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Antibacterial activity of bioactive compound produced by endophytic fungi isolated from *Mangifera casturi* Kosterm endemic plant from South Kalimantan, Indonesia

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ABSTRACT The endophytic fungi that live in endemic plants are a promising bio-prospect as the producers of antibacterial compounds. This research is aimed to evaluate the endophytic fungi antibacterial compound from *Mangifera casturi*. The bioactive compounds of 13 endophytic fungi were extracted using ethyl acetate and evaluated for antibacterial activity using disk diffusion assay. The minimum inhibitory concentration (MIC) was measured using the serial broth dilution method. Scanning Electron Microscopy (SEM) was used to examine cell damage because of the extract. The antibacterial compounds were then detected using GC-MS analysis. The endophytic fungi were identified morphologically and molecularly based on ITS rDNA sequencing Among the 13 isolates, the endophytic fungi identified as *Botryosphaeria rhodina* AK32 produced the antibacterial compounds that exhibited the highest activity and a broad spectrum. Moreover, they were capable against resistant bacteria, Methicillin Resistant *Staphylococcus aureus* (MRSA) with an MIC value of 1.56% for all the test bacteria. The mechanism of action of AK32 ethyl acetate extract seemed to affect the condition of bacterial cell walls, causing morphological alteration such as shrinkage of the cell, warted cells, and hollow cells. Based on GC-MS, the antibacterial compounds of AK32 ethyl acetate extract were di-n-octyl phthalate, benzyl alcohol, high-oleic (CAS) safflower oil, benzene acetonitrile, and benzotriazole.

KEYWORDS Antibacterial activity, *Botryosphaeria rhodina*, Endophytic fungi, *Mangifera casturi*, MRSA

1. Introduction

Bacteria are one of the causes responsible for many diseases in the world for a long time ago, and some of the diseases are lethal to a human being. The scientist has found a solution to this problem with the first discovery of antibacterial, sulphonamides in 1935 and penicillin in 1940 (Li and Webster 2018). However, bacterial mutations occur continuously resulting in resistance to the existing antibacterial compounds. This situation forced us to looking for the antibacterial compound that is able to overcome the resistant of bacteria (Silver 2011).

One of the sources of organisms that could produce antibacterial compounds is endophyte, especially endophytic fungi (Deshmukh et al. 2014; Nisa et al. 2015). Fungi were the most diverse and abundant group of endophytic organisms known as the most diverse producer of natural product (Tiwari 2015). From total bioactive compounds produced by microorganisms, as much as 47% were produced by fungi (Bills and Gloer 2016). As a microorganism that lives within plant tissue, endophytic fungi have known could produce secondary metabolites similar to the host plant (Stierle and Stierle 2015). Endophytic fungi also known work together with the host plant to produce important compound such as antimicrobial compound that help the host plant to improve their disease-resistance ability (Ludwig-Müller 2015). Strobel and Daisy (2003) said that there was specific rationale for the collection host plant for endophyte isolation and secondary metabolite discovery. One of the criteria was host plant should be endemic plant that has been known to have a special feature. Endemic plant that occupied an unique habitat seem to be more potential for endophytic fungal isolate source that produce novel bioactive compound with unique structure (El-Deeb et al. 2013).

Mangifera casturi or commonly called Mangga Kalimantan is an endemic plant that only can be found in South Kalimantan, Indonesia (Kostermans and Bompard 1993). Besides its endemicity, *M. casturi* also reported having

antibacterial compound. Many studies have reported that many part of *M. casturi* contained antibacterial compound. A study conducted by Rosyidah et al. (2010) revealed that *M. casturi*'s bark possesses antibacterial activity against *E. coli* and *S. aureus*. Extract and fraction of *M. casturi* fruit also reported possess antibacterial compound against *Pseudomonas aeruginosa*, *Bacillus cereus* (Meliana et al. 2021) and *Streptococcus* mutant (Suhendar et al. 2019) as well. Moreover, another part of *M. casturi* such as leaf extract either known to have antibacterial activity against *S.* mutant (Khairiyah et al. 2019) and *Streptococcus sanguinis* (Sari et al. 2020). For those reasons, *M. casturi* is a suitable candidate to be explored for its endophytic fungi producing antibacterial compounds. In addition, this exploration will also contribute to conservation efforts because it can reduce the exploitation of the *M. casturi* plant, considering that this plant is listed as extinct in the wild by IUCN. To date, there is still no research done for antibacterial compounds produced by endophytic fungi isolated from *M. casturi*.

2. Materials and Methods

2.1. Endophytic fungi isolates

The endophytic fungi isolates used in this study were an unidentified endophytic isolates collection of FMIPA UNS Biology Laboratory isolated in April 2019 from *M. casturi*'s root, stem, and leaves. The plant was obtained from several places at Padang Batung Sub-district, Kandangan, Hulu Sungai Selatan District, South Kalimantan. A total of 31 isolates of endophytic fungi were sorted and selected based on isolate's growth rate. Thirteen isolates of endophytic fungi that had quick growth rate were selected andcultured on PDA media and incubated at 28±2 °C for further analysis.

2.2. Bioactive compounds extraction

Bioactive compounds extraction from endophytic fungi was carried out based on Sharma et al. (2016) with slight modification. The endophytic fungi were cultivated in potato dextrose broth (PDB) and incubated for two weeks at room temperature in a shaker incubator at 90 rpm. After incubation, the culture broths were separated from the mycelium by filtration using Whatman filter paper. Fungal mycelia were homogenized by breaking the cell using mortar and pestle prior to extraction using ethyl acetate as the organic solvent. The extraction then conducted by adding 100 mL of ethyl acetate to mycelium and 100 mL of ethyl acetate to culture broths, and macerated for 24 h. Filter paper then used for filtering and separating Mycelium from ethyl acetate. Ethyl acetate was added into culture broth 1:1 volume ratio in separating funnel, shaken for 10 min and left for 5 min until two separated layers formed. The upper layers that contain bioactive compounds were collected and lower layer were discarded. For maximum extract yield, the extract obtained from mycelia and fermentation broth then mixed and concentrated by evaporating the solvent under a rotary evaporator at 40 °C to yield ethyl acetate extract for further assay.

2.3. Antibacterial screening

Screening of the antibacterial activity from ethyl acetate extract of endophytic fungi was carried out using the disc diffusion assay method. Crude extracts were evaluated for their antibacterial activity against Gram-negative bacteria (*Escherichia coli*), Gram-positive bacteria (*Staphylococcus aureus*), and resistant bacteria (methicillin-resistant *Staphylococcus aureus*). Those were obtained from the microbiology laboratory, Faculty of Medicine, Universitas Sebelas Maret. The bacteria were grown in Muller Hinton agar (MHA) media. As much as 20 µL of crude extract was dripped to the paper disc using ethyl acetate. Antibacterial activity detected after incubation at 37 °C for 24 h. The activity of extract to inhibit bacterial growth was shown with the appearance of the inhibition zone. Chloramphenicol (20 mg/mL) and vancomycin (0.03 mg/mL) were used as the positive control, whereas ethyl acetate was used as the negative control.

2.4. Determination of minimum inhibitory concentration (MIC)

Ethyl acetate extract possessed the highest diameter of inhibition zone obtained from the preliminary assay was selected to determine the MIC by the serial broth dilution method. Selected ethyl acetate extract of endophytic fungi was diluted twofold for ten times in sterile tubes aseptically. Final concentrations of ethyl acetate extract at each tube after dilution were 25% to 0.098% (v/v). Each tube then inoculated with an equal volume of overnight grown bacterial culture and incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration at which no visible growth observed after incubation.

2.5. Scanning electron microscope (SEM) analysis

Ethyl acetate extract with the highest antibacterial activity was investigated its effect on bacteria using SEM analysis based on Supaphon et al. (2013). The bacteria were treated with ethyl acetate extract at four times of MIC concentration and incubated for 18 h. After incubation, the pellet was collected and fixed with 2.5% glutaraldehyde ($C_3H_8O_2$) in phosphate buffer (PBS) for 2 h, then washed using PBS twice. Pellet then was post-fixed in 1% of osmium tetraoxide for 1 h and washed using PBS. Afterward, the pellet was dehydrated using alcohol series (50%, 70%, 80%, 90%, and 100%). The pellet then was freeze-dried and analyzed using SEM at Research Unit for Natural Product Technology, Indonesian Institute of Sciences (LIPI), Yogyakarta.

2.6. GC-MS Analysis

The most active extract was further subjected to GC-MS analysis to be identified as the chemical composition in the extract. The GC-MS analysis of the extract was performed using Shimadzu GC-MS-QP 2010S Plus fitted with an RTX-5 MS (30 m x 0.25 mm x 0.25 mm) capillary col-

umn in Organic Chemistry Laboratory of Mathematics and Science Faculty, Gadjah Mada University. The instrument temperature program was initially set at 70 °C and maintained for 5 min and gradually risen up to 300 °C at the end of the period at a rate of an increment by 5 °C/min, and maintained for 19 min. Pure helium gas was used as the carrier gas with a flow rate at 0.5 mL/min. Splitless injection port temperature was set and ensured at 300 °C. The mass spectrometer was performed in an electron ionization of 70 eV, while the mass spectral scan range was set at 28-600 (m/z). The identification of chemical compounds present in the extracts was based on their mass spectra with data comparison to NIST12 (National Institute of Standards and Technology, US) and WILEY229 libraries.

2.7. Morphologically and molecularly identification of endophytic fungi

The endophytic fungal isolate from *M. casturi* with the strongest antibacterial activity was identified morphologically and molecularly. Morphological identification was carried out by observing the macroscopic and microscopic characteristics of the endophytic fungi. Macroscopic characteristics observation included surface and reverse the colour of the colony. Whereas the microscopic identification was conducted using slide culture method to observe the characteristics for the hypha, conidiophore, conidia, and other certain characteristics. Morphological identification in this study referred to Watanabe (2002).

Molecular identification was carried out based on ITS rDNA sequence. Fungal genomic DNA was isolated using Quick-DNATM Fungal / Bacterial Miniprep Kit with cell-breaking treatment using sonication priory and continued by following the manufacturer's procedure. The ITS region was subjected and amplified using the polymerase chain reaction (PCR) with primer pairs ITS1 (5'TCCGTAGGTGAACCTGCGG'3) and ITS4 (5'TCCTCCGCTTGATATGC'3) (White et al. 1990). Targeted Pcr product was 550 bp, validated using electrophoresis with gel agarose and visualized by using GelDoc (González-Teuber et al. 2017). Amplified DNA then was subjected to DNA sequencing fand this DNA sequence was compared with already existing DNA sequences in NCBI GenBank using BLASTN program (http://blast.ncbi.nlm.gov/).

3. Results and Discussion

3.1. Endophyte from Mangifera casturi

As much as 13 isolates with rapid growth rate were selected from 31 endophytic fungi of *M. casturi* isolates collection. The selected isolates were isolated from different part of *M. casturi* plant, three from the root, five from the stem, and five were isolated from the leave of *M. casturi*. Those isolates have a growth range from 3-9 cm of diameter when grown in PDA media for 7 d. Among thirteen isolates, each isolate have a different characteristics of the colony.

TABLE 1 Antibacterial activity of ethyl acetate extract against test bacteria.

Name of Isolate	Diameter of inhibition zone (mm)		
	E. coli	*S. aureus*	MRSA
AK22	-	-	-
AK32	23.97±1.79	35.60 ±2.68	34.43±5.30
AKF1	12.55±3.60	12.85±2.07	13.23±1.23
BKF	-	-	-
BK1A	-	-	-
BK31	-	9.16±0.65	-
BK32	20.45±2.89	28±1.84	15.20±0.50
BK2	-	9.20 ±0.98	11.64±0.19
DK82	-	-	-
DK21	-	10.75±1.06	12.73±0.52
DKD	-	8.11±0.13	15.65±1.20
DK5	-	6.83±1.17	9.75±0.35
DK7	8.21±0.56	9.05±0.49	14.55±0.07

*data shown as mean ± SD
(-) no zone of inhibition observed

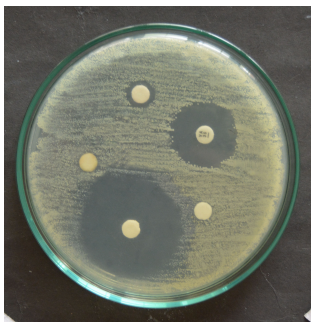

FIGURE 1 Inhibition zone of AK32, DK5, and BK31 ethyl acetate extract against MRSA. Positive and negative controls used were vancomycin and ethyl acetate, respectively.

3.2. Antibacterial activity of endophytic fungi

The result exhibited that from 13 selected isolate, four ethyl acetate extract of endophytic fungi were having a broad spectrum antibacterial activity due to their ability to inhibit Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) test bacteria. Five extracts have narrow spectrum (only against *S. aureus*) and another four did not showed any antibacterial activity at all. In the ability to inhibit MRSA, among nine extracts which had activity against Gram-positive test bacteria, there were eight extracts which had the activity to inhibit MRSA, and the highest antibacterial activity of ethyl acetate extract was shown by AK32 ethyl acetate extract which could inhibit all of the test bacteria (Table 1).

Ethyl acetate extract from several endophytic fungi showed antibacterial activity by forming an inhibition zone. The inhibition zone formed due to the sensitivity of bacteria to the antibacterial agent within the ethyl acetate extract. The wider zone formed the higher antibacterial

activity of the ethyl acetate extract. As shown in Figure 1, the inhibition zone formed by AK32 against MRSA was the widest zone, indicating that AK32 has the highest activity; whereas DK5 has a smaller zone indicated smaller activity and BK31 with no inhibition zone formed indicated no antibacterial activity.

3.3. Minimum inhibitory concentration of endophytic fungal ethyl acetate extract

Ethyl acetate extract of AK32 isolate that has the highest antibacterial activity was assayed for its MIC to test bacteria. The result exhibited that ethyl acetate extract of AK32 isolate has a MIC value 1.56% (v/v) to all of the test bacteria (Table 2). This information indicates that as much as 1.56% of the ethyl acetate extract of AK32 isolate is more than enough to inhibit the growth of *E. coli*, *S. aureus*, and MRSA.

TABLE 2 Minimum inhibitory concentration of AK32 ethyl acetate extract.

No	Test Bacteria	MIC % (v/v)
1	*E. coli*	1.56
2	*S. aureus*	1.56
3	MRSA	1.56

3.4. Antibacterial mechanism of action of the ethyl acetate extract

SEM analysis showed the appearance of test bacterial morphology after treated with ethyl acetate extract of AK32 isolate with four times MIC concentration. The microscopic display indicated morphological differences in treated and untreated of all three test bacteria. In untreated bacteria, all of the test bacteria showed an intact and smooth structure (Fig.2a-c). Whereas for bacteria treated with ethyl acetate extract of AK32 isolate, they showed an observable morphological change (Fig.2d-f). On treated *S. aureus* and MRSA, there are considerable morphological alteration such as deformation, irregular cell shape, loss of their coccal shape, hollowed cell walls, and wrinkled cell surface. The same thing occurred on treated *E. coli* there are observable morphological changes such as, broken of the cell membrane, shrinkage of cells, and forming wrinkled cells, and lysis in some cells. This deformation and morphological changes of test bacteria apparently affect the fungal cell wall as well as cell membrane (Khameneh et al. 2019). Mai-Prochnow et al. (2016)

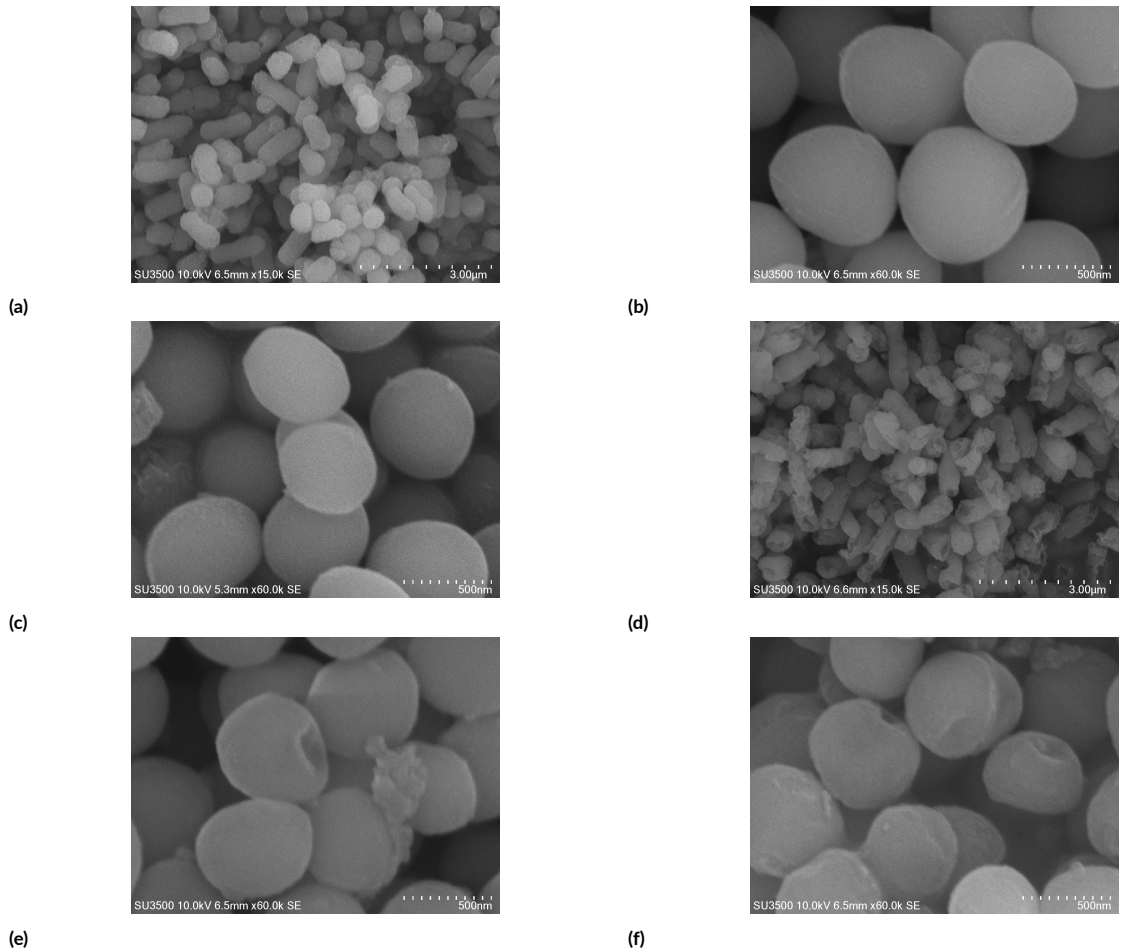

FIGURE 2 Scanning Electron Micrograph. A-C: untreated bacterial cells of E.coli (A), S. aureus (B), MRSA (C); D-F: treated bacterial cells of E.coli (D), S. aureus(E), MRSA (F) with AK32 ethyl acetate extract.

said that the main component of cell wall was peptidoglycan which is found in almost of all bacteria and responsible to maintain the integrity of the cell itself. Damage occurred in that might cause cytoplasmic leakage and lead to cell death (Vollmer et al. 2008).

3.5. GC-MS analysis

GC-MS chromatogram (Fig. 3) shows the presence of 89 compounds identified on different retention times and % area from AK32 ethyl acetate extract. The results of GC-MS analysis show that in AK32 ethyl acetate extract there are various classes of compounds such as fatty acids, phenolic, lipids, alkaloids, terpenoid, and volatile compounds. The major compounds in the extract were identified as di-n-octylphthalate (7.75%), benzyl alcohol (7.26%), Hi-oleic safflower oil (CAS) safflower oil (6.65%), benzeneacetonitrile (6.05%), and benztriazole (4.98%) as shown in peak 86, 6, 54, 17, and 27, respectively. Beside those major compounds, there were other minor compounds had been identified such as β-caryophyllene and farnesol as shown in peak 59 and 88 respectively

3.6. Morphology and Molecular Identity of Potential Ethyl Acetate Endophytic Fungi Isolate

AK32 isolate has a greyish-black surface colour with cottony-like texture, whereas the reverse colour was black

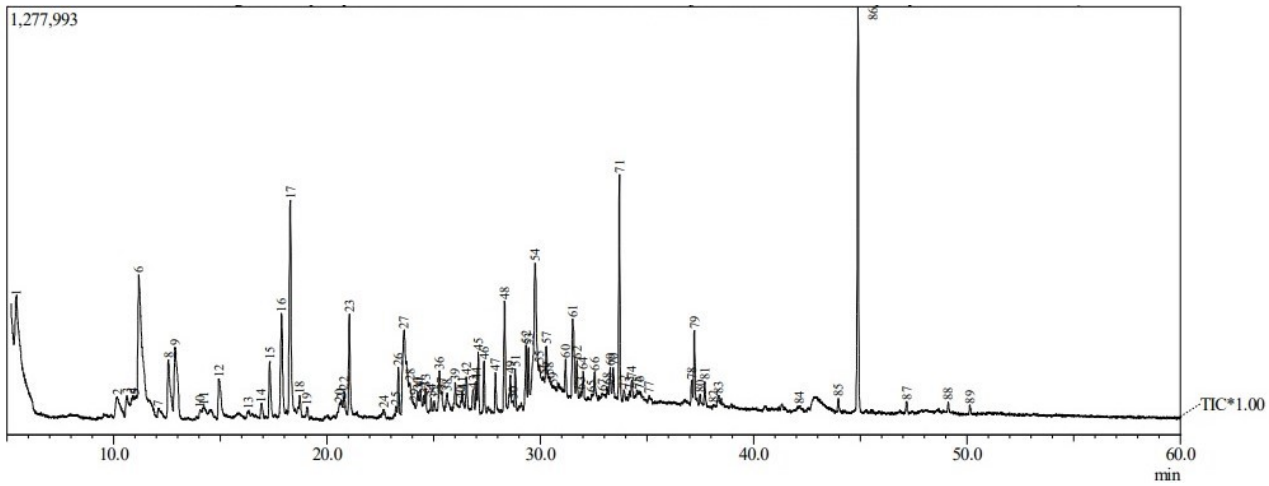

FIGURE 3 GC-MS chromatogram of AK32 ethyl acetate extract showed different retention time and % area.

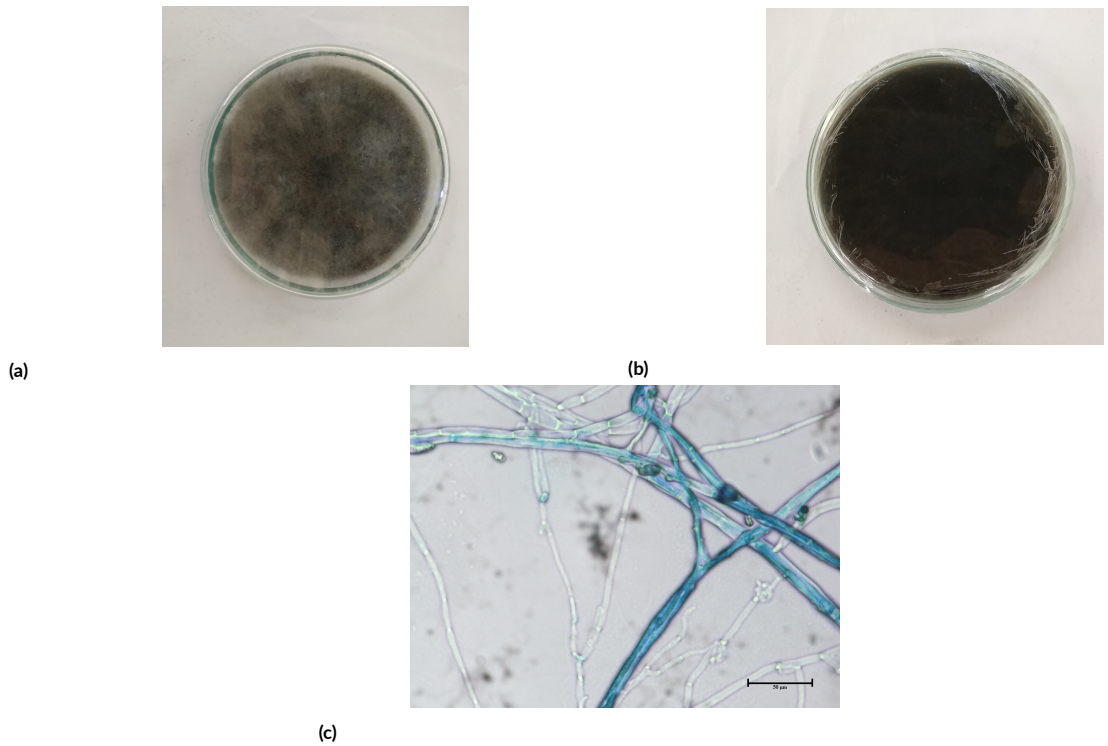

FIGURE 4 Morphological characteristic of AK32 isolate. A: surface colour and B: reverse colour of AK32 isolate after 7 days incubation on PDA; C: AK32 showed sterile mycelia.

(Fig. 4a-b). Microscopically, AK32 isolate showed sterile mycelia, so that AK32 isolate was classified as non-sporulating isolate (Fig. 4c). Strobel (2018) said that endophytic fungi frequently showed non-sporulating hyphae on PDA media, this occurred due to the inability of the endophytic to developed spore in the media. This indicates that AK32 isolate could not be identified based on its morphological characteristics because sterile mycelia do not show species-specific characteristics (Sun and Guo 2012).

The molecular identification based on the ITS rDNA region was used to validate the result from morphological identification. The amplicon of the ITS rDNA region was ± 550bp (Fig. 5).

Based on BLAST analysis, the potential antibacterial producing isolates have a similarity percentage of 99.60% with the Gene Bank database (Table 3). The ≥99% similarity to the database from Gene Bank indicates similarity at a species level (Liu 2011).

3.7. Discussion

Intensive search for new effective antimicrobial agents is required, which is facilitated by exploring new resources, especially endophytic fungi which have not been widely explored (Taufiq and Darah 2019). The antibacterial compound finding from endophytic fungi associated with endemic plants is a promising endeavor to answer the challenge in increasing the need for antibacterial drugs. In this study, nine isolates show antibacterial activity, and four of them (AK32, BK32, AKF1, and DK7) exhibited an antibacterial activity not only against Gram-positive bacteria, but also against Gram-negative bacteria with strong activity. These broad spectra of activity were affected by the existence and synergistic activity of various chemical compounds within the extract which have various mechanisms of action against Gram-positive and Gram-negative bacteria.

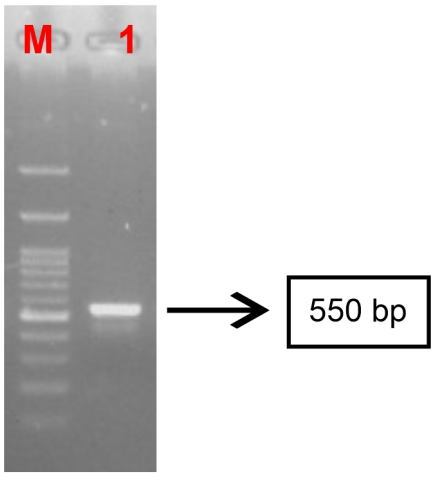

FIGURE 5 Electrophoregram ITS rDNA region amplicon of AK32 isolate, M: Marker DNA ladder 100bp, 1: AK32 isolate.

Another five ethyl acetate extracts of endophytic fungi displayed a narrow spectrum which effective only for Gram-positive bacteria. This indicated that the compound contained in this extract did not have mechanisms of action against Gram-negative bacteria. The more effectiveness of the extract against Gram-positive bacteria was also due to its morphological constituent of Gram-positive bacteria that have a simpler cell wall structure contained only the peptidoglycan layer as the outer barrier (Breijyeh et al. 2020). On the other hand, Gram-negative bacteria have a more complex barrier with additional protection afforded by the outer membrane contained lipopolysaccharide (LPS) which acts as barrier (Epand et al. 2016). This made the bioactive compounds that have no ability to damage the LPS layer, could not able to penetrate the inner cell of Gram-negative bacteria.

Among nine ethyl acetate extracts of endophytic fungi which had antibacterial activity against Gram-positive bacteria (*S. aureus*), eight of them have activity against resistant bacteria (MRSA). The mechanisms of action of the activity of the extract against MRSA was clearly demonstrated by SEM photograph's observation that there were morphological alteration with collapsed cell, and this could be do to the leak in it's cell wall or there were some changes occurred in the cell's membrane permeability. In this case, the damaged occurred by the extract of endophytic fungi could be cause by the extract had permeabilized the bacterial cell membrane that influenced the condition of bacteria. This probability was in line with study conducted by Taufiq and Darah (2019) who reported the extract of *Lasiodiplodia pseudotheobromae* IBRL OS-64 exhibited antibacterial activity by spoil the cell wall of bacterial cells which led to damaged of the cell. As shown by the SEM photograph, the most possible mechanisms of action involved could be the interaction of compound with membrane of the cell and might be linked with the ability of compound contained in the extract to inhibit the peptidoglycan synthesis process and penetrate the cell wall by after binding with penicillin-binding protein 2a (PBP2a). As known, MRSA has an altered PBP structure that is PBP2a caused by the existence of mecA gene, resulting in affinity reduction of β-lactam and being resistance to β-lactam antibiotics (Stapleton and Taylor 2002).

The highest antibacterial activity against all test bacteria was displayed by AK32 ethyl acetate extract. This extract had small MIC value (1.56%) indicating that AK32 only need a very small amount of bioactive compound to inhibit the bacteria. Bacterial cell treated with AK32 extract showed sign of damage, demonstrated by the shape

TABLE 3 BLASTn analysis of ITS rDNA gene coding sequence from potential antibacterial producing fungi.

No	Name of Isolate	Closest Species Identity	Identity	Query Cover	Accecion Number
1	AK32	*Botryosphaeria rhodina*	99.60%	99%	FJ941882.1

deformation and inward indention of the cell.

Through GC-MS analysis, ethyl acetate extract of AK32 showed several major compounds with different retention times. Di-n-octyl phthalate was one of the major compounds with the highest % area (7.75%). This compound belongs to the phthalate compound. Phthalate group of compound have known before as plasticizer and contaminant. Nevertheless, there are many study that reported phthalate compounds and their derivatives have isolated from natural sources (Thiemann 2021). Di-n-octyl phthalate were reported could be produced by various natural sources such as microbe like *Streptomyces parvusand* (Abd-Elnaby et al. 2016), *Trichoderma asperellum* (Bhardwaj and Kumar 2017), *Memnoniella* (Hande and Kadu 2015), and plant such as *Plumbago zeylanica* Linn. (Ira et al. 2015), *Pachygone ovata* (Poir). Miers (Amalarasi and Jothi 2019) and from *Coleus foskhohlii* (Takshak and Agrawal 2016). Thiemann (2021) also said that these compound have benefit for human health either. Boudjelal et al. (2011) in his study reported that di-n-octyl phthalate was a compound produced by the genus Actinoalloteichus and had antibacterial activity against *Bacillus subtilis*. Another study was conducted by Amalarasi and Jothi (2019) found that di-n-octyl pththalate isolated from *Pachygone ovata* (Poir). Miers possessed a cytostatic activity against breast cancer cell. Another major compounds were benzyl alcohol (7.26%), benzeneacetonitrile (6.05%), benzotriazole (4.98%), octadecanoic acid, methyl ester (3.85%); cyclopentenone (3.48); 2,4-decadienal, (E,E)- (3.00%); 1,2-ethanediol, diacetate (2.87%); and 9,12,15-octadecatrienal (2.41%) also have been reported had antibacterial activity (Yano et al. 2016; Wei et al. 2011; Wu et al. 2018; Trombetta et al. 2002; Halstead et al. 2015; Boudjelal et al. 2011).

In addition to those compounds, minor compounds found in AK32 extract such as β-caryophyllene and farnesol also reported having antibacterial activity. These both compounds belong to terpenoid group. Dahham et al. (2015) said that β-caryophyllene is a terpene that not only has high antibacterial activity but also has antifungal and antioxidant activities. Whereas, farnesol is a sesquiterpene compound that is known to have antibacterial activity against *S. aureus* (Inoue et al. 2016). Based on these findings, AK32 ability to inhibit test bacteria may be due to the synergetic collaboration between major and minor compounds within the extract.

From morphological and molecular identification, it is known that AK32 isolate is *Botryosphaeria rhodina*. *B. rhodina* is a teleomorph of *Lasidioplodia theobromae*. This fungus has been known as an endophyte and opportunistic pathogen. As endophytic fungi *B. rhodina* has various pharmaceutical activities, including antibacterial. Rukachaisirikul et al. (2009) in his research found that *B. rhodina* had antibacterial activity against *S. aureus* ATCC 25922 and MRSA by producing g-lactone, one dihydronaphthalene-2,6-dione, one hexahydro indeno furan, one cyclopentanone, and lasiodiplodine. Our study has revealed that *B. rhodinia* besides having antibacterial against *S. aureus* and MRSA, it also has the same effect on *E. coli* by producing different bioactive compounds. The difference between these compounds might be affected by the difference of the host plant. *B. rhodina* is also known has another activity. Abdou et al. (2010) revealed that *B. rhodina* isolated from medical plant *Bidens pilosa* has antifungal activity and cytotoxic depsidones by producing *Botryorhodines* A-D. The finding in this study provides a basis for further research such as isolation that focuses the target antibacterial compound to be developed as a new drug.

4. Conclusions

This study concludes that the endophytic fungal extracts isolated from *M.casturi*, endemic plant of South Kalimantan, Indonesia exhibit antibacterial activity due to the bioactive natural compounds production. AK32 isolate was the highest isolate produced antibacterial compound such as di-n-octyl phthalate, phenol, 2-methyl-, 4-pentadecyne, 15-chloro-, benzene acetonitrile, and benztriazole which had mechanism of action by damaging cell wall in gram-positive bacteria and damaging the outer membrane layer in gram-negative bacteria. AK32's compounds found by GC-MS analysis provided new information for developing new antibacterial agents from endophytic fungi. These active compounds can then be used as a source of antibacterial substance to support *M. casturi* conservation endeavor.

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Authors' contributions

FRN, RS, AS designed the study. FRN carried out the laboratory work. FRN, RS, AS analyzed the data. FRN, RS, AS wrote the manuscript. RS provided funding in the study. All authors read and approved the final version of the manuscript

Competing interests

The author attested that there were no conflicts of interest concerning in this study.

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