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Bioactivity of Endophytic Fungi *Fusarium brachygibbosum* Isolated from the Stem of *Avicennia marina* as a Candidate for New Drug Source

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ABSTRACT

Indonesia's biodiversity holds significant potential as a source of medicinal compounds, one of which is *Avicennia marina*, known for its antioxidant and antibacterial properties. However, due to its protected conservation status, its utilization is limited. An alternative approach to harness its benefits is through symbiotic microorganisms such as endophytic fungi. This study aimed to isolate and evaluate the bioactivity of the endophytic fungus *Fusarium brachygibbosum* isolated from the stem of *A. marina* as a potential source of novel therapeutic agents. The methodology involved fungal isolation, morphological identification, and evaluation of antibacterial activity using the disc diffusion method and antioxidant activity using the DPPH assay. The results demonstrated that the obtained isolate (coded ADM2) exhibited strong antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Bacillus subtilis*. Moreover, the antioxidant assay of the ADM2 isolate revealed a very strong potential (IC_{50} < 20 µg/mL), indicating its potent ability to scavenge free radicals. Based on these findings and the literature on *F. brachygibbosum*, this species shows great potential as a source of bioactive compounds with medical applications, particularly in treating infections and preventing degenerative diseases. Further in vivo studies are necessary to confirm the therapeutic potential of this fungus as a safe and effective drug candidate.

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1. INTRODUCTION

Indonesia, with its extraordinary biodiversity, harbors thousands of plant species with medicinal properties [1]. With more than 30,000 plant species distributed across the archipelago, many have long been used in traditional medicine by local communities [2]. This diversity includes a wide range of plants with the potential to serve as sources of bioactive compounds for the development of new drugs, from tropical rainforest species to coastal vegetation [3]. However, the sustainability of these natural resources is increasingly under threat due to land conversion and overexploitation, compelling us to find new ways to utilize medicinal plants sustainably without disrupting their ecosystems [4]. One such plant with significant therapeutic potential but facing conservation challenges is *Avicennia marina* [5].

Avicennia marina, commonly known as the grey mangrove, is widely distributed along Indonesia's coastlines and is recognized for its significant biological activities, including antioxidant and antibacterial

properties [6]. Recent studies have revealed that extracts from its roots and leaves contain bioactive compounds such as flavonoids, saponins, and terpenoids, which can be used in the treatment of various diseases [7]. However, the protected conservation status of *A. marina* limits its widespread utilization, particularly with the increasing demand for natural raw materials in the pharmaceutical industry [8]. Therefore, finding alternative strategies to explore its potential without harming its natural population has become essential, one of which is through the symbiotic relationship between *A. marina* and its associated microorganisms, such as endophytic fungi [5], [9].

Endophytic fungi, which live within plant tissues without causing disease, have emerged as an intriguing solution in the search for bioactive compounds [10]. In addition to playing a role in plant defense against pathogens, endophytic fungi are known to produce biologically active metabolites comparable to those of their host plants [11]. Several studies have reported that these fungi can synthesize antioxidant, antibacterial, antifungal, and anti-inflammatory compounds that are highly valuable for drug development [12]. In some cases, the compounds produced by endophytic fungi are even more potent than those found in their host plants [13]. This opens a significant opportunity to utilize endophytic fungi as a novel source of therapeutic agents that can complement or replace conventional medicine [14]. One particularly promising endophytic fungus is *Fusarium brachygibbosum* [15].

The endophytic fungus *F. brachygibbosum* is relatively rare. Although generally known as a plant pathogen, it possesses the ability to produce secondary metabolites with antibacterial, antifungal, antioxidant, and even cytotoxic activities [16]. Recent research has shown that compounds produced by *F. brachygibbosum* can be effective against infections caused by antibiotic-resistant bacteria and exhibit strong antioxidant potential. Therefore, *F. brachygibbosum* isolated from the roots of *A. marina* holds great promise for development as a source of more effective and environmentally friendly pharmaceutical agents, while also unlocking the hidden potential of Indonesia's coastal ecosystems rich in biodiversity. Thus, this endophytic fungus represents a valuable alternative for sustainable utilization of Indonesia's natural wealth, offering benefits for both local and global health.

2. RESEARCH METHOD

Isolation and Identification of Endophytic Fungi Based on Morphological Characteristics

The endophytic fungi were isolated from the stem of *Avicennia marina*. Fresh samples were washed with distilled water and surface-sterilized using 70% ethanol for 1 minute, followed by sodium hypochlorite solution for 1 minute, and then rinsed with sterile distilled water. The sterilized samples were cut into small segments and placed on Potato Dextrose Agar (PDA) medium. The plates were incubated at room temperature for 3–7 days. Fungal hyphae emerging from the plant tissues were transferred to fresh PDA plates to obtain pure isolates [17].

Morphological identification of the endophytic fungi was performed by observing both macroscopic and microscopic characteristics. Macroscopic observations included colony color, texture (cottony, granular, or mucilaginous), and radial or concentric growth patterns on PDA medium [18]. For microscopic observation (SEM), the slide culture method was employed to examine the hyphae and spores. The obtained morphological data were compared with existing literature for fungal species identification. Endophytic fungi exhibiting the most potent bioactivity were further subjected to molecular identification [18], [19].

Cultivation and Extraction of Endophytic Fungi

The isolates were initially grown on Potato Dextrose Agar (PDA) and incubated for 5–7 days. After sufficient growth, the isolates were transferred into Potato Dextrose Broth (PDB) and cultured under static conditions for 30 days to promote secondary metabolite production. The extract profile was compared with a negative control, which contained only PDB without the fungal isolates. Following incubation, the culture broth was extracted using ethyl acetate to obtain the crude extract, which was subsequently concentrated using a rotary evaporator [14].

Antioxidant Activity Test

The antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Crude extracts for the antioxidant assay were prepared by first dissolving 4 mg of the extract in 4 mL of methanol to obtain a stock solution of 1000 µg/mL. Serial dilutions were then performed to achieve concentrations of 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL. Each concentration was prepared in triplicate for the assay. Each sample (0.2 mL) was mixed with 3.8 mL of 0.5 mM DPPH solution and incubated in the dark for 30 minutes. The decrease in absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage of free radical scavenging activity was calculated using the following formula [17], [20].

$$\% \textbf{ Inhibition} = \frac{\mathbf{A_k - A_s}}{\mathbf{A_s}}$$

A_k = Absorbance of control
A_s = Absorbance of samples

Antioxidant activity was categorized based on the IC_{50} values (in µg/mL) as follows: an IC_{50} value of <20 µg/mL indicates very strong antioxidant activity; an IC_{50} value of <100 µg/mL represents strong antioxidant activity, and an IC_{50} value ranging from 100 to 500 µg/mL is classified as moderate antioxidant activity. These categories help in assessing the potency of the extract in scavenging free radicals, with lower IC_{50} values indicating higher antioxidant efficiency [21].

Antibacterial Activity Test

The antibacterial activity was evaluated using the disc diffusion method. Sterile paper discs (6 mm in diameter) were impregnated with the endophytic fungal extract (400 µg/mL) and placed onto Mueller-Hinton Agar (MHA) plates previously inoculated with bacterial cultures. The plates were incubated at 37°C for 24 hours. The diameter of the inhibition zones around the discs was measured to assess antibacterial effectiveness. Tetracycline (30 µg/disc) was used as a positive control, while ethyl acetate served as the solvent control [22].

3. RESULT AND DISCUSSION

A single endophytic fungal isolate (code ADM2) was successfully isolated from the stem of *A. marina*. Identification was performed based on its morphological characteristics (Table 1). The morphological features are presented in Figure 1.

Table 1. Macroscopic and Microscopic Characteristics of Isolate ADM2

Observation	Parameter	Description
Macroscopic	Surface colony	Whitish-cream to yellowish surface, with the central area showing a deeper yellow (a).
	Reverse colony	Deep golden yellow at the center, fading to white towards the margin (b).
	Structure	Compact colony with slightly floccose (fine fibrous) surface.
	Elevation	Slightly raised at the center, gradually sloping towards the edges.
	Pattern	Distinct concentric rings with a denser central zone.
	Exudate drops	No visible exudate droplets observed on the colony surface.
	Radial line	Radial lines not prominent.
	Concentric line	Concentric rings clearly visible from center to margin.
Microscopic	Shape	Interwoven hyphal network forming a dense mycelial mat c(1).
	Hyphae	Septate, thin-walled, irregularly branched hyphae c(1).
	Spora	Macroconidia falcate (crescent-shaped), mostly 3–5 septa, with tapering apical cells c(3); microconidia oval to ellipsoid, borne on monophialides c(2).
	Distinctive characteristics	Simple conidiophores with monophialides; medium to large-sized smooth-walled macroconidia.

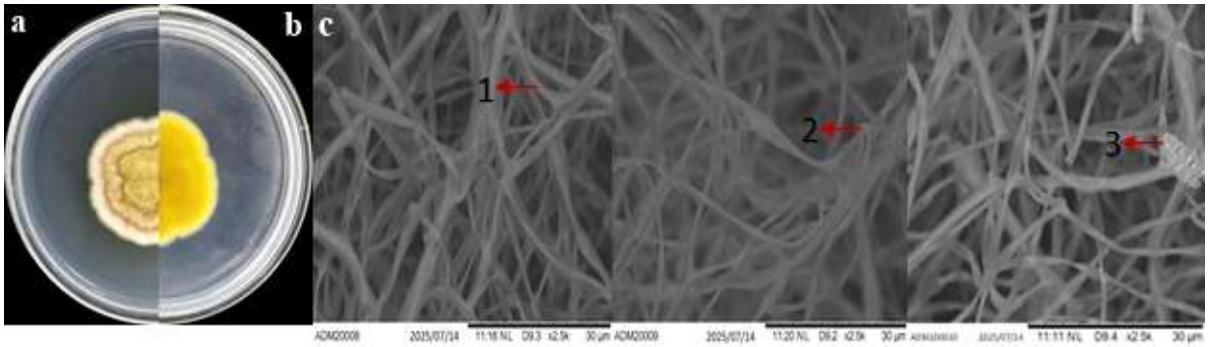

Figure 1 Macroscopic and Microscopic Characteristics of *ADM2*. a: front view, b: riverse view, c (1): hyphae, (2): microconidia, (3): macroconidia (Scale bar = 30 µm).

Figure 1 shows the morphological characteristics (front view, reverse view, and microscopic structure) of isolate ADM2 obtained from the stem of *A. marina*. Macroscopic observations revealed that the fungal colony exhibited a white to cream color on the upper surface and yellow on the reverse side, with irregular colony margins. The growth pattern displayed concentric rings and radial lines, indicating distinct growth zones. Microscopically, the fungus produced conidia (both microconidia and macroconidia) with septate hyphae. Based on these characteristics, isolate ADM2 was identified as belonging to the genus *Fusarium*. Subsequently, the bioactivity of isolate ADM2 was evaluated, as presented in Table 2 and Figure 2.

Table 2. Antioxidant and Antibacterial Activity of ADM2 Extract

Sample	Antioxidant Activity IC_{50} (µg/mL)	(%) Antibacterial Activity			
		E. coli	***S. typhi***	***S. aureus***	***B. subtilis***
ADM2	19,394****	76.3 ± 0.68***	75.6 ± 0.39***	81.8 ± 0.64***	80.8 ± 0.13***
Positive control	Ascorbic acid 10,083****	Tetracycline 100***	Tetracycline 100***	Tetracycline 100***	Tetracycline 100***

Note: Antibacterial activity percentage: *** ≥ 70% (strong), **50–70% (moderate), *< 50% (weak). Antioxidant activity IC_{50} (μg/mL): ****very strong <20 μg/mL; ***strong <100 μg/mL; **moderate 100–500 μg.

Table 2 presents the antibacterial and antioxidant activities of the endophytic fungal extract ADM2 isolated from the stem of *A. marina*. The antibacterial data demonstrated strong activity against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Bacillus subtilis*. The antioxidant activity was determined based on the IC_{50} value, indicating a very strong free radical scavenging capacity.

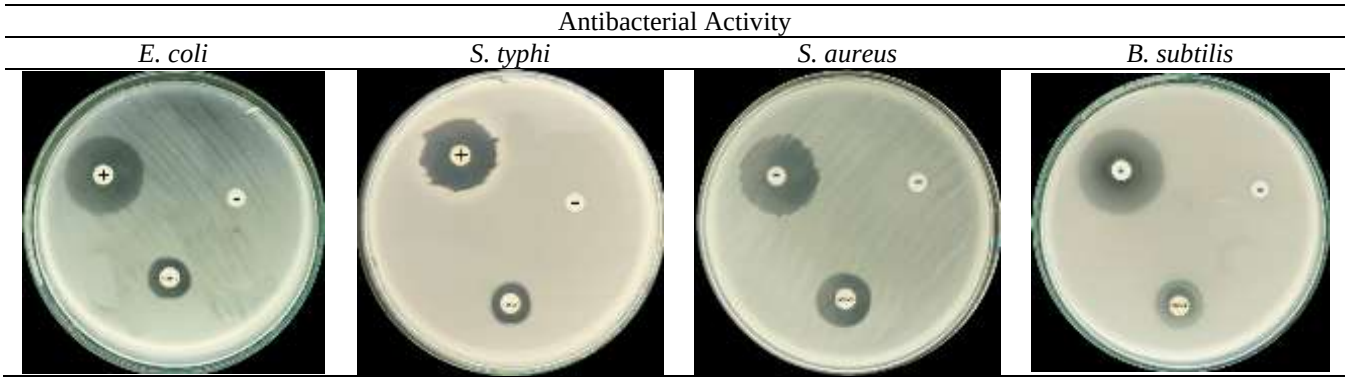

Figure 2 Antibacterial activity test results of the endophytic fungal extract *Fusarium brachygibbosum* isolated from the stem part of *Avicennia marina* using the disc diffusion method (a: positive control, b: negative control, c: ADM2 extract of *F. brachygibbosum*).

Based on its highly promising bioactivity, molecular identification was carried out on isolate ADM2 and it was found that the isolate was closely related to *Fusarium brachygibbosum*. The primers used for molecular identification of the endophytic fungus were ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') targeting the Internal Transcribed Spacer (ITS) region. The molecular identification results are shown in Figure 3, with the sequence as follows:

TCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCC
CTGTGAACATACCTTTATGTTGCCTCGGCGGATCAGCCCGCGCCCCGTAATACGGGACGGCCCGCC
GCAGGAACCACAAAACTCTGATTTTAGTGTAACTTCTGAGTCTAAAAAACAAATAAATCAAAACTT
TCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAACTAATGTGA
ATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGC
ATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCCCCGGGTTTGGTGTTGGGGATCGGGCTGTACT
CCAGCCCGGCC

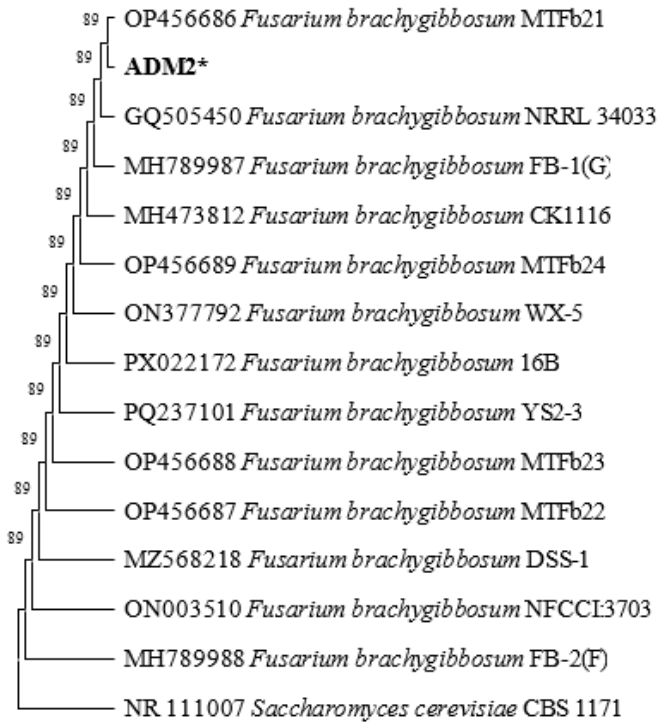

Figure 3. Neighbour-Joining was used to Create a PhylogeneticTree for ADM2* with a Bootstrap Value of 1000

Fusarium brachygibbosum belongs to the genus *Fusarium*, which is widely recognized for its ability to act as a pathogen in various plants. The genus itself has a broad spectrum, with many species responsible for plant diseases, particularly in staple crops such as maize and wheat [23]. However, not all species within this genus are pathogenic. Some, such as *F. brachygibbosum*, live as endophytes, meaning they interact with their host plants without causing visible disease symptoms [24]. This fungus is commonly found in tropical and subtropical plants, and its presence in diverse plant ecosystems indicates a high level of adaptation to natural environments [25]. Its occurrence as an endophyte in tropical plants suggests its ability to thrive under various environmental conditions, contributing to the host plant's resilience against physical and biotic stress factors. In many cases, this fungus not only survives within its host but also produces metabolites that benefit the plant, such as reducing infections caused by other pathogens or enhancing tolerance to drought and salinity.

As an endophytic fungus, *F. brachygibbosum* produces a variety of secondary metabolites with significant therapeutic potential, including strong antioxidant and antibacterial activities. In this study, the bioactivity assays revealed very strong antioxidant activity (IC_{50} < 20 µg/mL) and strong antibacterial activity against four tested bacteria: *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Bacillus subtilis*. These secondary metabolites are by products of the fungal metabolic pathways, not directly involved in its growth, but essential for the defense of the host plant against pathogens, predators, and environmental stress [26]. As an endophytic fungus, *F. brachygibbosum* produces biologically active compounds that are beneficial both for its host plant and for potential medical applications. Several secondary metabolites produced by *F. brachygibbosum* are known to exhibit strong antioxidant activity. This activity is attributed to compounds such as flavonoids, phenolics, and terpenoids, which can scavenge free radicals in the body [27]. Free radicals are molecules that can damage cells and contribute to the development of various degenerative diseases, including cancer, cardiovascular diseases, and premature aging [28]. The antioxidant compounds from *F. brachygibbosum* help combat oxidative stress caused by the accumulation of free radicals, thereby protecting cells from damage and maintaining overall health.

In addition to its antioxidant activity, *F. brachygibbosum* also produces metabolites with strong antibacterial properties. The antibacterial compounds produced by this fungus can inhibit the growth of various pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Bacillus subtilis*, which are known to cause infections that are difficult to treat, especially with conventional antibiotics. Studies on *F. brachygibbosum* extracts indicate that its antibacterial compounds hold potential for treating bacterial infections, not only limited to skin infections but also more severe internal infections.

Overall, the secondary metabolites produced by *Fusarium brachygibbosum* demonstrate remarkable potential in the development of new pharmaceuticals with strong antioxidant and antibacterial activities. These compounds may be used not only for treating bacterial infections but also for preventing oxidative stress-related diseases, such as cancer and cardiovascular disorders [29]. The ability of *F. brachygibbosum* to produce such

bioactive compounds makes it a highly promising candidate in pharmaceutical research, where the discovery of effective and safe new compounds is essential to address current global medical challenges. Further in vivo studies are required to strengthen the evidence for the therapeutic potential of these compounds and to explore their possible development into safe and effective pharmaceutical products. In vivo testing will help determine the proper dosage, safety profile, and long-term effectiveness of these bioactive compounds before they can be further developed into drugs for human use.

4. CONCLUSION

Fusarium brachygibbosum isolated from the stem of *Avicennia marina* demonstrated remarkable bioactivity, with very strong antioxidant capacity and significant antibacterial effects against several pathogenic bacteria. These findings highlight its potential as a promising natural source of bioactive compounds for future drug development, particularly in addressing challenges such as oxidative stress-related diseases and antibiotic resistance. While the results are encouraging, further in vivo and toxicity studies are required to validate its safety and therapeutic efficacy.

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