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WORD COUNT	7145	TIME SUBMITTED	14-DEC-2022 11:44AM
		PAPER ID	93973198

Trop J Nat Prod Res, November 2022; 6(11):1765-1772

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Bioactivity Endophytic Fungi Isolated from the Leaf Stalk of *Syzygium jambos* L. Alston

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ARTICLE INFO

Article history:
Received 22 May 2022
Revised 26 September 2022
Accepted 02 November 2022
Published online 01 December 2022

ABSTRACT

Antibiotic resistance and free radicals are a problem to be overcome by using antibiotics and antioxidants from natural ingredients. This study aimed to investigate the antibacterial and antioxidant activity of endophytic fungi (EF) isolated from the leaf stalks of jambu mawar (*Syzygium jambos* (L.) Alston). EF isolated from the leaf stalk of *S. jambos* was identified microscopically (shape of hyphae and spores) and macroscopically (growth patterns, colony color, texture, margin, and other characteristics). Antibacterial activity was tested on *Salmonella typhi, Escherichia coli, Staphylococcus aureus*, and *Bacillus subtilis* bacteria using the Kirby Bauer method. Antioxidant test using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The molecular identified of EF with high antioxidant and antibacterial activity to determine the species and continued with the isolation of pure compounds. Identification of pure compounds was carried out by spectroscopic methods including 1D NMR (^{1}H-NMR and C-NMR). The results of the isolation of EF from the leaf stalk of *S. jambos* found four isolates, namely SJS1-SJS4. The results of the antibacterial test represented that the EF isolate SJS1 had strong activity against *S. aureus* and *B. subtilis* bacteria. The antioxidant activity test showed IC50 value of 29.29 µg/mL. Molecular identification results showed that SJS1 was identified as *Lasiodiplodia theobromae*. Spectroscopic results of the pure compound identified as 3,5-dihydroxy-4-(4-hydroxyphenyl)tetrahydro-2H-pyran-2-one. The endophytic fungus *Lasiodiplodia theobromae* was isolated from the leaf stalk of *S. jambos* (L.) Alston has the potential as a source of antioxidants and antibacterial bioactive compounds that can be developed through further research, including in vitro and in vivo tests.

Keywords: Antibacterial, Antioxidants, *Lasiodiplodia theobromae*.

Introduction

The use of synthetic antibiotics with inappropriate doses causes pathogenic bacteria to become resistant and cause side effects. Therefore, research is needed to find alternative natural antibiotic ingredients. Endophytic fungi (EF) are reported to have metabolites that can inhibit bacterial growth. Some of EF such as *Fusarium*,[1] *Cladosporium*, *Chaetomium*, and *Ceratobasidim*[2] have antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. EF *Thanatephorus cucumeris* exihibits antibacterial activity.[3] *Fusarium verticillioides* isolated from *Syzygium jambos* contains 3-hydroxy-4-(hydroxy(4-hydroxyphenyl)methyl)dihydrofuran-2-on compound which has antibacterial activity against *S. aureus* and *S. typhi*.[4] Oxidation is a chemical reaction that results in the loss of electrons from atoms and produces free radicals.[5] Free radicals are groups of atoms, molecules, or ions whose electrons are unpaired, unstable, and active in chemical reactions with other molecules.

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Citation: Aini K, Elfita E, Widjajanti H, Setiawan A. Bioactivity Endophytic Fungi Isolated from the Leaf Stalk of *Syzygium jambos* L. Alston. Trop J Nat Prod Res. 2022; 6(11):1765-1772.
http://www.doi.org/10.26538/tjnpr/v6i11.4

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

These unpaired electrons will seek and capture electrons from other substances to stabilize themselves. These free radical molecules are referred to as reactive oxygen species (ROS).[6] Antioxidants play a role in reducing, preventing, and treating diseases caused by ROS. ROS are produced by metabolic pathways in cells, which play a role in the response to abiotic and biotic stresses.[7,8] Antioxidant compounds have been widely used in the food, pharmaceutical, agricultural, and beauty product industries. The necessity for natural antioxidants is currently the main focus of several studies. EF produce bioactive compounds that have biological activity as antioxidant,[5,9,10] such as *Fusarium oxysporum* from *S. jambos*.[11] To overcome synthetic antibiotic resistance and prevent free radicals, natural sources of antibiotics and antioxidants are required. Many studies have been carried out to find sources of medicinal crude materials and antioxidants derived from medicinal plants, one of which is jambu mawar (*Syzygium jambos*). *S. jambos* is known to contain active ingredients that have activity against pathogenic bacteria and as antioxidants.[12] The methanol extract of the *S. jambos* leaves and stem bark showed activity against *Staphylococcus aureus*.[13,14] The leaves' ethanol extracts [15–18] and fruit of the *S. jambos*[19] have antioxidant activity. However, the existence of this jambu mawar is difficult to find and requires long cultivation periods to be used as raw material. Therefore, the alternative is to isolate the EF associated with *S. jambos* plants. Antioxidant activity is all about the elimination of free radicals and nascent oxygen [O]. On the other hand, many bacteria can also depend on this nascent oxygen to survive. The removal of free radicals or oxidants will affect antimicrobial activity. This could partially explained that antioxidant

properties correlate positively with antimicrobial activity. This correlation is the basis for finding new natural source that plays a role as antibacterial and antioxidant, such as the use of endophytic fungi.[20–22] EF associates and live in plant tissues without causing symptoms, disturbances, or diseases in their host plants, and is a promising source of bioactive agents.[23–25] EF associated with medicinal plants have the potential to produce bioactive compounds. Recently, many researchers have conducted research on the potential of EF as a source of bioactive compounds. EF are important sources of bioactive compounds with unique chemical and biological activities.[26] EF produce metabolic compounds that are beneficial to humans as bioactive ingredients against pathogenic bacteria, viruses, cancer, treat diabetes, and antioxidants.[27,28] The types of endophytic fungi obtained cannot be predicted due to environmental conditions that affect the diversity of endophytic fungi on the host plant. Therefore, this study is to determine the antibacterial activity, antioxidant, and compounds contained in endophytic fungi isolated from the *S. jambos* leaf stalk.

Materials and Methods

Sampling

A sampling of *Syzygium jambos* (L.) Alston leaf stalks was taken in August 2021 at the versatile ground of Perumahan Kencana Damai, Sukamaju, Sako, Palembang. The sample was obtained from the same tree, where the leaf stalks were from five leaves. The sample was identified morphologically in the Biosystematics Laboratory, Department of Biology, Faculty of Mathematics and Natural Science, Universitas Sriwijaya, Palembang with letter number 233/UN9.1.7/4/EP/2021.

Isolation and identification of endophytic fungi

Syzygium jambos (L.) Alston leaf stalks were washed with clean water and drained until the water dried. Surface sterilization began by immersing each sample in 3% sodium hypochlorite (NaOCl) for ± 1 min. They were soaked in 70% alcohol for ± 1 min, rinsed with sterile distilled water for ± 1 min, [29,30] placed in a petri dish containing potato dextrose agar (PDA, Merck®), which was added with chloramphenicol (0.2 g/L), and incubated at room temperature for 48 hr.[11]

Pure isolates of EF were identified macroscopically and microscopically. Morphological characteristics were identified based on growth patterns, colony color, texture, margin, and other characteristics. Microscopic characteristics include the shape of hyphae and spores observed under a microscope (Hirox MXB-2500REZ), with the slide culture method. The results of microscopic and macroscopic observations will be compared with identification books for fungi [29,30] and relevant journal articles.

Cultivaton and extracton of endophytic fungi

Pure isolates of EF *S. jambos* leaf stalk were cultivated as much as 5 x 300 mL in potato dextrose broth by placing 6 blocks of pure culture in bottles. Then the cultures were incubated for four weeks (±28 days) at room temperature under static conditions. After the incubation period, the endophytic fungal mycelia were separated from the liquid culture and partitioned in ethyl acetate at a ratio of 1:1 three times. The ethyl acetate extract was separated from the liquid culture, then evaporated using a rotary evaporator until a thick extract was obtained.[31]

Antibacterial activity test of endophytic fungi

Antibacterial activity was tested using the paper disc method with 400 µg/mL by using Dimethylsulfoxide (DMSO) as solvent and positive control using tetracycline antibiotic 30 µg/mL. The tested bacteria were represented by Gram-negative and Gram-positive bacteria, namely *Salmonella typhi* (ATCC 1408), *Escherichia coli* (InaCCB5), *Staphylococcus aureus* (InaCCB4), and *Bacillus subtilis* (InaCCB4) grown on Nutrient Agar media (NA). Antibacterial activity was indicated by the clear zone around the paper disc after being incubated for 24 hr at room temperature. The clear zone formed indicates the sensitivity of bacteria to antibacterial ingredients from EF extract. The criteria for antibacterial activity consist of strong, moderate, and weak which are determined by the following equation.[32] With A = zone of inhibition of the test sample (mm); B = zone of inhibition standard antibiotic (mm).

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

Antioxidants activity assay of endophytic fungi

An antioxidant activity test was performed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. A total of 1.97 mg of DPPH was dissolved in 100 mL of 90% methanol solution so that a solution of 100 mL of 0.05 mM DPPH was obtained. The ethyl acetate extract of EF and ascorbic acid was dissolved to obtain concentrations of 50, 25, 12.5, 6.25, and 3.125 ppm. A total of 0.2 mL of EF extract of *S. jambos* leaf stalk was added with 3.8 mL of 0.05mM DPPH solution. The sample was placed in a dark place (without light) for 30 min. After that, the solution was analyzed using a UV-Vis spectrophotometer (Shimadzu UV-1900) λ_{maks} = 517 nm so that the absorbance value was obtained.[33] The absorbance results were obtained from the sample EF of *S. jambos* leaf stalk (a) and negative control (b), then the % inhibition was calculated using the formula:

$$\%\, inhibition = \frac{control\ absorbance - sample\ absorbance}{control\ absorbance} x\ 100\ \%$$

The linear regression equation, $y = b + ax$, is obtained where the x-axis is the sample concentration and the y-axis is the % inhibition. The IC50 value is the concentration value that can inhibit 50% of a test. Mathematically it can be seen as follows:

$$IC_{50} = \frac{50-b}{a}$$

Molecular identification of endophytic fungi

EF isolates that have strong antibacterial and antioxidant activity were identified molecularly to determine the species of the endophytic fungi. The molecular test of EF was carried out at the laboratorium of Genetika Science Indonesia, Banten, Indonesia using method of Genomic DNA extraction with Quick-DNA Fungal Miniprep Kit (Zymo Research, D6005), polymerase chain reaction (PCR) amplification (Bioline, BIO-25048) with MyTaq HS Red Mix twice. Standard PCR primers using ITS1 dan ITS4.[34] Analysis of DNA structure using Molecular Evolution Genetics Analysis Version 11.[35]

Isolation of chemical compound

Ethyl acetate extract of endophytic fungi exhibited strong antibacterial and antioxidant activity, then isolated the bioactive compounds. The bioactive compounds was analyzed using a thin-layer chromatography (TLC) silica gel G-60 F254 using *n*-hexane: EtOAc (5:5). The concentrated extract was separated by column chromatography over silica gel 60 (70–230 mesh) at the stationary phase (1:30) and eluent. The chosen eluent with increased polarity was *n*-hexane: EtOAc at a ratio of 10:0 to 0:10 (v/v). An eluate was collected and then tested using thin-layer chromatography with mixed eluent *n*-hexane: EtOAc (5:5). Similar chromatogram patterns were combined, evaporated, and rinsed with n-hexane to obtain the purified compound. The chemical structure of the compounds was determined using the following spectroscopy methods: 1DNMR (^{1}H-NMR and ^{13}C-NMR).[36]

Results and Discussion

Isolation and identification of endophytic fungi

The results of the isolation of EF from *S. jambos* leaf stalk found four isolates (SJS1 – SJS4). EF isolates were identified macroscopically by observing the front and bottom surfaces of the colonies, and colony color (Figure 1 and Table 1). Microscopic observations were carried out to determine the hyphae, shape, and type of spores (Figure 2 and Table 2). Four different genera were found: *Lasiodiplodia*, *Bipolaris*, *Aureobasidium*, and *Cladophialophora*.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

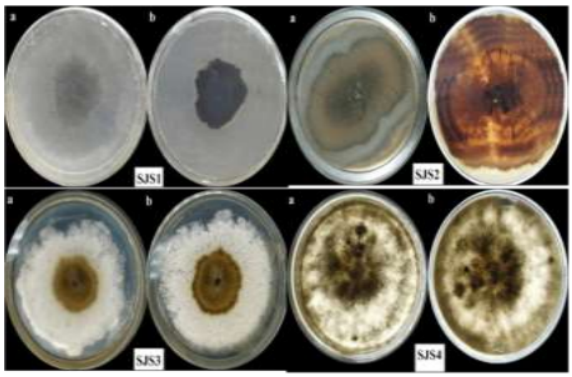

Figure 1: EF 7 days on PDA of *S. jambos* leaf stalks; a) Isolate colony surface; b) Isolate colony reverse.

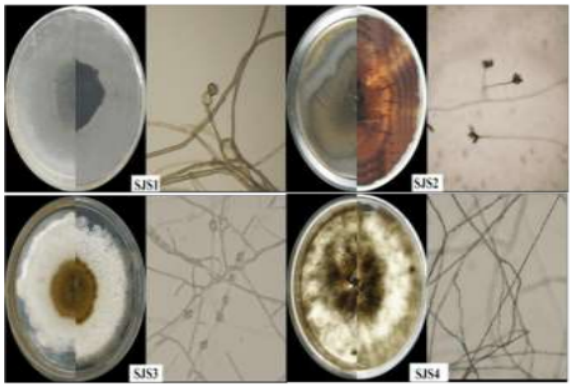

Figure 2: Microscopic characteristics (magnification: 1000x) of the EF 7 days on PDA of *S. jambos* leaf stalks

Antibacterial activity

The antibacterial activity of EF from *S. jambos* leaf stalk was evaluated using the paper disc diffusion method by measuring the zone of inhibition. The results of the antibacterial test are shown in Table 3. The results showed that EF SJS1 had strong activity against *S. aureus* and *S. typhi* bacteria, and moderate activity against *E. coli* and *B. subtilis*. The EF SJS4 had moderate activity against the four tested bacteria. SJS2 and SJS3 isolates had weak antibacterial activity against *E. coli* bacteria and moderate activity against *S. typhi*, *S. aureus* and *B. subtilis*.

Antioxidants activity

Antioxidant activity was tested using the DPPH method with parameter IC50. The antioxidant test results of the five isolates of EF showed that SJS1 isolates had a strong activity with an IC50 value of 29.29 g/mL, while the other three isolates were very weak (Table 4 and Fig. 3). Many studies have been on the diversity of EF and their biological activities, especially in the genus *Syzygium*. Antibacterial activity was shown by EF isolated from the stem bark of *Syzygium jambos,*[4] from leaves, bark, and root bark of *S. malaccense*[37] from root bark of *S. zeylanicum,*[38] from leaves and leaf stalks of *S. aqueum*.[33] Antioxidant activity was also demonstrated by EF isolated from *S. cummini* (*Eugenia jambolana*),[39] and from *S. aqueum*.[40] The antioxidant compound found in EF isolated from *S. aqueum* was 3-(hidroksil (3,4,5-trihidroksifenil)metil)-3,4-dihidro-2H-piran-4,5,6-triol, the antioxidant power was close to the activity of gallic acid with IC50 value of 11.4 µg/mL.[40] Alkaloids, phenols, flavonoids, saponins, and terpenes isolated from the EF *Eugenia jambolana* have antioxidant potential. These compounds exhibit antioxidant activity by breaking free radical chains through the donation of hydrogen atoms.[39]

The isolate of SJS1 (*Lasiodiplodia*) exhibited strong antibacterial and antioxidant activity. It is known that *L. theobromae* has antioxidant [41,42] and antibacterial activity.[43,44] EF *L. theobromae* contain secondary metabolites in the form of Chloropreussomerin A – B and preussomerin K, H, G, F that have antibacterial activity against bacteria *S. aureus* and *B. subtilis;*[45] also contain compounds cyclohexenes and cyclohexenones, indoles, jasmonates, lactones, melleins, phenols, lasiodiplodins,[46] L-asparaginase compounds as anti-leukemia.[47]

Table 1: Macroscopic characteristics of EF isolated from *S. jambos* leaf stalks

Isolate	Colony color	Reverse colony color	Texture	Topography	Pattern	Exudate drops	Radial line	Concentric circle
SJS1	Black and White	Black and White	Cottony	Umbonate	Zonate	-	-	√
SJS2	Dark brown and White	Dark brown	Velvety	Rugose	Zonate	-	√	-
SJS3	Light green and White	Light green and White	Cottony	Raised	Radiated	-	-	-
SJS4	White to gray	White to gray	Cottony	Raised	Radiated	-	-	√

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

Table 2: Microscopic characteristics EF isolated from *S. jambos* leaf stalks

Isolate	Type of spore	Shape of spore	Hyphae	Specific characteristic	Genus / species
SJS1	Conidia	Globose	Septate	showing characteristic 2-celled, pigmented, longitudinally striate pycnidioconidia	*Lasiodiplodia* sp.
SJS2	Conidia	Globose	Septate	Conidia porosporous, solitary, pale brown, ellipsoidal, usually 4-celled.	*Bipolaris* sp.
SJS3	Conidia	Globose	Hyaline	Conidiophores lacking. hyaline, cylindrical, 1-celled.	*Aureobasidium* sp.
SJS4	Conidia	Subglobose	Aseptate	Conidial chains	*Cladophialophora*

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: Antibacterial Activity EF of *S. jambos* leaf stalk

Sample (400 µg/disc)	*S. typhi*	*E. coli*	*S. aureus*	*B. subtilis*
Tetracycline	20.9 ± 0.29	21.9 ± 0.21	20.6 ± 0.34	20.9 ± 0.29
	100***	100***	100***	100***
SJS1	14.2 ± 0.29	14.8 ± 0.21	14.5 ± 0.08	14.8 ± 0.34
	67.9**	67.6**	70.4***	70.8***
SJS2	11.1 ± 0.25	12.1 ± 0.16	10.5 ± 0.46	10.2 ± 0.25
	53,1**	55,3**	51,0**	48,8*
SJS 3	11.9 ± 0.29	11.1 ± 0.17	12.8 ± 0.21	10.5 ± 0.16
	56.9**	50.7**	62.1**	50.2**
SJS4	11.2 ± 0.26	11.0 ± 0.08	10.9 ± 0.21	7.8 ± 0.25
	53.6**	50.2**	52.9**	37.3*

Note: *) Weak; **) Moderate; ***) Strong

Table 4: Antioxidants Activity EF of *S. jambos* leaf Stalk

Test Sample	IC50 (µg/mL)
SJS1	29.29 ± 0.45
SJS2	166.42 ± 0.98
SJS3	199.21 ± 0.71
SJS4	149.17 ± 0.91
Ascorbic Acid	8.09 ± 0.09

Lasiodiplactone A has potential as an antidiabetic and anti-inflammatory agent.[48] Jasmonic acid produced by *L. theobromae* is the main ingredient in cosmetic products.[49] In this study, the compound produced by *L. theobromae* from *S. jambos* was different from the compound *L. theobromae* that had been published. It was found that compounds belonging to the phenolic group have antibacterial and antioxidant activity. Different host plants cause different secondary metabolites in fungi of the same species. This is caused by the presence of fungi on the host plant that can copy the compounds of host plant.

Bipolaris (code SJS2) found in *S. jambos* leaf stalk also had moderate antibacterial activity. Bipolaris is a fungal pathogen that causes a leaf spot on palms.[50] Bipolarins A–H compounds, derivatives of tetracyclic ophiobolin-type sesterterpenes produced by Bipolaris have antibacterial activity.[51] Hybrid polyketide–terpenoid, 1-alkylated-3,5-dihydroxyphenyl derivative coupled to a farnesyl pyrophosphate unit (FPP), isolated from the fungus *Bipolaris zeicola* has immunosuppressive and cytotoxic activity,[52] sesquiterpenoids and xanthones compounds have anti-pathogenic activity of microorganisms.[53] *Bipolaris eleusines* produces chromone (*S*)-5-hydroxyl-2-(1-hydroxyethyl)-7-methylchromone compounds which have inhibitory power against *S. aureus,*[54] Ophiobolin A compounds have anticancer activity by inducing cell death.[55] *Aureobasidium* (code SJS3) has moderate activity against all four bacteria. Based on a literature study, *Aureobasidium* produces liamocins compounds that have antibacterial against *Streptococcus* spp.,[58] and yield volatile organic compounds that have antifungal against *Botrytis cinerea*.[56] *Aureobasidium pullulans* produce enzymes that are useful in industry, such as β-glucosidase, amylases, cellulases, lipases, proteases, xylanases and mannanases which have cytotoxic, antioxidant, and antibacterial properties.[57] It also produces pullulan (poly-α-1,6-maltotriose biopolymer) which is applied in the food, cosmetic and pharmaceutical industries,[58] poly(β-L-malic acid) (PMA) heavy oils and β-1,3-glucan,[59] hydroxydecanoic acid derivatives have antibacterial activity against *E. coli*, *S. aureus*, and *B.subtilis*.[60,61]

Molecular identification

Molecular identification was carried out to determine the EF species that have vigorous antibacterial and antioxidant activity. DNA sequences resulting from PCR amplification of SJS1 isolates were compared with DNA sequences available in the GenBank database, to analyze the phylogenetic relationship. The results showed the DNA sequence of SJS1 isolates was, GGAGCTCGAA AACTCGGTAA TGATCCTTCC GTAGGTGAAC CTGCGGAAGG ATCATTACCG AGTTTTCGAG CTCCGGCTCG ACTCTCCCAC CCTTTGTGAA CGTACCTCTG TTGCTTTGGC GGCTCCGGCC GCCAAAGGAC CTTCAAACTC CAGTCAGTAA ACGCAGACGT CTGATAAACA AGTTAATAAA CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CCCTTGGTAT TCCGGGGGGC ATGCCTGTTC GAGCGTCATT ACAACCCTCA AGCTCTGCTT GGAATTGGGC ACCGTCCTCA CTGCGGACGC GCCTCAAAGA CCTCGGCGGT GGCTGTTCAG CCCTCAAGCG TAGTAGAATA CACCTCGCTT TGGAGCGGTT GGCGTCGCCC GCCGGACGAA CCTTCTGAAC TTTTCTCAAG GTTGACCTCG GATCAGGTAG GGATACCCGC TGAACTTAAG CATATCAATA AGCGGA, with 566bp, GenBank code OM095454. Molecular characterization combined with morphological identification can identify fungi until to the species level. Molecular characterization was carried out on the area of rDNA Internal Transcribed Spacer (ITS). Currently, there are more than 90,000 fungi sequences in the ITS region, where this area is most widely used as a barcode area for fungi. PCR analysis used a pair of universal primers, namely ITS1 for forwarding primers and ITS4 for reverse primers. The amplification results of the ITS rDNA region varied by ±500 bp (Fig. 4). Sequence readings from each of the forward and reverse primers were processed by cutting the ends of the sequences with low peaks using the Bioedit program. Furthermore, the sequences are straightened so that the compilation sequences of the forward and reverse primers from the ITS rDNA area are obtained. Based on BLAST, the ITS isolate sequence SJS1 showed 98–100% similarity to the sequences of the *Lasiodiplodia theobromae*.

The construction of the EF SJS1 phylogeny tree is shown in Figure 3. The SJS1 phylogenetic tree showed that the isolate sequence was close to *Lasiodiplodia theobromae* with a bootstrap value of 1000. The results of the phylogenetic analysis of the SJS1 isolate are in line with the phenotypic characters that have been discussed previously. In this study, EF with strong antibacterial and antioxidant activity were carried out by molecular tests and identified as *Lasiodiplodia theobromae* (code SJS1). *L. theobromae* is a member of the *Botryosphaeriaceae* as an EF and latent pathogen that survives in plant vascular tissues for a certain period of time without showing symptoms under suitable environmental conditions.[62–67] With changes in environmental conditions inside or outside the host, some fungi can change their lifestyle from endophytes to pathogens.[62,68] Pathogenic symptoms caused by *L. theobromae* such as cancer, dieback, and rotting of fruit and roots in nearly 500 plant species globally.[69,70]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

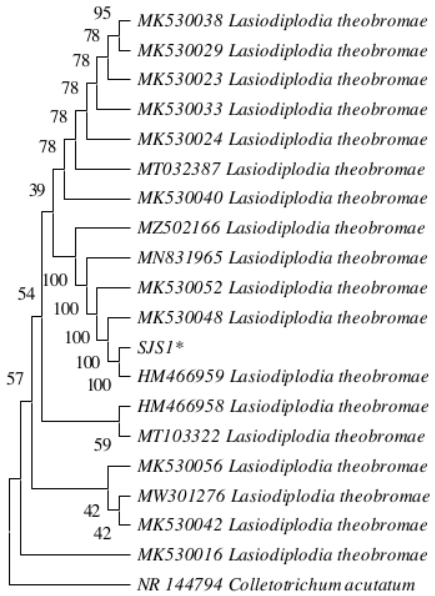

Figure 3: Phylogenetic analysis of endophytic fungi SJS1 (isolated from leaf stalk of *S. jambos*). This phylogenetic tree was constructed by using neighbor-joining method (1000 bootstrap replication) in MEGA11. (*: isolate target).

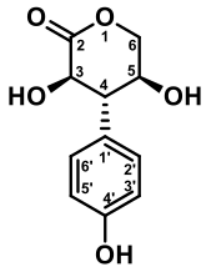

Figure 4: Compound 1 as 3,5-dihydroxy-4-(4 hydroxyphenyl) tetrahydro-2H-pyran-2-one.

Pure compound isolation

The ^{1}H-NMR spectrum of compound 1 (Figure 1 in Suplementary data) shows the presence of six proton signals of which four are signals for proton sp^3 which is a methylene signal at δ_H 3.61-3.81 ppm (2H, m), a proton methane signal at δ_H 4.14 ppm (1H, m), and two oxygenated methane signals at δ_H 6.23 (1H, s) and 5.16 (1H, d, J=3 Hz). Two other signals that appear on the spectrum are in the aromatic chemical shift region, namely at δ_H 7.64 and 8.17 ppm. In the spectrum of each signal appears doublet with the same plot constant, namely J = 8.0 Hz which has the integration of two protons. This indicates that compound 1 is a para-substituted aromatic compound, so it has two pairs of equivalent aromatic protons. Based on the analysis of the ^{1}H-NMR spectrum, compound 1 is a para-subtituted aromatic compound with nine protons bonded to eight carbon atoms. 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 (Figure 2 in Suplementary data) showed the presence of nine signals. There are four sp^3 carbon signals, all of which are in the oxygenated carbon region, namely δ_C 58.6, 62.3, 67.5, and 71.4 ppm. Five other signals appear at δ_C > 100 ppm. Two high-intensity signals indicate that compound 1 has two pairs of equivalent aromatic carbons (δ_C 124.2 and 128.4 ppm). Three carbon signals in the low field indicate the presence of oxyaryl carbon (δ_C 148.7 ppm), aromatic quaternary carbon (δ_C 151.7 ppm), and ester carbonyl carbon (δ_C 166.7 ppm). The analysis of the proton and carbon NMR spectra is reinforced by the data on the HMQC spectrum shown in Figure 2 (Suplementary data) 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 methylene proton correlation. The HMBC spectrum (Figure 3 in Suplementary data) showed a ^{1}H-^{13}C correlation through two or three bonds. The aromatic proton signal at δ_H 8.17 ppm ppm indicates that there are three correlations, namely to carbon δ_C 124.2 ppm which is the carbon equivalent, δ_C 151.7 ppm which is aromatic quaternary carbon, and δ_C 148.7 ppm which is oxyaryl carbon. Another aromatic proton, namely at δ_H 7.64 ppm also has three correlations, namely to carbon at δ_C 128.4, 148.7, and 71.4 ppm which are carbon equivalents, oxyaryl carbon, and oxygenated carbon in their substituents, respectively. Furthermore, oxygenated methine protons at δ_H 5.16 ppm have four correlations, namely to carbon at δ_C 58.6, 62.3, 128.4, and 151.7 ppm, respectively. The correlation indicates that the oxygenated methine group is bound via three bonds with a para-substituted aromatic ring with a hydroxyl group. The proton methine at δ_H 4.14 ppm is triple-bonded with methylene carbon (δ_C 62.3 ppm) and carbonyl ester carbon (δ_C 166.7 ppm). The methylene proton at δ_H 3.61-3.81 ppm has two correlations, namely with oxygenated carbon at δ_C 71.4 ppm and methine carbon at δ_C 58.6 ppm. The proton hydroxyl signal does not appear on the spectrum because the pure compound is measured with the solvent $CDCl_3$. Spectrum data for 1D NMR and 2D compounds are listed in Table 5.

Table 5: The NMR data of compound 1, recorded at ^{1}H-500 MHz; ^{13}C-125 MHz in $CDCl_3$

No. C	δ_C ppm	Type of C	δ_H ppm (ΣH. multiplicity. *J* (Hz))	HMBC
2	166.7	C		
3	67.5	CH	6.23 (1H, s)	166.7
4	58.6	CH	4.14 (1H, m)	62.3; 166.7
5	71.4	CH	5.16 (1H, d, J=3 Hz)	58.6; 62.3; 128.4; 151.7
6	62.3	CH_2	3.61-3.81 (2H, m)	58.6; 71.4
1’	148.7	C		
2’	128.4	CH	7.64 (1H, d, J=8 Hz)	71.4; 128.4; 148.7
3’	124.2	CH	8.17 (1H, d, J=8 Hz)	124.2; 148.7; 151.7
4’	151.7	C		
5’	124.2	CH	8.17 (1H, d, J=8 Hz)	124.2; 148.7; 151.7
6’	128.4	CH	7.64 (1H, d, J=8 Hz)	71.4; 128.4; 148.7

Based on the spectrum analysis of ^{1}H-NMR, ^{13}C-NMR, HMQC, dan HMBC and HMBC, it can be explained that compound 1 has a substituted benzene ring at the para position respectively is a hydroxyl group and a pyran ring i.e. 3,5-dihydroxytetrahydro-2H-pyran-2-one. Thus, the proposed chemical structure of compound 1 is 3,5-dihydroxy-4-(4-hydroxyphenyl)tetrahydro-2H-pyran-2-one as shown in Figure. 4.

Conclusion

Four EF were found isolated from *Syzygium jambos* leaf stalk, namely *Lasiodiplodia*, *Bipolaris*, *Aureobasidium* dan *Cladophialophora*. These four EF were tested for antibacterial and antioxidant. The EF SJS1 -- *Lasiodiplodia theobromae* has high antibacterial activity against *S. aureus* and *B. subtilis* bacteria. Antioxidant activity of *L. theobromae* showed strong activity with an IC50 value of 29.29 µg/mL. Isolation of pure compound from *L. theobromae* obtained compound 3,5-dihydroxy-4-(4-hydroxyphenyl)tetrahydro-2H-pyran-2-one, this compound belongs to the class of phenolic.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors thank to the DRPM Kemenristek Republik Indonesia, which provided research funding through Hibah Disertasi Doktor 2022 as the third output, with contract no. 0166.05/UN9/SB3.LP2M.PT/2022.

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

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