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Bioactivity of Endophytic Fungi Isolated from Branch of Jambu Mawar (*Syzygium jambos* (L.) Alston)

Kurratul 'Aini[1,2], Elfita[3,*], Hary Widjajanti[4], Arum Setiawan[4]**

[1]Graduate School of Sciences, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Palembang 30139, Indonesia.
[2]Universitas Islam Negeri Raden Fatah, Palembang, Palembang 30126, South Sumatra, Indonesia
[3]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Indralaya, Ogan Ilir 30662, South Sumatra, Indonesia
[4]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Indralaya, Ogan Ilir 30662, South Sumatra, Indonesia

*Corresponding author email: elfita.elfita.69@gmail.com; **: kurratulaini_uin@radenfatah.ac.id

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ABSTRACT. Endophytic fungi isolated from medicinal plants have high diversity and the secondary metabolites produced have biological activity. Endophytic fungi isolated from medicinal plants have been the subject of many studies on their diversity and biological activity, one of which is jambu mawar (*Syzygium jambos* (L.) Alston). *S. jambos* has long been used as a traditional medicine to treat infections caused by pathogenic bacteria in many parts of the world, including South Sumatra. This study purposed to explore the diversity of endophytic fungi isolated from the branches of *S. jambos*, the antibacterial activity of endophytic fungi extracts, the determination of the structure of the compounds, and the activity of the active compounds of the selected endophytic fungi. Observations of the morphological characteristics of endophytic fungi were macroscopically and microscopically. The endophytic fungal extracts were then tested for antimicrobial activity against test bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, and *Escherichia coli* using the Kirby-Bauer paper disk diffusion method. Isolation of pure compounds using a gravity chromatography column, the determination of the structure of the compound is determined based on ^{1}H-NMR, ^{13}C-NMR, HMQC, and HMBC spectroscopy. Four endophytic fungi, code SJC1–4, were isolated from a branch of *S. jambos*. The results of macroscopic and microscopic morphological characterization showed three genera of *Botryosphaeria*, *Trichothecium*, and *Aspergillus*. The endophytic fungal isolate SJC1 exhibited strong activity against Gram-positive bacteria and moderate activity against Gram-negative bacteria, while SJC2–4 showed moderate activity against the four bacteria. Molecular identification of SJC1 revealed that it was *Botryosphaeria mamane*. Isolation of SJC1's pure compound yielded compound 1, which was identified as 5-acetyl-6-hydroxy-3-methyl-2H-pyran-2-one. This compound is thought to have antibacterial properties.

Keywords: Antibacterial activity, *Botryosphaeria mamane,* Endophytic fungi, *Syzygium jambos*

INTRODUCTION

Endophytic fungi are a type of fungus in plants that do not cause significant plant disease (Jia et al., 2016). These fungi and their host plants have a very complex relationship. Some endophytic fungi can produce hormones that promote plant growth, such as antiphagocytes; help the host resist biological feeding; develop medicinal ingredients; and produce many plant by products in their biological activity (Khan et al., 2013). Endophytic fungi also possess natural antimicrobial activity that can compensate for plant resource shortages, limit the length of the regeneration cycle, and feature in industrial fermentation to mass produce natural active compounds at low cost and without pollution (Yuan et al., 2017). Endophytes associated with medicinal plants have been extensively studied. These fungi grow through natural openings in plants, such as stomata and natural wounds. Endophytes also belong to the group of dark septate endophytes and play an important role in the production of secondary metabolites and enzymes. Endophytes and the secondary metabolites they produce have great potential for medicine and pharmacy (Devi et al., 2020; Tiwari & Bae, 2022). In therapeutic applications, endophytic fungi act as antibacterial agents (Deshmukh et al., 2015; Gagana & Shivanna, 2020; Pansanit & Pripdeevech, 2018). For instance, the antibacterial activity of the endophyte *Tritirachium oryzae* isolated from *Syzygium malaccense* contains 2-(4-hydroxyphenyl) -4-methoxytetrahydrofuran-3-ol (Hapida et al., 2022); *Fusarium verticillioides*, isolated from the stem bark of *S. jambos,* contains 3-hydroxy-4-(hydroxy(4-hydroxyphenyl)methyl)dihydrofuran-2-on

(Aini et al., 2022); and *Penicillium brefeldianum* from the root bark of *Syzygium zeylanicum* contains p-hydroxybenzaldehyde (Syarifah et al., 2021).

Antibacterial resistance in turn occurs when microorganisms do not react to antibiotics that previously actively treated infections caused by bacteria. Resistance can occur through DNA mutations during replication, when bacterial species are naturally resistant to certain antibiotics, or the overuse of antibiotics (Mancuso et al., 2021). Natural sources of antibiotics are thus needed to overcome resistance to synthetic antibiotics. Numerous studies have been conducted to derive raw natural antibiotic materials from medicinal plants, including jambu mawar (*S. jambos* (L.) Alston). *S. jambos* commonly known as rose apple or jambu mawar, belongs to the family Myrtaceae and is widespread across sub-Saharan Africa, Central America, and Asia (Sharma, et al., 2013).

This plant is used for various ailments given its many active substances against pathogenic bacteria (Lim, 2012). All parts of the plant are reported to have medicinal value. In Indo-China, the whole plant is used to treat for digestive and dental ailments. Decoction of the leaves is used as a diuretic, medicine for sore eyes, and rheumatism. The seeds are used to treat diarrhea, dysentery, diabetes, and similar to nasal mucus. A decoction of the bark can also relieve asthma and bronchitis (Sharma et al., 2013). Methanol extracts of *S. jambos* leaves and stem bark have shown activity against *S. aureus* (Fathima et al., 2017; Wamba et al., 2018). However, *S. jambos* is difficult to find and requires a long period of cultivation before it can be used as a raw material. Therefore, another approach is to isolate the endophytic fungi associated with it. Studies on the isolation of endophytic fungi from specifically the stem bark (Aini et al., 2022; Roux et al., 2020), from leave and root bark (Aini et al., 2022) of *S. jambos* have been previously performed, which have antibacterial activity. Other endophytic fungi can also be found in *S. aqueum*, such as *Aspergillus*, *Trichoderma*, *Penicillium*, and others, which have antibacterial properties (Habisukan et al., 2021). Accordingly, this study explored diverse endophytic fungi isolated from *S. jambos* branches, their antibacterial and isolated pure compounds.

EXPERIMENTAL SECTION

Sampling

Samples of *S. jambos* branches were collected at the Kencana Damai Housing Complex multipurpose yard, Sako, Palembang in March 2021. The samples' morphologies were identified at the Biosystematics Laboratory, Department of Biology, Faculty of Mathematics and Natural Science, Universitas Sriwijaya, Palembang with the letter number 233/UN9.1.7/4/EP/2021.

Isolation and Identification of Endophytic Fungi

To prepare the samples, the *S. jambos* branches were rinsed 5–10 times with sterile water, with 70% ethanol for 3 minutes. They were rinsed again with sterile water before being set in a sodium hypochlorite solution for 5 minutes. Sterile specimens were cut into approximately 1–2 cm pieces and cultured in a potato dextrose agar (PDA) medium for 6 days (Manias et al., 2020; Shi et al., 2013).

The endophytic fungal isolates were then identified via macroscopic and microscopic morphology. In macroscopic morphology, each sample's surface and reverse colony color, shape, texture, and morphology were observed. Microscopic observation was carried out under a microscope (Hirox MXB-2500REZ) with glass slide cultures to confirm the type and shape of the spores, hyphae, and specifics characteristics. These observations were then compared to fungus identification books from Walsh et al. (2018) and Watanabe (2010); and related articles.

Cultivation and Extraction of Endophytic Fungi

After the endophytic fungus samples' maturation, up to 5 x 300 ml of pure isolate in potato dextrose broth (PDB) was placed in 6 pure culture flasks (1x1 cm). The PDB's composition was 20 g monohydrate dextrose, 200 g potato, and 1000 mL aquadest (Gustianingtyas et al., 2020). The cultures were then incubated under static conditions at room temperature for 4 weeks (± 28 days). After the incubation period, the endophyte mycelium was separated from the liquid culture and dissolved in ethyl acetate at a 1:1 ratio. The ethyl acetate extract was further isolated from the liquid culture and then evaporated using a rotary evaporator until a thick extract was obtained (Nagarajan, 2019), which weigh SJC1 3.9 g, SJC2 3.7 g, SJC3 3.9 g, and SJC4 3.2 g, respectively.

Antibacterial Activity of Endophytic Fungi Extract

The endophyte isolates were generated by screening for antibacterial activity using the Kirby-Bauer paper disk diffusion method. The endophyte extract was at a concentration of 400 µg/mL, and the antibiotic tetracycline was used at a concentration of 30 µg/mL for comparisons. The test bacteria are represented by Gram-negative – *E. coli* (InaCCB5) and *S. typhi* (ATCC 1408), while Gram-positive – *S. aureus* (InaCCB4) and *B. subtilis* (InaCCB4). A clear area around the tray indicated antibacterial activity. The isolates' antibacterial activity characterization was determined by comparing the clear zones of the endophytic fungus extract (A) to the clear zone of the antibiotic (B) and reported as weak $< 50\% <$ moderate $< 70\% <$ strong (Aini et al., 2022).

$$Antibacterial\ Activity\ (\%) = \frac{A}{B} x100\%$$

Molecular Identification

Endophytic fungus isolates that exhibited strong antibacterial activity were identified at the molecular

level at the Genetika Science Indonesia Laboratory using genomic DNA extraction with a Quick-DNA Fungal Miniprep Kit (Zymo Research, D6005) and twice via polymerase chain reaction (PCR) amplification with MyTaq HS Red Mix (Bioline, BIO-25048). ITS1 and ITS4 were used as standard PCR primers (Singha et al., 2016). The samples' DNA structures were analyzed using Molecular Evolution Genetics Analysis Version 11 (Tamura et al., 2021).

Isolation of Chemical Compounds of Endophytic Fungi

The mycelium was removed from the endophytic fungal cultures after 8 weeks of culture, with the medium then filtered. The medium was extracted 3 times with ethyl acetate (1:1), then evaporated under a vacuum to obtain a concentrated extract. The concentrated extracts (1.0 g) were separated via column chromatography on silica gel 60 (70-230 mesh) in the stationary phase (1:30). The eluent had been previously determined via thin layer chromatography using silica gel 60 F254. The selected eluent, according to increasing polarity, was *n*-hexane:EtOAc at a ratio of 10:0 to 0:10 (v/v). The eluates were collected and then combined into column fractions using thin layer chromatography. Each fraction was evaporated and purified using chromatographic techniques to obtain purified compound. The chemical structures of the compounds were determined via ^{1}H-NMR, ^{13}C-NMR, HMQC, and HMBC spectroscopy (Fadhillah et al., 2019).

RESULTS AND DISCUSSION

The isolation of the endophytic fungi from the *S. jambos* branches yielded 4 isolates encoded as SJC1–4. Based on macroscopic (**Figure 1, Table 1**) and microscopic (**Figure 1, Table 2**) characterization, they fell under three different genera, namely *Botryosphaeria, Trichothecium,* and *Aspergillus*. Isolate SJC3 had erect hyphae, septate, and black conidia on the tip. The inverted colonies were white, the phialides were scattered around the vesicles and twins, and the conidia were rough and dark. SJC3 was then identified as *A. niger* (Walsh et al., 2018). SJC4's surface color was yellow-green with yellow spots, and the reverse color was brown. The hyphae had septa, and the conidia were long. Additionally, phialides uniserat and bisserat were formed in the vesicles, a trait identified in *A. flavus*. SJC2 was characterized by septate hyphae and long conidiophore, and was unbranched. The conidia were two-celled and pear-shaped, with attachment points cut into "legs." This feature indicated it was *Trichothecium roseum*. Lastly, SJC1 was identified as purposive *Botryosphaeria* with *Diplodia* (Watanabe, 2010); *B. mamane* is bio-synonymous with *Cophinforma mamane* (Phillips et al., 2013) and morphologically anamorphic with *Fusicoccum mamane* (Crous et al., 2006; Mohali et al., 2007).

The antibacterial activity of the ethyl acetate extracts of SJC1–4 were tested by forming a clear area around the paper disk. The results showed that SJC1–4 all exhibited antibacterial activity (**Table 3**). SJC1 showed strong activity against Gram-positive bacteria and moderate activity against Gram-negative bacteria. SJC2 had moderate activity against the four test bacteria (*E. coli* 53.6%, *S. typhi* 52%, *S. aureus* 51.9%, *B. subtilis* 52%). Based on its characterization, SJC2 was identified as *Trichothecium* sp. Several *Trichothecium* sp species produce trichothecinol-A, which has antifungal, anticancer, and antimetastatic effects (Taware et al., 2014). The compounds 3β-hydroxyartemisinic acid, 3β,15-dihydroxyartemisinic acid, and 3-Oxoartemisinic acid produced by the biotransformation of *T. roseum*'s artemisinic acid have antifungal properties as well (Singh et al., 2019). Additionally, it produces cuspidatol compounds belonging to the sesquiterpene group, trichomide cyclodepsopeptides which exhibit immunosuppressive activity (Zhou et al., 2018). However, *T. roseum* can cause crop damage to apples, melons, oranges, and other crops by secreting harmful mycotoxins (Zhang et al., 2020).

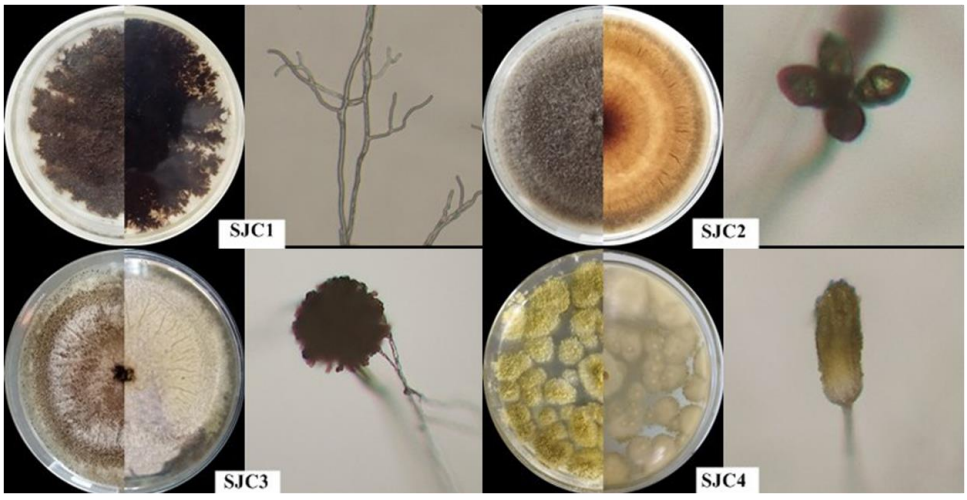

Figure 1. Microscopic and macroscopic characterization

Table 1. Macroscopic characteristics of endophytic fungi isolated from jambu mawar branches

Isolate	Colony color	Reverse colony color	Texture	Topography	Pattern	Radial line	Concentric circle
SJC1	Black	Black	cottony	Umbonate	Zonate	-	-
SJC2	Grey	Dark brown	cottony	Rugose	Zonate	√	√
SJC3	Grey and	White	cottony	Raised	Radiated	-	√
SJC4	Golden yellow	White	Powdery	Umbonate	Spread	-	-

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

Table 2. Microscopic characteristics of endophytic fungi isolatied from jambu mawar branches

Isolate	Type of spore	Shape of spore	Hyphae	Specific characteristic	Genus
SJC1	Conidia	Globose	Aerial	Pycnidia are globose, black, no observed of conidiospores	*Botryosphaeria* sp.
SJC2	Conidia	Globose	Septate	Conidiosphores are long, unbranched. Conidia with two celled	*Trichothecium* sp.
SJC3	Conidia	Globose	Septate	Codiosphores long. Phialides radiate around entire vesicle and are bisertae. Conidia are dark	*Aspergillus* sp.
SJC4	Conidia	Globose	Septate	Phialides are uniserate with club-shaped vesicle on surface. Conidia are oval	*Aspergilus* sp.

Table 3. Antibacterial activity of ethyl acetate extract of endophytic fungi SJC1 – SJC4

Sample	Genus/Species	Antibacterial Activity (%)			
		E. coli	*S. typhi*	*S. aureus*	*B. subtilis*
Tetracycline	-	21.3 ± 0.17 100.0	21.67 ± 0.56 100.0	21.77 ± 0.25 100.0	22.1 ± 0.16 100.0
SJC1	*Botryosphaeria* sp.	14.57 ± 0.21 68.9 **	15.13 ± 0.17 69.8**	15.57 ± 0.42 71.5***	15.73 ± 0.09 71.2***
SJC2	*Trichothecium* sp.	11.33 ± 0.74 53.6**	11.27 ± 0.65 52.0**	11.30 ± 0.16 51.9**	11.5 ± 0.24 52.0**
SJC3	*Aspergillus* sp.	11.97 ± 0.25 56.6**	11.17 ± 0.21 51.5**	12.10 ± 0.16 55.6**	11.23 ± 0.17 50.8**
SJC4	*Aspergilus* sp.	11.0 ± 0.22 52.1**	11.77 ± 0.12 54.3**	11.17 ± 0.21 51.3**	11.47 ± 0.31 51.9**

SJC3 and SJC4 both demonstrated moderate activity against all four bacteria. Based on macroscopic and microscopic characterization, these isolates were identified as *Aspergillus* varieties. Some of *Aspergillus* species, such as *A. flocculus* contains diorcinol and 3-Hydroxymellein compounds inhibit the parasite *Trypanosoma brucei brucei* (Tawfike et al., 2019). In turn, secondary metabolites of *A. aculeatus* are ergosterol peroxide, secalonic acid D and F, variecolin, variecolactone, and ergosterol, all of which have antimalarial (Yodsing et al., 2018). The bioactive compounds eicosane, eicosane 2-methyl, phenol, 2,6-bis(1,1-dimethyl ethyl)-4-methyl, hexadecane 2, and 11-octadecenoic acid, the methyl ester produced by *A. fumigatus*, show antibacterial activity against *S. aureus* and *E. coli* (Octarya et al., 2021). Alkaloid compounds from the genus *Aspergillus* are generally antimicrobial, cytotoxic, and antioxidant as well (Youssef et al., 2021). Bioactive compounds 1,2,3,4,6-Penta-trimethylsilyl glucopyranose, Fmoc-L-3-(2-Naphthyl)-alanine, D-(-)-fructopyranose, pentakis (trimethylsilyl) ether, bis (2-ethylhexyl) phthalate,

trimethylsilyl ether-glucitol, octadecanamide, and N-(2- methylpropyl)-N-nitroso, as produced by *A. niger*, are antioxidants that prevent genotoxicity (Abdel-Wahhab et al., 2020).

SJC1 had strong antibacterial activity against *S. aureus* (71.5%) and *B. subtilis* (71.2%) and moderate activity against *E. coli* (68.9%) and *S. typhi* (69.8%). SJC1 was also selected for molecular identification. Its evolutionary history was derived using the Neighbor-Joining method (Saitou & Nei, 1987). The percentage of replicate trees (1000 replicates), in which related taxa are clustered together in a bootstrap test, is given next to the branches (Felsenstein, 1985). Each tree is 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-difference method (Nei & Kumar, 2000) and measured in the number of base differences per sequence. The analysis included 20 nucleotide sequences. All positions with blank and missing information were removed. The final dataset contained a total of 455 locations and underwent evolutionary analysis in MEGA11 (Tamura et al., 2021). Based on PCR amplification, the result was 539bp, percentage identity > 97.5%, similar with KF531822, then SJC1 is identified as *B. mamane* (**Figure 2**).

It is known that *Botryosphaeria* sp. contains phenyl derivatives (Ju et al., 2016) and botryoisocoumarin-A inhibitors COX-2 (Ju et al., 2015). *B. dothidea* contains pycnophorin which has antibacterial activity against *B. subtilis* and *S. aureus;* compound stemphyperylenol, which exhibits antifungal activity against *Alternaria solani;* and the antioxidant compounds altenusin and djalonensone (Xiao et al., 2014). Druzian et al. (2020) and Valente et al. (2018) reported that *B. dothidea* contains antioxidants. *B. mamane* also contains sesquiterpen (Oliveira et al., 2015), thiodiketopiperazines (botryosulfuranols A-C) (Barakat et al., 2019), botryomaman, 2,4-dimethoxy-6-pentylphenol, (*R*)-(-)-mellein, primin, *cis*-4-hydroxymellein, *trans*-4-hydroxymellein and 4,5-dihydroxy-2-hexenoic acid which show antibacterial properties against *S. aureus* (Pongcharoen et al., 2007). *B. fabicerciana* contains mellein and b-orcinaldehyde compounds, which are useful against *B. cereus, S. aureus, B. subtilis* bacteria and have antioxidants properties (Silva et al., 2021). Isocumarin and tryptamin are antifungals produced by *B. ramose* (Hu et al., 2020; Wu et al., 2019).

Pure compound isolation (1.0 g) was performed to determine the active ingredient of the endophytic fungus isolate with the strongest antibacterial activity, which in this case was SJC1. The ^{1}H-NMR spectrum of compound 1 (**Figure 3**) showed three singlet signals, namely one vinyl proton signal at δ_H 5.92 ppm (1H, s) and two methyl proton signals at δ_H 2.26 (3H, s) and 2.66 ppm (3H, s). Also according to this spectrum, compound 1 was not an aromatic compound. The ^{13}C-NMR spectrum showed another eight signals: two methyl carbon signals at δ_C 20.8 and 30.1 ppm; one methane sp^2 carbon signal at δ_C 101.5; and five sp^2 quaternary carbons. There were also three sp^2 quaternary carbon compounds, δ_C 169.1, 181.2, and 205.3 ppm, in a low-field chemical shift, indicating the presence of cyclic ester carbonic carbon and ketone carbonyl.

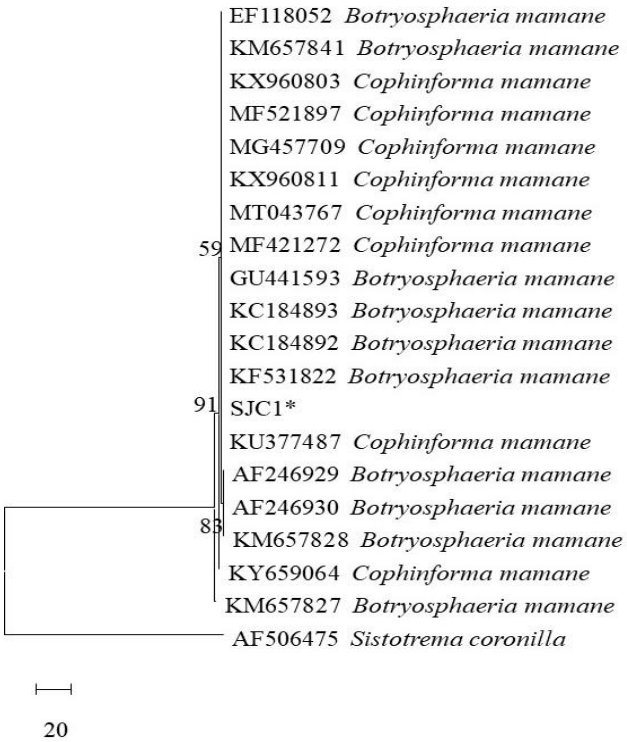

Figure 2. Phylogenetic tree of SJC1

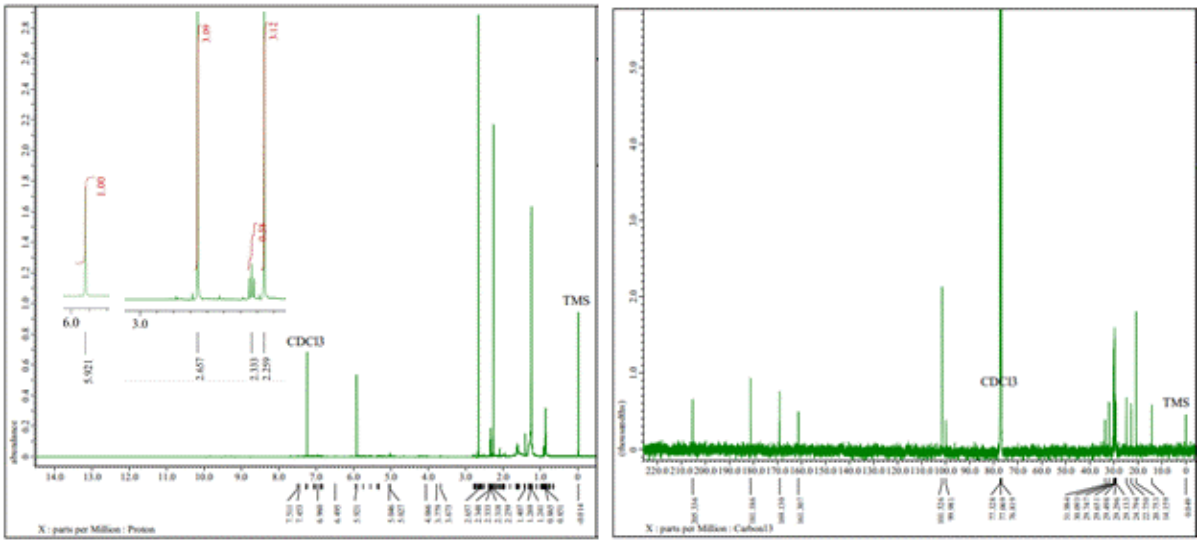

Figure 3. The ^{1}H-NMR (**A**) and ^{13}C-NMR (**B**) spectra of compound 1 (^{1}H-500 MHz; ^{13}C-125 MHz in $CDCl_3$

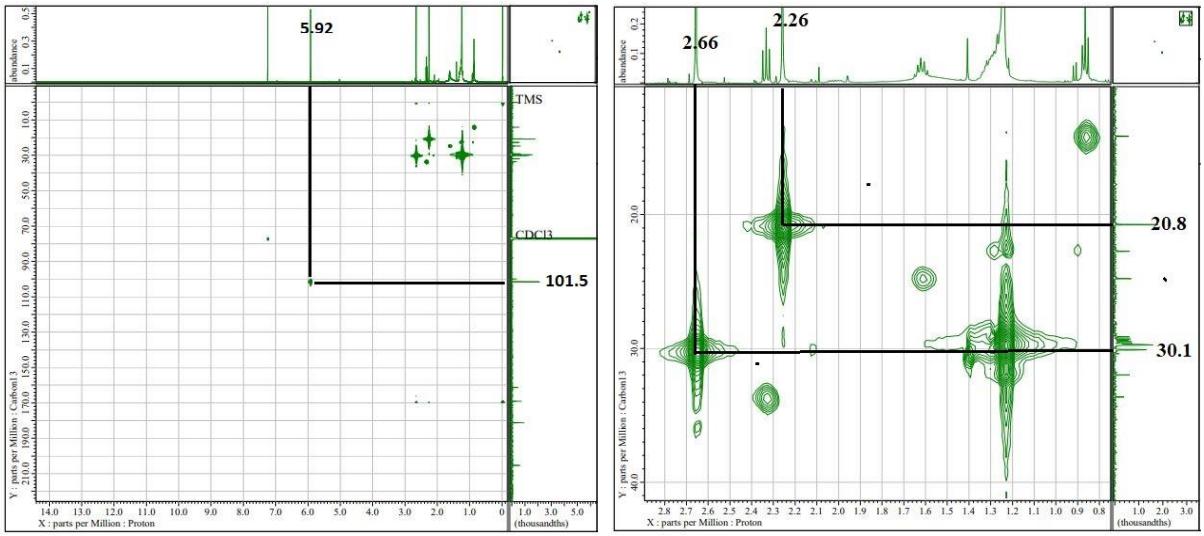

Figure 4. The HMQC spectra of compound 1

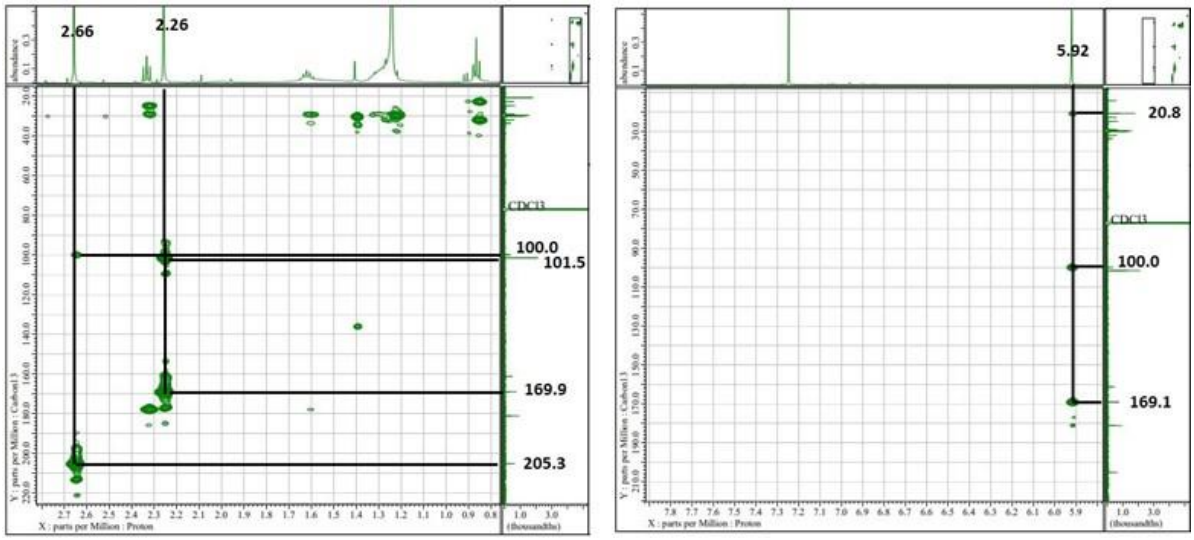

Figure 5. The HMBC spectra of compound 1

Table 4. 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	169.1	C		
3	161.3	C		
4	101.5	CH	5.92 (1H, s)	169.1; 181.2; 20.8; 100.0
5	100.0	C		
6	181.2	C		
7	205.3	C		
8	30.1	CH_3	2.66 (3H, s)	205.3; 100.0
9	20.8	CH_3	2.26 (3H, s)	101.5; 169.1

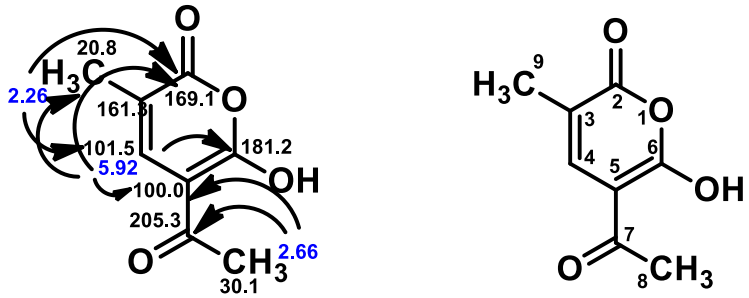

Figure 6. The structure of compound 1 as 5-acetyl-6-hydroxy-3-methyl-2H-pyran-2-one

Table 5. Antibacterial activity of pure compound

Sample	Concentration (ppm)	Diameter of inhibitor zone (mm)			
		S. typhi	*E. coli*	*B. subtilis*	*S. aureus*
Compound 1	500	7.25	7.30	7.05	7.05
	250	7.00	6.65	6.35	6.28
	125	6.68	-	-	-
	62.5	6.43	-	-	-
	31.25	6.20	-	-	-
	15.625	-	-	-	-

The HMBC spectrum of compound 1 (**Figure 5, Table 4**) showed a ^{1}H-^{13}C three-bond correlation between two or three methyl proton bonds at δ_H 2.26 ppm, carbonyl ester carbon, and carbon methine sp^2. The proton methine vinyl sp^2 5.92 ppm was correlated with three bonds to the carbonyl ester (169.1 ppm) and carbondioxy (181.2 ppm) in the alkyl ester; methyl carbon (20.8 ppm); and quaternary carbon sp^2 through two bonds (100.0 ppm). Furthermore, there was a triple bond correlation between methyl proton (δ_H 2.66 ppm) and both ketone carbonyl carbon (205.3 ppm) and quaternary carbon sp^2 (100.0 ppm). Based on the spectroscopic ^{1}H-NMR, ^{13}C-NMR, HMQC, and HMBC data, compound 1 had a cyclic lactone or pyran ring attached to methyl, hydroxyl, and acetyl groups. The structure of the compound was thus identified as 5-acetyl-6-hydroxy-3-methyl-2H-pyran-2-one (**Figure 6**).

The antibacterial activity of pure compound was determined using Kirby-Bauer's disc paper diffusion method at concentrations of 500, 250, 125, 62.25, 31.25 and 15.625 ppm. The test bacteria were again *E. coli*, *S. typhi*, *B. subtilis*, and *S. aureus*. The antibacterial activity test also determined the minimum inhibitory concentration (MIC) of compound (**Table 5**). MIC is the lowest concentration of an antimicrobial that will inhibit the growth of microorganisms after the incubation period (Andrews, 2001). The MIC parameter value uses a breakpoint based on a modification from EUCAST (1998) with the categories of susceptible (≤ 62.5), resistant (> 62.5) ppm, and area of technical uncertainty (ATU). The MIC value of compound 1 was at a concentration of 31.25 ppm for *S. typhi* and 250 ppm for all other bacteria. This means that *S. typhi* is more susceptible and other three bacteria is more resistant to compound 1.

Based on spectroscopic ^{1}H-NMR, ^{13}C-NMR, HMQC, and HMBC data, compound 1 had a cyclic lactone or pyran ring attached to methyl, hydroxyl, and acetyl groups. The structure of the compound was then identified as 5-acetyl-6-hydroxy-3-methyl-2H-pyran-2-one (**Figure 6**), confirming it as a lactone.

Lactone compounds are widely distributed in nature as various biologically active substances and metabolic intermediates. Lactones are cyclic compounds that have intramolecular ester bonds (Kataoka et al., 2007). Compounds in the lactone group are known to exhibit antibacterial activity (Sartori et al., 2021). Accordingly, the lactone penicilactones A compound produced by *Penicillium* sp. showed antibacterial activity against *S. aureus* (Bai et al., 2019). However, lactone compounds' antibacterial activity depends on if the structure contains substituent fluorine and the length of the bacterial lipopolysaccharide (Kowalczyk et al., 2021). As such, unsaturated and hydroxy lactone compounds have antibacterial activity against *S. aureus* (Wińska et al., 2018). ε-lactone compounds also show antibacterial activity (Mazur & Maslowiec, 2022). From the results of this study, compound 1 can be used as a drug candidate in the future.

CONCLUSSIONS

This study provides important knowledge about endophytic fungi derived from the plant *S. jambos* and their secondary metabolites as promising sources of alternative medicine. Four endophytic fungi were isolated from stem branches of *S. jambos*, namely *Botryosphaeria, Trichothecium*, and two *Aspergillus* species. Antibacterial tests showed that the four species exhibited antibacterial activity, with the strongest shown by *B. mamane*. *B. mamane* was found to contain 5-acetyl-6-hydroxy-3-methyl-2H-pyran-2-one, which is show antibacterial activity. This study therefore plays an important role in future examinations of herbal antibacterial medicines, with further clinical trials already underway.

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