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Research Article

Bioactivity of secondary metabolite of endophytic fungi extract isolated from root of Jambu Mawar (*Syzygium jambos* (L.) Alston)

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Abstract: This research aims to isolate endophytic fungi from *Syzygium jambos* and identify their active compounds. Endophytic fungi were isolated from the roots of *S. jambos* and cultured on Potato Dextrose Agar media. Antibacterial activity using the Kirby–Bauer method was tested on four Gram-positive and Gram-negative bacteria. Molecular identification was carried out on selected isolates to determine the species of endophytic fungi and isolate their active compounds. Column chromatography was used for compound isolation. The pure compounds were then analyzed spectroscopically using ^{1}H-NMR, ^{13}C-NMR, DEPT 135, HMQC, HMBC, COSY. The results of the isolation of endophytic fungi found four isolates SJR1 – 4, which show antibacterial activity. The strongest antibacterial activity was demonstrated by isolate SJR1, so it was continued with molecular identification. Molecular identification of SJR1 indicates that it is *Lasiodiplodia iranensis*. The pure compound *L. iranensis* was isolated and found to be 3-butyl-3,4-dihydroxy-6-((2-hydroxy-5-oxocyclopentyl) methyl)tetrahydro-pyran-2-one, which belongs to the phenolic group and has potential as an antibacterial. This compound can be used as an alternative medicinal ingredient.

1. INTRODUCTION

Endophytic fungi are known to produce many new metabolites that have the potential as natural agents in the pharmaceutical, agricultural, and environmental fields (Selim *et al*., 2012). Secondary metabolites produced by endophytic fungi are not needed for the growth of microorganisms, but play an important role in the health, nutritional needs, and economy of society (Abdel-Aziz *et al*., 2017). Secondary metabolites of endophytic fungi have various chemical compounds, such as terpenoids, polyketides, non-ribosomal peptides, or a

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combination of both. These secondary metabolites function as antibiotics or medical drugs, but there are also mycotoxins which are dangerous to health (Avalos & Limón, 2021). Secondary metabolites are natural products in the form of molecules with low molecular weight, having diverse chemical structures and biological activities produced by plants, bacteria, and fungi. Secondary metabolites (SM) are byproducts whose function is to defend an organism from environmental stress (Mosunova *et al*., 2021).

Endophytic fungi are organisms that live in all healthy plant tissues without showing signs of disease or morphological changes in either part or all of the plant's life cycle. The term endophyte was introduced by De Bary, namely organisms that reside in plant tissue (Khiralla *et al.,* 2016). Based on studies, endophytic fungi isolated from medicinal plants are known to be able to help their hosts in synthesizing secondary metabolite compounds whose effects are the same as natural medicines synthesized directly from plant tissue (Zheng *et al*., 2021).

There have been many studies on the discovery of new secondary metabolites produced by endophytic fungi and their bioactivity which has the same properties as compounds produced by their hosts. *Penicillium griseofulvum* isolated from the medicinal plant *Mentha pulegium* has activity against *Escherichia coli* bacteria (Amina *et al*., 2018). *Curvularia papendorii i*solated from *Vernonia amygdalina* contains kheiric acid which has activity against Methicillin-resistant *Staphylococcus aureus* (Khiralla *et al*., 2020). The endophytic fungi *Nigrospora, Diaporthe* and *Epicoccum* isolated from *Leucas martinicensis* have activity against *E. coli*, *S. aureus* (Ruth *et al*., 2020). Drug-resistant bacteria, such as *Staphylococcus aureus* resistant to Methicillin, *Streptococcus pneumonia* resistant to penicillin (Deshmukh *et al*., 2015).

Plants from the genus *Syzygium* Gaertn. (Myrtaceae) are known to have medicinal properties. *Syzygium* is known for having very strong aromatics because it is rich in essential oils, flavonoids, flavonols, anthocyanins, tannins, and phenolic acids (Sobeh *et al*., 2018). Jambu mawar (*Syzygium jambos* (L.) Alston) is one of the genera *Syzygium* which has medicinal properties. *S. jambos* leaves contain phenolic compounds which have antidiabetic potential (Gavillán-Suárez *et al*., 2015), alkaloid, flavonoid, and steroid compounds that have antioxidant and cytotoxic activity (Devakumar & Sudha, 2017). According to Wamba *et al*. (2018) the leaves and bark of *S. jambos* contain saponin, triterpenoid, anthraquinone, and tannin compounds which have antibacterial activity. However, this species is difficult to find, especially in South Sumatra, as an alternative, endophytic fungi have been isolated to overcome the need for compounds needed as medicinal ingredients. Previously, research had been carried out on the diversity and activity of endophytic fungal compounds from *S. jambos*.

A diversity of endophytic fungi isolated from *S. jambos* was found on the skin (Roux *et al*., 2020), as well as on leaves and root bark (Aini *et al.*, 2022b). Besides that, several pure compounds that have biological activity were also found in the endophyte function of *S. jambos*, namely the compound 3-hydroxy-4-(hydroxy(4hydroxyphenyl)methyl)dihydrofuran-2-on which was isolated from *Fusarium verticillioides* (Aini *et al*., 2022), the compound 3,5-dihydroxy-4-(4-hydroxyphenyl) tetrahydro-2H-pyran-2-one which was isolated from *Lasiodiplodia theobromae* (Aini *et al.*, 2022a) and 5-acetyl-6-hydroxy-3-methyl-2H-pyran-2-one which was isolated from *Botryosphaeria mamane* (Aini *et al*., 2023). These compounds have antibacterial and antioxidant activity.

A variety of endophytic fungi have also been isolated from other *Syzygium* genera. Endophytic fungi isolated from *S. zeylanicum* (L.) DC. have antioxidant and antibacterial activity (Syarifah *et al*., 2022). Endophytic fungi isolated from *S. aqueum* (Burm.f.) Alston have antibacterial and antifungal activity (Habisukan *et al*., 2021). Endophytic fungi isolated from *S. malaccense* (L.) Merr. & L.M.Perry have antibacterial potential (Hapida *et al*., 2021). Therefore, this research aims to identify the diversity of endophytic fungi isolated from the roots of the *S. jambos* plant and discover new compounds synthesized by endophytic fungi and their activities.

2. MATERIAL and METHODS

2.1. Isolation and Identification of Endophytic Fungi

Syzygium jambos root samples were taken in March 2021 at the multipurpose field, Jalan Sakura 3, Kencana Damai housing complex, Sako, Palembang 2°54'49.8"S - 104°45'43.9"E (-2.913824, 104.762194).

Isolation of endophytic fungi using a modified method from Elfita *et al*. (2014). *S. jambos* root samples were washed with sterile water 5-10 times, then used 70% ethanol for 3 minutes, rinsed again with sterile water, and finally washed in sodium hypochlorite (NaOCl) solution for 5 minutes. Sterilized root samples were cut approximately 1 - 2 cm and placed on Potato Dextrose Agar (PDA) media for 1-2 weeks in a dark room at room temperature 27 ± 2ºC. The hyphae that have grown are transferred to new PDA media to obtain pure isolates.

The growing endophytic fungal isolates were identified morphologically macroscopically and microscopically. Macroscopic characterization through observing the color of the top and bottom surface colonies, colony shape, texture, and topography. Microscopic characterization through observation under a microscope (Hirox MXB-2500REZ), namely the type and shape of spores, hyphae, and other distinctive characteristics. The results of these observations were then compared with fungal identification books and relevant articles.

2.2. Cultivation and Extraction

The pure isolate of the root endophytic fungus of *S. jambos* was cultivated in 5 x 300 mL in potato dextrose broth (PDB) media by placing 6 blocks of pure culture agar in a bottle. Then the culture was 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 (R300+ V-300 with interface I-300 Pro+ F305) until a thick extract was obtained (Supaphon & Preedanon, 2019)

2.3. Antibacterial Activity of Fungal Endophytes

The endophytic fungal isolates obtained were screened for antibacterial activity using the paper disk diffusion method with an ethanol extract concentration of 400 µg/mL, positive control using 30 µg/mL tetracycline antibiotics. The test bacteria were represented by Gram-negative – *Escherichia coli* and *Salmonella typhi*, while Gram-positive – *Staphylococcus aureus* and *Bacillus subtilis*. Antibacterial activity was shown by the clear zone around the paper disc. Criteria for antibacterial activity are determined by comparing the clear zone of endophytic fungal extract (A) and the clear zone of tetracycline antibiotics (B). For the criteria, $weak < 50\%$; $50\% < moderat < 70\%$; $strong > 70\%$. Calculations based on the following equation (Elfita *et al*., 2019):

$$Clear\ zone\ percentage\ (\%) = \frac{A}{B} x100\%$$

2.4. Molecular Identification

Endophytic fungal isolates that have strong antibacterial activity are subjected to molecular identification to determine the species of the endophytic fungus. Molecular testing of endophytic fungi was carried out at the Genetic Science Indonesia laboratory, Banten, Indonesia using the Genomic DNA extraction method with Quick-DNA Fungal Miniprep Kit (Zymo Research, D6005), PCR amplifcation with My Taq HS Red Mix (Bioline, BIO-25048) at twice. Standard PCR primers use ITS1 and ITS4 (Singha et al., 2016). Analysis of DNA structure using Molecular Evolution Genetics Analysis Versi 11 (Tamura *et al*., 2021).

2.5. Isolation of Chemical Compounds of Endophytic Fungi

Endophytic fungi that have strong antibacterial activity will have their active compounds isolated. Filtrate containing active secondary metabolites by liquid-liquid fractionation

(partition) with the organic solvent ethyl acetate. The fraction filtrate was then concentrated using a rotary evaporator until a thick ethyl acetate fraction was obtained. The concentrated extract was separated through column chromatography using silica gel 60 (70–230 mesh) as the stationary phase (1:30) and eluent. The eluent was chosen based on previous determination through thin-layer chromatography using silica gel 60 F254. The selected eluent with increased polarity was *n*-hexane:EtOAc in a ratio ranging from 10:0 to 0:10 (v/v). An eluate was collected and then combined via thin-layer chromatography into column fractions. Each fraction was evaporated and purified through chromatography to isolate the pure compound. The chemical structure of the compound was determined through various spectroscopy methods: Spectrum of ^{1}H-NMR, ^{13}C-NMR, DEPT 135, HMQC, HMBC and COSY (Elfita *et al*., 2012, 2016).

3. FINDINGS

3.1. Isolation of Endophytic Fungi

The results of the isolation of endophytic fungi from the roots of *S. jambos* found four isolates, namely SJR1 - 4 (Figure 1). This endophytic fungal isolate was then subjected to macroscopic (Figure 1a, Table 1) and microscopic morphological characterization (Figure 1b, Table 2). The results of macroscopic characterization identified four genera, namely *Lasiodiplodia*, *Rhizopus*, *Mortierella*, and *Cylindrocladium*. These four genera have the same type and shape of spores, namely conidia and globose. However, the hyphae of the genus *Rhizopus* are rhizoid, and the other three genera are septate.

Figure 1. Macroscopic (a); Microscopic (b) morphological characterization of endophytic fungi.

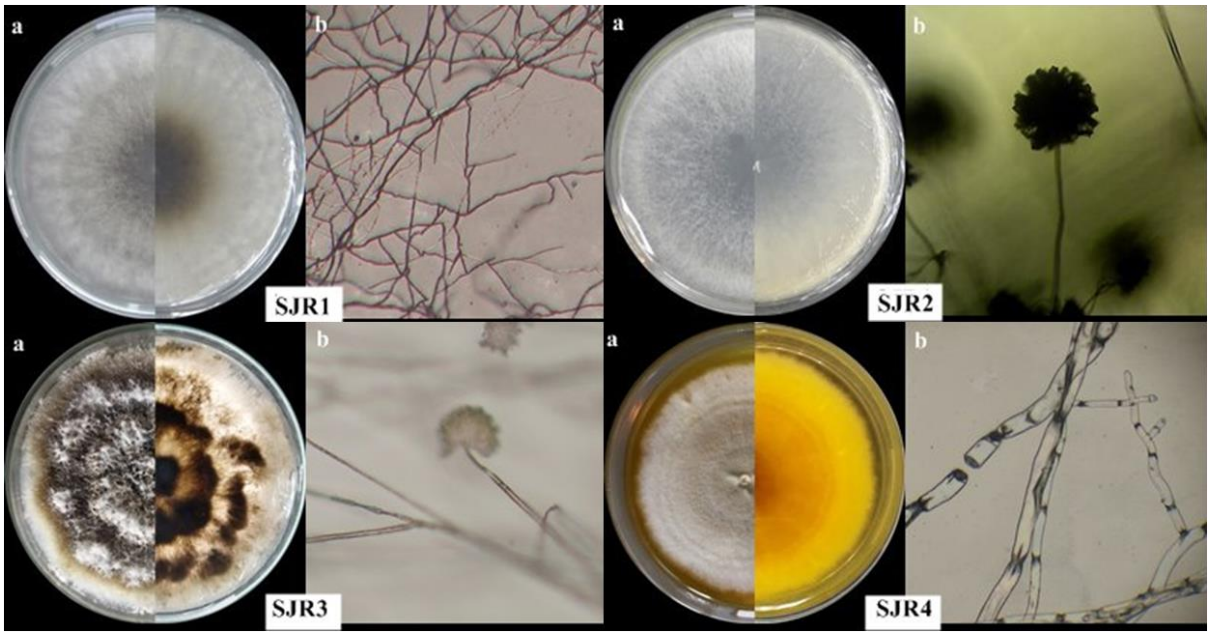


Table 1. Macroscopic characteristics of endophytic fungal isolation from *S. jambos* roots.

Isolate	Colony color	Reverse colony color	Texture	Topography	Pattern	Exudate drops	Radial line	Concentric circle
SJR1	White	Black and White	cottony	Raised	Zonate	-	√	√
SJR2	White	White	cottony	Raised	Zonate	-	-	-
SJR3	Black and White	Black and White	cottony	Raised	Flowery	-	-	√
SJR4	White	White to yellow pigmented	velvety	Umbonate	Radiated	-	√	√

Table 2. Microscopic characteristics of endophytic fungal isolation from *S. jambos* roots.

Isolate	Type of spore	Shape of spore	Hyphae	Specific characteristic	Genus / species
SJR1	Conidia	Globose	Septate	Hyaline conidia, usually two	*Lasiodiplodia* sp.
SJR2	Conidia	Globose	Rhizoids	Numerous stolon run among the mycelia	*Rhizopus* sp.
SJR3	Conidia	Globose	Septate	Sporagiosphores branched, long	*Mortierella* sp.
SJR4	Conidia	Globose	Septate	Hyaline conidia, conidiophores tapering towards the apex, branched, phialides terminal	*Cylindrocladium* sp.

3.2. Bioactivity of Endophytic Fungi

The results of antibacterial activity screening against the bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Salmonella typhi*, showed that the extract of endophytic fungi isolated from *S. jambos* roots had antibacterial activity (see Table 3). SJR1 isolate has strong activity against *B. subtilis* and *S. aureus* with percentages of 71.8% and 70.6%, and moderate activity against *S. typhi* (69.2%) and *E. coli* (69.4%). The isolates SJR3 and SJ4 have moderate activity toward four testing bacteria. The isolate SJR2 has moderate activity against *S. typhi*, *E. coli*, and *B. subtilis*, weak toward *S. aureus* (49.8%). Of the four fungi found, SJR 1 had the highest percentage of antibacterial activity, so it was chosen to continue with molecular identification.

Table 3. Antibacterial activity of endophytic fungal extracts.

Isolate	Ethyl acetate extract (gr)	Antibacterial Activity (%)			
		S. typhi	*E. coli*	*B. subtilis*	*S. aureus*
Tetracycline	-	100	100	100	100
SJR1	4.1	69.2**	69.4**	71.8***	70.6***
SJR2	3.8	54.8**	56.6**	51.7**	49.8*
SJR3	3.2	52.8**	55.8**	52.2**	53.8**
SJR4	3.2	52.9**	53.5**	51.4**	51.8**

Notet: < 50% = weak*; 50 – 70% = moderate**; > 70% = strong***

3.3. Molecular Identification

Molecular identification of SJR1 isolates using PCR amplification with universal primers of the ITS1 - ITS4 rDNA region varied by ± 500 - 600bp the result of assembling the PCR amplification sequence for SJR1 is 540bp (GenBank OM746696) (Figure 2). The results of phylogenetic analysis (Figure 3) SJR1 has a very close distance with a value of 99, this shows that these three isolates are closely related. Isolate SJR1 has a bootstrap value of 1000 and is in the genus *Lasiodiplodia*.

Figure 2. Electrophoresis of SJR1 isolates. M (Marker 100 bp DNA ladder).

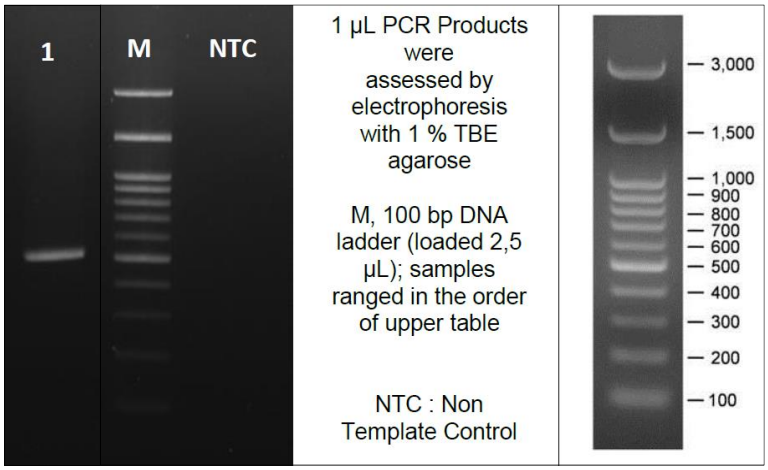
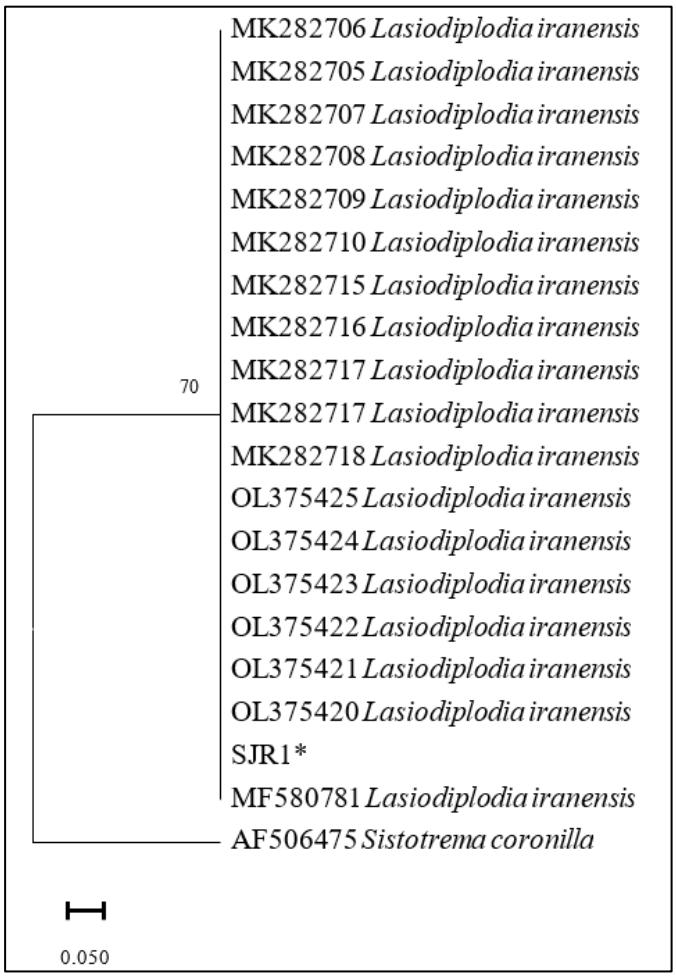


Figure 3. Phylogenetic tree of SJR1 endophytic fungal isolates.

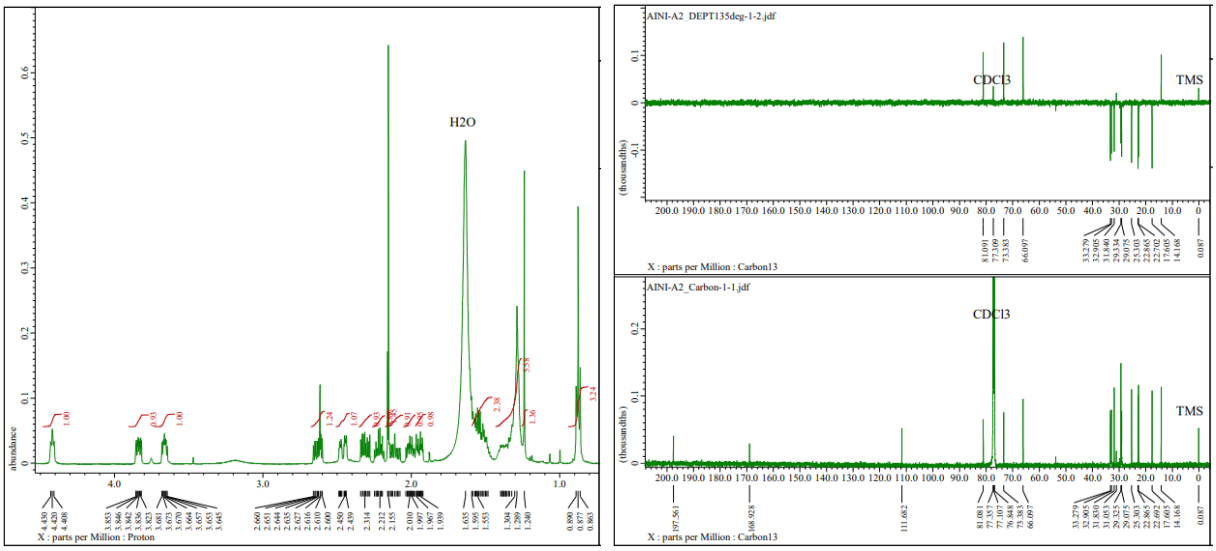


3.4. Isolation of Chemical Compounds of Endophytic Fungi

The chemical structure of compound **1** was determined based on ^{1}H-NMR, ^{13}C-NMR, DEPT 135, HMQC, HMBC, and COSY spectrum analysis. The spectrum of ^{1}H-NMR of compound **1** (Figure 4A) showed there are 17 proton signals. All proton signals are at δ_H < 4.5 ppm which indicates that all protons of compound **1** are sp^3. Proton at δ_H 3.5 – 4.5 ppm are sp^3 protons in oxygenated carbon. In the spectrum it can be seen that there are two triplet signals, namely at δ_H 0.88 (3H, J= 6.5 Hz); 4.42 ppm (1H, J= 5 Hz), and the other is a multiplet signal. Based on ^{1}H-NMR, spectrum analysis, compound **1** is non-aromatic which has an oxygenated carbon atom in the form of either a hydroxyl group or a cyclic ester.

The ^{13}C-NMR spectrum of compound **1** (Figure 4B) shows the presence of 15 signals. There are three carbon signals at δ_C > 100 ppm, three carbon signals at δ_C 65-85 ppm, and another at δ_C < 33.0 ppm. The spectrum of DEPT 135 compound **1** (Figure 4B) shows that all three signals at δ_C > 100 ppm are quaternary carbon. Two carbon signals at low fields, namely at δ_C 197.6 and 168.9 ppm indicate the presence of carbonyl ketone and carbonyl ester groups. The three oxygenated carbon signals at δ_C 65-85 are methine carbons, and all carbons at δ_C < 33.0 ppm are methylene carbons, except for one carbon at δ_C 29.3 ppm which is methine carbon and 14.2 ppm which is methyl carbon.

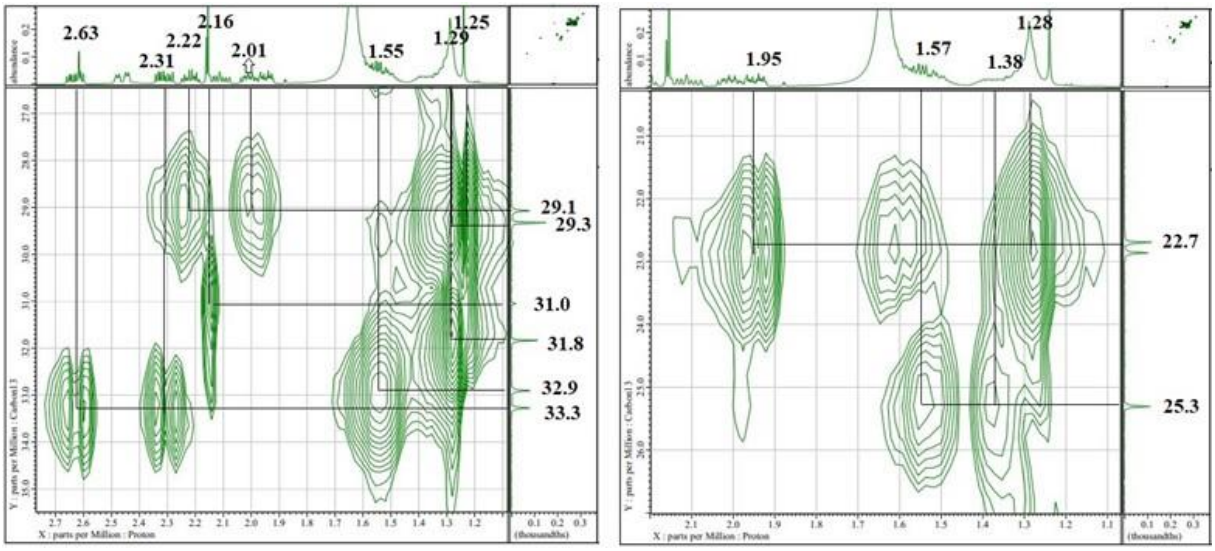
Figure 4.[1]H-NMR (A), [13]C-NMR and DEPT 135 (B) spectrum compound **1** ([1]H-500 MHz; [13]C-125 MHz in $CDCl_3$).



A B

Analysis of the NMR spectrum of protons and carbon is strengthened by data on the HMQC spectrum shown in Figure 5 and Table 4, namely the correlation of [1]H-[13]C through one bond.

Figure 5. The spectrum of HMQC of compound **1**.



The HMQC spectrum shows 17 correlations, there are 10 proton signals that correlate with five methylene carbon atoms and two other proton signals correlate with two methylene carbon atoms. Furthermore, three proton signals at δ_H 3.5 – 4.5 ppm correlate with three oxygenated methine carbons. Apart from that, there is also a correlation of a proton signal with methine carbon and a proton signal with methyl carbon.

The HMBC spectrum (see Figure 6) shows the correlation of [1]H-[13]C through two or three bonds. There is a methylene group that correlates with the ketone carbonyl carbon and two methylene groups that correlate with the ester carbonyl carbon. Methylene protons at δ_H 2.63 (1H, m) and 2.31 (1H, m) correlate two and three bonds with carbon at δ_C 197.6; 66.1; 29.1 ppm. Methylene protons at δ_H 2.22 (1H, m) and 2.01 (1H, m) correlate with carbon at δ_C 168.9; 197.6; 66.1; 33.3 ppm. Methylene protons at δ_H 2.46 (1H, m) and 2.11 (1H, m) correlate with carbon at δ_C 168.9; 81.1; 111.7; 22.7 ppm. This correlation indicates that there is a methylene group that is three bonds away from the ester carbonyl carbon and two methylene groups that are two and three

bonds away from the ketone carbonyl carbon. Furthermore, there is a correlation of the methyl proton with two methylene carbons at δ_C 22.7; 31.8 ppm, methylene proton correlation δ_H 1.25 (2H, m) with carbon at δ_C 29.1 ppm, and methylene proton correlation δ_H 1.95; 1.28 ppm with carbon at δ_C 111.7; 17.6; 31.8 ppm. This correlation indicates the presence of a straight carbon chain group in compound **1**.

Figure 6. The Spectrum of HMBC of compound **1**.

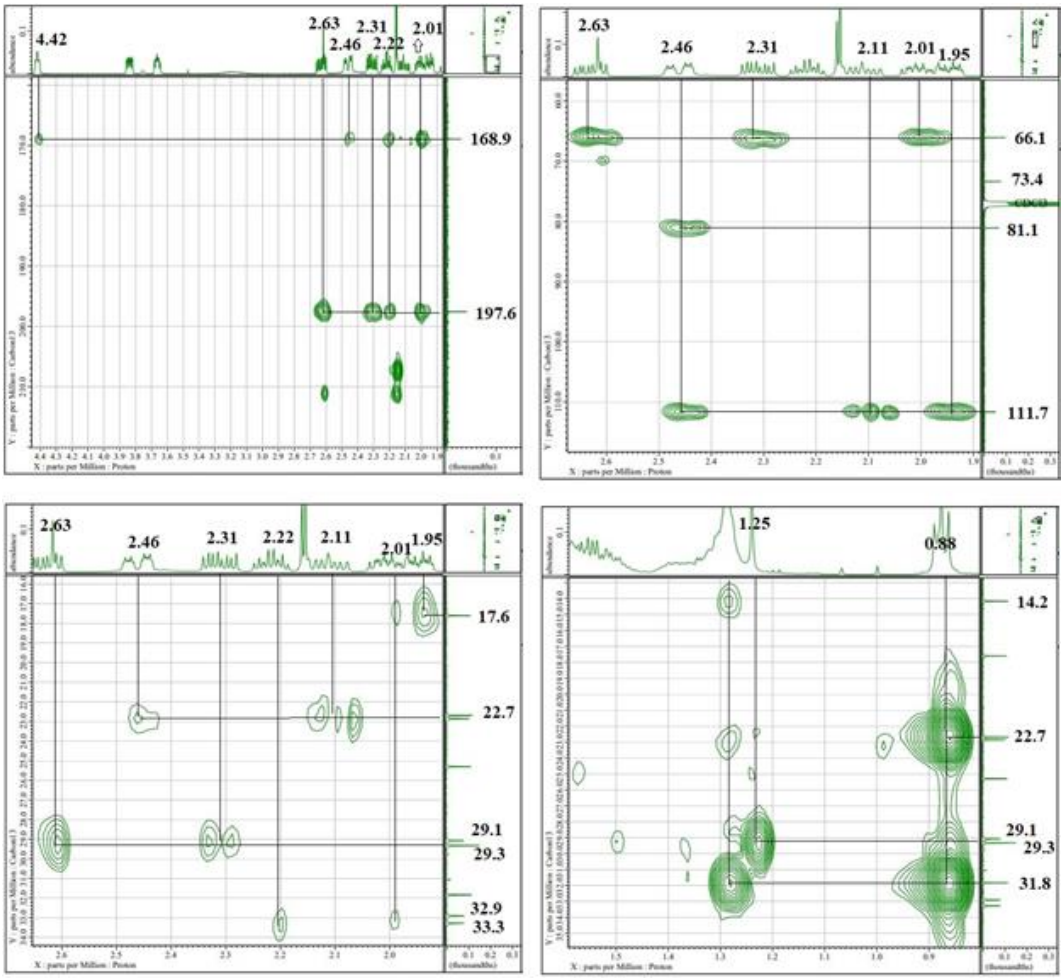
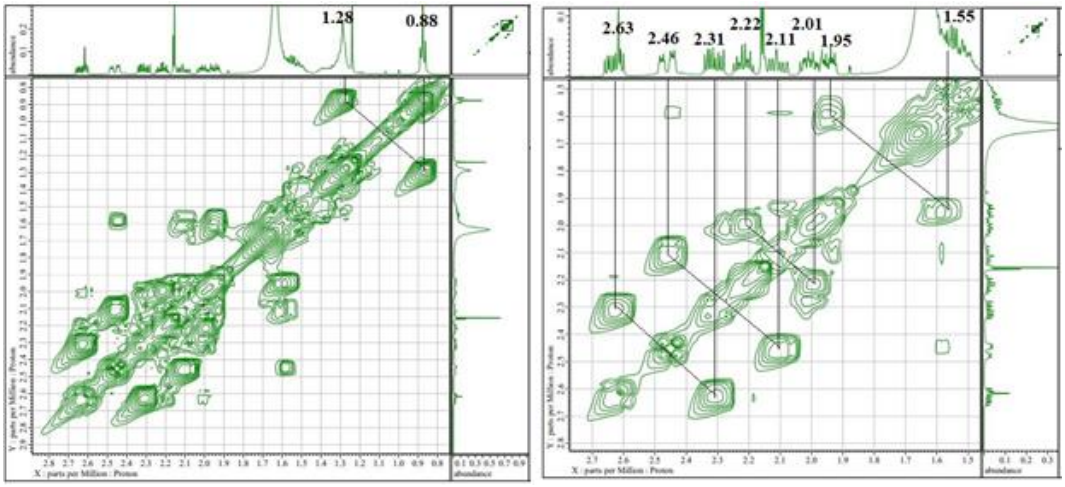


Figure 7. COSY spectrum of compound **1**.

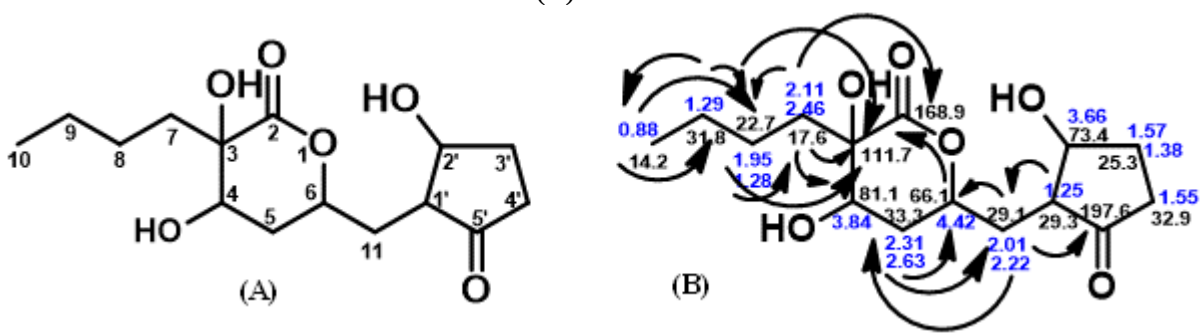


The COSY spectrum (see Figure 7) shows a ^{1}H-^{1}H correlation through two or three bonds. There are three ^{1}H-^{1}H correlations through two bonds, namely the correlation between methylene protons bound to the same carbon but with different chemical shifts. Apart from that, there is also a correlation between methyl protons and methylene protons through three bonds. The 1D and 2D NMR spectrum data for compound **1** are listed in Table 4. Based on the ^{1}H-NMR, ^{13}C-NMR, DEPT 135, HMQC, HMBC and COSY spectrum analysis, it can be explained that compound **1** has a straight chain consisting of four carbons (n-butyl), three hydroxyl groups, a carbonyl ketone group, a carbonyl ester, a methyl group, seven methylene groups (two methylene groups are on the butyl cyclo). Furthermore, there are four methine groups, including three oxygenated methine groups, and a quaternary carbon. The molecular formula of compound **1** is $C_{15}H_{24}O_6$ with the equivalent double bond being 4. Thus, the proposed chemical structure of compound **1** is 3-butyl-3,4-dihydroxy-6-((2-hydroxy-5-oxocyclopentyl) methyl)tetrahydro-pyran-2-one as shown in Figure 8.

Table 4. NMR data for 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	COSY
2	168.9	C			
3	111.7	C			
4	81.1	CH	3.84 (1H, m)		
5	33.3	CH_2	2.63 (1H, m)	66.1; 29.1	2.31
			2.31 (1H, m)	66.1; 29.1	2.63
6	66.1	CH	4.42 (1H, t, J= 5 Hz)	168.9	
7	17.6	CH_2	2.46 (1H, m)	168.9; 81.1; 111.7; 22.7	2.11
			2.11 (1H, m)	111.7; 22.7	2.46
8	22.7	CH_2	1.95 (1H, m)	111.7; 17.6; 31.8	
			1.28 (1H, m)		
9	31.8	CH_2	1.29 (2H, m)	14.2; 22.7	0.88
10	14.2	CH_3	0.88 (3H, t, J= 6.5 Hz)	22.7; 31.8	1.29
11	29.1	CH_2	2.22 (1H, m)	168.9; 197.6; 33.3	2.01
			2.01 (1H, m)	168.9; 197.6; 66.1; 33.3	2.22
1’	29.3	CH	1.25 (1H, m)	29.1	
2’	73.4	CH	3.66 (1H, m)		
3’	25.3	CH_2	1.57 (1H, m)		
			1.38 (1H, m)		
4’	32.9	CH_2	1.55 (2H, m)		
5’	197.6	C			

Figure 8. Structure of compound **1**: 3-butyl-3,4-dihydroxy-6-((2-hydroxy-5-oxocyclopentyl) methyl)tetrahydro-pyran-2-one with carbon atom numbering (A) and placement of proton and carbon chemical shifts as well as HMBC correlation (B).



4. DISCUSSION and CONCLUSION

The results of the isolation of endophytic fungi found four isolates, namely SJR1 – SJR4, based on microscopic and macroscopic characterization identified as the genus *Lasiodiplodia* (SJR1), *Rhizopus* (SJR2), *Mortierella* (SJR3), and *Cylindrocladium* (SJR4). These four isolates have antibacterial activity against the bacteria *B. subtilis*, *S. aureus*, *E. coli* and *S. typhi* with the strongest activity being SJR1 (*Lasiodiplodia*). The antibacterial activity of these endophytic fungi is due to the secondary metabolite content they produce. It is known that endophytic fungi are an alternative source of plant-derived bioactive compounds (Wen *et al*., 2022). Secondary metabolites produced by endophytic fungi act as antimicrobial, insecticides, anticancer, cytotoxic, and antioxidant (Bano *et al*., 2016). Apart from that, it also has the ability to act as an immunosuppressive, antidiabetic, antimalarial, antituberculosis and antiviral agent (Singh & Kumar, 2023).

Isolate SJR2 is characterized by white top colonies and white bottom colonies. Microscopically, the hyphae appear like roots or rhizoids, long sporangiopores and at the tip there is a round, dark colored sporangium. Based on these characteristics SJR2 was identified as *Rhizopus* (Walsh *et al*., 2018). The *Rhizopus* genus plays a role in increasing the nutrition of food ingredients through the fermentation process (Endrawati & Kusumaningtyas, 2018). *Rhizopus* can also be a pathogen that causes rot in plants (Hartanti *et al*., 2020). *Rhizopus oryzae* has antioxidant properties (Kang *et al*., 2016; Massarolo *et al*., 2017), antibacterial against *Salmonella typhi* (Jannah *et al*., 2020). *Rhizopus oligosporus* as an antioxidant (Dulf *et al*., 2018; Starzyńska-Janiszewska *et al*., 2020; de Lima *et al*., 2021), produces linoleic acid, α-linoeat acid and monolinolenins as antibacterial (Kusumah *et al*., 2020). Monohexosylceramides compounds inhibit the growth of *Bacillus terrae*, *Micrococcus luteus*, *Pseudomonas stutzeri* and antibiofilm from MRSA bacteria (Vieira *et al*., 2018).

The endophytic fungus SJR3 was identified as *Mortierella*, belonging to the order Mortierellales. Members of Mortierellales have a very high ecological and physiological diversity that allows them to be distributed throughout the world (Voigt & Kirk, 2014). *Mortierella parvispora* as antiparasitic activities, *Mortierella* has herbicidal activity (Vaca & Chávez, 2019), as bioremediation (Cui *et al*., 2017). *Mortierella alpine* as an antioxidant and antimicrobial (Goyzueta *et al*., 2020), synthesizes indoleacetic acid, gibberellic acid and ACC-deaminase (Ozimek *et al*., 2018). *Mortierella isabellina* is a type of fungi that produces compounds that have been found to have lower inhibitory effects than those detected in the dexamethasone-treated group. However, one of the compounds 15-ene steviol showed better effects than dexamethasone in reducing the release of monocyte chemoattractant protein (MCP)-1, 2, and 3, which LPS induces. Furthermore, three specialized products similarly showed better effects than dexamethasone in inhibiting the secretion of regulated on activation, normal T cell expressed and secreted (RANTES) in response to LPS. None of the tested compounds showed any cytotoxicity or triggered cell apoptosis, and none affected the protein integrity of toll-like receptor 4 (TLR4) or MyD88. This suggests that these compounds may exert the anti-inflammatory activity downstream of membrane-associated TLR4 and MyD88 molecules (Chang *et al*., 2021).

The SJR1 isolate has strong antibacterial activity, which was then identified molecularly to determine the species. Phylogenetic tree construction analysis using the Neighbor-Joining method (Saitou & Nei, 1987) with bootstrap 1000x repetitions (Felsenstein, 1985). This analysis involved 20 nucleotide sequences. Evolutionary analysis was performed in MEGA11 software (Tamura *et al*., 2021). Trees are drawn to scale and the units of branch length are the same as the evolutionary distances used to derive the phylogenetic tree. Evolutionary distances were calculated using the number-of-differences method (Nei & Kumar, 2000) and is measured in the number of basic differences per sequence. The analysis included 42 nucleotide sequences. From the SJR1 phylogenetic tree, the isolate sequence with a bootstrap value of 1000 shows 98–100% similarity to the species sequence, namely *Lasiodiplodia iranensis* (Figure 3).

Figure 3 shows that almost all samples have Bootstrap values in phylogenetic tree construction ranging >80. A bootstrap value of >80 means that the species are identical or have almost the same nucleotide base sequence and have a high level of similarity. According to that a branch of a phylogenetic tree is declared stable if the Bootstrap value is >80 and if it is <50 it is declared unstable. The similarity percentage can also influence the closeness of positions in the phylogenetic tree construction.

L. iranensis is found on *Arachis hypogeae* (Isalar *et al*., 2021), but is pathogenic on *Dioscorea* spp. causes rot in plant (Jibrin *et al*., 2022), causes death in *Coffea canephora* (Ramos *et al*., 2023). Based on research by Gagana & Shivanna (2020), *L. iranensis* found in *Memecylon umbellatum* has antibacterial activity, but the compounds contained in it have not been reported. Shen *et al*. (2022) reported that *L. iranensis* can produce jasmonic acid, a plant hormone that controls development, growth, photosynthesis, protects plants against insects and is applied in agriculture, industry and other fields. *Lasiodiplodia* sp. isolated from flower of *Viscum coloratum* produces essential oils in the form of cyclo-(Trp-Ala), indole-3-carboxylic acid (ICA), indole-3-carbaldehyde, mellein and 2-phenylethanol (Qian *et al*., 2014). *L. iranensis* isolated from the mangrove of *Avicenna ger-minans* was found to contain the compounds 11,12-didehydro-7-iso-jasmonic acid, 4,5-didehydro-7-iso-jasmonic acid, cyclo-(L-Leu-L-Pro), jasmonate-threonine, and abscisic acid (Delgado Gómez *et al*., 2023). In research Li *et al*. (2023), *L. iranensis* synthesizes 1,3,6,8-tetrahydroxynaphthalene, dimethylcoprogen, and (R)-melanin.

Based on this research, it is known that the four isolates found had antibacterial activity. The endophytic fungus *L. iranensis* produces the compound 3-butyl-3,4-dihydroxy-6-(3-(2-hydroxycyclobutyl)-3-oxopropyl) tetrahydro-2H-pyran-2-one which is included in the phenolic group. Phenolic compounds form a broad group of compounds derived from the secondary metabolism of plants found in various natural sources such as fruits, vegetables, tea, wine and honey (Lima *et al*., 2019). The antibacterial activity of phenolic compounds is related to the hydroxyl groups located in the cytoplasmic membrane of bacterial cells (Gyawali & Ibrahim, 2014). According to Griffin *et al*. (2005) and Figueiredo *et al*. (2008) said that the number and position of hydroxyl groups play a role in antimicrobial activity. The main antibacterial mechanism of phenolics such as eugenol is to disrupt the cytoplasmic membrane of bacteria, which increases their non-specific permeability (Li *et al*., 2015). In this study, compound **1** was found to have a hydroxyl group. It is suspected that this hydroxyl group is what disrupts the bacterial cytoplasmic membrane.

The presence of hydrophobic phenolic groups in the lipid bilayer disturbs interactions between lipids and proteins and leads to an increase in membrane permeability. This causes alterations in the structure of the membrane and speeds up the release of intracellular contents, ultimately leading to the disruption of membrane integrity. As a result, this process allows for the entry of substantial quantities of antibacterial agents (Char *et al*., 2010). Carvacrol and thymol, which are phenolic isomers, are non-polar compounds that insert themselves into the bacterial cell membrane, disrupting its normal function. The hydroxyl groups and the presence of a double bond in carvacrol and thymol enable them to function as proton exchangers, leading to a reduction in the gradient across the cytoplasmic membrane. This ultimately leads to the breakdown of the proton motive force and a decrease in the ATP pool, resulting in the death of the cell (Kachur & Suntres, 2020).

The start of disruption to the bacterial cell is suggested by shifts in cell membrane potential, which manifest as hyperpolarization of the membrane. This hyperpolarization is a result of pH changes or heightened movement of K^+ ions, leading to outward diffusion in order to maintain membrane potential balance (Whiteaker *et al*., 2001; Wu *et al*., 2016). Ensuring the balance of ions is crucial for the growth of cells and is involved in essential cellular functions like transporting substances, regulating metabolism, managing turgor pressure, and facilitating movement (Cox *et al*., 2001). The permeability of bacterial membranes is indicated by relative

electrical conductivity to explain how antimicrobial action works. Small ions like K^+, Na^+, and H^+ are crucial for supporting cell membrane function, enzyme activity, and normal metabolism, but the bacterial cytoplasmic membrane blocks their access. When electrical conductivity increases, it leads to cytolysis and bacterial death (Diao *et al*., 2014).

Maintaining the internal conditions of metabolism and energy transduction of cells relies on the integrity of the cell membrane, which is an important factor. (Sánchez *et al*., 2010). The functioning of the cell metabolism can be impacted by damage to the integrity of the cell membrane, leading to the inhibition of cell growth and potentially causing cell death. (Cox *et al*., 2001). The interactions between chemicals and membrane lipids can impact the fluidity of the membrane by affecting the order, shape, packing, and curvature of the lipids (Mykytczuk *et al*., 2007). Certain types of essential oils have the potential to harm bacterial cells and enhance the fluidity of the core of the lipid bilayer membrane. Changes in fluidity can lead to an increase in cell membrane permeability and result in harm to cellular contents, impacting cellular processes (Cherrat *et al*., 2016).

Compound **1** produced by *L. iranensis* in this research is expected to be an alternative source of medicinal ingredients, due to the need in the health sector. The limitation of this research is that it has not been tested *in vivo*, so further research needs to be carried out by applying it to test animals

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Kurratul 'Aini: Investigation, Resources, Software, Writing, original draft preparation, editing. **Elfita Elfita**: Methodology, Supervision, Validation, Analysis of new compound, Writing, original draft preparation, editing. **Hary Widjajanti**: Methodology, Validation and Analysis of Endophytic fungi. **Arum Setiawan**: Methodology, Validation and Analysis of Endophytic fungi. **Rian Oktiansyah**: Methodology, Analysis of Endophytic Fungi and Discussion.

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