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Determination Of Sun Protection Factor (SPF) Value Of Endophyte Fungi From Sendok Leaves (*Plantago Major L.*)

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Abstract. Excessive exposure to UV rays can cause adverse effects on the skin, such as erythema, pigmentation, premature aging, and skin cancer. The use of sunscreen is an important protection for the skin from UV exposure. Sunscreen works by reflecting or absorbing UV rays, preventing them from reaching the skin. However, synthetic sunscreens may have potential side effects such as irritation and allergic reactions. Therefore, sunscreens made from natural ingredients are an alternative to synthetic sunscreens. One source of natural ingredients to replace synthetic sunscreen is the utilization of secondary metabolites from endophytic fungi. Endophytic fungi have potential as natural active ingredients in sunscreen because they can produce secondary metabolites similar to their host plants. This study evaluated the potential of endophytic fungi on spoon leaf plants as a natural sunscreen alternative using UV-VIS spectrophotometry. Endophytic fungi were cultivated in a PDA medium and then propagated in a PDB medium before extraction with ethyl acetate. Macroscopic and microscopic identification results showed that the two fungal isolates obtained belonged to the genus *Aspergillus*. Phytochemical tests confirmed the presence of flavonoids, alkaloids, and steroids in the extracts. Ethyl acetate extracts of *Aspergillus* sp. from spoon leaves showed that the extracts protected against ultraviolet rays, with the highest SPF value at a concentration of 500 ppm, namely 38.47 and 40, which falls into the ultra-protection category.

Keywords : Endophytic Fungi, *Plantago major L.*, Sunscreen, SPF

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Introduction

Indonesia is located on the equator with a tropical climate that is susceptible to sun exposure and global warming, thus increasing the risk of UV exposure, especially for people who are active outdoors [1]. UV-A rays (320-400 nm) and UV-B (280-320 nm) reach the Earth's surface, while the ozone layer absorbs UV-C (200-290 nm) [2]. UV-A and UV-B rays cause changes in skin pigment, premature aging, and even cause skin cancer. Skin cancer is the third most common cancer in Indonesia, with almost 90% caused by UV exposure [3]. Therefore, preventive measures against skin cancer are essential, one of which is using sunscreen.

Sunscreen protects the skin by reflecting or absorbing UV rays. It can be synthetic chemicals, such as titanium dioxide and benzophenone. However, these ingredients can cause adverse skin effects, such as irritation, burning sensations, and allergic reactions [4]. Therefore, natural sunscreen can be an alternative to synthetic sunscreen because it contains secondary metabolites that are effective as UV protectors [5]. Various sources of secondary metabolites as sunscreen ingredients can be obtained from plants, microorganisms, or other organisms. One microorganism with great potential is endophytic fungi, which can provide a solution for producing secondary metabolites sustainably.

Endophytic fungi live within plant tissues in a mutualistic symbiosis and can produce bioactive compounds similar to their hosts through genetic transfer [6]. Its reproductive speed and bioactivity make it a potential source of natural materials as a source of endophytic fungal isolates [7]. The Sendok leaves contain alkaloids, flavonoids, saponins, steroids and tannins [8]. These compounds are secondary metabolites with chromophore groups that can absorb UV-A and UV-B rays, thereby reducing the impact of UV radiation on the skin [9]. Research by Wempi et al. [10] found that ethyl acetate extract of Sendok leaves from Bantul has high antioxidant activity with an IC₅₀ value of 16.81 ± 0.11 µg/mL. The presence of antioxidants in a sunscreen product or formula can increase the effectiveness of the sunscreen. The content of bioactive compounds that can function as antioxidants can absorb UV rays and protect the skin from the adverse effects of sunlight [11]. Therefore, this

study tested the sunscreen activity of ethyl acetate extracts of endophytic fungi from Sendok leaves in vitro method.

Experimental

Isolation of Endophytic Fungi. The leaves of the plant were washed with running water for 5 minutes, then cut into 1 × 1 cm² pieces. The isolation process was carried out under sterile conditions in a LAF. The samples are sterilised by sequentially wiping their surfaces with 70% alcohol for 1 minute, 5% sodium hypochlorite solution for 5 minutes, 70% alcohol for 1 minute, and rinsing with distilled water for approximately 3 minutes, repeated twice. The samples are dried using tweezers and planted in Petri dishes containing PDA (Potato Dextrose Agar) medium. The endophytic fungi were incubated at 25°C–27°C for 7 days, and their growth was observed daily until live endophytic fungi were visible in the PDA isolation medium. The endophytic fungi obtained were then inoculated into Petri dishes containing PDA medium, stored at 25°C for 5 days until new colonies were obtained.

Extraction of Endophytic Fungi. Glassware and media are sterilized using an autoclave at 121°C for 15–20 minutes, while loop needles are sterilized using a Bunsen burner. PDA (Potato Dextrose Agar) medium is prepared by dissolving 9.36 g of instant PDA in 240 ml of distilled water, then adding 125 mg of chloramphenicol. The solution is heated on a hot plate at 60°C, sterilized in an autoclave, and then poured into Petri dishes aseptically. PDB (Potato Dextrose Broth) medium is prepared by boiling 1.125 kg of potatoes in 4.5 L of distilled water for 30 minutes, adding 90 g of dextrose and 625 mg of chloramphenicol, then sterilizing the solution. The isolated endophytic fungi were reactivated by planting mycelium on PDA medium using sterile inoculation needles. The endophytic fungi were incubated for 7 days and then observed for their macroscopic and microscopic characteristics. The endophytic fungi were prepared using the slide culture method for genus identification with a Hyrox digital microscope. Next, endophytic fungi were cultivated by inoculating endophytic fungal isolates into 500 ml of PDB medium for 3–4 weeks at room temperature (25–27°C). Secondary metabolites were extracted using ethyl acetate solvent (1:1) for 7 days with daily shaking for 15 minutes. The extract was separated using a separatory funnel and evaporated using an evaporator until a concentrated extract

was obtained. The extract was then dried in an oven at 45°C [12].

Phytochemical Screening. Phytochemical tests were conducted to identify the content of secondary metabolites in endophytic fungal extracts. Alkaloid tests used 2 drops of Dragendorff's reagent, with positive results indicated by an orange solution or brown sediment. Tannin tests were conducted with 1% FeCl₃ solution, indicating positive results if the solution turned dark blue or greenish black. Flavonoid tests used 2 mg of magnesium and 3 drops of concentrated HCl, with a positive indication of a color change to red, yellow, or orange. Saponin tests were conducted by adding hot water, where stable foam lasted for 30 minutes and did not disappear after adding 2 drops of 2 N HCl, indicating a positive result. Meanwhile, steroid tests were conducted with a mixture of chloroform, 0.5 ml + 0.5 ml of acetic anhydride, plus 2 drops of concentrated H₂SO₄, with positive results indicated by a bluish green color for sterols and the appearance of a brown or purple ring for triterpenoids [13].

SPF Testing. In vitro testing was conducted using a UV-Vis spectrophotometer. Fifty milligrams of sample was dissolved in 50 ml of ethyl acetate to create a 1000 ppm stock solution, which was then diluted to 100-500 ppm. Absorbance was measured at wavelengths of 290-320 nm with a difference of 5 nm. The SPF value was calculated using Mansur's equation 1.

SPF Spectrophotometric =

$$CF \times \sum \frac{320}{290} EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad (1)$$

Where, CF : Correction Factor; I : Light Intensity Spectrum; EE : Erythema Effect; and Abs : Absorbance.

Table of Values EE x I given (Table 1),

Table 1 Determination of EE x I Values

Wavelength (nm)	EE x I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

used for the calculation of SPF by multiplying each value $EE(\lambda) \times I(\lambda)$ with $Abs(\lambda)$ and summing up the results.

Result and Discussion

Results of Endophytic Fungi Rejuvenation.

Endophytic fungi were rejuvenated by restoring the culture using Potato Dextrose Agar (PDA) media containing carbohydrates from potatoes and dextrose for fungal growth. Chloramphenicol was add-

The appearance of fine threads

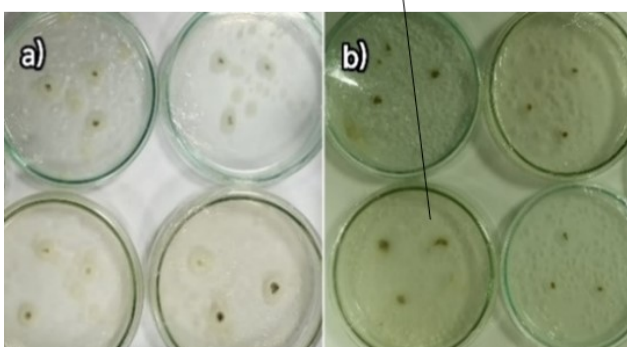


Figure 1 . Results of endophytic fungal rejuvenation on day 3 RU1(a) and RU2 (b)

Colony spreads (white patches)

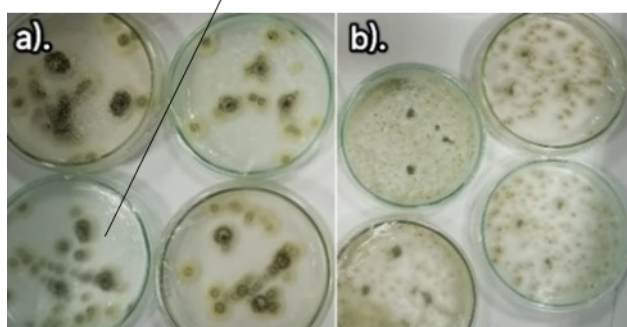


Figure 2 . Results of endophytic fungus rejuvenation on the 5th day of RU1(a) and RU2 (b)

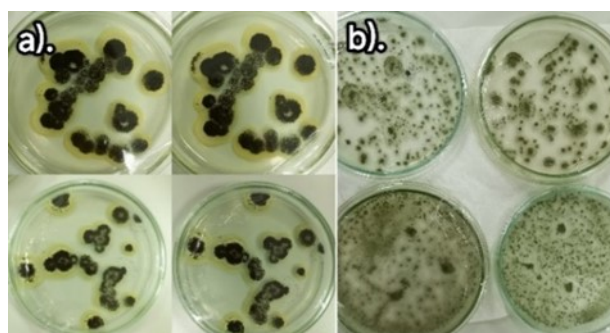


Figure 3 . Results of endophytic fungal rejuvenation on the 7th day RU1(a) and RU2 (b)

ed to prevent bacterial contamination. The two endophytic fungal isolates produced were coded RU1 and RU2 for easy identification. The two endophytic fungal isolates showed rapid colony growth and no contamination after 7 days, as can be seen in Figures 1, 2, and 3.

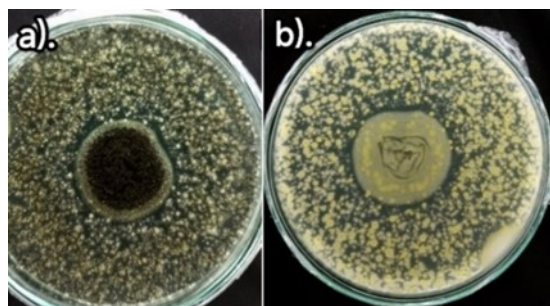


Figure 4. Macroscopic results of endophytic fungus RU1, front view (a) and back view (b)

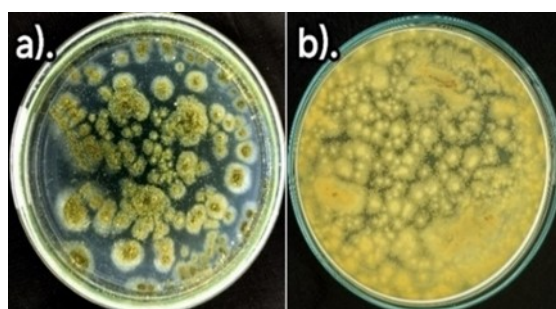


Figure 5 . Macroscopic results of the endophytic fungus RU2, front view (a) and back view (b)

Identification of Endophytic Fungi. Identification of endophytic fungi of sendok leaves was carried out macroscopically and microscopically. Macroscopically, identification includes observing the colony's colour, surface, and edge. The results of macroscopic identification are shown in Figures 4 and 5.

Figures 4 and 5 show that endophytic fungi RU1 and RU2 have different macroscopic characteristics. Endophytic fungus RU1 from the front has black colonies with fibrous characteristics, white borders, rough surfaces, and looks dusty due to the large number of spores. From the back, the initial white colour changes to yellowish. Meanwhile, endophytic fungi RU2 from the front are green with a few white borders, while they are golden yellow from the back. The surface is rough and fibrous because it also has many spores.

Microscopic identification was carried out using the slide culture method at Mid-Range magnification. Endophytic fungi can be identified

by their characteristics, seen from the hyphae and conidia. The results of the microscopic identification of endophytic fungi RU1 and RU2 can be seen in Figures 6 and 7.

Based on Figures 6 and 7, microscopic observations of endophytic fungi RU1 and RU2 show several differences. Endophytic fungi RU1 had hyaline, septate, and branched hyphae, with small conidia that form round chains and spread to cover vesicles that form jets. The conidiophores were hyaline, pale brown at the apex, with rounded tips emerging from the hyphae. Meanwhile, endophytic fungi RU2 also have septate and branched hyphae, but their conidiophores have rough walls, especially at the apex. The conidia are small, round, and form chains.

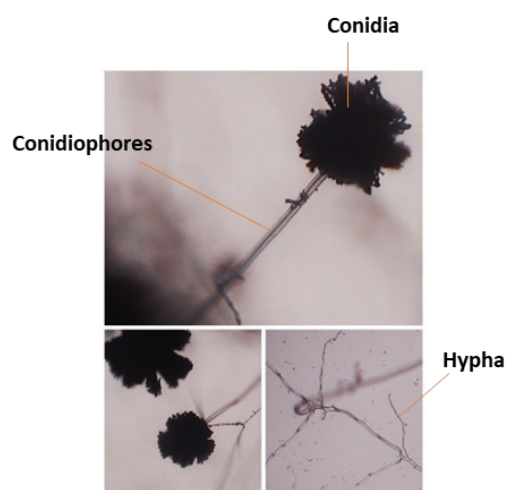


Figure 6. Microscopic isolate of endophytic fungus RU1

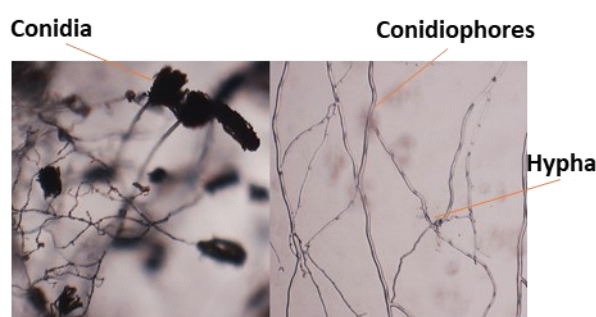


Figure 7. Microscopic Isolate of Endophytic Fungus RU2

Macroscopic and microscopic characteristics are referenced from the book *Fungi and Food Spoilage and Pictorial Atlas of Soil and Seed Fungi* [14] [15]. Therefore, both isolates can be categorised as *Aspergillus* sp. This study selected two fungal isolates from the same genus to evaluate the potential content and bioactivity of the two.

Cultivation and Extraction. Some literature states that using Potato Dextrose Broth (PDB) medium for cultivating endophytic fungi for 3-4 weeks at a temperature of 25-27°C can effectively produce biomass and secondary metabolite compounds. During this period, the fungi enter a stationary phase, which is characterised by a change in the colour of the medium as an indication of metabolite formation [16] [17]. In addition, the pH of the medium was set at 5 to support optimal fungal growth. This was explained in a study emphasizing the important role of pH in the metabolic process of endophytic fungi, which is optimal in environments with a pH below 7 [18].

After the cultivation process, endophytic fungi were extracted using ethyl acetate solvent to obtain soluble compounds. Ethyl acetate, as a semipolar solvent, is capable of extracting semipolar compounds from fungal cultures. The resulting extract is then evaporated using a rotary evaporator and dried in an oven at 45°C. The concentrated extract obtained is ready for further analysis [19].

Phytochemical Screening. The qualitative test results of ethyl acetate extracts from endophytic fungi and host plants are indicated by changes in the colour of the reagents used during testing. The results of phytochemical compound screening can be seen in the table 2.

Table 2. Phytochemical screening results

Isolate Code	Test Results		
	Plant Host	Isolate RU1	Isolate RU2
Alkaloid	(+)	(+)	(+)
Tannin	(+)	(-)	(-)
Flavonoids	(+)	(+)	(+)
Saponins	(-)	(-)	(-)
Steroid	(+)	(+)	(+)
Terpenoid	(-)	(-)	(-)

Screening results of phytochemicals in plant hosts and isolating *Aspergillus* sp. identify positive flavonoid compounds, alkaloids, and steroids. Flavonoid compounds with a double-conjugated bond, potential as an active material sunscreen [6]. Tannin is compound polar phenolic with characteristic astringent that works as defense to pathogen [20]. Compounds that form a hydrogen bond with the protein in polar solvents late in the process. Screening results simi-

lar phytochemicals between extract endophytes fungi and plant hosts show that endophytes fungi can produce the same compound, possibly because coevolution and genetic transfer make endophytes fungi a source alternative for metabolite secondary [7]. Changes in production metabolite secondary, which is influenced by internal plant factors or environmental stress, can trigger the endophyte to activate genes as an adaptation to climate change [21].

Endophytic fungi extract *Aspergillus* sp. also contains compounds similar to flavonoids, alkaloids, and steroids, with colour fluorescence showing similarities to plant hosts. Although there are differences in colour fluorescence at UV 365 nm, different colour patterns emerge due to variations in composition and biochemical conditions in each organism. The similarity in Rf values of 0.62 in the *Aspergillus* sp extract and the host plant indicates that the tested samples contain the same group of compounds, namely alkaloids [22].

SPF Test Results. Determination of SPF value of extract mould endophyte sendok leaves was done in vitro with a UV-Vis spectrophotometer at long wavelengths 290-320 nm, which includes UV-B rays, causing erythema. SPF value of the extract can be seen in the table 3.

Table 3. SPF value of endophytic fungal extract of Sendok leaves

Concentration	SPF Value And Category	
	RU1	RU2
100 ppm	6.44	13.75
	(Medium Protection)	(Maximum Protection)
200 ppm	14.24	22.98
	(Maximum Protection)	(Ultra Protection)
300 ppm	22.68	33.7
	(Ultra Protection)	(Ultra Protection)
400 ppm	32.7	40
	(Ultra Protection)	(Ultra Protection)
500 ppm	38.47	40
	(Ultra Protection)	(Ultra Protection)

The average measurement of SPF values is presented in Table 4. A concentration of 500 ppm provides the highest SPF value. SPF value increases along with improved concentration because the amount of the compound metabolite increases. Extract isolate RU2 showed the highest SPF value compared to RU1, with category maximum at 100 ppm

and ultra category at a higher concentration. Although TLC and phytochemical tests show similarity in glow colours and groups of compounds, differences in SPF value can be caused by variations in concentration or chemical structure of chemicals, which affect their effectiveness as a material active sunscreen [23].

Flavonoids, alkaloids, and steroids in endophytic fungal extracts, especially flavonoids with aromatic rings and chromophore groups, namely conjugated double bonds, play a role in light absorption so that they can absorb UV rays and prevent the occurrence of adverse effects on the skin. When UV rays are absorbed, electron excitation and delocalisation occur, which then release energy at a lower level when returning to a stable state [24]. The flavonoid structure, with two aromatic rings and an oxygenated heterocyclic ring, allows UV light absorption and structural changes.

The effectiveness of sunscreen is measured by the SPF value, which indicates the level of protection from UV rays. The higher the SPF value, the better its protection. A compound with an effective active can give optimal protection even at low concentrations. Durability sunscreen is rated with multiple SPF values, with 10, where skin blushes after 10 minutes without protection [25]. In this study, the highest SPF value was obtained from the ethyl acetate extract of the endophytic fungi of the Sendok leaves, namely at a concentration of 500 ppm, with an ultra protection category that can protect the skin for 6-7 hours. The high SPF value on both isolates of mould endophytes and the similarity results. Phytochemistry test showed relatedness component active with UV protection, supporting the potential isolation of *Aspergillus* sp as a material active for effective UV protection.

Conclusion

This study concluded that ethyl acetate extract from endophytic fungi of *Plantago major* L. leaves was identified as *Aspergillus* sp. based on macroscopic and microscopic observations. The endophytic fungal extract demonstrated protective properties against sunlight. This makes the endophytic fungal extract a potential active ingredient for sunscreen. SPF testing on the fungal extract isolate RU1 yielded the highest SPF value at 500 ppm, which was 38.47, while isolate

RU2 had a value of 40.

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