

The Potency of endophytic fungi from the bark of *Syzygium cumini* (L.) Skeels as antifungal agents

Nurhaida Nurhaida*¹, Syarifah A. Rashid², and Darah Ibrahim²

¹ Department of Chemistry, Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Aceh, Indonesia.

² Industrial Biotechnology Research Laboratory, School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia

Abstract. The bark of *Syzygium cumini* is potential as an antimicrobial agent, so this bark is a reservoir of endophytic fungi. The purpose of this study was to isolate endophytic fungi and determine its antifungal activity. Several methods used in this research are surface sterilization, endophytic isolation on plain media and media containing host plant, morphology identification of endophytic fungi and determine antifungal activity. Thirteen endophytic fungi have been isolated from the bark of *S. cumini*, in which seven and six endophytic fungi grow on the plain media and media containing host plants, respectively. Based on the morphology identification, these endophytic fungi include the genus *Phomopsis*, *Colletotrichum* and *Aspergillus*. Seven endophytic fungi displayed antifungal activity against pathogenic microorganisms. The results demonstrated that endophytic fungi isolate from *S. cumini* bark could be a source of antifungal agents.

Keywords: endophytic fungi, *Syzygium cumini*, antifungal.

1 Introduction

Currently, the frequency of infectious diseases, especially caused by pathogenic fungi has increased worldwide. Moreover, a serious global concern is the overuse of antibiotics, so that pathogen microorganisms become resistant to the drug [1]. Therefore the search for new antibiotics, especially antifungal agents still needs to be done. Many sources of bioactive compounds are found in nature, but currently, many researchers devote attention to microorganisms, such as endophytes, especially endophytic fungi. Endophytic microorganisms reside in tissues of living plants without causing any negative effect on its host plant [2]. Every plant is host to one or more endophytic microorganisms, which they able to produce secondary metabolites. Compounds produced by endophytes can be the same or different from its host [3]. Bioactive compounds from fungal endophytes have been becoming an inspiration for the researcher since immense structure diversity and complexity, therefore this will be an opportunity to find novel drugs [4].

In Aceh, *Syzygium cumini* is known as Jambee kleng and in English, it is called Java plum. *S. cumini* belongs to family Myrtaceae and all parts of this plant such as seed, fruit, leaves, flower, and bark are used as folk medicine in many countries [5]. This is because every part of *S. cumini* possesses medicinal properties such as antioxidant, anti-allergic, anti-inflammatory, antimicrobial, anti-diarrheal, antiviral, anticancer, antidiabetic, etc [6]. Based on the medicinal properties shown by *S. cumini*, that the opportunity to find endophytic fungi is great. In this study will be focused to

isolate endophytic fungi from the bark of *S. cumini* and determine its antifungal activity against pathogenic fungi.

2 Materials and Methods

2.1 Chemical and Media

The chemicals used to sterilize the bark are sodium hypochlorite (NaOCl) and ethanol. In this study, the media used are potato dextrose agar (PDA), water agar (WA) and sabouraud dextrose agar (SDA). To prevent contamination in endophytic isolation, the antibiotic used is chloramphenicol, while ketoconazole is used as a positive control.

2.2 Collecting Plant Sample

The barks of *S. cumini* (L.) Skeels was collected from Syiah Kuala District, Banda Aceh, Indonesia. The bark samples were taken using the hand-picking method, then they were brought to the laboratory for cleaning and sterilization.

2.3 Isolation of Endophytic Fungi

Surface sterilization of the bark samples was carried out using a modified method (Tong *et al.*, 2011). Briefly, the barks of *S. cumini* were washed with running tap water for 10 minutes, then they were cut into 2 cm x 2 cm length. Furthermore, the barks were soaked in 70% ethanol and 3% sodium hypochlorite for 90 seconds, respectively. Finally, they were rinsed in sterile distilled water for 1- 2

* Corresponding author: haida_yusuf@unsyiah.ac.id

minutes. The sterilized barks were cut into 1 cm x 1 cm length and they were placed in Petri dishes containing PDA and PDA containing host plant (10 g/L), which medium supplemented with chloramphenicol (0.2 g/L). Plates were sealed using parafilm, then incubated at $30 \pm 2^\circ\text{C}$ for 14 days under dark condition. To check the fungal growth from bark segments, the plates were monitored every day. Individual hyphal tips were transferred onto WA and incubated at 30°C for 24 – 48 hours, then the single spore was put onto fresh PDA (containing host plant) and incubated for 7 days. The pure cultures were numbered and maintained in PDA slants and kept at 4°C .

2.4 Identification of Endophytic Fungi

The characterization of endophytic fungal isolates morphology was carried out using the stained method with lactophenol blue reagent. The endophytic fungal cultures were put onto slides, then added 1 drop of lactophenol blue reagent. Furthermore, these slides were examined using a light microscope. The morphological characteristics were identified based on the growth pattern, hyphae, colony color and colony diameter [7].

2.5 Screening for Antifungal Activity by Agar Plug Diffusion Assay

Four test fungi were obtained from Industrial Biotechnology Research Laboratory, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia and used for the antifungal assay. These fungi microorganisms include *Aspergillus niger*, *Microsporium fulvum*, *Trichophyton rubrum* and *Fusarium solani*. The agar plug diffusion method was used to test the antifungal activity of endophytic fungi isolates. The inoculum size of test fungi cultures was determined by hemocytometer (under a light microscope), then adjusted to 1×10^6 spores/mL. The test fungi were then seeded on SDA for using the swab streaking method. Potato dextrose agar plates containing host plant extract were used to inoculate endophytic fungal isolates. After incubation for 20 days at 30°C , the endophytic fungal agar plug was cut using a sterile cork-borer into 1.0 cm diameter and 0.4 cm thickness. Further, the bioactive compounds secreted by mycelial (stationary phase) agar plugs were aseptically placed on the SDA medium which was previously sowed with test fungi. The plates were kept at 4°C for 7 days, to allow diffusion of bioactive compounds in the agar. Furthermore, all the plates were observed for the zone of inhibition after incubation at 30°C for 96 hours. The presence of an inhibition zone displayed antifungal activities. The sensitivity levels of antifungal activity were determined by measuring the zone of inhibition in millimeters. The positive control used was agar plug containing $30 \mu\text{g}/\text{disc}$ of ketoconazole.

3 Results and Discussion

3.1 Isolation and Identification of Endophytic Fungi

The growth of endophytic fungi isolates was indicated by the appearance of hyphae around bark segments (Figure 1). The use of a water agar medium aims to obtain a single spore so that the pure endophytic fungal isolates can be produced.



Figure 1. The appearance of hyphae around bark segments

Thirteen endophytic fungi isolates have been isolated from the barks of *S. cumini* (L.) Skeels and they can be cultured on artificial media. Seven endophytic fungi isolates can grow on PDA medium, while six endophytic fungi isolates can grow on PDA containing host plant. The use of conventional methods in the isolation of endophytic fungi would produce faster-growing culturable fungi and some of those endophytes are never been isolated before [8][9]. The macroscopic endophytic fungi isolates were shown in Figure 2. Based on the morphological identification, the endophytic fungi isolates comprise some genera, namely *Phomopsis*, *Collectotrichum*, and *Aspergillus*. The genus *Aspergillus* have also been isolated from leaf, petiole and stem tissue of *S. cumini*, which these endophytic fungi isolates demonstrated antioxidant activity [10].

3.2 Screening for Antifungal Activity by Agar Plug Diffusion Assay

Based on the screening using agar plug assay, seven endophytic fungi isolates demonstrated antifungal activity (Table 1). Bioactive compounds produced by endophytic fungi from barks of *S. cumini* can inhibit pathogenic fungi. Therefore, these endophytic fungi were suggested to produce antifungal agents. The range of inhibition zones of compounds produced by these endophytic fungi against tested fungi were 12 – 25 mm. The inhibition zone in disc diffusion breakpoints can be interpreted in resistant (≤ 16 mm), intermediate (17 – 20 mm) and susceptible (≥ 21 mm) [11]. *A. niger*, *T. rubrum*, and *M. fulvum* demonstrated susceptible against compounds produced by endophytic fungi isolates of BJ-1, BJ-2, BJ-4, BJ-9, and BJ-12, while *T. rubrum* showed intermediate against endophytic fungi isolates of BJ-9 and BJ-12. Compounds produced by endophytic fungi isolates of BJ-6, BJ-7, and

BJ-12 can also inhibit *A. niger* and *M. fulvum*, however, these tested fungi only displayed resistant.

Endophytic fungi isolates of BJ-3, BJ-5, BJ-8, BJ-10, BJ-11, and BJ-13 did not show antifungal activity. This is because these isolates might be potential as

another agent such as antitumor, anticancer, antioxidant, antidiabetes or as enzyme producer. Endophytic fungi reside in living plant tissue as their host and act as chemical synthesizers [12] such as antiviral, anticancer, antidiabetic and antimicrobial effects [13].

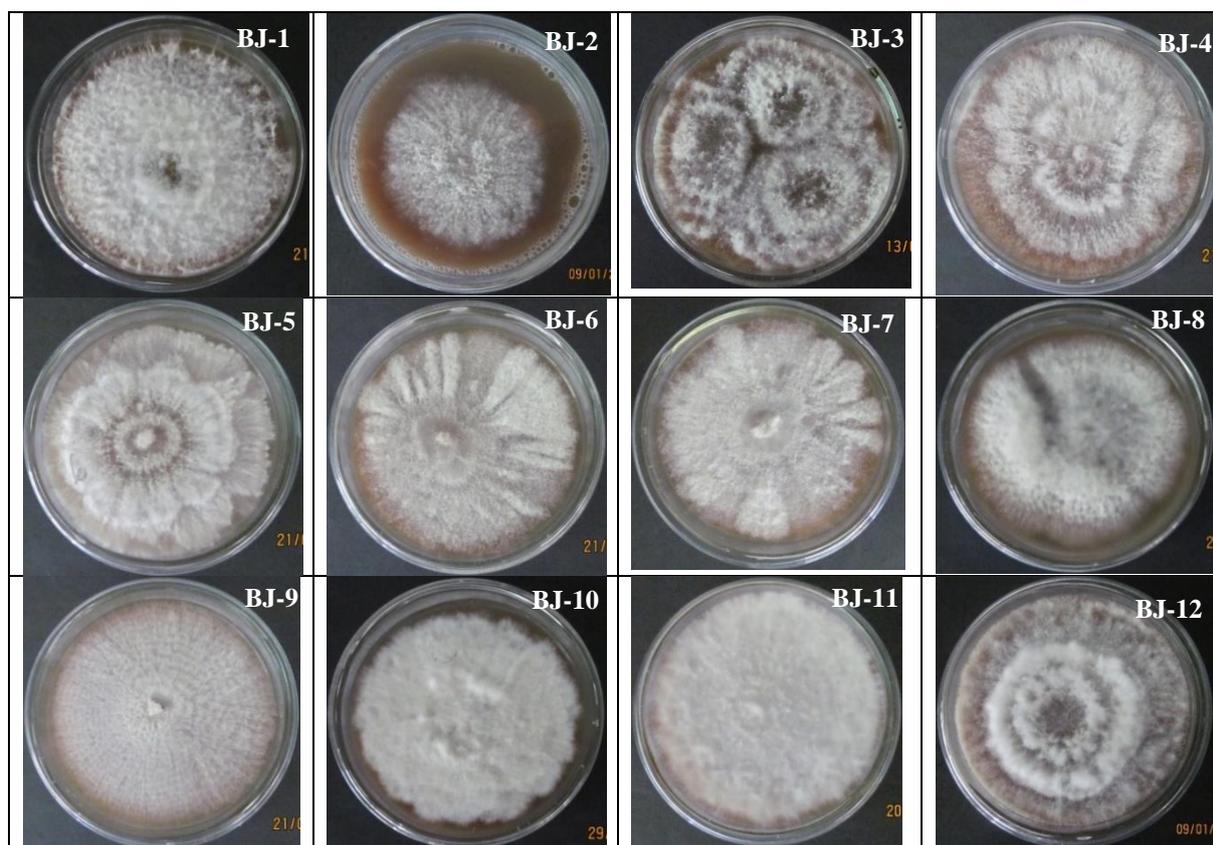


Figure 2. Some endophytic fungi isolated from the bark of *S. cumini* on PDA medium plates at 7 days

Table 1. Endophytic fungal isolates from barks of *S. cumini* and their antifungal activity

Endophytic fungi isolates	Antifungal activity (mm)			
	<i>A. niger</i>	<i>T. rubrum</i>	<i>M. fulvum</i>	<i>F. solani</i>
BJ-1	–	–	25 ± 0.7	–
BJ-2	–	–	24 ± 0.4	–
BJ-3	–	–	–	–
BJ-4	–	24 ± 0.5	–	–
BJ-5	–	–	–	–
BJ-6	15 ± 0.0	–	15 ± 0.0	–
BJ-7	12 ± 0.0	–	–	–
BJ-8	–	–	–	–
BJ-9	25 ± 0.0	20 ± 0.0	15 ± 0.0	–
BJ-10	–	–	–	–
BJ-11	–	–	–	–
BJ-12	15 ± 0.0	20 ± 0.4	25 ± 0.4	–
BJ-13	–	–	–	–
Positive control	15 ± 0.0	14.7 ± 0.5	30 ± 0.0	12 ± 0.8
Negative control	–	–	–	–

– : no inhibition zone

4 Conclusion

This study has shown that endophytic fungi isolated from barks of *S. cumini* (L.) Skeels may be potential as antifungal agents. Further investigation can be focused on the isolation of bioactive compounds from these endophytic fungi and purification of its pure compounds.

Acknowledgments

The author would like to thank for assistance or encouragement from colleagues, special work by financial support from Industrial Biotechnology Research Laboratory, School of Biological Sciences, Universiti Sains Malaysia.

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