

Isolation and Screening of Endophytic Fungi from Soursop (*Annona muricata* L.) Leaves: Potential Sources of Antimicrobial Compounds

NURHAIDA^{1*}, MURNIANA,¹ BELLA SEPTIE AMRINA¹, SYARIFAH AB RASHID²

¹Chemistry Department, Universitas Syiah Kuala, Banda Aceh, Indonesia

²School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang Malaysia

Abstract. Endophytic fungi, which reside within plant tissues, offer a unique ecological perspective due to their mutually beneficial relationship with host plants. Soursop (*Annona muricata* L.) leaves, known for their medicinal properties, contain bioactive compounds that may be produced by endophytic fungi. This study aimed to isolate endophytic fungi from soursop leaves and evaluate their potential as antimicrobial compound producers. Twenty endophytic fungal isolates were obtained from soursop leaves using Potato Dextrose Agar (PDA) supplemented with leaf extract. Out of these isolates, eight demonstrated antimicrobial activity against the tested microorganisms. Notably, the extract produced by SLE16 exhibited inhibitory effects against Gram-negative bacteria, while isolates SLE7, SLE8, and SLE14 showed antifungal activity against *Candida albicans*. Furthermore, isolates SLE18, SLE19, and SLE20 displayed significant inhibition of *Trichophyton rubrum* growth. The extracellular extracts derived from these endophytic fungi exhibited hydrophilic properties and formed inhibition zones larger than 15 mm, indicating their potential as antimicrobial compounds. These findings highlight the potential of endophytic fungi isolated from soursop leaves as valuable sources of novel antimicrobial compounds.

Keyword: Endophytic fungi, Soursop leaves, Antimicrobial activity

INTRODUCTION

Endophytic fungi are a group of fungi that inhabit plant tissues without causing harm to their host plants. These fungi can be found in various plant parts such as leaves, stems, flowers, fruits, and seeds [1]. The interaction between endophytic fungi and their host plants is characterized by a symbiotic mutualism, where both parties benefit from the relationship. Plants provide shelter and nutrients to the endophytes, while the endophytes produce secondary metabolites that help plants survive in stressful abiotic and biotic environments [2]. Some endophytic fungi have the ability to produce toxic compounds that protect their host plants from pathogenic microbes, insects, nematodes, and herbivores [3]. When their host plants die, endophytic fungi can also live as saprophytes [4]. Medicinal plants are a potential source of endophytic fungi.

One such medicinal plant is soursop (*Annona muricata* L.). Soursop is known for its ease of cultivation without requiring special care. Various parts of the soursop plant, including the fruit, leaves, root, seed, flower, and bark, have been traditionally used to treat ailments such as wounds, ulcers, boils, seizures, acne, and head lice [5]. Soursop leaves contain bioactive compounds such as annonaceous acetogenins, tannins, phytosterols, calcium oxalate, murisin alkaloids, and essential oils [7, 8]. Isolating endophytic fungi from soursop leaves could potentially lead to the discovery of novel antimicrobial compounds. This study aims to isolate endophytic fungi from soursop leaves and screen these isolates for their potential as producers of antimicrobial compounds.

METHODOLOGY

Isolation of Endophytic Fungi

Soursop leaf samples were washed with running water and cut into 2 x 2 cm pieces. The leaf pieces were then sterilized by immersing them in 70% ethanol (EtOH) for 60 seconds, followed by 2% sodium hypochlorite (NaOCl) for 30 seconds. Afterward, the sterilized leaf pieces were dried on sterile tissue and further cut into 1 x 1 cm pieces using a sterile knife. These sterile leaf pieces were placed on two types of media: Potato Dextrose Agar (PDA) and PDA supplemented with leaf extract. Both media contained 0.2 g/L chloramphenicol to inhibit bacterial growth. As a control, distilled water used to rinse the leaf pieces was spread onto PDA. Successful isolation of endophytic fungi was confirmed if no fungi grew on the control media. The plates were then incubated at room temperature for 7 days and observed daily. The growing hyphae were transferred to water agar. After incubating for 24-48 hours, the endophytic fungal isolates were transferred to PDA media containing leaf extract and incubated at room temperature for 7 days. The endophytic fungal isolates were stored at 10°C for future use.

Extraction of Secondary Metabolites from Endophytic Fungal Isolates

Endophytic fungal isolates were cultured in Potato Dextrose Broth (PDB) containing leaf extract and incubated in the dark at room temperature. After 14 days of incubation, the biomass was separated from the fermentation broth using muslin cloth and Whatman filter paper No. 1. The fermentation broth represents the crude extract produced extracellularly by the endophytic fungal isolates.

Screening for Antimicrobial Activity

Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (Agar) media were used to evaluate the antimicrobial activity of the extracellular extracts. MHA was used for screening antibacterial activity, while SDA was employed for testing antifungal activity. The extracellular extracts were tested against Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Klebsiella pneumoniae*), and four pathogenic fungi (*Candida albicans*, *Candida utilis*, *Trichophyton rubrum*, and *Microsporum fulvum*). Sterile blank discs with a diameter of 6 mm were impregnated with 20 microliters of each extracellular extract. These discs were placed on the MHA and SDA media, which had been previously inoculated with the test bacteria and fungi. The plates were then incubated at 37°C for 18-24 hours for bacteria and at 30°C for 48-96 hours for fungi. Chloramphenicol and ketoconazole were used as positive controls for bacteria and fungi, respectively, while the media containing leaf extract served as the negative control. The antimicrobial activity was determined by the presence of a clear zone around the disc, and the size of the inhibition zone was measured in millimeters.

RESULTS AND DISCUSSION

Isolation of Endophytic Fungi from *A. muricata* Leaves

Figure 1 illustrates the twenty endophytic fungal isolates obtained from soursop leaves. Approximately nine isolates were obtained from the PDA media, while eleven isolates were obtained from the PDA media supplemented with leaf extract. The addition of leaf extract in the isolation medium created a favorable environment resembling the conditions inside the host plants, facilitating the growth of endophytic fungal isolates. Endophytes typically have a symbiotic relationship with their host plants [9], and the addition of plant extracts in the isolation medium promotes the proliferation of endophytic fungi [10]. Similar increases in the number of endophytic isolates have been observed with the addition of extracts from *Taxus* stem bark [11]. However, it should be noted that morphological similarities alone may not indicate the same species, as each isolate has the potential to produce different extracts. This is evident from the variations in antimicrobial activity exhibited by the isolates against the tested microbes.

Screening of Endophytic Fungal Isolates

The fermentation broth produced by the endophytic fungal isolates represents the extracellular extract secreted into the growth media. The extracts are considered polar compounds due to the use of water in the growth media. These extracellular extracts were tested for their antimicrobial activities against pathogenic microbes. Table 1 presents the antimicrobial activities of the extracellular extracts.

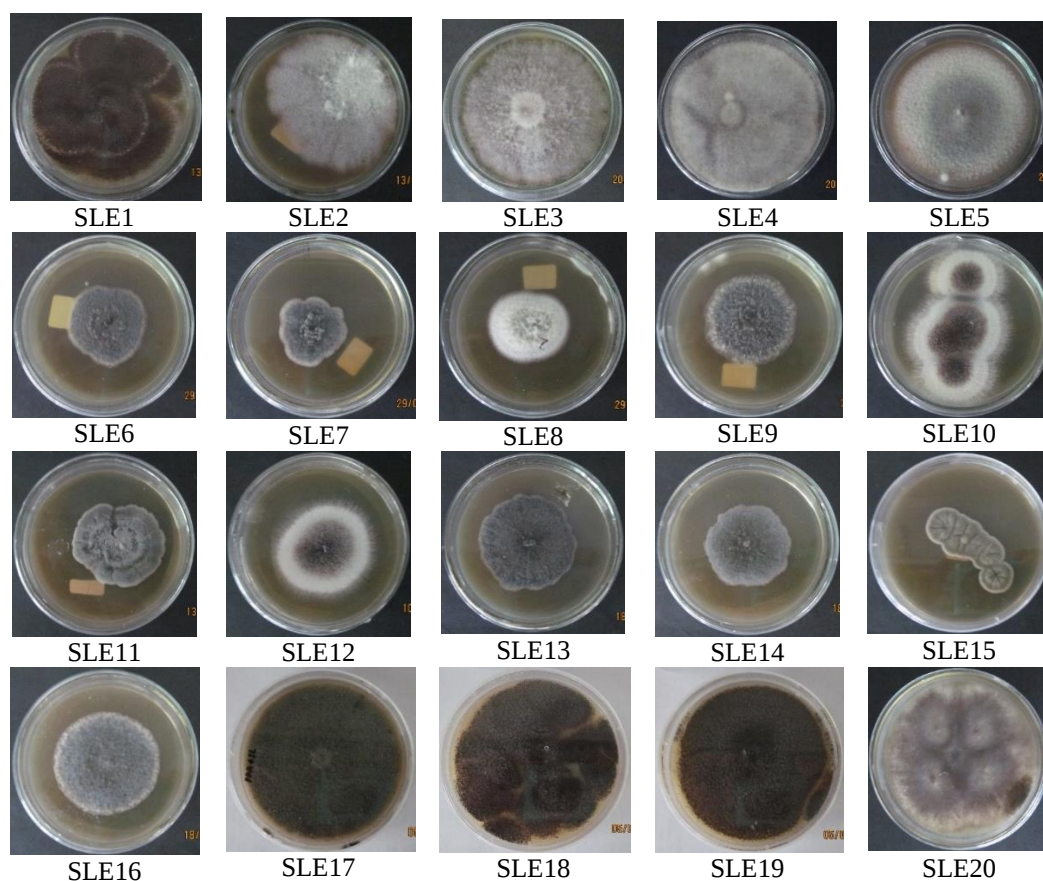


Figure 1. Morphological characteristics of endophytic fungal isolates obtained from *A. muricata* leaves after 14 days of incubation on PDA supplemented with host plant extract.

Out of the 20 endophytic fungal isolates, only 8 isolates exhibited antimicrobial activity against the tested microorganisms, but none of them showed activity against *S. aureus*. This may be attributed to the absence of compounds in these extracts that can inhibit the growth of Gram-positive bacteria. The crude extract produced by isolate SLE16 demonstrated inhibitory effects against Gram-negative bacteria (*K. pneumoniae*). The efficacy of an antimicrobial agent in inhibiting growth is influenced by factors such as the nature of the microorganism, concentration of the agent, and duration of contact [12]. Additionally, the formation of an inhibition zone depends on the amount of extract applied to the disc, solubility of the antimicrobial compound in the media, diffusion coefficient, and effectiveness of the antimicrobial agent [13]. Isolates SLE7, SLE8, and SLE14 exhibited antifungal activity by inhibiting the growth of *Candida albicans*. Isolate SLE19 demonstrated inhibitory effects against both *C. utilis* and *M. fulvum*, while isolates SLE18 and SLE20 inhibited the growth of *T. rubrum*, with their inhibition zones being larger than the positive control. This suggests that these extracts have the potential to act as anti-*T. rubrum* compounds.

Tabel 1. Antimicrobial activities of the extracellular extract isolated from endophytic fungi against test microbes

Microbes test	Crude extract of endophytic fungi isolates / Diameter of inhibition zone (mm± SD)								Positive control	Negative control
	SLE5	SLE7	SLE8	SLE14	SLE16	SLE18	SLE19	SLE20		
<i>S. aureus</i>	—	—	—	—	—	—	—	—	30.0±0.0	—
<i>K. pneumoniae</i>	—	—	—	—	10.0±0.0	—	—	—	30.0±0.0	—
<i>C. albicans</i>	—	20.0±0.0	15.0±0.0	15.0±0.0	—	—	—	—	29.0±0.0	—
<i>C. utilis</i>	—	—	—	—	—	—	20.0±0.0	—	31.0±0.0	—
<i>T. rubrum</i>	15.0±0.0	—	—	—	—	20.0±0.0	—	20.0±0.0	15.0±0.0	—
<i>M. fulvum</i>	—	—	—	—	—	—	20.0±0.0	—	30.0±0.0	—

The hydrophilic properties of the extracts are crucial for ensuring that the antimicrobial compounds dissolve in the aqueous phase, which is abundant in microbial cells. On the other hand, when these compounds target hydrophobic cell membranes, they require lipophilic properties. Hence, achieving an appropriate hydrophilic-lipophilic balance (HLB) is necessary to maximize their activity [14, 15]. The extracellular extracts produced by the endophytic fungal isolates displayed inhibition zones larger than 15 mm. Such activities indicate susceptibility or easy inhibition of the test microorganisms, as the inhibition zones exceeded 15 mm [16]. Consequently, the aqueous extracts derived from endophytic fungi isolates from *A. muricata* leaves exhibit potential as producers of antimicrobial compounds.

CONCLUSION

In this investigation, 20 endophytic fungi were isolated from soursop (*A. muricata* L.) leaves and designated as soursop leaves endophytic (SLE 1-20). Among these, eight isolates (SLE5, SLE7, SLE8, SLE14, SLE16, SLE18, SLE19, and SLE20) demonstrated antimicrobial activity against the tested microorganisms. The aqueous extracts of these eight endophytic fungal isolates from soursop leaves have the potential to yield antimicrobial compounds.

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