Isolation and Identification of Endophytic Fungi Associated with Indonesian *Sesbania grandiflora* Plant

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Abstract. In the present study, endophytic fungi associated with Indonesian *Sesbania grandiflora* plant were isolated and identified for the first time. The objective of this study was to report new data regarding the endophytic fungi found in *S. grandiflora* as one of Indonesian medicinal plant. Six isolates of endophytic fungi were isolated from the leaves, bark, seed, flower, and root of *S. grandiflora* collected from Labuhan Ratu, Kedaton, Bandar Lampung, Indonesia. Based on the prediction of their morphological characteristics visually, four isolates from all parts of plant were identified as *Fusarium* sp, while two isolates obtained from the bark and the seed were identified as *Hormiscium sp* and *Penicillium* sp, respectively. In addition, TLC profile result of secondary metabolites extract of endophytic fungi indicated that more than one major compound was observed. Furthermore, the antibacterial screening of isolated endophytic fungi did not show inhibition growth against resistant *E. coli*. However, the phytopharmacological study on the isolated fungi associated with *S. grandiflora* as well as their biological properties are still in progress. The results of this study revealed that *S. grandiflora* plant is reliable source of endophytic fungi for the future investigation.

Keyword: Endophytic Fungi, *Hormiscium sp.*, *Fusarium sp.*, *Penicillium sp.*, *Sesbania grandiflora*.

1. Introduction

S. grandiflora, one of the medicinal plants belonging to the Fabaceae family that can be found in the tropical dry and moist forest areas such as Indonesia, Malaysia, India, Myanmar and Philippines. All parts of *S. grandiflora* are used as a traditional herbal remedy since the ancient times to cure various disorders including cough, fever, rheumatic swellings, smallpox, worms, biliousness, gout, itchiness, leprosy, anaemia, and gastric, as well as some diseases caused by bacterial infection[1-4]. The pharmacological study on the various extracts of *S. grandiflora* have been done by many researchers. Panda et al [5] has been reported that *S. grandiflora* leaves exhibited an antioxidant activity. A subsequent research by Ramesh et al [6] have evaluated the restorative effects of *S. grandiflora* on

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oxidative damage induced by cigarette smoke exposure in the brain of rats. The results showed that *S. grandiflora* restores the brain from cigarette smoke induced oxidative damage.

Our investigation on the phytochemical study of the Indonesian *S. grandiflora* plant has been started since more than one decade. In the our previous research, we successfully separated some isolated compounds including flavonoids, terpenoid, and phenolic from *S. grandiflora* roots. Most of the constituents isolated demonstrated moderate antituberculosis property [7,8]. Recently, we have reported a number of 2-arylbenzofuran-3-carbaldehydes obtained from the stem bark of *S. grandiflora.* All the isolated compounds displayed moderate cytotoxicity against HeLa, HepG2, and MCF-7 cancer cell lines. None of the compounds showed antibacterial activity against *several bacterial strains.* However, two compounds exhibited moderate activity against *Mycobacterium tuberculosis* H37Rv [9,10]. Additionally, we have published several new derivatives of major isolated compound from *S. grandiflora* stem bark. We found that among them, a synthetic diester showed moderate antibacterial activity against the plant pathogen *Rhodococcus fascians* with a MIC of 0.1 mg/mL [11].

Based on the above results, both isolated and synthetics compounds revealed moderate activity against certain cancer cell lines and did not show growth inhibitory activity against most bacteria. Due to their limited biological activities, therefore we attempted to refocuse our research on the phytochemical study of endophytics associated with *S. grandiflora*, including the isolation and screening of biological properties. We report herein the extraction and isolation of endophytic fungi associated with different parts of *S. grandiflora* (the leaves, bark, seed, flower and root) along with their antibacterial activity against resistant *Escherchia coli*.

2. Experimental

2.1. General experimental procedures

All chemicals and solvents used for the extraction and isolation of the isolated compound were reagent grade. TLC has been conducted on silica gel 60 GF_{254} plates (Merck; 0.25 mm), potato, agar, dextrose, 70% alcohol, distilled water, ethyl acetate (EtOAc), *n*-hexane, methylene blue, ciprofloxacin antibiotic, tissue, cotton, filter paper, aluminum foil, and plastic wrap.

2.2 Materials

The healthy and fresh of all parts of *S. grandiflora* were collected in 18th February 2008 in Labuhan Ratu, Bandar Lampung, Indonesia. The specimen of plant was authenticated at the Herbarium Bogoriense, LIPI Bogor, Indonesia. Resistant *Escherchia coli* strain was obtained from the Abdul Muluk Hospital, Lampung.

2.3 Extraction and isolation

Freshly different parts of samples (leaves, stem bark, and root) were subjected to surface sterilization following procedures as described by [12] with minor modification. The samples were cleaned by rinsing under running tap water followed by washing with 70% ethanol for 3 min and then finally rinsed in sterile distilled water. After sterilization, each part of plant materials was further cut (aseptically) in to 1 cm × 1 cm length then placed onto the agar medium in petri dishes. All dishes were sealed with parafilm and incubated for 3–7 days at room temperature. During the incubation period, observations are conducted to ascertain the rate of growth of endophytic fungi. The isolation of endophytic fungi from these dishes was carried out by transferring of hyphal tips to fresh potato dextrose agar (PDA) plates to yield pure cultures for identification.

2.4 Identification of endophytic fungi

All isolated endophytic fungi obtained were identified based on their cultural characteristics and morphological, including growth pattern, hyphae, colour of colony and medium, surface texture, margin character, aerial mycelium, mechanism of spore, production and characteristics of the spore [13]. The characteristics and morphological data observed from isolated endophytic fungi were then compared with the identified endophytic fungi from the same genus of Fabaceae that reported by previous researchers [14,15].

2.5 Fermentation of endophytic fungi

The isolated endophytic fungi was fermented to produce large-scale fungi on the solid medium using procedures described by McNeil et al and Gasong et al [16,17] with modification. Briefly, one ose of isolated endophytic fungi was inoculated into 100 mL of potato dextrose broth (PDB) and potato dextrose yeast (PDY) and then the media was incubated for 7-14 day at room temperature. After incubation, mycelia and fermentation broth were separated by filtration. The fermentation broth was then extracted by using liquid–liquid partition method with ethyl acetate solvent (v/v:1/1) followed by reducing the volume of ethyl acetate with rotary evaporator under reduce pressure. The concentrated extract obtained was monitored by thin layer chromatography (TLC) technique with the appropriate eluent. After elution, the spots obtained on the TLC plate were observed under UV light at a wavelength of 254 nm and 366 nm, then the TLC plate was sprayed using cerium sulfate as staining reagent.

2.6 Antibacterial screening of endophytic fungi

The profile of secondary metabolites obtained from endophytic fungi was screened using the immersion bioautographic method that is similar to the one used in agar diffusion methods [18]. Initially, the TLC plate is first immersed in or cover with agar medium, which after solidification is seeded with the tested microorganisms (resistant *E.coli*) and then incubated. After 24 h, the inhibition zones of growth was observed.

3. Results and discussion

The ability of the plants to produce secondary metabolites with a variety of biological activities is related with the role of endophytes within living tissues of the host plants. The endophytes associated with medicinal plants are known to acquire the potential bioactive compounds. Preliminary study on the endophytic fungi associated with different parts of Indonesian *S. grandiflora* will be discussed in this paper.

After extraction and isolation processes, six endophytic fungi were successfully obtained from different parts *S. grandiflora* (leaves, stem bark, and root). The diversity of endophytic fungi in a host plant is highly influenced by a number of factors, including growth condition, pH at which plants grow, as well as the host type [19]. The identification of isolated endophytic fungi was determined by comparing visually the morphological characteristic of the fungi with the previously reported. Figure 1 displayed the morphological character of the isolated fungi of different parts *S. grandiflora*.

Figure 1. Morphological characteristics of the isolated fungi of different parts *S. grandiflora*

According to Bernet [13], the isolated endophytic fungi from the leaves, stem bark, and root of *S. grandiflora* were early identified as *Fusarium sp., Hormiscium sp., Penicillium sp* as tabulated in Table 1. From the leaves, stem bark, seed, and flower tissues of *S. grandiflora*, producing the same fungi that is identified as *Fusarium sp.* The existence of *Fusarium sp* in most of the tissues of plant due to its role to control soil-borne pathogens [20]. Fusarium species can help prevent crop diseases and large yield losses. The bioprospecting endophytic fungi from *Fusarium* genus as sources of bioactive metabolites was reviewed by Toghueo [21]. It was reported that these endophytic microbes may provide protection and survival strategies in their host plants with production of chemically diverse and structurally unprecedented secondary metabolites including antimicrobial, anticancer, antiviral,

antioxidants, antiparasitics, immunosuppressants, immunomodulatory, antithrombotic, and biocontrol ability against plants pathogens and nematode [21].

Hormiscium sp., *Penicillium sp* were isolated from the stem bark and seed, respectively. *Penicillium* genus are important in human life and they commonly appear as soil, storage, indoor and airborne fungi or as food contaminants. This genus was reported to be able to produce a number of secondary metabolites that possessed interesting biological activities such as antioxidant, antibacterial, antifungal, antiviral, antibacterial, antitumor, antifungal, immune-suppressants, and cholesterol-lowering properties [22-28].

Among three endophytic fungi obtained from *S. grandiflora*, one isolated fungi has been fermented to produce its secondary metabolites. The secondary metabolites produced were assessed by TLC technique using *n*-hexane: ethyl acetate (7:3) as eluent (Figure 2), following assayed their antibacterial activity against resistant *E. coli* (Figure 3). The results of TLC profile indicated that at least one major compound was performed, while the antibacterial result showed that no inhibition growth was observed from the extracts tested. However, the phytochemical investigastion on the endophytic fungi associated with *S. grandiflora* is still in progress for further recognizing the profile of bioactive compounds obtained as an important and novel naturally resources that may lead the potential applications in the agricultural, medical, and food industries.

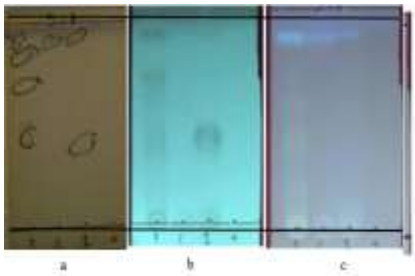

Figure 2. The chromatogram of TLC profile of endophytic fungi obtained from *S. grandiflora*; (a) sprayed with cerium sulfate; (b) UV observation at a wavelength of 254 nm (c) UV observation at a wavelength of 365 nm

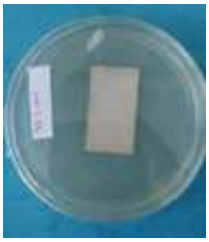

Figure 3. Bioautographic assay result of endophytic fungi obtained from *S. grandiflora*

4. Conclusions

Six endophytic fungi associated with different parts of *S. grandiflora* have been isolated in this research. Among them, three endophytic fungi were identified as different genus based on the morphological characteristic. One out of three isolated did not show inhibition against resistant *E.coli* strain. However, further phytochemical study on the endophytic fungi of *S. grandiflora* is still in progress to better understanding the role of the living microorganism associated with *S. grandiflora.*

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