazole, a-(N-formyl) carboxamide moiety, racemic imidazolidin-2,4-dione moiety, polyketide isoquinoline alkaloid fusarimine, fusaravenin, bisindole enantiomers, (±)-fusaspoid A, 7-desmethylscorpinone and 7-desmethyl-6- methylbostrycoidin, apicidin derivatives: apicidin, apicidin A, apicidins B, C, D1, D2 and D3, apicidin E, apicidins F, J and K, apicidin G–I (Li et al. 2020). Wei and Wu (2020) isolated metabolite secondary, including γ-Pyrone derivative, α-pyrones, fupyrones A and B, anthraquinones and naphthoquinones, isocoumarins (karimunone B), macrolides (β-resorcylic macrolides, 7-hydroxy-14-de-Omethyl-lasiodiplodin), ethyl-3,5-dihydroxy-7-(6,8-dihydroxynonyl) benzoate, and 7-ethylbenzoate-7-heptanoic acid, γ-methylidene-spirobutanolide core, fusaspirols A-D, fusarielins. There are several groups of compounds produced by endophytic fungi of the *Fusarium* genus that have antibacterial abilities, including from the sesquiterpene group, such as 3β-hydroxy-β-acorenol and fusariumins D, the terpene group (fusariumins C), naphthalenone derivatiev, pyrone derivative, namely pyrano[2,3-g]indole moieties and amoenamide C. There are also group of compounds derived from naphthoquinone, 6-hydroxy-astropaquinone B dan astropaquinone D. Other compounds, such as sesquiterpenoids derivative, emericellins A-B, phenolic bisabolane sesquiterpenoids, tremulane sesquiterpene, furan derivatives, poliketides also have antibacterial ability (Zheng et al. 2021).

Some endophytic fungi also produce the same bioactive compounds as their host, such as paclitaxel (taxol), baccatin III, podophyllotoxin, hypericin, camptothecin, vinblastine, huperzine a, diosgenin, a-irone, toosendanin produced by fungi isolated from *Taxus* sp (Zhao et al. 2010). Based on the Dictionary of Natural Products database on 9 Oktober 2021, 3-hydroxy-4-(hydroxy(4-hydroxyphenyl)methyl)dihydrofuran-2-on were isolated from the *Fusarium verticillioides* are new phenolic compounds. Phenolic compounds are the best source of antibiotic. Several phenolic compounds were antibacterial activity against *S. epidermidis* and *P. aeruginosa*, such as tannic acid, epigallocatechin gallate, etc. (Mandal et al. 2017). Phenolic compounds are known antioxidants and other bioactive agents that have benefits for human health and many diseases (Tungmunnithum et al. 2018). However, based on the literature, there are no compounds similar to those found in this study. The compound produced by the endophytic fungus *F. verticillioides* is different from the compounds found in its host *S. jambos*, although it is still in the phenolic compound group.

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