

The Potential of Endophytic Fungi from Jeruju Stems (*Acanthus ilicifolius*) as a Source of Antioxidant and Antibacterial Activity

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ABSTRACT

The mangrove biodiversity of Indonesia is rich and offers great potential for the discovery of bioactive compounds. The mangrove plant known as jeruju (*Acanthus ilicifolius*) has long been used in medicine and is recognized to include endophytic fungus capable of generating biologically active secondary metabolites. This investigation's goal was to distinguish and assess endophytic fungi from the stem of *A. ilicifolius* as possible sources of antibacterial and antioxidant substances. After being isolated using surface sterilization techniques and cultivated on suitable media, endophytic fungi were characterized both macroscopically and microscopically. Liquid fermentation and solvent extraction were used to produce fungal extracts. Antioxidant activity was measured using the DPPH method, while antibacterial activity against many harmful bacteria was measured using the disc diffusion method. According to the results, the isolated endophytic fungus displayed a range of morphological characteristics and bioactivities. Among the isolates, BR6, which was identified as *Penicillium* sp., showed the greatest antioxidant activity with an IC₅₀ value of 28.1 µg/mL. This isolate also shown strong antibacterial activity, has over 70% inhibition rates against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Salmonellat yphi*. These findings imply that endophytic fungi from *A. ilicifolius*, particularly *Penicillium* sp., have significant potential as natural sources of antioxidant and antibacterial compounds for use in pharmaceutical and medical applications.

Keywords: *A. ilicifolius*, Antioxidant, Antibacterial, Endophytic fungi, *Penicillium* sp.

INTRODUCTION

Indonesia is a country with extremely high biodiversity, particularly in mangrove ecosystems, which play a vital ecological and pharmacological role (Prasetya et al., 2024). One mangrove plant commonly found in coastal areas is the jeruju (*Acanthus ilicifolius*), which has traditionally been used as a medicinal plant due to its content of various bioactive compounds such as flavonoids, alkaloids, phenolics, and terpenoids

(Altarriba & Varona, 2007; Deandra et al., 2024). Biological activities, such as antioxidant and antibacterial qualities, are known to be present in these compounds. (Mustaqimah et al., 2024; Pringgenies et al., 2020).

In addition to bioactive compounds produced directly by host plants, attention to endophytic microorganisms, particularly endophytic fungi, has increased in recent decades (Moubarek & Halat, 2020). Microorganisms known

as endophytic fungus coexist harmoniously with plant tissues without producing symptoms of illness (Howes et al., 2018; Pratiwi et al., 2024). Secondary metabolites produced by these fungus are reported to resemble or even more potent than, compounds produced by their host plants (Ogofure et al., 2025; Ortega & Torres-mendoza, 2025). This makes endophytic fungi a promising alternative source for the discovery of new bioactive compounds (Habisukan et al., 2024; Oktiansyah, Habisukan, et al., 2025).

Acanthus ilicifolius, or jeruju, is a plant that may support a variety of endophytic fungi in its stems (Rahmaniah et al., 2019). The extreme environmental conditions of mangroves, such as high salinity and tidal fluctuations, are thought to encourage endophytic fungi to produce secondary metabolites with strong biological activity as an adaptive mechanism (Noviyanto, Oktiansyah, et al., 2025; Zhang et al., 2017). These metabolites potentially possess antioxidant activity that plays a role in scavenging free radicals and antibacterial activity that is effective against pathogenic bacteria (Noviyanto, Widjajanti, et al., 2025; Onyekachukwu et al., 2024).

This description calls for further investigation into the potential of endophytic fungus isolated from the stem of the jeruju (*Acanthus ilicifolius*) as a source of antibacterial and antioxidant chemicals. By offering scientific data information about the potential of mangrove endophytic fungi as a source of new bioactive chemicals, this study seeks to improve the use of Indonesian biodiversity in the pharmaceutical and health industries.

MATERIAL AND METHOD

Sample Preparation

Fresh, disease-free jeruju (*Acanthus ilicifolius*) stems were utilized in this investigation. They were acquired from Sungsang IV, which is situated at GPS point 2.34921191S 104.90601444E 300°NW, elevation: 16.3m, Banyuasin II District, Banyuasin Regency 30971, South Sumatra.

Isolation and Purification of Endophytic Fungi

Samples of *A. ilicifolius* stems were washed with running water until clean for approximately 5 minutes. Surface sterilization was then performed by cleaning each sample with 70% alcohol for approximately 3 minutes using cotton wool, followed by rinsing with sterile distilled water for approximately 1 minute. The stems were then cut as needed and soaked in sodium hypochloride (NaOCl) for approximately 30 seconds, washed once more with 70% alcohol for around 30 seconds, followed by a minute or so of sterile distilled water. The sterilized plant parts were aseptically cut into sections approximately 3 x 1 cm. After that, the samples were placed on Petri dishes with Potato Dextrose Agar (PDA) medium and let to sit at room temperature for three to seven days (Mavani et al., 2020).

Observations were made daily until fungal growth was visible. Fungal colonies growing on PDA medium that exhibited distinct morphological characteristics (shape, color, and size) were then purified. The colonies were purified by inoculating to new PDA media and incubating at room temperature for two 24-hour periods. Following purification, the fungal colonies were cultivated into stock cultures (on PDA slants in test tubes) and working cultures (on PDA medium in a petri dish) (Novianti et al., 2025; Yun et al., 2009).



Endophytic Fungal Cultivation and Extraction

Potato Dextrose Broth (PDB) medium was used to culture pure isolates of endophytic fungus. Twenty fungal colonies were collected using a 5 mm diameter cork borer (*agar plug*) then added to 300 milliliters of PDB medium for inoculation. After that, the cultures were maintained at room temperature under static circumstances for 30 days, which is the ideal amount of time for fungal growth and metabolite formation. Filter paper was used to separate the mycelia from the cultures following the incubation period. After adding ethyl acetate solvent to the culture medium (1:1), the extraction was carried out via partitioning: ethyl acetate was added until two liquid phases formed, which were then separated using a separatory funnel. This process was repeated three times. To get a concentrated extract, After being removed from the liquid culture, the ethyl acetate extract was evaporated using a rotary evaporator (Firdausi, 2020; Habisukan et al., 2021).

Characterization of Endophytic Fungal

The following traits of endophytic fungal colonies have been noted: a) the hue of the colony and its opposite side, b) Colony surface: smooth, granular, or powdery c) whether exudate droplets are present or absent; d) whether radial furrows from the colony edge center are present or absent; and e) whether concentric circles are present or absent. The slide culture method was used for microscopic inspection. A preparation was prepared by placing a 1 x 1 cm² piece of sterile PDA media on a sterile glass slide placed on a support rod in a Petri dish. The fungal isolate was inoculated onto the media by scratching, then covered with a coverslip and incubated for a few days at room temperature.

The resulting preparation was observed under a microscope to identify microscopic characteristics such as spore shape and color, and the presence of septa in the hyphae (Walsh et al., 2018; Watanabe, 2010).

Test for Antioxidant Activity

Testing for antioxidant activity involved mixing 0.2 mL of sample in methanol with 3.8 mL of 0.05 mM DPPH solution at different concentrations (1000, 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL). After homogenizing the solution mixture, it was permitted to stand in the dark for half an hour. A UV-Vis spectrophotometer was used to determine absorbance at λ max. By calculating the percentage inhibition of DPPH uptake, the amount of DPPH radical absorption in the sample was used to determine its antioxidant activity (Budiono et al., 2019; Molyneux, 2004).

A linear regression equation ($y = ax + b$) was produced by graphing the percentage inhibition and sample concentration on the x and y axes. By setting y at 50% and computing the x value as the IC₅₀, this equation was utilized to find the IC₅₀ value. The sample concentration needed to decrease DPPH radicals by 50% is indicated by the IC₅₀ value. Parameters a and b were obtained from the formula data at various relevant concentrations, by linear regression calculations using the Statistica 7.0 program (Oktiansyah, et al., 2025).

Test of Antibacterial Activity

The extract was tested for antibacterial activity against *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* using the disc diffusion method on Mueller-Hinton Agar (MHA) medium. Paper discs containing endophytic fungal extract (400 µg/disk), tetracycline positive control (30

µg/disk), and a negative control of 10% DMSO were applied to the medium surface that had been infected with the test bacteria. A caliper was used to measure the diameter of the inhibition zone that formed after all plates were incubated for 24 hours at 37°C (Irawan et al., 2024; Sayed et al., 2022; 2023).

RESULT AND DISCUSSION
Characterization of Endophytic Fungal

The endophytic fungi that were effectively separated from the jeruju tree's stem tissue showed distinct character diversity, both based on visual observation of colony morphology and microscopic characteristics (Figure 1). Based on these isolation results, six distinct endophytic fungal isolates were obtained. A description of the characteristics of each isolate is presented in Tables 1 and 2, based on macroscopic and microscopic observations.

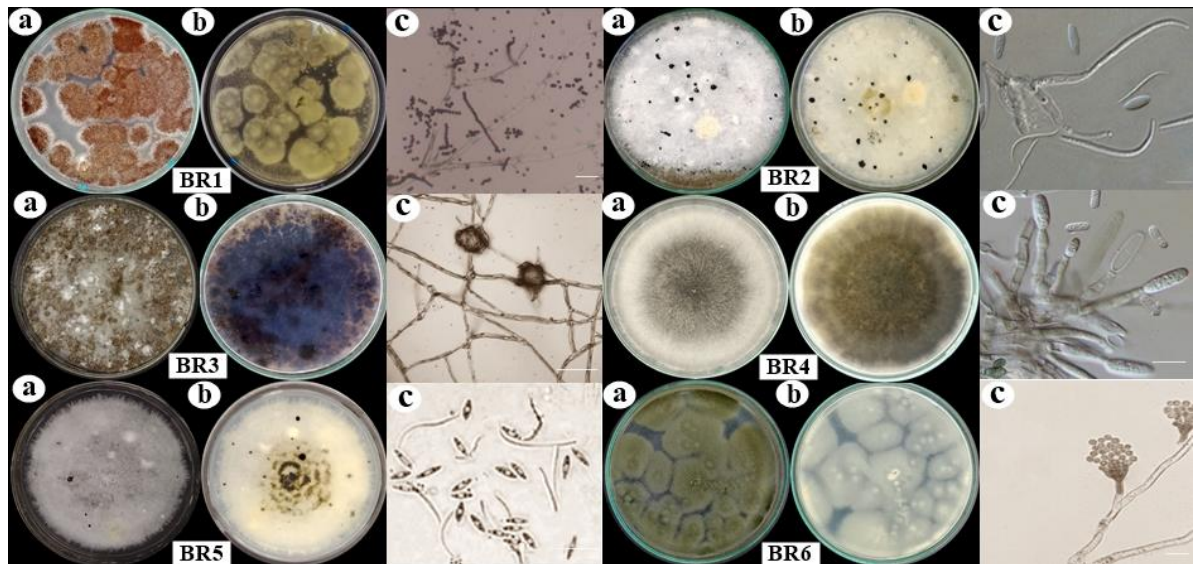


Figure 1. Characteristics makroskopis and mikroskopis of endophytic fungi from Jeruju Stems (*A. ilicifolius*) (a. front view, b. reverse view, c. mikroskopis).

Table 1. Macroscopic traits of endophytic fungi from Jeruju stems (*A. ilicifolius*)

Code	Surface Colony	Colony Reverse	Surface Texture	Elevation	Form	Margin	Exudate Drops	Line Radeal	Concentric Circle
BR1	Dark brown	White	Powdery	Raised	Irregular	Lobate	-	-	✓
BR2	White	Yellowish white	Velvety	Flat	Circular	Entire	✓	✓	-
BR3	Gray	Black	Cottony	Raised	Circular	Undulate	-	-	✓
BR4	Whitish gray	Gray	Cottony	Umbonate	Circular	Curled	-	✓	-
BR5	White	White	Cottony	Convex	Circular	Undulate	✓	-	✓
BR6	Greenish white	White	Powdery	Flat	Irregular	Lobate	-	-	✓

Table 2: Microscopic Features of Jeruju Stem Endophytic Fungi (*A. ilicifolius*)

Code	Spore	Shape	Hyphae	Characteristics	Identification
BR1	Conidia	Globose	Septate, hyaline	Conidia head with vesicle	<i>Aspergillus</i> sp.
BR2	Conidia	Elipsoid	Septate, hyaline	Short fusiform with rounded ends	<i>Diaporthe</i> sp.
BR3	Chlamydiospore	Globose	Septate, thick-walled	Thick-walled spores	<i>Papulaspora</i> sp.
BR4	Conidia	Falcate	Septate, hyaline	Acervuli with setae	<i>Colletotrichum</i> sp.
BR5	Conidia	Elipsoid	Septate, hyaline	Conidophore simple, short	<i>Diaporthe</i> sp.
BR6	Conidia	Subglobose	Septate, hyaline	Brush-like conidiophore	<i>Penicillium</i> sp.

Based on both macroscopic and microscopic features, endophytic fungi that were separated from the jeruju (*Acanthus ilicifolius*) stem tissue exhibited a significant degree of morphological variation. The effective acquisition of six endophytic fungal isolates (BR1–BR6), indicating that the jeruju stem tissue is a habitat that supports the colonization of various types of endophytic fungi. The results of further characterization showed a fairly high taxonomic diversity, which includes the genera *Aspergillus*, *Diaporthe*, *Papulaspora*, *Colletotrichum*, and

Penicillium, which are known as endophytic fungi groups with high adaptability and the potential to produce bioactive secondary metabolites.

Test of Antioxidant and Antibacterial Activity

Antioxidant and antibacterial activity assays were carried out to assess the bioactive potential of the variety of endophytic fungi that were isolated from *A. ilicifolius* stems. Table 3 displays each fungal isolate's antioxidant and antibacterial activity test findings.

Table 3: Endophytic fungus from *A. ilicifolius* exhibit antibacterial and antioxidant properties

Code Extracts	Weight (g)	Antioxidant Activity IC ₅₀ (µg/mL)	% Antibacterial Activity			
			<i>E. coli</i>	<i>S. typhi</i>	<i>B. subtilis</i>	<i>S. aureus</i>
BR metanol	1,9	24,5***	70,2***	70,7***	68,4**	69,8**
BR1	1,6	45,1***	73,5***	74,1***	70,4***	71,9***
BR2	0,9	37,4***	72,1***	75,9***	71,8***	73,3***
BR3	0,7	198,9**	45,1*	45,9*	51,1**	53,2**
BR4	1,3	64,7***	70,9***	71,5***	69,7**	70,2***
BR5	1,1	441,6**	68,2**	65,9**	62,3**	64,9**
BR6	1,5	28,1***	78,2***	76,6***	74,8***	77,6***
Positive control		AA 10,08****	TT 100***	TT 100***	TT 100***	TT 100***

Note: (AA. Ascorbic Acid, TT. Tetrasiklin)

Antioxidant activity IC₅₀ (µg/mL): ****very strong < 20 µg/mL, ***strong 20 ≤ IC₅₀ < 100 µg/mL; **moderat 100-500 µg/mL; * weak > 500 µg/mL. Antibacterial activity percentage: *** ≥ 70% (strong), ** 50-70% (moderat), * ≤ 50% (weak).

The results of the antioxidant and antibacterial activity tests in Table 3 show that isolate BR6 has the best biological potential compared to other endophytic fungal isolates. Isolate BR6, identified as *Penicillium* sp., exhibited strong antioxidant activity with an IC₅₀ value of 28.1 µg/mL, as well as high antibacterial activity against all tested bacteria, both Gram-positive and Gram-negative. This ability indicates that endophytic *Penicillium* sp. from jeruju stems has great potential as a producer of bioactive compounds.

The high antioxidant activity of the *Penicillium* sp. (BR6) isolate is thought to be related to its ability to produce secondary metabolites such as phenolic compounds, flavonoids, and polyketide derivatives that act as free radical scavengers (Visagie et al., 2021; Yaderets et al., 2021). Various studies have reported that the *Penicillium* genus is known as one of the fungi that produces effective natural antioxidant compounds (Ghalem & Mohammed, 2021; Sunani & Hendriani, 2023), thus this finding strengthens the potential of the BR6 isolate as an alternative source of antioxidants derived from endophytic fungi (Al-Rajhi et al., 2022; Visagie et al., 2014).

Apart from its antioxidant properties, the *Penicillium* sp. (BR6) isolate also demonstrated strong antibacterial activity with inhibitory percentages of 78.2% against *Escherichia coli*, 76.6% against *Salmonella typhi*, 74.8% and 77.6%, respectively, against *Bacillus subtilis* and *Staphylococcus aureus*. The relatively even activity against both Gram-positive and Gram-negative bacteria indicates that the antibacterial compounds produced by *Penicillium* sp. are broad-spectrum (Manisha et al., 2025; Yanti et al., 2021). This aligns with the characteristics of the

Penicillium genus, which is known to produce various antibiotic and antibacterial compounds (Kola et al., 2016; Mamangkey et al., 2024). According to these findings, *Penicillium* sp. (BR6) plays a significant role as a bioactive endophyte in *A. ilicifolius* and highlights its potential for further purification and characterization of active compounds (Dominggus Kalor et al., 2025).

CONCLUSION

This study shows that the stem of jeruju (*Acanthus ilicifolius*) harbors diverse endophytic fungi with promising bioactive potential. Several endophytic fungal genera were successfully isolated and characterized, indicating that mangrove plants provide a suitable environment for fungi capable of producing biologically active secondary metabolites.

The antioxidant and antibacterial evaluations revealed that the endophytic fungal extracts exhibited varying biological activities. *Penicillium* sp. had the most antioxidant and broad-spectrum antibacterial activity among the isolates. These results emphasize the potential natural supply of antioxidant and antibacterial chemicals found in endophytic fungi from *A. ilicifolius*. It is advised to conduct compound isolation to determine the active ingredients and investigate their potential uses in the pharmaceutical and medical industries.

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