

Antibacterial Activity of the Endophytic Fungus *Fusarium solani* Isolated from the Stem of *Bauhinia fulva* Blume Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Endophytic fungi are microorganisms that live within plant tissues and can produce secondary metabolites with potential antibacterial, antifungal, and anticancer activities. This study aimed to isolate, identify, and evaluate the antibacterial activity of endophytic fungi from the stem of *Bauhinia fulva* Blume through isolation, morphological identification, fermentation, ethyl acetate extraction, phytochemical screening, and antibacterial testing using the disk diffusion method. The results showed that the isolated endophytic fungus was identified as *Fusarium solani*, producing 1.4095 g of ethyl acetate extract. Phytochemical screening revealed the presence of alkaloids, flavonoids, quinones, and triterpenoids/steroids. The ethyl acetate extract of *F. solani* exhibited antibacterial activity against *Staphylococcus aureus* with a maximum inhibition zone of 20.85 mm and lower activity against *Escherichia coli* with a maximum inhibition zone of 5.56 mm, indicating its potential as a natural source of antibacterial compounds.

Keywords: Antioxidant, *Bauhinia fulva* Blume, *Fusarium solani*, endophytic fungi, secondary metabolites

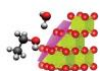
1 Introduction

Biodiversity represents the entirety of life forms on Earth, encompassing genetic, species, and ecosystem diversity, and plays a crucial role in maintaining ecological balance. High levels of biodiversity within a region not only enrich the ecosystem but also provide substantial potential for the discovery of new natural resources of value, particularly in the health sector. However, development activities and environmentally unfriendly exploitation of natural resources may threaten the preservation of biodiversity, including sources of bioactive compounds from plants [1]. Therefore, alternative and sustainable approaches to the utilization of biological resources are required, one of which is the exploration of endophytic microorganisms.

Endophytic microorganisms are microbes that live within the tissues of their host plants in a mutualistic association [2,3]. Endophytic fungi, one of the major groups of these microorganisms, possess the ability to produce bioactive compounds, either identical or non-identical to those of their host plants, yet often

exhibiting similar biological activities to the bioactive compounds produced by the host [4–6]. This similarity is presumed to occur due to coevolution and genetic transfer processes between the fungi and their host plants. Another advantage of endophytic fungi lies in their ease of cultivation, short life cycles, and ability to produce large quantities of bioactive compounds through fermentation. Numerous studies have demonstrated that the secondary metabolites produced by endophytic fungi exhibit important biological activities, including antibacterial, antifungal, antioxidant, and anticancer properties [7–11].

The genus *Bauhinia* (Family *Fabaceae*) comprises approximately 300 species that predominantly grow in tropical and subtropical regions, including Indonesia, which hosts around 10 species [12]. Several *Bauhinia* species have been reported to possess pharmacological activities, such as antibacterial, antioxidant, and anticancer properties in *B. variegata*, as well as antifilarial activity in *B. racemosa* [12,13]. Extracts of *Bauhinia purpurea* have also been shown to exhibit antipyretic, anti-



inflammatory, antifungal, analgesic, and antitumor effects. The differences in biological activities among species within this genus are generally attributed to variations in the secondary metabolite composition, which are influenced by environmental factors, plant parts, and geographical conditions [14].

Several studies have reported diverse endophytic fungi associated with *Bauhinia* species such as *B. forficata*, *B. racemosa*, *B. malabarica*, and *B. vahlii*, including genera such as *Aspergillus*, *Penicillium*, *Curvularia*, *Colletotrichum*, and *Fusarium*, many of which are known to produce bioactive antimicrobial compounds [15]. Among these fungi, *Fusarium solani* has attracted considerable attention due to its ability to biosynthesize various secondary metabolites, including polyketides, alkaloids, terpenoids, quinones, peptides, and phenolic compounds with significant antimicrobial activities against both Gram-positive and Gram-negative bacteria [16]. Previous studies also demonstrated that endophytic *F. solani* isolated from medicinal plants produces antibacterial metabolites with promising pharmaceutical potential [17,18]. There have been limited scientific reports on the isolation and identification of endophytic fungi from *Bauhinia fulva* Blume or on their potential antibacterial activity. Therefore, this research was conducted to address this knowledge gap and to provide new scientific contributions regarding the relationship between *Bauhinia fulva* Blume and its associated endophytic fungi.

Based on the above description, this study aims to isolate and identify endophytic fungi from the stem of *Bauhinia fulva* Blume, determine their colony growth rates, examine their secondary metabolite content, and evaluate their antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

2 Methods

2.1 Cultivation and Extraction

Fresh stems of *B. fulva* Blume were washed, cut into pieces of approximately $1 \times 1 \text{ cm}^2$ segments, and aseptically surface-sterilized using 70% ethanol and 5.25% NaOCl [14,19]. The tissue segments were dried on sterile filter paper and inoculated onto PDA media, followed by incubation at room temperature for 14 days. The resulting endophytic fungal colonies were purified through repeated subculturing until single isolates were obtained. Molecular identification was conducted based on ITS rDNA sequences using an automated DNA sequencer and BLAST analysis [20].

Fermentation was carried out in 500 mL of PDB medium for 21 days. Following incubation, the mycelia were separated from the liquid medium via

filtration. The resulting filtrate was evaporated using a rotary evaporator to yield 1.4095 g of concentrated extract, which was subsequently used for phytochemical screening and antibacterial activity assays.

Fermentation was conducted in 500 mL of liquid PDB medium at room temperature for 21 days. The fermentation filtrate was extracted three times with ethyl acetate (1:1 v/v) [21]. The liquid extracts were evaporated using a rotary evaporator to obtain concentrated ethyl acetate extract.

2.2 Phytochemical Screening

Phytochemical screening was performed on the ethyl acetate extract of *B. fulva* to identify the presence of secondary metabolites [22,23]. The chemical profile of the ethyl acetate extract was monitored using thin-layer chromatography (TLC). The concentrated extract (1.4095 g) was spotted on silica gel plates. The separation was performed using an optimized mobile phase system (7:3 n-hexane:ethyl acetate). The resulting spots were visualized under ultraviolet (UV) light at wavelengths of 254 nm and 366 nm, as well as by spraying with specific reagents to confirm the presence of secondary metabolites. This analysis was conducted to monitor the production of metabolites across different fermentation periods (7, 14, 21, and 28 days).

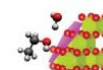
The alkaloid test was conducted using Mayer's and Dragendorff's reagents, where the formation of white/red, brown, or yellow precipitates indicated a positive result. The flavonoid test was carried out by adding Mg powder, 37% HCl, ethanol, and amyl alcohol; the appearance of red, yellow, or orange coloration indicated a positive reaction. The saponin test involved shaking the test solution followed by the addition of 2 N HCl; the formation of stable foam indicated a positive result. The quinone test was performed by adding 1 N NaOH, where a red coloration signified a positive result. The terpenoid and steroid tests were conducted using acetic anhydride and concentrated H_2SO_4 ; a red color indicated the presence of terpenoids, while blue/green coloration indicated steroids. The tannin test was performed using 5% FeCl_3 , and the appearance of dark blue or dark green coloration indicated a positive result.

2.3 Antibacterial Assay

Antibacterial activity was evaluated using the agar diffusion method [24–27].

2.3.1 Preparation of test bacteria

The test microorganisms used in this study



consisted of the Gram-positive bacterium *Staphylococcus aureus* ATCC 6538 and the Gram-negative bacterium *Escherichia coli* ATCC 11229. The cultures were grown in 5 mL of MHB medium and incubated at 37 °C for 24 hours. All microorganisms were obtained from the Central Laboratory of Universitas Padjadjaran and maintained on appropriate culture media prior to use. The turbidity was adjusted to an absorbance of 0.1 at 600 nm using a UV-Vis spectrophotometer.

2.3.2 *Inhibition assay*

The antibacterial assay was conducted by dissolving the ethyl acetate extract in 10% DMSO, followed by the preparation of serial concentrations of 10%, 5%, 2.5%, 1.25%, and 0.625%. Each concentration was then tested against *Staphylococcus aureus* and *Escherichia coli*. Bacterial cultures were inoculated onto the surface of Mueller-Hinton Agar (MHA) media. Sterile paper discs were impregnated with the ethyl acetate extract of the endophytic fungus at a certain concentration and placed onto the inoculated agar surface. Chloramphenicol (500 ppm) was used as a positive control, while discs containing solvent were used as a negative control. The plates were incubated at 37 °C for 24–48 hours, and inhibition zones were observed.

2.3.3 *Measurement of inhibition zones*

The diameter of the inhibition zone surrounding the paper discs containing the ethyl acetate extract was measured from the bottom of the Petri dish using a ruler. The clear zones indicated antibacterial activity of the tested endophytic fungi.

3 Results and Discussion

3.1 *Cultivation of Endophytic Fungi*

Isolation of endophytic fungi from the stem of *B. fulva* Blume was carried out using a surface sterilization method. This method involves immersing explants in chemical sterilizing agents at appropriate concentrations, functioning as disinfectants to obtain plant tissues free from surface contaminants [22], and serves as a procedure to separate or isolate each fungal type and its population [28]. The sterilization process was performed using 70% ethanol and 5.25% NaOCl to eliminate surface microorganisms. Sterile stem segments were then placed onto PDA medium containing chloramphenicol to promote the growth of endophytic fungi from *B. fulva* Blume. PDA medium was used because it is unsuitable for the growth of bacteria and pathogenic molds, thereby reducing the likelihood of contamination. According to Nurdin and Nurdin, culture media can support microbial growth

effectively if the pH is appropriate, the medium contains no inhibitory substances, is sterile, and provides sufficient nutrients for microorganisms [29].

The endophytic fungi were incubated for 14 days at room temperature. The resulting fungal colonies were purified to obtain single isolates. Macroscopic observations revealed white colony surfaces with brown reverse pigmentation. Based on the selection results, the isolated endophytic fungus was identified as belonging to the genus *Fusarium* and classified as *Fusarium solani* (Fig. 1).

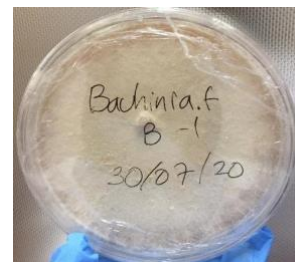


Figure 1. Endophytic fungus *Fusarium solani*

Molecular identification was carried out based on the Internal Transcribed Spacer (ITS) rDNA gene sequences using BLAST analysis. Molecular analysis using ITS rDNA sequences is a widely applied method for fungal identification up to the species level because this region exhibits both high conservation and significant genetic variation. The Basic Local Alignment Search Tool (BLAST) analysis of the ITS sequence from the endophytic fungal isolate *Bauhinia fulva* stem A1 showed a very high level of similarity with several *Fusarium* species, particularly *F. solani*, with a percent identity of 99.28–99.87%, a Query Cover of 100%, and an E-value of 0.0 (Fig. 2). A percent identity above 99% indicates that the isolate shares a very close genetic relationship with the reference species. Meanwhile, an E-value of 0.0 signifies that the observed similarity represents true homology between the isolate sequence and *F. solani* sequences available in the GenBank (NCBI) database. This reflects the accuracy of sequence alignment between unknown nucleotide sequences and reference sequences in GenBank. The Basic Local Alignment Search Tool (BLAST) analysis showed a very high similarity with *Fusarium solani* sequences available in GenBank. The higher the alignment score, the greater the similarity between the two sequences [20].

The BLAST results were further supported by phylogenetic analysis, which showed that the *B. fulva* stem isolate A1 clustered in a single clade with *F. solani*, with a high bootstrap value (>90%) (Fig. 3). This strongly confirms that the isolate shares a close evolutionary relationship and belongs to the genus

Fusarium. The close branching pattern indicates an evolutionary relationship and the likelihood of shared genetic characteristics [25].

[illegible]

Figure 2. Top Ten BLAST Hits with the Highest Scores against the Database.



Figure 3. Phylogenetic Tree of *B. fulva* Stem Isolate A1.

3.2 Antibacterial Activity Assay

The antibacterial activity of the ethyl acetate extract of the endophytic fungus *F. solani* was evaluated using the disk diffusion method against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. In the diffusion method, antibacterial activity is indicated by the formation of inhibition zones around the disk, as shown in **Fig. 4**, **Table 3**, and **Table 4**. The inhibition zone refers to the clear area surrounding the disk. According to Yanti and Mitika, inhibition zones measuring ≤ 5 mm are categorized as weak, 5–10 mm as moderate, 10–20 mm as strong, and ≥ 21 mm as very strong [26].

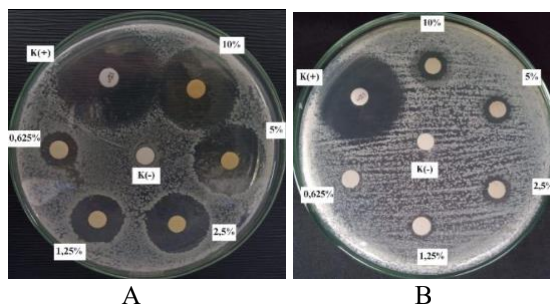


Figure 4. Antibacterial Assay Results: (A) against *S. aureus* and (B) against *E. coli*

The results showed that the ethyl acetate extract exhibited antibacterial activity against *S. aureus*, with inhibition zones ranging from 5.30 mm to 20.85 mm. The inhibitory activity increased with increasing extract concentration, where the 0.625% concentration showed moderate activity, concentrations of 1.25%, 2.5%, and 5% showed

strong activity, and the 10% concentration showed very strong activity. For comparison, the positive control (chloramphenicol) produced a 28.10 mm inhibition zone categorized as very strong, while the negative control (PDAC medium) showed no inhibition activity.

Table 3. Antibacterial Activity of Endophytic *F. solani* Against *S. aureus*

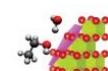
Treatment	Inhibition zone (mm)	Activity
Ethyl acetate fraction 0.625%	1.20	Weak
Ethyl acetate fraction 1,25%	1.35	Weak
Ethyl acetate fraction 2,5%	3.55	Weak
Ethyl acetate fraction 5%	4.26	Weak
Ethyl acetate fraction 10%	5.56	Moderate
Control (-) PDAC medium	0.00	No Inhibition
Control (+) Chloramphenicol	26.10	Very strong

Table 4. Antibacterial Activity of Endophytic *F. solani* Against *E. coli*

Treatment	Inhibition zone (mm)	Category
Ethyl acetate fraction 0.625%	5.30	Moderate
Ethyl acetate fraction 1.25%	10.70	Strong
Ethyl acetate fraction 2.5%	15.50	Strong
Ethyl acetate fraction 5%	17.65	Strong
Ethyl acetate fraction 10%	20.85	Very strong
Control (-) PDAC medium	0.00	No Inhibition
Control (+) Chloramphenicol	28.10	Very strong

Against *E. coli*, the antibacterial activity was lower, with inhibition zones ranging from 1.20 mm to 5.56 mm. Most extract concentrations showed weak inhibitory activity, while the 10% concentration showed moderate activity. The positive control produced an inhibition zone of 26.10 mm, categorized as very strong, whereas the negative control showed no inhibition zone.

The difference in antibacterial activity between *S. aureus* and *E. coli* is likely related to differences in bacterial cell wall structure. Gram-positive bacteria such as *S. aureus* have a simpler peptidoglycan layer that is more susceptible to antibacterial compounds, while Gram-negative bacteria such as *E. coli* possess an outer membrane containing lipopolysaccharides that can inhibit the penetration of bioactive



compounds. Therefore, the ethyl acetate extract of *F. solani* demonstrated greater effectiveness against Gram-positive bacteria than Gram-negative bacteria.

The results showed that the ethyl acetate extract of the endophytic fungus *Fusarium solani* exhibited antibacterial activity against *S. aureus*, with inhibition zones ranging from 5.30–20.85 mm, which were categorized as moderate to very strong activity depending on the extract concentration. The positive control (500 ppm chloramphenicol) produced an inhibition zone of 28.10 mm, categorized as very strong activity, while the negative control (PDAC medium) showed no inhibition activity (**Fig. 4A**). These results suggest that the ethyl acetate extract of *Fusarium solani* contains compounds capable of inhibiting the growth of *S. aureus*; however, the antibacterial activity observed in this study should be considered preliminary because the evaluation was limited to the disk diffusion method.

Against *E. coli*, the extract also exhibited antibacterial activity, although the inhibition zones were lower, ranging from 1.20 to 5.56 mm and categorized as weak to moderate activity. The positive control produced an inhibition zone of 26.10 mm, whereas the negative control showed no inhibition activity (**Fig. 4B**).

The phytochemical screening of the ethyl acetate extract of *F. solani* indicated the presence of alkaloids, flavonoids, quinones, and terpenoids/steroids (**Table 1**). To provide more robust evidence of the chemical composition, TLC analysis was performed (**Fig. 5**). The chromatogram revealed a distinct pattern of secondary metabolites, with the highest intensity and number of spots observed in the 21-day fermentation extract. This correlates with the biomass data, where the maximum mycelial weight (0.3027 g) was achieved on day 21. The TLC profile confirms that the observed antibacterial activity is attributed to a complex mixture of these secondary metabolites rather than a single compound. Furthermore, the consistency of the TLC spots across multiple replicates suggests that the metabolic production by *F. solani* is stable under the tested fermentation conditions.

The inhibitory activity against *S. aureus* indicates the presence of antibacterial bioactive compounds in the extract, which are presumed to originate from triterpenoid groups. Triterpenoids are known to exert potent antibacterial effects through several mechanisms, primarily by disrupting the bacterial cytoplasmic membrane. These lipophilic compounds can penetrate the phospholipid bilayer, leading to increased membrane permeability and the leakage of vital intracellular constituents such as proteins and ions [28]. The difference in response between *S.*

aureus and *E. coli* is likely due to differences in cell wall structure, in which Gram-negative bacteria are generally less susceptible to lipophilic compounds because of their outer membrane barrier derived from fungal secondary metabolites. Thus, the endophytic fungus *F. solani* isolated from the stem of *B. fulva* Blume has potential as a natural source of antibacterial compounds effective against Gram-positive bacteria, specifically *S. aureus*, but shows no activity against the Gram-negative bacterium *E. coli*.

Table 1. Phytochemical Test Results of Endophytic Fungal Extract from the Stem of *B. fulva* Blume

No.	Secondary Metabolite	Test Result on Extract	Positive Reaction
1.	Alkaloids	+	Formation of an orange-red solution
2.	Flavonoids	+	Formation of a pale yellow solution
3.	Quinones	+	Formation of a pale yellow solution
4.	Saponins	-	Formation of stable no foam
5.	Tannins	-	No dark green or blue-black coloration was observed.
6.	Triterpenes/steroids	+	Formation of a pale yellow solution

Note: (+) indicates a positive result, (–) indicates a negative result

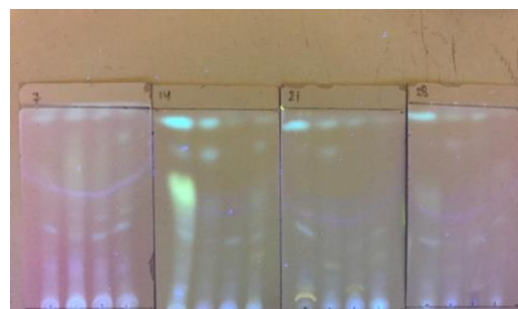
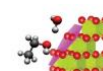


Figure 5. TLC profile of *Fusarium solani* ethyl acetate extract under UV light (254 nm and 366 nm) showing the optimization of secondary metabolite production from day 7 to day 28

4 Conclusion

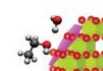
The endophytic fungus isolated from the stem of *B. fulva* Blume was identified as *F. solani* based on ITS rDNA gene analysis, showing a similarity level of 99.87%. The ethyl acetate extract contained alkaloids, flavonoids, quinones, and terpenoids/steroids. The results showed that the ethyl acetate extract exhibited antibacterial activity against both tested bacteria, with inhibition zones increasing as the concentration increased. Against *S. aureus* (Gram-positive bacteria), the inhibition zone ranged from 5.30 mm at 0.625% concentration (moderate category) to 20.85 mm at 10% concentration (very strong category), indicating strong antibacterial



activity. In contrast, against *E. coli* (Gram-negative bacteria), the inhibition zones ranged from 1.20 mm to 5.56 mm, which were mostly categorized as weak inhibition, except at the 10% concentration, which showed moderate inhibition activity. The negative control (PDAC medium) showed no inhibition activity in both tests, while the positive control (chloramphenicol) produced the highest inhibition zones of 28.10 mm and 26.10 mm, both categorized as very strong. Overall, these findings indicate that the ethyl acetate extract possesses concentration-dependent antibacterial activity and is more effective against Gram-positive bacteria than Gram-negative bacteria.

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