Science and Technology Indonesia
e-ISSN:2580-4391 *p*-ISSN:2580-4405
Vol. 10, No. 1, January 2025

Research Paper

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Biodiversity and Analysis of Antioxidant and Antibacterial Activity of Endophytic Fungi Extracts Isolated from Mangrove *Avicennia marina*

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Abstract
Avicennia marina, a mangrove species commonly found along coastal areas, plays both ecological and pharmacological roles, with its plant parts exhibiting antioxidant and antibacterial activities. This study aimed to investigate the diversity of endophytic fungi from various organs of *A. marina* collected from mangrove ecosystems and to explore and analyze their antioxidant and antibacterial activities. Endophytic fungi were isolated from the roots, stems, and fruits of *A. marina* using PDA medium and were morphologically identified. Each fungal isolate was cultivated in PDB medium for 4 weeks under static conditions, followed by extraction to obtain concentrated extracts. Antioxidant and antibacterial activities were assessed using the DPPH method and disk diffusion assay. A total of 23 fungal isolates were obtained from the roots, stems, and fruits of *A. marina*. The identification results showed that the root isolates had the highest genus diversity, followed by the stem and fruit isolates. The highest distribution of antioxidant and antibacterial activities was observed in the endophytic fungal extracts from fruits, followed by those from roots and stems. Notably, the majority of the 23 endophytic fungal extracts exhibited strong antioxidant and antibacterial activities. Isolates AMF3 and AMF6 showed the most potent antioxidant activity, classified as very strong, with IC_{50} values below 20 μg/mL. Morphological identification revealed AMF3 as *Neopestalotiopsis* sp. and AMF6 as *Aspergillus niger*. This study highlights the potential of *Neopestalotiopsis* sp. and *Aspergillus niger* endophytic fungi from *A. marina* fruits as sources of natural antioxidant and antibacterial compounds, offering valuable insights for biotechnological applications of mangrove-associated endophytes.

Keywords
Antibacterial, Antioxidant, *Avicennia marina*, Biodiversity, Endophytic Fungi

Received: 9 September 2024, Accepted: 1 November 2024
https://doi.org/10.26554/sti.2025.10.1.139-151

1. INTRODUCTION

Avicennia marina, a mangrove species commonly found in intertidal zones, has garnered significant attention for its biological adaptations to stressful environments (Omomowo et al., 2023; Yang et al., 2023). Previous research has demonstrated that various parts of *A. marina*, including the bark, roots, and fruits, exhibit antioxidant, antimicrobial, and anticancer properties (Alsaadi et al., 2022; Cerri et al., 2022). However, despite the extensive research on this plant, little is known about the endophytic fungi associated with *A. marina* and their bioactive potential, leaving a substantial gap for exploration (Al Husnain et al., 2023; Hashem et al., 2023).

Endophytic fungi are plant microbiomes that inhabit plant tissues without causing harm (Jha et al., 2023). These fungi have gained recognition for their ability to produce bioactive compounds, offering alternatives to synthetic drugs or agrochemicals (Chaachouay and Zidane, 2024; Oktiansyah et al., 2018). In particular, mangrove ecosystems provide a unique environment where endophytic fungi thrive due to harsh conditions, such as high salinity, elevated temperatures, and tidal influences (Dittmann et al., 2022; Tobing et al., 2022). The resilience of these fungi in such environments suggests their potential for producing novel bioactive secondary metabolites (Conrado et al., 2022). Studies have shown that endophytic fungi can exhibit superior bioactivity compared to their host plants, owing to their unique metabolic pathways influenced by the symbiotic relationship with the host. The limited understanding of endophytic fungi from *A. marina* constrains the development of potential biotechnological applications, such as novel drug discovery or agricultural biocontrol agents (Gupta et al., 2023; Song et al., 2023).

Recent research has revealed that endophytic fungi isolated from mangrove species are capable of producing a variety of bioactive secondary metabolites. For example, endophytic fungi from *Rhizophora mucronata*, another mangrove species, have demonstrated significant antimicrobial and anticancer activities (Eshboev et al., 2023). Similarly, endophytes from *Sonneratia apetala* have shown promising antioxidant and anti-inflammatory properties (Uddin et al., 2024; Wu et al., 2021). These findings underscore the potential of mangrove-associated fungi as sources of bioactive compounds with pharmaceutical relevance. Moreover, research on terrestrial plant endophytes has provided compelling evidence of the diverse metabolic capabilities of these fungi (Alam et al., 2021). Studies on fungi from plants such as *Taxus brevifolia* and *Catharanthus roseus* have shown that they can produce bioactive compounds in quantities equal to or greater than their host plants (Varghese et al., 2024). This evidence suggests that endophytic fungi residing in *A. marina* may also possess bioactive properties superior to those of the host plant (Kumari et al., 2023; Yang et al., 2023). Given the environmental pressures faced by mangroves, it is likely that endophytic fungi inhabiting *A. marina* tissues have developed unique metabolic adaptations, leading to the production of novel bioactive metabolites (El-Nagar et al., 2024; Tripathi et al., 2022).

Despite the promising findings from other mangrove species, the specific bioactivity of endophytic fungi within *A. marina* remains largely unexplored. While studies have focused on the plant's own bioactive properties, the endophytes that may contribute to or enhance these properties have not been thoroughly investigated. Therefore, isolating and evaluating these endophytes could provide valuable insights into their potential applications in the pharmaceutical field.

2. EXPERIMENTAL SECTION

2.1 Materials

This study utilized endophytic fungi isolated from *Avicennia marina* collected from a mangrove area at GPS coordinates 2.35717187S 10491981381E 74°E. The materials used for isolation included Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) from Oxoid to culture the fungi. Ethyl acetate was used as a solvent for extraction. Reagents such as methanol, physiological NaCl solution, and 70% alcohol from Sigma-Aldrich (D9132) were employed for sterilization and preparation processes. DPPH (2,2-diphenyl-1-picrylhydrazyl) from Sigma-Aldrich was utilized to assess antioxidant activity. For antibacterial activity testing, the bacteria used were *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Salmonella typhi*. Tetracycline was used as a positive control for comparison.

2.2 Instrumentation and Characterization

Antioxidant activity test used spectrophotometer UV-Vis (Shimadzu UV-1900) to check the absorption of DPPH while antibacterial activity test used Laminar Air Flow (LVG-4AG-F8) to avoid the contamination. Microscopic observation of endophytic fungal morphology using a Hirox 1000 digital microscope.

2.3 Isolation and Identification of Endophytic Fungi Based on Morphological Characteristics

Endophytic fungi were isolated from the bark, roots, and fruits 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. Endophytic hyphae growing around the tissues were transferred to fresh PDA plates to obtain pure isolates (Elfita et al., 2023).

The morphological identification of endophytic fungi was conducted by observing both macroscopic and microscopic characteristics. Macroscopic observations included colony color, texture (cottony, granular, mucilaginous), and radial or concentric ring patterns on the PDA medium. For microscopic observation, the slide culture method was used to examine hyphae and spores at 1000× magnification. These observations were compared with existing literature to identify fungal species (Oktiansyah et al., 2023a).

2.4 Cultivation and Extraction of Endophytic Fungi

The fungal isolates were cultured on PDA and incubated for 5-7 days. Once growth was observed, the isolates were transferred to PDB medium and cultured under static conditions for 30 days to produce secondary metabolites. After incubation, the liquid medium was extracted using ethyl acetate to obtain crude extracts, which were then concentrated using a rotary evaporator (Oktiansyah et al., 2023b).

2.5 Antioxidant Activity Test

Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Extracts at concentrations of 1000, 500, 250, 125, 62.5, 31.25, and 15.625 μg/mL (in triplicate) were mixed with 3.8 mL of 0.5 mM DPPH solution in 0.2 mL aliquots and incubated in the dark for 30 minutes. The decrease in absorbance was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. The percentage of free radical scavenging activity was calculated using the following formula (Oktiansyah et al., 2023b):

$$\%\text{Inhibition} = \frac{A_k - A_s}{A_s} \quad (1)$$

A_k = Absorbance of control

A_s = Absorbance of samples

2.6 Antibacterial Activity Test

Antibacterial activity was evaluated using the disk diffusion method. Paper disks (6 mm in diameter) were soaked with fungal extracts (400 μg/mL) and placed on Muller-Hinton Agar (MHA) plates inoculated with bacterial cultures. The plates were incubated at 37°C for 24-48 hours. The diameter of the inhibition zones around the disks was measured to assess antibacterial effectiveness. Tetracycline (30 μg/disk) was used as a positive control, while ethyl acetate served as the solvent control (Elfita et al., 2024; Hapida et al., 2021; Oktiansyah et al., 2024).

3. RESULT AND DISCUSSION

3.1 Identification Morphologically

Endophytic fungi were successfully isolated and identified from *A. marina*, consisting of 8 isolates from roots (AMA1-AMA8), 9 isolates from stems (AMB1-AMB9), and 6 isolates from fruits (AMF1-AMF6). Identification was performed by observing their morphological characteristics (Table 1 and Table 2). Each isolate exhibited varying morphological features, as shown in Figures 1-4.

The figures display the morphological characteristics of endophytic fungi from *A. marina*. The identification results revealed a diverse range of endophytic fungal species from different parts of *A. marina*. The root had 8 isolates, including *Trichoderma, Colletotrichum, Mortierella, Diaporthe, Pythium, Aspergillus*, and *Neopestalotiopsis* (Figure 1). The stem contained 9 isolates belonging to the genera *Colletotrichum, Fusarium, Aspergillus, Trichoderma, Penicillium*, and *Paecilomyces* (Figure 2). The fruit contained 6 isolates from the genera *Eurotium, Aspergillus, Neopestalotiopsis, Pythium,* and *Colletotrichum* (Figure 3).

Microscopic characteristics of each isolate endophytic fungi can be seen in Figure 4. Based on these findings, two genera, *Aspergillus* and *Colletotrichum*, were found in all organs. The endophytic fungi *Aspergillus* and *Colletotrichum* are frequently found in various plant organs due to their high adaptability to environmental conditions and host tissues (Khan et al., 2024; Paulussen et al., 2017). *Aspergillus* is known for its ability to thrive in diverse environmental conditions and utilize a wide range of nutrient sources available within plant tissues. Meanwhile, *Colletotrichum* possesses the capacity to interact with various plant species, functioning both as a pathogen and an endophyte, which allows it to persist and develop in different plant tissues without causing significant damage to its host (Cannon et al., 2012; Khodadadi et al., 2020). These physiological and biochemical adaptations make both genera highly common as endophytes in various plant organs.

3.2 Bioactivity of Endophytic Fungi Isolated from *Avicennia marina*

The endophytic fungi isolated from *A. marina* exhibited varying antioxidant and antibacterial activities (Table 3). Several extracts from the endophytic fungal isolates demonstrated both antioxidant effects and antibacterial activity against the four tested microorganisms. The extract from isolate AMF3 displayed the most potent bioactivity compared to the other isolates.

Table 3, Figure 5, and Figure 6 present the results of antioxidant and antibacterial activities from methanol extracts of the host plant and endophytic fungal extracts isolated from various parts of *Avicennia marina* (roots, stems, and fruits). The antibacterial data demonstrate effectiveness against *Escherichia coli, Staphylococcus aureus, Salmonella typhi*, and *Bacillus subtilis*. Antioxidant activity was measured using IC_{50} values, indicating the extract's ability to scavenge free radicals. In antibacterial tests, endophytic fungal isolates showed more prominent results compared to the host plant extracts. Isolates AMA4, AMF3, and AMF6 exhibited the highest activity, with antibacterial activity exceeding 80% against all tested bacteria, making them highly potent for inhibiting bacterial growth. In contrast, the host plant extracts, such as those from the roots and stems of *A. marina*, showed moderate antibacterial activity, ranging from 50% to 70%, with some exceptions like the fruit extract, which exhibited slightly higher activity.

In antioxidant testing, several endophytic fungal isolates displayed very strong activity, particularly isolates AMF3 and AMF6, with IC_{50} values below 20 μg/mL. These IC_{50} values are significantly lower compared to the host plant extracts, where the IC_{50} of root, fruit, and stem extracts of *A. marina* ranged between 24.316 and 30.972 μg/mL, indicating strong antioxidant activity. Based on the antibacterial and antioxidant results, AMF3 and AMF6 were identified as the most promising isolates. Morphological identification revealed that isolates AMF3 and AMF6 correspond to *Neopestalotiopsis* sp. and *Aspergillus niger*, respectively.

The endophytic fungus *Neopestalotiopsis* sp. exhibits excellent antioxidant and antibacterial activities due to its ability to produce various bioactive compounds that play a crucial role in these biological activities (Gouda et al., 2016; Hashem et al., 2023; Omomowo et al., 2023). Recent studies have demonstrated that *Neopestalotiopsis* sp. is capable of synthesizing secondary metabolites such as alkaloids, flavonoids, phenolics, and terpenoids, which have potential as antioxidant and antibacterial agents. These compounds function by neutralizing free radicals and preventing oxidative damage to cells, which is the primary mechanism behind their antioxidant activity (Zandavar and Babazad, 2023).

The strong antioxidant activity of *Neopestalotiopsis* sp. is also attributed to its ability to produce antioxidant enzymes such as catalase and superoxide dismutase, which protect host cells from oxidative stress (Forman and Zhang, 2021; Losada-Barreiro et al., 2022; Tumilaar et al., 2023). Other research has revealed that the phenolic compounds produced by this endophytic fungus can inhibit lipid peroxidation and enhance free radical scavenging activity, significantly contributing to its potential as an antioxidant agent (Zandavar and Babazad, 2023).

Moreover, the antibacterial activity of *Neopestalotiopsis* sp. is highly significant due to its production of antimicrobial metabolites, such as pestalotins, which are effective against various pathogenic bacteria, including *Escherichia coli, Staphylococcus aureus*, and *Bacillus subtilis*. These compounds function by disrupting bacterial cell walls, inhibiting protein synthesis, and modulating bacterial resistance mechanisms, leading to microbial cell death. The strong antibacterial activity of *Neopestalotiopsis* sp. has been documented in several studies, highlighting its potential as a source of novel antimicrobial compounds (Darapanit

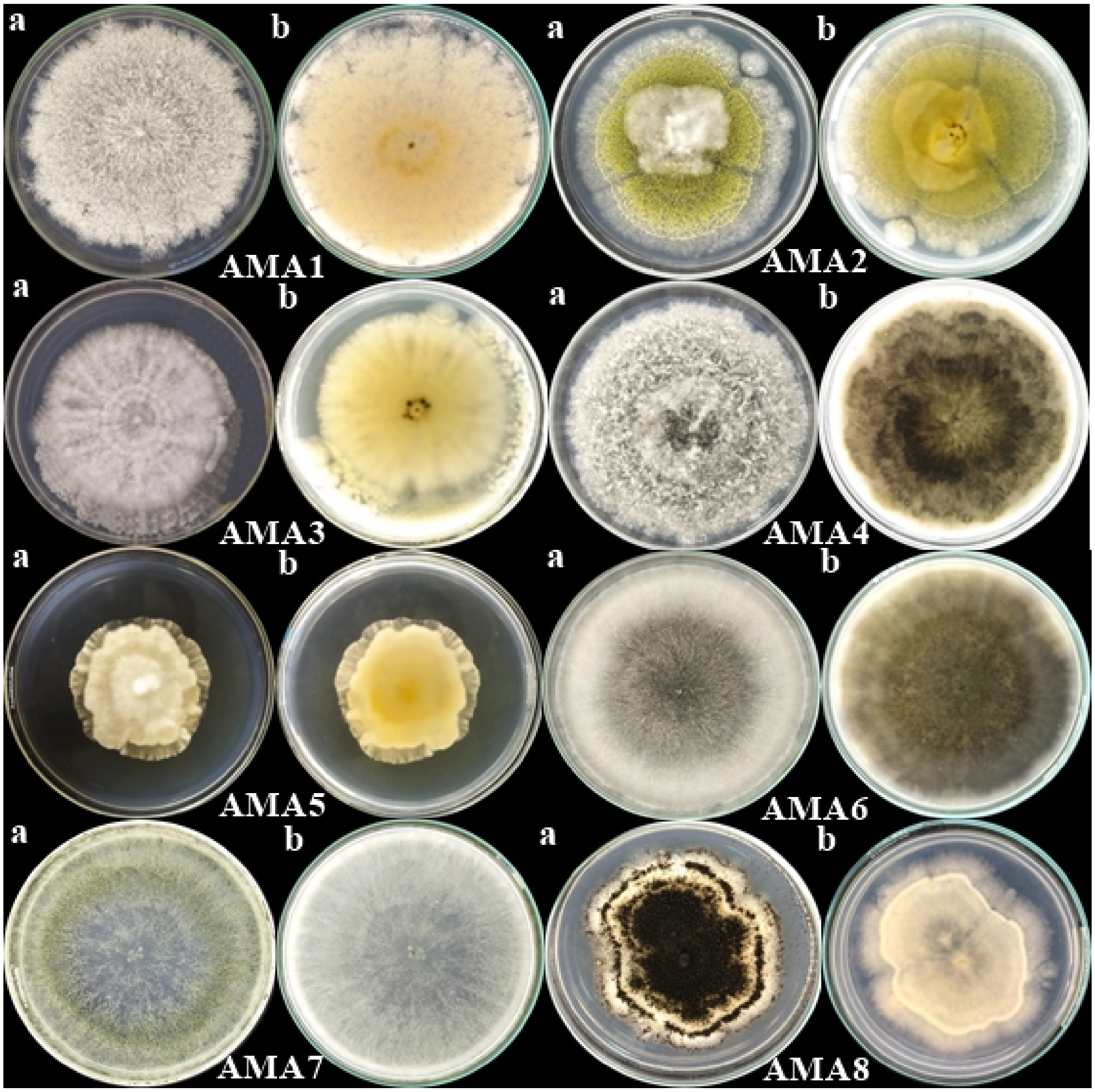


Figure 1. Colony Characteristics of Endophytic Fungi from the Roots of *Avicennia marina* (a: front view; b: reverse view)

et al., 2021). Therefore, the combination of *Neopestalotiopsis* sp.'s ability to produce secondary metabolites with antioxidant and antimicrobial properties makes this fungus a promising candidate for the development of natural-based drugs that are effective against oxidative stress and bacterial infections (He et al., 2022)).

The endophytic fungus *Aspergillus niger* exhibits excellent antioxidant and antibacterial activities due to its production of various bioactive secondary metabolites. The remarkable antioxidant and antibacterial activities of *Aspergillus niger* are closely linked to the production of characteristic compounds such as citric acid, oxalate, and 4-hydroxy-α-pyrone (Ebadi et al., 2024; Umaru et al., 2020). Citric acid, a prominent secondary metabolite of *A. niger*, possesses the capability to chelate metal ions, thereby inhibiting oxidative processes and establishing an acidic environment that hampers bacterial growth. Additionally, citric acid functions as a chelating agent, preventing oxidative cell damage, which significantly enhances its antioxidant potential (Adamczak et al., 2020).

In addition to citric acid, *Aspergillus niger* also produces unique antimicrobial compounds such as 4-hydroxy-α-pyrone, which has been shown to exhibit significant antibacterial activity, particularly against pathogenic bacteria like *Staphylococcus aureus* and *Escherichia coli* (Ababutain et al., 2021; Wei et al., 2022). These compounds function by damaging bacterial cell walls and disrupting membrane integrity, leading to cell lysis. Oxalic acid produced by *A. niger* also exerts similar inhibitory effects by creating an unfavorable environment for the growth of pathogenic bacteria. Other compounds, such as polyketides produced by *A. niger*, play a crucial role in suppressing the growth of other microorganisms. Polyketides not only act as antibacterial agents but also serve as potent antioxidants, offer-

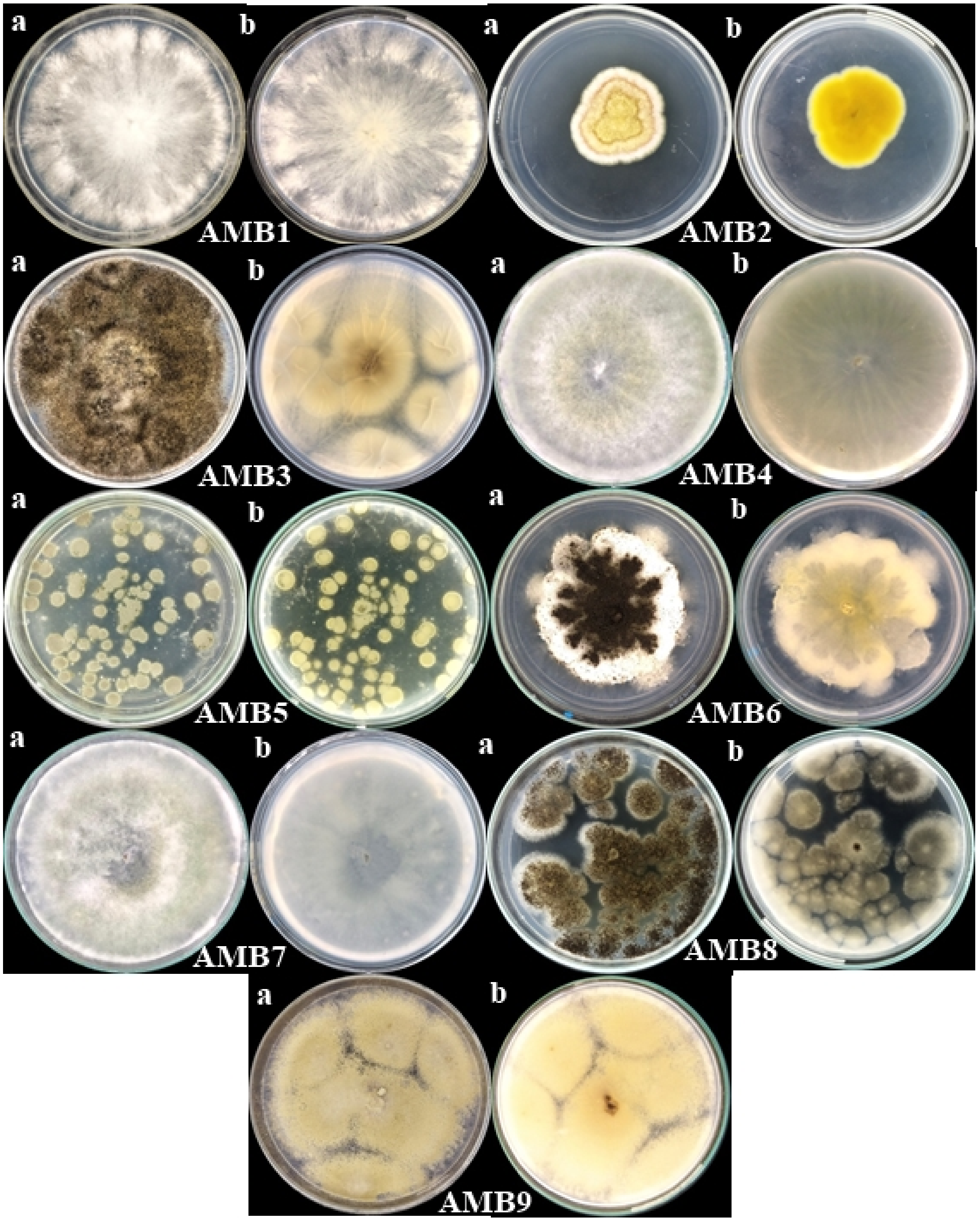


Figure 2. Colony Characteristics of Endophytic Fungi from the Stems of *Avicennia marina* (a: front view; b: reverse view)

ing protection against free radicals that cause cellular damage (El-Zahar et al., 2022; Ragavendran et al., 2019). Thus, the combination of these distinctive compounds not only enhances *A. niger*'s potential as a natural source of antioxidants but also makes it an effective agent in combating bacterial infections that are resistant to conventional antibiotics.

3.3 Biodiversity of Endophytic Fungi Isolated from *Avicennia marina*

Endophytic fungi were successfully isolated from three parts of *Avicennia marina*, namely the roots, stems, and fruits. A total of 23 fungal isolates were obtained, representing 10 different genera. The distribution of the endophytic fungal genera found in each plant organ is presented in Table 4.

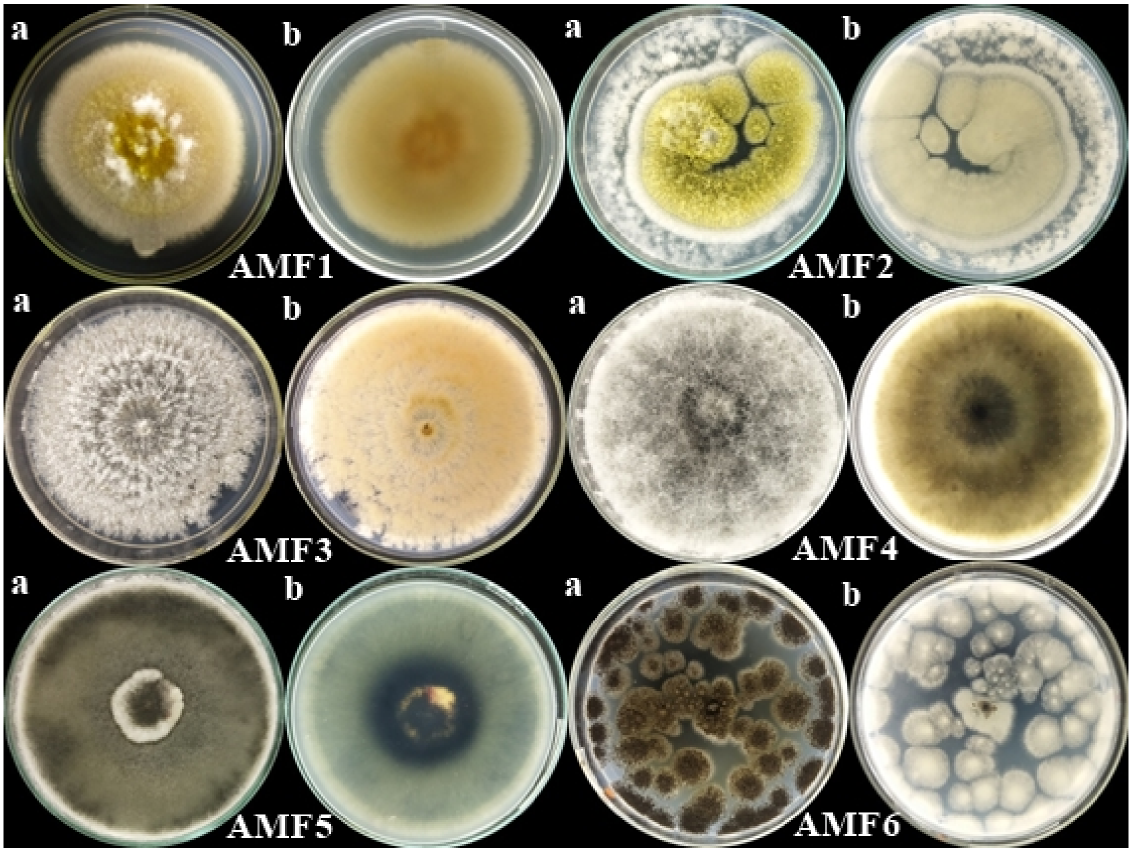
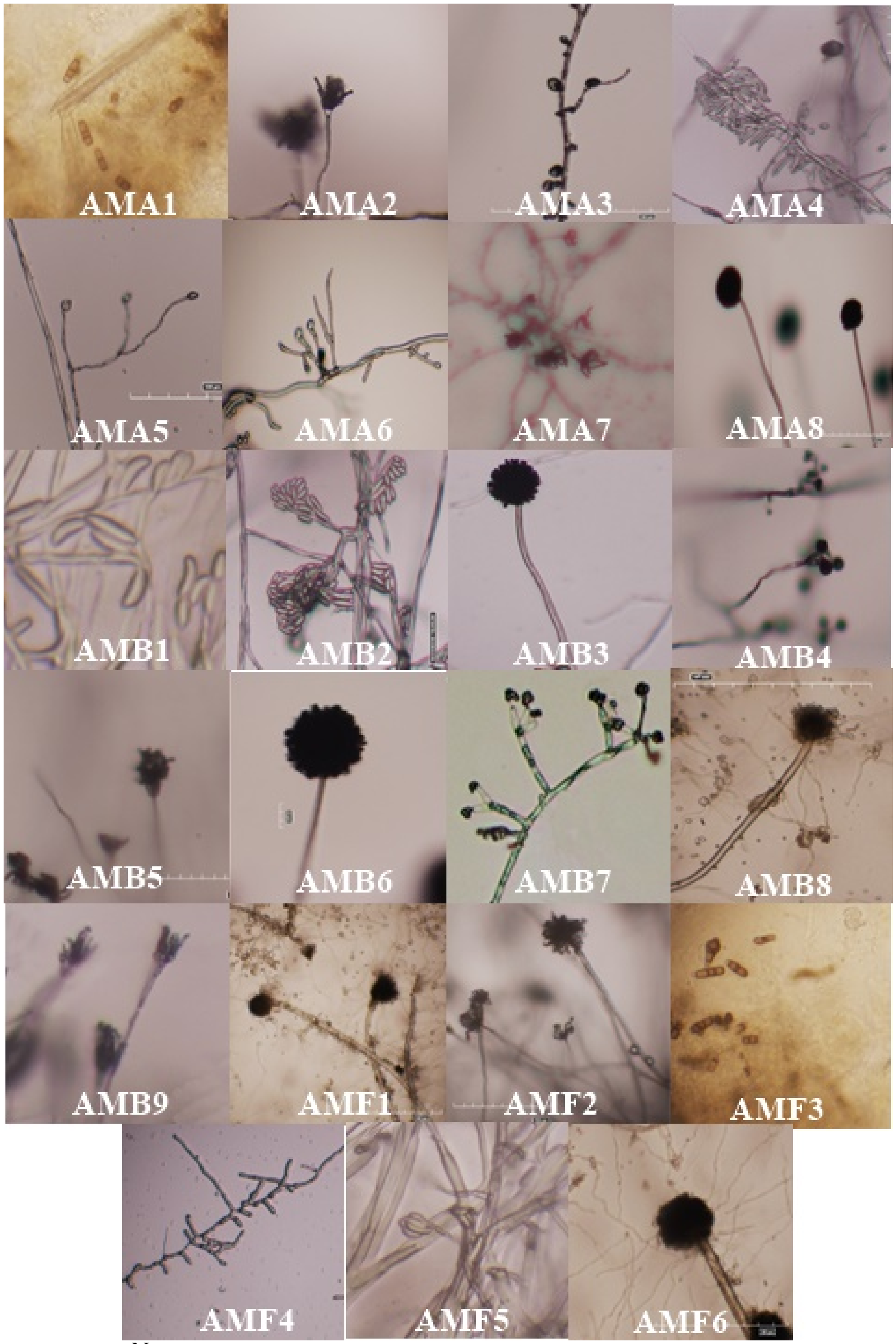


Figure 3. Characteristics of Endophytic Fungal Colonies from *A. marina* Fruit (a: front view; b: reverse view)

Table 1. Colony Characteristics of Endophytic Fungi from Roots, Stems, and Fruits of *A. marina*

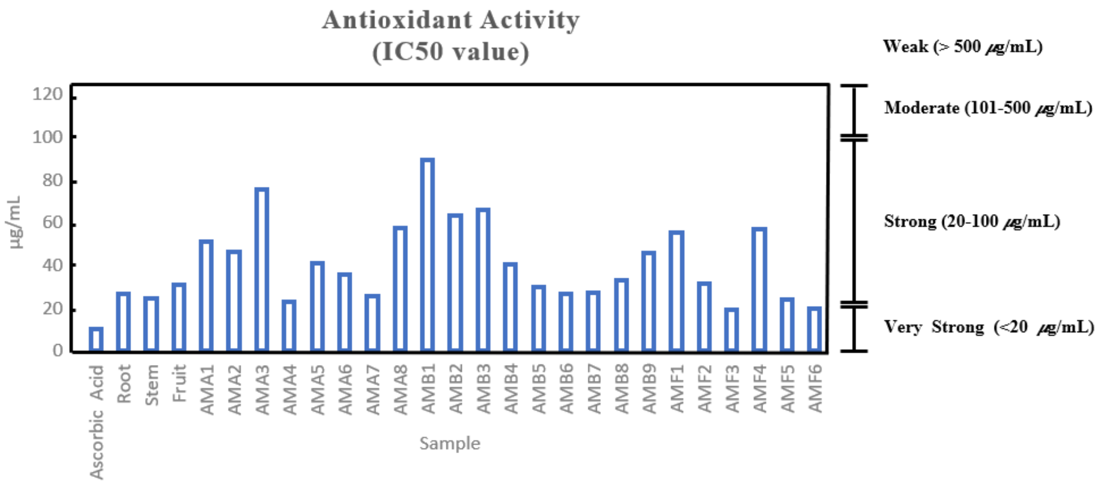
Code	Surface Colony	Reverse Colony	Structure	Elevation	Pattern	Exudate Drops	Radial Line	Concentric Circle
AMA1	White	Beige	Cottony	Umbonate	Radiate	-	-	✓
AMA2	Yellow with white	Yellow	Cottony	Rugose	Zonate	-	-	✓
AMA3	White	White	Cottony	Verrucose	Flowery	-	✓	✓
AMA4	White	White to grey	Cottony	Umbonate	Radiate	-	-	✓
AMA5	White	White	Cottony	Rugose	Zonate	-	-	✓
AMA6	White to grey	White to grey	Cottony	Umbonate	Zonate	-	-	✓
AMA7	White to green	White	Cottony	Rugose	Zonate	-	✓	✓
AMA8	Black	White	Velvety	Unbonate	Radiate	-	✓	-
AMB1	White	White	Cottony	Rugose	Zonate	-	-	✓
AMB2	Orange	Orange	Cottony	umbonate	zonate	-	-	✓
AMB3	Tan to brown	White	Velvety	Unbonate	Radiate	-	-	-
AMB4	White	White	Cottony	Rugose	Zonate	-	-	✓
AMB5	Grey	Grey	Cottony	umbonate	Spread	-	-	-
AMB6	Black with white border	White	Velvety	Unbonate	Radiate	-	-	-
AMB7	White	White	Cottony	Rugose	Zonate	-	-	✓
AMB8	Black	White	Velvety	Unbonate	Spread	-	-	-
AMB9	White to cream	White	Powdery	Flat	Spread	-	-	-
AMF1	White to cream	White to cream	Cottony	Umbonate	Zonate	-	-	✓
AMF2	Yellow	White	Powdery	Umbonate	Zonate	-	-	-
AMF3	White	Beige	Cottony	Umbonate	Radiate	-	✓	✓
AMF4	White	White to grey	Cottony	Umbonate	Radiate	-	-	✓
AMF5	Grey	White to grey	Cottony	Umbonate	Radiate	-	-	✓
AMF6	Black	White	Velvety	Unbonate	Spread	-	-	-

Table 4 demonstrates the diversity of endophytic fungi isolated from three parts of *Avicennia marina*-roots, stems, and fruits. Fungal diversity analysis across these plant parts revealed distinct colonization patterns. A total of 23 isolates, representing 10 different genera, were identified, with *Aspergillus* emerging as the most dominant genus, present in all sections



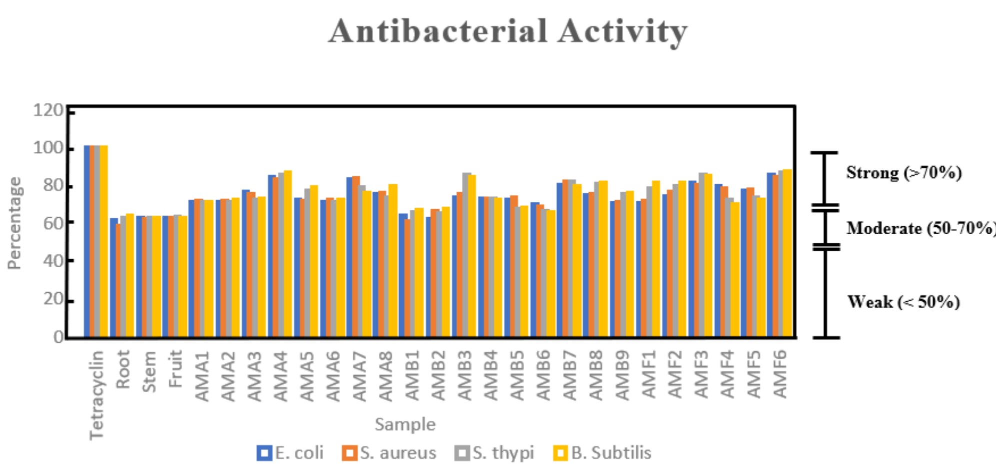
Note:
AMA1-AM8 = 8 endophytic fungal isolates from roots,
AMB1-AMB9 = 9 endophytic fungal isolates from stems,
AMF1-AMF6 = 6 endophytic fungal isolates from fruits.

Figure 4. Microscopic Characteristics of Endophytic Fungi from the Roots, Stems, and Fruits of *Avicennia marina*



Note: AMA1-AMA8 = Roots endophytic fungal; AMB1-AMB9 = Stems endophytic fungal; AMF1-AMF6 = Fruits endophytic fungal.

Figure 5. Antioxidant Activity of Methanol Extracts from Roots, Stems, and Fruits of *A. marina* and Endophytic Fungal Extracts (μg/mL) Based on IC_{50} Values



Note: AMA1-AMA8 = Roots endophytic fungal; AMB1-AMB9 = Stems endophytic fungal; AMF1-AMF6 = Fruits endophytic fungal.

Figure 6. Antibacterial Activity of Methanol Extracts from Roots, Stems, and Fruits of *A. marina* and Endophytic Fungal Extracts (μg/mL) Against *E. coli*, *S. aureus*, S. *typhi*, *B. subtilis*

(roots, stems, and fruits), accounting for a total of 8 isolates. Other genera, such as *Colletotrichum*, were consistently found in all tissues, while genera like *Fusarium* and *Penicillium* were restricted to specific tissues (stems). These findings emphasize the tissue-specific associations of endophytic fungi, suggesting that the ecological niches provided by different plant tissues play a role in influencing fungal colonization.

The diversity index further supports these observations, with the Simpson index of diversity (1-D) indicating that the roots (0.8438) have the highest fungal diversity, followed by the stems (0.7901), and the fruits (0.6667). These values suggest that the roots provide a more favorable environment for a diverse fungal community, likely due to their closer interaction with the soil and nutrient-rich nature, in contrast to the relatively exposed and nutrient-deficient stems and fruits. The Shannon diversity index (H') reflects the same trend, with the highest diversity in the roots (1.906) and the lowest in the fruits (1.242), further confirming that the roots offer a more complex habitat for fungal colonization. It is well-established that endophytic fungal communities are significantly influenced by plant tissue type, with roots often supporting greater diversity due to their direct exposure to a wide array of soil microbes (Hashem et al., 2023; Vimal et al., 2024). The high diversity observed in the roots is consistent with findings from studies on other mangrove species, where roots serve as a hotspot for microbial interactions (Sui et al., 2023). However, the presence

Table 2. Microscopic Characteristics of Endophytic Fungi from Roots, Stems, and Fruits of *A. marina*

Isolate	Spore	Shape	Hyphae	Characteristic	Species of Identification
AMA1	Conidia	Subglobose	Septate	Conidiophores hyaline.	*Neopestalotiopsis* sp.
AMA2	Conidia	Subglobose	Septate	Hyphae hairy colony texture.	*Aspergillus* sp.
AMA3	Sporangia	Subglobose	Septate	Sporangial and antheridia types.	*Pythium longifolium*
AMA4	Conidia	Globose	Septate	Emits droplets of conidia.	*Diaporthe* sp.
AMA5	Sporangia	Globose	Septate	Sporangia terminally.	*Mortierella* sp.
AMA6	Conidia	Subglobose	Septate	Conidiophores phialides short.	*Colletotrichum acutatum*
AMA7	Conidia	Subglobose	Septate	Conidiophores apically at irregularly.	*Trichoderma pseudokoningii*
AMA8	Conidia	Subglobose	Septate	Phialides radiate around the entire vesicle.	*Aspergillus* sp.
AMB1	Conidia	Subglobose	Septate	Short phialides conidiophores.	*Colletotrichum* sp.
AMB2	Conidia	Ovoid	Septate	Body structure in the form of mycelium.	*Fusarium* sp.
AMB3	Conidia	Subglobose	Septate	Phialides radiate and are biseriate.	*Aspergillus niger*
AMB4	Sporangia	Subglobose	Septate	The hyphae are in the early phases of growth.	*Trichoderma* sp.
AMB5	Conidia	Subglobose	Septate	Conidiophores erect, slightly rough.	*Penicillium* sp.
AMB6	Conidia	Subglobose	Septate	Phialides radiate around the entire vesicle.	*Aspergillus* sp.
AMB7	Sporangia	Subglobose	Septate	The hyphae are in the early phases of growth.	*Trichoderma* sp.
AMB8	Conidia	Subglobose	Septate	Phialides radiate and are biseriate.	*Aspergillus niger*
AMB9	Conidia	Phialides	Septate	The phialides are swollen at the base.	*Paecilomyces varioti*
AMF1	Conidia	Subglobose	Septate	Conidia phialosporous.	*Eurotium rubrum*
AMF2	Conidia	Globose	Septate	Conidiophores rough especially at apex.	*Aspergillus flavus*
AMF3	Conidia	Subglobose	Septate	Conidiophores hyaline, branched.	*Neopestalotiopsis* sp.
AMF4	Sporangia	Cylindrical	Septate	Sporangia form a new mycelium.	*Pythium* sp.
AMF5	Conidia	Subglobose	Septate	Short phialides conidiophores.	*Colletotrichum* sp.
AMF6	Conidia	Subglobose	Septate	Phialides radiate and are biseriate.	*Aspergillus niger*

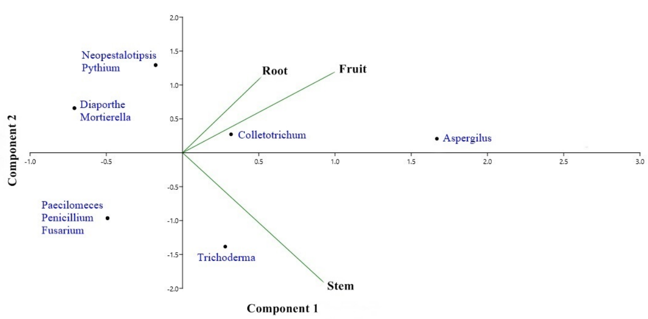


Figure 7. Principal Component Analysis (PCA) of Endophytic Fungi Isolated from *Avicennia marina*

of *Aspergillus* in all parts of the plant may indicate its role as a core member of the *A. marina* microbiome, capable of adapting to various plant tissues, aligning with previous reports from coastal ecosystems (Heo et al., 2019). The low diversity in the fruit may be attributed to the seasonal nature of the fruit and the limited time available for endophytic fungal colonization. For a graphical representation of the diversity data, refer to Figure 4.

Figure 7 presents the results of Principal Component Analysis (PCA) of the endophytic fungi isolated from different parts of *Avicennia marina*-roots, stems, and fruits. PCA was employed to identify relationships between various fungal species and plant parts, and to reduce data dimensionality, making it easier to identify key patterns. In this figure, *Aspergillus* is distinctly separated from other genera, positioned far along the first component axis (Component 1), indicating its significant contribution to total variation. This suggests that *Aspergillus* is the most dominant genus with a unique distribution pattern compared to other fungal genera. Its position far to the right on Component 1 also shows a stronger association with the fruit, consistent with its distribution in Table 4. In contrast, genera such as *Neopestalotiopsis*, *Pythium*, and *Diaporthe* are located in the upper left quadrant of the plot, indicating a stronger connection to the roots. Genera like *Penicillium* and *Fusarium* are found in the lower left, suggesting their association with the stems. These positions illustrate the differing distributions of fungal species across various plant parts, confirming the presence of specific relationships between fungal types and the plant tissues they inhabit. The analysis also confirms that roots and fruits harbor more diverse fungal communities compared to stems, as some genera like *Colletotrichum* are located in the center of the plot, indicating a more even distribution across all plant parts. Thus, this PCA supports the conclusion that fungal colonization patterns are influenced by the specific plant parts analyzed.

These findings are crucial for understanding the ecological role of endophytic fungi in mangrove ecosystems. The tissue-specific colonization patterns suggest functional specialization of these fungi, with potential implications for nutrient cycling, plant health, and ecosystem stability in coastal environments (Jacob et al., 2023; Zhu et al., 2024). The higher diversity in the roots may enhance plant resilience by facilitating nutrient

Table 3. Antibacterial and Antioxidant Activities of Endophytic Fungal Extracts from Roots, Stems, and Fruits of *Avicennia marina*

Sample	Extract	Weight (g)	% Antibacterial Activity				Antioxidant Activity
			E. coli	*S. aureus*	*S. typhi*	*B. subtilis*	IC_{50} (μg/ml)
Host Plant	Methanol of *A. marina* root	1.8	61.4 ± 0.42**	58.3 ± 0.54**	61.6 ± 0.37**	64.2 ± 0.89**	$2^{6.485}$***
	Methanol of *A. marina* stem	1.9	62.9 ± 0.16**	62.2 ± 0.58**	62.6 ± 0.73**	62.7 ± 0.65**	24.316***
	Methanol of *A. marina* fruit	2.0	63.0 ± 0.90**	62.9 ± 0.90**	63.2 ± 1.18**	62.6 ± 0.73**	30.972***
Endophytic Fungi	AMA1	2.1	71.4 ± 0.16***	71.7 ± 0.56***	71.4 ± 0.60***	71.1 ± 0.48***	51.272***
	AMA2	1.9	70.9 ± 0.44***	71.8 ± 0.31***	71.3 ± 0.65***	72.1 ± 0.16***	46.425***
	AMA3	1.4	76.3 ± 0.64***	75.6 ± 0.35***	72.2 ± 0.60***	72.8 ± 0.39***	75.760***
	AMA4	1.2	84.3 ± 0.18***	83.4 ± 0.40***	85.8 ± 0.16***	86.6 ± 0.68***	22.968***
	AMA5	1.5	72.3 ± 0.89***	71.9 ± 0.35***	76.9 ± 0.34***	78.9 ± 0.65***	40.870***
	AMA6	1.5	71.3 ± 0.18***	72.4 ± 0.51***	71.1 ± 0.38***	72.1 ± 0.74***	35.783***
	AMA7	0.9	82.9 ± 0.90***	83.7 ± 0.36***	78.8 ± 0.69***	76.1 ± 0.64***	25.504***
	AMA8	1.8	75.4 ± 0.78***	76.1 ± 1.18***	73.7 ± 0.25***	79.8 ± 0.89***	57.340***
	AMB1	1.5	63.7 ± 0.14**	60.9 ± 0.80**	65.9 ± 0.62**	67.2 ± 0.44**	89.511***
	AMB2	1.6	62.3 ± 0.73**	66.2 ± 0.13**	65.4 ± 0.63**	67.7 ± 1.28**	63.377***
	AMB3	2.1	73.3 ± 0.39***	75.3 ± 0.63***	85.0 ± 0.51***	84.2 ± 0.57***	65.937***
	AMB4	2.4	73.1 ± 0.19***	72.9 ± 0.36***	72.0 ± 1.14***	72.4 ± 0.69***	40.369***
	AMB5	2.4	72.3 ± 0.52***	73.3 ± 0.18***	67.4 ± 0.74**	68.1 ± 0.54**	30.145***
	AMB6	1.5	69.7 ± 0.74**	68.9 ± 0.73**	66.4 ± 1.18**	65.8 ± 0.39**	26.491***
	AMB7	1.4	80.0 ± 1.14***	82.2 ± 0.36***	81.8 ± 0.69***	79.6 ± 0.64***	27.126***
	AMB8	1.6	74.5 ± 0.72***	75.6 ± 0.28***	80.8 ± 0.31***	81.3 ± 1.05***	32.904***
	AMB9	1.5	70.6 ± 0.31***	71.1 ± 0.65***	75.0 ± 0.16***	76.1 ± 0.44***	45.863***
	AMF1	1.1	70.8 ± 1.18***	71.5 ± 0.31***	78.6 ± 0.16***	81.1 ± 0.56***	55.215***
	AMF2	2.0	74.0 ± 0.27***	76.5 ± 0.28***	79.8 ± 0.15***	81.1 ± 1.05***	31.362***
	AMF3	2.0	81.3 ± 0.44***	80.4 ± 0.36***	85.7 ± 0.18***	84.7 ± 0.74***	19.042****
	AMF4	1.5	79.6 ± 0.43***	78.3 ± 0.30***	72.2 ± 1.12***	70.1 ± 0.44***	57.105***
	AMF5	1.8	77.1 ± 0.43***	77.7 ± 0.47***	73.6 ± 1.23***	72.1 ± 1.27***	23.832***
	AMF6	1.8	85.3 ± 0.16***	84.4 ± 0.80***	86.7 ± 0.42***	87.1 ± 0.54***	19.678****
	Positive Control		Tetracycline 100***	Tetracycline 100***	Tetracycline 100***	Tetracycline 100***	*Ascorbic Acid* 10.083****

Note: AMA1–AMA8 = 8 endophytic fungal isolates from root;
AMB1–AMB9 = 9 endophytic fungal isolates from stem; AMF1–AMF6 = 6 endophytic fungal isolates from fruit.
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 4. Diversity of Endophytic Fungi Isolated from Roots, Stems, and Fruits of *Avicennia marina*

Genera	Part of Plat *Avicennia marina*			Total
	Root	Stem	Fruit	
Aspergilus	2	3	3	8
Colletotrichum	1	1	1	3
Diaporthe	1	0	0	1
Fusarium	0	1	0	1
Mortierella	1	0	0	1
Neopestalotiopsis	1	0	1	2
Paecilomeces	0	1	0	1
Penicillium	0	1	0	1
Pythium	1	0	1	2
Trichoderma	1	2	0	3
Number of isolates	8	9	6	23
Simpson index (D)	0.1563	0.2099	0.3333	-
Simpson index of diversity (1-D)	0.8438	0.7901	0.6667	-
Shannon index of diversity (HI)	1.906	1.677	1.242	-

uptake and providing protection against pathogens (de Andrade et al., 2023). This study not only expands our understanding of fungal diversity in mangrove ecosystems but also opens new opportunities to explore the biotechnological potential of these endophytes, particularly in bioremediation and agriculture.

4. CONCLUSIONS

A total of 23 endophytic fungal isolates from the roots, stems, and fruits of *Avicennia marina* were successfully identified, representing 10 genera. The highest fungal biodiversity was observed in the roots, with an H' value of 1.906. Isolates AMF3 and AMF6 were the most promising due to their excellent bioactivity. Morphological identification revealed that AMF3 and AMF6 were *Neopestalotiopsis* sp. and *Aspergillus niger*, respectively. These endophytic fungi hold potential for development as natural sources of antioxidant and antibacterial compounds through systematic follow-up research.

5. ACKNOWLEDGMENT

The authors thank to the Direktorat Riset, Teknologi, dan Pengabdian kepada Masyarakat, Direktorat Jendral Pendidikan Tinggi, Riset, dan Teknologi as the funder through the Penelitian Tesis-Magister with contract number 0018.008/UN9/SB1. LP2M.PT/2024.

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